ANTIBIOTICS AND THE NUTRIENT REQUIREMENTS OF THE CHICK

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ABSTRACT

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A study has been made on the growth stimulatory effect of antibiotics when fed to chicks as supplements to different rations. A total of 9 biological tests employing 1280 chicks were carried out.

Chicks fed the Connecticut ration and modifications thereof showed a significant growth response to aureomycin supplementation of the rations. The efficiency of feed utilization was improved by the addition of aureomycin to the rations in this experiment.

The effect of aureomycin has been tested in rations in which the protein level has been adjusted to 16%, 17.9%, 19.5% and 22% respectively. The basal rations used consisted of ground corn and wheat as the cereal grain and soybean oil meal and fishmeal as the protein supplements along with additional vitamins and minerals to balance the ration. The results showed that aureomycin did not lower the dietary requirement of the chick for protein.

A test was made on the effect of antibiotics on growth of chicks when fed in rations which were deficient in specific amino acids. A wheat ration deficient in lysine

and a corn ration deficient in tryptophan have been used. The results showed that antibiotics did not lower the dietary requirement of the chick for either lysine or tryptophan.

Antibiotics were fed in chick rations containing different amounts of available carbohydrates. It was found that antibiotics did not lower the dietary requirement of the chick for carbohydrate.

A comparison of aureomycin and penicillin showed no difference in growth stimulating properties between the two antibiotics under the conditions of the different experiments. Aureomycin supplement (aurofac) promoted the same growth response at 0.05 and 1.0% levels.

The greatest growth response to antibiotics was obtained when the chicks were fed a diet adequate in available carbohydrates and protein and balanced in amino acids.

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I. INTRODUCTION

The discovery that antibiotics were of value in enhancing growth rate and feed efficiency in broiler production has created an entirely new field of research in nutrition. Whether or not the old concepts of protein, energy, vitamin and mineral requirements for growing birds will be affected thereby, has become a leading question in the minds of those who are concerned with nutritional problems.

It has been found by various investigators that increases in rate of growth varying from 10-15% have been possible by adding antibiotics to a ration. Furthermore, it has been found that feed efficiency has also been increased in varying degrees with the use of antibiotics. This in turn has meant a further reduction in costs to the producer. Reports have indicated moreover, that the incidence of disease has been lowered and that the less vigorous birds in the flock which formerly were unable to compete with the healthier and stronger birds, are now able to survive and attain better growth. As a result a greater degree of uniformity is found in the birds in the broiler industry

since the addition of antibiotics has become standard practice in feeding.

It has been noted that there is considerable variation in the response of chicks to antibiotics. This variation is apparently the result of differences in the composition of the rations fed. An investigation was undertaken to determine the effect of antibiotics on the dietary requirements of the chick for carbohydrates, protein and the amino acids, lysine and tryptophan.

The mode of action of antibiotics has not yet been definitely established. There are a number of ways in which they could promote growth. It has been suggested by *Cuthbertson that they may act by inhibiting the micro-organisms that destroy or inactivate growth factors. Some support has been given to this hypothesis by the observations that vitamin B_{12} can be removed from intestinal contents by bacterial action, that Escherichia coli can inactivate vitamin B_{12}, and that complete elimination of bacterial flora may occur on feeding aureomycin. The decreased utilization of vitamins by bacteria caused in this way would lead to increased availability to the host. Antibiotics could encourage growth of micro-organisms that synthesize growth

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* Cuthbertson, W. F. J. 1952. "Antibiotics in Nutrition". J. Sci. Food Agric., February 3; 49.

factors; for example, oral streptomycin leads to an increased population of Bacterium megatherium in the gut of the mouse and this organism has been shown to produce vitamin B_{12}. Antibiotics again could act by elimination of bacteria normally, or, under certain conditions, proliferating in the gut and producing toxins that decrease the growth potential of the organism. It has been shown that Clostridium perfringens can be eliminated from the caecae of turkeys and the faeces of pigs when these animals are given penicillin or terramycin. This organism is known to be responsible for enterotoxaemia in sheep, and consequently the elimination of this bacterium might well increase the growth rate of animals.

It is highly probable that no single effect of oral antibiotics is sufficient to explain their action, which will depend on circumstances; it may be due to the elimination of toxic organisms or of bacteria that inactivate growth factors, or even to enhanced proliferation of vitamin synthesizing bacteria.

II. REVIEW OF LITERATURE

(a) Nature of antibiotics.

Antibiotics have been defined as chemical substances produced by micro-organisms which have the capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms. They are characterized by certain properties which distinguish them sharply from the ordinary chemical antiseptics and disinfectants.

Antibiotics are produced primarily by micro-organisms, which are designated as "antagonists" since they have the property of antagonising or inhibiting the growth of other organisms. Some are readily secreted into the culture medium, whereas others are retained largely in the microbial cells.

One of the most distinguishing properties of antibiotics is their selective action upon bacteria and other micro-organisms. Some are active largely upon Gram-positive and only upon few Gram-negative bacteria, whereas others affect alike various bacteria within each of these groups. Some act upon fungi, whereas others do not. Some are also active against rickettsia. Most antibiotics have very little effect upon viruses. The differences in the antimicrobial action of antibiotics are both qualitive and quantitive in nature. One may thus speak of a

bacteriostatic or an antibiotic spectrum, to designate the range of selective antimicrobial action of a given substance upon a number of representative bacteria and other micro-organisms.

Antibiotics vary greatly in their physical and chemical properties. A large number of chemical compounds which possess antibiotic properties have now been isolated. These may either belong to distinctly different groups of chemical compounds, or they may show close similarity to one another. The latter is true, for example, of the different conditions of culture.

Certain micro-organisms are capable of producing more than one antibiotic. Penicillin notatum and P. chrysangenum produce not only different types of penicillin, but also a different antibiotic designated as penatin, penicillin B or notatin; Aspergillus flavus produces aspergillic acid and penicillin, the latter having been described under a number of different names. Streptomyces griseus produces two forms of streptomycin, actidone, and an antibiotic present in the mycelium of the organism. The culture filtrates of Penicillin notatum and Streptomyces griseus are active against certain bacterial toxins (antidotic properties), whereas the purified antibiotics produced by these organisms, namely penicillin and streptomycin do not possess that activity. Again, some antibiotics are produced by more than one organism. Penicillin is formed by numerous

strains of Penicillin notatum, P. chrysogenum and Aspergillus flavus, and by a variety of other fungi. Streptomycin is produced by S. griseus and S. bikiniensis; streptomycin-like substances are produced alone, or in mixture with streptothricin and possibly with other antibiotics, by a variety of other Streptomyces species. Since antibiotics are usually named after the organism producing them, considerable confusion has frequently resulted from the duplication of names for the same antibiotic, which has been studied in crude preparations isolated from different organisms.

The effect of the medium or of the substrate upon the antibacterial activities of different antibiotics varies greatly. Some substances have no effect at all upon the antibacterial activities of a given antibiotic; others may reduce considerably such activity by neutralizing the antibiotic action by some constituent of the medium such as peptone, glucose, serum or salt. Some substrates, like blood, may adsorb the antibiotic and completely inactivate it.

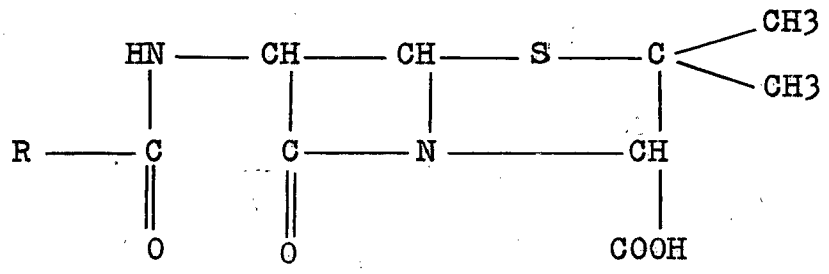
The mechanism of antibacterial action of one antibiotic is distinct from that of another. Some antibiotics interfere with the growth of micro-organisms and with their cell division; some influence microbial respiration; others affect the utilization of essential metabolites. Although antibiotics are often spoken of as primarily bacteriostatic agents, their bactericidal properties may also be very

pronounced; the nature of the bacteria affected, their stage of growth, and the composition of medium or substrate are of great importance in this connection.

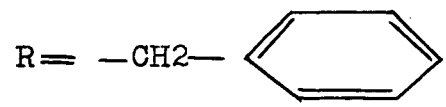
Different strains of a given bacterial species vary greatly in their sensitivity to a given antibiotic. Sensitive organisms can gradually develop resistance to certain antibiotics if allowed to be in contact with them for any length of time. Different antibiotics vary greatly, however, in the mechanism with which such resistance develops. The loss of resistance also differs with the antibiotic and the sensitive bacteria.

The bacterial action of the more common antibiotics is fairly well established.

Penicillin in the free state, is an organic acid but is usually administered as the calcium or sodium salt. The effectiveness of this drug depends upon several factors, some of which are: variation in bacterial strain susceptibility, number of organisms present and the pH of the medium. Penicillin as pointed out above is biosynthesized by several specia of fungi. The structural formula is:



The most commonly used form is benzyl penicillin (penicillin G) where the R group has the structure:



Bacteria under the influence of bactericidal concentrations of penicillin undergo distortion, swelling and ultimate lysis. The over-all effect can be interpreted in terms of inhibition of mononucleotide catabolism. Inhibition of this catabolism prevents the mononucleotide from liberating a base that maintains a redox potential. If this potential is lowered by penicillin, the -SH group of amino acids are protected from irreversible dehydrogenation. This interruption of cell metabolism causes the death of the bacterium.

Since the -SH groups act as donors for the "H" to be ultimately accepted by atmospheric oxygen, the dehydrogenation will proceed more rapidly as there is more O_2 available and therefore penicillin acts more effectively on anaerobic organisms when they are freely exposed to oxygenation.

Streptomycin is similar to penicillin in possessing low toxicity to animals but differing in that it acts on the gram negative organisms and is very slowly absorbed from the intestinal tract upon oral administration.

Streptomycin has the emperical formula of $C_{21}H_{37-39}O_{12}N_7$. This substance is a salt of an organic base which can be dissociated and form a complex with

ribonucleic acid. It is this complex which is believed to block the metabolism of the organism, along with inhibition of the polynucleotide catabolism resulting in a lowering of the redox potential causing death and lysis of the organism.

Aureomycin is a yellow crystallizable substance biosynthesized by Streptomyces aureofaciens. It has a wide antibacterial spectrum which includes a number of gram-positive and gram-negative organisms. The formula of this substance is not known but it is known to contain C, H, N, and O_2. The mechanism of the antibacterial action remains unsolved.

Bacitracin is a neutral compound, soluble in water and organic solvents. It is highly active against certain gram-positive bacteria, has very limiting toxicity to animals, and exerts, clinically, a marked effect in the treatment of infections caused by sensitive bacteria.

Terramycin has a calculated formula for the free, amphoteric base of $C_{22}H_{24-26}N_2O_9$. It has been shown that a phenyl group and a carbohydratic moiety in the amphoteric terramycin are present. Terramycin appears to be stable in aqueous solutions in the range of pH3 to 9 at 5°; and in the dry, crystalline state, terramycin and its hydrochloride are not inactivated on prolonged storage at room temperature. Terramycin salts are readily absorbed. The amphoteric form

is highly insoluble and is therefore only slowly absorbed. The antibacterial spectra and the blood concentrations produced by oral administration of terramycin and aureomycin are very similar.

Terramycin is active against a variety of micro-organisms, including gram-positive and gram-negative bacteria.

(b) Effect of antibiotics on growth.

One of the early reports of the growth stimulating effect of antibiotics was made by Moore and co-workers, (1) in 1946. In their work they were looking for a drug or combination of drugs that would completely inactivate all bacteria in the intestinal tract and provide a sterile animal so that vitamin requirements could be studied uncomplicated by "intestinal vitamins" or toxic substances. Their results showed that sulfasuxidine and streptomycin singly or in combination lead to increased growth responses in chicks when added to a purified diet. Unfortunately, these investigators did not carry on further experiments resulting from this growth response.

In 1949 Stokstad, et al (2), reported experiments in which it was found that crude aureomycin (product of Streptomycin aureofaciens fermentation), or a fraction prepared from it, would produce growth response in chicks when added to an all-vegetable diet containing 70% soybean oil meal and supplemented with an adequate quantity of Vit. B_{12}.

More marked increases with aureomycin fraction were obtained in experiments with pigs by Cunha and co-workers (3), who used a diet containing corn and peanut meal and supplemented with minerals, vitamin A and D, thiamine, riboflavin, niacin, pantothenic acid, folic acid and choline. In one experiment, pigs receiving an aureomycin fermentation supplement grew at a rate which was 2.5 times the gain of those fed on the basal ration during a five week test period.

In another experiment, Catron (4), studied the effect of aureomycin fermentation products and found an increased growth rate in pigs and preventation of bloody diarrhea. Various batches of supplements prepared from Streptomyces aureofaciens fermentation product were fed to 195 pigs. These animals showed an average daily gain in body weight of 1.64 pounds per pig with feed efficiency of 3.47 pounds of feed per pounds of gain. Three per cent of the animals were removed from the experiment due to enteritis. In contrast, 50 pigs not receiving this supplementation, showed an average dailty gain of 1.30 pounds and feed consumption of 3.74 pounds per pound of gain. Twenty per cent of the latter pigs were removed from the experiment due to enteritis. Carpenter (5), obtained similar results with pigs.

Using turkeys, McGinnis and co-workers (6), obtained extra growth response with supplements obtained

from aureomycin fermentation. A basal diet containing 56% of soybean oil meal was used. When this was supplemented with liverpaste containing B_{12}, McGinnis obtained poults weighing 386 grams at four weeks of age. When an aureomycin fermentation product was added at a level of 0.5% poults weighing 586 grams were obtained. Extra growth response in poults with aureomycin fermentation supplements has also been noted by Singsen and Matterson (7).

Berg (8), studied the effect on growth of fryers by adding aureomycin fermentation product to a high energy fryer ration. The addition of 0.25% of aureomycin fermentation to a high-energy diet containing 66% maize or wheat, 1% alfalfa meal, 6.8% herring meal, 20% soybean oil meal, 1% livermeal and mineral supplements, gave a significant growth response. When the herring meal was omitted and the protein level corrected by additional soybean oil meal, the growth was the same as in the control group without the aureomycin fermentation product.

In a further study by Stokstad and Jukes (9), 1950, it was shown that chicks fed on a corn-soybean diet produced growth responses with crystalline aureomycin hydrochloride supplementation. Succinylsulfathiazole, streptomycin and 3-nitro-4-hydroxyphenylarsonic acid also gave growth activity but these substances appeared less potent than aureomycin. A study was also made on different fractions of aureomycin

mash. Some fractions were obtained from fermented mash which was also devoid of aureomycin but which still retained growth activity. Furthermore, an extract prepared by heating a fraction with 0.5 N-NaOH gave a growth response in chicks although all the aureomycin had been destroyed by the treatment. Aureomycin fermented whole mash was also heated with NaOH without destroying the growth promoting activity, although aureomycin had been inactivated. On the basis of a large number of experiments crystalline aureomycin did not appear to give uniformly as great a response as aureomycin whole mash.

Whitehill, Oleson and Hutchings (10), showed that the addition of 25mg. aureomycin HCl per Kg. feed had a slightly better growth promoting effect on chicks than 1000 ml. Streptomyces aureofaciens mash per Kg. of feed. Growth response was also produced when streptomycin and penicillin were fed. Leuke, et al, (11), reported that the addition of Vitamin B_{12} and streptomycin (0.05%) to a corn-soybean diet for young pigs resulted in a 40% increase in average daily gains.

McGinnis, et al, (12), in their experimental work on turkey poults showed that aureomycin, streptomycin, terramycin and penicillin stimulated the growth rate of turkey poults fed a practical ration; 3-nitro-4-hydroxy-benzene arsonic acid was also effective in stimulating

growth. Davies and Briggs (13), showed that chick growth stimulation resulted in most cases, but not in all, when a practical corn-soybean ration was supplemented with either aureomycin HCl, procaine penicillin G, bacitracin or terramycin.

Although much of the work that has been done shows that there is a marked growth increase in feeding antibiotics, there are reports of instances where the addition of antibiotics had no effect on growth rate. Speer, et al, (14), reported an experiment in which the addition of aureomycin to a ration fed to young pigs had no effect on growth rate. The authors explained the lack of response to aureomycin by the "disease level" and that healthy, well-fed pigs, may not respond to aureomycin if fed under sanitary and disease free conditions. Scott and Glista (15), using chicks on a corn-soybean diet noted only a slight, insignificant response to aureomycin for the first few weeks with ad libitum feeding and no response when intake was limited. The chicks in his experiment "originated from a well fed group of breeding hens and were not depleted of A.P.F. reserves". Oleson, Hutchings and Whitehill (16), found that depleted chicks showed no response to aureomycin in the absence of vitamin B_{12} in the diet although there was a marked response to the antibiotic when vitamin B_{12} was present in sub-optimal or optimal amounts. Biely and March (17), have reported an experiment in which chicks fed a corn ration plus vitamins

at levels above those recommended by the N.R.C. resulted in no increase in the rate of growth with the addition of aureomycin.

Scott et al, (18), tested an all-vegetable ration with aureomycin (15 mg/lb.) and without aureomycin plus a number of supplements. The test rations contained ground corn, soybean oil meal, crude soybean oil, DL-methionine and minerals plus the known vitamins. Corn distillers' solubles and defatted liver meal were the only supplements which improved the basal ration without aureomycin, nor did aureomycin alone provide any improvement. When aureomycin was fed, all the supplements used, except dehydrated alfalfa, defatted liver meal, dried brewers yeast, and dried cereal grass (rye), gave a definite growth response when added to the basal ration. These include corn distiller's solubles, butyl fermentation solubles (grain base), butyl fermentation solubles (molasses base), all fed at the 5% level and also 2% of a grass juice concentrate. These results support the belief that the response from an antibiotic is dependent, in part, on the makeup of the ration with which it is fed.

A number of reports in the literature show that certain ingredients added to a chick ration will produce growth stimulus whether antibiotics are present or not. To date this growth factor is unidentified. Hill (50), reported that the addition of dried whey, fish meal or fish

solubles to chick rations containing soybean oil meal as a source of supplementary protein increased growth. Fish meal was more effective in promoting growth than the highest level of whey fed. Hill also reported that the magnitude of the growth response obtained from whey or fish meal was governed to a marked extent by the quality of the soybean oil meal. Using a purified type of diet with no source of animal protein, Carlson et al, (51), obtained evidence which indicated that dried brewers' yeast as well as fish meal contained a hitherto unrecognized growth factor. In their experiments, the growth response to yeast and fish meal was approximately equal, while the response to vitamin B_{12} was very poor, indicating that their diet was deficient in a factor other than B_{12}. Wiese et al, (52), showed that the addition of fish meal to an all-vegetable soybean type of chick ration supplemented with vitamin B_{12} gave an additional growth response in chicks at ten weeks of age. Sunde et al, (53), concluded that the addition of fish solubles or liver products to an all-vegetable type of chick ration gave an increase in growth over that obtained with vitamin B_{12} supplements. These results were obtained using a practical type of corn-soybean ration. Hill and Branion (54), also found that the addition of fish meal or dried whey to a chick ration containing adequate B_{12} gave a small additional growth response. Supplementation of the basal diet with

methionine in some of their experiments failed to give a growth increase comparable to that given by fish meal and whey. Berg et al, (55), found that supplementation of an all-vegetable type of chick diet with vitamin B_{12} or with 5% of fish meal gave approximately the same growth response. In contrast, when these same supplements were added in combination with an aureomycin containing fermentation product, chicks receiving aureomycin plus the fish meal grew significantly better than those fed aureomycin plus vitamin B_{12}. Similar results have been reported by McGinnis et al, (56), with turkey poults. They observed that a combination of fish meal and antibiotic gave a much greater response than either one alone when added to a turkey starting diet composed largely of corn and soybean oil meal. In another report McGinnis et al, (57), studied the effects of fish meal, fish solubles, liver meal and dried whey on growth of chicks and turkey poults fed different types of rations. They found that terramycin with whey and liver L gave greater growth response than any of the supplements alone. The use of a purified soybean protein as a substitute for soybean oil meal failed to amplify the deficiency of an unidentified growth factor for the chick. This is in contrast to the report by Hill (50). The addition of vitamin free casein or amino acids (methionine, lysine and tryptophan) to the basal diets failed to give a growth

response. Differences were obtained in response to fish solubles to different soybean oil meals, however, these differences were not statistically significant. In a later report, McGinnis (58), reported evidence is accumulating that fish meal, liver L faction, or dried whey produce greater growth response with chicks or poults in the presence of antibiotics than is the case when antibiotics are not added; and conversely that antibiotics produce a greater response when added to rations containing these animal protein sources than when added to an all-vegetable type ration. At least one additional unidentified factor, present in fish meal, fish solubles, certain liver fractions and dried whey, is required for maximum growth of chicks and turkey poults.

(c) Antibiotics and vitamins.

Davies and Briggs (13), reported that there was no apparent sparing action by an antibiotic on B_{12} for either the chick or poult when fed a practical corn-soybean ration. Atkinson and Couch (25), showed that injection of crystalline B_{12} into poults fed an all-vegetable protein (soybean oil meal-corn) diet failed to have any effect on growth, although such an injection did promote the growth of poults fed aureomycin. Reynolds, et al, (26), using good commercial chick rations, in a study involving 500 birds from 1 day to 4 weeks found that 1/3 of an ounce of terramycin to a ton of

feed can cut B_{12} requirements by 40%. A higher level in the feed did not stimulate further gains. It was found that even as terramycin showed a B_{12} sparing effect, the vitamin also showed an antibiotic sparing effect. They recommended that the optimum fortification of 10 grams of terramycin and 6 mg. of vitamin B_{12} in each ton of chick starter be used.

Craviato-Munoz, et al, (27) found that aureomycin was able to replace vitamin B_{12} in the rat maintained on a synthetic diet. When aureomycin was allowed to stand at room temperature or was autoclaved, the antibiotic potency was destroyed. If such aureomycin was given intraperitoneally or was fed, it was inactive for the growth promoting effect. It was postulated that aureomycin may decrease the number of *Escherichia coli* in the intestinal tract of the rat and allow the increase of other bacteria such as *Bacillus megatherium* which can produce adequate quantities of vitamin B_{12} for rat growth. That *E. coli* has a marked affinity for vitamin B_{12} has also been demonstrated by Davis and Mingioli (28).

Stokstad and Jukes (29) conducted several experiments in which graded levels of B_{12} were fed to the chicks in the presence and absence of aureomycin. A sparing action of aureomycin on vitamin B_{12} was noted in some experiments but not in others. When 10 micrograms of vitamin B_{12} was given in the presence of aureomycin, almost maximum response was obtained with the vitamin, but in the

absence of aureomycin, 50 micrograms of vitamin B_{12} per kilogram of diet produced a markedly greater response than 10 micrograms. Aureomycin often produced little or no growth response but markedly decreased mortality in chicks when on the basal vitamin B_{12} deficient diet or when inadequate levels of B_{12} were added. The chicks at hatching were markedly deficient in B_{12} due to the fact that they were obtained from eggs laid by hens which were on a diet deficient in this vitamin. A summary of all experiments pertaining to the effect of aureomycin on the mortality of vitamin B_{12} deficient chicks showed that in every case the mortality was lower when aureomycin was added. Two explanations are possible to explain this reduction of mortality. First, aureomycin may reduce the requirement of the chick for vitamin B_{12}. Second, the deficient chicks may have a higher susceptibility than normal chicks to the deleterious effect of certain microorganisms in the intestinal tract and aureomycin may decrease the number of these organisms.

An experiment by Davis and co-workers (30) was carried out to determine the content of radioactive vitamin B_{12} in the feces of rats fed CO_{60} and aureomycin. The fecal vitamin B_{12} of the rats fed a soybean diet, with or without aureomycin, was estimated by extracting aqueous homogenate of stool specimens with butanol. Addition of aureomycin to the basal diet produced an increase in the vitamin B_{12}

content, at a given intake of radioactive CO_{60}. Considerably smaller increase in vitamin B_{12} content could be brought about by the addition of a larger amount of CO_{60}. These results, therefore, indicate that the growth enhancement effect of aureomycin might be due to the increase in the production of vitamin B_{12} and perhaps other accessory factors by the bacterial organisms in the intestinal flora.

Linkswiler, Baumann and Snell, 1951 (31) made a study on the effect of aureomycin on the response of rats to various forms of vitamin B_6. It was found that aureomycin increased the growth of rats fed low levels of pyridoxine. This increase was sufficient to cause over-all errors of 10 to 100% in a bioassay for vitamin B_6 but largely disappeared when high levels of the vitamin were fed. Aureomycin caused a marked increase in the growth of rats fed limiting amounts of pyridoxal or pyridoxamine. In the presence of the antibiotic the three forms of vitamin B_6 were approximately equal in growth promoting activity, whereas, rats fed pyridoxal or pyridoxamine in the basal ration grew less than rats fed an equimolar amount of pyridoxine.

Lih and Baumann (32) 1951, set up an experiment using groups of 5 weanling rats which were given appropriate diets deficient in thiamine, riboflavin or pantithenic acid and then graded doses of the vitamin were fed. When antibiotics were added it was found that penicillin, aureomycin,

and streptomycin stimulated the growth of rats receiving limiting amounts of thiamine, riboflavin or pantothenic acid. The antibiotics were most effective in diets that contained enough vitamin for half-maximum growth, and the growth responses due to the antibiotics were approximately equal to those observed when the vitamin content of the diet was doubled in the absence of antibiotics. The relative effectiveness of the antibiotics varied with the vitamin deficiency. In thiamine deficiency penicillin produced the greatest increases in growth; aureomycin and streptomycin produced definite but lesser increases; while chloromycetin, terramycin and 3 nitro-4-hydroxyphenyl arsonic acid were inactive; some stimulation was noted with streptomycin, chloromycetin and terramycin in the presence of a moderate level of riboflavin; 3-nitro-4-hydroxyphenyl arsonic acid and sulfasuxidine appeared to be inactive. In pantothenic acid deficiency aureomycin and streptomycin were best, while penicillin was less active; the antibiotics also stimulated growth in the absence of added pantothenic acid.

Biely and March (17) in a study of the effect of aureomycin and vitamins on the growth rate of chicks, found that a chick ration containing vitamins at levels above those recommended by N.R.C. resulted in no increase in rate of growth when aureomycin was added. In a folic acid deficient diet without aureomycin, it was found that chicks at 4 weeks of age averaged only 308 grams in weight and were

poorly feathered. The birds receiving aureomycin, in spite of a suboptimal amount of folic acid in the diet, averaged 410 grams and were well feathered. It would appear, therefore, that the feeding of aureomycin to chicks lowered their dietary requirement for folic acid. In a further experiment, rations that were deficient in riboflavin, nicotinic acid, and folic acid respectively, were fed with and without crystalline aureomycin. When riboflavin, nicotinic acid or folic acid were omitted from the basal ration, there was a significant decrease in the rate of growth. The addition of aureomycin to these vitamin deficient rations stimulated the growth rate in each case to the extent that the chicks fed the vitamin deficient rations containing aureomycin attained weights similar to those fed the complete basal ration. It would appear from these experiments that dietary levels of nicotinic acid, folic acid or riboflavin that are suboptimal for maximum growth rate of the chick under normal conditions may be adequate when aureomycin is fed.

Sunde, et al, (33) in a report, presented evidence to show that one function of the antibiotics is the sparing of vitamin B_{12} and thus the indirect sparing of methionine and choline requirements of the chick. Chicks were fed a grain ration containing peanut meal as the protein supplement. The addition of either methionine, choline or

vitamin B_{12} to the basal ration, brought a significant stimulation of growth. The magnitude of the growth response from vitamin B_{12} additions (0-200 gamma/kg.) depended upon the quantity of the vitamin added with increases in growth up to the 200 gamma per kg. level. No additional growth response was observed by combinations of choline, methionine, vitamin B_{12} and streptomycin above that obtained with (1) 30 micrograms vitamin B_{12} per kg. plus streptomycin, (2) choline and methionine or (3) a high level of vitamin B_{12}.

Coates, et al, (34) report a study of the effect of antibiotics on the growth of chicks deprived of vitamins of the B complex. They fed penicillin (2.5mg./100g.) as the procaine derivative to 12-16 day old chicks fed vitamin deficient diets. Results showed that the presence of antibiotic had no effect on the degree of deficiency of riboflavin, thiamine, pyridoxine or pantothenic acid. It lessened that of biotin or folic acid and increased that of nicotinic acid. Although there were indications that the diets were slightly deficient in vitamin B_{12}, the effects of penicillin were essentially the same when vitamin B_{12} was added.

As part of a larger investigation concerning biochemical responses in chicks to antibiotics, liver vitamin A and serum carotenoid levels have been determined

by Burgess, et al, (35) when penicillin was included in the diet. The basal diet had the following composition (lb./100 lb.): ground yellow corn 54, solvent extracted soybean oil meal 17, wheat middlings 5, alfalfa meal 5, fish meal 6, meat meal 4, brewers yeast 5, limestone 1.5, tricalcium phosphate 1.4, salt (iodized) 0.5, manganese sulfate 0.3 oz., choline chloride 209, fish oil (2400 units A, 400 units Vit. D.) 50 gms. Penicillin was added at the rate of 30 mg. Na penicillin G per kg. diet. It was found that Vitamin A per gram fish liver and total serum-carotenoids was significantly elevated by administration of dietary penicillin. These increases were independent of feed consumption and of body weight response to the antibiotic. Liver weight per 100g. body weight was less in the penicillin-treated group. Total liver Vitamin A, on the other hand, was increased by dietary treatment.

(d) Antibiotics and Protein.

Burnside, et al, (36) fed rations to pigs in which peanut meal, soybean oil meal and fish meal made up 41.5, 44.5, and 30%, respectively. The remainder of the ration in each lot consisted of corn, bone meal 0.5, limestone 0.5 and salt 0.5% plus all the known vitamins which a pig has been shown to need. The addition of the A.P.F. supplement to the corn-peanut meal ration resulted in 2.13 times the rate of

gain obtained on the control ration. The addition of the A.P.F. supplement to the corn-soybean oil meal ration increased the rate of gain approximately 30% thus showing that A.P.F supplement is much more beneficial when added to a corn ration containing peanut meal than when it contains soybean oil meal. The addition of the A.P.F. supplement to the corn-fish meal ration was beneficial to a small extent. The results showed that the A.P.F. supplement increased the feeding value of peanut meal and soybean oil meal so that these plant protein supplements were similar in feeding value to the fish meal.

Sherwood and Couch (37) initiated a study to determine the effect of supplementing a low vitamin B_{12} diet (vegetable protein only) with crude A.P.F. concentrate* on the growth of chicks up to eight to ten weeks of age. The results showed that soybean oil meal and cottonseed meal of low gossypol content may be used as the sole source of protein when properly supplemented. A.P.F. concentrate produced a marked increase in growth.

Matterson, et al, (19) in their study on growth response to practical-type chick starting rations with antibiotics, found that a plant-protein ration in which 2.5

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* Lederle's A.P.F. concentrate - a fraction derived from aureomycin fermentation.

per cent fish meal replaced a like amount of high protein soybean oil meal, was superior to an all-plant protein ration. The greatest percentage growth response with antibiotics was obtained on the poorer quality rations (all-plant protein). However, the greatest weight is obtained on the better quality rations (all-plant protein plus fish meal) supplemented with antibiotics. The results as a whole suggested that the better the quality of the ration with respect to protein and vitamins, the less growth response obtained by supplementing the ration with antibiotics.

Berg (38) has reported that with the advent of the first experiments with antibiotic feedstuffs, it appeared that the growth response resulting with this material when added to an all soybean oil meal protein diet was so great as to make the use of animal protein from fish meal or meat meal no longer a necessity in high-energy broiler feeds. Subsequent trials have shown, however, that even better gains will be made on a ration containing fish meal and antibiotics than will be made on the all-vegetable protein diet plus antibiotics and B_{12}. This might be attributed to a more balanced protein with fish meal or addition of an unknown factor. As yet the level of fish meal to be fed has not been established since the requirement varies according

to the type of fish meal used or according to different samples of soybean oil meal. In tests conducted to determine the level of fish meal necessary for maximum growth with antibiotics, variable results in the range of 2 to 7 per cent have been found. Berg has also reported tests made on comparing rations containing different levels of protein. In three trials it was found that 19 per cent protein promoted as good growth as 21 per cent.

McGinnis (39) in a series of three experiments on turkeys for a period of four weeks, studied the effect of penicillin on diets containing different protein levels. The rations used were of a practical type and all diets contained the same amount of fish meal, vitamin and mineral supplements. The level of protein in the various diets was varied by changing the relative amounts of soybean oil meal and ground corn. It was found that the addition of penicillin (5 p.p.m.) to the experimental diets containing different amounts of protein did not lower the requirement of the turkey poult for protein. Best results were obtained at the 27% level of protein. The response from penicillin at the various levels was reasonably uniform except for the lowest level used. In this particular case, a greater response was obtained from penicillin.

Machlin, et al, (40) studied the effect of dietary antibiotic on protein requirement of growing chicks when

fed a corn-soybean ration at different protein levels. The protein content was lowered by reduced levels of corn and soybean meal in constant proportions and replacing them with glucose (cerelose). It was found in each of three experiments at four and six weeks of age, maximal and near maximal weight was obtained at the 19 per cent protein level with aureomycin. Without the aureomycin greater weight was obtained at 21 or more per cent of protein in every case.

The literature reveals very little information on the effect of antibiotics in rations where a deficient amino acid occurs. Jones and Combs (41) fed practical type rations to chicks suboptimal in lysine and tryptophan. Aureomycin supplementation appeared to spare dietary requirement for tryptophan but not for lysine. In another experiment, using a corn-soybean meal type ration, dietary procaine penicillin G did not spare the requirement for methionine. Sherwood and Couch (37) found by using cottonseed meal as a protein supplement it was necessary to add dl-lysine to make up the amino acid deficiency even when aureomycin was present in the ration. Machlin, et al, (42) studied the effect of the addition of an aureomycin fermentation product to a cottonseed meal diet. It was found that there was a significant response when aureomycin fermentation product was added to the basal diet or to a lysine-supplemented

diet. Best results were obtained when both lysine and the aureomycin fermentation product were added. The addition of an A.P.F. supplement to an all poult starting mash containing 29% protein and deficient in lysine (0.9%) has been studied by Slinger, et al, (43) in relation to the severity of white feather condition. When the diet contained 29% protein and 1.8% lysine, no cases of white feathers appeared as a result of including the A.P.F. supplement. Supplementing a diet containing 28% protein and 1.9% lysine with penicillin, aureomycin or A.P.F. resulted in much greater response in growth and feed efficiency than when such additions were made to a diet containing 20% protein and 1.2% lysine. Furthermore, the addition of these materials to the latter diet produced a high incidence of white feathers, while when added to the former diet no white feathers were noted. These results indicate that certain A.P.F. concentrates and antibiotics increase the lysine requirement of the poult for normal feather pigmentation. It is also suggested that these materials may increase the requirement of this species for other amino acids.

(e) Antibiotics and feed efficiency.

Reports on the effect of aureomycin fermentation product or crystalline aureomycin on the efficiency of feed utilization of chickens, when added to diets containing a

source if vitamin B_{12}, are not consistent. Biely, et al, (1951) (44) showed a definite increase in feed efficiency when an aureomycin fermentation product was added to a 17 per cent protein diet containing fish meal. The feed efficiency was improved to the extent that it was better than that of 21 per cent protein all-vegetable rations containing aureomycin fermentation. Berg, et al, (1950) (8) found no difference in feed conversion rates when this fermentation product was added to a high energy diet containing fish meal and fed to chicks. Neither Scott and Glista (1950) (15) nor Groschke and Evans (1950) (45) showed an increased efficiency when crystalline aureomycin was added to diets containing all the known vitamins including B_{12}. Machlin, et al, (1951), (40) found that aureomycin increased efficiency of feed utilization when added to a corn soybean diet containing vitamin B_{12}. The effect of the aureomycin on feed efficiency was more pronounced with increasing protein levels.

Atkinson and Couch, (1951), (46) using a soybean-corn ration on turkey poults found an improvement in feed utilization when an antibiotic supplement was added. This was verified by subsequent tests. Davies and Briggs, (1951), (13) found an improved feed efficiency in chicks and poults when the diet was supplemented with a variety of antibiotics. The ration used in this instance was a practical corn-

soybean type.

In studies with swine Bowland, et al, (1950), (47) reported a higher efficiency of gain with an aureomycin fermentation product than with a vitamin B_{12} concentrate. Other trials showed a greatly increased rate and efficiency of gain when aureomycin fermentation was fed to unthrifty weanling pigs over a six week period. Rations used in these experiments were both the all-vegetable and mixed animal-vegetable protein types. Carpenter (1950), (48) found similar results in pigs and noted that feed efficiency was increased from 6.4 to 3.1 lb. of feed/lb. gain by aureomycin supplementation to an animal-vegetable diet. Carpenter was also able to demonstrate an increase in feed efficiency when crystalline aureomycin (1.25g./100lb of feed) was added to diets containing B_{12}. Beeson (1950), (49) on the other hand, using 20% protein corn-soybean meat alfalfa rations plus added vitamins was unable to find any such difference in feed efficiency.

(f) Action of antibiotics on the intestinal flora.

In view of the fact that it has been found that antibiotics generally stimulate growth, research workers have tried to determine the exact reason for this response. To date, nothing conclusive has been found although considerable investigational work is in progress. Research has involved

several theories. Some of these theories are as follows:

1. Factor synthesis theory - in this theory it is postulated that chick growth can be greatly stimulated by a vitamin-like factor as yet undiscovered, and that one way to provide this factor is by feeding an antibiotic. The antibiotic is supposed to inhibit other bacteria in the intestine allowing a type to flourish which synthesizes the postulated growth factor.

It is doubtful if such a vitamin-like factor is the reason for added growth, since, in order to produce the results claimed by antibiotics, it would have to be much more potent than vitamin B_{12}, or anything occurring in distillers' solubles, fish meal, fish solubles, dried whey, dried brewers' yeast, liver, or other natural feed ingredients.

2. Competition theory - in this theory it is stated that both the animal and its intestinal microbes are competing for the same supply of vitamins from the diet. The intestinal bacteria may flourish to such an extent that they rob the animal of vitamins and thus retard growth. Feeding an antibiotic is supposed to decrease the bacterial count, thus relieving the animal from competition and permitting more rapid growth.

3. Enterotoxism theory - states that antibiotics do not really stimulate growth at all, but rather through their antibiotic action prevent a retardation of growth by a slow

poison which is produced by harmful bacteria in the intestinal tract.

In order to arrive at a comparative basis for study of the intestinal flora of the chicken, some understanding of the normal intestinal bacteria as against the modified intestinal bacteria with antibiotics, is necessary.

The kinds and numbers of microorganisms in the so called normal intestinal flora of the chicken have been studied by Shapiro and Sarles (59). This investigation covered a period from hatching to the age of one year. Two chicks were selected at randon every 2 - 3 days up to 45 days, then every 7 - 14 days up to the age of 30 weeks. After this, selections were made at irregular intervals up to the age of one year. The birds were decapitated and the entire intestinal tract exposed. Specific segments were removed to obtain intestinal contents for sampling. The bacteria present were divided into seven groups by selective media. The results obtained revealed that newly hatched chicks harbour very few microorganisms intestinally. After 21 hours an increase of gas producing organisms appeared. No other increase was noted until the birds were fed (24 hours after hatching). A rise in microorganisms was noted until 40 hours after hatching when a peak was reached. The highest number of organisms appeared in the caecum then a

progressively lower number in the colon and especially high in the caecal pouches. The coliform were the dominant group in the colon. E. coli was the predominant organism in the coliform group but was of numerical significance only in the contents of the colon and caecal pouches. It was found that the enterococcus and coliform counts were very similar in the duodenum, ilium, and colon. In the caecal pouches, the number of enterococci was lower than the number of coliforms. Lactobacilli were found to be the most numerous group of bacteria in most areas of the intestinal tract. Since the vitamin requirements of these organisms are known to be quite extensive, it is likely these bacteria play an important role in the vitamin economy of the host.

Although the literature presents a variety of opinions as to the action of antibiotics, it is generally conceded that there is some relation between the intestinal microflora and these substances. Mention has already been made (27, 28, 29, 30) suggesting that the sparing effect of aureomycin on vitamin B_{12} (theory 2) or the inhibition of toxin producing microorganisms (theory 3). Lih and Baumann (32) in their experiment on rats with different antibiotics in diets deficient in the B vitamins, support theory 2. They also suggest that since different antibiotics affect the various types of organisms to different degrees, it is

not surprising that their growth promoting power should vary with the vitamin that happens to be limiting in the diet. Biely and March (17) in showing the effect of aureomycin in lowering the dietary requirement for certain B vitamins support theories 2 and 1. Atkinson and Couch (25) found that supplementing an all-vegetable protein diet with eight crystalline vitamins promoted the growth of poults in some instances but failed to have an appreciable effect in the presence of streptomycin and vitamin B_{12} or when aureomycin A.P.F. was fed. They explain this by supporting theory 2.

Speer, et al, (14) support the idea of inhibiting toxin producing bacteria (theory 3) in pigs. Sieburth, et al, (60) presented evidence for theory 3 when they reported that terramycin and penicillin completely prevented the growth of Clostridium (probably welchii) in the intestinal tract of turkeys. Terramycin also had a similar effect in an experiment with pigs. This organism or one closely related to Clostridium welchii has been shown to be the causative agent of an entertoxemia in sheep. These workers further hypothesized that such an organism could be producing toxins in small quantities in the intestine of young animals which have a growth depressing effect.

March and Biely (61) found that those bacteria to be first affected by aureomycin feeding were the lactobacilli.

At the level of aureomycin customarily added to chick starting rations with A.P.F. supplements there was a significant depression in the number of lactic acid bacteria present in the feces. Increasing the amount of aureomycin in the diet again lead to a significant decrease in the fecal lactic acid bacteria and a suppression of the total aerobic and coliform bacteria in the feces. However, the higher level of aureomycin fed did not stimulate growth to any greater extent than the lower level. They conclude that the suppression of the lactic acid bacteria in the intestine may be a factor in the growth stimulating effect of aureomycin for the chick.

Dixon and Thayer (62) found that functional ceca in the chick were not essential for the growth promoting action of antibiotics. The numbers of enterococci bacteria in penicillin fed lots were lower than in the controls, while the numbers in the aureomycin fed lots were higher than in the controls. Lactic acid bacteria counts were consistently higher in the aureomycin fed lots. The coliform count showed no consistent trend among lots which could be correlated with growth response.

Anderson, et al, (63) studied the effect of antibiotics on the intestinal flora of chickens using diets containing 17, 20, 23, and 26 per cent protein. In the first

experiment an inverse relationship existed between pH and protein; penicillin effected a lowering of pH. Anaerobic and microaerophilic counts were greater than aerobic, the former decreasing, the latter remaining constant with increasing protein. Both groups were influenced by penicillin up to 23 per cent protein, followed by a decided decrease with 26 per cent. Aciduric were predominant over proteolytic types, penicillin exerting the same influence as on the above types. Penicillin enhanced the coliform and lactobacilli counts. Lowest counts were obtained with enterococci. Penicillin reduced these types. When higher energy diets were fed and aureomycin used as the antibiotic, the results obtained with pH, anaerobes, aerobes, and enterococci were similar to those in the first experiment. Aciduric and proteolytic counts were reduced in the presence of aureomycin, the latter more than the former. Coliform counts were again increased by antibiotics but the significance was reduced at 23 per cent protein followed by a slight increase with 26 per cent. Lactobacilli were restricted by aureomycin up to the third week, after which they increased.

The growth response observed when streptomycin and other antibiotics are fed to turkeys was studied by Kratzer, et al, (64) in relation to the numbers of yeast organisms present in the intestinal tract. Poults fed streptomycin-containing diets exhibited a 5 to 10 fold

increase in numbers of intestinal yeast organisms over control poults. Cultures of a yeast isolated from intestinal contents were fed as supplements (1% of the diet) to adequate chick and poult diets and the growth rates obtained were compared with those of birds fed normal diets or supplements of antibiotics. One strain of yeast isolated from the intestinal tract of streptomycin fed poults produced a slightly more rapid growth than normal diets in some, but not in all, chick and poult feeding trials. This slight growth response was probably different from that obtained from streptomycin feeding. Day old chicks given access for the first 3 days of life to droppings of normal or aureomycin fed month old chicks grew slower than control chicks. Autoclaved droppings produced no growth depression. Droppings from birds fed aureomycin produced less growth depression than droppings from normal birds. Aureomycin improved growth of the chicks fed either fresh or autoclaved droppings.

(g) Comparison of antibiotics as growth stimulants.

A study of a comparison of several antibiotics as growth stimulants in practical chick starting rations was made by Matterson, et al, (19) 1951. Five antibiotics, aureomycin, streptomycin, penicillin, terramycin and bacitracin, were used as supplements to an all-plant protein ration and an all-plant protein ration in which 2.5 per cent

fish meal replaced a like amount of high protein soybean oil meal. It was found that streptomycin was the least effective in promoting growth response in chicks. It was also noted that although the greatest percentage growth response with antibiotics was obtained on the poorer quality all-plant protein rations, the greatest weight was obtained on the better quality, all-plant protein plus fish meal rations. Approximately 9 grams of antibiotics per ton of feed were used so that no effort was made to determine the least quantity of antibiotic necessary to obtain a given response.

Branion and Hill (20) found the addition of aureomycin hydrochloride, penicillin G potassium, terramycin hydrochloride and streptomycin sulfate at a level of 25 milligrams per kilogram of a diet containing 15 per cent animal protein supplements or an all-plant protein diet supplemented with crystalline vitamin B_{12} resulted in a growth response in turkey poults up to 8 weeks of age. The relative ability of these antibiotics to stimulate growth was not the same from 0 to 4 weeks as from 4 to 8 weeks. At 8 weeks of age terramycin gave the greatest growth response, followed, in the case of an animal protein diet, by aureomycin, streptomycin and penicillin and in the case of a plant protein diet by streptomycin, penicillin and aureomycin in that order. However, at four weeks of age,

penicillin gave the greatest growth response with both diets, followed in the case of the animal protein diet, by aureomycin, terramycin and streptomycin and in the case of the plant protein diet, by terramycin, aureomycin and streptomycin, in that order. They suggest that these results may be due to a variation in the level of antibiotics required at various ages, a loss of potency upon storage in a mixed feed, a reduced effect with continuous feeding or a specific influence on definite microorganisms which are present at certain stages but not at others, or whose influence on the host nutrition may be more marked at some stages than others.

In a report by McGinnis (21) data on the critical amounts of the various antibiotics required for maximum growth of chicks and turkeys has been presented. In his experiments for aquisition of this data, he used practical rations and fed them under practical conditions. Results showed that the minimum level of a particular antibiotic in grams per ton of feed necessary for maximum chick or poultry growth appeared to vary with the different antibiotics. Procain penicillin appeared to be more effective than terramycin, aureomycin or bacitracin in promoting chick growth. This was particularly true at the level of 2 grams per ton of feed. Aureomycin and terramycin appeared to be approximately equivalent in value for chick growth.

A level of 2 grams of these antibiotics per ton of feed was as effective as a 5 gram level. Procain penicillin appeared to be somewhat more effective than terramycin, aureomycin or bacitracin in promoting turkey poult growth.

In a further report McGinnis (22) showed the effect of removing penicillin from the diet of poults at different ages. It was found that turkeys which received penicillin throughout the entire four week period had the highest average weights at the end of the trial. The feeding of penicillin for the first week of the poults' life caused a marked increase in average weight at four weeks even though it was removed from the diet when the poults were one week of age. Results from previous experiments with both chicks and poults indicated that the antibiotic supplement could be removed from the diet when they were eight weeks of age without materially affecting average weight at twenty weeks of age.

In a report on high versus low level antibiotic feeding, McGinnis (22) fed a practical turkey ration to which was added 50 p.p.m. of three antibiotics. Increasing the level of diamine penicillin from 2 to 50 p.p.m. did not improve growth. A similar finding was made with the bacitracin supplement. In contrast, increasing the level of terramycin from 5 to 50 p.p.m. did appear to give an improved growth response. A comparison of pure terramycin hydrochloride

and terramycin contained in the antibiotic feed supplement at a level of 50 p.p.m. showed these two materials to give approximately the same growth. As was pointed out previously in a report by McGinnis, terramycin and aureomycin fed at graded levels produced no effect in growth when these antibiotics were increased. Under laboratory conditions, therefore, increasing the level of the three different antibiotics tenfold did not materially alter the growth responses obtained. Under field conditions where desease may be encountered, the results obtained by feeding high levels of antibiotics may be entirely different.

Kramke and Fritz (23) fed graded levels of several antibiotics in practical starting rations to chicks and poults in an effort to determine the minimum quantities which would afford good growth stimulation. Aureomycin, bacitracin, penicillin, and terramycin all gave essentially optimum growth stimulation when used at the rate of 10 grams per ton of feed. At lower levels of intake penicillin proved to be the most effective, giving practically as good results with 5 grams of antibiotic per ton. Streptomycin at comparable levels was much less effective. The quantitative requirements were similar for chicks and poults. Maximum percentage gains due to antibiotics were observed at about 4 weeks of age.

Reynolds, et al, (24) made a study of the comparison of terramycin and penicillin at various levels to broilers. They fed a complete practical type broiler ration containing 5 per cent fish meal, 2.5 per cent dried whey and no B_{12} supplement. The diets were supplemented with 2 grams penicillin, 5 grams penicillin, 2 grams terramycin, 5 grams terramycin, 10 grams terramycin and a combination of 2.5 grams terramycin and 2.5 grams penicillin, per ton respectively. Results showed that terramycin produced the greatest gain in body weight and most efficient utilization of feed. No statistically significant difference between groups were revealed. Penicillin and terramycin were approximately equivalent gram for gram in their growth promoting properties and two grams/ton of either antibiotic appeared to be near the optimum level for growth promoting properties. No statistically significant differences between groups were revealed.

III. EXPERIMENTAL

Experiment 1

Introduction

The first study was undertaken to compare the effect of antibiotics on chick growth when fed in three different rations. The first ration was the standard Connecticut ration containing ground yellow corn as the cereal grain and fishmeal, meat meal, soybean oil meal and liver meal as the protein supplements. This ration is noted for its high energy, low fibre content. The second ration was an all-plant protein ration containing ground ground yellow corn as the cereal grain and soybean oil meal as the protein supplement. The third ration also contained ground yellow corn as the cereal grain but fishmeal as the protein supplement. Further ingredients were added to each of the three rations in order to balance them for vitamins and minerals.

Supplements composed of vitamins and minerals were added to each of the above basal rations in order to determine whether any additional growth response would result. Antibiotics were added to these supplemented rations to study their response on growth of chicks as

compared to the unsupplemented rations with antibiotics

TEST NO. 1

Purpose

The purpose of this experiment was to determine the effect on growth of chicks of adding antibiotics to three different practical rations and to make a comparison of the growth response to each ration. Also, to study the effect of adding vitamins and minerals to these rations and to determine if any additional response could be gained when antibiotics were added with these supplements.

Methods and Procedure

Day old, male, New Hampshire chicks were wing-banded and distributed at random in twelve compartments of electrically heated, Jamesway battery brooders. Twenty chicks were distributed to each compartment. The chicks were held in these batteries until the end of the experiment at five weeks. The temperature was adjusted as the birds grew older.

The birds were fed mash and water ad lib. Granite grit was fed to them weekly. The feed on hand was recorded at the end of the third, fourth and fifth weeks for the purpose of calculating feed efficiency. A record of the feed efficiency is presented in Table 11.

The birds were weighed at the end of each week and a statistical analysis made on the weights at five weeks. The weights are presented in Table 11 and graphically in Figures 1, 2 and 3. The analysis is presented in Table 1V.

The original Connecticut ration was used as the first test ration. Dried brewers' yeast, however, was used to replace the butyl fermentation solubles and in addition 0.2 gms. of riboflavin were added to assure a sufficient amount of this vitamin. The choline chloride (25%) was increased from 32 gms. to 200 gms. (0.44 lb.) to be assured of a good methylating donor.

In the soybean ration 59.8 lb. of corn and 34 lb. of soybean were used. In view of the fact that it was an all-vegetable ration one pound of limestone and 2 lb. of bonemeal were added to balance the minerals. This ration needed an additional supply of the B complex vitamins. To supply these 2 lb. of distillers' solubles, along with small quantities of choline chloride (25%), niacin, Ca pantothenate, riboflavin and vitamin B_{12} were added. Since soybean is low in methionine 0.1 lb. were added to make up the deficiency.

The fishmeal ration contained 74.8 lb. of corn and 21.0 lb. of fishmeal. One pound of limestone was added to balance the calcium level. The same B-complex vitamins were added as in the soybean ration except that vitamin B_{12} was excluded since the fishmeal supplied an adequate amount.

Methionine was also excluded since fishmeal supplies enough of this amino acid.

The supplementary value of an antibiotic fermentation product was tested by supplementing the three basal rations with 0.3% of the preparation i.e. ration #2 (Connecticut), ration #6 (Soybean) and ration #10 (Fishmeal). The preparation was also combined with a supplement of vitamins and minerals and added to the basals i.e. ration #4 (Connecticut), ration #8 (Soybean) and ration #12 (Fishmeal).

The supplementary value of seven vitamins and six minerals was tested. The vitamin and mineral supplement was added to the three basals i.e. ration #3 (Connecticut), ration #7 (Soybean) and ration #11 (Fishmeal). To three more basal rations the vitamins and minerals were added with the antibiotic preparation and fed in rations as described in the preceding paragraph. Fifty milligrams of thiamin hydrochloride, 5 grams of inositol, 4.54 grams of para amino benzoic acid, 22.7 milligrams of folic acid, 22.7 mgs. of menadione and 136 milligrams of alpha-tocopherol (vitamin E) per hundred pounds of feed were the vitamins added. Five grams of ferric citrate, 500 milligrams of copper sulphate, 25 milligrams of nickel carbonate, 50 milligrams of cobalt acetate, 25 milligrams of hydrated aluminium sulphate and 25 milligrams of zinc acetate per hundred pounds of feed

were the minerals added.

The composition of the basal rations and the supplements to the basal rations are shown in Table 1.

Results

At five weeks of age the chicks receiving the Connecticut basal ration weighed an average of 461 grams or 27 grams less than the average weight of the birds on the soybean basal ration (#5). The birds on the fishmeal basal weighed an average of 426 grams or 35 grams less than those on the Connecticut ration and 52 grams less than those on the soybean ration. These results indicate the greatest response to growth with the soybean ration.

When the aureomycin fermentation product was added to the Connecticut basal ration (#2) the average weight of the birds at five weeks was 560 grams. These weights were 99 grams more than the basal ration (#1). The soybean ration with the aureomycin supplement (#2) produced an average weight of 525 grams or 37 grams more than the basal ration (#5) and 35 grams less than the Connecticut ration with the aureomycin supplement (#2). The fishmeal ration plus the aureomycin supplement (#10) produced chicks weighing an average of 473 grams or 53 grams more than the average of the chicks on the basal ration (#9). The average weights from this supplemented ration were 87 grams

less than the Connecticut supplemented ration (#2) and 52 grams less than the soybean supplemented ration (#6). These results indicate that the greatest response was gained from the aureomycin supplementation to the Connecticut basal.

The vitamin and mineral mixture when added to the Connecticut basal ration (#3) resulted in average weights of 474 grams or 13 grams more than the basal ration (#1). When this mixture was added to the soybean basal ration (#7) the average weights were 452 grams or 36 grams less than the basal ration (#5). When added to the fishmeal basal ration (#11), the average weights were 471 grams or 45 grams more than the basal ration (#9). These results indicate, therefore, that the greatest response from the vitamin and mineral supplement was to be gained when added to the fishmeal basal ration.

The vitamin and mineral supplement and aureomycin when added to the Connecticut basal resulted in an average weight of 551 grams or 9 grams less than the aureomycin supplement ration (#2) and 77 grams more than the vitamin and mineral supplemented ration (#3). This combination added to the soybean basal (#8) resulted in an average weight of 523 grams or 2 grams less than the aureomycin supplemented basal (#6) and 71 grams more than the vitamin and mineral supplemented ration (#7). The combination

added to the fishmeal basal ration (#12) resulted in an average weight of 530 grams or 57 grams more than the aureomycin supplemented ration (#10) and 59 grams more than the vitamin and mineral supplemented ration (#11). These results indicate that there was no advantage in feeding a supplement of vitamins, minerals and aureomycin fermentation product over the addition of aureomycin fermentation product alone. Some advantage was gained when the vitamin and mineral supplement and aureomycin was added to the fishmeal ration. The fact that vitamins and minerals added to the fishmeal ration also gave a response to growth indicated that fishmeal must be limiting in some factor that is supplied by these additions.

The statistical analysis on the fifth week average weights indicated a significance at the 5% level for the interaction of rations, antibiotics, and vitamins and minerals. Since this figure represented the variation, it was used as the testing error. The results then showed that the addition of antibiotics gave a test of significance.

The results from the feed utilization data indicate that the Connecticut and fishmeal basal rations are the most efficient. The addition of the aureomycin fermentation to the basals produced an improved efficiency in all rations (#2, 4, 6, 8, 10, 12) where it was used. On the other hand,

vitamins and minerals when used as a supplement (#3, 4, 7, 8, 11, 12) had little effect on feed utilization.

Conclusions

1. Aureomycin fermentation product, when added to the Connecticut, soybean and fishmeal basal rations resulted in a significant increase in growth in chicks. The highest increase was obtained with the Connecticut ration.
2. The addition of vitamins and minerals to these basal rations produced no significant weight increases in the Connecticut and soybean rations. Some increase was noted when these supplements were added to the fishmeal basal ration.
3. The vitamin and mineral supplement and aureomycin fermentation product resulted in no increase in weight over the supplementation with aureomycin fermentation alone when added to the Connecticut and soybean basal rations. Again, however, an increase in weight was noted when this combination was added to the fishmeal basal ration.
4. The Connecticut and fishmeal rations were most efficiently utilized by the chicks. The aureomycin fermentation supplement improved the efficiency in all rations but this was not further improved

when added with the vitamin and mineral supplement. The vitamin and mineral supplement alone did not improve the feed efficiency.

Table 1

Composition of Basal Rations

	Basal Ration #1 Connecticut lb./100lb.	Basal Ration #4 Soybean lb./100lb.	Basal Ration #8 Fishmeal lb./100lb.
Ground yellow corn	69.0	59.8	74.8
Fishmeal	8.0	-	21.0
Meatmeal	8.0	-	-
Soybean oil meal (solvent)	8.0	34.0	-
Livermeal	3.0	-	-
Ground Limestone	-	1.0	1.0
Bonemeal	-	2.0	-
Alfalfa leaf meal	1.0	-	-
Dried Brewers' Yeast	2.0	-	-
Dried Distillers' Solubles	-	2.0	2.0
Iodized Salt	0.5	0.5	0.5
Manganese Sulphate	0.025	0.025	0.025
Feeding Oil (2250A; 300D)	0.25	0.25	0.25
Choline chloride (25%)	0.44	0.44	0.44
Nicotinic acid	0.9 gm.	0.9 gm.	0.9 gm.
Calcium pantothenate	0.5 gm.	0.5 gm.	0.5 gm.
Riboflavin	0.2 gm.	0.2 gm.	0.2 gm.
dl-Methionine	-	0.1 lb.	-
Vitamin B12	-	1.0 mg.	-

Ration #2 - #1 plus antibiotic.*
" #3 - #1 " vitamin and mineral.
" #4 - #1 " antibiotic and vitamin and mineral.
" #6 - #5 " antibiotic.
" #7 - #5 " vitamin and mineral.
" #8 - #5 " antibiotic and vitamin and mineral.
" #10 - #9 " antibiotic.
" #11 - #9 " vitamin and mineral.
" #12 - #9 " antibiotic and vitamin and mineral.

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* Lederle's A.P.F. 0.3% - an aureomycin fermentation preparation.

Table 11

Weekly Average Weights

Connecticut Ration

Ration No.	Ration	Weeks 1	2	3	4	5
1	Basal	75 g.	142 g.	218 g.	330 g.	461 g.
2	Antibiotic	80	162	267	396	560
3	Vitamins & Minerals	75	145	239	357	474
4	Vitamins, Minerals & Antibiotic	83	163	267	399	551

Soybean Ration

Ration No.	Ration	Weeks 1	2	3	4	5
5	Basal	72 g.	140 g.	234 g.	344 g.	488 g.
6	Antibiotic	80	150	248	380	525
7	Vitamins & Minerals	70	133	228	361	452
8	Vitamins, Minerals & Antibiotic	79	149	248	374	523

Fishmeal Ration

Ration No.	Ration	Weeks 1	2	3	4	5
9	Basal	73 g.	129 g.	206 g.	315 g.	426 g.
10	Antibiotic	79	150	232	350	473
11	Vitamins & Minerals	71	130	207	348	471
12	Vitamins, Minerals & Antibiotic	78	157	258	384	530

Table 111

Feed Efficiency

Connecticut Ration

Ration No.	Ration	Weeks 3	4	5	Feed efficiency for test period
1	Basal	1.91	2.29	2.38	2.15
2	Antibiotic	1.85	2.24	2.22	2.06
3	Vitamins & Minerals	1.86	2.29	2.91	2.27
4	Vitamins, Minerals & Antibiotic	1.78	2.28	2.64	2.16

Soybean Ration

Ration No.	Ration	Weeks 3	4	5	Feed efficiency for test period
5	Basal	2.02	2.91	2.78	2.53
6	Antibiotic	1.84	2.29	2.39	2.12
7	Vitamins & Minerals	2.21	2.34	3.51	2.54
8	Vitamins, Minerals & Antibiotic	1.97	2.59	2.62	2.33

Fishmeal Ration

Ration No.	Ration	Weeks 3	4	5	Feed efficiency for test period
9	Basal	2.18	2.55	2.55	2.38
10	Antibiotic	1.81	2.19	2.30	2.05
11	Vitamins & Minerals	2.08	2.06	2.62	2.23
12	Vitamins, Minerals & Antibiotic	1.81	2.13	2.33	2.05

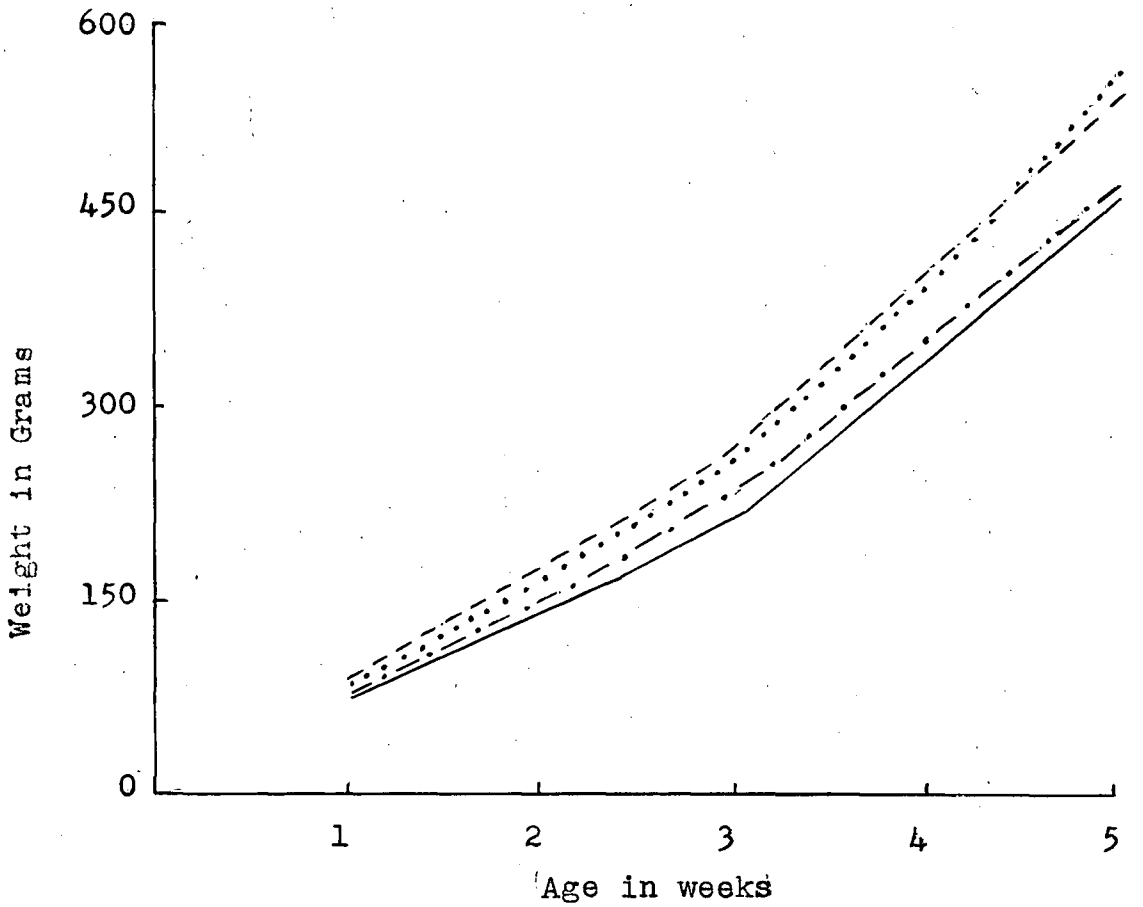
Table IV

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Variance
Total	988,922	224	
Rations	44,270	2	22,135
Antibiotics	227,897	1	227,897*
Vitamins & Minerals	5,418	1	5,418
Rations x Antibiotics	23,161	2	11,580
Rations x Vitamins & Minerals	43,547	2	21,773
Antibiotics x Vitamins & Minerals	2,936	1	2,936
Rations x Antibiotics x Vitamins & Minerals	79,762	2	39,881
Error	561,931	231	2,639

* Significant at p = 0.05 when triple interaction (rations x antibiotics x vitamins and minerals) is used as the testing error.

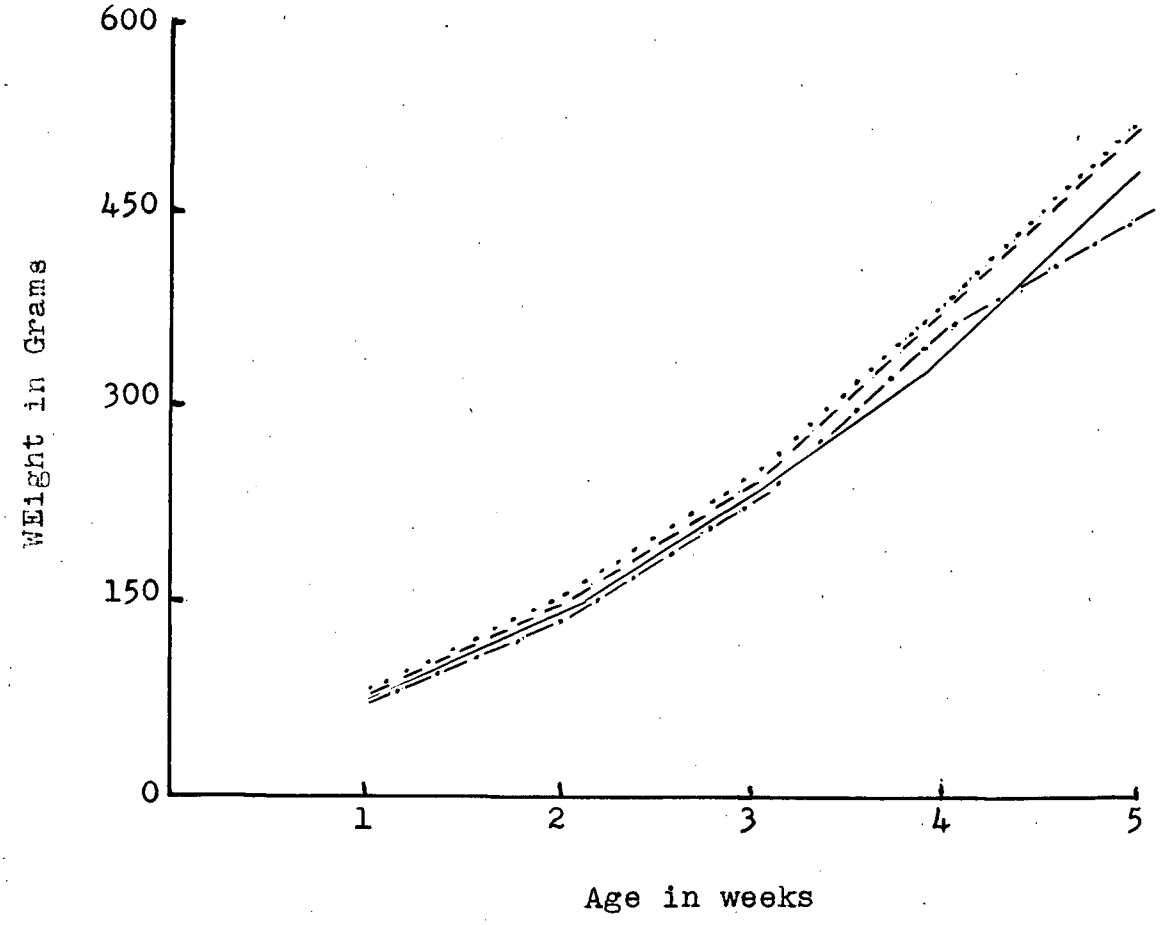
CONNECTICUT RATION



——————— Basal
. . . . . Antibiotics
—.— . — . — Vitamins and Minerals
— — — — — — Vitamins, Minerals and Antibiotics

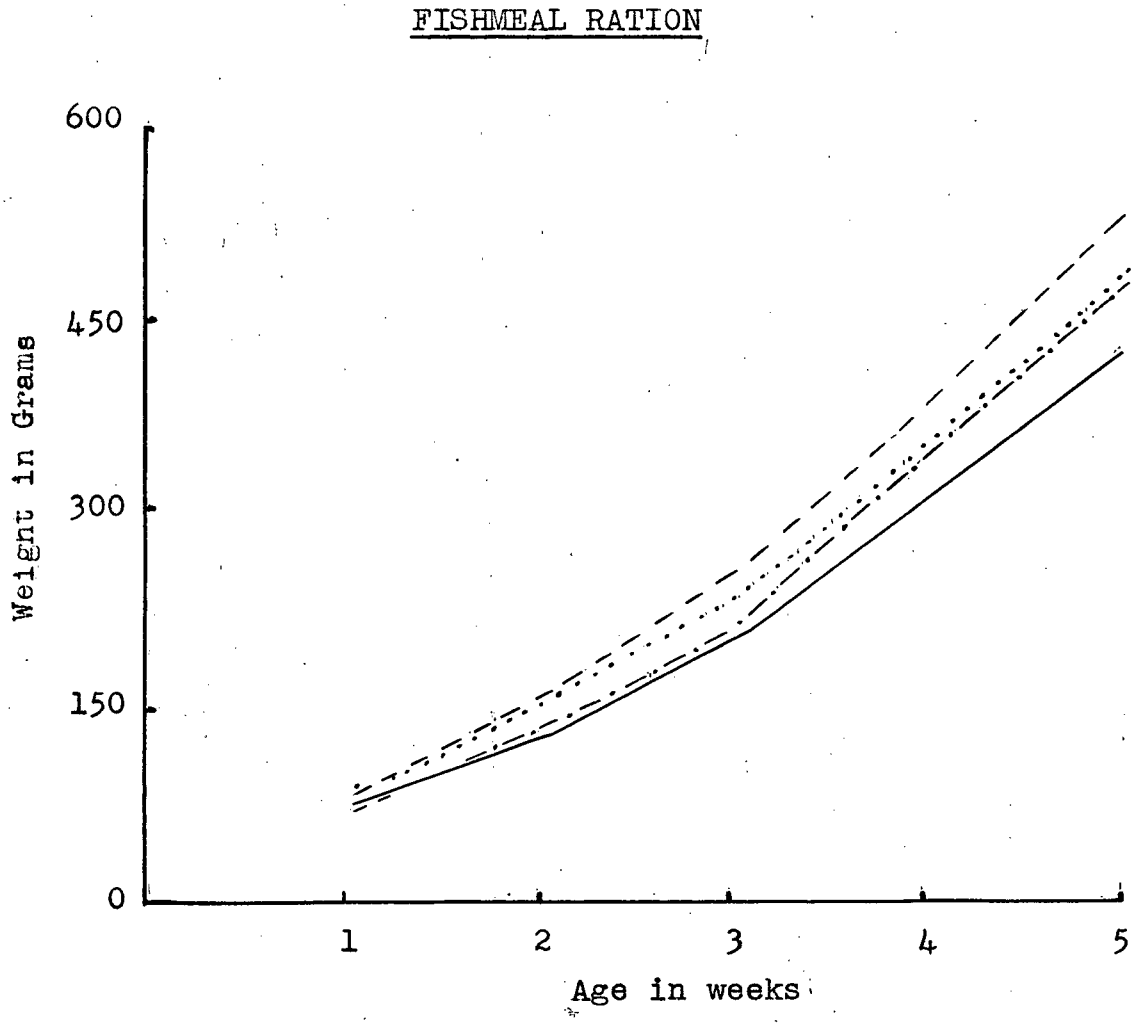
Figure 1. The Growth response of chicks receiving different supplements.

SOYBEAN RATION



——————— Basal
. . . . . Antibiotics
—.—.—.— Vitamins and Minerals
— — — — — Vitamins, Minerals and Antibiotics

Figure 1. - Growth response of chicks receiving different supplements.



———— Basal
. . . . . Antibiotics
—.—.—.— Vitamins and Minerals
– – – – – – Vitamins, Minerals and Antibiotics

Figure 1. Growth response of chicks receiving different supplements.

Experiment 11

Introduction

The second study was conducted to determine whether or not antibiotics would lower the protein requirement of the chick. Rations containing different protein levels were formulated with the same basic ingredients in each ration. The protein levels were lowered by increasing the ground wheat and ground yellow corn and decreasing the soybean oil meal and fishmeal used in the rations. Two different levels of antibiotics were tested with these basal rations. All rations were fed to both male and female chicks to determine sex difference in growth response to antibiotics.

TEST NO. 2

Purpose

The purpose of this test was to determine the effect of antibiotics on the growth rate of chicks when fed in rations with a lower protein level than that recommended for optimum growth.

Methods and Procedure

Day old, female, White Leghorn chicks were used in this experiment. Twenty chicks were distributed at random to each of sixteen brooder compartments. The equipment used

and the method of handling the chicks was the same as outlined in Test 1. Each ration was tested in duplicate. The birds were weighed at 7, 14, 29, 41 and 54 days and the amount of feed consumed was recorded for the test period. A record of the average weights is presented in Table 11. and the food consumption in Table 111. The weights at 54 days are presented in Figure 1. A statistical analysis also was made on the weights of the chicks at 54 days and the results are presented in Table 1V.

Four basal rations were used for this test and the protein level for each ration adjusted to 14, 16, 18 and 20% protein levels. A chemical analysis of these rations showed the protein levels to be 16.0, 17.9, 19.5, and 22.0 respectively. The ingredients used in these rations are shown in Table 1. Equal parts of wheat and corn were used as cereal grains. The protein supplements were soybean and fishmeal which were also added in equal parts. To decrease the protein level the cereal grains were increased and the protein supplements decreased. As the protein level was lowered, the amount of bonemeal was increased due to the fact that the fishmeal was reduced and hence it was necessary to adjust the calcium-phosphorus ratio. Vitamins were added to each ration in the same amounts regardless of the protein level.

The effect of adding an antibiotic was tested with 0.5% of an antibiotic preparation containing 1.8 gms. per lb. of aureomycin hydrochloride.

Results

The weights at 54 days showed that those birds on the 16% level or protein averaged 536 grams without aureomycin and 586 grams with aureomycin or an increase of 5.6% due to the effect of aureomycin. The chicks on the 17.9% protein level averaged 662 grams without aureomycin and 712 grams with aureomycin or an increase of 7.5% due to the effect of aureomycin. The chicks on the 19.5% level averaged 748 grams without aureomycin and 728 grams with aureomycin showing a decrease in weight when aureomycin was added. The chicks on the 22% level averaged 770 grams without aureomycin and 784 grams with aureomycin or an increase of 1.9% due to the effect of aureomycin.

It is evident from the growth data that the addition of aureomycin to the basal rations containing the different protein levels did not lower the requirement of the chick for protein. Although a greater response was obtained by adding aureomycin to the rations at the 16 and 17.9% protein levels than at the 19.5 and 22% levels, still in no instance did the antibiotic bring the weights up to the weights of the chicks at the 22% level. The statistical

analysis on the average weights at 54 days showed that there were no significant differences due to the addition of the antibiotic to the different rations, the only real differences being due to the varied protein levels.

The feed efficiency results showed a lowered feed utilization at the lower protein level. The addition of aureomycin to the 16 and 17.9% protein rations did not improve the feed efficiency over the basal rations. Rations fed at the 17.9 and 19.5% levels were equal in value with and without aureomycin in so far as feed utilization was concerned.

Conclusions

1. When the protein level of chick rations was lowered, it was found that the addition of an antibiotic increased growth response at lower levels but not sufficiently to bring the average weights up to those at the 22% protein level. This showed that adding an antibiotic to rations containing lower levels of protein does not lower the requirement of the chick for protein.
2. Feed efficiency was lessened with the lower protein levels. The addition of aureomycin did not improve the efficiency at any of the levels.

Table 1

Composition of Basal Rations

Ingredient	16% Basal	17.9% Basal	19.5% Basal	22% Basal
	- pounds per 100 pounds -			
Ground wheat	41.1	39.0	37.2	35.4
Ground yellow corn	41.1	39.0	37.2	35.4
Soybean oil meal	3.7	6.0	8.0	10.0
Fishmeal	3.7	6.0	8.0	10.0
Dehydrated cereal grass	5.0	5.0	5.0	5.0
*BY	1.0	1.0	1.0	1.0
Iodized salt	0.5	0.5	0.5	0.5
Feeding oil (2250A: 300D)	0.25	0.25	0.25	0.25
Ground limestone	1.0	1.0	1.0	1.0
Bonemeal	2.0	1.75	1.25	1.0
Manganese sulphate	0.025	0.025	0.025	0.025
Choline chloride (25%)	0.4	0.4	0.4	0.4
Nicotinic acid	0.8 gm.	0.8 gm.	0.8 gm.	0.8 gm.
Calcium pantothenate	0.5 gm.	0.5 gm.	0.5 gm.	0.5 gm.

Rations #1 - #4 inclusive - basals.
" #1a - #4a inclusive - basals plus 0.5% aureomycin aurofac.**

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* BY - commercial product used mainly for riboflavin supplement.

** Aureomycin aurofac - commercial preparation containing aureomycin hydrochloride as an antibiotic.

Table 11

Average Weights

Ration No.	Protein level-%	Days 7	14	29	41	54
1	16.0	69 gm.	105 gm.	224 gm.	376 gm.	532 gm.
1a	16.0	71	109	250	410	586
2	17.9	80	130	299	478	662
2a	17.9	83	138	311	499	712
3	19.5	84	138	326	524	748
3a	19.5	84	140	321	510	728
4	22.0	83	138	240	540	770
4a	22.0	86	144	338	549	784

Table 111

Feed Efficiency

Ration No.	Feed efficiency for test period
1	3.0
1a	3.2
2	2.5
2a	2.8
3	2.5
3a	2.5
4	2.4
4a	2.4

Table lV

Analysis of Variance

Source of variation	Sum of Squares	d.f.	Variance
Total	3,480,844	313	
Blocks	30,275	1	30,275
Antibiotics	34,175	1	34,175
Protein	2,081,198	3	693,732*
Blocks x Antibiotics	413	1	413
Blocks x Protein	3,911	3	1,303
Antibiotic x Protein	74,854	3	24,931
Blocks x Antibiotic x Protein	110,042	3	36,680
Error	1,145,976	298	3,846

* Significant at $p = 0.01$ when triple interaction (blocks x antibiotic x protein) is used as the testing error.

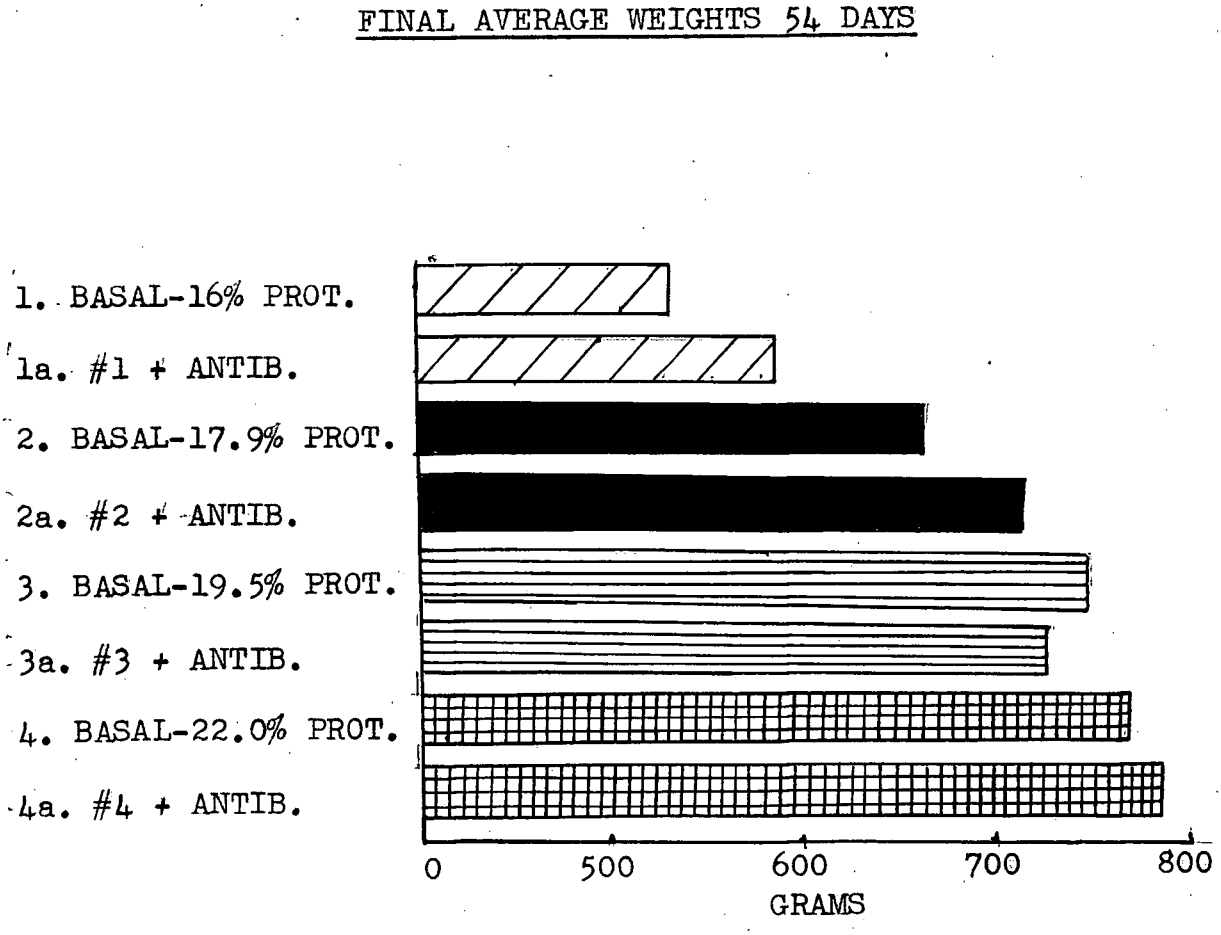

Figure 1. Average weights of chicks fed 16, 17.9, 19.5 and 22.0 per cent protein rations supplemented with antibiotics.

TEST NO. 3

Purpose

The purpose of this experiment was to confirm the results obtained in Test No. 2 by again lowering the protein level of the ration. Aureomycin was again added to the basal rations to determine if it had any effect on lowering the protein needs of the chick.

Methods and Procedure

Day old, female, New Hampshire chicks were used in this experiment. Distribution and handling of the birds was the same as in the previous tests. All tests were in duplicate.

The chicks were weighed each week to the end of the fourth week; a record of their average weights is shown in Table 1. Feed consumption was kept for the trial period and the feed efficiency is recorded in Table 11.

The ingredients in the basal rations used in this experiment were the same as in Test No. 2. Two basal rations were made up with protein levels at 22 and 16%. To each basal was added 0.5% of an antibiotic preparation containing 0.9 gms. of aureomycin hydrochloride.

Results

The results in growth from this experiment showed that the chicks fed at the 22% level without aureomycin

averaged 351 grams and with aureomycin averaged 352 grams. In other words, no increase was to be noted from the use of an antibiotic in this ration. The chicks fed at the 16% level without aureomycin averaged 280 grams and with aureomycin averaged 294 grams or 5% increase. These results, therefore, confirm those obtained in Test No. 2 in that although a greater response was obtained at the lower protein level, with the addition of antibiotics, there was not a lowering of the requirement of the chick for protein.

The addition of antibiotics had little or no effect on feed efficiency in this experiment. Efficiency was lower at the lower protein level.

Conclusions

This test was conducted to confirm the results of Test No. 2. The data again showed that the addition of antibiotics did not lower the protein requirements of the chick.

Table 1

Average Weights

Ration No.	Protein level-%	Weeks 1	2	3	4
1	22.0	82	155	237	351
1a	22.0	83	155	242	352
2	16.0	73	125	192	280
2a	16.0	74	130	201	294

Table 11

Feed Efficiency

Ration No.	Feed efficiency for test period
1	2.17
1a	2.12
2	2.52
2a	2.57

Ration 1 and 2 - basals
Rations 1a and 2a - basals plus 0.5% aureomycin aurofac.

TEST NO. 4

Purpose

The purpose of this test was again to study the effect of antibiotics on a lowered protein intake and in addition to determine the effect of antibiotics as to sex. The response when a higher level of antibiotic was fed in the ration was also studied.

Methods and Procedure

Day old, male and female, White Leghorn chicks were used in this experiment and the method of distribution and handling was the same as in the previous tests.

The composition of the rations were the same as in the previous tests and were fed at 16 and 22% protein levels. An antibiotic was added to the basal rations at 0.5% and 1.0% levels to determine the effect of a higher level of feeding. Again an aureomycin preparation was used as in the previous tests.

The chicks were weighed at the end of each week up to the end of four weeks and the results are presented in Table 1 and the fourth week results in Figure 1. Feed efficiency data was recorded and is presented in Table 11.

Results

The results of the chicks weights at four weeks of age show that the female chicks fed the 22% basal ration averaged 366 grams and the males averaged 405 grams without antibiotic. When the antibiotic was added at the 0.5% level, the females averaged 399 grams or 9.01% increase over those without antibiotic. When added at the same level, the males averaged 436 grams or a 7.6% increase over those without antibiotic. The female chicks fed antibiotic at the 1.0% level averaged 403 grams or 10.1% increase over those chicks without antibiotic. The male chicks fed antibiotic at the same level averaged 436 grams or 13.5% increase over those chicks without antibiotic. These results suggest that there is no difference in growth by sexes when antibiotics are added. The higher level of aureomycin had no added effect on growth in either sex. The female chicks fed on the 16% protein basal ration averaged 282 grams without antibiotic and the males averaged 297 grams. The addition of 0.5% antibiotic resulted in average weights of 309 grams in the females or a 9.5% increase over those chicks without antibiotic. The addition of 0.5% antibiotic resulted in average weights of 337 grams in the males or a 13.5% increase over those chicks without antibiotic. When 1.0% antibiotic was added the female chicks averaged 305 grams or a 8.1% increase over those chicks without antibiotic. When 1.0%

antibiotic was added the male chicks averaged 336 grams or a 13.1% increase over those chicks without antibiotic. The results here again are the same as those obtained when 22% protein was fed.

The addition of antibiotics to the basal rations improved the feed efficiency to some extent except when 0.5% antibiotic was fed to the 16% basal. There appears to be some error in this result.

Conclusions

1. Adding an antibiotic to a ration in which the protein level was lowered to 16% resulted in no effect on lowering the protein needs of the chick when compared to a ration containing 22% protein.
2. There was little difference in response to growth between sexes when an antibiotic was added. The males appeared to grow faster than the females at the 16% protein level when compared with the growth response at the 22% protein level when antibiotic was added to both.
3. Increasing the antibiotic level from 0.5 to 1.0% did not increase the rate of growth. The antibiotic again as in previous tests was aureomycin.

Table 1

Average Weights

Ration No.	Protein Level-%	Weeks 1		2		3		4	
		Female	Male	Female	Male	Female	Male	Female	Male
1	22	75	79	201	204	265	274	366	405
2	22	77	84	206	218	283	305	399	436
3	22	78	84	216	226	291	304	403	436
4	16	64	66	146	142	201	202	282	297
5	16	70	71	161	163	228	238	309	337
6	16	67	73	153	167	220	242	305	336

Table 11

Feed Efficiency

Ration No.	Feed efficiency for test period
1	2.35
2	2.63
3	2.22
4	2.97
5	2.81
6	2.75

Rations 1 and 4 - basals.
Rations 2 and 5 - basals plus 0.5% aurofac.
Rations 3 and 6 - basals plus 1.0% aurofac.

FINAL AVERAGE WEIGHTS AT 4 WEEKS

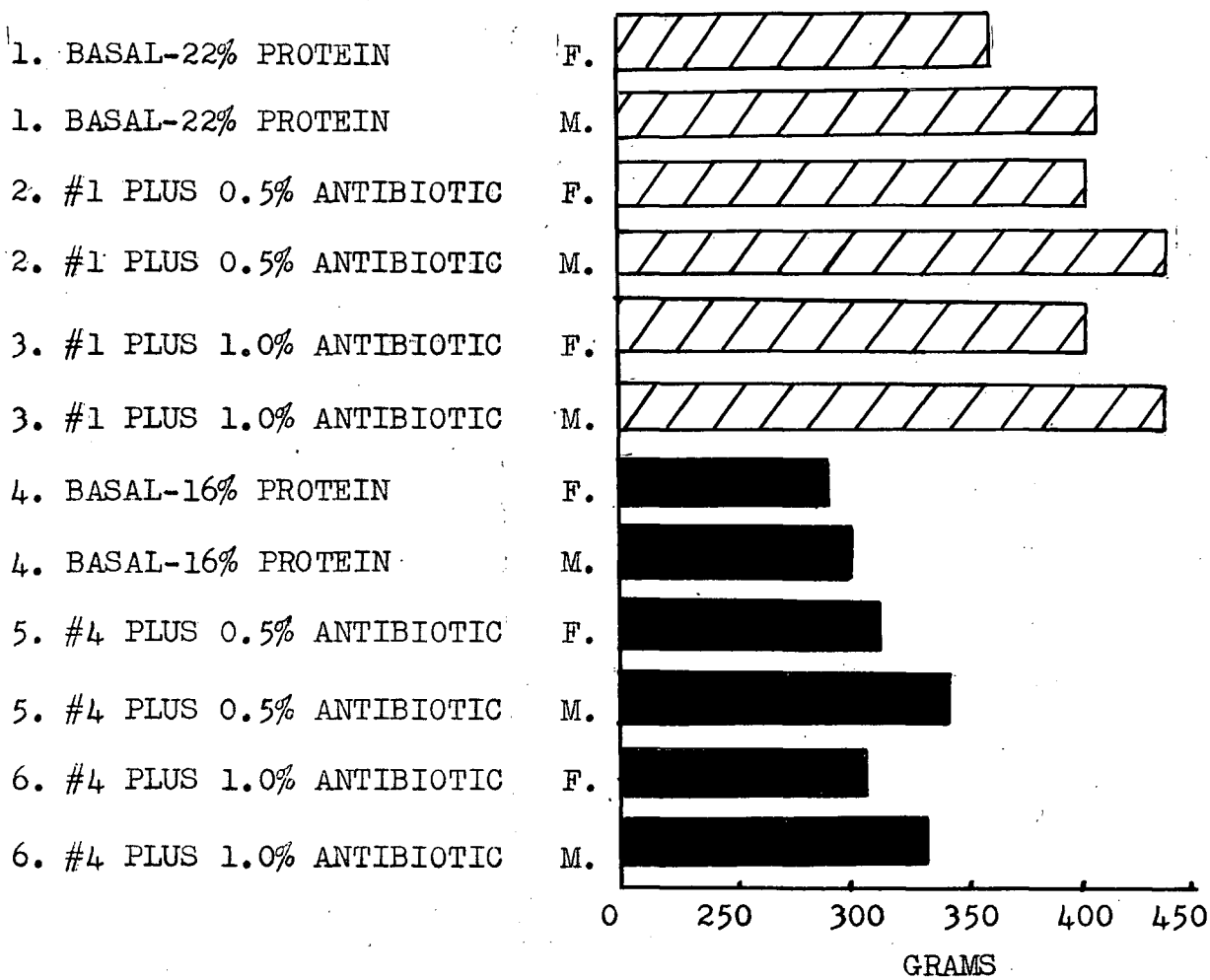

Figure 1. Average weights of male and female chicks fed 22% and 16% protein rations supplemented with two different levels of antibiotics.

Experiment 111

Introduction

The third study was designed to determine whether or not antibiotics would reduce the requirement of the chick for specific amino acids. The effect was determined of antibiotics on chick growth in a basal ration composed of wheat as the main ingredient. This ration was deficient in lysine. Again the effect of antibiotics on chick growth in a tryptophan deficient basal ration was determined by using corn as the main ingredient in the ration. Crystalline lysine and tryptophan were added to the respective basal rations to determine their effect on growth response with and without the addition of antibiotics.

TEST NO. 5

Purpose

1. To study the effect of antibiotics on growth in chicks when added to a wheat ration which is deficient in lysine and also the effect when added to a corn ration which is deficient in tryptophan.

2. To compare this growth response to the basal rations to which have been added crystalline amino acids to make up the deficiencies.

3. To compare two antibiotics in so far as their growth promoting strength is concerned when added to the basal rations.

Methods and Procedure

Day old, male, New Hampshire chicks were used in these experiments which were distributed and handled as in the previous tests. Twenty chicks were distributed to each compartment.

The chicks were weighed at weekly intervals and the experiment was carried for three weeks. Results of the growth response are presented in Table 11 and Figure 1. A statistical analysis was made on the average weights at three weeks and the results are shown in Table 111.

The wheat ration was comprised of 88.83 pounds of ground wheat to which was added 5 pounds of cereal grass to increase the fibre, and protein to provide some of the essential vitamins and minerals. The minerals were balanced by adding 1 pound of limestone and 2 pounds of bonemeal. The vitamins were balanced by adding choline, vitamins A and D, riboflavin (BY), nicotinic acid, pantothenic acid and vitamin B_{12}. This ration, however, was deficient in the animo acid lysine. The corn ration was made up of 80.83 lb. of ground corn along with the same ingredients that were used in the wheat ration except that nicotinic acid was omitted.

The reason for this was due to the fact that without nicotinic acid, tryptophan cannot be utilized in the body. Since tryptophan was the deficient amino acid being studied, nicotinic acid was omitted. Ten pounds of gelatin was added to this ration to raise the protein level and balance the other amino acids. The protein level for the wheat ration was 14.79% and 18.37% for the corn as determined by chemical analysis.

The wheat basal ration (#1) was supplemented with 0.5% l-lysine HCl (ration #2) per 100 lb. Ration #1 was again supplemented with 1.0% aureomycin aurofac (Ration #3) and with 1.5 gm. of penicillin (Ration #4) per 100 lb. The corn ration (#5) was supplemented with 0.2% dl-tryptophan (ration #7) and again with 1.0% aureomycin aurofac and 1.5 gm. of penicillin (ration #8) per 100 lb. In ration #9 nicotinic acid was added to the corn ration to study the effect on chick growth. To this ration was added 0.2% dl-tryptophan (ration #10) and again 1.0% of aureomycin aurofac (ration #11) and 1.5 gm. of penicillin (ration #12) per 100 lb.

Results

The average weights at four weeks for the wheat ration showed the chicks fed the basal ration (#1) weighed 91 grams. Those fed dl-lysine (ration #2) weighed 131 gm.

while those fed aureomycin weighed 109 gm. and penicillin weighed 106 gm. These results show that although the antibiotics did improve the basal ration (#1), the improvement was not significant since they failed to reach the weight of the amino acid supplemented ration (#2). The average weights for the corn ration showed that the chicks fed the basal ration (#5) weighed 57 grams while the tryptophan supplemented ration (#6) weighed 83 grams. Again, however, the chicks supplemented with aureomycin (#7) weighed only 59 grams and those fed penicillin (#8) weighed 60 grams. The same interpretation as in the wheat ration must be concluded. The addition of nicotinic acid increased the average weights to 65 gm. when fed with the corn ration (#9). The addition of tryptophan (#10) again improved growth with average weights of 116 grams. Aureomycin (ration #11) fed to ration #9 resulted in average weights of 73 grams while penicillin (ration #12) fed to ration #9 resulted in average weights of 75 grams. Again, however, although the addition of the antibiotics improved the average weights they were not equal to the tryptophan supplemented ration. The addition of nicotinic acid, however, did improve average weights indicating its synergistic effect with tryptophan. The addition of penicillin or aureomycin to the basal rations resulted in equal responses to growth indicating that their value as antibiotics under these conditions were the same.

A statistical analysis on the weights at three weeks of age showed the differences that were significant for the different treatments. No significant difference was obtained when antibiotics were added to the corn basal ration (#5).

Mortality was high in the corn basal ration (#5) resulted in 16 deaths. When tryptophan was added (#6) there were no deaths while only 4 deaths occurred in the aureomycin fed ration (#7) and the same number in the penicillin fed ration (#8). This indicates that the antibiotics have a value in decreasing mortality. Only one death occurred among the chicks when nicotinic acid was added to the corn basal and supplemented rations (#9, 10, 11, 12).

Conclusions

1. The addition of antibiotics to wheat and corn rations deficient in lysine and tryptophan respectively had no effect in lowering the need of these amino acids for the chick.
2. There was no difference between penicillin and aureomycin in their growth promoting effect under the conditions of this experiment.
3. Antibiotics appeared to have an effect on reduction of mortality in the corn, tryptophan deficient diet.

Table 1

Composition of Basal Rations

Ingredient	Wheat Basal #1 lb./100 lb.	Corn Basal #5 lb./100 lb.
Ground wheat	88.83	-
Ground corn	-	80.83
Gelatin	-	10.0
Dehydrated cereal grass	5.0	5.0
Ground limestone	1.0	1.0
Bonemeal	2.0	2.0
Iodized salt	0.5	0.5
Manganese sulphate	0.025	0.025
Choline chloride (25%)	0.4	0.4
Feeding oil (2250A: 300D)	0.25	0.25
BY	2.0	2.0
Nicotinic acid	0.8 gm.	-
Calcium pantothenate	0.5 gm.	0.5 gm.
Vitamin B_{12}	0.001 gm.	0.001 gm.

Ration #1 - basal.
" #2 - #1 plus l-lysine HCl.
" #3 - #1 plus aureomycin.
" #4 - #1 plus penicillin.
" #5 - basal.
" #6 - #5 plus dl-tryptophan.
" #7 - #5 plus aureomycin.
" #8 - #5 plus penicillin.
" #9 - #5 plus nicotinic acid.
" #10 - #5 plus nicotinic acid & dl-tryptophan.
" #11 - #5 plus nicotinic acid & aureomycin.
" #12 - #5 plus nicotinic acid & penicillin.

Table 11

Average weights at 3 weeks

Ration No.	Ration	Average weight
1	Wheat	91 gm.
2	Wheat plus lysine	131
3	Wheat plus aureomycin	109
4	Wheat plus penicillin	106
5	Corn	57
6	Corn plus tryptophan	83
7	Corn plus aureomycin	59
8	Corn plus penicillin	60
9	Corn plus nicotinic acid	65
10	Corn plus nicotinic acid & tryptophan	116
11	Corn plus nicotinic acid & aureomycin	73
12	Corn plus nicotinic acid & penicillin	75

Table 111

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Variance	Minimum Significant Difference	
				5%	1%
Total	157,544	211			
Treatment	116,023	11	10,548*	13	10
Error	41,521	200	208		

* Significant at the 1% level.

FINAL AVERAGE WEIGHTS AT 3 WEEKS

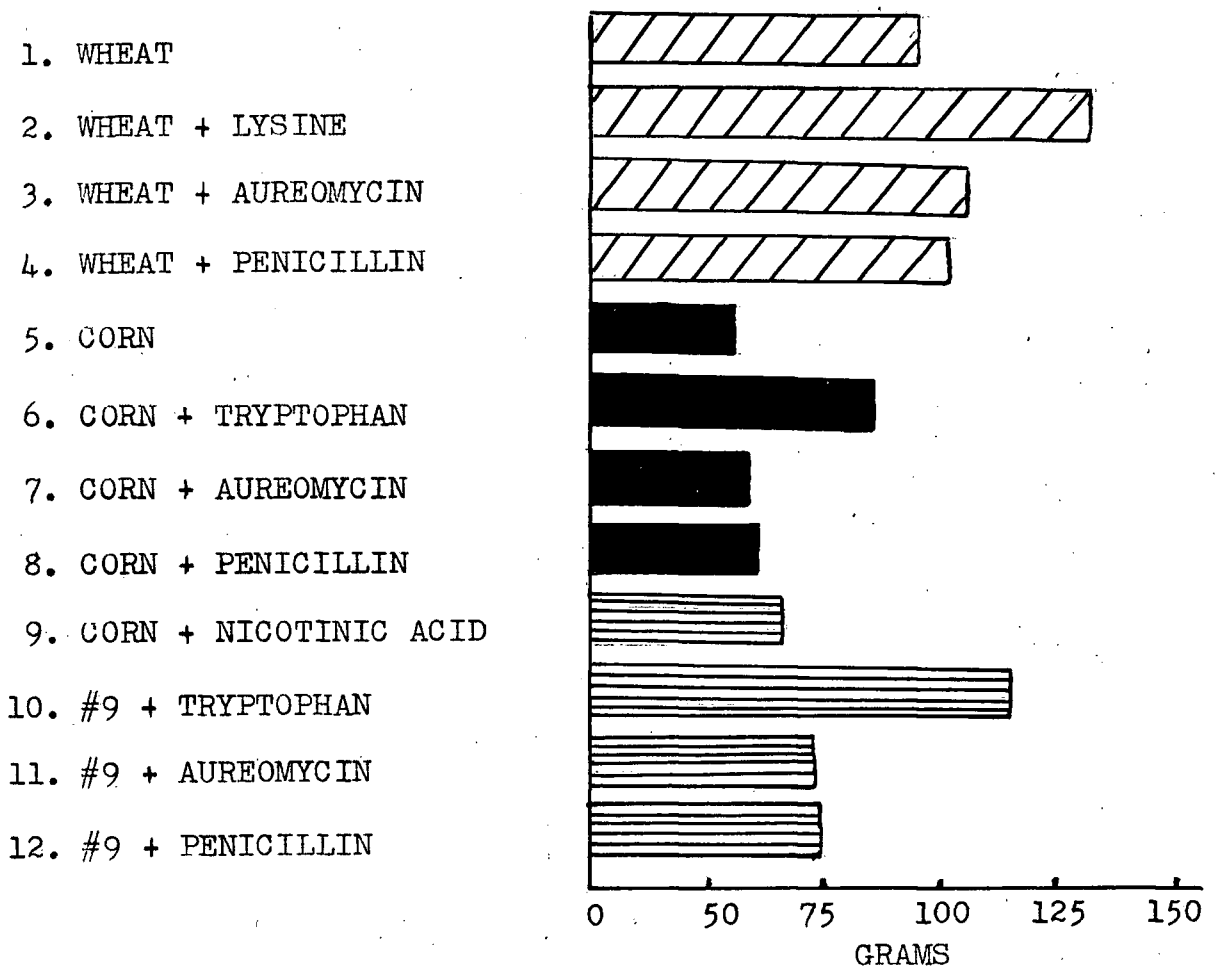

Figure 1. Average weights of chicks fed wheat and corn rations supplemented with amino acids, antibiotics and a vitamin.

TEST NO. 6

Purpose

The purpose of this experiment was to determine the effect on chick growth of adding a supplement of vitamins to a wheat ration deficient in lysine. Also to determine the effect of these vitamins with lysine and/or penicillin added to the basal ration.

Methods and Procedure

Day old, male, New Hampshire chicks were used in this experiment and were distributed and handled as in previous experiments.

The experiment was carried out for three weeks and the chicks weighed at the end of each week. A record of the average weights are presented in Table 1. A statistical analysis was made on the weights at 3 weeks and the analysis is shown in Table 11. Feed consumption was recorded for the test period and the feed efficiency record is also presented in Table 1.

The basal ration used in this experiment was composed of the same ingredients as in the previous test. Rations were supplemented with 0.5% dl-lysine HCl per 100 lb. A vitamin supplement composed of 0.05 gm. thiamin, 0.10 gm. pyridoxine, 5.0 gm. para-amino benzoic acid, 0.025 gm. folic

acid, 0.025 gm. 2-4 naphthoquinone, 5.0 gm. inositol, 0.01 gm. biotin per 100 lb. was used. Penicillin was fed at the rate of 1.5 mg. per 100 lb.

Results

The average weights of the chicks at 3 weeks of age showed that the chicks on the basal wheat ration (#1) weighed 92 grams while those chicks on the ration to which lysine was added (#2) averaged 115 grams. When the vitamins were added alone to the basal (#3) the chicks weighed 93 grams or no increase over the weights of the chicks on the basal ration. When added with lysine, (#4), however, the weights indicated an average of 136 grams. The addition of penicillin (#5) resulted in no improved growth with average weights of 91 grams. Penicillin and lysine combined (#6) resulted in a substantial increase with an average weight of 154 grams. Penicillin combined with the vitamin supplement (#7) resulted in chicks weighing 102 grams on an average. When lysine, vitamins and penicillin were added (#8) the average weights were 177 grams.

The results from this test indicate that neither the vitamin supplement nor penicillin when added in combination or alone resulted resulted in any growth increase. When lysine was added to either the vitamin supplemented ration (#3) or the penicillin ration (#5) there was a significant increase in growth. The greatest response was obtained with the combination of all three added to the basal (#8). These

results suggest, therefore, that in order to obtain a response to either additional vitamins and/or penicillin, the amino acid deficiency must be made up in the ration. It further suggests that neither a vitamin supplement nor an antibiotic will have any effect on lowering the needs of specific amino acids for the chick.

Feed efficiency data showed an improvement whenever lysine was included as a supplement to the basal ration.

The statistical analysis on treatment of rations showed that only real differences were found when the lysine deficient diet was supplemented with crystalline lysine.

Conclusions

A vitamin supplement and/or penicillin added to a wheat basal diet had no effect on reducing the needs of the amino acid, lysine for the chick.

Table 1

Average Weights at three weeks and Feed Efficiency

Ration No.	Ration	Average Weights	Feed Efficiency
1	Basal	92 gm.	4.73
2	#1 plus lysine	115	4.01
3	#1 plus vitamins	93	4.18
4	#1 plus lysine and vitamins	136	2.55
5	#1 plus penicillin	91	4.88
6	#1 plus lysine and penicillin	154	2.85
7	#1 plus vitamins and penicillin	102	3.50
8	#1 plus lysine, vitamins & penicillin	177	2.41

Table 11

Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Variance	Minimum Significant Difference	
				5%	1%
Total	192,077	158			
Treatment	148,982	7	21,283*	10.57	13.95
Error	43,095	151	285		

* Significant at the 1% level.

TEST NO. 7

Purpose

In this experiment it has been assumed that arginine is a second limiting amino acid in a wheat ration. The purpose was to test the effect of antibiotics on growth of chicks when arginine was added to a wheat basal ration to which lysine and a vitamin supplement were included.

Methods and Procedure

Day old, male, New Hampshire chicks were distributed and handled as in previous experiments. Twenty chicks were distributed to each of four compartments.

The chicks were kept for three weeks and weighed at the end of each week. The average weights over the period are presented in Table 1. Feed efficiency was also kept for the trial period as shown in Table 11.

The basal ration used was composed of the same ingredients as in Tests 5 and 6 except that 0.5% of l-lysine HCl was added along with the vitamin supplement. This supplement was described in Test 5. Ration #2 was supplemented with penicillin at the rate of 15 mg./lb. Ration #3 was supplemented with 0.5% arginine and ration #4 was supplemented with a combination of arginine and penicillin at the same levels as above. During the last week of the

experiment 0.3% methionine was added to rations #3 and #4.

Results

The average weights at three weeks of age resulted in 131 grams for the chicks fed the basal ration (#1). Chicks fed the penicillin supplemented ration (#2) averaged 180 grams. When arginine was added to the basal ration (#3) the average weights were 115 grams. This showed that arginine did not improve growth when used as a supplement. Ration #4, to which penicillin and arginine had been added, resulted in average weights of 171 grams. This again showed that arginine had no effect in improving chick growth when combined with penicillin as a supplement. In view of the lack of response to arginine it was decided to add methionine to rations #3 and #4 during the last week of the experiment. This supplement, however, had no effect on chick growth.

Conclusions

A wheat basal ration to which had been added lysine and a vitamin supplement did not produce added growth with a supplement of arginine and penicillin over penicillin alone. This showed that arginine was not a second limiting factor when an antibiotic was added.

Table 1

Average Weights

Ration No.	Ration	Weeks 1	2	3
1	Basal	57	84	131
2	#1 plus penicillin	65	115	180
3	#1 plus arginine	56	80	115
4	#1 plus penicillin and arginine	66	109	171

Table 11

Feed Efficiency

Ration No.	Feed efficiency for test period
1	4.04
2	3.15
3	4.56
4	3.25

Experiment IV

Introduction

The fourth study was made to determine what effect antibiotics might have in lowering the carbohydrate requirement of the chick. In the basal ration, corn starch was added to a ration composed of ground yellow corn and ground wheat as the cereals and fishmeal, soybean oil meal and cereal grass as the protein supplements. The available carbohydrate level was lowered by replacing the corn starch with an equal amount of ground cellulose. As in the protein tests, two levels of antibiotics were compared by chick growth response. The effect of antibiotics on male and female chicks was also determined.

TEST NO. 8

Purpose

To study the effect of antibiotics on chicks fed a ration in which the available carbohydrates had been lowered and to determine whether or not antibiotics had any effect on lowering the carbohydrate needs of the chick.

Methods and Procedure

Day old, male, New Hampshire chicks were used in this experiment. Twenty chicks were distributed at random in each of eight compartments, the test being run in duplicate.

Handling of the chicks was the same as in previous tests.

The experiment was carried out over a four week period and the chicks weighed at the end of each week. Average weights at the end of each week are presented in Table 1. A record of feed consumption was kept for the trial period and the results are presented in Table 11.

The same ingredients were used in the basal ration as were used in the protein tests. The wheat and corn were reduced and 15% corn starch was added to make up the carbohydrate level (ration #1). The protein level was re-adjusted by increasing the soybean and fishmeal. In order to lower the available carbohydrate level 15% ground cellulose was substituted for the starch (ration #2). An aureomycin preparation (0.5%) was added to both the starch ration and the cellulose ration.

Results

The basal ration (#1) to which corn starch had been added resulted in average weights of 338 grams in chicks at 4 weeks of age. The addition of aureomycin to the basal (#3) resulted in average weights of 350 grams or an increase of 3.5%. The basal ration (#2) to which ground cellulose had been added produced average weights of 33.6 grams. The addition of aureomycin to this basal (#4) resulted in average weights of 346 grams or an increase of 2.9%. These results, therefore, indicate that antibiotics had no effect on

lowering the carbohydrate level requirements of the chick since the average weights of the cellulose and antibiotic ration (#4) failed to reach the average weights of the starch and antibiotic ration (#3).

The feed efficiency was lowered when 15% cellulose was fed in the ration (#2) as compared to the starch basal ration (#1). Some response to antibiotics was noted however, when this ration (#2) was supplemented with aureomycin (#4). Chicks on the higher carbohydrate ration and antibiotic (#3) showed no improvement over the basal starch ration (#1).

Conclusions

1. Antibiotics had no effect on lowering the carbohydrate needs of the chick when added to a ration low in available carbohydrate.
2. Feed efficiency was lowered when the lower carbohydrate ration was fed. The addition of antibiotics resulted in some improvement in this ration.

Table 1

Average Weights

Ration No.	Ration	Weeks 1	2	3	4
1	Basal - starch	84 gm.	152 gm.	234 gm.	338 gm.
2	Basal - alphacel*	85	148	226	336
3	Basal #1 plus antibiotic**	86	157	244	350
4	Basal #2 plus antibiotic	87	156	239	346

* Ground cellulose.
** Aureomycin aurofac.

Table 11

Feed Efficiency

Ration No.	Feed efficiency for test period
1	1.99
2	2.47
3	1.98
4	2.25

TEST NO. 9

Purpose

To repeat the test as outlined in Test No. 8 except in addition to 0.5% of antibiotic, a higher level (1.0%) of antibiotic was fed. Both male and female chicks were used in this experiment.

Methods and Procedure

Day old, male and female, White Leghorn chicks were used in this experiment. The chicks were divided at random into each of 12 brooder compartments with 15 chicks in each compartment. The chicks were handled the same as in the previous experiments.

The birds were kept on experiment for four weeks and weighed at the end of each week. A record of the average weights is presented in Table 1 and a graph showing the final weights is shown in Figure 1. Feed consumption was recorded for the period and the results are presented in Table 11.

The composition of the rations was the same as in the previous test again using 15% corn starch and 15% ground cellulose for the carbohydrate study. The basal rations were supplemented with 0.5% aureomycin preparation and 1.0% aureomycin preparation to determine any difference in growth response to the higher level of feeding.

Results

The average weights of the male and female chicks fed the starch basal ration (#1) were 380 grams and 394 grams respectively. When 0.5% aureomycin preparation was added to this basal ration (#2) the females weighed 400 grams or an increase of 5.2% and the males averaged 449 grams or an increase of 13.9%. When 1.0% aureomycin preparation was fed to this basal (#3) the female chicks averaged 406 grams or an increase of 6.8% over the basal and 466 grams for the males or an increase of 17.5%. The average weights of the female and male chicks fed the cellulose basal ration (#4) were 348 grams and 353 grams respectively. The addition of 0.5% aureomycin preparation to this basal ration (#5) resulted in average weights of 353 grams for the females or 1.4% increase and 377 grams for the males or 6.7% increase. When 1.0% aureomycin preparation was added to this basal ration (#6) the females averaged 355 grams or 2.01% increase and the males 368 grams or 4.2% increase. The chicks on the cellulose basal ration supplemented with antibiotic did not weigh as much as the chicks fed the starch basal and supplemented with antibiotic. This again indicated that antibiotics had no effect on lowering the carbohydrate needs of the chick. There appeared to be a greater response in growth to antibiotics from the males in the starch fed ration. There was no difference between the two levels of antibiotic fed in-so-far as growth

was concerned.

The feed efficiency results indicated that feed efficiency was lowered when 15% ground cellulose was added to the ration. The efficiency was improved by the addition of aureomycin in each ration although no difference was observed between the two levels of antibiotic fed.

Conclusion

1. Lowering the available carbohydrate level of the chick ration, did not lower the carbohydrate needs of the chick when antibiotics were added.
2. Efficiency of feed utilization was lowered when the carbohydrate level was lowered. The addition of antibiotics improved the feed efficiency although no difference occurred between the two levels fed.
3. Male chicks showed more growth response to antibiotics than female chicks when the 15% starch basal ration was fed.

Table 1

Average Weights

Ration No.	Ration	Sex	Weeks 1	2	3	4
1	Basal - starch	F*	76	197	269	380
		M*	75	196	264	394
2	Basal #1 plus 0.5% antibiotic	F	77	201	287	400
		M	86	213	311	449
3	Basal #1 plus 1.0% antibiotic	F	81	206	292	406
		M	85	220	329	466
4	Basal - alphacel	F	73	178	250	348
		M	70	177	255	353
5	Basal #4 plus 0.5% antibiotic	F	73	189	255	353
		M	79	194	268	377
6	Basal #4 plus 1.0% antibiotic	F	75	181	257	355
		M	78	178	259	368

* F - female.
M - male.

Table 11

Feed Efficiency

Ration No.	Feed efficiency for test period
1	2.32
2	2.11
3	2.19
4	3.03
5	2.73
6	2.73

FINAL AVERAGE WEIGHTS AT 4 WEEKS

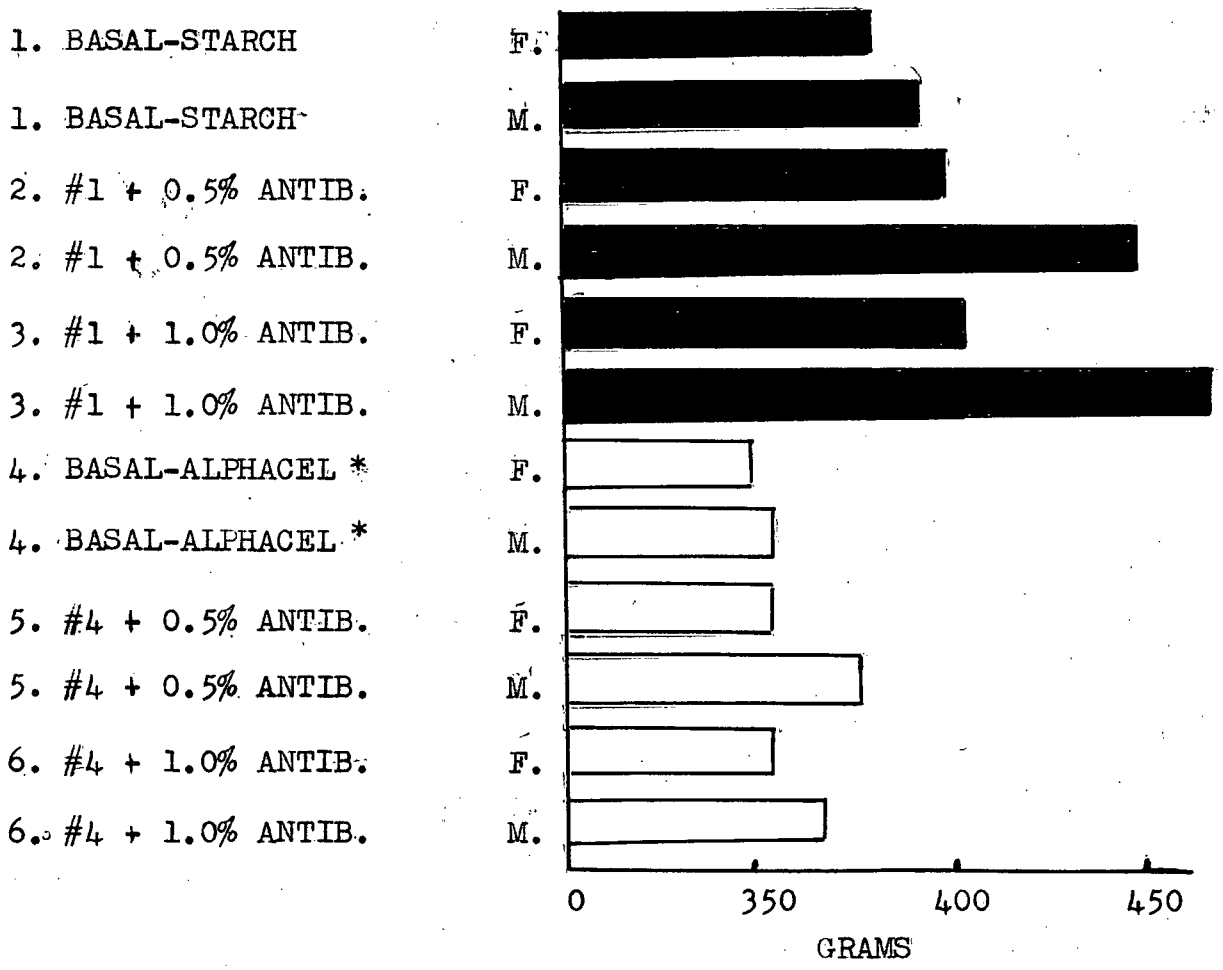

Figure 1. Average weights of male and female chicks fed rations at two carbohydrate levels supplemented with two levels of antibiotics.

* Ground cellulose.

IV. DISCUSSION

A series of nine tests was designed to demonstrate the effect of antibiotics fed at different levels and with different chick rations. A brief summary of the different experiments is as follows:

Experiment #1.

The first experiment was designed to show the effect of antibiotics on growth of chicks when added to a Connecticut ration, a soybean ration and a fishmeal ration and to make a comparison of the growth response to each ration. In addition, the effect of adding vitamins and minerals to these basal rations was studied both with and without antibiotics.

Experiment #2.

Tests 2, 3 and 4 involved a study of the effect of antibiotics on the growth rate of chicks fed rations containing different levels of protein. The study was designed to determine whether antibiotics had any effect on lowering the protein needs of the chick. A test was also conducted on different levels of antibiotics in the rations to determine any difference in growth response.

Experiment #3.

Tests 5, 6 and 7 involved a study to show the effect of antibiotics on rate of growth of chicks when used in

rations in which specific amino acids were deficient. The object of these tests, therefore, was to determine if antibiotics had any effect on lowering the dietary requirements for these limiting amino acids. Tests, again, were made with different levels of antibiotics to determine any difference in rate of growth at the levels used. The effect of adding additional vitamins was also determined in some of the rations.

Experiment #4.

Tests 8 and 9 were designed to study the effect of antibiotics on rate of growth of chicks fed rations containing different levels of available carbohydrates. The object was to determine whether or not antibiotics had any effect on lowering the requirement of the chick for carbohydrate.

The results from the first experiment showed that the addition of antibiotics to each of the rations described, produced a significant growth increase. The highest growth increase was obtained with the Connecticut ration when antibiotics were fed. The Connecticut and soybean rations supplemented with vitamins and minerals produced no additional growth whereas the fishmeal ration when thus supplemented did produce additional growth. Similar results were obtained when antibiotics were fed in these rations. Fishmeal, for example, is known to be deficient in folic acid (65) which

may be the reason for the added growth. The fact that the greatest response to antibiotic was gained with the Connecticut ration is in agreement with work done by Berg (8) working with a high energy ration. When he substituted the herring meal in the ration and added more soybean oil meal the growth response was lowered.

These results seem to indicate that the response to antibiotics is dependent, in part at least, on the composition of the ration with which it is fed. This is verified by work done by Scott et al (18) in testing an all-vegetable ration containing ground corn, soybean oil meal, crude soybean oil, dl-methionine and minerals plus the known vitamins. They found that only supplements including corn distillers' solubles, and butyl fermentation at the 5% level and also 2% of a grass juice factor concentrate gave a definite growth response in the test ration when aureomycin was included. Other supplements such as dehydrated alfalfa meal, defatted liver meal, dried brewers' yeast, and dried cereal grass (rye) had no effect when aureomycin was added. McGinnis (58) has reported that fishmeal, liver L-fraction or dried whey produced greater growth response with chicks or poults in the presence of antibiotics than was the case when antibiotics were not added and also that antibiotics produced a greater response when added to rations containing these animal protein sources than when added to an all-vegetable type ration.

At least one additonal unidentified factor, present in fishmeal, fish solubles, certain liver fractions and dried whey, was required for maximum growth of chicks and turkey poults. Other reports (19, 38) showed that the presence of an animal protein supplement in a ration improved the growth response when antibiotics were included in the ration.

The second series of tests (Experiment 11) on the effect of antibiotics on chicks fed diets containing different levels of protein showed that the antibiotics had no effect on lowering the protein needs of the chick. The basal rations used were composed of equal parts of wheat and cornmeal, equal parts of soybean oil meal and fishmeal along with additional ingredients to balance the rations. The protein levels were adjusted by altering the relative amounts of soybean and fishmeal to the corn and wheatmeal. These results are in contrast to reports by Berg (38) in comparing rations containing different levels of protein. In three trials it was found that 19% protein promoted as good growth as 21% protein. The results, however, are in agreement with work done by McGinnis (39) on turkeys when fed penicillin in practical rations at different protein levels. The fishmeal, however, was kept at a constant level, and the protein levels adjusted by changing the relative amounts of soybean oil meal and ground corn. He found that the addition of penicillin did not lower the requirement of the turkey poult for protein.

Machlin et al (40) on the other hand found that the protein level in chick rations could be lowered from 21 to 19 per cent. They used a corn-soybean ration and reduced the corn and soybean meal in constant proportions to reduce the protein level. The reductions were replaced with glucose (cerelose).

An aureomycin supplement fed at 1.0% levels (1.8 gms. aureomycin HCl per 100 lb. ration) resulted in no added growth over 0.5% level (0.9 gms. aureomycin HCl per 100 lb. ration). McGinnis (21) found that 0.1 gm. of aureomycin per 100 lb. ration was the minimum amount to be used in rations. Kramke and Fritz (23) reported that 0.5 gm. per 100 lb. ration of aureomycin gave optimum growth.

In the third series of tests (Experiment 111) on antibiotics and amino acids, it was found that antibiotics had no effect in lowering the needs of the chick for specific amino acids. This result was shown in a wheat ration deficient in lysine to which an antibiotic was added and also in a corn ration deficient in tryptophan to which an antibiotic was again added. No difference was found between penicillin (1.5 gm. per 100 lb. ration) and aureomycin (1.8 gm. aureomycin HCl per 100 lb. ration) in their growth promoting activity when used in these rations. Jones and Combs (41) found that aureomycin appeared to spare the dietary requirement for tryptophan but not for lysine. They fed practical type rations to chicks which were suboptimal in lysine and

tryptophan. Sherwood and Couch (37) found aureomycin did not lower the requirements in a ration supplemented with cottonseed meal as a protein. This was again verified by Machlin et al (42) while Slinger et al (43) found similar results in studying lysine deficiencies and antibiotics in relation to white feathers in turkeys. They suggested that antibiotics may even increase the requirement of turkeys for lysine and perhaps other amino acids. In comparing penicillin and aureomycin McGinnis (21) found that procaine penicillin appeared to be more effective than aureomycin in promoting chick and turkey growth. Branion and Hill (20) found that turkeys on an animal protein diet at 8 weeks of age gave the greatest growth response to aureomycin and in the case of a plant protein diet at the same age a greater response to penicillin. However, at four weeks of age, penicillin gave the greatest growth response with both diets.

In the fourth series of tests it was found that antibiotics had no effect in lowering the carbohydrate requirement of the chick. Again, ground corn and wheat and soybean oil meal and fishmeal were the main ingredients used along with added ingredients to balance the ration. The available carbohydrate level was lowered by substituting 15% alphacel for a part of the wheat and corn meal in the ration. Starch (15%) was used in place of the alphacel to restore the available carbohydrate in the basal ration.

The results also showed that aureomycin, when fed at two different levels (o.5 and 1.0%), did not lower the carbohydrate requirement of the chick.

Considerable evidence has been found in the literature to indicate that there is a relationship between antibiotics and the vitamins. Atkinson and Couch (25) showed that antibiotics were necessary in all-vegetable rations to promote growth when poults were injected with vitamin B_{12}. The injection of B_{12} alone had no effect on growth rate. Reynolds, et al, (26) found that feeding terramycin in good commercial rations reduced the requirement for B_{12}. They found that even as terramycin had a B_{12} sparing effect, the vitamin also showed an antibiotic sparing effect. Craviato-Munoz, et al, (27) found aureomycin was able to replace vitamin B_{12} in the rat maintained on a synthetic diet. They postulated that aureomycin may decrease the number of Escherichia coli in the intestinal tract and allow the increase of bacteria such as Bacillus megatherium which can synthesize adequate vitamin B_{12} for rat growth. Stokstad and Jukes (29) found that aureomycin spared vitamin B_{12} in some cases but not in others. They found that aureomycin was effective in lowering mortality especially in the absence of vitamin B_{12}. It was suggested that aureomycin may have a sparing action on vitamin B_{12} or reduce the number of certain microorganisms in the intestinal tract which are

injurious to the bird. Linkswiler, Baumann and Snell (31) found that aureomycin improved the growth of rats when fed the three forms of vitamin B_6 (pyridoxal, pyridoxamine and pyridoxine) whereas pyridoxine was the only effective form in the absence of this antibiotic. Lih and Baumann (32) found that antibiotics stimulated growth in young rats receiving limiting amounts of thiamine, riboflavin or pantothenic acid. Biely and March (17) found that aureomycin fed to chicks lowered the dietary requirement for folic acid, nicotinic acid and riboflavin. A report by Sunde, et al, (33) showed that one function of antibiotics was the sparing of vitamin B_{12} and the indirect sparing of methionine and choline requirements of the chick.

The results from experiment 1 indicated that the addition of aureomycin improved the feed efficiency when fed in the Connecticut, soybean and fishmeal rations. Tests in experiment 11, however, showed that the feed efficiency was lowered when antibiotics were fed in rations with sub-optimal protein levels. Similar results were obtained in the tests in experiment 111 when amino acids were deficient and in experiment 1V when the carbohydrate level was lowered. Biely and March (44) showed that aureomycin increased feed efficiency in a 17 per cent protein diet containing fish meal over a 21 per cent protein all-vegetable ration containing aureomycin. Some reports (18, 15, 45, 49) found that antibiotics did not

improve feed efficiency when fed in different rations, whereas other reports (40, 46, 47, 48) found a definite improvement in feed efficiency.

The action of antibiotics has still not been definitely established. Reports (17, 27, 28, 29, 30, 32) indicate the sparing effect of antibiotics on the B vitamins and suggest that feeding an antibiotic may decrease the bacterial count in the intestine, relieving the animal from competition for these vitamins and permitting more rapid growth. There is also the possibility that the antibiotics might inhibit certain bacteria in the intestine and allow a type to flourish which synthesizes a growth factor. Speer, et al, (14) and Sieburth, et al, (60) support the view that the antibiotics act by inhibiting toxin producing bacteria and thus prevent a retardation of growth.

A study by Biely and March (61) has shown that aureomycin feeding lowered the lactobacilli in the intestine of chicks. Since these bacteria are vitamin consuming, this may be a factor in the growth stimulating effect of aureomycin. Shapiro and Sarles (59) have shown that lactobacilli are the most numerous group of bacteria in most areas of the intestinal tract of normal birds.

There is still a great deal to be learned concerning the action of antibiotics on the microbe population of the intestine. Much interest is being shown at the present

time in the results being obtained by Reyniers and his associates at the University of Notre Dame in research on germ-free life in chicks. Chicks can be reared in special equipment absolutely germ-free and develop as well as control chicks on the same diet. Perhaps more work of this nature resulting in more fundamental information will reveal the mode of action of antibiotics.

V. SUMMARY

A study has been made on the growth stimulatory effect of antibiotics when fed to chicks as supplements to different rations. A total of 9 biological tests employing 1280 chicks were carried out. The results obtained are summarized as follows:

1. Chicks fed the Connecticut ration and modifications thereof showed a significant growth response to aureomycin supplementation of the rations. The efficiency of feed utilization was improved by the addition of aureomycin to the rations in this experiment.

2. The effect of aureomycin has been tested in rations in which the protein level has been adjusted to 16%, 17.9%, 19.5% and 22% respectively. The basal rations used consisted of ground corn and wheat as the cereal grain and soybean oil meal and fishmeal as the protein supplements along with additional vitamins and minerals to balance the ration. The results showed that aureomycin did not lower the dietary requirement of the chick for protein.

3. A test was made on the effect of antibiotics on growth of chicks when fed in rations which were deficient in specific amino acids. A wheat ration deficient in lysine and a corn ration deficient in tryptophan have been used. The results showed that antibiotics did not lower the dietary requirement of the chick for either lysine or tryptophan.

4. Antibiotics were fed in chick rations containing different amounts of available carbohydrates. It was found that antibiotics did not lower the dietary requirement of the chick for carbohydrate.

5. A comparison of aureomycin and penicillin showed no difference in growth stimulating properties between the two antibiotics under the conditions of the different experiments. Aureomycin supplement (aurofac) promoted the same growth-response at 0.05 and 1.0% levels.

6. The greatest growth response to antibiotics was obtained when the chicks were fed a diet adequate in available carbohydrates and protein and balanced in amino acids.

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