EFFECT OF CHLORTETRACYCLINE AND MINERAL SUPPLEMENTATION ON GRAZING STEER PERFORMANCE

By

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INTRODUCTION - CHLORTETRACYCLINE

The beneficial effects of antibiotic addition to poultry and swine rations to promote growth has been well established. Many scientists have demonstrated that ruminants obtain only limited benefits from supplemental feeding of antibiotics. However, under certain conditions, others have observed very positive effects. These effects have not been completely described or understood, but during the past 20 years it would have been difficult for the livestock and poultry industries to economically supply the high quality meat, milk and eggs that we have today without the use of antibiotics and other antibacterial agents.

Experiments since 1951 have shown that chlortetracycline added to the rations of cattle will stimulate gain and feed efficiency. However, there have been some studies where either no significant affect or a decreased digestibility were obtained with a concurrent reduction in weight gain and efficiency of feed utilization. Phillison (1970) postulated that physiological and biochemical responses of the caudal portion of the ruminant digestive tract, beginning with the abomasum, acts similarly to the same portion of the digestive tract of monogastrics. Newman (1951) observed that total bacterial counts in the rumen ingesta of chlortetracycline-fed heifers were about the same as in control heifers, although the types of microorganisms were less diverse when chlortetracycline can inhibit some of the naturally-occurring rumen flora. However, Russof (1954) demonstrated that the mode of action of chlortetracycline in stimulating growth in young calves was not because of its action in the rumen. It was also suggested that bone metabolism may be the area in which chlortetracycline exerts its influence; furthermore, some studies indicate that general growth was stimulated, and it is possible that the pituitary growth hormone is involved.
It would be beneficial to determine if, by incorporation of an antibiotic into the diet of grazing ruminants, an improved rate of gain and efficiency of production could be obtained as presently observed in monogastric animals. This could potentially help in those situations where high levels of production are desired even though poor environmental conditions may exist.

There has been a reluctance to feed antibiotics to mature cattle, based on the adverse effects of chlortetracycline on rumen fermentation and function. But during the past 30 years, extensive research has been conducted and response criteria as cited by Riley (1983), have been: (1) increased gains, (2) decreased feed required per kilogram of gain, (3) reduced incidence of liver abscesses, (4) reduced incidence of shipping fever, (5) disease control and prevention, and (6) increased ease of adaptation of cattle to feedlot or pasture conditions after shipment. Chlortetracycline has been utilized extensively in beef cattle production, mainly in concentrates and protein supplements. However, it has recently been shown that cattle grazing diverse pastures benefit from the administration of chlortetracycline even without supplemental feeding of protein or grain.

Since chlortetracycline has been studied extensively in growing and finishing cattle, and only limited data is available for grazing animals, this investigation was conducted to determine if the administration of supplemental levels of chlortetracycline, the most used antibiotic in ruminant nutrition, would improve the daily growth of steers grazing native range.
INTRODUCTION - CALCIUM AND PHOSPHORUS

Much information has been accumulated on mineral functions in the animal body, efficiency of mineral utilization, requirements of minerals for various purposes, interactions among minerals, symptoms that develop when mineral requirements are not satisfied by the food supply, and mineral supplements which will avert diseases associated with mineral deficiencies or imbalances.

The two most studied minerals are calcium and phosphorus, but there are still many processes and mechanisms not fully understood. Optimum levels of calcium and phosphorus have not been precisely established, but sufficient experiments have been conducted to determine the recommended dietary levels. It is known that calcium and phosphorus requirements vary continuously, but animals can not, in practical feeding, be nourished in a correspondingly changing fashion, because of the time and cost involved.

It is important to provide an adequate mineral supply, mainly calcium and phosphorus, in the diets of farm animals, otherwise appetite will be impaired, inefficient food use will result, followed, in severe cases, by pathological symptoms which may cause death.

When mineral supplements are required, the choice should be made among those minerals known to be highly available to the animal. Total mineral content of the diets should be kept as low as possible, not only because of the deleterious effect of excesses, but also because minerals contribute no food energy to the animal. Farm animals must have minerals, but minerals in moderation.

Numerous experiments and observations have shown that grazing beef cattle require calcium and phosphorus in their diet for normal processes of growth, fattening and reproduction. Because of the difficulty of exerting close control on
mineral intake of grazing cattle, scientists have been trying to establish the optimum and minimum amounts of calcium and phosphorus required for fattening and growing grazing cattle.

Considering all the aspects mentioned above, an experiment was designed to determine the effect of calcium and phosphorus supplementation on weight gain of grazing steers.
LITERATURE REVIEW - CHLORTETRACYCLINE

A revolution in agriculture has taken place since 1930 in the United States. A steady improvement in livestock production has been necessary to meet increasing demand for animal products, even though there has been a continuous decline in farms and farmers.

Part of the improvement has resulted from breeding for increased production. Another has been farm mechanization which enables one person to care for more animals than previously. A third key factor has been the tremendous improvement in nutritional quality of today's cattle diets when compared to those of twenty five years ago. This improvement in the nutritional quality has been achieved mainly by the processing of feeds, but a very important aspect has been antibiotic usage.

Since the discovery of chlortetracycline (CTC) in 1948 and terramycine in 1950, the literature has been replete with literally thousands of papers describing the value of these drugs in a large number of clinical and subclinical bacterial and rickettsial disease situations. However, specific activities and functions of an antibiotic in the digestive system of the ruminant have not been completely established with clearly defined experimental procedures.

CTC (7-Chlortetracycline) has been reported to be primarily produced by many strains of *Streptomyces aurofaciens*, but the species *sayamaensis*, *lusitanus*, *omiyaensis* and *viridifaciens* also produce the antibiotic (Weinstein, 1978). CTC is an amphoteric compound with a yellow color in all its crystalline forms. The structural form shown in Figure 1 is: 7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-11-dioxo-2-naphthacenecarboxamide.
(Weinstein, 1978). The chlortetracyclines are primarily bacteriostatic, and are believed to exert their antibiotic effect by inhibition of protein synthesis.

(A) THE ANTIBIOTIC RESPONSE.

The term "antibiotic response", when used to describe results observed following the feeding of antibiotics to animals, is generally accepted to mean the difference in weight gains and feed conversion between control animals and antibiotic-fed animals.

The veterinarian, the nutritionist and the livestock producer, while curious about the basic mechanism of antibiotic action, are more interested in learning about how antibiotics improve the performance of animals. The fact that antibiotics are strikingly dissimilar chemically is probably the strongest argument against considering the possibility that the antibiotic response results from a direct action on the tissues when fed to animals. It is hard to imagine that all antibiotics would affect the same metabolism process or that the same end results would be observed if antibiotics affected different processes. Finally, the lack of toxicity of some of the most effective antibiotics suggests that these drugs have little or no direct affect on the metabolism of the host (Maddock, 1956).

All antibiotics have a common denominator; that is, the ability to suppress the growth of many microorganisms. It is on this fact that the explanation for an antibiotic response probably rests. An antibiotic which has a direct suppressive effect on a certain species of bacteria would have a beneficial or positive effect if that particular species were injurious or pathogenic. Some investigations have suggested that the antibiotic effect occurs indirectly. That is, the various species of bacteria in the digestive tract probably exist in intense competition with each other. If an antibiotic were to attack the competitor of the presumably good
FIGURE 1

CHLORTETRACYCLINE
-(Aureomycin)

Adapted from "Antibiotics" by Weinstein et. al. (1978).
species, this latter species might be permitted to flourish and thus might produce more nutrients for the host (Maddock, 1956).

As a summary, Stokstad (1954) proposed the following possibilities regarding the actual mechanism involved:

1) Increased bacterial synthesis of essential or stimulatory growth factors.
2) Inhibition of bacteria which compete with the host for essential nutrients.
3) Inhibition of microorganisms which are deleterious because they produce toxic compounds or damage the intestinal tissues.

(B) MODE OF ACTION OF ANTIBIOTICS IN NUTRITION

I.) Disease Control Effect.

Weiner (1972) referred to Groskke's work in 1950 which suggested that antibiotics stimulate growth indirectly by changing the intestinal flora from undesirable to desirable types, which synthesize unknown growth factors. Radison et. al. (1956) demonstrated that the feeding of low level CTC induced increased sensitivity of intestinal bacteria to the host defense mechanism. In other words, he stated that phagocytic activity of leucocytes was determined by their ability to phagocytize bacteria in vitro. Bacteria isolated from calves receiving CTC were more sensitive to phagocytosis than bacteria isolated from calves fed control rations. In a later work, MacFadden and Bartley (1959) inferred that a relationship might exist between age of the calf, development of a defense mechanism and the resultant growth response to antibiotic feeding. In their experiment they found that feeding CTC aided the host defense mechanism, so by the fifth week a near maximum phagocytic activity against intestinal coliform bacteria was reached. They postulated that the improved growth and feed efficiency was, at least in part, a result of the effect of the antibiotic on the alteration of the virulence of
the microbes due to contact and hence the increased susceptibility to host phagocytosis.

Rusoff et al. (1953) concluded that the growth stimulating effect of CTC in calves apparently is not due to selective inhibition of the common bacterial groups of the intestine, namely, coliforms, enterococci, and Clostridium perfringens. In an other study, Rusoff (1953) reported that the increased growth obtained from the parenteral administration of CTC to young calves could not accrue from its action in the rumen, since it has been suggested that injected antibiotics enter the intestine via the bile, thus by-passing the rumen. Injected CTC is excreted mainly in the urine, whereas oral CTC is excreted primarily in the feces. The fact that CTC is present in the bile and urine of the oral-fed calves indicated that some of the antibiotic is being absorbed and excreted by these pathways. It should be pointed out that no CTC was detected in the blood of either group of calves.

Finally, Jukes and Williams (1953) state three possible ways in which an antibiotic may favorably affect the intestinal flora and hence promote growth:

1) Antibiotics may inhibit or destroy organisms which produce subclinical infections.
2) Antibiotics may produce an increase in the number and activity or organisms that synthesize known or unknown vitamins or growth factors which are eventually made available to the host.
3) Antibiotics may inhibit organisms which compete with the host for available nutrients.

A very important aspect of antibiotic usage was established by Catron (1952), who defined level of disease control as the degree or amount in which feedlot contamination with bacteria and/or virus infection decreased after
antibiotic treatment. He further stated that the higher the disease level on a farm, the greater the response to antibiotic feeding.

II.) Vitamin Sparing Effect.

Manson et. al. (1954) found that CTC supplementation stimulated the production of folic acid in the intestines of poultry by favoring the establishment of the coliform organisms that synthesize the vitamin. Guggenheim et. al. (1953) reported that certain antibiotics stimulated the growth of rats receiving suboptimum amounts of thiamine, riboflavin, pantothenic acid or pyridoxine. These workers concluded that antibiotics aid the absorption of limiting vitamins by suppressing intestinal organisms that would keep the vitamin from being absorbed by the host. If this view is correct, then the animals receiving antibiotics would be expected to excrete larger amounts of vitamins in their urine, and perhaps in their feces as well, and to accumulate larger amounts in their livers. There are many conflicting views regarding a vitamin sparing effect in antibiotic supplemented rations. Jukes and Williams (1953) reported that CTC had a sparing effect for one of the B vitamins in five out of seven experiments, while penicillin had a sparing effect for one of the B vitamins in four out of eleven experiments. Guggenheim et. al. (1953) established that the vitamin sparing effect of antibiotics may either be brought about by their effect on the intestinal flora or it may be a result from a direct action on tissue metabolism.

Certainly, CTC does not spare vitamins by replacing them in biochemical reactions in the body. This can be supported by: 1) Antibiotics completely different in structure have exerted similar effects. 2) The chemical structure of antibiotics differ completely from the structure of vitamins, and 3) The same sparing effects have been noted for nearly all the B vitamins (Maddock, 1956).
III.) Protein Sparing Effect.

Williamson et al. (1972) observed that the practice of including low levels of antibiotics in the feed of livestock and poultry has been firmly established over the last 20 years. The specific mode of action of antibiotics when used at growth stimulating levels is still debated, but it is generally recognized that improved animal performance does occur. This may be the result of sparing essential nutrients such as protein.

Hogue et al. (1957) fed milk at two levels to young dairy calves. They showed that the calves given the poorer quality diet showed a better response to added CTC (11.3 vs. 5.2 percent). Although calves on the higher milk intake had better growth, they concluded that the amount of milk fed to the calves could influence the response to antibiotics.

Klopfenstein et al. (1964), working with sheep, concluded that composition of the microbial population was altered by antibiotic treatment. They found that protozoal concentrations were significantly increased by feeding CTC. Treated animals had a higher gas production, mainly ammonia. These workers used a ration high in nitrogen, because it was believed that quantitative differences in the magnitude of protozoal metabolism could be detected more easily at high levels. Hogue et al. (1954) used high nitrogen intakes with calves, and found significantly greater apparent protein digestion and nitrogen retention when CTC was fed.

Since the net utilization of protozoa protein has been proven superior to rumen bacteria when fed to rats (McNaught et al., 1954), and since rumen protozoa have not been shown to be susceptible to antibiotics at moderate concentrations (Williams et al., 1951); it is believed that CTC can create a sparing effect of protein by favoring rumen protozoa development.
IV.) Effect on Metabolism.

Hays (1969) expressed this mode of action as pertaining to "the direct effect on the rate and pattern of metabolic processes in the host animal." This view was supported by Franklin (1963), who stated that CTC inhibits the transfer of amino acids from the transfer-RNA complex to the ribosomal protein in both, animal and bacterial systems. Allison and Barry (1955) showed that the feeding of CTC at 10 mg/454 g of feed, depressed the respiration of rumen microorganisms as well as the number of microorganisms sampled. Jukes and Williams (1953) cited work done by others that indicated penicillin prevents the uptake of glutamic acid by bacterial cells and CTC uncouples oxidative phosphorilation reaction.

Hag (1952) found that feeding CTC to lactating cows had no affect on the flora of the milk, which meant that the center of activity of CTC in the digestive system may be in the intestines of the ruminant. Against this theory, there are a number of papers showing that undoubtly some changes occur in the stomach system of the ruminant receiving an antibiotic. Newman et. al. (1951) obtained results that indicated a much less diverse flora for CTC-treated steers than for the controls.

Hester (1954) found a definite pattern of antibiotic distribution in the intestinal tract of CTC-treated calves. The antibiotic increased in concentration from the upper to the lower portion of the small intestine and to the anal end of the large intestine.

Mann (1954) presented evidence that CTC-fed calves had a larger quantity of rumen contents but less acid than controls. This provided a more suitable environment for intensive rumen bacteria and protozoa action at an earlier age.

Rusoff et. al. (1954) presented evidence which indicates that CTC stimulates the pituitary gland to produce more growth hormone, resulting in
greater bone metabolism and growth. However, Louisiana workers (Hester, 1954) did not find any measurable amounts of CTC in the pituitary gland. Lassiter (1955) theorized that one might expect to find CTC in this gland if that was the means by which the antibiotic improves growth.

Hibbs et. al. (1954) suggested that the mode of action of antibiotics was an alteration in energy metabolism, possibly involving the microflora of the rumen. These workers found that CTC-treated calves had significantly higher blood sugar levels than control calves during the 8 to 12 week age period. By chromatographic separation, it was found that the 12-week old CTC-fed calves had a lower percentage of propionic acid and higher percentage of butyric acid in the rumen juice than controls.

Hibbs and Conrad (1953) observed that CTC had no effect on total volatile fatty acids (VFA) or acetic acid content in rumen juice of 12-week old calves. Rumen propionic acid was slightly lower and butyric acid slightly higher in CTC-fed calves. In another study, Hibbs (1954) demonstrated that CTC did not affect dry matter, cellulose or protein digestion, but calves fed CTC had slightly higher retention of nitrogen. Chandler and Bliss (1948), Paine et. al. (1948), Johansson et. al. (1953) and Saz et. al. (1955) reported that certain microorganisms developed a resistance to antibiotics. All these workers based their theory on the observation of an initial shock that decreased digestibility, then as the flora recovered, digestibility was equal or slightly lower to the digestibility figures observed prior to CTC supplementation.

Murley et. al. (1951) found that CTC did not affect reducing sugars or urinary nitrogen content of calves. Fecal excretions of dry matter, reducing sugars, nitrogen, ether extract and ash were also not affected. Murley et. al. (1952) observed that when calves were fed a restricted diet, CTC produced a
slight improvement in the utilization of carbohydrates, nitrogen, ash and ether extract.

Loosli (1951) and Jacobson et. al. (1952) have proposed that antibiotic fed calves grow faster because of increased appetite. The question arises as to whether such calves eat more because of the action of the antibiotic or because of their larger size (obtained during the trial). When feed consumption for the experiment was stated in terms of feed per unit of body weight, on a weekly basis, there was little difference in feed consumption by antibiotic-fed calves and by control calves (Lassiter, 1955). Bartley (1953) found that CTC-fed calves consumed more hay and grain during the first seven weeks of age, but not from birth to 12 weeks of age. Mochrie et. al. (1953) presented data on calves from birth to five weeks of age as well as from birth to 16 weeks of age. In the 16-week study, CTC improved calf growth approximately 5%. The results obtained by these workers do not answer the original question, but suggest that much of the increased feed consumption by antibiotic-fed calves may result from larger calves. If antibiotics affected the appetite of calves directly, those fed antibiotics would consume more feed per unit of body weight.

Chance (1953) showed that the intake level of an antibiotic apparently determines the rate at which food passes through the digestive system. It has been observed that the rate of passage of dry matter, crude fiber, crude protein and nitrogen-free extract was increased when 500 mg of Aureomycin (CTC) was fed for a period of 15 days. These workers stated that the rumen pH of steers was lower when the control ration was fed that when the ration was supplemented with CTC. In addition, rumen bacteriological data showed a definite increase in total count when 500 mg of CTC was fed and a further increase in total count when the CTC was increased to 1 g per day. In the same experiment, these Michigan workers
found a reduction in the number of rumen streptococci and a slight increase in the coliform groups.

It appears that the effect of CTC on blood sugar content and rumen fatty acid levels is secondary rather than a primary reason why CTC improves growth rate. The only other clearly demonstrated effect of CTC on metabolic processes is a depressing effect on blood non-protein-nitrogen content as reported by Lassiter et al. (1955).

(C.) EFFECT OF ANTIBIOTICS ON BLOOD CONSTITUENTS.

Various workers, Fincham (1953), Mochrie (1953) and Rusoff (1954) have studied the effect of antibiotics on such blood constituents as erythrocyte count, hemoglobin percentage, packed cell volume, red blood cell counts, corpuscular volume, corpuscular hemoglobin concentration, plasma "Allen" fat levels, plasma calcium and inorganic phosphorus levels, and the blood levels of various vitamins, but have found none of these constituents to be affected significantly by the feeding of CTC. Russof et al. (1954) reported a lowered body temperature in Holstein calves receiving CTC, but no apparent differences in erythrocyte counts, hematocrit value, plasma Ca or plasma inorganic P values. There was a slight decrease in leucocyte counts for CTC supplemented calves.

(D.) ANTIBIOTIC CONTENT IN TISSUE AND DIGESTA.

There are two routes through which the use of antibiotics in animal feed may pose a threat because of possible emergence of resistant bacteria. One is that there might be a direct transmission from animals to man. This most likely would be seen among people intimately involved with the raising and care of animals: veterinarians, animal handlers, farmers and ranchers. The other route is an
apparent risk of indirect transmission; that is resistant bacteria present in the intestinal tract of animals passing through the food chain, being consumed, and then establishing themselves in the intestine of the human that consumes the meat.

Bird (1952) stated that results thus far indicate no significant accumulation of antibiotics in flesh. Jukes and Williams (1953) stated that the amount of CTC in the diet of calves required to give detectable amounts in the tissues was at least 20 times as great as that commonly used for nutritional purposes, and that cooking destroyed CTC in all the tissues tested. Luther et. al. (1953, 1954) did extensive work determining the antibiotic content in livestock tissues. They concluded that residual antibiotic activity is not found in the tissues of animals receiving the normal level of antibiotic supplementation given for growth stimulation.
LITERATURE REVIEW - CALCIUM AND PHOSPHORUS

Researchers and producers recognize the importance of providing adequate levels of nutrients, including minerals, when developing supplemental feeding programs for cattle. Special attention must be given to calcium and phosphorus when balancing diets. Mitchell et. al. (1947) indicated that grains and grain products are inadequate in calcium, but less often inadequate in phosphorus. Protein concentrates of plant origin are again inadequate in calcium, but adequate in phosphorus; while those of animal origin, to the extent that they contain bone, are rich in both calcium and phosphorus. Legume forage and hay crops are rich in calcium, but of variable phosphorus content depending on the available phosphorus level in the soils upon which they are grown. Non-legume pasture crops and hays are not particularly reliable sources of either calcium and phosphorus, again depending upon soil conditions.

In addition to the variations in the calcium and phosphorus concentrations because of the type of feedstuff, there is a seasonal variation. Calcium and phosphorus levels in range grass are low in the fall, but during the winter and early spring these levels may be even lower because of the weathering processes. This introduced the idea of continuous feeding of calcium and phosphorus supplement to grazing cattle (Knox, 1942).

(A) THE ROLE OF MINERALS IN THE ANIMAL BODY.

Minerals and trace elements often received little attention in practical feeding and nutrition research. However, this is an important and complex area, since 21 elements have been established as essential or probably essential for animals. While many similarities exist in functions, metabolism, supply, and practical importance between two or more mineral elements, the differences are sufficient to make each element a separate story requiring individual consideration.
The functions of mineral nutrients are varied and numerous, but they may be classified, according to Mitchell et. al. (1947) under four categories:

1) They contribute to the structure of the body. For example calcium, magnesium and phosphorus are important constituents of bone and therefore constitute a growth requirement.

2) They aid in maintaining the status quo of the tissues already formed thereby guarding against the constant erosion of the life processes. This is a maintenance requirement.

3) They participate in the functional activities of the body, such as muscular activity. This may or may not lead to an increased requirement in the food supply. Reproduction, lactation, and egg production will increase mineral requirements in proportion to the mineral content of the products formed.

4) They function as integral parts of the enzyme systems, and aid materially in metabolizing the organic matter making up the bulk of animal diets.

Mitchell et. al. (1947) also stated that minerals are of such importance in the utilization of carbohydrates, fats and proteins, that the amounts of individual minerals required by the animal will parallel the amounts of organic nutrients consumed.

Hubbert (1958) indicated that only limited attention had been given to the inorganic nutrient requirements of the rumen microflora, although the ash of such materials as alfalfa, and molasses had been found to stimulate both in vitro and in vivo cellulose digestion. These Iowa workers found that the addition of 50 to 300 mcg. of calcium per ml of fermentation medium, appeared to increase cellulose digestion by approximately 10%. This increase made it appear that the rumen microorganisms have a requirement for calcium. Burroughs et. al. (1951) included calcium among the minerals required by the rumen flora. Furthermore, it was
shown that rumen microorganisms have a considerable tolerance to excess levels of calcium in the fermentation medium.

(B) FUNCTIONS OF CALCIUM IN THE ANIMAL BODY.

As cited by Wasserman (1960), calcium has several important functions within the animal:
1) An essential element in the conversion of prothrombin to thrombin in the blood clotting mechanism.
2) A necessary ion (but replaceable by strontium) in promoting normal cardiac, smooth and skeletal muscle function.
3) Essential for the normal functioning of nervous tissue (in a low calcium environment, the nerve becomes hyperexcitable and leads to the formation of spontaneous impulse transmissions associated with clinical "tetany").
4) Maintenance of normal environment and in the development of the bio-electrical potential at the cell surface.
5) A major constituent of the skeleton and teeth.

Wasserman et. al. (1960) pointed out that selecting the most important physiological function of calcium would be meaningless contemplation, since calcium is essential for productive metabolism and for life itself; on a quantitative basis, emphasis is usually given to the hard tissues of the body because about 99% of the body calcium is found in the bone and teeth.

The functional roles of calcium in the soft tissues of the body are so critical that calcium level of the blood plasma is one of the most closely regulated (Miller, 1970). It has been said that the regulation of plasma calcium content is quite complex with many practical implications. The regulation involves several hormones, including the parathyroid hormone, calcitonin, estrogens, and others. When plasma calcium starts to decline, calcium is mobilized from the bone, bringing plasma calcium back to normal. Bones provide substantial reserves of
calcium when dietary intake is deficient. Miller (1979) mentions that calcium withdrawal from bones over relatively short periods with subsequent replacement is not harmful. Even though, the calcium reserves of the bone serve a very vital function, they are exhaustible. Becker et. al. (1953) says that if calcium depletion is sufficiently advanced, the bones become very soft and easily broken. Perry et. al. (1981) mentioned that when there is either too little calcium in the diet or poor utilization, growing animals develop a condition known as Ricketts. If this condition is sufficiently severe, there may be bending and turning of the long bones.

The physiological mechanisms controlling the absorption of calcium are not completely known, but Schachter and Rosen et. al. (1959) have shown that Ca can be transported against a concentration gradient from the mucosal to the serosal side of the intestinal membrane. Prior to the studies of Schachter and Rosen, others had postulated the existence of a calcium-transport system, based upon the observation that calcium absorption is narrowly controlled and is somewhat independant of dietary calcium intake. Also, it has been observed that animals on low calcium diets are more efficient absorbers of dietary calcium than animals on high calcium diets; and this adaptation may be mediated through an "active" transport system (Gershoff, 1958). This is in accordance with the findings of the NRC (1978) which stated that when dietary calcium is low, cattle absorb a higher percentage of that ingested. Likewise, with an excess in dietary calcium, the percentage absorbed declines.

Miller et. al. (1979) stated that all the changes in absorption of calcium occur fairly quickly, enabling animals to meet their calcium needs over a wide range of intakes without accumulating excess calcium in the body. Besides variations in the absorption of calcium because of different intakes of the mineral, it is generally recognized that as an animal grows older, there is less efficient
utilization of ingested calcium (Hansard, 1954). It has not been clear, however, whether this decreased efficiency with age is because of poor absorption from the gastrointestinal tract or poor retention by the tissues or both.

Excretion of calcium increases with increasing age. Hansard et al. (1954) observed after an oral administration of labeled calcium, that the total excretion ranged from 3% in the young calf to 62% in six-month old calves, and 83% in 160-month cattle. This indicated there was little absorption in aged animals, even when large amounts of available calcium were present, and that oral therapy of additional calcium, even when adequate phosphorus was present, did not compensate for the loss of calcium from the body.

Hansard (1957) found that calves absorbed 90% of the calcium in milk. In older cattle, true absorption was quite variable, ranging from 22 to 55%, but averaging 45%. Calcium in milk-fed calves is more efficiently absorbed and retained than calcium supplied in rations consisting of forages and concentrates.

Hansard et al. (1957) also found evidence that inorganic sources of calcium are more available than organic sources. However, differences due to animal age were greater than those associated with differences in feed source.

Oltjen (1975) reported that calcium absorption may be influenced by other factors including vitamin D, phosphorus intake, and acid-base balance. High fat diets reduce calcium availability by increasing fecal calcium losses through the formation of calcium soaps. Viseck et al. (1953) found that endogenous fecal calcium was not significantly changed when dietary calcium varied from normal to a high level, and in some animals, a low calcium diet decreased endogenous fecal calcium.

Drake (1964) cited studies done by Robinson and Huffman in 1925, in which plasma calcium for cattle ranged from 7.7 to 14.7 mg percent with an average of 11 mg percent and a daily variation of 2 mg percent. Later research by Henderson
and Weakly (190) found that the blood of calves fed rations low in calcium had just as much calcium as animals fed rations rich in calcium. In general, as the animals became older, there was a slight lowering of the calcium concentration in the blood.

Huffman (1933) noted that blood calcium values were highest when phosphorus values were lowest in the presence of a narrow Ca:P ratio and elevated when dietary calcium was high in relationship to dietary phosphorus. However, in no case was serum calcium level markedly changed. Dietary calcium level did not influence serum calcium level.

Greaves et al. (1934) found that the calcium content of the blood ranged from 12.5 to 13.13 mg percent. These workers noted a low negative correlation between inorganic phosphorus and calcium of the blood. Stanley (1938) reported that the calcium content of blood serum varied from 10 to 12 mg percent in grazing cattle.

Jorgensen (1976) cited that normal plasma concentration of calcium in dairy cows was 8.5 to 11.5 mg percent.

Wasserman (1960) said that although you can find opponents and proponents, the function of vitamin D in the metabolism of calcium has reached a general agreement, that the primary physiological action of vitamin D is the enhancement of calcium absorption by the intestinal tract.

(C) FUNCTIONS OF PHOSPHORUS IN THE ANIMAL BODY.

There is an enormous amount of information showing that cattle require phosphorus in their diet to accomplish normal development.

Without supplementation, except for salt, phosphorus deficiency is the most widespread and economically important mineral problem in grazing cattle or in those fed forages (Underwood, 1966).
Wasserman (1960) mentioned the following phosphorus functions:

1) Essential for anabolic and catabolic reactions in the body, as exemplified by the role of phosphorus in high energy linkage formation.

2) A precursor for biologically significant compounds such as phospholipids, which are important in the formation of cell membranes and cell permeability.

3) A precursor in the synthesis of genetically significant substances, particularly deoxyribonucleic acid (DNA).

4) A contributor to the buffering capacity of the body fluids and cells.

5) A major constituent of bones and teeth.

Like calcium, the majority of the body phosphorus is located in the hard tissues. In the adult, about 85% of the total body phosphorus is found in bones and teeth, and the balance in the soft tissues. Black et al. (1943) noted that about 1% of the skeletal phosphorus was "labile" phosphorus, and this "labile" pool was fifteen times as large as the total circulating phosphorus in the plasma.

Knox et al. (1942) reported that the feeding of phosphorus-bearing minerals to livestock in phosphorus deficient areas may be expected to produce some, if not all of the following beneficial results:

a) More regular breeding of females.

b) More living calves.

c) Young animals that are stronger at birth.

d) Heavier calves and lambs at weaning time, largely as a result of greater milk flow of their dams.

Black et al. (1943) studied the effects of supplemental phosphorus for cattle grazing phosphorus deficient areas. One hundred Brahman-Shorthorn and Brahman-Hereford heifers were used. Heifers receiving phosphorus had better health, vigor and thrift than the control groups. The supplement-fed groups produced, on the average for a two-year period, 60% more beef (7,208 pounds) than
the controls as measured by weight of the calves at weaning time. The primary advantage in feeding phosphorus appeared to be an increase in calf crop percent and greater weights attained by the offspring.

The mechanism of phosphorus absorption by the gastrointestinal tract is not completely known, but investigators have been hesitant to postulate a transport system. McHardy and Parsons (1956) have shown the increasing levels of phosphate ions in the intestinal lumen, linearly enhanced net absorption, which is a typical response of a diffusion-like process. From the data obtained by these workers, Wasserman (1960) concluded that the phosphate ion is readily absorbable. Cohn and Greenberg (1939) found that vitamin D did not enhance phosphate absorption in rats raised on a vitamin D deficient, high calcium-low phosphorus diet. Wasserman (1960) established that phytate phosphorus may not be as available as inorganic phosphate, and that vitamin D seems to enhance the availability of phytate phosphorus.

Animals do not have hormones to readily mobilize phosphorus from the bone in response to the animal's needs. In fact, phosphorus is withdrawn along with calcium from bone in response to calcium needs (Miller, 1979). The inability to finely regulate inorganic phosphorus in blood plasma results in more deficiency signs at earlier stages than occur with insufficient calcium. Eckles et al. (1932) found that when too little dietary phosphorus is given to dairy animals, appetite declines, growth rate is reduced, milk production is lowered, and the efficiency of feed utilization decreases. Beeson et al. (1941) found that animals given ample phosphorus utilize their feed better and gain more per unit of feed consumed than do animals on a low-phosphorus diet. A very important aspect of this experiment was that, on the same food intake, the low-phosphorus animals gained less per unit of feed consumed. This indicates that a low-phosphorus regime interferes with the utilization of feed, even before the appetite has decreased.
The inefficient utilization of food in the first stages of aphosphorosis is due to some disturbed metabolic process and not to low food intake. The exact physiological action associated with the loss of appetite is not known, but this phenomenon is common to most nutritional deficiencies (Miller, 1979).

In another experiment by Beeson et. al. (1941) steers fed a low-phosphorus ration had a lack of appetite after 112 days. Also associated with the deprived appetite was wood chewing and eating of soil.

Miller et. al. (1979) also found that deprived appetite manifested chewing wood, bones, and hair in phosphorus-deficient animals. However, cows may suffer from severe phosphorus deficiency without exhibiting a deprived appetite. Therefore, the early effects in cows of a phosphorus-deficient diet is poor utilization of feed and reduced gain. Continued deficiencies adversely affect reproductive performances.

Burroughs et. al. (1956) suggested that phosphorus requirements for weight gain and feed efficiency are greater than for bone development or maintenance of plasma inorganic phosphorus level.

Drake et. al. (1964) believed that phosphorus may be involved with the conversion of carotene to vitamin A and the storage of vitamin A in the liver of cattle. Gallup et. al. (1953) found that plasma carotene levels were consistently higher in phosphorus deficient steers and cows, and that a decreased vitamin A storage in the liver was indicated. However, in a similar study (Gallup, 1953) with sheep, the results were opposite of those obtained in cattle. This would indicate potential species differences concerning the relationship of vitamin A and phosphorus.

Phosphorus may function as a buffer for volatile fatty acids (VFA) produced in the rumen of cattle and sheep (Smith et. al., 1956). Parthasarthy et. al. (1952)
found that the rumen liquor may contain as much as 25 mg percent of inorganic phosphorus.

Farris et al. (1958) noted that the addition of one thousand mcg of phosphorus per ml of rumen fermentation medium did not depress cellulose digestion. It was necessary to deplete the microorganisms of phosphorus by incubating them for 24 hours in a phosphorus-deficient medium before the addition of phosphorus would increase cellulose digestion. Hall (1961) used washed suspensions of rumen microorganisms to study various levels of phosphorus in different chemical forms upon cellulose digestion. Marked increases in cellulose digestion occurred when levels of 20 to 120 mcg of phosphorus per ml were added.

Ortho and phytin forms of phosphorus have been shown to enhance rumen microbial activity by Raun et al. (1956). However, very little is known about the availability of meta and pyro forms to rumen microbes. Ammerman et al. (1957) showed that phosphorus in gamma calcium pyrophosphate was almost totally unavailable while that supplied by vitreous calcium metaphosphate appeared to be only 50% as available as phosphorus from monocalcium phosphate. However, Tillman and Brethour (1958) using radioactive isotope techniques, found that phosphorus was equally as available in acid sodium pyrophosphate as it was in monosodium phosphate.

Phosphorus is so largely concerned in the utilization of the main organic nutrients (Mitchell, 1947) that it is logical to assume that the requirements for this element by cattle should parallel the amount of organic nutrients consumed.

The most effective means of diagnosing a phosphorus deficiency appears to vary somewhat in different circumstances. For example, as cited by Miller (1979), it was suggested by Underwood in 1966 that the most sensitive and earliest biochemical measure of a phosphorus deficiency was reduced serum inorganic phosphorus. In contrast, the Netherlands Committee on Mineral Nutrition (1973) did
not consider serum inorganic phosphorus to be sensitive enough to recommend it in diagnosing deficiencies in cattle.

Tillman and Brethour (1959) determined with cattle, that when criteria were weight gains, feed consumption, efficiency of feed utilization, digestibility or net retention, 2 g of phosphorus per 45.4 kg body weight did not meet the phosphorus requirements of these animals. When response criteria were bone growth, as measured by autoradiographs and plasma inorganic phosphorus level, 2 g of phosphorus per 45.4 kg of body weight, met the phosphorus requirement.

In Cornell experiments, Wise et al. (1958) demonstrated that 0.22% phosphorus in the air dry ration was sufficient for maximum growth rate in young dairy animals, but bone ash was higher when 0.30% was fed. Mitchell (1947) stated that in early growth about 18.16 g of phosphorus was required per 454 g of protein, while in the mature steers the ratio was approximately 9.08 g (or less) of phosphorus per 454 g of protein.

The recommended nutrients by the NRC (1978) are based on phosphorus availability. The percentage of phosphorus availability declines from 90% for young calves, to 55% in cattle over 400 kg. For finishing cattle, maintenance requirements were estimated on the basis of 2 g of phosphorus and 1 g of calcium per 100 g of maintenance protein. The phosphorus content of the expected gain was calculated as 43.7 g of phosphorus per kg of protein deposited, and calcium was assumed to be 1.7 times the phosphorus content (NRC, 1976).

Except for the limited research indicating adverse effects on performance when the diet contains more phosphorus than calcium, information on excess or toxic levels of phosphorus and on the maximum safe level is meager. One reason may be the relatively high cost of phosphorus. Since large amounts of phosphorus are required, the cost of providing phosphorus is substantially greater than for any other mineral element (Miller, 1979). Two of the most often used phosphorus
supplements are dicalcium phosphate and defluorinated rock phosphate. Smaller amounts of steamed bone meal are utilized. In manufactured feeds and liquid feed supplements, phosphoric acid and/or ammonium polyphosphates are commonly used (Miller and Stake, 1972).

Robinson and Huffman (1925) showed that normal cattle blood contained an average of 5.87 mg inorganic phosphorus per 100 ml of blood, with a range of 3.00 to 8.99. The maximum 24-hour variation was 1.87 mg inorganic phosphorus per 100 ml of blood.

Malan et al. (1928) studied the composition of bovine blood while grazing phosphorus deficient pasture and found a major characteristic to be low inorganic phosphorus with a correlated reduction in total phosphorus. He also demonstrated that the total phosphorus and inorganic phosphorus were about twice as high in lamb blood as in maternal blood.

Palmer et al. (1926) noted that inorganic blood phosphorus in individual cattle may vary markedly from day to day and even from hour to hour. Exercise caused a dramatic change in blood phosphorus of cattle with a definite increase followed by a steady decrease which persisted for several hours. Feeding had a small replenishing affect on blood inorganic phosphorus. The value rose within the first hour and apparently did not return to normal until approximately 3 hours later. Normal water consumption had no significant affect.

Huffman et al. (1933) observed that inorganic blood phosphorus of dairy cattle maintained on a low-phosphorus ration dropped from an average value of 6.9 mg to 4.2 mg per 100 ml within one month. Dairy cattle provided supplemental phosphorus had an inorganic phosphorus level of approximately 8.0 mg per 100 ml.

Haag and Jones et al. (1935) found that the normal decline of blood plasma inorganic phosphorus with age may continue well into the third or possibly fourth year, and that the average normal value for mature cattle was approximately 5.2
mg %. VanLandingham et al. (1935) also demonstrated that there was a definite decline in blood phosphorus. They noted that the inorganic phosphorus in blood of dairy animals fed a normal phosphorus ration showed a slight increase from the second to the fourth month, after which it remained fairly constant until the tenth month, and then gradually declined as the animals grew older. The inorganic whole blood phosphorus levels ranged from 5.0 to 7.0 mg %.

Stanley (1938) reported inorganic serum phosphorus in range cattle, which ranged from 3.96 to 8.31 mg % with an average of about 5.0 mg %. The phosphorus content of the range was found to be 0.178%.

Knox et al. (1941) supplemented range cattle with dicalcium phosphate and observed inorganic blood phosphorus levels to range from 1.2 to about 4.2 mg % in control cattle, while the cattle receiving phosphorus supplement also had low phosphorus levels (1.2 to 4.8 mg %), but remained in excellent condition, which caused the authors to question the commonly accepted theory that 4.0 mg % of phosphorus should be the lower limit. These workers believed that serum phosphorus level was a good diagnosis for phosphorus deficiency in range cattle.

Beeson et al. (1941) noted that steers fed rations containing 0.15% phosphorus (or less) showed a definite lowering of the inorganic blood phosphorus to deficient levels of around 3.58 mg %. A phosphorus intake of 1.46 mg (or less) per 45.4 kg of live weight did not support normal blood plasma phosphorus levels. Inorganic blood phosphorus values for steers on rations containing 0.18% or more phosphorus, corresponding to an intake of 2.0 g or more phosphorus per 45.4 kg live weight were all within the normal range. The optimum daily phosphorus requirement as determined by rate of gain, feed utilization, appetite and inorganic blood phosphorus level was shown to be approximately 10 g daily for a 227 kg steer calf.
Moxon et al. (1947) reported inorganic blood phosphorus of range cattle ranged from 1.19 to 9.57 mg % with a mean value of 4.77. These results were collected during a five-year period, which probably explains the wide range in values.

Watkins and Knox (1948) observed seasonal fluctuations in the blood inorganic phosphorus content in range cattle of New Mexico. They obtained an average 3.53 mg % phosphorus, but the range indicated lower levels in the spring and higher blood phosphorus levels during the fall. They found that the blood phosphorus content of their cattle was less than recommended, however they said it appeared extremely doubtful that additional phosphorus would have increased production with the energy and protein supplied by the range.

Long et al. (1952) studied the blood composition of beef cows of different breeds and found no obvious breed differences, but rather a relationship to age, dietary and seasonal factors. In the same experiment, the average inorganic phosphorus level was 7.5 mg % for heifers and 4.5 mg % for cows. Marsh and Swingle (1960) also noted blood phosphorus to be higher in heifers than cows.

Rusoff et al. (1954) detected little difference in blood inorganic phosphorus between bulls of different breeds. Average values ranged from 3.55 to 6.15 mg %.

(D) CALCIUM AND PHOSPHORUS INTERACTIONS.

Many experiments have been conducted to determine the relationships of calcium, phosphorus, and vitamin D in animal nutrition and physiology. Most of the work has been concerned more with qualitative than quantitative information (Chandler, 1962). It has been concluded that there is no simple relationship between the metabolism of calcium and phosphorus, although the interaction of these elements in certain instances can be explained primarily on the basis of physio-chemical concepts. This relationship becomes extremely complex as biological factors are introduced into a given system (Wasserman, 1960).
Considerable emphasis has been placed on the ratio of Ca:P in the diet as an indication of nutritional status. Although the Ca:P ratio should be considered, one should be cognizant of the absolute levels of these minerals in the diet.

Calcium seems to possess, to a preeminent degree, the property of disturbing the assimilation of other minerals. Its relation to phosphorus utilization is well known (Mitchell, 1947).

Hansard and Plumlee (1954) observed increased urinary phosphorus excretion when the intake of calcium was low and suggested that when insufficient calcium is present in the blood for bone calcification to proceed, a part of the excess phosphorus is excreted via the urine. Tillman and Brethour (1959) found that a part of the excess calcium is excreted via the urine if an insufficient amount of phosphorus is present.

Except for prepartum feeds, ruminants are able to tolerate a much higher ratio of calcium to phosphorus, than are monogastrics. Calcium to phoshorus ratios below 1:1 probably should be avoided in ruminants; while for simple stomach animals it is generally agreed that the optimum Ca:P ratio lies between 1:1 and 2:1 (Wise, 1963).

Strangely enough, optimal ratios of Ca:P for ruminants has received little attention. In 1933, Huffman observed satisfactory growth among dairy calves fed a ration based on alfalfa hay and containing calcium and phosphorus ratios between 4:1 and 5:1. Theiler (1937) showed that a Ca:P ratio of 4:1 did not significantly affect the performance of growing beef heifers and steers when an adequate daily amount of phosphorus was supplied.

Miller (1979) cited research done by Cornell University, which demonstrated that growth rate and feed utilization of calves were satisfactory with calcium to phosphorus ratios ranging from 1:1 to 7:1. Decreased performance and nutrient conversions were noted at ratios above or below this range.
Ricketts et al. (1970) fed a ratio of calcium to phosphorus of 8:1 to Holstein steers and found that the growth and feed utilization were reduced. Smith (1966) did not find significant differences in lactating cows fed rations containing calcium and phosphorus ratios of 1:1, 4:1 and 8:1.

The responses of dietary calcium, phosphorus and vitamin D are not the result of the actual amount of the diet consumed. It was observed that dietary intake was reduced when rations contained a high level of calcium and/or phosphorus (Chandler, 1962).

Chandler (1962) agreed with Wasserman (1960) concluding that total calcium and phosphorus in the diet, as well as the ratio must be taken into consideration. The ratio is of less importance when the total amount of calcium and phosphorus is increased to higher levels.
MATERIALS AND METHODS.

One hundred and twenty crossbred steers were blocked by weight and assigned at random to six treatment groups: 1) 0 mg CTC/hd/day, no mineral supplement; 2) 75 mg CTC/hd/day, no mineral supplement; 3) 150 mg CTC/hd/day, no mineral supplement; 4) 0 mg CTC/hd/day, with mineral supplement; 5) 75 mg CTC/hd/day, with mineral supplement; 6) 150 mg CTC/hd/day, with mineral supplement. The steers were locally purchased within a 60-mile radius of the experimental pastures and were approximately 9-11 months of age (206.5 kg ave. weight).

The steers were processed prior to being assigned to treatment groups. They were dewormed, vaccinated against leptospirosis, IBR, BVD and black leg, and treated against lice and grubs. Each steer was implanted with Ralgro® at the beginning of the experiment, and reimplemented twice during the 9-month experimental period which began January 11 and ended October 14, 1983. During the entire period, steers were maintained in six different groups and rotated between pastures at 15-day intervals. The 540-acre area used for the experiment was divided in six pastures; Two of one hundred acres each, three of eighty four acres each, and one of eighty acres.

The steers were fed supplement in the mornings of Monday, Wednesday and Friday of each week throughout the trial. During the winter phase, the supplement provided a daily intake of 454 g of ground grain sorghum, 454 g of soybean meal, 120 mg of Rumensin®, 23 g of potassium chloride, and as treatments; 7.25 g of dicalcium phosphate, 4.5 g of trace mineral premix, plus the appropriate antibiotic treatment level.
For the summer period, the supplement was formulated to supply 136 g of ground sorghum grain, 200 mg of Rumensin® and, as mineral treatments, 6 g of Dical, 4 g of trace mineral and the appropriate antibiotic level. Salt was used as an intake controller with the amount of salt adjusted at weekly intervals in an attempt to stabilize consumption of supplement.

Feed left in the feeders was weighed each week to determine actual supplement consumption as shown in table 1.

Individual weights were taken at monthly intervals after being penned over night without feed or water.

Blood samples, by jugular puncture, were taken once on August 31 from eight steers selected at random from each treatment. Tubes were taken to the analytical laboratory in groups of eight and centrifuged immediately after arrival. Calcium, phosphorus, and glucose determinations were made utilizing the sequential multiple channel analyzer 12H60 (Technicon Instruments).

Hand clipped grass samples were collected twice to determine the mineral content for the period when native grass has its highest nutritional value (summer) and when values are low because of weathering (winter). The samples were dried at 50° C and ground. Proximate analysis was conducted on a mixture of samples from the six pastures. In addition to proximate analysis, samples were analyzed for magnesium, sodium, potassium, manganese, zinc, copper, iron, calcium and phosphorus.

Water samples were taken from each of the tanks and at selected points along the creek. Random soil samples were also collected. Water and soil samples were analyzed for the same minerals mentioned previously. The minerals chosen for analysis were based upon the composition of the mineral supplement (Appendix table 1).
Table 1. Daily Mineral and Antibiotic Supplement Consumption During Winter and Summer Native Bluestem Pasture Trials.

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<td>2</td>
<td>3</td>
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**Winter**

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<th>Rumensin®, mg</th>
<th>KCl, g</th>
<th>Dicalcium Phosphate, g</th>
<th>Trace mineral mix, g</th>
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**Summer**

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1. Jan. 11 - Apr. 29, 1983 - 108 days
Proximate analysis and mineral analysis was determined for the grain supplement by standard AOAC procedures.

RESULTS AND DISCUSSION.

Performance data:

During the Winter period, steers ate the supplement in a voracious manner; however, during Summer, supplement intake was quite variable and it was hard to determine the amount of salt necessary to regulate intake to the desired level (Figures 2-6). The steers were on lush grass which was responsible for some of the reduction in supplement intake.

Steers fed 150 mg CTC daily gained more \((P<.05)\) than those fed 75 mg or no antibiotic during the winter. Mineral supplementation did not affect winter gains. For the summer, neither mineral nor antibiotic feeding directly affected gains, but a significant \((P<.05)\) interaction occurred between the high antibiotic level and mineral supplementation; antibiotic alone improved gains while the combination lowered gains. The best performance for the entire 276 day trial was obtained from steers fed 150 mg CTC but given no supplemental mineral other than salt. Complete results are shown in Table 2. There was a notable difference in total gain and daily gain between the 108 day winter and 168 day summer periods. The daily gain for the six treatments during the winter period averaged .19 kg and for the summer .87 kg.
Figure 2. Treatment 1, 0 mg Ca/HD/day; no mineral

Comparison between salt content of supplement and consumption of total mixture

LEGEND

- Salt
- Supplement intake (g)

TIME (WEEKS)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

SALT CONTENT OF SUPPLEMENT (g)

SUPPLEMENT INTAKE (g)
Figure 3. Treatment 2. 75 mg CTC/40/day: no mineral content.

Graph showing the comparison between intake and consumption of total mixture.

Legend:
- Intake
- Salt
Figure A. Treatment 3, 150 mg CIC/HD/day: No Mineral

Legend

- SUPPLEMENT INTAKE (g)
- SALT CONTENT OF SUPPLEMENT (g)

Comparison between salt content of supplement and consumption of total mixture and supplementation of total mixture.
Figure 7. Treatment 6, 150 mg CTC/HO/HO: with mineral supplement intake and consumption of total mixture and comparison between salt content of supplement.
Table 2. Effect of Mineral and Antibiotic Supplementation on Performance of Steers Grazing Native Bluestem Pastures.

<table>
<thead>
<tr>
<th>Treatment:</th>
<th>Chlortetracycline, mg/hd/da</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral supplement number code</td>
<td>0</td>
<td>75</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>No. steers</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Average initial weight (kg)</td>
<td>205.4</td>
<td>208.2</td>
<td>209.2</td>
<td>206.6</td>
</tr>
<tr>
<td>Winter gain:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total, kg</td>
<td>15.6</td>
<td>18.2</td>
<td>16.7</td>
<td>14.8</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>.14b</td>
<td>.17b</td>
<td>.15b</td>
<td>.14b</td>
</tr>
<tr>
<td>Summer gain:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total, kg</td>
<td>146.5</td>
<td>151.1</td>
<td>143.8</td>
<td>153.8</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>.87ab</td>
<td>.90a</td>
<td>.85b</td>
<td>.91a</td>
</tr>
<tr>
<td>Total gain:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total, kg</td>
<td>162.1</td>
<td>169.2</td>
<td>160.6</td>
<td>168.6</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>.59b</td>
<td>.61ab</td>
<td>.58b</td>
<td>.61ab</td>
</tr>
<tr>
<td>Average final weight (kg)</td>
<td>367.5</td>
<td>377.4</td>
<td>369.8</td>
<td>375.2</td>
</tr>
</tbody>
</table>

Values in same row with different superscripts differ significantly (P<.05)
1 Jan. 11 - Apr. 29, 1983 - 108 days
2 Apr. 29 - Oct. 14, 1983 - 168 days
Results of this trial are contrary to those from a 129-day grazing trial by Riley et al. (1983) in which he obtained a 15.3 percent (16.3 kg) improvement in CTC-treated steers. His trial used a much higher level of CTC (437 mg/hd/day). The daily gain obtained by Riley (1983) for CTC-treated steers was .93 kg which is similar to the .87 kg obtained during the summer phase of this experiment. Steers used in Riley's trial were 23 kg heavier initially and grazed brome grass pasture instead of native range as used in this trial. Smith (1961) grazed steers on native range during the summer at the Kansas Agricultural Experiment Station and showed that 80 mg/head/day of CTC produced an improvement in daily gain of .07 kg. This also resulted in an extra 11.25 kg of total gain. Boggs et al. (1976) conducted a field trial in 1976 using a medicated (CTC) mineral mix. The daily intake of CTC was 443 mg for the 151-day grazing period. Steers receiving medicated mineral were an extra 13.6 kg heavier. One of the reasons for the differences in response between this trial and others is probably the marked difference in antibiotic intake. Results obtained by Smith (1961) were similar to the results of this experiment, which could be expected since the antibiotic consumption was approximately the same. Bartley (1954), after a series of experiments, proposed that the optimum level of CTC feeding should be 1 mg/kg of body weight daily for the first 12 weeks and 0.3 mg/kg from 13 to 25 weeks of age. However, Hogue et al. (1954) reported that feeding CTC at levels of 10, 20 and 40 mg per 45.4 kg of body weight did not bring about any improvements beyond the first 7 weeks of age. They concluded that there was no advantage to feeding calves antibiotics past 7 weeks of age.

Fincham and Voelker (1953) fed 80 mg of CTC daily from birth to 200 days of age and 240 mg daily until 2 1/2 years of age. They found most of the growth
stimulation of CTC occurred before the animals were 6 months old and that the advantage was maintained.

One of the most important questions concerning the feeding of antibiotics to beef cattle is the amount which must be fed to obtain a maximum growth response and a significant detrimental effect on the incidence of disease. This trial did not resolve that question but did indicate that the levels needed to be higher than 150 mg/head/day. The cost of supplementing 150 mg/head/day of CTC is $5.4 cents (determined based upon $1.78 per 40 g CTC premix).

The addition of CTC at levels ranging up to 600 mg/head/day in diets of confined feedlot cattle was found in early investigations to be followed by a loss of appetite, a depression of apparent digestibility of crude fiber and a decreased nitrogen retention (Riley et al., 1981).

In this grazing trial, the average daily intakes of CTC were 65.6 mg for the low level and 127.1 mg for the high level. Total weight gain per steer during the winter period for the 150 mg CTC treatments (Table 2) were 7 kg higher than the other treatments. This supports Catron's theory (1952) that the higher the disease or stress level, the greater the response to antibiotic feeding. Conditions during the winter period are more stressful and offer a potential for greater antibiotic response.

The differences in the response to CTC obtained by various researchers and this trial verify a need for further research to evaluate the effect of cattle type, dosage level, and grazing conditions on response to feeding a CTC-medicated supplement.

Corah (1978) cited work done by Boggs, Riley and Smith (1976) in which a complimentary response to implants and CTC feeding was clearly shown. A complimentary response between CTC feeding and Ralgro® implantation could not
be measured in this trial since all the animals were implanted for the duration of the experiment.

Table 3. Effect of Mineral and Antibiotic Supplementation on Blood Glucose Level in Grazing Steers.

<table>
<thead>
<tr>
<th>TREATMENT:</th>
<th>GLUCOSE (mg/100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
</tr>
<tr>
<td>0 mg CTC, No Mineral</td>
<td>73.38</td>
</tr>
<tr>
<td>75 Mg CTC, No Mineral</td>
<td>72.2</td>
</tr>
<tr>
<td>150 Mg CTC, No Mineral</td>
<td>79.75</td>
</tr>
<tr>
<td>0 Mg CTC, Mineral Supplement</td>
<td>75.88</td>
</tr>
<tr>
<td>75 Mg CTC, Mineral Supplement</td>
<td>68.43</td>
</tr>
<tr>
<td>150 Mg CTC, Mineral Supplement</td>
<td>72.13</td>
</tr>
</tbody>
</table>

There were no significant differences in blood glucose between the controls and the two levels of CTC supplementation. Blood glucose in this trial ranged from 68.5 to 79.8 mg % (Table 3). The controls averaged 74.7 mg % while 70.4 and 76 mg % were the averages for the 75 and 150 mg CTC treatments, respectively. It should be emphasized that 75 and 150 mg daily CTC were the projected levels of treatment, but in reality the steers consumed 65.6 and 127.1 mg, respectively.

Murley et. al. (1951) observed that blood glucose levels rose slightly more rapidly and exhibited a greater increase in CTC-fed calves than in control calves. Hibbs and Conrad (1953) observed that 8 to 12-week old calves fed CTC maintained a blood sugar level of 9 mg % higher than control calves. Voelker et.
al. (1952) studied 1500 blood samples from animals treated with 200-240 mg CTC and found that CTC-treated animals did not differ in blood glucose levels. These Ohio workers used growing cattle instead of young dairy calves referenced in the trial mentioned above and suggest that blood glucose depends more on the animal age than antibiotic intake.

In experiments with high levels of antibiotic, blood sugar content has not been measured. It would be beneficial to design an experiment with high antibiotic intakes and determine if CTC-feeding had an affect on blood glucose.

CALCIUM AND PHOSPHORUS

An important part of this experiment was to determine if calcium and phosphorus supplementation had any beneficial affect on grazing steer performance and blood serum mineral composition.

Supplementation of calcium and phosphorus was accomplished by dicalcium phosphate addition. Actual consumption of supplemental minerals is shown in Table 1. Supplemental mineral did not significantly improve weight gain. Total gain for the mineral supplemented groups averaged 167.4 kg, while the average for the non-mineral treatments was 165.7 kg.

As cited by Knox and Watkins (1942), a satisfactory mineral mixture is one which is economical and will be consumed in such quantities that ample salt and phosphorus will be supplied. Calcium may be disregarded, for nearly any mixture made with a calcium-phosphorus compound such as bone meal, bone black or dicalcium phosphate will furnish more calcium than is needed. In the same paper, these workers mentioned that the daily consumption of mineral mixtures, may be compared with the calculated need for phosphorus. Requirements for supplemental phosphorus are calculated by deducting the phosphorus provided by the forage. A
daily phosphorus requirement can be calculated by allowing 7 g/454 kg of live weight for maintenance, to which is added 0.67 percent of the weight gained.

The average weight during the winter period was 218.3 kg suggesting a maintenance requirement for phosphorus of 3.37 g. The summer average weight was 329.5 kg projecting a maintenance requirement for phosphorus of 5.05 g. The phosphorus required for gain, according to these authors, would be determined by 0.67 percent of the daily gains for each period. Daily gain for the winter was 79 g, which would mean a 0.53 gm phosphorus requirement for gain. The summer period daily gain was 398 g and the phosphorus requirement for this gain according to Knox and Watkins (1942) would be 2.67 g.

Adding the requirements for maintenance and gain, the total phosphorus needed would be of 3.9 g in the winter period and 7.7 g for the summer (Knox and Watkins, 1942) These figures do not agree with the requirements established by the NRC (1976), which is 13 g for each period.

The amount of supplemental minerals provided in this trial are shown in table 4. Unfortunately, due to a miscalculation, the supplement supplied only 10% of the desired daily requirements. The shortage of phosphorus might be a partial explanation for the low gains obtained in comparison with other trials. It has been shown that steers stop growing after a 6-month period on a diet containing 0.13 percent phosphorus. Probably a reason for this, is that the efficiency of energy utilization is decreased. According to the NRC (1976), the lowest phosphorus requirement would be a ration containing 0.18 percent.

BLOOD CALCIUM AND PHOSPHORUS LEVELS

Average plasma calcium (Table 5) for the six treatments was 10.19 mg % (range 9.98-10.65). Drake (1964) worked with cattle on native range at the Kansas
Table 4. Estimated Daily Phosphorus Intake During Different Periods of the Trial.

<table>
<thead>
<tr>
<th>(Period) Weight, kg.</th>
<th>NRC Requirements (g)</th>
<th>Phosphorus Consumption (g)</th>
<th>Grass % P (g)</th>
<th>Grain % P (g)</th>
<th>Dical % P (g)</th>
<th>Total (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Winter) 200</td>
<td>9</td>
<td></td>
<td>0.022 1.10</td>
<td>0.646 5.95</td>
<td>18.5 1.30</td>
<td>8.35</td>
</tr>
<tr>
<td>(Summer) 250</td>
<td>17</td>
<td></td>
<td>0.084 5.25</td>
<td>0.33 1.65</td>
<td>18.5 0.10</td>
<td>8.00</td>
</tr>
<tr>
<td>(Summer) 300</td>
<td>16</td>
<td></td>
<td>0.004 6.30</td>
<td>0.33 1.65</td>
<td>18.5 0.90</td>
<td>8.85</td>
</tr>
<tr>
<td>(Summer) 350</td>
<td>18</td>
<td></td>
<td>0.084 7.35</td>
<td>0.33 1.65</td>
<td>18.5 0.90</td>
<td>9.90</td>
</tr>
<tr>
<td>Mineral</td>
<td>Required* (NRC)</td>
<td>Winter treatment</td>
<td>Summer treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese, mg</td>
<td>35</td>
<td>24.1</td>
<td>24.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>138</td>
<td>108.5</td>
<td>108.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron, mg</td>
<td>**</td>
<td>35.4</td>
<td>35.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper, mg</td>
<td>22</td>
<td>17.7</td>
<td>17.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine, mg</td>
<td>.06</td>
<td>.73</td>
<td>.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt, mg</td>
<td>.5</td>
<td>.40</td>
<td>.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium, g</td>
<td>4.5</td>
<td>2.3</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, g</td>
<td>38</td>
<td>11.9</td>
<td>11.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus, g</td>
<td>13-16</td>
<td>1.28</td>
<td>1.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, g</td>
<td>14-18</td>
<td>3.32</td>
<td>3.32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Some values estimated.
**Minimum requirement has not been established.
Table 6. Effect of Mineral and Antibiotic Supplementation on Blood Calcium and Phosphorus Level in Grazing Steers.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CALCIUM mg %</th>
<th>PHOSPHORUS mg %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
</tr>
<tr>
<td>0 Mg CTC, No Mineral</td>
<td>10.14</td>
<td>0.38</td>
</tr>
<tr>
<td>75 Mg CTC, No Mineral</td>
<td>10.06</td>
<td>0.33</td>
</tr>
<tr>
<td>150 Mg CTC, No Mineral</td>
<td>9.98</td>
<td>0.28</td>
</tr>
<tr>
<td>0 Mg CTC, Mineral Supplement</td>
<td>10.65</td>
<td>0.43</td>
</tr>
<tr>
<td>75 Mg CTC, Mineral Supplement</td>
<td>10.20</td>
<td>0.24</td>
</tr>
<tr>
<td>150 Mg CTC, Mineral Supplement</td>
<td>10.09</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Agricultural Experiment Station and obtained an average blood calcium of 11.0 mg %. Stanley (1938) reported that the calcium content of blood serum varied from 10 to 12 mg % in grazing cattle. Results obtained in this trial would be considered in the normal range and it could be concluded that calcium was supplied in adequate amounts and was not a limiting factor for normal growth. It has been established that mineral levels in the blood are a good measurement of homeostatic ability of the organism and not necessarily of the adequacy of a ration when fed to animals.

Average blood phosphorus level (Table 5) was 6.3 mg %. The dicalcium phosphate supplemented animals averaged 6.27 mg % and the controls 6.30 mg %. These values are similar to those cited in the literature. Robinson and Huffman (1925) reported cattle blood contained an average of 5.87 mg % of phosphorus. Stanley (1938) studied inorganic serum phosphorus in range cattle and found a range from 3.96 to 8.31 mg % with an average phosphorus content of 5.0 mg %.

Blood samples in this trial were taken on August 31 at a time when phosphorus content of the range was probably at its highest point.

Underwood (1966) mentioned that the most sensitive and earliest biochemical measurement of a phosphorus deficiency was reduced serum inorganic phosphorus. Since the phosphorus consumption during the summer period was notably below the recommended requirements, the phosphorus blood contents from this trial, according to Underwood (1966), should have been lower than the figures reported in the literature, which was not the case. However, the Netherlands Committee on Mineral Nutrition (1973) does not consider serum inorganic phosphorus to be sensitive enough to be recommended in diagnosing deficiencies in cattle.

Even though a trace mineral premix was mixed into the supplement fed to steers in this trial, detailed consideration of the functions of micro-minerals in the
animal body is not included in this report. The grass supplement were expected to meet all the trace mineral requirements which would make it possible to evaluate the potential effects of calcium and phosphorus supplementation.
LITERATURE CITED - CHLORTETRACYCLINE


LITERATURE CITED - CALCIUM AND PHOSPHORUS.


Drake, C.L. 1964. Supplemental Calcium, Phosphorus and Protein for Steers on Bluestem Pasture, Ph.D. Dissertation, Kansas State University, Manhattan, Kansas.


## Appendix Table 1. Composition of Trace Mineral Premix

<table>
<thead>
<tr>
<th>ELEMENT</th>
<th>Label&lt;sup&gt;1&lt;/sup&gt; (%)</th>
<th>KSU Analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>0.53</td>
<td>0.417</td>
</tr>
<tr>
<td>Zinc</td>
<td>2.39</td>
<td>2.057</td>
</tr>
<tr>
<td>Iron</td>
<td>0.78</td>
<td>0.951</td>
</tr>
<tr>
<td>Copper</td>
<td>0.39</td>
<td>0.249</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.016</td>
<td>----</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.009</td>
<td>----</td>
</tr>
<tr>
<td>Magnesium</td>
<td>51.33</td>
<td>54.027</td>
</tr>
<tr>
<td>Sodium</td>
<td>----</td>
<td>0.013</td>
</tr>
<tr>
<td>Potassium</td>
<td>----</td>
<td>0.037</td>
</tr>
<tr>
<td>Calcium</td>
<td>----</td>
<td>0.813</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

<sup>1</sup>Huber Corporation, Calcium Carbonate Division.
Appendix TABLE 2. Mineral Composition of Winter Supplement (DM Basis)

<table>
<thead>
<tr>
<th>ELEMENT</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>1.765</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.001</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.553</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.008</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.025</td>
</tr>
<tr>
<td>Copper</td>
<td>0.004</td>
</tr>
<tr>
<td>Iron</td>
<td>0.042</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.410</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.646</td>
</tr>
</tbody>
</table>
### Appendix TABLE 3. Mineral Composition of Grass Samples (DM Basis)

<table>
<thead>
<tr>
<th>ELEMENT</th>
<th>March 20 (%)</th>
<th>August 12 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>0.137</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>0.351</td>
<td>0.485</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.022</td>
<td>0.084</td>
</tr>
</tbody>
</table>
### Appendix TABLE 4. Proximate Analysis of Grass Samples (DM Basis)

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>Sample date</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>March 20</td>
<td>August 12</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>8.97</td>
<td>7.24</td>
<td></td>
</tr>
<tr>
<td>Crude Protein</td>
<td>3.03</td>
<td>4.43</td>
<td></td>
</tr>
<tr>
<td>Ether Extract</td>
<td>1.61</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>37.86</td>
<td>31.13</td>
<td></td>
</tr>
<tr>
<td>Nitrogen Free Extract</td>
<td>48.53</td>
<td>54.04</td>
<td></td>
</tr>
</tbody>
</table>
Appendix TABLE 5. Proximate Analysis of Winter Supplement (DM Basis)

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>KSU Analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>9.45</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>23.08</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>2.00</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>4.89</td>
</tr>
<tr>
<td>Nitrogen Free Extract</td>
<td>60.58</td>
</tr>
</tbody>
</table>
EDUARDO GARCIA FRIAS

Birthdate: December 24, 1960
Birthplace: Tehuantepec, Oaxaca. Mexico.
Parents: Jesus and Carolina Garcia.
Permanent Mailing Address: Mariano Arce #13
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Mexico.

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Queretaro, Qro. Mexico.

Bachelor of Science in Animal Science, December 1981. Instituto Tecnologico y de Estudios Superiores de Monterrey-Unidad Queretaro, Queretaro, Qro. Mexico.

Publications:
EFFECT OF CHLORTETRACYCLINE AND MINERAL SUPPLEMENTATION ON GRAZING STEER PERFORMANCE

By

EDUARDO GARCIA-FRIAS
B.S., ITESM-U.Q., 1981

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984
ABSTRACT

One hundred and twenty crossbred steers were blocked by weight and assigned at random to six treatment groups: 1) 0 mg CTC/hd/day, no mineral supplement; 2) 75 mg CTC/hd/day, no mineral supplement; 3) 150 mg CTC/hd/day, no mineral supplement; 4) 0 mg CTC/hd/day, with mineral supplement; 5) 75 mg CTC/hd/day, with mineral supplement; 6) 150 mg CTC/hd/day, with mineral supplement. The steers were locally purchased within a 60-mile radius of the experimental pastures and were approximately 9-11 months of age (206.5 kg ave. weight).

Steers fed 150 mg CTC daily, gained more (P<.05) than those fed 75 mg or no antibiotic during the winter. Mineral supplementation did not affect winter gains. For the summer, neither mineral nor antibiotic feeding directly affected gains, but a significant (P<.05) interaction occurred between the high antibiotic level and mineral supplementation; antibiotic alone improved gains while the combination lowered gains. The best performance for the entire 276 day trial was obtained from steers fed 150 mg CTC but given no supplemental mineral other than salt.

There were no significant differences in blood glucose between controls and the two levels of CTC. Blood glucose values ranged from 68.5 to 79.8 mg %.

Supplemental mineral did not improve weight gain. Average plasma calcium for the six treatments was 10.19 mg % and would be considered in the normal range. Phosphorus level in the blood serum was 6.3 mg % which was similar to values cited in the literature.