1979

Effect of Sodium Sulfate, Calcium Level and Source and Phosphorus on the Potentiation of Chlortetracycline and Performance of Growing Pigs

John P. Balios

EFFECT OF SODIUM SULFATE, CALCIUM LEVEL AND SOURCE AND PHOSPHORUS ON THE POTENTIATION OF CHLORTETRACYCLINE AND PERFORMANCE OF GROWING PIGS

BY

JOHN P. BALICS

A thesis submitted
in partial fulfillment of the requirements for the
degree Doctor of Philosophy, Major in
Animal Science, South Dakota
State University

1979

EFFECT OF SODIUM SULFATE, CALCIUM LEVEL AND SOURCE AND PHOSPHORUS ON THE POTENTIATION OF CHLORTETRACYCLINE AND PERFORMANCE OF GROWING PIGS

This thesis is approved as creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Richard C. Wahlstrom
Thesis Adviser — Date

Joe A. Minyard
Head, Animal Science Department — Date

EFFECT OF SODIUM SULFATE, CALCIUM LEVEL AND SOURCE AND PHOSPHORUS ON THE POTENTIATION OF CHLORTETRACYCLINE AND PERFORMANCE OF GROWING PIGS

Abstract

JOHN P. BALIOS

Under the supervision of Professor Richard C. Wahlstrom

Experiments designed to study the potentiation of antibiotic in swine were conducted over a two-year period. During the two years, 344 crossbred pigs were used in three separate experiments to study the effect of calcium sources and levels, sulfate sources and levels, and phosphorus levels in diets containing different levels of chlortetracycline. The first experiment was divided into three two-periods. During the first two weeks 50 g/ton of chlortetracycline in the diets increased gain of pigs from 0.68 to 0.72 kg. per day. Pigs fed diets containing chlortetracycline gained significantly faster when the source of calcium was sulfate rather than carbonate. In the second period, pigs receiving a higher calcium diet with antibiotic grew significantly faster than those fed a low-calcium diet. During the third two-week period pigs fed a calcium carbonate diet achieved gains significantly greater than those fed a calcium sulfate diet. On an accumulative basis (0-6 weeks) there were no significant differences

among treatments in either average daily gain or feed efficiency. Diets containing three levels of calcium (0.3, 0.7 and 1.1%) with and without 100g/ton of chlortetracycline were used during the first four-week period of Experiment 2. In the second period (2 weeks) all diets contained 0.3% calcium. Pigs fed the antibiotic diets received 200 g/ton of chlortetracycline. Blood was collected from all pigs at the beginning of the experiment, at the end of the first period and at the end of the second period. Over the entire six-week period antibiotic fed pigs gained significantly ($P < .01$) faster and required less feed per Kg. gain than pigs fed diets without antibiotic. Pigs fed diets of 0.3 and 0.7% calcium responded significantly ($P < .05$) better to antibiotic than those fed the high calcium diet. Blood serum calcium was similar among all treatments at the first and third blood collections. At the second collection pigs on the lowest dietary calcium level showed blood serum calcium levels significantly higher than the others. Serum chlortetracycline at the second blood collection increased linearly with decreasing levels of dietary calcium. Serum chlortetracycline at the third collection followed the same pattern as the second and was closely correlated with the dietary calcium levels of the first period. Several methods of potentiation of chlortetracycline were studied in the third

experiment which was conducted for ten weeks. The diets included increasing levels of sodium sulfate, calcium sulfate and dietary phosphorus and also a low calcium diet. All diets contained 200 g/ton chlortetracycline. Blood was collected at the beginning of the experiment after pigs were fasted overnight and fed for five hours and again two weeks later. Chlortetracycline significantly ($P < .05$) increased daily gain. At both sampling periods the lowest values of blood serum calcium were found in pigs fed the very high (1.8%) phosphorus diet. At the first collection blood serum from all pigs, except those fed the calcium sulfate supplemented diets, contained chlortetracycline levels which were significantly higher ($P < .05$) than the controls. At the second collection only serum from pigs fed the low calcium diet and the two very high phosphorus diets had chlortetracycline values significantly higher ($P < .05$) than controls. Increasing amounts of sodium sulfate in the diet caused a linear increase of blood serum chlortetracycline in the blood serum. At both collections, blood serum chlortetracycline was also significantly ($P < .01$) higher when pigs were fed the high phosphorus diets.

ACKNOWLEDGMENTS

The author would like to take this opportunity to express his deep and sincere appreciation to Dr. R. C. Wahlstrom, Professor of Animal Science, for his advice, guidance, assistance and understanding throughout the studies and during the writing of this manuscript.

Appreciation is extended to Dr. G. Libal for his guidance and criticism throughout the studies.

Further appreciation is extended to Professors R. Baker and R. J. Emerick for their valuable assistance in the analytical procedures; Professor L. W. Tucker for his help in the statistical analysis; Mr. Bill Heylens and his co-workers at the Swine Research Unit for their help in various phases of these studies; and Mrs. Helen Schultz and Mrs. Jan Thompson for typing this thesis.

The author is indebted to his parents and parents-in-law for their continued consultation and assistance throughout his academic career.

I am most grateful to my wife, Mary, for her patience and understanding during the course of my graduate study and to my children, Anthoula and Peter, for making everything worthwhile.

JPB

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

INTRODUCTION

About 30 years have elapsed since the growth promoting properties of dietary antibiotics first became an object of extensive research. Since that time an inexhaustible number of papers and review articles have appeared, which cover in great detail the whole concept of antibiotics and their magic effects on promoting growth, improving feed efficiency, and preventing and treating diseases.

In the late 1940's the American swine producer was attempting to move from pasture to dry lot feeding. Catron (1949) described the situation as: "Many farmers have been turning to the use of concrete lots to overcome swine disease, parasites, water hauling and fencing problems. This definitely creates nutritional deficiencies, new experiences for many. Many rations which once gave excellent results in pasture feeding result in slow growth rate, scouring and high death losses under dry lot conditions."

The advent of the antibiotics very effectively enabled the industry to achieve performances and feed efficiency better than the good pasture rations used up until that time.

At first antibiotics were almost entirely restricted to their low level nutritional use. However, in the late 1950's and early 60's with the development of intensive methods of animal management, particularly in the pig and

poultry field, antibiotics were added to the feed for prolonged periods to control or prevent the development of clinical disease.

It has been known for some time that very high levels of antibiotics are effective against diseases other than those for which they are now incorporated into feeds. However, the antibiotic level which must be used for the prevention and treatment of these diseases is so high, that it is not economically feasible to use the antibiotics for these purposes.

Thus, the cost of antibiotic treatment under these conditions of concentrated production, became a very important factor, and attention was drawn to the cost of treatment, based on the biological availability of antibiotics.

In 1960 a new word was added to the vocabularly of poultry and feed men. The word was "potentiation" which means to make more active or powerful. Potentiation made it possible for the activity or power of broad-spectrum antibiotics such as chlortetracycline to be increased two or more times. The benefits, particularly to broilers and replacement chicks, were measured in improved health and more efficient production.

Most of the research regarding potentiation of antibiotics has been done in the area of poultry, with

conclusions, that the same principles and same results--to a certain extent--will apply to other livestock.

It was the object of this study to investigate the potentiation of antibiotics, and particularly chlortetracycline, in swine diets.

REVIEW OF LITERATURE

Antibiotics have been defined as a group of soluble organic substances that are produced by microorganisms and that are characterized by the ability to inhibit, at low concentrations, the growth, activity, or multiplication of other microorganisms. Some of the antibiotics widely used in animal production are penicillin, streptomycin, bacitracin, tylosin, chlortetracycline, and oxytetracycline. The latter two antibiotics are more commonly known by their trade names, Aureomycin and Terramycin.

Antibiotic Growth Effect in Pigs

The first indications of the profound effects of antibiotics on the growth of pigs appeared in the summer of 1949. Stokstad et al. (1949) and Jukes (1949) noted that it was possible, by assay with chicks, to standardize crude preparations from the chlortetracycline fermentation which supplied not only vitamin B_{12} but another growth factor as well.

These preparations, known as "Animal Protein Factor," were furnished to various investigators who were studying the effect of vitamin B_{12} in swine nutrition. Cunha and co-workers, (1949) reported that these preparations markedly improved the growth of pigs on an all-vegetable diet even when vitamin B_{12} was ineffective. In one of their

experiments, the gain on the basal diet was 0.13 Kg. per day, with vitamin B_{12}, 0.11 Kg. per day and with the chlortetracycline fermentation supplement, 0.33 Kg. per day.

In addition to obtaining the same results as Cunha, Catron (1949) and Carpenter (1950), based on the observation that these preparations were controlling diarrhea, concluded that the growth-promoting effects of chlortetracycline "Animal Protein Factor" preparation on pigs, were due primarily to its antibiotic content.

In 1953 Braude and co-workers reviewed the whole concept of antibiotics in swine nutrition. Table I, which is condensed from their review, summarizes the data published by various agricultural experiment stations with respect to the effects of various antibiotics on the growth rate and feed efficiency.

TABLE 1. GROWTH AND FEED EFFICIENCY OF PIGS RECEIVING VARIOUS ANTIBIOTICS

Antibiotic	Growth Index (unsupplemented = 100)		Feed Required Per Unit of Gain (unsupplemented = 100)	
Aureomycin	135.9	(187)*	90.2	(146)
Penicillin	110.6	(53)	94.3	(44)
Streptomycin	115.2	(50)	94.4	(41)
Terramycin	123.7	(23)	93.9	(17)
Bacitracin	109.0	(12)	103.0	(10)
Chloramphenicol	105.5	(6)	98.2	(6)
Neomycin	93.3	(4)	87.6	(3)
Polymyxin B	96.0	(1)	100.0	(1)
Subtilin	89.0	(1)	130.0	(1)

*Figures in parenthesis indicate number of comparisons

Factors Affecting Response to Antibiotics

Size of the Pig

The feeding of antibiotics to suckling pigs is a difficult field of experimentation mainly because very young pigs often will not eat enough creep to obtain maximum benefit from antibiotics in the feed.

Carpenter (1951) found that baby pigs which were allowed to eat a diet containing chlortetracycline supplementary to suckling during the first eight weeks, weighed

1.43 times as much at weaning as controls receiving the supplementary diet without antibiotic. If, instead of suckling, the baby pigs were fed an artificial milk, the response to antibiotics was also quite marked.

Wahlstrom and co-workers (1950) found that two day old pigs grew at the average rate of 0.34 Kg. per day for eight weeks on a diet of lard, glucose, soybean protein, methionine, minerals and vitamins. With the addition of chlortetracycline, 100 mg per Kg. of dry matter in the diet, the growth was 0.44 Kg. per day. No growth-promoting effect was obtained from penicillin or sulfathalidine. Noland et al. (1951) in a similar experiment reported similar results.

Under normal conditions of hygiene and management, the greatest response to antibiotics is obtained from weaning to about 45 Kg. body weight. Robinson et al. (1954) showed that there is little benefit beyond the 45 Kg. In another study the same workers (1952, 1953) reported that there is also little benefit by introducing the antibiotics to pigs that had reached the 45 Kg. weight. Lucas (1955) concluded that the growth response to antibiotics decreases as the starting weight increases.

State of Health

Pigs reared under normal conditions are subject to intestinal infections accompanied by diarrhea, sometimes

mixed with blood. This phenomenon is not uncommon in swine husbandry and may be the cause for undersized, unthrifty pigs in an otherwise normal litter. It appears that antibiotics may prevent or at least reduce this unthriftiness.

Catron and Cuff in 1951 conducted an experiment with "unthrifty" pigs weighing about 9 Kg. The control group gained 0.32 Kg. per day while a second group receiving a supplement which furnished 40 mg of chlortetracycline per Kg. of diet gained 0.52 Kg. per day during a 9-week period.

Becker et al. (1951) reported that a group of unthrifty pigs that served as controls grew at a rate of 0.25 Kg. per day and consumed 4.75 Kg. feed per Kg. gain for a period of five weeks, while another group of similar pigs fed 11 mg of chlortetracycline per Kg. of diet gained 0.75 Kg. per day and required 2.88 Kg. of feed per Kg. gain for the same period of time. These results are in agreement with the work by Carpenter (1950).

Method of Feeding

There are two contrasting methods of feeding pigs: "ad libitum" and "restricted." Because of the difference in appetite, time allowed for feeding, the quantity of food permitted and the palatability of feed, the differences in growth rate obtained between these two systems of feeding are as great as those due to feeding of antibiotics.

Often the question is asked, "Is the increased growth rate due to increased feed consumption, or is increased consumption of feed due to increased growth rate?"

Brown et al. (1952) and Wallace et al. (1955) reported that no antibiotic response was obtained with equalized feeding, and it was concluded that responses to antibiotics with ad libitum feeding are due to an increased consumption of food by the treated animals. On the other hand, Robinson et al. (1953) found little evidence of increased food intake due to appetite stimulation by penicillin, the slightly greater food intake of the treated pigs being attributable to their greater weights. Although these findings are contradictory, it seems very important in considering responses found with restricted and ad libitum feeding on similar diets, to keep in mind the greater overall weight gains obtained as a result of the greater feed intake when feed is not limited (Shorrock 1940, Hvidsten and Homb 1961).

Composition of Diet

Most of the experiments to study the antibiotic effect on the growth of pigs have been made with either an all-vegetable protein ration based on corn and soybean meal, or with a mixed protein diet of corn soybean meal and an animal protein such as fishmeal, meat scraps or tankage.

Bowland et al. (1951) and Lepley et al. (1950) reported that growth on the mixed protein diet was usually better than on the all-vegetable diet. In contradiction to these reports, many workers (Becker et al., 1952; Burnside et al. 1950; Cunha et al., 1950a,b,c,d 1951; Edwards et al., 1950, 1951) have reported that the growth response to antibiotics was generally greater with the all-vegetable protein diets than with the mixed protein diets.

Robinson et al. (1952) in an attempt to explain these differences, conducted an experiment with pigs for a period of 15 weeks. Pigs receiving the vegetable protein diet gained 58.5 Kg. and those given a diet with fishmeal, 68 Kg. The addition of penicillin increased the gains to 67.6 and 74.4 Kg. respectively. Although the percentage response is greater when antibiotics are added to all-vegetable protein diets, growth on such diets was improved only up to that obtained with an unsupplemented mixed protein diet.

Mode of Action of Antibiotics

Since the first reports of the effect of certain antibiotics on pig growth, a number of reviews have been devoted to the various mode of actions involved (Braude et al., 1953a,b; Jukes and Williams, 1953; Stokstad, 1954, Jukes, 1955; Combs, 1956, Luckey, 1959).

Among the various theories, the hypothesis of an action of the antibiotic on the flora of the digestive tract was suggested very early, because the only property that most antibiotics which promote growth have in common is their antibiotic power with regard to bacteria.

However, other evidence suggests that the bacterial flora is not the only site of action of antibiotics. Thus a second theory suggests that antibiotics might intervene in cell metabolism and by so doing, modify the utilization of the chemical substances introduced by the diet.

A third theory, which is a very promising one, says that antibiotics may act directly on one or several endocrine systems capable of influencing various metabolisms, and therefore affect growth or other psysiological functions.

These are the three theories that are the most prevalent at this time and which might very well be complementary to each other. It is possible, for example, that the products of bacterial metabolism formed in the intestine and absorbed by the portal blood, could act directly either on the cell or on various endocrine systems which could, in turn, affect intermediate metabolism.

Due to the fact that there is not much support for possibilities two and three, and the few reports available contradict each other, possibility one will be discussed in this review.

The possible ways in which an antibiotic may promote growth by affecting the intestinal flora have been listed by Moore (1946) and expanded by Jukes and Williams (1953), as follows:

1. The antibiotics may inhibit or destroy organisms which produce subclinical infections; that is, they suppress organisms which produce toxic reactions that cause a slowing of growth of the host animal.

2. Antibiotics may produce an increase in the number or activity of organisms which synthesize certain 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.

Romoser et al. (1952) found an increased number of Aerobacter aerogenes in the ceca of chicks during the feeding of penicillin. This organism (after isolation and growth in various media) when fed to chicks, gave a consistently light growth response. But additions of penicillin to the diet promoted further growth. Anderson et al. (1942, 1953a,b) observed that penicillin caused a shift in the cecal flora from normal E. coli to a mutant of E. coli. When this mutant E. coli was included in the diet, it increased growth rate of chicks, but again, when the antibiotic was included, it further promoted growth

Manten and Hoogerheide (1958) reported an increase in yeasts in the rat and mouse receiving 200 p.p.m. of chlortetracycline.

Raibaud et al. (1957) has shown an increased colibacilli/lactobacilli ratio exists in the stomach, small intestine and cecum of rats receiving chlortetracycline. In the same study he points out that a parallelism exists between the weight gain and the increase in the number of colibacilli and streptococci. In animals with a positive growth response to antibiotics, the flora was dominated by lactobacilli (pig, rat, mouse, suckling calf, chicken and turkey).

The possible role of the lactobacilli in the nutrition of the host was reported in 1952 by March and Biely, who noted in the chicken that chlortetracycline caused a reduction in the number of lactobacilli. These authors suggested that the diminution in the number of lactic acid bacteria could be a mode of action of the antibiotics because the lactobacilli are very demanding in such growth factors as vitamins. To support this hypothesis, Johansson et al. (1953a) noted a decrease in lactobacilli following feeding of chlortetracycline. However, Anderson et al. (1952) and Moore et al. (1946) found increases in lactobacilli following feeding of penicillin and streptomycin. Wahlstrom et al. (1951) reported no changes in the coliform

or lactic acid bacteria in the feces of baby pigs which had a marked weight increase as a result of feeding chlortetracycline, streptomycin or penicillin.

Lev et al. (1956) have shown that chicks raised in an infected environment have a flora rich in Clostridium from the first few days of life. In some experiments, animals receiving penicillin showed no Cl. welchii. The authors then suggested that the presence of Cl. welchii is responsible for a growth depression. This agrees with the results of Elam et al. (1953) who found that penicillin, chlortetracycline and bacitracin reduce the number of clostridia.

Kratzer et al. (1951) reported a five to ten times increase in the number of yeasts in the excreta of chicken-fed streptomycin. Williams et al. (1951) and Wahlstrom et al. (1951) found little or no change in the number of yeasts. Johanson et al.(1953b) observed an increase in intestinal enterococci from feeding chlortetracycline while Sieburth et al. (1952) noted a decrease in coliforms and enterococci.

Having in mind the theory that if the improvement in the growth of animals treated with antibiotics is exerted via the digestive flora, there should not be a response to antibiotics in germ-free animals, many researchers studied this field extensively.

Coates et al. (1951) reported that in a laboratory used for 10 years for rearing chicks, the weight of the control animals at three weeks of age was 167 g while with a penicillin-supplemented ration the weight reached 192 grams. In new quarters, the weights of the birds were 192 and 188 g with and without penicillin, respectively. Bird et al. (1952) and Hill et al. (1952, 1953) have also recorded similar results. Coates et al. (1953) in another experiment noted that addition of the intestinal contents of infected birds to the diet of chicks reared under very strict sanitary conditions caused a depression of growth which was partially counteracted by penicillin. After autoclaving, the intestinal contents caused no growth depression. On the other hand, introducing infected birds into a batch of non-infected birds resulted in a response to antibiotics.

From the above discussion it is clear that the bacterial environment as well as the bacteria themselves play an important role in depression of growth of the host animal. Lev et al. (1956 conducted a series of experiments to determine the microbial species responsible for growth depression. A preliminary trial in which germ-free chicks were infected with a pure culture of E. coli or Cl. perfringens isolated from the intestine of a conventional chick, showed that the bacterium responsible for

the growth depression was Cl. perfringens. The same study showed also that when birds from a clean environment were placed in an infected environment, their growth was depressed and Cl. welchii was found in their ceca. Addition of penicillin not only stimulated the growth of the birds in the infected environment, but also eliminated clostridia from the intestine.

In another study, Lev and Forbes (1959) reported that there was no growth retardation in chicks contaminated with E. coli, Lactobacillus lactis and Streptococcus liquefacien. However, Cl. welchii type A caused a depression of growth compared with germ-free chicks. Addition of penicillin at a rate of 45 mg/kg counteracted the depression due to Cl. welchii. These results led Lev and Forbes to conclude that Cl. welchii is a growth-depressing factor. They also concluded that penicillin either eliminates Cl. welchii or modifies the activity of its lecithinase.

Sieburth et al. (1951) and Elam et al. (1954) found that penicillin stimulated growth only when there was a reduction in the number of Clostridia in the feces. Smith (1959) found a marked reduction in the number of lecithinase positive Cl. welchii in pigs from herds where antibiotics were regularly used.

Chelation

The past 20 years more and more emphasis has been given to the fact that a great number of antimicrobial compounds contain sites at which chelation might occur. During that period an equally great number of reports have been published concerning the effect of metallic cations on various activities of these compounds.

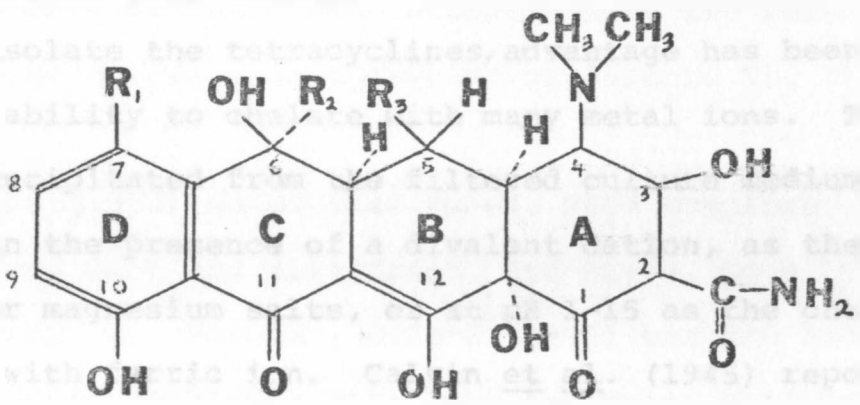
The possible consequences of the ability of an antimicrobial compound to combine with metallic ions have been summarized by Weinberg (1957) and include:

1. The compound may inhibit enzyme systems by (a) removing essential cations from the extra or intracellular environment, (b) facilitating the assimilation of toxic cations into the cell, or (c) combining with essential cations that are already attached to enzyme molecules.
2. The compound may stimulate enzyme systems by (a) removing toxic cations from the extra- or intracellular environment or (b) facilitating the assimilation of desirable cations into the cell.
3. The activity of the compound may be depressed or enhanced by quantitative or qualitative alteration of either the metallic cations or other metal binding substances in the environment.

4. The compound may be isolated, purified, assayed, assimilated, detoxified, or excreted more readily in the chelate form.

Chelation and Tetracyclines

The tetracyclines comprise a group of antibiotics characterized by their common hydronaphthacene skeleton and broad spectrum of antibacterial activity (Figure 1).



	R_1	R_2	R_3
Tetracycline	H	CH_3	H
7-Chlortetracycline	Cl	CH_3	H
5-Oxytetracycline	H	CH_3	OH

Figure 1.

Chlortetracycline (aureomycin), the first member of the group to be isolated, was discovered by Duggar in 1948 among the metabolic products of Streptomyces aureofaciens. Oxytetracycline (terramycin) was isolated two years later from Streptomyces rimosus fermentation by Finlay et al. (1950).

All tetracyclines are amphoteric each having three ionizable groups, with PK_as ranging from 3.3 to 9.7. Their common chromophoric system gives a yellow color to all members of this family.

To isolate the tetracyclines, advantage has been taken of their ability to chelate with many metal ions. They may be precipitated from the filtered culture medium at pH 8-10 in the presence of a divalent cation, as the calcium or magnesium salts, or at pH 1-15 as the chelate compound with ferric ion. Calvin et al. (1945) reported that perhaps the most important sites for chelation lie along the system marked by atoms 1 through 7 which consists of two 1,3 diketones with two of the keto groups in the enol form.

Regna et al. (1951) reported that oxytetracycline forms mixed salts with a number of pairs of divalent ions such as Ba++ and Ca++ or Ca++ and Mg++. The salts are insoluble at pH 8.5 to 9.5 and use was made of this property in the precipitation of the drug from fermentation broths.

Albert (1953, 1956) in his studies observed the formation of drug-metal complexes of 1:1 and, as the pH is increased, of 2:1. The cations tested (in order of decreasing stability) were: Fe+++, Al+++, Cu++, Ni++, Fe++, Co++, Zn++, and Mn++. Unfortunately Ca++ was not included in his studies. It was noted that the stability constants of both tetracyclines are similar. He also noted that the order of affinity for the metallic ions is similar to that shown by the common amino acids with one important exception. Fe++ is bound more strongly with the amino acids while tetracyclines show a greater preference for the Fe+++.

Albert (1953) noted that Fe+++, Fe++ complexes have a red color, Cu++ and Ni++ green, and Al+++, Co++, Zn++, and Mn++ yellow. However, Oxford (1953) was not able to obtain colored complexes with Zn++ and Mn++, while according to his test method, Ca++ and Co++ had the greatest affinity for the antibiotic.

Effect of Tetracycline-Metal Complex on Enzyme Systems

The first suggestion that the action of tetracyclines might be related to metal binding came from Van Meter et al. (1952), who noted that chlortetracycline inhibition of oxygen consumption in aged mitochondria was reversed by Mg++. It is believed that the effect of aging mitochondria

is to release endogenous magnesium from the centers of enzyme activity into the suspending medium. This would account for the fact that chlortetracycline is often ineffective in reducing the oxygen uptake when fresh mitochondria is used. Thus if the Mg^{++} concentration of the active centers is low (aged mitochondria), the addition of aureomycin is expected to reduce the oxygen uptake by holding the remaining magnesium in an inactive complex (Van Meter and Oleson, 1951; Van Meter et al., 1952). Brody et al. (1954) reported that the uncoupling action of all three tetracyclines is prevented if sufficient Mg^{++} is included in the medium.

Bernheim (1954a) found that chlortetracycline and exytetracycline inhibt the formation of a benzoic acid oxidase system in Pseudomonas aeruginosa. This inhibition was found to be reversed by the addition of Fe^{++} or Mn^{++} (Bernheim, 1954b).

Saz and Slei (1953, 1954a,b) in a series of studies observed that chlortetracycline and oxytetracycline inhibited the reduction of the nitro groups, of a cell-free enzyme system, to the corresponding arylamines. This inhibition was reversed by manganese. They also reported that after purification, the enzyme was found to be a pyridine nucleotide linked, loosely dissociable flavin mononucleotide-containing flavoprotein with a requirement for Mn^{++}.

Rokos et al. (1958, 1959) reported that bivalent cations were necessary for the inhibition of hydrolases by chlortetracycline.

Weinberg and Tolzmann (1955) speculated that since the tetracyclines combine with Ca^{++}, it could be possible that appropriate concentrations of the tetracyclines might inhibit the coagulation of blood, thus interfering with the heart activity.

Braude et al. (1953) have indeed found that oxy- and chlortetracycline are excellent anticoagulants, but Ca^{++} is able to reverse this action if the drugs are present in concentrations less than 10^{-4} M.

Swain et al. (1956) reported that the heart failure of isolated dog heart-lung preparations produced by large quantities of tetracyclines could be reversed by an equimolar concentration of Ca^{++}.

Effect of Tetracycline-Metal Complex on the Growth of Microbes

Weinberg (1954a,b, 1955a,b) in a series of papers, presented the results of his tests to determine the influence of inorganic salts and chelating agents on the ability of the tetracyclines to inhibit microbial growth. From these tests he concluded that in general the antimicrobial action of the tetracyclines is unaffected by the majority of the inorganic salts and chelating agents.

However, he found that multivalent cations, like Fe++, Mg++, Mn++, Ca++ and Al+++, were able to supress the inhibitory action of tetracyclines against some microorganisms. This suppresion depended upon the genus of the microorganism and the medium used. For example, he reported that with strains of Escherichia and Bacillus, Fe++ is very active in complex media, but inactive in a synthetic medium. Because of this observation, he went on to say that this might be a reflection of the alternate metabolic pathways used by cells in different nutritive environments.

To further prove this dependence of tetracycline to suppress microbial growth, Weinberg (1954a, 1955a,b,) conducted qualitative tests. Filter paper discs soaked in 0.1 per cent solutions of salts of different cations were placed on inoculated drug-containing media. Depending on the organism, the cation involved, the medium, and the tetracycline involved, the minimum inhibitory concentrations of the drug were increased from two to thirty times in areas around active ion discs as compared to controls.

Soncin (1953) (cited by Weinberg 1955) and Weinberg (1955) conducted a few studies where the addition of Mg++ to the drug-microbe was delayed from one to eight hours. Under these conditions, Mg++ was still capable of suppressing the antimicrobial activity. They also reported

that when the cells were exposed to Mg++ for one to two hours prior to addition of the tetracyclines, a lower concentration of Mg++ was required to suppress the drugs.

Mechanism of Action of the Tetracyclines as Explained by Chelation

It is evident from the above discussions that most of the activities of the tetracyclines (inhibition of microbial growth, inhibition of enzyme systems) are strongly affected by the metallic ions or other metal binding substances.

Many hypotheses have been offered over the years to explain the suppression of antimicrobial and other inhibitory activities of the tetracyclines by such ions as Fe++, Mg++ and Ca++. Of these, three are the most prevalent.

The first hypothesis is that the tetracyclines function by chelating or binding certain ions that are essential for specific functions. To support this hypothesis are the findings of Braude et al. (1953) that it is possible to prevent coagulation of the blood by chelation of the calcium with the use of very high levels of terramycin. Brody et al. (1954) have demonstrated that fatty acid oxidation by the liver mitochondria can be inhibited by oxytetracycline and chlortetracycline. However, additions of Mg++ reverses that inhibition by those tetracyclines. Their explanation of that reversal is that the

the tetracyclines chelate with Mg++ (when present at normal concentrations) thus depriving the system from an essential element. However, when the Mg++ concentration is high, the chelating ability of the antibiotic is overwhelmed. Van Meter et al. (1952) have also offered the same explanation to the reversal by excess magnesium of the inhibition of the oxidative phosphorylation in animal tissue by chlortetracycline.

On the other hand, work by Price et al. (1957b) showed that extremely high molar ratios of cation to antibiotics are required to obtain a significant decrease in antibiotic activity, which makes this hypothesis a little doubtful.

The second hypothesis is that cation-antibiotic complexes formed on or in the cell might render the antibiotic non-toxic (Weinberg, 1955c). However, Hunter and Lowry (1956) suggested that the possibility also exists that tetracyclines may act by combining with a metallic ion bound to the enzyme and the actual toxic entity is the drug-metal complex. As far as the excess quantity of metallic ion required to inhibit that toxicity, they offer the explanation that the drug-metal complex has a much greater affinity for the enzyme than does the non-chelated metal.

The third hypothesis is that of the preferential

adsorption of metallic cations by the bacterial cell. This hypothesis was adopted by Soncin (1953) to explain the magnesium interference with oxytetracycline and chlortetracycline. He also suggested that ions might also displace the antibiotic that has already reached its site of action. As it was mentioned above, both Soncin (1953) and Weinberg (1955) observed that the reversal of antibiotic activity could be accomplished by addition of magnesium as late as two hours after exposure of the bacterial cells to antibiotic.

Price et al. (1957a) in a series of experiments noted that when sufficient calcium or magnesium was added, oxytetracycline activity could be reversed even after prolonged exposure of cells to antibiotic. They then concluded that the antibiotic attaches itself at the surface of the bacterial cell. Further support was given to this hypothesis by the finding that delayed addition of sodium citrate or potassium oxalate also reversed the antibiotic inhibition.

The same workers (Price et al. 1957b) in another experiment observed that while Ca suppressed the uptake of Oxytetracycline by the cells, its uptake was not influenced. This also supports the hypothesis that cations interfere with the antibiotic as a result of their preferential adsorption on the cell surface.

McCalla (1940) demonstrated that bacterial cells do adsorb cations such as calcium. In 1941, the same worker in experiments with methylene blue, showed that one ion can be displaced from a bacterial cell surface by another. The replacing power of the cations studied is:
Na NH_4 K Mg Ca Ba Mn Al Fe.

Based on these observations, Price et al. (1957b) suggested that it is possible that tetracyclines (amphoteric by nature) may act as cations that are replaced by these positively charged ions. The degree of cationic inhibition for a given antibiotic should be proportional to its basicity. Data of one of the experiments of Price et al. (1957b) substantiated this observation. Highly basic antibiotics, such as neomycin and polymyxin B, were found to be markedly inhibited by divalent cations. In contrast to that, acidic or neutral antibiotics such as penicillin or chloramphenicol are highly resistant to divalent cation interference.

Potentiation

As it was mentioned in the introduction, potentiation is a relatively new term in animal research and means to make more active or powerful. Most of the research in the field of potentiation of antibiotics has been done with chickens, rats and humans.

To evaluate the potentiating effect of the different procedures used, many criteria have been employed by different workers. Among them the following are included:

1. Determination of effect of potentiation on the minimum inhibitory concentrations of antibiotic in nutrient broth.
2. Reversal of antibiotic inactivation following exposure of antibiotic to specific divalent cations.
3. Comparative and preferential antibiotic and calcium uptake by washed cell suspensions.
4. Blood levels following feeding trials (continuous feeding).
5. Blood levels following feeding trials (slug feeding).
6. Duodenal loop method--blood antibiotic concentration.
7. Duodenal loop method--liver antibiotic concentration.
8. Duodenal loop method--urine antibiotic concentration.
9. Disease challenge trials.
10. Tissue residue levels.

The disease challenge method--probably the most important one for measuring potentiation of antibiotics--has

shown that there is a definite positive correlation between improved disease control and increased blood levels of antibiotics.

This is probably the reason that most of the workers have adapted the method of measuring blood levels of antibiotics following feeding as a means of evaluating their potentiation.

From a practical standpoint of view, calcium is the only element which represents an important inhibitor in feeds, and since this mineral is present in relatively high levels in practically all feeds in order to meet the animal's requirement, most of the research on potentiation is centered around this element.

There are two ways to reverse the inhibition of antibiotics due to calcium. The first one is to reduce the calcium and the second to use calcium complexing substances such as chelating agents and certain acids and salts.

Price and Zolli (1959) using citric acid and Kojic acid as possible potentiators found that under low calcium conditions citric acid had rather limited effectiveness (at 0.4 and 0.8 mM concentrations) whether the criterion was serum level, liver level or urine level of oxytetracycline.

However, in the presence of calcium, addition of

citric acid at a 0.4 mM concentration, partially neutralized the deleterious effect of calcium, while at a 0.8 mM concentration it completely counteracted it. They concluded that although there are certain limitations of citric acid feeding, it is still a desirable potentiator when divalent cations act as inhibitors. Kojic acid, on the other hand, had no apparent potentiating effect of terramycin, under either normal or high calcium conditions.

Two other compounds that have been studied very extensively are glucosamine and Terephthalic acid (TPA). Unlike the other potentiators which have their effect by chelating calcium, TPA decreases the urinary secretion of the antibiotics.

Luther (1960),working with chickens, studied the potentiating effects of both glucosamine and Terephthalic acid. He reported that in the absence of added calcium, TPA increased absorption of terramycin in the blood by 34% while glucosamine increased absorption only 11 per cent. However, under high calcium conditions, glucosamine improved absorption by 44% indicating that glucosamine is a good potentiator in the presence of added calcium. When TPA and glucosamine were added together in the diet, TPA further increased the oxytetracycline blood levels by 39% over the 44% increase due to glucosamine.

English (1958) in his studies with purebred Beagle

dogs, found that D-glucosamine hydrochloride enhanced the serum levels of tetracycline from 76% at one hour to 34% at seven hours over those obtained after oral administration of tetracycline hydrochloride. Snell and Garkusha (1958) employing labeled C^{14} oxytetracycline and measuring it with liquid scintillation counting, reported that glucosamine HCl enhances the serum levels of oxytetracycline in Beagle dogs and strain A mice between 0 and 24 hours. Method of administration in these studies was by stomach tube feeding.

Luther (1960) in a complex type of experiment used oxy- and chlortetracycline from 100 to 1000 g/ton with low (0.18%), normal (1.2%), and high levels (2.25%) of calcium, normal (0.7%), and high levels (4.87%) of phosphorous and all these diets with and without 0.35% TPA. He reported that:

a) Terramycin gave a 0.112 and chlortetracycline a 0.054 mg/ml increase in blood antibiotic level for each additional 100 g/ton of antibiotic fed. Those increases represented a 39% increase for terramycin and 52% for chlortetracycline.

b) In the absence of TPA, calcium reduction gave an average 1.89 times greater actual increase in blood oxytetracycline compared to

chlortetracycline, but only 48% of the enhancement of chlortetracycline based on percentage increase value. When TPA was added to the reduced calcium diets, the figures were 2.18 times increase of oxytetracycline over chlortetracycline in actual blood values, but only 64% of the enhancement of chlortetracycline based on percentage increase.

c) The response to TPA, although somewhat less for both antibiotics than the response to reduced calcium, is additive to that of reduced calcium.

d) Increased level of phosphorous gave a 0.613 mcg/ml (110%) increase for oxytetracycline and 0.165 mcg/ml (80%) increase for chlortetracycline.

Cover et al. (1959) conducted a series of experiments to study the potentiating effect of TPA and low dietary calcium on the tetracyclines using the challenge disease method. They reported that both TPA and reduced dietary calcium increased the amount of antibiotic which is present in the serum. Proportionally there was a two-fold increase in the clinical activity of both oxy- and chlortetracycline against the disease infectious synovitis in chicken. The mortality rate when 100 grams per ton of antibiotic was used with potentiation was similar to that when 200 grams were fed without the potentiation. The

conclusion of their study was that in treating diseases which have antibiotic sensitivity similar to that of infectious synovitis, use of either low calcium diets or TPA over a short period of treatment will reduce by about half the feed level of antibiotic necessary to produce the beneficial clinical result. These results are in very close agreement with those obtained by Price and Zolli (1959).

Dorsey and Harshfield (1959) in disease-challenge trials (fowl cholera in chicken) showed that chlortetracycline at 1,000 g/ton of feed and oxytetracycline at 500 g/ton feed, gave the same protection, reducing mortality from over 80% to less than 10%. Price and Zolli (cited by Luther 1960) conducted a potentiating study with chickens infected with fowl cholera. Terramycin at 200 and 400 g/ton of feed was included in normal (1.26%) and low (0.165%) calcium diets. They reported that a decreased calcium intake for five days almost doubled the efficacy of oxytetracycline.

Work by Stokstad et al. (1959) and Pensack et al. (1959) indicated that not all calcium salts have the same inhibitory effect on broad-spectrum antibiotics. Their data suggests that it is possible to feed these less inhibitory forms of calcium in the diet, thereby meeting the animal's requirement without depressing the antibiotic blood level.

Stokstad et al. (1959) and Donovan et al. (1960) reported that the less soluble calcium salts would give a smaller calcium ion concentration and a slower release at the anterior portion of the intestinal track where antibiotic absorption is maximal.

Among those calcium sources with less inhibitory effect on the antibiotics is calcium sulfate which has been reported by Tyler and Wilcox (1942) and Heywang (1946) to be less available to the chicken. Watts and Miner (1959) reported that in colloidal (soft) phosphate, one of the materials showing potentiation of antibiotics in feeds, both calcium and phosphorous are showing reduced availability to the animal.

Work at Pfizer & Co., Inc. (Luther, 1960) showed that at the 0.16% calcium level, the availability of oxytetracycline (measured in blood) was 100% when calcium was supplied in the form of either carbonate or sulfate. However, at the 2.15% calcium level, the availability of oxytetracycline was 37% in the presence of calcium carbonate and 88% with calcium sulfate.

Camp (1960) investigating the use of colloidal phosphate as a means for enhancing antibiotic levels found that replacement of one-third of the dicalcium phosphate with soft phosphate resulted in a 64% potentiation. A two-thirds replacement gave a 96% enhancement.

Kaplan et al. (1957) conducted a study with 188 patients using tetracycline hydrochloride and a complex of tetracycline and sodium hexametaphosphate. He reported that absorption with tetracycline phosphate complex is about twice as efficient as that obtained with tetracycline hydrochloride. Welch et al. (1957a) in a triple crossover experiment with 90 human subjects, found that tetracycline phosphate complex and a mixture of tetracycline base and sodium metaphosphate are equally effective in increasing tetracycline blood levels compared to tetracycline base.

Welch et al. (1975b) using 30 volunteer human subjects, observed that tetracycline base plus sodium metaphosphate produced markedly higher blood concentrations than either tetracycline base or tetracycline hydrochloride. The same workers (1957c) in a quadruple crossover experiment with 20 human subjects, showed very conclusively that both chlortetracycline hydrochloride and chlortetracycline base, when mixed with sodium hexametaphosphate, produced higher serum concentrations than those obtained without the hexametaphosphate.

Dearborn et al. (1957) carried out a factorial experiment with rats to study the effects of citric acid, dicalcium phosphate, sodium metaphosphate, food, oil and sorbital on the serum activity of tetracycline hydrochloride

or tetracycline base. They reported that citric acid, when given in equal weight with tetracycline hydrochloride, gave the highest concentrations in the experiment. Dicalcium phosphate and food resulted in lower and sodium metaphosphate in higher serum activity than was observed in their absence. These results are in full agreement with those obtained by Sweeney et al. (1957).

In a triple crossover study with 15 human subjects, Welch et al. (1957d) reported that while citric acid when mixed with oxytetracycline hydrochloride increased blood concentration of oxytetracycline, sodium metaphosphate decreased it.

Eggert and Elliott (1959) conducted a series of experiments to determine if the results obtained with poultry would apply to swine. The first experiment used weanling pigs in a total of nine treatments. The first diet was a low (0.06%) calcium, low (0.33%) phosphorus diet, and the other two were supplemented with either dicalcium phosphate and ground limestone or calcium sulfate and liquid phosphoric acid to contain 0.70 per cent total calcium and 0.51 per cent phosphorus. Each of these diets was given with or without either 0.5 per cent TPA or 0.6 per cent tetrasodium ethylene diamine tetraacetate (EDTA). All diets contained 200 mg/Kg. of chlortetracycline. Their findings from this experiment were that both EDTA and TPA

were effective in increasing blood levels of chlortetracycline. They also found that calcium sulfate and phosphoric acid gave somewhat better results than the combination of calcium carbonate and TPA, but not as good as the low calcium diet.

In a similar type of experiment with baby pigs, they confirmed the findings of the previous experiment. Low calcium diets as well as diets supplemented with calcium sulfate gave values of blood chlortetracycline which were 80 per cent greater than when calcium carbonate was used in diets. This increase was comparable to that obtained when 0.5 per cent TPA was added in the diets. Looking at the bone ash, they reported that, as expected, low calcium rations definitely interfere with bone formation. On the other hand, there were no differences between calcium carbonate and calcium sulfate, indicating that calcium from calcium sulfate is available for bone formation. They observed that none of the treatments had any significant effect on serum calcium levels.

The results obtained from two experiments with growing-finishing swine were in accordance with those obtained from previous experiments with young pigs. There was an increased serum level of antibiotic with increasing levels of chlortetracycline, an additional boost from adding TPA and also an advantage of those rations in which calcium

sulfate was substituted for calcium carbonate. An interesting observation in one of their experiments, where the basal diet contained 0.3 per cent total calcium before supplementation, was that there was only a slight advantage with rations containing calcium sulfate compared with the calcium carbonate rations. Based on results obtained from previous experiments where total calcium of basal diets were less than 0.1 per cent before supplementation, they concluded that, for optimum potentiation of aureomycin in the blood serums through the substitution of calcium sulfate for calcium carbonate in pig rations, the calcium content of the basal ration must be lower than 0.1 per cent.

Workers at the American Cyanamid Company (1964) proposed another more efficient way to potentiate chlortetracycline by the use of sodium sulfate. This chemical compound will theoretically tie up calcium, allowing more chlortetracycline to be absorbed in the duodenum which probably is the principal site of absorption of chlortetracycline. The reason that addition of sodium sulfate is more efficient is because there is no need for reformulation of the diet.

A series of experiments were conducted with chickens to study the efficacy of using sodium sulfate. The results of these experiments are summarized as follows:

1. Addition of sodium sulfate to a feed containing calcium carbonate (1.0% total calcium) increased the blood levels of chlortetracycline to the same extent as did substituting an equivalent amount of calcium sulfate for calcium carbonate in the ration.
2. Addition of sodium sulfate to the rations permitted better absorption of chlortetracycline regardless of the source of phosphorus.
3. Addition of sodium sulfate to a low calcium feed increased the blood level of chlortetracycline by 33 per cent.
4. Sodium sulfate improved absorption of chlortetracycline to a greater extent than did equivalent quantities of sulfate ions from calcium sulfate.

MATERIALS AND METHODS

Three experiments were conducted over a period of two years to study the effect of different diets on the potentiation of chlortetracycline.

Experiment 1. The objective of this experiment was to study the effect of level and source of calcium on performance of growing pigs fed a conventional (corn plus soybean meal) type of diet with and without chlortetracycline. One hundred and twenty eight crossbred pigs of an average weight of 32.0 kg. were randomly allotted to eight treatments, each treatment replicated four times in a 2x2x2 factorial experiment. Four pigs were assigned to each pen and an attempt was made to allot equal numbers of barrows and gilts to each group. However, because of a shortage of barrows, three gilts and one barrow were assigned to each treatment in replications three and four.

The pigs were housed in an environmentally-controlled confinement building and had access to feed and water ad libitum. Records were kept on the weekly weights of each pig and feed was weighed back every two weeks to obtain feed consumption data. All diets were calculated to have a 1:1 ratio of calcium to phosphorus and to contain 14% crude protein.

The composition of the diets used is shown in Table 2.

TABLE 2. COMPOSITION OF DIETS (PERCENT). EXPERIMENT 1

Ingredient	Diet 1[a]	2	3[a]	4	5[a]	6	7[a]	8
Ground corn	84.2		85.1		81.4		83.0	
Soybean meal (48% C.P.)	13.4		13.2		13.9		13.6	
Calcium sulfate	1.6		---		2.9		---	
Calcium carbonate	---		0.9		---		1.6	
Monosodium phosphate	0.2		0.2		1.2		1.2	
Trace mineral salt (.8% Zm)	0.4		0.4		0.4		0.4	
Premix[b]	0.2		0.2		0.2		0.2	
Calculated minerals (%)								
Calcium	0.4		0.4		0.67		0.67	
Phosphorus	0.4		0.4		0.67		0.67	
Ca:P ratio	1:1		1:1		1:1		1:1	

a. Premix with chlortetracycline (50 g/ton).

b. Supply per kg. of diet: Vitamin A, 3,307 IU; Vitamin D, 331 IU; Vitamin E, 5.5 IU; Vitamin K, 2.2 mg; Riboflavin, 2.76 mg; Pantothenic acid, 11.0 mg; Niacin, 17.6 mg; choline, 110.0 mg; Vitamin B_{12}, 110.0 mcg.

Diets 1 through 4 were calculated to contain 0.40 percent calcium and 0.40 percent phosphorus, while diets 5 through 8 were calculated to contain 0.67 percent calcium and 0.67 percent phosphorus. In diets 1, 2, 5 and 6 supplemental calcium was supplied by calcium sulfate and in 3, 4, 7 and 8 by calcium carbonate. All diets were supplemented with monosodium phosphate to supply the required phosphorus. The experiment was conducted for six weeks.

Experiment 2. The objective of this experiment was the quantitative measurement of chlortetracycline and calcium in blood serum as well as growth performance of growing pigs fed different levels of calcium..

Ninety six pigs of an average weight of 21.2 kg. were utilized for this experiment. They were allotted according to weight and sex to six treatments of four pigs per treatment with each treatment replicated four times. Four pigs (two barrows and two gilts) were allotted to each pen and housed in an environmentally-controlled building. All pigs were fed and watered ad libitum.

The experiment consisted of two periods of four and two weeks for periods one and two respectively. During the first period the six treatments consisted of diets containing 0.3, 0.7, and 1.1 percent calcium with and without chlortetracycline (100 g/ton). The calcium to phosphorus ratio ranged from 1:1.2 to 1.2:1 with the supplemental.

phosphorus supplied by monosodium phosphate. At the end of the first period all pigs were changed to a low (0.3 percent) calcium diet for the second period. Pigs treated with chlortetracycline in the first period continued to receive the antibiotic but at a level of 200 g/ton. For both periods the conventional (corn plus soybean meal) type diet, shown in Table 3, was employed to provide a 16 percent crude protein diet.

The animals were weighed weekly for the first two weeks, at the end of the first period and again at the end of the second period. Feed was weighed back twice during the first period and at the end of the second period to obtain feed consumption data.

Blood was drawn from all pigs from the jugular vein at the beginning of the experiment, at the end of the first period, and at the end of the second period.

Experiment 3. The primary objective of this experiment was to study the potentiation of chlortetracycline. For this purpose, 104 pigs of an average weight of 21 kg. were allotted according to weight and sex to thirteen treatments, each treatment replicated twice. Four pigs (two of each sex) were included in each pen. The pigs were housed in an environmentally-controlled building and had access to feed and water at all times.

After the allotment, all pigs were allowed to become

TABLE 3. COMPOSITION OF DIETS (PERCENT). EXPERIMENT 2.

Ingredient	Diet 1[a]	2	3[a]	4	5[a]	6
	Calcium Level %					
	0.3		0.7		1.1	
Ground corn	79.3		79.5		74.3	
Soybean meal (48% C.P.)	18.5		19.0		19.4	
Calcium carbonate	0.6		1.6		2.6	
Monosodium phosphate	---		1.3		2.1	
Trace mineral salt (1% Zm)	0.4		0.4		0.4	
Premix[b]	1.2		1.2		1.2	

a. Diets with chlortetracycline (100 g/ton) for the first period and 200 g/ton for the second.

b. See Table 2.

c. Pigs on diets 3 and 5 were changed to diet 1 and those on diets 4 and 6 to diet 2 for the second period.

accustomed to their respective diets for three days. At the end of the third day they were fasted overnight and then given access to their diets the next morning for a period of approximately five hours. At that time twenty cubic centimeters of blood was drawn from the jugular vein of each pig. Blood was also obtained from each pig 14 days after the initiation of the experiment.

The diets, the composition of which is shown in Table 4, included two control diets--with and without chlortetracycline--three diets with increasing levels of sodium sulfate, a low calcium diet, two high calcium diets--with and without sodium sulfate--two high phosphorus diets, and three diets with increasing levels of calcium sulfate. All diets contained a 16 percent crude protein.

This experiment was terminated after a period of ten weeks during which period all animals were weighed every two weeks.

Analytical Procedures

Upon collection, blood samples were placed in plastic centrifuge tubes and immediately placed in an ice chest filled with ice. Upon arrival at the lab, the centrifuge tubes were placed in a refrigerator overnight to allow enough time for clotting and shrinkage of the clot. The next day the blood was centrifuged and the serum collected

TABLE 4. COMPOSITION OF DIETS (PERCENT). EXPERIMENT 3

Ingredient	DIET NO. 1	2	3	4	5	6	7	8	9	10	11	12	13[a]
Corn	75.6	75.1	74.3	73.4	78.2	73.0	70.5	72.8	70.0	75.3	74.5	73.8	75.6
Soybean meal (48% C.P.)	19.2	19.3	19.4	19.6	18.7	19.7	20.1	19.7	20.2	19.2	19.4	19.5	19.2
Calcium carbonate	1.9	1.9	1.9	1.9	0.7	2.9	2.9	1.9	1.9	1.5	0.75	----	1.9
Monosodium phosphate	0.9	0.9	0.9	0.9	---	2.0	2.0	3.2	5.5	0.9	0.90	0.9	0.9
Sodium sulfate	---	0.355	1.07	1.775	---	---	2.13	---	---	---	---	---	---
Calcium sulfate	---	---	---	---	---	---	---	---	---	0.7	2.05	3.43	---
Trace mineral salt (1% Zn)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Premix[b]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Calculated analysis (%)													
Calcium	0.8	0.8	0.8	0.8	0.35	1.2	1.2	0.8	0.8	0.8	0.8	0.8	0.8
Phosphorus	0.6	0.6	0.6	0.6	0.37	0.9	0.9	1.2	1.8	0.6	0.6	0.6	0.6
Sodium	0.23	0.35	0.58	0.81	----	0.64	1.3	0.69	1.04	0.23	0.23	0.23	0.23
Sulfates		0.24	0.73	1.21	----		1.45	---	---	0.39	1.15	1.92	---
Protein	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00

[a]Control diet without chlortetracycline

[b]See Table 2.

in plastic analyzer cups which were placed in a freezer at -5 C until analyzed.

Blood Calcium Analysis

The serum was diluted with 0.5% lanthanun solution 1:10 to prevent interference of phosphorus. Duplicate samples were thoroughly mixed, and along with a standard and a blank, were analyzed for calcium using the Perkin-Elmer 303 Atomic Absorption Spectrophotometer.

Blood Chlortetracycline Analysis

Microbiological Assay--Cylinder Plate Method:

This method is one of the most accurate for detecting antibiotics in biological fluids and feeds. It is based on the principle that when a cylinder filled with an antibiotic containing solution is placed on the surface of solidified agar which has previously been inoculated with an organism sensitive to a given antibiotic, the antibiotic will: 1) diffuse from the cylinder, 2) inhibit the growth of the test organism, and 3) form a circular zone of inhibition the diameter of which is directly proportional to the concentration of the antibiotic in the cylinder.

The method as described by Grove and Randall (1955) was used in these studies to determine the concentration of chlortetracycline in the blood serum.

Preparation of the test organism. The test organism used throughout the analysis was Bacillus cereus var. mycoides (ATCC 11778) obtained from the American Type Culture Collection. The organism was grown at a temperature of 30 C and stored under refrigeration. A suspension of the organism was prepared as follows: An agar slant of antibiotic medium No. 1 was streaked heavily with the test organism and incubated for 24 hours at 30 C. The growth was washed off with 5 ml. of sterile distilled water onto the surface of a Roux bottle containing 300 ml. of agar medium No. 1, and the suspension was spread over the entire agar surface with the aid of sterile glass beads and incubated for one week at 30 C. After incubating, the growth was washed from the agar surface with 30 ml. of sterile distilled water into a glass stoppered flask and the suspension was heat shocked for 30 minutes at 65 C. Following the heat shock, the suspension was washed three times with sterile distilled water, centrifuged between each washing, and heat shocked again as before. This suspension then was maintained under refrigeration and used as the stock spore suspension for all assay work.

To determine the spore suspension needed to give a sensitivity to chlortetracycline of 0.005 g/ml., a preliminary study was performed with concentrations of 0.1,

0.2, and 0.4%. Although they all gave the same results, the 0.4% concentration gave the clearest zone and was selected as the concentration to use in this study.

Preparation of plates. Flat bottom petri dishes with dimensions of 100 x 22mm were used in this analysis. Six milliliters of melted agar medium No. 8 was added to each petri dish, distributed evenly, covered and allowed to harden for 15 minutes.

For the second layer, 100 ml. of agar medium No. 8 was cooled to 55 C in an incubator and mixed well with 0.4 ml. of the spore suspension to give a concentration of 0.4%. Four milliliters of the seeded medium was added to the hardened first (base) layer, distributed evenly and allowed to harden for 15 minutes.

After the inoculated layer was hardened, six cylinders were placed on the agar surface on a 2.8 cm radius at 60 degree intervals. The cylinders used were stainless steel with an outside diameter of 8mm (± 0.1mm), an inside diameter of 6mm (± 0.1mm), and a length of 10mm (± 0.1mm).

Working standard and standard curve. Ten milligrams of chlortetracycline hydrochloride, which was kept at room temperature in a tightly stoppered vial inside a desicator, were diluted with double distilled water to give a concentration of 1mg/ml solution, which was diluted again with phosphate buffer No. 4, pH 4.5, to give a

concentration of chlortetracycline of 3.2 mcg/ml. This solution was further diluted with a diluent containing one part of 3.5 percent bovine albumin plus two parts of phosphate buffer No. 4, pH 4.5. The bovine albumin was used as a 3.5 percent solution to equal the protein binding power of normal serum.

To prepare the standard curve, the 3.2 mcg/ml solution of chlortetracycline was diluted 1:10 with the bovine albumin-phosphate buffer solution (1+2), to give a concentration of 0.32 mcg/ml. This solution was further diluted with the phosphate buffer-albumin solution by halves to obtain concentrations for the standard curve of 0.32, 0.16, 0.08, 0.04, 0.02, 0.01 and 0.005 mcg/ml.

Three plates for each concentration of the curve were used, except the 0.04 mcg/ml, which as a reference was included in all plates. On each plate, three cylinders were filled with the 0.04 mcg/ml concentration of chlortetracycline and the other three with the concentration of the curve tested, alternating reference and concentration under test. Thus, there were 45 of the 0.04 mcg/ml readings and 9 for each of the concentrations of the standard curve.

The plates were incubated for a period of 16-18 hours at 30 C, after which the diameter of each zone of inhibition was measured. The readings of the 0.04 mcg/ml

concentration under test for each set of three plates were averaged. An average figure for all 45 readings of the 0.04 mcg/ml concentration was obtained which was used as a correction point for the curve. If, for example, the average figure of all 45 readings was 12 mm and the average figure for the 0.04 mcg/ml concentration for a given set of plates was 11 mm, then 1 mm was added to the diameter of the zones of the concentration under test.

The corrected values including the average of the 45 readings of the 0.04 mcg/ml concentration were plotted on a two-cycle semilogarithmic paper, using the concentrations in mcg/ml as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa.

Preparation of blood serum. The frozen serum was allowed to thaw and 0.5 ml was transferred into a plastic analyzer cup where it was mixed with 1.0 ml of phosphate buffer No. 4 pH 4.5. This dilution is necessary because very small concentrations of tetracyclines in blood serum are inactive when added to assay plates (no zones of inhibition) but reveal activity when diluted.

Assay of serum. Two plates were used for each blood serum sample to be tested. Two cylinders in each plate were filled with 0.04 mcg/ml (reference) concentration, two were filled with the first serum under test, and two

were filled with the second, alternating always standard with the serums.

All the plates were incubated for 16-18 hours at 30 C after which the diameter of the zones of inhibition were measured. These diameters were then corrected according to the reference point in each plate and the concentration of chlortetracycline in mg/ml for each serum sample obtained from the standard curve.

Data were analyzed statistically by least squares analysis of variance procedures available at the South Dakota State University Computer Center. A probability level of less than 0.05 was accepted as being significant. To separate treatment mean differences, Duncan's new multiple-range test and Dunnett's test, where applicable, were used to separate treatment mean differences.

RESULTS AND DISCUSSION

Experiment 1. A general summary of the overall feed and growth performance data of this experiment is shown in Table 5 and average daily gains by two-week periods and on an accumulative basis are presented in Table 6. Statistical analysis of the data are shown in appendix Tables 1, 2 and 3. Overall there were no significant differences among treatments in average daily gain, feed consumption or feed per gain.

The effects of dietary antibiotic, calcium source and calcium level are presented in Table 7. During the first two-week period pigs fed the antibiotic-supplemented diets gained about 12% faster when the calcium source was calcium sulfate than when calcium carbonate was the source of calcium. However, when the diets did not contain antibiotic, the pigs gained 3% faster (0.69 vs. 0.67 kg/day) when fed the diets containing calcium carbonate. This interaction between antibiotic and calcium source was statistically significant ($P < .05$) and indicates a greater response to antibiotic, during the early growth phase, when calcium sulfate is used as a calcium source. Pigs receiving the antibiotic in their diets gained 0.72 kg. daily which was faster ($P < .05$) than the 0.68 kg. per day of pigs fed diets without antibiotic during the first two-week period.

During the second period (2-4 weeks), pigs receiving the antibiotic diets gained significantly faster when the diets contained 0.67% Ca derived from either calcium source, than when the diets contained 0.4% Ca. Gains of pigs fed diets without antibiotic were similar at both calcium levels. With both sources of calcium daily gains of pigs were less when fed the low calcium (0.4%) diets containing antibiotic than when these diets did not include antibiotic.

In the third period, pigs on the calcium carbonate containing diet achieved gains which were significantly ($P < .01$) better than the gains of pigs on a calcium sulfate containing diet (0.77 vs 0.70 kg/day). It is possible that increased feed consumption of sulfates by the pigs on the calcium sulfate diet caused an adverse effect on growth rate for the last period. Gains of pigs on either calcium source and calcium level with antibiotic were similar to the gains of the pigs on the same diets without antibiotic.

These findings are in agreement with those by Robinson *et al.* (1954) and Lucas (1955). In their studies they both concluded that there is little benefit from antibiotics beyond the weight of 45 kilograms.

Over the six-week period the only statistically significant difference obtained was between sexes ($P < .05$)

TABLE 5. GROWTH AND FEED PERFORMANCE DATA. EXPERIMENT 1

Item	Calcium source							
	$CaSO_4$				$CaCO_3$			
Calcium level (%)	0.4		0.67		0.4		0.67	
Antibiotic[a]	+	-	+	-	+	-	+	-
No. of Pigs	16	16	16	16	16	16	16	16
Avg. initial wt., kg.	32.3	32.3	32.3	32.3	32.3	32.4	32.3	32.3
Avg. final wt., kg.	62.7	61.8	63.3	61.5	61.5	62.8	64.5	63.0
Avg. daily gain, kg.	0.73	0.70	0.74	0.69	0.70	0.72	0.77	0.73
Avg. daily feed intake, kg.	2.38	2.37	2.46	2.35	2.29	2.34	2.50	2.39
Feed/gain	3.26	3.37	3.34	3.39	3.27	3.24	3.26	3.27

[a] + = with chlortetracycline (50 g/ton), - = without antibiotic

TABLE 6. AVERAGE DAILY GAINS (KG) PER PERIOD AND ACCUMULATIVE. EXPERIMENT 1.

Period (weeks)	Antibiotic	Ca Level	Calcium source			
			$CaSO_4$		$CaCO_3$	
			0.4	0.67	0.4	0.67
0 - 2	+		0.77	0.75	0.67	0.68
	-		0.68	0.66	0.70	0.67
2 - 4	+		0.70	0.77	0.70	0.83
	-		0.73	0.73	0.75	0.73
4 - 6	+		0.70	0.69	0.73	0.80
	-		0.70	0.69	0.73	0.80
0 - 4	+		0.73	0.76	0.68	0.75
	-		0.70	0.69	0.72	0.70
0 - 6	+		0.73	0.74	0.70	0.77
	-		0.70	0.69	0.72	0.73

TABLE 7. EFFECTS OF ANTIBIOTIC, CALCIUM SOURCE AND CALCIUM LEVEL ON AVERAGE DAILY GAINS.[a] EXPERIMENT 1.

Period (Weeks)	Antibiotic	Means for main effects and interactions					
		+	-	$CaSO_4$	$CaCO_3$	0.4	0.67
0 - 2	+	0.72		0.76	0.68	0.72	0.67
	-		0.68	0.67	0.69	0.72	0.67
	Mean	0.72[c]	0.68[d]	0.72	0.69	0.72	0.70
2 - 4	+	0.75		0.74	0.77	0.70	0.80
	-		0.74	0.73	0.74	0.74	0.73
	Mean	0.75	0.74	0.74	0.76	0.72[d]	0.77[c]
4 - 6	+	0.74		0.70	0.77	0.72	0.75
	-		0.74	0.70	0.77	0.72	0.75
	Mean	0.74	0.74	0.70[d]	0.77[c]	0.72	0.75
0 - 4	+	0.74		0.75	0.72	0.71	0.76
	-		0.71	0.70	0.72	0.71	0.76
	Mean	0.74	0.71	0.73	0.72	0.71	0.73
0 - 6	+	0.74		0.74	0.74	0.72	0.76
	-		0.71	0.70	0.73	0.71	0.71
	Mean	0.74	0.71	0.72	0.74	0.77	0.74

[c,d] Values with different superscripts within the same line differ significantly ($P < .05$)

[a] All values are in kilograms

as barrows outgained gilts by 0.75 to 0.70 kg/day. The fact that calcium sulfate, at the levels tested, did not affect the performance of growing pigs is supported by the findings of Combs and Wallace (1962). In their studies, neither rate nor efficiency of gain were significantly influenced by the source of supplementary calcium used. However, both dry matter and crude protein digestibility were significantly lowered when calcium sulfate rather than ground limestone or oyster shell supplied the supplementary calcium. Also, McCampbell and Aubel (1934) found that limestone and calcium sulfate are essentially equal as sources of calcium for swine.

Feed efficiency (feed/gain) data are reported in Table 8 while statistical analysis is shown in appendix Tables 2 and 3. Feed/gain followed the same pattern as the average daily gains as far as performance is concerned. For the first two weeks, pigs on a calcium sulfate diet, regardless of level of calcium and presence of antibiotic, required less feed (2.74 vs. 2.85) per unit of gain compared to pigs fed diets with calcium carbonate. Less feed was also required per unit of gain by pigs receiving diets containing calcium sulfate plus antibiotic at both levels of calcium compared to the pigs on the same diet without antibiotic or the pigs on a calcium carbonate diet with or without antibiotic. Also, regardless

TABLE 8. FEED/GAIN PER PERIOD AND ACCUMULATIVE EXPERIMENT 1.

Period (Weeks)	Antibiotic	Means for main effects and interactions					
		+	-	$CaSO_4$	$CaCO_3$	0.4	0.67
0 - 2	+	2.75		2.61	2.88	2.73	2.76
	-		2.86	2.88	2.83	2.81	2.90
	Mean	2.75	2.86	2.74	2.85	2.77	2.83
2 - 4	+	3.21		3.27	3.14	3.26	3.15
	-		3.14	3.11	3.16	3.05	3.23
	Mean	3.21	3.14	3.19	3.15	3.16	3.19
4 - 6	+	4.03		4.21	3.85	3.99	4.06
	-		4.01	4.24	3.78	4.13	3.89
	Mean	4.03	4.01	4.23^{b}	3.82^{a}	4.06	3.98
0 - 4	+	2.98		2.93	3.01	2.99	2.96
	-		3.00	2.99	3.00	2.93	3.07
	Mean	2.98	3.00	2.96	3.01	2.96	3.02
0 - 6	+	3.29		3.31	3.27	3.28	3.30
	-		3.32	3.38	3.26	3.31	3.33
	Mean	3.29	3.32	3.35	3.27	3.30	3.32

^{a,b} Values with different superscript differ significantly ($P < .05$).

of level and source of calcium, pigs receiving antibiotic were more efficient in converting feed to gain than non-antibiotic fed pigs.

For the second period, there were no significant differences, with pigs on all treatments achieving similar feed efficiencies. During the third period, pigs on a calcium carbonate diet required significantly less feed ($P < .05$) per unit of gain than those on a calcium sulfate diet. Feed per gain was 3.81 and 4.22 for pigs fed diets containing calcium carbonate and calcium sulfate respectively.

Overall, as with average daily gains, there were no significant differences among treatment for the total experiment of six weeks.

Experiment 2. The overall growth and feed performance data of this experiment are shown in Table 9. Statistical analysis is shown in appendix Tables 4, 5 and 6.

The average daily gains by periods and on an accumulative basis are reported in Table 10. During all periods there was a highly significant antibiotic effect ($P < .01$) as pigs fed diets with antibiotic, regardless of calcium level, gained faster than the non-antibiotic fed pigs by 0.62 to 0.52 kg., 0.67 to 0.53 kg., and 0.73 to 0.58 kg. for the first, second and third period respectively. Over the entire experimental period (6 weeks) the antibiotic

TABLE 9. GROWTH AND FEED PERFORMANCE DATA. EXPERIMENT 2

Item	Antibiotic[a]	Dietary calcium level (%)					
		0.3		0.7		1.1	
		+	-	+	-	+	-
No. of Pigs		16	16	16	16	16	16
Initial body wt., kg.		21.2	21.2	21.2	21.2	21.0	21.0
Final body wt., kg.		49.5	43.7	50.2	42.6	48.3	45.3
Average daily gain, kg.		0.68	0.54	0.69	0.51	0.65	0.58
Daily feed intake, kg.		1.78	1.55	1.75	1.46	1.75	1.62
Feed/gain		2.62	2.87	2.54	2.86	2.70	2.80

[a] + = with chlortetracycline (100 g/ton), - = without antibiotic.

TABLE 10. AVERAGE DAILY GAIN (KG) BY PERIODS AND ACCUMULATIVE. EXPERIMENT 2

Antibiotic	Dietary Calcium level (%) 0.3	0.7	1.1	Mean
	0 - 2 Weeks			
+	0.63[a]	0.64[a]	0.59	0.62[a]
-	0.51[b]	0.50[b]	0.56	0.52[b]
Mean	0.57	0.57	0.58	
	2 - 4 Weeks			Mean
+	0.70[a]	0.70[a]	0.61	0.67[a]
-	0.56[b]	0.52[b]	0.52	0.53[b]
Mean	0.63	0.61	0.57	
	4 - 6 Weeks			Mean
+	0.70[a]	0.75[a]	0.73	0.73[a]
-	0.54[b]	0.54[b]	0.67	0.58[b]
Mean	0.62	0.65	0.70	
	0 - 4 Weeks			
+	0.67[a]	0.67[a]	0.60	0.65[a]
-	0.54[b]	0.50[b]	0.54	0.53[b]
Mean	0.61	0.59	0.57	
	0 - 6 Weeks			Mean
+	0.68[a]	0.69[a]	0.65	0.67[a]
-	0.54[b]	0.51[b]	0.58	0.54[b]
Mean	0.61	0.60	0.62	

[a,b] Values within the same column and period with different superscripts differ significantly ($P < .01$)

fed pigs gained 0.67 kg. daily versus 0.54 kg. for those fed diets without antibiotics. This difference was highly significant ($P < .01$). There was also a significant calcium x antibiotic effect as pigs fed the two lower dietary calcium levels responded to antibiotics significantly better ($P < .05$) than those on the high calcium level. Average daily gains of pigs fed diets of 0.4 and 0.7% dietary calcium with antibiotic over the entire experimental period (6 weeks) were 0.68 and 0.69 kg., respectively, compared to 0.54 and 0.51 kg. for those pigs on the same levels of calcium without antibiotic. On the other hand, while pigs on the diet containing 1.1% calcium tended to grow slightly faster than their counterparts without antibiotic, these differences were not statistically significant at any of the periods. Over the entire experimental period, pigs on the high level of calcium with antibiotic gained 0.65 kg. daily as compared to 0.58 kg. for those fed the non-antibiotic diet.

These results may be compared with the results obtained by Costain and Lloyd (1962). In their studies they found that reducing calcium from 0.85% to 0.40% in diets of growing pigs did not effect gain, feed conversion or apparent digestibility of nutrients. Plumlee et al. (1956) reported an experiment in which four levels of calcium (0.2 to 1.1%) ± 100 ppm Zn were fed to pigs from

15 to 85 kg. Pigs fed diets of 0.2 and 0.5% calcium gained similarly with or without zinc. Pigs fed 0.8% or 1.1% calcium grew slower and less efficiently than those fed the lower levels. Zinc additions improved the performance of pigs on the higher levels of calcium.

There was also a significant sex effect ($P<.05$) for the entire six-week period with barrows achieving average daily gains of 0.68 kg. versus 0.58 kg. for gilts. These differences were a reflection of similar statistical significance ($P<.05$) obtained in periods two and three (Appendix Table 5).

In addition, there was a significant ($P<.05$) replication effect. This effect may have been partly due to weather changes, as the first replication was removed from experiment at the beginning of September while the fourth replication completed the experiment about mid-October, and partly to different starting weights of pigs used in the four replicates (47.5, 44.8, 46.8 and 48.3 kg. for the first, second, third and fourth replicates respectively). Also, the fact that pigs used in the fourth replicate were more variable in starting weight might have contributed to the replicate difference.

Feed efficiency (feed/gain) data for this experiment are shown in Table 11 while statistical analysis is reported in appendix Tables 5 and 6. Although pigs fed

TABLE 11. FEED PER GAIN PER PERIOD AND ACCUMULATIVE. EXPERIMENT 2.

Antibiotic		Dietary Calcium level (%) 0.3	0.7	1.1	
			0 - 2 Weeks		Mean
+		2.44	2.27	2.45	2.39[a]
-		2.75	2.51	2.56	2.60[b]
	Mean	2.60	2.39	2.51	
			2 - 4 Weeks		Mean
+		2.76	2.77	3.02	2.85[c]
-		3.22	3.29	3.13	3.21[d]
	Mean	3.00	3.03	3.07	
			4 - 6 Weeks		
+		2.69	2.57	2.63	2.63[e]
-		2.81	2.86	2.72	2.80[f]
	Mean	2.75	2.72	2.67	
			0 - 4 Weeks		
+		2.59	2.52	2.74	2.62[c]
-		3.00	2.87	2.83	2.90[d]
	Mean	2.79	2.69	2.79	
			0 - 6 Weeks		
+		2.62	2.54	2.70	2.62[e]
-		2.87	2.86	2.80	2.84[f]
	Mean	2.74	2.70	2.75	

[a,b] Values within the same column and period with different superscripts differ significantly ($P < .05$).

[c,d] Values within the same column and period with different superscripts differ significantly ($P < .025$)

[e,f] Values within the same column and period with different superscripts differ significantly ($P < .01$)

antibiotic diets consumed significantly more feed daily ($P < .025$ for the first two periods and $P < .01$ for the third), they were also more efficient in converting that feed to body weight gain. Feed per gain for the pigs fed the antibiotic diets were 2.39, 2.85 and 2.63 for the first, second and third periods respectively compared to 2.60, 3.21 and 2.80 for pigs fed diets without antibiotic during those same periods. These differences were significant during each period. Over the entire experiment (6 weeks), antibiotic fed pigs required 2.62 kg. of feed per kg. gain while the non-antibiotic fed pigs required 2.84 kg. of feed per kg. gain, a difference which was significant at the 0.1% level ($P < .001$). There was also a replication effect which was significant at the 2.5% probability level.

Blood serum calcium data are shown in Table 12. Serum calcium in the blood obtained at the beginning of the experiment was similar in all pigs and there were no significant differences among treatments. Four weeks later (second blood collection), there was a significant calcium ($P < .01$) effect with pigs on the lower dietary calcium level showing higher values of serum calcium. Those values were 11.19 mg/100ml for the 0.3% dietary calcium level versus 10.52 and 10.63 mg/100ml for the 0.7 and 1.1% dietary calcium respectively. The third blood collection

took place two weeks after the second. During this two-week period all pigs were fed a diet of 0.3% calcium and 200 g/ton chlortetracycline. Blood serum calcium was generally lower in all treatments compared to the previous blood collection. The most striking difference was the sharp decrease of serum calcium levels of the pigs fed the 0.3% calcium diet in the previous period. Serum calcium was 11.19 and 10.02 mg/100ml for the second and third blood collections respectively. This represented a 10.5% decrease.

It is well documented that if large quantities of calcium ions are removed from the circulating body fluids, the calcium ion concentration again returns to normal within minutes to hours. These effects result from the fact that body contains a type of exchangeable calcium that is always in equilibrium with the calcium ions in the extracellular fluids. A small portion of this exchangeable calcium is that calcium found in all tissue cells, especially in highly permeable types of cells such as those of the liver and the gastrointestinal tract. Newman et al. (1967) found in their studies with growing pigs that restriction of calcium intake increased efficiency of absorption and retention of calcium.

It is possible that in this study pigs fed the low calcium diet for the first four weeks responded by utiliz-

ing more of the exchangeable calcium, absorbing more from the gastrointestinal tract and retaining more to maintain homeostasis. Continuing the feeding of the low calcium diet for another two weeks probably depleted the exchangeable calcium and started mobilizing calcium from the bones, a process which is much slower than the previous, resulting in lower serum calcium values.

Feeding 100 g/ton chlortetracycline increased blood serum calcium levels significantly ($P<.01$) at the second collection period. Serum calcium values were 10.95 and 10.61 mg/100ml for antibiotic and non-antibiotic fed pigs, respectively which represented a 3.1% increase due to antibiotic.

In the third blood collection even though antibiotic fed pigs showed a 4.1% increase in serum calcium over non-antibiotic fed pigs (10.44 vs. 10.02 mg/100ml), this difference was not significant. The fact that the coefficient of variation for this third bleeding was 11.65, approximately double the 6.5 and 6.2 coefficient of variations for the first and second blood collections respectively, might have contributed to the failure to detect a statistically significant difference.

Significant differences were also found among replicates for blood serum calcium at all three blood collection periods. This difference was mainly due to the fact that

TABLE 12. BLOOD SERUM CALCIUM. EXPERIMENT 2.

Blood Sample[a]	Antibiotic	Dietary Calcium level (%) 0.3	0.7	1.1	
		------mg/100 ml-------			Mean
1	+	10.23	10.42	10.33	10.33
	-	10.46	10.12	10.26	10.28
	Mean	10.35	10.27	10.29	
					Mean
2	+	11.31	10.58	10.96	10.95[c]
	-	11.08	10.46	10.30	10.61[d]
	Mean	11.19[c]	10.52[d]	10.63[d]	
					Mean
3[b]	+	10.25	10.60	10.47	10.44
	-	9.79	10.20	10.07	10.02
	Mean	10.02	10.40	10.27	

[a] First blood collection took place at the beginning of the experiment, second four weeks later, and third at the end of the experiment.

[b] Between second and third blood collection all pigs were fed a 0.3% calcium diet and 200 g/ton chlortetracycline.

[c,d] Values with different superscript within the same line or column differ significantly ($P < .01$).

the height and density of the flame of the spectrophotometer was never the same between any two days of analysis, thus changing the absolute values of blood calcium but not the relative ones.

At this point it is appropriate to say that despite the fact that some statistical differences were obtained in blood serum calcium none of these differences appear to be of any practical importance since all the values of blood serum calcium obtained in this experiment fall within the normal range of 9 to 12 mg/100ml as reported by Ullrey et al. (1967).

Blood serum levels of chlortetracycline as affected by decreasing dietary calcium levels and increasing chlortetracycline levels in the diet are reported in Tables 13 and 14 respectively. Mean squares for blood serum chlortetracycline are shown in appendix Table 9. Since the first blood collection took place at the beginning of the experiment, blood serum chlortetracycline concentration was very small and is not reported in the tables.

The concentrations of chlortetracycline in the blood from the second blood collection were 0.263, 0.360 and 0.482 mcg/ml for pigs fed 1.1, 0.7, and 0.3% dietary calcium respectively. There was a highly significant calcium effect at the 1% probability level. In addition, chlortetracycline concentrations were linearly correlated

with a correlation coefficient of r =-0.998. The regression equation which describes the regression line best fitted to these data is:

$$Y = 0.548 - 0.244X$$

where X represents the dietary calcium levels and Y the chlortetracycline concentrations in the serum.

Potentiation of chlortetracycline or increased serum chlortetracycline concentration due to decreased calcium in the diet is also reported in Table 13. It can be seen from this table that a decrease in calcium from 1.1 to 0.7% in the diet, resulted in an increase in serum chlortetracycline of 0.097 mcg/ml or 26.9 percent. A further decrease of 0.4% in dietary calcium to a level of 0.3% increased serum chlortetracycline by 0.122 mcg/ml or an additional 25.3 percent. Totaling the above results, a 0.8% decrease in dietary calcium increased serum chlortetracycline by 45.4 percent. These results agree with the findings of Eggert and Elliot (1959). In one of their experiments, baby pigs fed chlortetracycline (100 gm/ton) with low calcium diet (0.17% Ca) for five weeks showed serum chlortetracycline levels of 0.260 mcg/ml while those on a 0.95% calcium diet showed values of 0.140 mcg/ml. This represented a decrease of 46.2% for a 0.8% increase in dietary calcium. In another study with weanling pigs, a 0.7% decrease in dietary calcium brought a 50% increase

TABLE 13. BLOOD SERUM CHLORTETRACYCLINE AS AFFECTED BY DECREASING LEVELS OF DIETARY CALCIUM. EXPERIMENT 2.

Diet	Dietary Ca	Dietary P	Dietary Ca:P	Serum levels Blood sample 2 [a]	Potentiation from decreasing dietary calcium	
	-----%----			mcg/ml	mcg/ml	%
5	1.1	0.92	1.2:1	0.263^{de}		
3	0.7	0.70	1:1	0.360^{ce}	0.097	26.9
1	0.3	0.37	1:1.2	0.482^{be}	0.122	25.3

[a]See Table 12.

[b,c,d]Values with different superscripts within the same column differ significantly ($P < .01$)

[e]Regression line and correlation coefficient for dietary calcium on serum chlortetracyclines, diets 5, 3 and 1. $Y = 0.548 - 0.244X$, $r = 0.998$.

in serum chlortetracycline.

The serums concentrations of chlortetracycline in the blood from the third blood collection (Table 14) which took place two weeks following the second, were 0.560, 0.662 and 0.695 mcg/ml for diets 5, 3 and 1 respectively. Although all diets contained the same amount of calcium (0.3%) and chlortetracycline (200 g/ton) for the second experimental period, serum concentration followed the same pattern as in the first period, with pigs receiving diet 5 having the lowest concentration of serum chlortetracycline and those on diet 1 showing the highest concentration.

When serum chlortetracycline values from the third blood collection were plotted against the dietary calcium levels of the first period, a linear correlation was found with a correlation coefficient of $r = -0.920$. The linear regression which best fits these data is:

$$Y = 0.757 - 0.169X$$

where Y represents the serum chlortetracycline concentrations and X dietary calcium level.

The fact that serum levels of chlortetracycline in the blood from the third blood collection were not the same, but linearly correlated with the dietary calcium levels of the first experimental period, indicates that there was a carry over effect of dietary calcium level.

TABLE 14. EFFECT OF INCREASING DIETARY CHLORTETRACYCLINE ON BLOOD SERUM LEVELS. EXPERIMENT 2.

Blood Sample[a]	Diet	Dietary Ca	Dietary Chlortetracycline	Serum levels	Increase	
		%	mcg/ml	mcg/ml	mcg/ml	%
	5	1.1	100	0.263[d]		
2	3	0.7	100	0.360[c]		
	1	0.3	100	0.483[b]		
	5	0.3	200	0.560[ce]	0.297	53.0
3	3	0.3	200	0.662[b]	0.302	45.6
	1	0.3	200	0.695[b]	0.212	30.5

[a]See Table 12.

[b,c,d]Values with different superscripts within the same period differ significantly ($P < .01$).

[e]Regression line and correlation coefficient for dietary calcium levels of the first period on blood serum chlortetracycline of the second period. $Y = 0.757 - 0.169X$, $r = 0.920$

On a percent basis, serum chlortetracycline increased by 30.5, 45.6 and 52.0% for pigs on diets 1, 3 and 5 which contained 0.3, 0.7 and 1.1% calcium during the first period.

Since pigs fed diet 1 received a 0.3% calcium diet throughout the experiment, the 30.5% increase of serum chlortetracycline can be attributed to the increased dietary chlortetracycline from 100 to 200 g/ton. Eggert and Elliott (1959), based on their data with weanling baby pigs, made the following statement: "With increasing levels of chlortetracycline in the diet there is a stepwise increase in serum level of the antibiotic." It is possible that increased antibiotic in the diet will bring an increase in serum chlortetracycline of the same absolute magnitude in all pigs. If that assumption is true, the additional increase in serum chlortetracycline in pigs fed diets 3 and 5 might be due to decreased dietary calcium from 0.7 to 0.3 and 1.1 to 0.3% Ca for pigs on diets 3 and 5 respectively.

Experiment 3. Growth and feed performance data, blood serum calcium and chlortetracycline are reported in Tables 15 through 20 while statistical analyses are shown in appendix Tables 10 through 12. During the course of the experiment one pig on treatment 7 and one on treatment 10 died. Necropsy performed by a staff veterinarian at the Animal Disease Research and Diagnostic Laboratory at South Dakota State University indicated that the possible

cause of death of both pigs was stomach ulcers. Two pigs on treatment 1 were removed after 56 days on the experiment because of very poor performance and data on these pigs are not included in the results. Because of the very low calcium level in diet 5, pigs on this treatment developed severe rickets and they were removed from the experiment after 8 weeks, although their growth rate did not appear to be affected at that time.

Table 15 summarizes the growth and feed performance data of this experiment. Diet 13 was the only diet without antibiotic, it was included to serve as a control to determine antibiotic effect on growth and feed performance. Diet 1 was an identical to 13 with the addition of antibiotic to serve as control to determine the potentiating effect of chlortetracycline in the other diets.

To compare the average daily gains of pigs on treatments 1 through 12 with those fed diet 13 (control), Dunnett's test was applied. With the exception of pigs on diet 1, which was the same as the control with the addition of antibiotic, all pigs performed significantly better ($P < .05$) than those on the control diet. Pigs on diet 1 fed antibiotic grew faster than the negative controls (0.61 vs. 0.53 kg/day); however, this difference was not significant. Statistical analysis of the data by three week intervals (Table 10) showed that pigs on both

diets (1 and 13) gained slower than the pigs on all other diets throughout the experiment.

At a time when many farmers-especially in South Dakota-are attributing the poor performance of their pigs to high sulfates in the water, it is interesting to note that pigs on this study with as high as 19,000 ppm of sulfates in their diet performed above average for their age. National Academy of Science considers an average daily gain of around 0.70 kg/day as normal. In this study pigs fed diets 2, 3, 4, 7, 10, 11, and 12 with sulfates of 2,400, 7,300, 12,100, 14,500, 3,900, 11,500 and 19,200 ppm respectively achieved daily gains of 0.78, 0.77, 0.79, 0.79, 0.83, 0.70 and 0.77 kg/day. Sulfates in the water in South Dakota seldom exceed 6,000 ppm. Although, water consumption will generally be approximately twice that of feed consumption, the sulfate intake of pigs receiving the high sulfate diet was probably higher than the sulfate intake of pigs on most waters that have been thought to reduce performance of pigs. It is also possible, however, that other minerals contained in high concentrations in the water in combination with the sulfates might be the cause of reduced pig performance in certain instances.

Extensive research by scientists at the American Cyanamid Company (1964), showed that broiler-chicks can be fed a diet containing 2.8% sodium sulfate for three

TABLE 15. GROWTH AND FEED PERFORMANCE DATA[a]. EXPERIMENT 3.

DIETARY	DIET[b] 1	2	3	4	5[c]	6	7	8	9	10	11	12	13[d]
Ca, %	0.80	0.80	0.80	0.80	0.35	1.20	1.20	0.80	0.80	0.80	0.80	0.80	0.80
P, %	0.60	0.60	0.60	0.60	0.37	0.90	0.90	1.20	1.80	0.60	0.60	0.60	0.60
Na, %	0.23	0.35	0.58	0.81	----	0.64	1.30	0.69	1.04	0.23	0.23	0.23	0.23
SO_4, %	----	0.24	0.73	1.21	----	----	1.45	----	----	0.39	1.15	1.92	----
No. of pigs	8	8	8	8	8	8	8	8	8	8	8	8	8
Initial body wt. kg.	20.8	20.9	20.9	20.9	20.9	20.8	20.9	20.8	20.9	20.4	20.9	20.9	20.9
Final body wt. kg.	63.5	75.7	75.0	76.5	60.7	70.8	76.2	76.4	70.4	78.4	70.8	75.0	57.7
Avg. daily gain, kg.[e]	0.61	0.78	0.77	0.79	0.71	0.71	0.79	0.79	0.71	0.83	0.78	0.77	0.53
Daily feed intake, kg.[f]	----	2.20	2.28	2.26	1.95	2.18	----	2.27	2.09	----	2.05	2.31	1.72
Feed/gain[f]	----	2.82	2.96	2.86	2.75	3.07	----	2.87	2.94	----	2.89	3.00	3.25
Firmness of feces[g]	1	2	3	4	2	1	5	1	3	1	4	5	3

[a]The length of the experiment was 70 days.

[b]All diets contained chlortetracycline (200 g/ton).

[c]Data of pigs on treatment 5 were calculated on a 56-day basis.

[d]Diet 13 did not contain antibiotic.

[e]Average daily gain for pigs on treatments 1,7 and 10 was calculated on a basis of 6,7 and 7 pigs respectively.

[f]Average daily feed intake and feed/gain were not calculated for pigs on treatments 1,7 and 10.

[g]The scale for feces firmness was 1 = normal, 3 = soft, 5 = loose.

weeks or 1.5% sodium sulfate for nine weeks without any adverse effect on growth rate or feed efficiency. Higher levels of sodium sulfate for extended periods of time caused high mortality due to wet litter. Table 15 shows the firmness of feces of pigs in this experiment. Although the feces of pigs on the high sulfate diets (either sodium or calcium sulfate) were loose throughout the experiment, no effect was noted on growth and feed performance. Eggert and Elliott (1959) working with baby pigs of an average weight of 6.5 pounds, reported that diets with as high as 3.5% calcium sulfate did not significantly effect growth rate and feed efficiency when fed for a period of five weeks. In another study, growing-finishing pigs were fed diets with as high as 2.0% calcium sulfate for seventy days. Again no significant differences were noted in growth rate and feed efficiency.

The effects of dietary treatment on blood serum calcium values are presented in Table 16 and mean squares for blood serum calcium are reported in appendix Table 11. At both blood collection periods there was a highly significant treatment effect ($P<.01$) on serum calcium level. The lowest values of serum calcium were recorded in the blood of pigs on diets that contained 0.8% calcium and 1.8% phosphorus.

It is possible that the imbalance of calcium to phos-

TABLE 16. BLOOD SERUM[a] CALCIUM. EXPERIMENT 3.

Diet	Dietary Ca	Dietary P	Blood sample[b] 1	Blood sample[b] 2
	------%-----		---mg/100ml---	
1 (Control)	0.80	0.60	10.38[d]	10.95[d]
2	0.80	0.60	10.70[d]	11.03[d]
3	0.80	0.60	10.68[d]	11.39[d]
4	0.80	0.60	10.42[d]	11.01[d]
5	0.35	0.37	10.54[d]	11.47[d]
6	1.20	0.90	10.54[d]	10.68[d]
7	1.20	0.90	10.43[d]	11.11[d]
8	0.80	1.20	10.11[d]	10.45[d]
9	0.80	1.80	9.51[c]	10.30[c]
10	0.80	0.60	10.83[d]	11.20[d]
11	0.80	0.60	10.69[d]	11.30[d]
12	0.80	0.60	10.36[d]	11.11[d]
13	0.80	0.60	10.34[d]	10.67[d]

[a] Each value is an average of serum calcium from 8 pigs.

[b] Blood sample 1 was taken at the beginning of the experiment after fasting the pigs overnight and then allowing them to eat for 5 hours. Blood sample 2 was taken two weeks later.

[c,d] Values with different superscript within the same column differ significantly ($P < .05$).

phorus might have caused the decrease in blood serum calcium. National Research Council recommends a dietary calcium to phosphorus ratio of between 1:1 and 1.2:1. Diet 9 had a ratio of 1:2.25. It is well documented that a great excess of either calcium or phosphorus interferes with the absorption of the other, a fact which helps to explain why a certain ratio between them in the diet is desirable for their best absorption. But even though the blood calcium values of pigs on diet 9 are statistically significantly lower than the others, they still fall within the normal range of 9-12 mg/100ml reported by Ullrey et al. (1967). There was also a significant difference among replications in blood serum calcium at both collection periods.

Blood serum chlortetracycline values are reported in Table 17. Statistical analysis of these data showed that there was a highly significant treatment effect ($P < .01$) at both sampling periods. To make individual comparisons with the control, Dunnett's test was applied. In the first blood sample (taken at the beginning of the experiment) blood from all pigs, with the exception of those treated with calcium sulfate (diets 10, 11 and 12), contained chlortetracycline levels which were significantly higher ($P < .01$) than the controls. In the second sample (two weeks after the first) only blood from pigs fed the very low calcium diet and the two very high phosphorus

TABLE 17. BLOOD SERUM CHLORTETRACYCLINE. EXPERIMENT 3.

Diet[c]	Serum levels[a] Blood Sample[b] 1	2
	-------mcg/ml-------	
1 (Control)	0.383[e]	0.673[g]
2	0.558[d]	0.688[g]
3	0.661[d]	0.679[g]
4	0.818[d]	0.734[g]
5	0.740[d]	0.883[f]
6	0.638[d]	0.621[g]
7	0.752[d]	0.702[g]
8	0.848[d]	0.929[f]
9	0.692[d]	0.867[f]
10	0.487[e]	0.700[g]
11	0.251[e]	0.623[g]
12	0.333[e]	0.607[g]

[a]Each value is an average of serum from 8 pigs.

[b]See Table 16.

[c]For composition of diets, see Table 5.

[d,e]Values with different superscript differ significantly ($P < .01$). All means were compared with the control with Dunnett's test.

[f,g]Values with different superscript differ significantly ($P < .05$).

diets showed chlortetracycline values significantly higher ($P < .05$) than controls.

Since several potentiating procedures were used in this experiment, the data are presented in different tables to study each method separately.

The effect of sodium sulfate on blood serum chlortetracycline is summarized in Table 18. Increasing amounts of sodium sulfate in the diet caused a linear increase of blood serum chlortetracycline in blood sample 1. The correlation coefficient can many times be misleading because it does not take into consideration dietary calcium content. A better variable to be correlated with blood serum chlortetracycline would be a sulfate to calcium ratio. On that basis an even better correlation coefficient was obtained with $r = 0.9795$. The regression line best fitted to these data is:

$$Y = 0.4242 + 0.1820X$$

where X = sulfate to calcium ratio and Y = blood serum chlortetracycline levels.

The actual increases of blood serum chlortetracycline at the first sampling when the diet of the pigs was supplemented with 0.355, 1.07, 1.775 and 2.13% sodium sulfate were 0.175, 0.278, 0.435 and 0.369 mcg/ml respectively compared to the chlortetracycline in blood serum of pigs fed diet 1 that was not supplemented with sodium sulfate

TABLE 18. BLOOD SERUM[a] CHLORTETRACYCLINE AS AFFECTED BY SODIUM SULFATE. EXPERIMENT 3.

Diet	Dietary Ca	Dietary Sodium sulfate	Dietary SO_4:Ca	Blood sample[b] 1[c]	Blood sample[b] 2	Potentiation due to sodium sulfate, Blood sample 1 mcg/ml	Potentiation due to sodium sulfate, Blood sample 1 %	Potentiation due to sodium sulfate, Blood sample 2 mcg/ml	Potentiation due to sodium sulfate, Blood sample 2 %
	%	%		mcg/ml	mcg/ml	mcg/ml	%	mcg/ml	%
1	0.80	----	----	0.383[g]	0.673[e]				
2	0.80	0.355	0.44	0.558[f]	0.688[e]	0.175	31.4	0.015	2.2
3	0.80	1.07	1.34	0.661[ef]	0.679[e]	0.278	42.1	0.006	1.0
4	0.80	1.775	2.22	0.818[d]	0.734[d]	0.435	53.2	0.061	8.3
7	1.20	2.13	1.775	0.752[de]	0.702[de]	0.369	49.1	0.064	9.1

[a] See Table 17.

[b] See Table 17.

[c] Regression line and correlation coefficient of SO_4:Ca vs. serum chlortetracycline $Y = 0.4242 + 0.18197X$, $r = 0.9705$.

[d,e,f,g] Values with different superscripts within the same column differ significantly ($P < .01$)

(Table 18). The only significant effect of sodium sulfate on blood serum chlortetracycline in blood sample 2, which was taken two weeks after the first sample, was for pigs fed diet 4 which contained 1.775% sodium sulfate and 0.8% calcium. Blood serum chlortetracycline of pigs fed this diet was greater ($P < .01$) than that of pigs fed diets 1, 2, or 3.

Although no published data were found relative to the effects of sodium sulfate on chlortetracycline potentiation in the swine field, research with chickens at the American Cyanamid Company (1964) showed results similar to these obtained in this study. In one of their studies, five uniform groups of 12 chicks each were fasted overnight, fed for three hours a constant calcium diet with different increments of sodium sulfate and then bled by cardiac puncture. They reported that each added level of sodium sulfate progressively increased the amount of chlortetracycline in the blood serum. A level of 0.355% sodium sulfate in the diet caused a 22% increase in blood serum chlortetracycline while 1.42% sodium sulfate resulted in an increase of 50% in serum chlortetracycline.

Blood serum chlortetracycline as affected by calcium sulfate is reported in Table 19. None of the blood serums from pigs under test had chlortetracycline levels significantly different than the controls (unpotentiated). At

TABLE 19. BLOOD SERUM CHLORTETRACYCLINE AS AFFECTED BY CALCIUM SULFATE. EXPERIMENT 3.

Diet	Dietary			Serum levels[a]	
	Ca	Calcium Sulfate	Calcium from Calcium Sulfate	Blood Sample[b] 1	2
				-------mcg/ml-------	
1	0.80	----	----	0.383[cd]	0.673
10	0.80	0.70	0.15	0.487[c]	0.700
11	0.80	2.05	0.43	0.251[d]	0.623
12	0.80	3.43	0.72	0.333[cd]	0.607

[a]See Table 17.

[b]See Table 17.

[c,d]Values with different superscripts differ significantly ($P < .01$).

the first blood sampling period, there was a highly significant ($P < .01$) treatment effect on blood serum chlortetracycline of pigs fed the three levels of calcium sulfate. Serum from pigs fed the 0.70% calcium sulfate diet contained 0.487 mcg/ml chlortetracycline which was significantly greater than the 0.251 mcg/ml of chlortetracycline in serum of pigs fed the 2.05% calcium sulfate diet. The chlortetracycline level of 0.333 mcg/ml in blood serum of pigs fed diets containing 3.43% calcium sulfate was intermediate and not significantly different from either of the other levels of calcium sulfate. The fact that blood serum chlortetracycline was the lowest in blood obtained from pigs on diet 11 may have been due to the reduced feed intake of pigs fed this diet.

Serum chlortetracycline from the second blood collection was similar for all pigs fed diets 1, 10, 11 and 12.

After extensive research with calcium sulfate as a calcium supplement for swine diets, Eggert and Elliott (1959) concluded that: "for optimum potentiation of chlortetracycline in the blood serum through the substitution of calcium sulfate for calcium carbonate in pig rations, the calcium content of the basal ration must be lower than 0.1 percent total initial calcium." They also reported that, even when calcium sulfate supplemented a diet with lower than 0.1 percent total initial calcium, it

did not have as great a potentiating effect as a low calcium diet for the first and second week, but thereafter there was no significant difference between the calcium sulfate and low calcium treatment results in regard to serum levels of chlortetracycline.

Although the basal (corn-soybean meal) diets in this study contained less than 0.1% calcium, addition of calcium carbonate raised the initial total calcium before supplementation of calcium sulfate to 0.65 and 0.37% for diets 10 and 11 respectively (Table 19). According to the findings of Eggert and Elliott (1959) only diet 12 with total initial calcium of 0.08% before supplementation of calcium sulfate, should have given a potentiation of chlortetracycline in the blood serum. Yet, no potentiation was noted in this study in both blood collections. The fact that both blood collections took place within two weeks after the initiation of the experiment might have been a factor for not detecting a potentiation. According to Eggert and Elliott (1959) more than two weeks of continuous feeding of calcium sulfate are probably required to obtain a potentiation equivalent to that of a low calcium diet.

As it was mentioned in the review of literature the theory behind the potentiating power of sodium sulfate is based on the fact that sulfates supplied by sodium sulfate

tie up much of the dietary calcium (forming calcium sulfate) allowing more chlortetracycline to be absorbed in the duodenum which is probably the primary site of absorption of chlortetracycline. If this is true it is difficult to explain why when calcium sulfate supplements the diet there is no immediate potentiating effect such as the one noted in this study when sodium sulfate was used.

Blood serum chlortetracycline as affected by increasing levels of dietary phosphorus at constant levels of dietary calcium is shown in Table 20. Dietary calcium for diets 1, 8 and 9 was 0.80% while phosphorus content was 0.60, 1.20 and 1.80% respectively, in these three diets. The calcium to phosphorus ratio was 1:0.75, 1:1.50 and 1:2.25 for diets 1, 8 and 9 respectively.

At both collection periods blood serum chlortetracycline was significantly ($P<.01$) higher when pigs were fed the high phosphorus diets. At the first sampling period the diets containing 1.2 and 1.8% phosphorus gave serum chlortetracycline increases of 54.8 and 35.3%, while two weeks later (blood sample 2) the increases in serum chlortetracycline were 27.6 and 22.4% respectively. Blood serum chlortetracycline levels of pigs fed the higher phosphorus (1.8%) diet were significantly ($P<.01$) lower than those pigs fed diet of 1.2% phosphorus, at the first collection period.

This indicates the probability that there is an optimum dietary phosphorus increase which will give the best potentiating effect. A further increase will either not change it or have a negative effect compared with the increase obtained with the optimum dietary phosphorus increase. It is possible that the use of more diets with dietary phosphorus levels below and above the 1.8% phosphorus used in this study, would have indicated if there is indeed an optimum dietary phosphorus level for maximum potentiation of chlortetracycline.

A very large number of feeding trials with chickens at the Pfizer Research Station (Luther, 1960) showed results similar to those obtained in this study. In one study, two groups of chicks were fed a constant calcium diet (1.2% Ca) with two levels of phosphorus (0.7 and 4.08%). Blood obtained from chicks which were fed the 0.7% phosphorus diet contained 0.206 mcg/ml chlortetracycline, while blood from those chicks on the 4.08% phosphorus diet contained 0.371 mcg/ml chlortetracycline, an increase of 0.165 mcg/ml. Unfortunately, a diet with an intermediate dietary phosphorus increase was not included. It is possible that with a more moderate increase in dietary phosphorus a greater potentiation would have been obtained.

It was mentioned in the introduction that this study was undertaken to investigate the possible potentiation of

TABLE 20. BLOOD SERUM CHLORTETRACYCLINE AS AFFECTED BY INCREASING LEVELS OF DIETARY PHOSPHORUS WHEN DIETARY CALCIUM LEVEL IS KEPT CONSTANT. EXPERIMENT 3

Diet	Dietary Ca	Dietary P	Dietary Ca:P	Serum levels[a] Blood sample[b] 1	Serum levels[a] Blood sample[b] 2	Potentiation from increased dietary phosphorus Blood sample 1 mg/ml	Potentiation from increased dietary phosphorus Blood sample 1 %	Potentiation from increased dietary phosphorus Blood sample 2 mg/ml	Potentiation from increased dietary phosphorus Blood sample 2 %
	---------%---------			----mcg/ml-----					
1	0.80	0.60	1:0.75	0.383[f]	0.673[d]				
8	0.80	1.20	1:1.50	0.848[c]	0.929[c]	0.465	54.8	0.256	27.6
9	0.80	1.80	1:2.25	0.692[d]	0.867[c]	0.209	35.3	0.194	22.4

[a] See Table 17.

[b] See Table 17.

[c,d,f] Values with different superscript within the same column differ significantly ($P < .01$).

chlortetracycline in swine, employing different methods. The results of this study point out that a low calcium diet, a diet high in phosphorus and addition of sodium sulfate in the diet, will markedly increase the concentration of chlortetracycline in the blood serum, especially shortly after introducing these diets to growing pigs. Of course, the ultimate target of increasing chlortetracycline concentrations in the blood serum is to increase the effectiveness of this antibiotic to prevent or treat diseases.

From a practical standpoint, in order for increased blood serum chlortetracycline to be of value, it is necessary to study the potentiating procedures used in this study in combination with disease challenge trials. If these trials prove that increased levels of chlortetracycline in the blood reduce mortality, scours or other symptoms, then recommendations can be made on their use.

To theorize, it is possible that whatever the amount of an antibiotic given to an animal orally, a certain part of it will be tied up by calcium. This part of the antibiotic will be confounded to the alimentary tract reducing the deleterious bacteria and thus improving the growth rate or "health" of the animal. When the binding capacity of the calcium is surpassed any excess antibiotic that is left unbound will be absorbed by the blood to combat the pathologic bacteria responsible for most of the diseases.

CONCLUSIONS

1. There was a direct correlation between calcium level in the diet and blood serum chlortetracycline. Decreased levels of dietary calcium increased blood serum chlortetracycline.

2. A low calcium diet was a very effective chlortetracycline potentiator for the periods tested. However, prolonged feeding of this diet to growing pigs for periods of over five weeks caused rickets.

3. Within the levels and periods tested, sodium sulfate in a diet for growing pigs did not affect the growth performance of the pigs. Shortly after its introduction to the pigs, sodium sulfate significantly increased blood serum chlortetracycline levels.

4. Increased levels of dietary phosphorus significantly increased blood serum chlortetracycline. A level of 1.2% appears to be optimum when calcium level is 0.60%.

5. For the periods and levels tested, calcium sulfate appears to be a good source of calcium to support a normal growth performance of growing pigs. However, it did not increase blood serum chlortetracycline for the first two weeks after its introduction to the pigs.

SUMMARY

Three experiments designed to study the potentiation of antibiotic in swine were conducted over a two-year period. During the two years, 344 crossbred pigs were used in three separate experiments to study the effect of calcium sources and levels, sulfate sources and levels, and phosphorus levels in diets containing different levels of chlortetracycline.

The first experiment was divided into three two-week periods. During the first two weeks 50 g/ton of chlortetracycline in the diets increased gain of pigs from 0.68 to 0.72 kg. per day. Pigs fed diets containing chlortetracycline gained significantly faster when the source of calcium was sulfate rather than carbonate. In the second Period, pigs receiving a higher calcium diet with antibiotic grew significantly faster than those fed a low calcium diet. During the third two-week period pigs fed a calcium carbonate diet achieved gains significantly greater than those fed a calcium sulfate diet. On an accumulative basis (0-6 weeks) there were no significant differences among treatments in either average daily gain or feed efficiency.

The second experiment consisted of two periods. During the first period (4 weeks) three levels of calcium

(0.3, 0.7 and 1.1%) with and without 100 g/ton of chlortetracycline were used for a total of six treatments. During the second period (2 weeks) all diets were changed to a low (0.3%) calcium. Pigs fed the antibiotic containing diets for the first period continue to receive antibiotic at a higher level (200 g/ton). Blood was collected from all pigs at the beginning of the experiment, at the end of the first period and at the end of the second period. During all periods there was a highly significant antibiotic effect ($P < .01$) as pigs fed diets with antibiotic gained faster than the non-antibiotic fed pigs by 0.62 to 0.52 kg., 0.67 to 0.53 kg. and 0.73 to 0.58 kg. for the first, second and third period respectively. There was also a significant calcium X antibiotic effect. Pigs fed the two lower dietary calcium levels responded to antibiotic significantly better ($P < .05$) than those on the high calcium level. Feed per gain for the pigs fed the antibiotic diets was 2.39, 2.85 and 2.63 for the first, second and third period respectively compared to 2.60, 3.21 and 2.80 for pigs fed diets without antibiotic. These differences were significant for each period. Blood serum calcium was similar among all treatments at the first and third blood collections. At the second collection pigs on the lowest dietary calcium level showed blood serum calcium levels significantly higher than the others. Serum chlortetracycline at

the second blood collection increased linearly (0.263, 0.360 and 0.482 mcg/ml) with decreasing (1.1, 0.7 and 0.3%) levels of dietary calcium. At the third collection serum chlortetracycline followed the same pattern as the second and was closely correlated with the dietary calcium levels of the first period.

In the third experiment several methods of potentiation of chlortetracycline were studied. The diets used included increasing levels of sodium sulfate (0.355, 1.07, 1.775 and 2.13%), calcium sulfate (0.7, 2.5 and 3.43%) and dietary phosphorus (0.6, 1.2 and 1.8%) and also a low calcium diet. All diets contained 200 g/ton chlortetracycline. To determine the growth and potentiating effect of these diets two control diets were also included one with antibiotic and one without. The experiment was conducted for ten weeks. Blood was collected two times. At the beginning of the experiment all pigs were fasted overnight, fed for five hours the next morning and then bled via the jugular vein. The second blood collection took place two weeks later.

Pigs fed the diets with the antibiotic grew significantly faster ($P < .05$) than those fed the diet without antibiotic. At both sampling periods the lowest values of blood serum calcium were found in pigs fed the very high (1.8%) phosphorus diet. At the first collection blood

serum from all pigs, except those fed the calcium sulfate supplemented diets, contained chlortetracycline levels which were significantly higher ($P < .05$) than the controls. Increasing amounts of sodium sulfate in the diet caused a linear increase (0.175, 0.278, 0.435 and 0.369 mcg/ml) of blood serum chlortetracycline in the blood serum. Blood serum chlortetracycline was also significantly ($P < .01$) higher when pigs fed the high phosphorus diets. At the second collection only serum from pigs fed the low calcium diet and the two very high phosphorus diets had chlortetracycline values significantly higher ($P < .05$) than controls. At both collections, blood serum chlortetracycline levels of pigs fed the calcium sulfate containing diets were not different from those fed the control (unpotentiated) diet.

LITERATURE CITED

Albert, A. 1953. Avidity of terramycin and aureomycin for metallic cations. Nature. 172:433.

Albert, A. and C. W. Rees. 1956. Avidity of the tetracyclines for the cations of metals. Nature. 177:433.

American Cyanamid Company. 1964. Aureomycin program for starting chickens. Cyanamid Technical Bulletin 22.

Anderson, G. W., S. J. Slinger, and W. F. Pepper. 1952. Effect of dietary microorganisms on the growth and cecal flora of chicks. Poul. Sci. 31:905.

Anderson, G. W., S. J. Slinger, and W. F. Pepper. 1953a. Bacterial cultures in the nutrition of poultry. I. Effect of dietary bacterial cultures on the growth and cecal flora of chicks. J. Nutr. 50:35.

Anderson, G. W., S. J. Slinger, W. F. Pepper, and M. M. Hauser. 1953b. Bacterial cultures in the nutrition of poultry. II. Effect of dietary coliform cultures on the growth and cecal flora of poultry. J. Nutr. 50:47.

Becker, R. E., S. W. Terrill, R. O. Nesheim, and R. J. Meade. 1951. Comparison of antibiotics for growing-fattening pigs. Mimeograph AS215 University of Illinois.

Becker, D. E., S. W. Terrill, D. E. Ullrey, and R. J. Meade. 1952. The growth response of the pig to a dietary source of various antibacterial agents and to intramuscular injections of procaine penicillin. Antibiotics and Chemotherapy 2:260.

Bernheim, F. 1954a. The effect of certain antibiotics on the formation of an adaptive enzyme in a strain of Pseudomonas aeruginosa. J. Pharmacol. Exp. Therap. 110:115.

Bernheim, F. 1954b. The effect of certain metal ions and chelating agents on the formation of an adaptive enzyme in Pseudomonas aeruginosa. Enzymologia 16:351.

Bird, H. R., R. J. Lillie, and J. R. Sizemore. 1952. Environment and stimulation of chick growth by antibiotics. Poul. Sci. 31:907.

Bowland, J. P., S. E. Beacom, and L. W. McElroy. 1951. Animal protein factor and antibiotic supplementation of small grain rations for swine. J. Anim. Sci. 10:629.

Braude, A. I., P. Davey, and J. Siemienski. 1953. Prevention of blood coagulation by antibiotics in vitro. Proc. Soc. Exp. Biol. Med. 83:160.

Braude, R., S. K. Kon, and J. W. G. Porter. 1953a. Antibiotics for nutrition. Nutri. Abstr. Rev. 23:473.

Braude, R., H. D. Wallace, and T. J. Cunha. 1953b. The value of antibiotics in the nutrition of swine. Antibiotics and Chemotherapy. 3:271.

Brody, T. M., R. Hurwitz, and J. A. Bain. 1954. Magnesium and the effect of the tetracycline antibiotics on oxidative processes in mitochondria. Antibiotics and Chemotherapy. 4:864.

Brown, P. B., D. E. Becker, S. W. Terrill, and L. E. Card. 1952. The effect of aureomycin on the growth and metabolism of the pig. Arch. Biochem. Biophys. 41:378.

Burnside, J. E., T. J. Cunha, H. M. Edwards, G. B. Meadows, G. A. Lamar, A. M. Pearson, and R. S. Glasscock. 1950. Response of the pig to APF, B_{12} and B_{12b}. Proc. Soc. Exp. Biol. Med. 74:173.

Calvin, M. and K. W. Wilson. 1945. Stability of chelate compounds. J. Amer. Chem. Soc. 67:2003.

Camp, A. A. 1960. South Agric. Workers' Conf. Birmingham, Alabama.

Carpenter, L. E. 1950. Effect of aureomycin on the growth of weaned pigs. Arch. Biochem. 27:469.

Carpenter, L. E. 1951. Effect of APF concentrate containing aureomycin on gestating, lactating, and growing swine. J. Anim. Sci. 10:657.

Catron, D. V. 1949. Recent development in swine nutrition. Vet. Med. 44:215.

Catron, D. V. and P. Cuff. 1951. Runt pigs can be saved. Iowa Farm Sci. 5:119.

Coates, M. E. 1953. Mode of action of antibiotics in animal nutrition. Chemistry and Industry 1333.

Coates, M. E., C. D. Dickinson, G. F. Harrison, S. K. Kon, S. H. Cummins and W. F. J. Cuthbertson. 1951. The mode of action of antibiotics in stimulating growth of chicks. Nature. 168:332.

Combs, G. E. and H. D. Wallace. 1962. Growth and digestibility studies with young pigs fed various levels and sources of calcium. J. Anim. Sci. 21:734.

Combs, G. F. 1956. Mode of action of antibiotics in poultry. First Intern. Conf. Antib. in Agric. 397:107.

Costain, R. A. and L. E. Lloyd. 1962. Effect of ration calcium level, oxytetracycline and terephthalic acid upon the performance of growing pigs. J. Anim. Sci. 21:751.

Cover, M. S., W. J. Benton, L. M. Greene and F. D. D'Armi. 1959. Potentiation of tetracycline antibiotics with terephthalic acid and low dietary calcium. Avian Diseases. 3:353.

Cunha, T. J., J. E. Burnside, D. M. Buschman, R. S. Glasscock, A. M. Pearson and A. L. Shealy. 1949. Effect of vitamin B_{12}, animal protein factor and soil for pig growth. Arch. Biochem. 23:324.

Cunha, T. J., J. E. Burnside, H. M. Edwards, G. B. Meadows, R. H. Benson, A. M. Pearson, and R. S. Glassock. 1950a. Effect of animal protein factor on lowering the protein needs of the pig. Arch. Biochem. 25:455.

Cunha, T. J., J. E. Burnside, H. M. Edwards, G. B. Meadows, R. H. Benson, A. M. Pearson, and R. S. Glassock. 1950b. Effect of APF supplement on efficiency of feed utilization for the pig. J. Anim. Sci. 9:615.

Cunha, T. J., H. M. Edwards, G. B. Meadows, R. H. Benson, A. M. Pearson and R. S. Glassock. 1950c. APF, B_{12}, B_{13} and related factors for the pig. J. Anim. Sci. 9:653.

Cunha, T. J., G. B. Meadows, H. M. Edwards, R. F. Sewell, A. M. Pearson, and R. S. Glasscock. 1951. A comparison of aureomycin, streptomycin, penicillin and aureomycin B_{12} feed supplement for the pig. Arch. Biochem. 23:510.

Cunha, T. J., G. B. Meadows, H. M. Edwards, R. F. Sewell, C. B. Shawyer, A. M. Pearson and R. S. Glassock. 1950d. Effect of aureomycin and other antibiotics on the pig. J. Anim. Sci. 9:653.

Dearborn, E. H., J. T. Jr. Litchfield, H. J. Eisner, J. J. Corbett and C. W. Dunnett. 1957. The effects of various substances on the absorption of tetracycline in rats. Antibiotic Medicine and Clinical Therapy. 4:627.

Donovan, G. E., K. E. Price, W. C. Sherman and W. M. Reynolds. 1960. Antibiotic potentiation. I. Influence of various anions in lowering calcium inhibition of intestinal absorption. Poul. Sci. Meeting, p. 17.

Dorsey, T. A. and G. S. Harshfield. 1959. Studies on control of fowl cholera. Tech. Bull. 23, South Dakota State College Agric. Mech. Arts, Agric. Exp. Sta. May.

Duggar, B. M. 1948. Aureomycin: A product of the continuing search for new antibiotics. Annals N. Y. Acad. Sci. 51:177.

Edwards, H. M., T. J. Cunha, G. B. Meadows, R. F. Sewell, and C. B. Shawyer. 1950. Observations on aureomycin and APF for the pig. Proc. Soc. Exp. Biol. and Med. 75:445.

Ewards, H. M., T. J. Cunha, G. B. Meadows, C. B. Shawyer and A. M. Pearson. 1951. Effect of APF in supplying multiple factors for the pig. Proc. Soc. Exp. Biol. and Med. 76:173.

Eggert, R. C., W. T. Akers and C. N. Huhtanen. 1959. Chlortetracycline absorption and calcium utilization in growing swine as affected by terephthalic acid and calcium source. J. Anim. Sci. 18:1505.

Eggert, R. G. and R. F. Elliott. 1959. The potentiation of aureomycin in swine. 10th Annual Feed Symposium.

Elam, J. F., R. L. Jacobs, J. Fowler and J. R. Couch., 1954. Effect of dietary clostridia upon growth-promoting responses of penicillin. Proc. Soc. Exp. Biol. and Med. 85:645.

Elam, J. F., R. L. Jacobs, J. Fowler, F. Hale and J. R. Couch. 1953. Effect of feeding chloromycetin mycelial meal upon the growth and fecal microflora of swine. J. Anim. Sci. 12:819.

English, A. R. 1958. Enhancement of tetracycline serum levels with glucosamine hydrochloride as adjuvant. Cross-over studies in beagle dogs. Proc. Soc. Exp. Biol. Med. 99:208.

Finlay, A. C., G. L. Hobloy, S. Y. Pan, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sabin, I. A. Solomons, J. W. Vinson and J. H. Kane. 1950. Terramycin, a new antibiotic. Science 111:85.

Grove, D. C. and W. A. Randall. 1955. Assay methods of antibiotics, a laboratory manual. Medical Encyclopedia Inc. New York.

Heywang, B. W. 1946. Sources of calcium for laying chickens during hot weather. Poul. Sci. 25:215.

Hill, D. C., H. D. Branion and S. J. Slinger. 1952. Influence of environment on the growth response of chicks to penicillin. Poul. Sci. 31:920.

Hill, C. H. and J. W. Kelly. 1953. The effect of antibiotics on the growth of chicks raised in new quarters. J. Nutr. 51:463.

Hvidsten, J. and T. Homb. 1961. The effect of supplementary terramycin and aureomycin at low and high levels in long-term experiments with bacon pigs. Acta. Agric. Scand. 11:121.

Hunter, F. E. Jr. and O. H. Lowry. 1956. The effects of drugs on enzyme systems. Pharmacol. Reviews. 8:89.

Johansson, K. R., G. E. Peterson and E. C. Dick. 1953a. Effects of dietary aureomycin upon the intestinal microflora and the intestinal synthesis of vitamin B_{12} in the rat. J. Nutr. 49:135.

Johansson, K. R., G. E. Peterson and E. C. Dick. 1953b. Effect of parenteral and oral administration of antibiotics to rats fed a vitamin B_{12}-deficient diet. Fed. Proc. 12:418.

Jukes, T. H. 1949. Promotion of growth of chicks by aureomycin fermentation mash. Poultry Nutrition Conference, Federation Meetings, Chicago.

Jukes, T. H. 1955. Antibiotics monograph, 4: Medical Encyclop. Inc., Interci. Publ., 128.

Jukes, J. H. and W. W. Williams. 1953. Nutritional effects of antibiotics. Pharmacol. Reviews. 5:381.

Kaplan, M. A., H. L. Dickinson, K. A. Hubel and F. H. Buckwalter. 1957. A new, rapidly absorbed, complex salt of tetracycline. Antibiotic Medicine and Clinical Therapy. 4:99.

Kratzer, F. H., C. R. Grau, M. P. Starr and D. M. Reynolds. 1951. Growth-promoting activities of antibiotics and yeast cultures for chicks and turkey poults. Fed. Proc. 10:386.

Lepley, K. C., D. V. Catron and C. C. Culbertson. 1950. Dried whole aureomycin mash and meat and bone scraps for growing-fattening swine. J. Anim. Sci. 9:608.

Lev, M., C. A. E. Briggs and M. E. Coates. 1956. Bacteriological studies of "infected" and "uninfected" chicks in relation to antibiotic growth stimulation. Nature. 178:1125.

Lev, M. and M. Forbes. 1959. Growth response to dietary penicillin of germ-free chicks and of chicks with a defined intestinal flora. Brit. J. Nutr. 13:78.

Lucas, I. A. M. 1955. The growth of pigs kept to one level of feeding, in two environments, and fed diets with and without antibiotic. J. Agr. Sci. 46:307.

Luckey, T. D. 1959. Antibiotics, their chemistry and non-medical uses. D. Van Nostrand Co. Inc., New York, 174.

Luckey, T. D. 1955. Mode of action of antibiotics evidence from germ-free birds. Antib. in Agric. 397:135.

Luther, H. G. 1960. Antibiotic potentiation and its practical application in the prophylaxis and therapy of poultry diseases. Pfizer European Agricultural Research Conference, Proceedings. Lucerne April 28.

Manten, A. and J. C. Hoogerheide. 1958. The influence of a new antifungal antibiotic, Pimaricin, on the yeast flora of the gastrointestinal tract of rats and mice during tetracycline administration. Antibiotics and Chemotherapy. 8:381.

March, B. and J. Biely. 1952. The effect of feeding aureomycin on the bacterial content of chick feces. Poul. Sci. 31:177.

McCalla, T. M. 1940. Cation adsorption by bacteria. J. Bact. 40:23.

McCalla, T. M. 1941. The adsorption of H^+ by bacteria as measured by the glass electrode. J. Bact. 41:775.

McCampbell, C. W. and C. E. Aubel. 1934. Calcium carbonate vs. calcium sulfate in swine rations. Amer. Soc. Animal Prod. 34:189.

Moore, P. R., A. Evenson, T. D. Luckey, E. McCoy, C. A. Elvhjem and E. B. Hart. 1946. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J. Biol. Chem. 165:437.

Newman, C. W., D. M. Thrasher, S. L. Hansard, A. M. Mullins and R. F. Boulware. 1967. Effects of tallow in swine rations on utilization of calcium and phosphorus. J. Anim. Sci. 26:479.

Noland, P. R., E. L. Stephenson, T. S. Nelson and D. L. Tucker. 1951. Response of baby pigs to antibiotics. J. Anim. Sci. 10:1059.

Oxford, A. E. 1953. A colorimetric method, based on metallic complex formation, for the detection of aureomycin in presence of amino acids and proteins. Nature. 172:395.

Pensack, J. M., C. N. Huhtanen, W. L. Williams, E. L. R. Stokstad and T. M. Jukes. 1959. Factors affecting the rate of chlortetracycline absorption and excretion in poultry. 48th Meet. Poultry Sci. August.

Plumlee, M. P., J. H. Conrad, M. H. Kennington and W. M. Beeson. 1956. Levels of calcium with and without zinc for growing-fattening swine in drylot. Purdue Agr. Exp. Sta. A. H. Mimeo 187.

Price. K. E. and Z. Zolli. 1959. The influence of terephthalic acid on oxytetracycline serum levels in chickens. Studies N mode of action I. Avian Diseases 3:157.

Price, K. E., Z. Zolli Jr., J. C. Atkinson and H. G. Luther. 1957a. Antibiotic inhibitors. I. The effect of certain milk constituents. Antibiotics and Chemotherapy 7:672.

Price, K. E., Z. Zolli Jr., J. C. Atkinson and H. G. Luther. 1957b. Antibiotic inhibitors. II. Studies on the inhibitory action of selected divalent cations for oxytetracycline. Antibiotics and Chemotherapy. 7:689.

Raibaud, P. and M. Gaullet. 1957. Les lactobacillus dominants dans le tube digestif du pore adulte. C. R. Acad. Sci., 244:816.

Regna, P. P., I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier. 1951. The isolation and general properties of terramycin and terramycin salts. J. Amer. Chem. Soc. 73:4211.

Robinson, K. L., W. E. Coey and G. S. Burnett. 1952. Some effects of procaine penicillin and of certain feed supplements on food consumption and live-weight gain of pigs. J. Sci. Food & Agric. 3:448.

Robinson, K. L., W. E. Coey and G. S. Burnett. 1953. The response of pigs to dietary procaine penicillin. Emp. J. Exp. Agric. 21:275.

Robinson, K. L., W. E. Coey and G. S. Burnett. 1954. Use of antibiotics in the food of fattening pigs. J. Sci. Food Agr. 5:541.

Rokos, J., M. Burger and P. Prochazka. 1958. Effect of calcium ions on the inhibition of hydrolases by chlortetracycline. Nature. 181:1201.

Rokos, J., P. Malek, M. Burger, P. Prochazka and J. Kolc. 1959. The effect of divalent metals on the inhibition of pancreatic lipase by chlortetracycline. Antibiotics and Chemotherapy. 9:600.

Romoser, G. L., M. S. Shorb and G. F. Combs. 1952. Studies on mechanism of antibiotics in promoting chick growth. Poul. Sci. 31:932.

Saz, A. K. and R. B. Slie. 1953. Manganese reversal of aureomycin inhibition of bacterial cell-free nitro-reductase. J. Amer. Chem. Soc. 75:4626.

Saz, A. K. and R. B. Slie. 1954a. The inhibition of organic nitro-reductase by aureomycin in cell-free extracts. II. Cofactor requirements for the nitro-reductase enzyme complex. Arch. Biochem. Biophys. 51:5.

Saz, A. K. and R. B. Slie. 1954b. Reversal of aureomycin inhibition of bacterial cell-free nitro-reductase by manganese. J. Biol. Chem. 210:407.

Shorrock, R. W. 1940. The "restricted" feeding of bacon pigs. J. Agric. Sci. 30:598.

Sieburth, J. McN., J. Gutierrez, J. McGinnis, J. R. Stern and R. H. Schneider. 1951. Effect of antibiotics on intestinal microflora and on growth of turkeys and pigs. Proc. Soc. Exp. Biol. and Med. 76:15.

Sieburth, J. M., J. McGinnis and C. E. Skinner. 1952. The effect of terramycin on the antagonism of certain bacteria against species of proteus. J. Bact. 64:163.

Smith, H. W. 1959. The effect of continuous administration of diets containing tetracyclines and penicillin on the number of drug-resistant and drug sensitive Clostridium welchii in the feces of pigs and chickens. J. Pathol. and Bacteriol. 77:79.

Snell, J. F., and R. Garkuscha. 1958. Radioactive oxytetracycline. III. Effect of glucosamine HCl on serum concentrations. Proc. Soc. Exp. Biol. and Med. 98:148.

Soncin, E. 1953. Fenomeni di interferenza tra elettroliti e antibiotici. III. Ione magnesio e aureomicina, terramicina, chloramfenicolo. Arch. Internat. Pharmacodynamie. 94:346.

Stokstad, E. L. R. 1954. Antibiotics in animal nutrition. Physiol. Rev. 34:25.

Stokstad, E. L. R., T. H. Jukes, J. V. Pierce, A. C. Page, Jr. and A. L. Franklin. 1949. The multiple nature of the animal protein factor. J. Biol. Chem. 180:647.

Stokstad, E. L. R., J. M. Pensack and C. N. Huhtanen. 1959. The effect of calcium salts on chlortetracycline absorption. Antibiotics Ann. 879.

Swain, H. H., G. F. Kiplinger and T. M. Brody. 1956. Action of certain antibiotics on the isolated dog heart. J. Pharmacol. Exp. Therapeutics. 117:151.

Sweeney, W. M., S. M. Hardy, A. C. Dornbush and J. M. Ruegsegger. 1957. Absorption of tetracycline in human beings as affected by certain excipients. Antibiotic Medicine and Clinical Therapy. 4:642.

Tyler, C. and J. S. Wilcox. 1942. Calcium and phosphorus balances with laying birds. J. Agric. Sci. 32:43.

Ullrey, D. E., E. R. Miller, B. E. Brent, B. L. Bradley and J. A. Hoefer. 1967. Swine hemotology from birth to maturity. IV. Serum calcium, magnesium, sodium, potassium, copper, zinc and inorganic phosphorus. J. Anim. Sci. 26:1024.

Van Meter, J. C. and J. J. Oleson. 1951. Effect of aureomycin on the respiration of normal rat liver homogenates. Science 113:273.

Van Meter, J. C., A. Spector, J. J. Oleson and J. H. Williams. 1952. In vitro action of aureomycin on oxidative phosphorylation in animal tissues. Proc. Soc. Exp. Biol. Med. 81:215.

Wallace, H. D., J. I. McKigney, A. M. Pearson and T. J. Cunha. 1955. The influence of chlortetracycline on the growth and carcass characteristics of swine fed restricted rations. J. Anim. Sci. 14:1095.

Wahlstrom, R. C. and B. C. Johnson. 1951. Growth effect of various antibiotics on baby pigs fed synthetic rations. Fed. Proc. 10:397.

Wahlstrom, R. C., S. W. Terrill and B. C. Johnson. 1950. Effect of antibacterial agents on growth of baby pigs fed a "synthetic" diet. Proc. Soc. Exp. Biol. and Med. 75:710.

Watts, A. B. and J. J. Miner. 1959. Soft phosphate in poultry rations. 48th Med. Poultry Sci. August.

Weinberg, E. D. 1957. The nutual effects of antimicrobial compounds and metallic cations. Bacteriol. Rev. 21:46.

Weinberg, E. D. 1954a. The influence of inorganic salts on the activity in vitro of oxytetracycline. Antibiotics and Chemotherapy. 4:35.

Weinberg, E. D. 1954b. The reversal of the toxicity of oxytetracycline by multivalent cations. J. Infect. Dis. 95:291.

Weinberg, E. D. 1955a. The effect of metallic cations on the anti-Streptomyces activity of anhydrochlortetracycline. J. Pharm. and Exp. Therap. 115:61.

Weinberg, E. D. 1955b. The effect of Mn^{++} and antimicrobial drugs on sporulation of Bacillus subtilis in nutrient broth. J. Bact. 70:289.

Weinberg, E. D. 1955c. Further studies on metallic ion reversal of oxytetracycline; reversal of drug-inactivated cells by Mg^{++}. Antibiotics Annual 1954-1955, Medical Encyclopedia, Inc., New York, N. Y., P. 169-173.

Weinberg, E. D. and M. B. Tolzmann. 1955. The mutual effects of Ca^{++} and antimicrobial drugs on coagulation of plasma. Proc. Indiana Acad. Sci. 64:53.

Welch, H., C. N. Lewis, A. W. Staffa and W. W. Wright. 1957a. Blood concentrations of three tetracycline capsule preparations following a single oral dose in man. Antibiotic Medicine and Clinical Therapy. 4:215.

Welch, H., W. W. Wright and A. Kirshbaum. 1957b. A comparison of blood concentrations following oral administration of tetracycline hydrochloride, tetracycline base, and tetracycline base mixed with metaphosphate. Antibiotic Medicine and Clinical Therapy. 4:293.

Welch, H., W. W. Wright and C. N. Lewis. 1957c. Blood concentrations following oral administration of chlortetracycline hydrochloride and chlortetracycline base, with and without sodium hexametaphosphate. Antibiotic Medicine and Clinical Therapy. 4:414.

Welch, H. and W. W. Wright. 1957d. Effects of citric acid and sodium hexametaphosphate on serum concentrations from orally administered oxytetracycline hydrochloride. Antibiotic Medicine and Clinical Therapy. 4:735.

Williams, W. L., R. R. Taylor, E. L. R. Stokstad and T. H. Jukes. 1951. Mechanism of the growth promoting effect of aureomycin in chicks. Fed. Proc. 10:270.

APPENDIX TABLES

TABLE 1. MEAN SQUARES OF AVERAGE DAILY GAINS. EXPERIMENT 1

Source	df	Period 1	2	3
Total	127			
Replication (Rep)	3	0.67473*	0.07328	0.69648**
Sex	1	0.00361	0.37736	0.45006**
Rep x Sex	3	0.07281	0.02478	0.03077
Calcium (Ca)	1	0.03781	0.34341*	0.13455
Rep x Ca	3	0.13417	0.00129	0.04121
Ca x Sex	1	0.09138	0.08050	0.07752
Rep x Ca x Sex	3	0.05971	0.01807	0.12902
Antibiotic (Ant)	1	0.27565*	0.03283	0.00002
Rep x Ant	3	0.04485	0.11695	0.01218
Ant x Sex	1	0.03920	0.05404	0.00416
Rep x Ant x Sex	3	0.01453	0.04841	0.00166
Ca x Ant	1	0.01901	0.43129*	0.00018
Rep x Ca x Ant	3	0.08449	0.09999	0.00349
Ca x Ant x Sex	1	0.00383	0.01341	0.00001
Rep x Ca x Ant x Sex	3	0.12878	0.05107	0.05434
Source (So)	1	0.21945	0.05569	0.70063**
Rep x So	3	0.26054*	0.13433	0.06459
Sex x So	1	0.01280	0.00131	0.00416
Rep x Sex x So	3	0.03819	0.03299	0.11406
Ca x So	1	0.00245	0.00619	0.32301*
Rep x Ca x So	3	0.07411	0.05309	0.08789
Ca x Sex x So	1	0.07703	0.09084	0.03219
Rep x Ca x Sex x So	3	0.10309	0.03944	0.00239
Ant x So	1	0.36765*	0.01106	0.00066
Rep x Ant x So	3	0.04448	0.02078	0.00907
Ant x Sex x So	1	0.01051	0.00041	0.00439
Rep x Ant x Sex x So	3	0.06355	0.01995	0.01808
Ca x Ant x So	1	0.00845	0.05486	0.00131
Rep x Ca x Ant x So	3	0.05876	0.02830	0.08746
Ca x Ant x Sex x So	1	0.08303	0.00049	0.06346
Rep x Ca x Ant x Sex x So	3	0.03224	0.05853	0.01029

*$P < .05$

**$P < .01$

TABLE 2. MEAN SQUARES OF AVERAGE DAILY GAINS. EXPERIMENT 1 (CONTINUED)

Source	df	Period	
		1+2	1+2+3
Replication (Rep)	3	0.21004*	0.00837
Sex	1	0.11701	0.20721*
Rep x Sex	3	0.04423	0.02402
Calcium (Ca)	1	0.03611	0.06257
Rep x Ca	3	0.03318	0.02574
Ca x Sex	1	0.09191	0.08873
Rep x Ca x Sex	3	0.01783	0.02947
Antibiotic (Ant)	1	0.12189	0.05653
Rep x Ant	3	0.07578	0.03153
Ant x Sex	1	0.05080	0.03094
Rep x Ant x Sex	3	0.00988	0.00623
Ca x Ant	1	0.15610	0.06799
Rep x Ca x Ant	3	0.07358	0.03757
Ca x Ant x Sex	1	0.00049	0.00028
Rep x Ca x Ant x Sex	3	0.05979	0.04962
Source (So)	1	0.01424	0.03816
Rep x So	3	0.14762*	0.10563
Sex x So	1	0.00619	0.00075
Rep x Sex x So	3	0.02877	0.04486
Ca x So	1	0.00330	0.04922
Rep x Ca x So	3	0.04778	0.02811
Ca x Sex x So	1	0.07752	0.01781
Rep x Ca x Sex x So	3	0.06325	0.02193
Ant x So	1	0.06615	0.02850
Rep x Ant x So	3	0.01862	0.01129
Ant x Sex x So	1	0.00219	0.00001
Rep x Ant x Sex x So	3	0.01222	0.00983
Ca x Ant x So	1	0.02231	0.01260
Rep x Ca x Ant x So	3	0.00816	0.00419
Ca x Ant x Sex x So	1	0.01926	0.03032
Rep x Ca x Ant x Sex x So	3	0.00819	0.00188

*$P < .05$

TABLE 3. MEAN SQUARES FOR FEED/GAIN. EXPERIMENT 1.

Source	df	Period				
		1	2	3	1+2	1+2+3
Total	31					
Calcium (Ca)	1	0.02475	0.01087	0.05120	0.02703	0.00428
Antibiotic (Ant)	1	0.09570	0.03713	0.00180	0.00428	0.00690
Ca x Ant	1	0.00383	0.15820	0.19531	0.05368	0.00003
Calcium source (So)	1	0.09353	0.01403	1.32845*	0.01575	0.05527
Ca x So	1	0.00878	0.09790	0.00281	0.01575	0.00113
Ant x So	1	0.21288	0.06390	0.01901	0.00945	0.01163
Ca x Ant x So	1	0.00113	0.02258	0.02880	0.00577	0.00340
Replication (Rep)	3	0.45764	0.92814	1.77627*	0.52693**	0.10074
Rep x Ca	3	0.06197	0.00878	0.18733	0.01422	0.02934
Rep x Ant	3	0.01004	0.02289	0.05222	0.00765	0.00231
Rep x Ca x Ant	3	0.02958	0.03244	0.02960	0.02814	0.00270
Rep x So	3	0.10184	0.02874	0.03561	0.01609	0.00504
Rep x Ca x So	3	0.19031	0.01962	0.18930	0.05073	0.01312
Rep x Ant x So	3	0.07106	0.03255	0.06114	0.03461	0.01584
Error	3	0.05806	0.10776	0.12626	0.01630	0.01201

*$P < .05$

**$P < .01$

TABLE 4. MEAN SQUARES FOR AVERAGE DAILY FEED CONSUMPTION. EXPERIMENT 1.

Source	df	Period 1	2	3	1+2	1+2+3
Total	31					
Calcium (Ca)	1	0.00037	1.04763	0.29070	0.26281	0.27195
Antibiotic (Ant)	1	0.03063	0.26463	0.01240	0.11045	0.06938
Ca x Ant	1	0.03603	0.28313	0.30615	0.12251	0.17258
Calcium source (So)	1	0.00300	0.00113	0.00633	0.00151	0.00300
Ca x So	1	0.06938	0.09353	1.11378**	0.00020	0.11400
Ant x So	1	0.03850	0.08100	0.03063	0.05281	0.00813
Ca x Ant. x So	1	0.00877	0.00525	0.01665	0.00845	0.00945
Replication (Rep)	3	0.31109***	1.78077*	1.36237**	0.21279	0.21639
Rep x Ca	3	0.07299	0.01784	0.10596	0.02500	0.02448
Rep x Ant	3	0.00924	0.40513	0.05301	0.11481	0.06948
Rep x Ca x Ant	3	0.07994	0.21964	0.24791	0.11118	0.10739
Rep x So	3	0.13479*	0.23877	0.30864	0.18525	0.21588
Rep x Ca x So	3	0.09483*	0.07084	0.20700	0.02781	0.02654
Rep x Ant. x So	3	0.09395*	0.01484	0.14447	0.03342	0.00519
Error	3	0.00916	0.13024	0.05431	0.02876	0.02931

*P<.05
**P<.025
***P<.01

TABLE 5. MEAN SQUARES FOR AVERAGE DAILY GAINS. EXPERIMENT 2.

Source	df	Period 1	2	3	1+2	1+2+3
Total	95					
Calcium (Ca)	2	0.00213	0.01453	0.01626	0.00565	0.00216
Antibiotic (Ant)	1	1.03750***	1.39925***	2.55454***	1.32305***	2.12415***
Ca x Ant	2	0.21666*	0.25823*	0.32404*	0.23768*	0.18985
Sex	1	0.21956	0.35309*	0.39343*	0.16566	0.23780*
Ca x Sex	2	0.05076	0.13567	0.25816	0.10922	0.09472
Ant x Sex	1	0.07333	0.06893	0.00930	0.00128	0.00738
Ca x Ant x Sex	2	0.05620	0.06858	0.01894	0.01834	0.08240
Rep	3	0.22121**	0.29782*	0.23319*	0.23962**	0.17964*
Rep x Ca	6	0.02748	0.07292	0.02478	0.08633	0.00653
Rep x Ant	3	0.10545	0.10745	0.05975	0.09287	0.07238
Rep x Ca x Ant	6	0.04414	0.00500	0.03004	0.00542	0.01684
Rep x Sex	3	0.00480	0.01005	0.01985	0.00540	0.04749
Rep x Ca x Sex	6	0.13228	0.11586	0.08734	0.10525	0.00977
Rep x Ant x Sex	3	0.01871	0.03519	0.21393	0.02034	0.02419
Error	54	0.05569	0.07989	0.08389	0.07456	0.05659

*P < .05
**P < .025
***P < .001

TABLE 6. MEAN SQUARES FOR FEED/GAIN. EXPERIMENT 2.

Source	df	Period				
		1	2	3	1+2	1+2+3
Total	23					
Calcium (Ca)	2	1.62295	0.01445	0.01132	0.02412	0.00541
Antibiotic (Ant)	1	0.23404	0.80667*	0.17002**	0.47320**	0.28820**
Ca x Ant	2	0.83501	0.09828	0.02087	0.05532	0.02358
Replication	3	0.65486	0.78659*	0.03524	0.21646*	0.14886**
Rep x Ca	6	1.05959	0.02278	0.02489	0.02274	0.01523
Rep x Ant	3	1.52670	0.06120	0.00699	0.04602	0.01729
Error	6	1.08206	0.08603	0.00781	0.03279	0.00710

*P < .025
**P < .01

TABLE 7. MEAN SQUARES FOR AVERAGE DAILY FEED CONSUMPTION. EXPERIMENT 2.

Source	df	Period				
		1	2	3	1+2	1+2+3
Total	23					
Calcium (Ca)	2	0.09912	0.02345	0.20318	0.05720	0.04727
Antibiotic (Ant)	1	0.49594**	1.71735*	2.54150**	1.00042**	1.50500**
Ca x Ant	2	0.13745	0.11285	0.10090*	0.07703	0.07262
Replication (Rep)	3	0.30155*	0.11954	0.66908**	0.15008	0.26182*
Rep x Ca	6	0.03531	0.31896	0.06092	0.11132	0.04156
Rep x Ant	3	0.05575	0.18329	0.13668*	0.06201	0.06243
Error	6	0.03631	0.16114	0.01965	0.06828	0.04146

*P$<$.05
**P$<$.01

TABLE 8. MEAN SQUARES FOR BLOOD SERUM CALCIUM. EXPERIMENT 2.

Source	df	Period 1	Period 2	Period 3
Total	95			
Calcium (Ca)	2	0.04313	3.75578**	1.51582
Antibiotic (Ant.)	1	0.07993	3.77230**	4.84651
Ca x A t.	2	0.75174	0.51838	0.05851
Sex	1	0.48232	0.05963	0.02964
Ca x Sex	2	0.58648	0.38594	1.14752
Ant. x Sex	1	0.49853	0.75939	0.21944
Ca x Ant. x Sex	2	1.20557	0.24854	0.08153
Replication (Rep)	3	2.38749**	2.92287**	9.04468**
Rep x Ca	6	0.67272	0.38232	0.39563
Rep x Ant.	3	0.52797	0.42973	0.80601
Rep x Ca x Ant.	6	1.25445	1.02119*	1.85385
Rep x Sex	3	0.22799	0.27758	1.77419
Rep x Ca x Sex	6	0.17691	0.85634	1.95852
Rep x Ant x Sex	3	0.23287	0.22926	3.61684
Error	54	0.45609	0.45017	1.41593

*P $<$ .05
**P $<$ .01

TABLE 9. MEAN SQUARES FOR BLOOD SERUM CHLORTETRACYCLINE. EXPERIMENT 2.

Source	df	Period 2	Period 3
Total	47		
Calcium (Ca)	2	0.19133**	0.87867**
Sex	1	0.01139	0.00519
Ca x Sex	2	0.00142	0.01367
Replication (Rep)	3	0.02638**	0.03240*
Rep x Ca	6	0.00211	0.06571
Rep x Sex	3	0.00092	0.00393
Rep x Ca x Sex	6	0.00410	0.00885
Error	24	0.00288	0.00930

*$P < .05$
**$P < .01$

TABLE 10. MEAN SQUARES FOR AVERAGE DAILY GAINS. EXPERIMENT 3.

Source	df	Period 1	Period 2	Period 1+2
Total	102			
Rep	1	0.50408**	0.06871	0.13151
Trt	12	0.22204**	0.48223**	0.25288**
Rep x Trt	12	0.03389	0.08172	0.02149
Sex	1	0.05486	0.22817	0.09746
Rep x Sex	1	0.01022	0.28167	0.03071
Trt x Sex	12	0.02399	0.06495	0.03097
Rep x Trt x Sex	12	0.02727	0.10638	0.04385
Error	51	0.05112	0.11759	0.06091

*P $<$ .05
**P $<$ .01

TABLE 10. CONTINUED

Source	df	Period 3	Period 2+3	Period 1+2+3
Total	91			
Rep	1	0.73802**	0.39392*	0.03680
Trt	11	0.29244**	0.32582**	0.24229**
Rep x Trt	11	0.09850	0.05324	0.03478
Sex	1	0.14615	0.12438	0.10711
Rep x Sex	1	0.11895	0.00996	0.02032
Trt x Sex	11	0.01329	0.01131	0.01553
Rep x Trt x Sex	10	0.02255	0.16300	0.01034
Error	45	0.07089	0.06857	0.05261

*P $<$ .05
**P $<$ .01

TABLE 11. MEAN SQUARES FOR BLOOD SERUM CALCIUM. EXPERIMENT 3.

Source	df	Period 1	Period 2
Total	102		
Rep	1	3.29049**	1.87055*
Trt	12	0.87865**	0.87040**
Rep x Trt	12	0.13033	0.28829
Sex	1	0.14891	0.02652
Rep x Sex	1	0.07001	1.53402*
Trt x Sex	12	0.34967*	0.09283
Rep x Trt x Sex	12	0.14131	0.22831
Error	51	0.15972	0.28558

*P < .05
**P < .01

TABLE 12. MEAN SQUARES FOR BLOOD SERUM CHLORTETRACYCLINE. EXPERIMENT 3.

Source	df	Period 1	Period 2
Total	94		
Rep	1	0.18442**	0.25368**
Trt	11	0.32419**	0.11599**
Rep x Trt	11	0.01648	0.01635
Sex	1	0.00359	0.00155
Rep x Sex	1	0.00045	0.04229
Trt x Sex	11	0.01015	0.01631
Rep x Trt x Sex	11	0.01017	0.00916
Error	47	0.00866	0.01274

**$P < 0.1$