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Effects of Different Feed Additives on the Growth of Turkeys

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EFFECTS OF DIFFERENT FEED ADDITIVES ON THE GROWTH OF TURKEYS

By

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ON THE GROWTH OF TURKEYS

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

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EFFECTS OF DIFFERENT FEED ADDITIVES ON THE GROWTH OF TURKEYS

Abstract

Hossein Samie

Under the supervision of Professor C. W. Carlson

A total of 3780 male and 216 female Large White Nicholas turkeys were used in four experiments to determine the effect of different feed additives on turkey performance. In the first experiment, the effect of 60, 120 or 240 ppm Cu on the growth of male turkeys was examined as affected by 75, 100 or 125% of the suggested NRC (1977) levels of sulfur amino acids (S-AA). The second experiment was designed to study the effect of 60 ppm Cu, Neo-Terramycin at 200 gm per ton, (80 gm Terramycin after 12 weeks) and Zn-bacitracin at 50 gm per ton (25 gm after 8 weeks) on the growth of male turkeys. The turkeys received either a low protein dietary series or a high protein series. The low protein diets provided either 75 or 125% of the NRC (1977) levels of S-AA and the high protein diet contained 125% of the NRC levels of S-AA. In the third experiment, the effects of Neo-Terramycin at 200 gm per ton (80 gm after 12 weeks) and a combination of Neo-Terramycin and 120 ppm Cu on the growth of male turkeys were studied. In this experiment, turkeys received either a low protein dietary series or low protein series which contained 20% wheat bran.

These two series were isonitrogenous but not isocaloric. In the last experiment, both male and female turkeys were used. The effects of virginiamycin at 20 gram per ton in the presence of 0.025% amprolium added to normal protein diets were studied. Individual weights and group feed consumption data were obtained at 4-week intervals. Liver and blood samples were taken from turkeys in Experiment 1 to determine the effect of copper on the copper content of liver and blood.

The results from these experiments show that 75% of the NRC recommended levels of S-AA did not support optimum growth rate of poults up to 12 weeks of age. Poults receiving 100% of S-AA performed as well as those receiving the 125% level. After 12 weeks of age, the 75% NRC level of S-AA allowed turkey body weights to be comparable to those receiving 100 or 125% S-AA. Addition of 60 ppm Cu stimulated the growth rate at 8 weeks of age 120 or 240 ppm Cu caused a growth depression up to this age. No significant differences due to the addition of any level of copper were observed after 8 weeks of age. The liver or blood copper content were not affected by any levels of copper used in this experiment.

In the second experiment, turkeys on the high protein diet were heavier than those on the low protein diet. Of those poults receiving the low protein diets, those fed 125% of the NRC recommended levels of S-AA were heavier

than those fed the 75% level of S-AA up to 16 weeks of age. Addition of Cu did not have any effect on turkey body weights. Neo-Terramycin up to 12 weeks of age and bacitracin up to 8 weeks of age were effective in improving growth.

The results from the third experiment showed that turkeys on diets without wheat bran were significantly heavier than those on diets containing 20% wheat bran up to 24 weeks of age. Addition of Neo-Terramycin or a combination of Neo-Terramycin and copper significantly stimulated growth up to 8 weeks of age. There were no differences between the weights of poults receiving Neo-Terramycin alone or the weights when Neo-Terramycin combined with copper.

The results of the last experiment show that virginiamycin in the presence of amprolium improved body weights of both sexes of turkeys up to 16 weeks of age with no effect on male body weights after this age.

Although there were beneficial trends, no significant differences were demonstrated in overall feed conversion due to the addition of any of the feed additives used in these studies. Turkeys on high protein diets converted feed 21% more efficiently than those on low protein diets.

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INTRODUCTION

In the last forty years there have been many great achievements in medicine and the biological sciences among which, one of the most impressive, has been the discovery and development of antibiotics. It is common place to remark that antibiotics have revolutionized the treatment of infectious diseases, and to point to their contribution as tools for basic biological research. Most of the antibiotics were discovered before any detailed knowledge was available of the biochemistry underlying their effects.

One of the great economic developments in the field of antibiotics is the use of these drugs in feeds for the promotion of animal growth. The use of antibiotics in the control of harmful bacteria was a culmination of years of searching for what has been termed a "magic bullet" by P. Ehrlich in 1913 at the 17th International Congress of Medicine in 1913-1914 (Gale et al., 1972). Antibiotics when added to the food of animals are able to improve their nutrition by producing changes in the intestinal bacteria. This effect was discovered accidentally; the "magic bullet" reached an unseen target. The growth rate of young chickens was found to be increased by adding streptomycin (Moore et al., 1946) and that of turkeys by chlortetracycline (Stokstad et al., 1949) to their diets. The work with these antibiotics led to studies with other species and it

was found, perhaps not unexpectedly, that the growth-promoting effect was even more marked when certain intestinal infections were present. It is now well established that the growth rate of young animals is usually increased by adding relatively small amounts of certain antibiotics to the diet. This effect on growth occurs in apparently healthy animals fed diets that are adequate in all known nutritional factors and the effect is also observed with certain deficient diets.

The objective of this study was to investigate the effects of different feed additives on the rate of gain and feed efficiency of Nicholas White turkeys.

LITERATURE REVIEW

I - Antibiotics

Significance of Antibiotics

The antibiotics are defined as a group of soluble organic substances that are produced by microorganisms and that are characterized by their property of inhibiting at low concentrations the growth, activity, or multiplication of other microorganisms. Antibiotic-like substances are also produced by the flowering plants and by animals. Such plants as garlic, hops and tomatoes contain antibacterial compounds termed respectively "Alliine", "lupulon" and "tomatine" while lysozyme, a protein with antibacterial properties, is present in egg white and in the secretions of the eye (Jukes, 1955). These substances are not included among the antibiotics because the definition of this term is restricted to compounds that are produced by microorganisms. However, the extensive occurrence of antibacterial substances in more complex organisms serves to emphasize their widespread nature.

The discovery and development of the useful antibiotics have been based on empirical screening procedures that were designed to find an antibacterial substance that was harmless to animals. It may be presumed that such a substance must block certain essential enzyme systems in the bacterial cell without appreciably affecting any of the

essential systems in the cells of the host. The mechanisms of this biochemical selectivity has been studied from various standpoints.

Penicillin exerts its toxic effects on microorganisms only under conditions where their growth is possible (Work, 1952). It inhibits the formation of combined glutamate in Staphylococcus aureus cells while peptide glutamate accumulates in the medium. Bacitracin was found to behave similarly to penicillin (Pine, 1951). In contrast, chlortetracycline tended to inhibit the formation of peptides rather than the uptake of glutamate (Gale, 1951). Streptomycin appears to inhibit the oxaloacetate-pyruvate condensation in E. coli and Umbreit et al. (1951) have suggested that animal cells are not similarly affected because they are less permeable to the antibiotic. Other biochemical reactions in bacterial cells were unaffected even by high concentration of streptomycin.

It is evident from these and other examples that differences between the antibiotics exist with respect to their antibacterial mechanisms as well as to their chemical structures. The only biochemical property common to the antibiotics under consideration is their antibacterial effect. They do not, for example, all possess a single and distinctive chemical group which might have a growth-promoting effect on animals. The strong inference is that any

such effect is secondary to the antibacterial action of antibiotics on the microorganisms in the digestive tract.

Before the discovery of the growth-promoting effect of antibiotics on animals, many investigations were made of the results produced by adding sulfonamides to purified diets. It was found that under such conditions, deficiencies of biotin, folic acid and vitamin K were produced (Black et al., 1942; Light et al., 1942; Welch, 1942). However, these deficiencies did not appear when these three substances were added to purified diets or when rats received diets of natural foods to which sulfonamides were added. The results were taken to indicate that rats were able to obtain a supply of biotin, folic acid and vitamin K from a nondietary source when the rats were maintained on a purified diet that was deficient in these three vitamins and that this nondietary source originated in a bacterial fermentation that took place in the intestinal tract. It could be deduced that sulfonamides could depress or change the intestinal bacteria in a manner that interfered with the production of these vitamins, which are needed by the host animal. However, the sulfonamides do not interfere with the assimilation of vitamins that are added to the diet. The reason that deficiencies of biotin, folic acid and vitamin K developed was because they were not added to purified diets for rats, since deficiencies of these vitamins had not appeared before sulfonamides were added. In

contrast, thiamine, riboflavin, pyridoxine and pantothenic acid were added to the diets because, if these additions were not made, deficiencies developed even in the absence of sulfonamides.

The concept of a symbiotic relationship between animals and the bacteria in their gastrointestinal tract is known very well. The studies with rats on diets with sulfonamides emphasized this relationship and experiments with ruminants showed that these animals were even more versatile, for it was found that they could largely dispense with certain water-soluble vitamins, which were shown to be produced by fermentation in the rumen. Furthermore, simple nitrogenous compounds, such as urea, can be transformed by the rumen microorganisms into amino acids, which could replace part of the protein requirement of ruminants.

In contrast with these beneficial activities of the microorganisms of the digestive tract, there are many familiar examples of pathogenic bacteria and protozoa that can invade the alimentary canal to produce clinical disease. The sulfonamides and antibiotics have made some of their greatest contributions to medicine in controlling enteric diseases produced by organisms of this type.

A few investigators in the 1940's described experiments with rats and chicks in which antibacterial substances produced increases in the growth rate of the

animals. Moore and co-workers (1946) found that succinylsulfathiazole and streptomycin increased the growth of chicks on a purified diet. These authors made the important suggestion that the dietary supplements inhibited intestinal bacteria that were producing toxic materials or were rendering certain dietary vitamins unavailable to the animal but that the possibility that the agents were acting systematically could not be overlooked. Morehouse and Mayfield in 1946 found that 3-nitro-4 hydroxyphenylarsonic acid increased the growth of chicks and turkeys when added to natural diets. This observation was made during studies of the coccidiostatic effect of this substance and it was attributed to the elimination of some infection. All these studies in which the growth rate of animals was improved by antibacterial substances appeared to indicate that the effect was due to suppression of harmful microorganisms, and indeed such an effect would not be surprising in view of the association of weight loss with clinical disease.

The apparent beneficial effect of antibiotics on growth rate and feed efficiency observed in the late 1940's caused many investigators to conduct more experiments on the effect of antibiotics on the growth of animals and there is a vast number of such reports during the 1950's.

Stokstad and Jukes (1950) found that Aureomycin* hydrochloride, at comparatively low levels, produced great response in turkey poults. Branion and Hill (1951) reported that Aureomycin, penicillin, Terramycin** and streptomycin, when added at a level of 25 mg per kg of diet, resulted in a growth response in turkey poults up to 8 weeks of age, with Terramycin having the greatest response. They also found that birds fed plant protein showed better response to antibiotics than those birds fed animal protein. Groschke (1950) stated "antibiotics stimulate growth indirectly by changing the intestinal microflora from `undesirable' to `desirable' types and that unknown factors synthesized by the desirable types are responsible for the growth effect".

Some reports indicate that different breeds might show different resonses to antibiotics. Nordskog and Johnson in 1953 found that the difference in growth response to antibiotics of nine groups of chickens from different breeds was highly significant, ranging from 6.3 to 22.6 percent. Daghighian and Waibel (1982) fed bacitracin to Nicholas and British United turkeys. They reported greater and more consistent growth responses were observed from Nicholas male turkeys than from the other strain at 8 weeks of age.

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* The trade name of Lederle Laboratories Division, American Cyanamide Co., for chlortetracycline is Aureomycin.
** The trade name of Chas. Pfizer and Co., for oxytetracycline is Terramycin.

MacGregory et al. (1954) studied the effects of antibiotics on the growth rate of turkeys of various ages. In their study, turkey poults which had received diets with and without penicillin during the first 8 weeks were fed diets with and without penicillin from 8 to 20 weeks of age. They observed that the addition of penicillin at 8 weeks had no effect on growth rate of these poults not receiving this supplement from 1 to 8 weeks of age. The addition of penicillin at 8 weeks to the diets of poults which had received pencillin from 1 to 8 weeks of age had no effect on the growth rate of males to 20 weeks of age, but significantly increased the growth rate of females. Scott and Jensen (1952) reported that the growth advantage obtained by feeding Aureomycin to turkey poults to 8 weeks of age was rapidly lost when the antibiotic was withdrawn from the feed after the eighth week.

Moran and McGinnis (1965) studied the effect of antibiotics on the growth of turkeys fed diets containing either corn or barley as the cereal grain. They reported that enzyme and antibiotic supplementation had essentially no effect on growth of poults when added to corn diet unless the protein to energy relationship were extensively altered. Poults fed barley-containing diets responded to enzyme and antibiotic supplementation in all trials, but did not respond to increased energy alone. Their proposed hypothesis was that the beta-glucan of barley supported the

establishment of "undesirable" microflora which in turn was detrimental to poults growth. This "undesirable" microflora may be modified either directly by the use of an antibiotic or indirectly through the use of supplemental enzyme which can degrade the beta-glucan.

Carlson et al. (1956) reported that greater and more consistent growth responses were achieved from antibiotics with lower energy diet than with higher energy diets. Slinger et al. (1951) reported that penicillin did not affect protein requirements of broiler chickens.

It has been well established that the antibiotic growth response can be produced by certain non-absorbed antibiotics such as streptomycin or bacitracin and has been observed in disease-free as well as conventional animals, but not maintained in germ-free animals. McGinnis et al. (1950) reported that unlike vitamin B 12, streptomycin was completely ineffective in promoting growth when given by injection into the breast muscle. Bacitracin has been found to produce a growth response when added to the diet but not when implanted subcutaneously (Branion et al., 1952). Eagle et al. (1947) reported that injected bacitracin is excreted in the urine rather than bile so that it is possible that implanted bacitracin may not reach the intestine. In contrast, those antibiotics such as Aureomycin and penicillin, which have been found to produce growth responses when injected (Dixon and Thayer, 1951; Elam et

al., 1951; Hester et al., 1954), are known to be excreted into the intestine via the bile (Cook et al., 1952).

As mentioned before, the growth response of animals due to the addition of antibiotics was attributed to the suppression of some harmful microorganisms. Moore et al. (1946), even before the impact of antibiotic use in agriculture, suggested that unidentified intestinal bacteria could have an inhibitory effect upon growth without causing clinical disease.

One species of bacteria involved in the depression of growth and sensitive to nearly all known feed additive antimicrobial agents is Clostridium perfringens. In 1951 Sieburth et al. reported that penicillin and Terramycin inhibited the growth of Clostridium perfringens in the ceca and increased the growth of turkeys. Lev and Forbes (1959) demonstrated that C. perfringens caused a significant reduction in growth of germ free chicks and that penicillin largely overcame the growth depression. Penicillin had no effect on the growth of germ free chicks. According to Elam et al. (1953), several antibiotics, including penicillin, bacitracin, Terramycin and Neomycin produced significant increase in growth and significant decrease in the total numbers of clostridia per gram of feces of chicks. Jacobs et al. (1953) showed that several antibiotics failed to stimulate growth and to reduce fecal clostridium of chicks reared in very clean conditions where

the clostridia population was low. Eyssen and DeSomer in 1963 reported that the growth-stimulating effect of antibiotics is most likely due to suppression of Gram-positive intestinal bacteria which interfere with the absorption of nutrients. Stutz et al. (1983a) have lately reported that organisms detected in the intestinal of chicks fed a practical diet in descending numbers were lactobacilli, streptococci, staphylococci, coliforms and clostridia. They also concluded that bacitracin and efrotomycin had no effect on the population of the intestinal organisms, other than Clostridia perfringens, in the ileum of chicks. These investigators, in another study (Stutz et al., 1983b), found that a thiopeptin antibiotic caused a significant improvement in weight gain and feed efficiency. They also found that this antibiotic significantly decreased the number of C. perfringens organisms in the ileal content of the chicks.

Virginiamycin is another antibiotic produced by Streptomyces virginiae, isolated by DeSomer and VanDijck in 1955. This antibiotic is active against Gram-positive bacteria of the gut. Several reports have shown that virginiamycin will increase growth and improved feed efficiency in chicken and turkeys (Combs and Bossard, 1963; March et al., 1978; Miles et al. 1984). Yates and Schaible in 1962 compared virginiamycin with Terramycin which have "broad" and Zn-bacitracin which has "narrow" spectras.

They reported that virginiamycin was comparable to Terramycin and Zn-bacitracin in its effectiveness on growth, feed utilization and livability. Eyssen *et al*. (1962) reported that virginiamycin was active against lactobacilli in the chicken crop both *in vivo* and *in vitro* thus it was important as a factor in the growth-promoting effect of antibiotics.

Biosynthesis of Antibiotics

Over the years, as more and more antibiotics have been discovered, it has been found that most of them, especially those of medical importance, fall into the general category of secondary metabolites. Among microorganisms, their taxonomic distribution is restricted and among bacteria we find them to be produced largely by certain spore-forming groups. During their normal life cycle such organisms will grow until they have produced the maximum number of cells they are capable of forming under the particular conditions. Once the culture has stopped growing, it enters the stationary phase, followed by death or, alternatively, by spore formation. Usually after the cells have stopped dividing, secondary metabolites begin to be produced. Their production continues for a certain length of the time, which may be longer or shorter than the active growth period, and then production ceases (Zahner and Maas, 1972). Secondary metabolites are often produced in large amounts and for the most part they are excreted into the culture medium.

In contrast to primary metabolites, secondary metabolites are not essential for growth of the organism. Primary metabolites are either building blocks for macromolecules, intermediates in reactions generating energy-rich compounds such as ATP, or parts of coenzymes. Secondary

metabolites protect the cell against environmental or internally generated hazards that arise during the resting phase (Zahner and Maas, 1972).

One characteristic of secondary metabolites that distinguishes them from primary metabolites is frequent "uncontrolled" production by the organism. Microorganisms do not produce primary metabolites (amino acids, purines) in amounts greater than needed for growth. This economy is brought about mainly through metabolic regulatory mechanisms, especially enzymes and feedback inhibition (Lehninger, 1982). Accumulation of secondary metabolites suggests that the cells are inefficient in the regulation of synthesis of these substances.

To elucidate biosynthetic pathways of antibiotics the isotopic tracer technique is used most commonly. With isotopes it is relatively easy to determine the origin of the building block of an antibiotic molecule. In the usual experiment the antibiotic-producing strain is grown with a given radioactive nutrient, the antibiotic is isolated and the distribution of radioactivity in different parts of the antibiotic molecule is determined (Gale et al., 1972).

Several primary metabolites have been identified to be used for antibiotic synthesis. Fatty acids, amino acids, carbohydrates, purine and pyrimidine bases are among these metabolites (Gale et al., 1972; Zahner and Maas, 1972).

Short-chain Fatty Acids Antibiotics

Step wise head-tail condensation of active acetate and malonate units leads to the formation of β-polyketomethylene chains. Substances that are biosynthetically derived from β-polyketones are called polyketides. Actually the starter molecule of polyketide synthesis is not always acetyl-CoA. It may be propionyl-CoA or more complicated CoA derivations. For example, in the synthesis of tetracyclines the starter is malonamoyl-CoA. Moreover, the units to be added to the starter are not always acetate unit but may, for example, be propionate units, as in the synthesis of erythromycin (Zahner and Maas, 1972).

These intermediary β-polyketones undergo several types of further modifications. For example, reduction may lead to formation of fatty acids, but it does not occur in the formation of antibiotics. Introduction of double and triple bonds results in the formation of polyenes and polyacetylenes, the former being found in polyene antibiotics and the latter being found among a variety of compounds occurring among basidiomycetes (Gale et al., 1972). Repeated cyclization of β-polyketones lead to the formation of polyaromatic compounds such as tetracyclines.

The starter molecule for tetracycline formation is malonamoyl-CoA to which acetate units are added via malonyl-CoA to form a β-polyketomethylene chain. This is converted via a polycyclic intermediate to the final products.

Peptide Antibiotics

Many antibiotics utilize amino acids as building blocks. In some cases amino acids are the sole constituents, whereas, in others they are joined to other metabolites such as sugars and fatty acids. Some antibiotics are derived from only one or two amino acids (cycloserine, penicillin), others from a larger number (actinomycin). Some of the amino acids found in antibiotics are quite different from those found in proteins (Gale et al., 1972; Zahner and Maas, 1972). Thus antibiotics often contain D-amino acids, β-methylated amino acids, β-amino acids, and precursor amino acids such as ornithine. Although there are common features, the synthesis of antibiotics is fundamentally different from that of protein synthesis. Antibiotic synthesis does not require the presence of ribosomes, tRNA or mRNA. Any code for the assembly of amino acids is imprinted only into the biosynthetic enzymes (Zahner and Maas, 1972). It should be noted here that peptide antibiotics are much smaller than proteins, having a range of molecular weights from 350 to 3000. Other features that distinguish antibiotic synthesis from protein synthesis are:

1. Relaxation in the specificity of assembly resulting usually in the production of a family of closely related substances rather than a unique polypeptide chain.

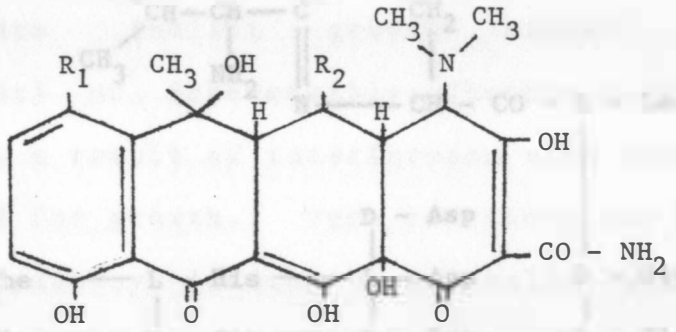
2. Formation of cyclic structures with no free α - amino or α - carboxyl group.

3. Insensitivity to protein-synthesis inhibitors such as chloramphenical and puromycin.

4. *In vivo* production during a late stage of the growth cycle, after protein synthesis has ceased.

Antibiotics Containing Sugars, Purines and Pyrimidines

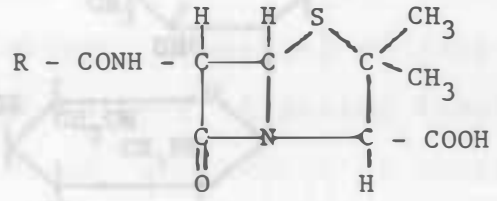
Sugars, purines or pyrimidines can also be found as constituents of antibiotic molecules. Sugars occur widely among antibiotics produced by actinomycetes. Neomycin and puramycin are among this category of antibiotics. Among fungal antibiotics the occurrence of sugar is rare. No sugar-containing antibiotics have been found among Aspergillales (Zahner and Maas, 1972). The structural formulas of commonly used antibiotics, tetracyclines, streptomycin, penicillin and bacitracin are shown in Figure 1.



Tetracycline $R_1 = H$, $R_2 = H$

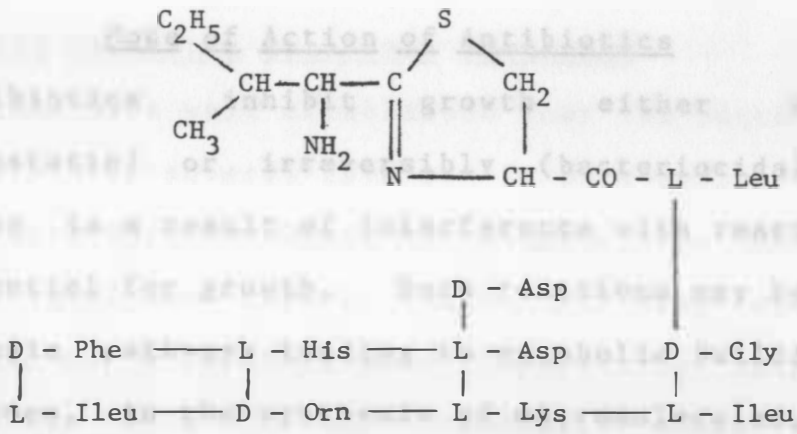
Chlortetracycline $R_1 = Cl$, $R_2 = H$

Oxytetracycline $R_1 = H$, $R_2 = OH$

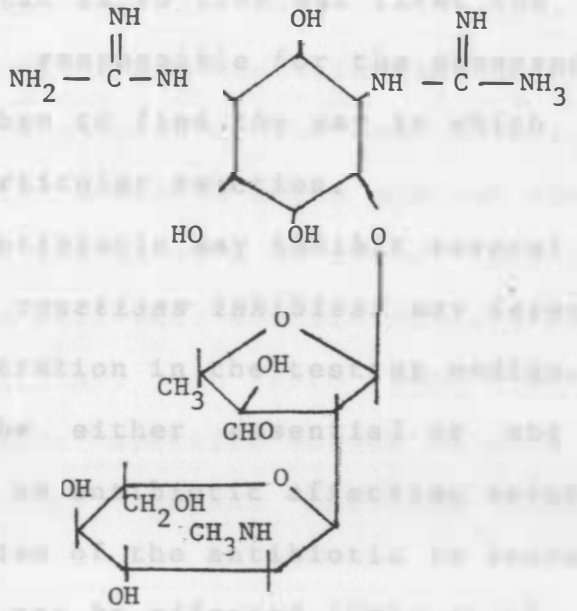


Penicillin

Figure 1. Structural formulas of Tetracycline, Penicillin, Bacitracin A and Streptomycin.



Bacitracin A



Streptomycin

Figure 1. Continued

Mode of Action of Antibiotics

Antibiotics inhibit growth either reversibly (bacteriostatic) or irreversibly (bacteriocidal). This inhibition is a result of interference with reactions that are essential for growth. Such reactions may be in the biosynthesis pathways leading to metabolic building blocks or coenzymes, in the synthesis of macromolecules, such as proteins and nucleic acids, or in the maintenance and synthesis of cellular structures, such as the cell membrane (Freeman, 1979). To determine the mode of action of an antibiotic is to find out first the reaction whose inhibition is responsible for the observed inhibition of growth and then to find the way in which the antibiotic blocks that particular reaction.

A given antibiotic may inhibit several reactions, and the number of reactions inhibited may depend on the antibiotics concentration in the testing medium. The reaction blocked may be either essential or not essential for growth. With an antibiotic affecting several reactions as the concentration of the antibiotic is increased, more and more reactions may be affected (Gale et al., 1972). Also, the blocking of one reaction may secondarily lead to inhibition of other reactions, this being another way in which an antibiotic may affect several reactions.

Antibiotics Affecting Cell-Wall Formation

It has been well established that the bacterial cell wall consists of several layers. In Gram-positive bacteria the wall structure is relatively simple. The main layer is the peptidoglycan layer, which in many species is surrounded by a teichoic acid layer. In Gram-negative bacteria the wall is more complex, consisting of a peptidoglycan layer surrounded by layers of lipoproteins and lipopolysaccharides (Freeman, 1979). In both Gram-positive and Gram-negative bacteria the wall surrounds the cytoplasmic membrane and lends rigid support to an otherwise fragile cell. Most bacteria live in environments that are relatively hypotonic to the cell interior and peptidoglycan normally protects cells against the high osmotic pressure of the inside of a bacterial cell (Freeman, 1979).

All known cell-wall antibiotics act on the formation of the peptidoglycan. As there is no comparable structure in animal cells, specific action on this layer ensures selective toxicity against bacteria (Zahner and Maas, 1972).

Penicillins and bacitracin are among antibiotics that prevent cell wall formation. Penicillins, the most famous and the most useful of all the antibiotics used in medicine are inhibitors of peptidoglycan synthesis (Pine, 1951; Work, 1952). They prevent the incorporation of glutamic acid into peptidoglycan (Gale and Pine, 1950). Penicillin

requires growth of cells in order to kill them; the faster the growth, the more effective is its action. Bacitracin, a mixture of cyclic polypeptides consisting of D- and L-amino acids, produced by Bacillus subtilis, inhibits peptidoglycan synthesis and also has an effect on membranes. Concentrations of bacitracin which have no effect on protein synthesis inhibit the incorporation of lysine into peptidoglycan and cause accumulation of peptidoglycan precursors (Freeman, 1979; Gale, et al., 1972).

Antibiotics Affecting Protein Synthesis

A variety of antibiotics have been found to be inhibitors of protein synthesis. Antibiotics can inhibit the synthesis of nucleic acids and proteins at different sites such as replication of DNA, transcription, formation of aminoacyl-tRNA and translation (Zahner and Mass, 1972).

A majority of the antibiotics affecting protein synthesis act at the level of translation. Chemically they form a very heterogeneous group (Gale et al., 1972). This attests to one or more differences in the mechanism of translation between bacteria and cells of higher forms, which permits them to exhibit selective toxicity. One difference is in the structure of ribosomes. Bacterial ribosomes have a semidentation value of 70S and are composed of 30S and 50S subunits. Mammalian ribosomes have a semidentation value of 80S and are composed of 40S and 60S

subunits (Lehninger, 1982). Table 1 summarizes information about the mode of action of the principle antibiotics affecting translation.

Table 1. Action of antibiotics affecting translation.

Antibiotic	Subunit affected	Mode of action
Streptomycin	30S	Distorts configuration of 30S subunit
Tetracyclines	30S	Blocks binding of aminoacyl-tRNA
Puromycin	50S	An analog of aminoacyl-tRNA, clears p site of peptidyl-tRNA

From Zahner, H. and W.K. Maas, 1972. Biology of antibiotics.

Tetracyclines are classical broad-spectrum antibiotics causing a variety of apparently unrelated effects when added to growing cells. Gale and Pine (1950) first showed that chlortetracycline was a specific inhibitor of protein synthesis in Staphylococcus aureus. Since then the tetracyclines have been shown, in a wide variety of tests, to be effective inhibitors of protein synthesis at drug concentrations similar to those required to inhibit growth. Tetracyclines bind to DNA (Kohn, 1961), proteins (Kohn, 1961; Salgarello, 1963), synthetic polynucleotides (Connamacher and Mandel, 1965) and to ribosomes. Day in 1966 reported

that only that drug bound to ribosomes is inhibitory. The binding of tetracyclines to ribosomes is dependent upon the concentration of Mg^{2+} and K^{+} ions and upon the drug concentration (White and Cantor, 1971). Under optimum conditions of binding, up to 300 drug molecules were bound per 70S ribosomes of Bacillus megateritum (Maxwell, 1968). Other results support the general conclusion that binding of tetracycline occurs principally (but not exclusively) on the 30S subunits of bacterial ribosomes. It is suggested that the tetracyclines inhibit some function occurring on 30S subunits (Day, 1966; Connamacher and Mandel, 1965; Freeman, 1979).

Bacterial Resistance to Antibiotics

Not all bacterial species are equally sensitive to a given antibiotic. The range of organisms inhibited by an antibiotic is called its antibacterial spectrum. Some antibiotics inhibit growth of a wide variety of organisms ("broad spectrum" antibiotics), whereas others have a narrower antibacterial spectrum. In a population that is sensitive to a given antibiotic, resistant forms can arise. As each antibiotic has been introduced, a short period, during which the antibiotic is used effectively on a large scale, has generally been followed by the appearance of a higher and higher proportion of resistant organism up to a point at which the value of the antibiotic, as a therapeutic agent, has often been severely undermined. Resistance of Staphylococcus aureus to penicillin provides an example. Soon after the introduction of benzyl penicillin into widespread use, a large-scale World Health Organization survey showed that the resistant strain had increased dramatically (Fig. 2) and a similar pattern has been found with most other antibiotics (Gale et al., 1972).

Resistance to antibiotics may be due to:

1. Modification of the target enzyme in the cell so that it is insensitive to the inhibitors yet still able to carry out normal physiological function.

2. Prevention of access of the inhibitor to the target.

3. Synthesis by the bacteria of an enzyme capable of inactivating the inhibitor.

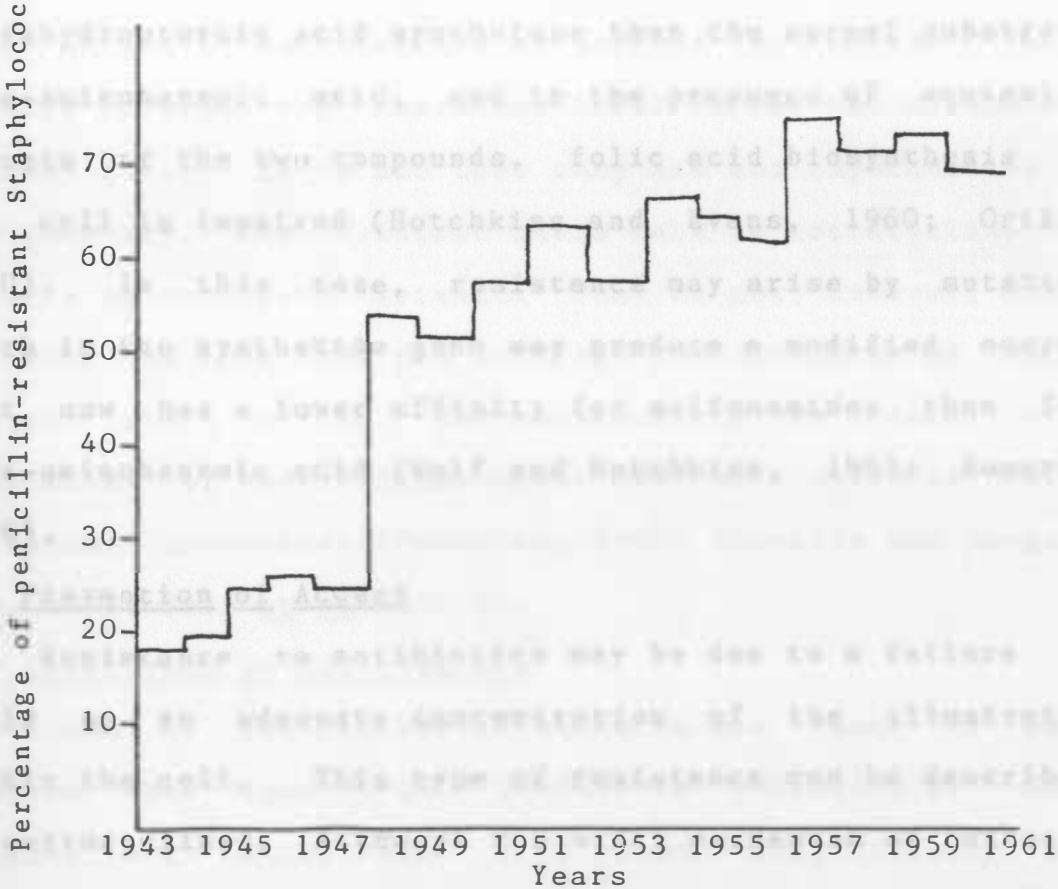


Figure 2. The incidence of penicillin-resistant staphylococci among clinical isolates during the period 1943 to 1959. From Gale et al., 1972. The Molecular basis of Antibiotic Action.

1. Modification of the target

The majority of targets in microbial cells are enzymes and there is competition between the inhibitor and the normal substrate. The affinity of the enzyme for the

inhibitor has to be appreciably higher than that of the substrate to have much effect on cell growth. A typical example of antibacterial action of this type is that of sulfonamides. Most sulfonamides have a higher affinity for tetrahydropteroic acid synthetase than the normal substrate para-aminobenzoic acid, and in the presence of equimolar amounts of the two compounds, folic acid biosynthesis in the cell is impaired (Hotchkiss and Evans, 1960; Ortiz, 1970). In this case, resistance may arise by mutation which in the synthetase gene may produce a modified enzyme that now has a lower affinity for sulfonamides than for para-aminobenzoic acid (Wolf and Hotchkiss, 1963; Nomura, 1970).

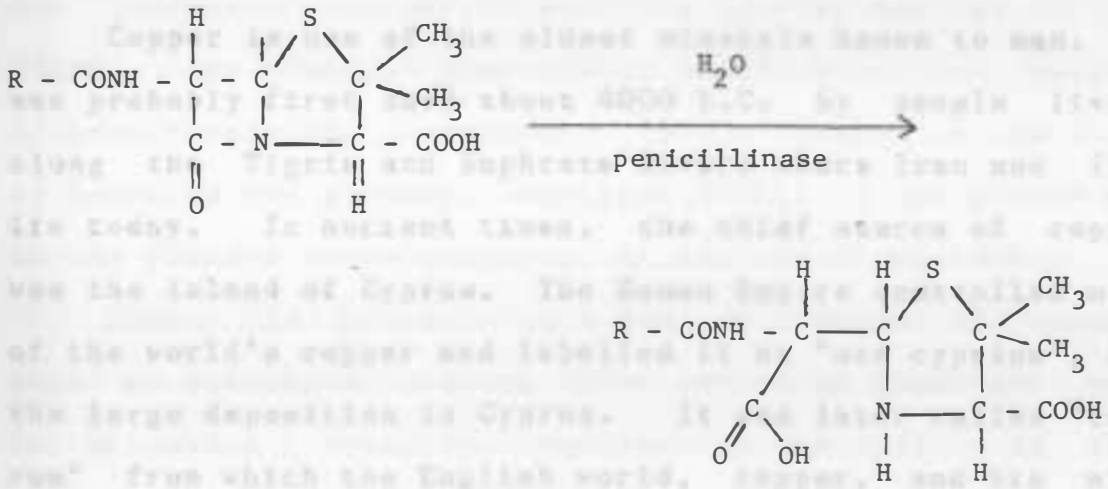
2. Prevention of Access

Resistance to antibiotics may be due to a failure to build up an adequate concentration of the illustrated within the cell. This type of resistance can be described by tetracyclines. Although the exact mechanism of tetracycline resistance is not known, many investigators postulate that the resistant cell does not build up enough antibiotic to kill the bacteria. Franklin (1967) has shown when a sensitive cell first meets tetracycline, some passes through the cell membrane but seems free to leave again immediately; that is, initially there is a free flow in and out of the cell. The presence of a low concentration of tetracycline in the cell, however, seems to induce a change

in the cell's permeability to tetracycline, so that outflow is inhibited while inflow is unchanged (Franklin, 1967; Franklin and Higginson, 1969). The result is an accumulation within the cell which is lethal because of the inhibitory effect of tetracycline on protein synthesis. These authors reported that resistant bacteria can not accumulate tetracycline. It is not that they are not impermeable; on the contrary, the resistant cells take in tetracycline as readily as sensitive variants. They do not, however, adapt to stop the loss when the internal concentration of antibiotics start to rise, and consequently the internal concentration of tetracycline never reaches a point at which it becomes inhibitory (Franklin, 1967; Franklin and Hogginson, 1969; Gale et al., 1972).

3. Resistance by Inactivation

Many of the classes of bacteria are found to make enzymes that specifically inactivate antibiotics. Such enzymes act in two ways: (1) either they destroy the antibiotic by opening one or more covalent bonds in their structure or (2) they chemically substitute key residues, thus making them inactive. Penicillinase enzyme, which destroys penicillin, and chloramphenicol transacetylase, which destroys chloramphenicol, are examples of these types of enzymes (Gale et al., 1972). Destruction of penicillin is shown below.


H
S
CH3
R - CONH - C - C
C
CH3
C - N — C - COOH
O
H
H2O
penicillinase
H
H
S
CH3
R - CONH - C — C
C
CH3
C
N — C - COOH
O
OH
H
H

II Copper

Copper is one of the oldest minerals known to man. It was probably first used about 8000 B.C. by people living along the Tigris and Euphrate Rivers where Iran and Iraq lie today. In ancient times, the chief source of copper was the island of Cyprus. The Roman Empire controlled much of the world's copper and labelled it as "aes cyprium", for the large deposition in Cyprus. It was later called "cuprum" from which the English world, copper, and its elemental symbol, Cu were derived.

Today, the United States is the largest Cu producing country in the world. In 1976 the United States produced 1,605,586 tons of Cu valuing 2,235 million dollars (Cromwell et al., 1981). Approximately 2% of the copper in the United States is made into copper sulfate ($Cu\ SO_4 .\ 5\ H_2O$), commonly called "bluestone" or "blue vitriole". Copper sulfate has many industrial and agricultural uses. It is used as a pesticide, as a fertilizer, and as a supplement to animal and poultry feeds.

Functions of Copper

Although the presence of copper in animal tissue was demonstrated by Boutigny in 1833, its importance in nutrition was not recognized until 1928 when Hart and co-workers demonstrated that copper was required along with iron for the synthesis of hemoglobin in rats (Scott et al., 1982).

A large portion of the body copper is in the liver and its concentrations varies with the species and age of the animal, the chemical composition of the diet, and various disease conditions (Underwood, 1977). Copper in the blood is bound to the protein, ceruloprotein, in the plasma and to the protein erythrocuperin, in the red blood cells.

Copper is known to be a part of a number of enzymes such as cytochrome oxidase which serves an important role in oxidation - reduction reactions in the body. In this enzyme, copper atoms undergo cyclic Cu (II) - Cu (I) valence transition as they participate in carrying electrons to oxygen (Lehninger, 1982).

Copper is also present in the active group of lysyl-oxidase, an enzyme that makes the cross-linkage between polypeptide chains in collagen and elastin. Chicks hatched from copper-deficient dams showed no amine oxidase in the aorta or liver. If these chicks were maintained on a copper- deficient diet no enzyme could be detected during the first four weeks of life. Those receiving copper showed high amino oxidase levels after the third day of life (Scott et al., 1982). Amine oxidase increases the incorporation of lysine into the desmosine (elastin) of the aorta. Scott et al. (1982) state "the primary role of copper in elastin formation in the chicken appears to be the need for this ion by the amine oxidase involved in the oxidative deamination of the epsilon amino group of lysine.

A copper deficiency reduces the number of oxidized lysine residues available to condense for the formation of desmosine".

Deficiency of Copper

Copper deficiency causes a different clinical syndrome which varies with the age, sex and species of animal and the severity and duration of the deficiency (Underwood, 1977). Besides anemia which is a general symptom for all species, depressed growth, depigmentation of hair, fur, wool or feathers, and gastrointestinal disturbances (diarrhea or scours) have been observed in copper deficiency (Scott et al., 1982).

Copper is required for proper iron metabolism in the body. Red blood cells require copper for their production and for the maintenance of their integrity in the circulation (Underwood, 1977). In 1956 Bush et al. concluded that copper deficiency anemia in pigs resulted from both shortened erythrocyte survival time and limited capacity of the bone marrow to produce red cells. Different species have shown different types of anemia due to deficiency of copper. In rats, rabbits, pigs and lambs this anemia is hypochromic and microcytic. Sheep and cattle have shown hypochromic and macrocytic anemia while in Cu-deficient chicks and dogs the anemia has been reported as normocytic and normochromic (Underwood, 1977).

Growth Promotion by Copper and its Relationship to Sulfur-Containing Amino Acids

Since the 1950's when it was discovered that the addition of copper to the diet of pigs usually promoted growth (Cromwell et al., 1981), the use of this feed additive at levels well above the nutritive requirements has been practiced to large extent in both swine and poultry rations. Copper in the form of cupric sulfate ($CuSO_4.5H_2O$) has been used in turkey diets generally not only as a growth stimulant like that of antibiotics but also is used as a treatment for a fungal infection of the crop known as "trush" and a crop protozoan infection (Guenthner et al., 1978; Robbin and Baker, 1980a). Smith (1969) reported a 2.3 percent increase in chick body weights on a diet containing 100 ppm Cu and a 7.3 percent decrease in body weights due to the addition of 350 ppm Cu. King (1975) reported copper at 100 ppm resulted in significant increase in body weight and significantly smaller ceca both as a proportion of body weight and as the weight of a unit length in ducklings. At the South Dakota State University Poultry Research Unit, 120 ppm Cu in the form of copper sulfate or copper oxide was shown to stimulate growth rate of turkeys up to 10% with a reduction in feed requirements (Guenthner et al., 1978). This level of Cu also reduced the incidence of aortic rupture by about one-half. Doerr et al. (1980) reported that the addition of copper sulfate

to a broiler diet increased their body weight and decreased the mold count in the litter. Kashani and Carlson (1980) reported a 2.3% growth response in turkeys due to the addition of 120 ppm copper.

Copper is also used in swine rations very extensively especially in England and other European countries. Cromwell et al. (1981) summarized the results of 18 experiments. They reported 250 ppm copper to increase body weights by 3.1% and that swine required 2.5% less feed per unit of gain.

High levels of copper in the poultry diet has been shown to be harmful and interferes with the metabolism of the sulfur-containing amino acids, methionine and cystine. Christmas and Harms (1979) reported that 500 or 750 ppm copper in the diet significantly produced gizzard erosion, decreased feed intake and resulted in a reduction in final body weight of turkeys. The addition of 0.4 percent methionine did tend to increase feed consumption and growth rate in this study. Robbins and Baker (1980a) found that chicks fed a purified diet were more susceptible to copper toxicity than those fed a corn-soybean meal diet. They reported that addition of sulfur-containing amino acids (methionine and cystine) alleviated copper toxicity to some extent. In another study, Robbins and Baker (1980b) used a soy-protein semipurified diet to determine the effect of sulfur on the alleviation of copper-induced growth depres-

sion in two breeds of chickens. The diet was complete in every respect except for the deficiency of sulfur-containing amino acids. Copper at 0 and 500 ppm levels and DL-methionine at 4 levels of 0.06, 0.12, 0.18 and 0.24 percent were fed to the birds. Using 20-day weights, the sulfur-containing amino acids requirements were calculated by "estimating the requirements as the abscissa of the point an the fitted curve whose ordinate was 95% of the upper asymptote" as described by Robbins et al. (1979).

Sulfur-containing amino acid requirements were estimated 0.55% at zero copper in both breeds. Copper at 500 ppm increased the sulfur-containing amino acid requirements to greater than 0.67% and gain increased linearly with each methionine addition. The growth response at the 0.24% level of methionine; however, was greater for the heavier breed than for the light breed with the 500 ppm Cu level in that the heavier chicks reached about 90% of maximum weight achieved by control birds compared with 75% of maximum performance for light breeds. This difference, however, was reported to be due to the differences in feed consumption. Addition of sulfur-containing amino acids in the diet did not decrease the concentration of copper in the liver of birds receiving 500 ppm Cu. In another experiment, these investigators found that the requirements for sulfur-containing amino acids for birds receiving 0, 250 or 500 ppm of copper were estimated at 0.59, 0.64 and 0.77

percent, respectively. Copper additions linearly increased erosion score of the gizzard lining and copper concentration. Methionine slightly decreased copper content of the gizzard lining in chicks fed high amounts of Cu but it did not correct the gizzard erosion.

English workers reported that the addition of 250 ppm copper to a wheat-fish meal diet with 0.75 percent methionine and cysteine has been shown to increase chicks' growth by five percent but this level of copper significantly decreased growth when added to corn-soybean meal diet containing 0.65 percent sulfur-containing amino acids (Jenkins et al., 1970).

These results indicate that the sulfur containing amino acids requirements were increased by 8% with the addition of 250 ppm Cu and by 30% with the addition of 500 ppm Cu. An eight percent increase of sulfur-containing amino acids requirements with 250 ppm Cu is interesting and is particularly important in the poultry industry since corn-soybean meal diets limited in S-AA are typical for poultry and therefore high levels of Cu could cause growth depression.

Copper-Antibiotic Relationship

Although the growth response from the feeding of copper is generally thought to be similar to that of feeding antibiotics, the response of these two feed additives could be additive. Beames and Lloyd (1965) reported that a

combination of 250 ppm Cu and 110 ppm Tylosin caused better growth rate than when these two compounds were fed singly. Stahly et al. (1980) reported that the single addition of Cu, Aureomycin or virginiamycin improved daily gains in early-weaned pigs by 22 and 17%, respectively during 28-day trials. Dietary inclusion of both copper and an antibiotic (Aureomycin or virginiamycin) further improved daily gains by 10 to 11% and feed efficiency by 2 to 5% compared with the single addition of each antimicrobial agent. The addition of copper to the diet also increased postweaning pig survival in this study. Cromwell et al. (1981) combined the results of several studies on feeding combination of copper and antibiotics and reported that the combination of copper and antibiotics will improve growth rate and feed efficiency greater than when these compounds are fed singly (Fig. 3).

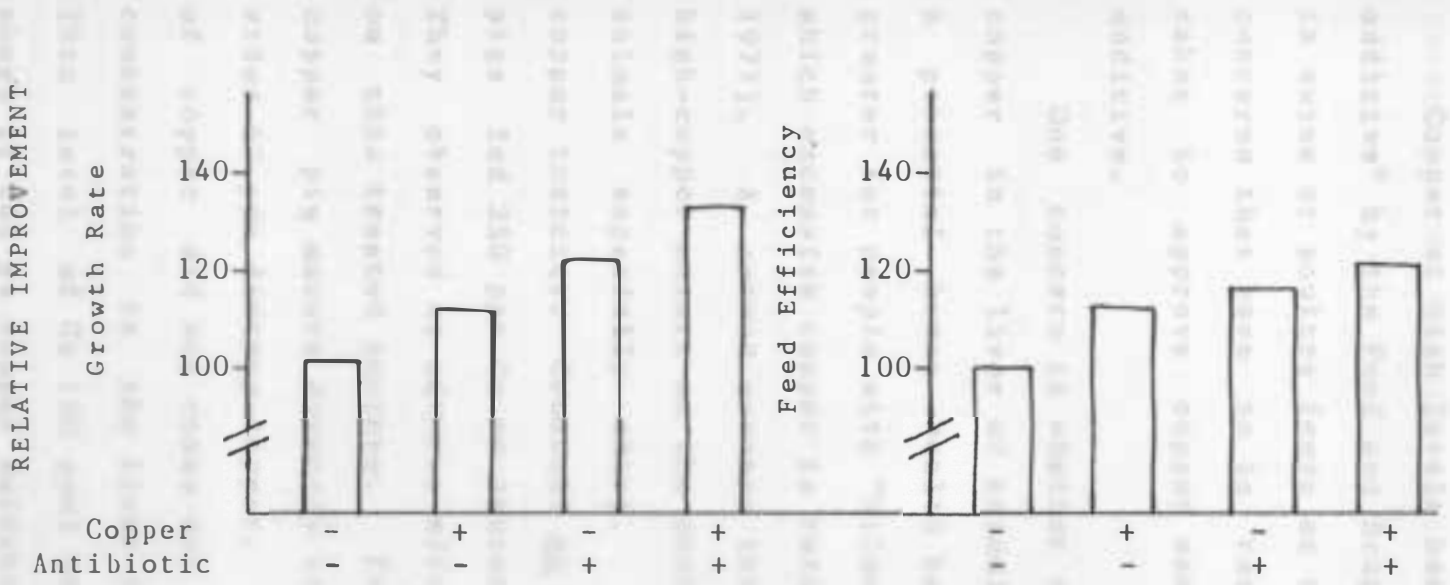


Figure 3. Relative improvements in performance of weaning pigs from single addition and the combined addition of copper (250 ppm) and antibiotics to the diet. (From Cromwell et al., 1981).

Status of Copper

Copper at high levels has not been cleared as a "feed additive" by the Food and Drug Administration (FDA) for use in swine or poultry feeds at this time. There are several concerns that have to be resolved before action will be taken to approve copper usage at high levels as a feed additive.

One concern is whether or not an increased level of copper in the liver of animals fed high copper constitutes a potential human health hazard. This risk would be greater for people with "Wilson's Disease", a condition in which excessive copper is retained in the body (Underwood, 1977). A second concern involves the effect of spreading high-copper manure on the pasture and its effect on grazing animals especially sheep. Sheep are very sensitive to copper toxicity. Cromwell et al. (1981) spread manure from pigs fed 250 ppm Cu on pasture over a three-year period. They observed no adverse effect on sheep allowed to graze on this treated pasture. Prince et al. (1975) fed high-copper pig manure directly to sheep at a level that provided 60 ppm dietary copper. They reported that this level of copper did not cause any toxicity in sheep. Even Cu concentration in the liver did not rise in this study. This level of Cu (60 ppm) has shown to be very toxic to sheep if fed as copper sulfate (Kline et al., 1971). These

investigators postulated that the copper in the feces is largely in an unavailable form.

MATERIALS AND METHODS

A total of four experiments were conducted to examine the effect of copper, Zn-bacitracin, Neo-Terramycin and virginiamycin on the growth of turkeys. A total of 3780 male and 216 female Nicholas White turkeys obtained from a commercial hatchery were used in this study. Female turkeys were used only in the last experiment. All birds were debeaked and detoed at one day of age at the hatchery. All experiments were carried out in two windowless houses with a forced ventilation system. Birds were randomly distributed in both houses. In each of the first 3 experiments, 26 toms were allotted to each pen in one house (west house) containing 24 pens and 52 toms were placed in each pen in the other house (east house) containing 12 pens. Only the west house with 24 pens was used for the 4th experiment. Feed and water were provided ad libitum and fresh wood shavings were used as bedding for each experiment. Individual weights and group feed consumption data were obtained at 4-week intervals. For the first three experiments the number of birds were reduced to 20 and 40 birds per pen in the west and east house after eight weeks of age by removing the weight extremes. These numbers were again similarly reduced to 15 and 30 birds per pen after 12 weeks of age. Data were subjected to statistical analysis

for differences among treatments according to analysis of variance (Steel and Torrie, 1980).

Experiment 1

This experiment was designed to determine the effect of copper on the sulfur-containing amino acid requirements of turkeys. This decision was based on a previous study at this laboratory which showed that the addition of 120 ppm Cu as copper sulfate caused a slight decrease in body weight at 8 and 16 weeks of age when low protein diets containing 75, 85 or 100% of the NRC (1977) recommended sulfur-containing amino acid levels were used (Kashani et al., 1980). This level of Cu (120 ppm) was suspected of decreasing sulfur amino acids (S-AA) utilization and causing growth depression. Thus, this factorial experiment was designed to determine the effect of 60, 120 or 240 ppm Cu on the growth rate of turkeys as affected by three different levels of sulfur containing amino acids (75, 100 or 125% of NRC requirements).

A total of 1200 day-old tom turkeys were used in this experiment. The low protein dietary series provided 23, 20, 18, 16, 14 and 12% protein, dropped at 4 week intervals, as recommended by Guenthner et al., (1978). The compositions of the low protein diets are shown in Table 2. These diets provided only 75% of the NRC recommended levels of S-AA. Additional DL-methionine was added to provide diets containing 100 or 125% of S-AA. Copper in the form

of copper sulfate was used in this experiment. The turkeys received 0, 60, 120 or 240 ppm Cu. Three replicates were used for each treatment. To study whether or not addition of copper would increase blood and/or liver copper content, at the termination of this experiment, blood and liver samples from two turkeys from each pen were obtained for copper analysis. Blood samples were drawn through cardiac puncture prior to slaughter of the birds. Heparin was used as an anticoagulant. Plasma was separated and diluted with five parts of 0.1% aqueous Triton X-100. Copper concentration was then determined using a model 503 Perkin-Elmer atomic absorption spectrophotometer equipped with a model 2100 heated graphite atomizer and background correction.

Whole liver was detached from the gall bladder, cut into small pieces, mixed and an aliquote was dried overnight at 100 C. A 0.5 gram sample of the dried liver was boiled with 10 ml concentrated nitric acid to reduce volume to 2 ml. The digest was diluted to 10 ml with water and copper content was determined by flame atomic absorption spectrophotometry (model 503 Perkin-Elmer). All copper analysis procedures were done under the direction and supervision of Dr. R. J. Emerick at South Dakota State University Biochemistry Station. This experiment started on 1-12-82 and ended 6-28-82.

Table 2. Composition of low protein diets (%).

Ingredient	Age of birds (weeks)					
	0-4	4-8	8-12	12-16	16-20	20-24
Corn	49.50	57.20	65.25	70.74	76.18	81.50
SBM (47%)	38.50	30.70	25.21	19.96	14.62	9.35
Alfalfa meal (17.5%)	2	2	2	2	2	2
Fish meal	2	2	--	--	--	--
Yellow grease	1	1	1	1	1	1
Limestone	3	3	3	3	3	3
Dicalcium phosphate	2	2	2	2	2	2
TM - salt*	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix**	1	1	0.5	0.5	0.5	0.5
Lysine	0.22	0.38	0.16	0.115	0.13	0.08
DL-methionine	0.29	0.22	0.38	0.185	0.07	0.07
Calculated Analysis						
Crude protein (%)	23	20	18	16	14	12
ME (Kcal/kg)	2791	2865	2950	3011	3067	3120
Calcium (%)	1.61	1.40	1.30	1.30	1.30	1.30
Phosphorus (%)	0.83	0.80	0.72	0.72	0.70	0.70

* TM - salt mix contained in percent, not less than 0.250 Mn, .033 Cu, .0025 Co, .005 Zn, 97 NaCl, .1 S, .2 Fe and .007 I.

** Vitamin premix contained per Kg, 1,056,000 IU, Vit. A; 275,000 IU, Vit. D ; 4,400 IU, Vit. E; 1.76 mg, Vit B_{12}; 1.320 mg, riboflavin; 8.8 gm niacin; 1.76 mg d-pantothenic acid; 76.384 mg choline; 217.8 mg mendione; 220 mg folic acid and 22 mg d-biotin.

Experiment 2

In this experiment, the effect of three different feed additives (Cu, Zn-bacitracin and Neo-Terramycin) on the growth rate of 1200 day-old Nicholas White tom turkeys were compared to each other in another factorial design. Birds received either low protein diets, identical to those used in Experiment 1 or a high protein diet (32, 29, 24, 21, 18 and 16% protein, dropped at 4-week intervals) recommended by Waibel, 1975. Birds on the low protein diet received either 75 or 125% of the NRC (1977) recommended S-AA levels. Compositions of high protein diets are shown in Table 3. These diets provided 125 of the NRC (1977) recommended levels of S-AA. Supplements of 60 ppm Cu, 200 gram per ton Neo-Terramycin (80 gram per ton Terramycin after 12 weeks) and 50 gram per ton of Zn-bacitracin (25 gram per ton after 8 weeks) were used in this experiment. Three replicates were used for each treatment. This experiment was conducted for 24 weeks, starting 9-22-82 and ending 3-8-83.

Table 3. Composition of high protein diets (%).

Ingredient	Age of birds (weeks)					
	0-4	4-8	8-12	12-16	16-20	20-24
Corn	21.27	28.50	47.25	53.77	60.70	64.40
SBM (48%)	58.37	52.40	37.15	30.73	25.00	20.60
Fish meal	3.03	2.60	2.23	1.80	1.40	1.00
Yellow grease	10.98	10.50	8.50	9.20	9.30	10.50
Limestone	1.54	1.50	1.33	1.22	.64	.97
Dicalcium phosphate	3.01	2.80	2.46	2.15	1.85	1.50
TM - salt*	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix**	1	1	0.5	0.5	0.5	.50
DL-methionine	0.3	0.23	0.18	0.15	0.09	0.03
Calculated Analysis						
Crude protein (%)	31.73	29.25	23.88	20.82	18.24	16.24
ME (Kcal/kg)	3007	3080	3185	3290	3380	3475
Lysine (%)	2.08	1.88	1.42	1.21	1.03	0.86
S-AA (%)	1.30	1.16	0.96	0.83	0.70	0.56

*, ** See Table 2.

Experiment 3

Another 1200 day-old tom turkeys were used in this experiment to investigate the effects of Neo-Terramycin and copper on the growth rate of turkeys and to study if the effects of these two compounds were additive. One half of the turkeys received the low protein dietary series similar to those which were used in Experiment 1 (Table 2) and the other half received low protein series diets which contained 20% wheat bran. The protein percentages in these diets were similar to diets used in Experiment 1 but they were not isocaloric. Table 4 shows the composition of these diets. Birds received either no feed additives at all as a control group or 200 gram/ton Neo-Terramycin (80 gram/ton Terramycin after 12 weeks) or 120 ppm as copper sulfate in addition to Neo-Terramycin. Birds were kept on the experiment for 24 weeks from 12-4-83 to 6-17-84. Three replicate groups for each treatment were used in this experiment.

Table 4. Composition of low protein diets including wheat bran.

Ingredients	Age of birds (weeks)					
	0-4	4-8	8-12	12-16	16-20	20-24
Corn	30.44	38.21	46.27	51.79	57.19	62.46
SBM (47%)	35.50	27.66	22.13	16.86	11.55	6.32
Wheat bran	20	20	20	20	20	20
Alfalfa meal	2	2	2	2	2	2
Fish meal	2	2	--	--	--	--
Grease	1	1	1	1	1	1
Limestone	3	3	3	3	3	3
DiCal-PO4	2	2	2	2	2	2
TM - salt*	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix**	1	0.5	0.5	0.5	0.5	0.5
Ameri-bond	2	2	2	2	2	2
Lysine	0.27	0.40	0.41	0.21	0.16	
DL-methionine	0.29	0.23	0.20	0.15	0.10	
Calculated Analysis						
Crude protein (%)	23	20	18	16	14	12
ME (Kcal/kg)	2328	2400	2485	2545	2600	2635
Calcium (%)	1.43	1.4	1.3	1.3	1.3	1.3
Phosphorus (%)	1.0	0.96	0.90	0.86	0.85	0.85

*, ** See Table 2.

Experiment 4

In this experiment only the west house with 24 pens was used. A total of 180 males and 216 females were used. Each pen contained either 15 males or 18 females. This experiment started on 1-10-84. Females were on experiment for 16 weeks and were off test on 5-4-84. Males were on the experiment for 20 weeks and were off the test on 5-29-84. This experiment was designed to investigate the effects of a combination of virginiamycin and amprolium, a coccidiostatic drug, on the growth of turkeys. All birds received 0.025% amprolium in their diet. One half of birds also received 20 gm/ton virginiamycin in addition to amprolium.

A normal protein series diets recommended by NRC (1977) provided 28, 26, 22, 19 and 16% protein for each successive 4-week period (Table 5). Six replicates for each treatment were used in this experiment.

Table 5. Composition of normal protein diet used in experiment 4.

Ingredients	Age of birds (weeks)				
	0-4	4-8	8-12	12-16	16-20
Corn	41.35	47.55	57.6	65.8	73.6
SBM (47%)	51.0	45.4	35.2	27.2	19.4
Alfalfa meal	2	2	2	2	2
Limestone	2	2	2	2	2
Dicalcium phosphate	2	2	2	2	2
TM - salt*	0.5	0.5	0.5	0.5	0.5
Vitamin premix**	1.0	0.5	0.5	0.5	0.5
DL-methionine	0.15	0.05	--	--	--
Calculated Analysis					
Protein (%)	28	26	22	19	16
ME (Kcal/kg)	2690	2760	2860	2950	3025
Lysine (%)	1.73	1.58	1.67	1.03	0.8
S-AA (%)	1.05	0.90	0.75	0.65	0.55

*, ** See Table 2.

RESULTS AND DISCUSSION

Experiment 1

Average data for body weights at 4 and 8 weeks of age are shown in Table 6. Up to 8 week of age, poults receiving the 75% of NRC (1977) recommended levels of sulfur-containing amino acids had significantly ($P<0.05$) lower body weights compared to those on the 100 or 125% S-AA diets. Addition of 120 or 240 ppm copper significantly ($P<0.05$) decreased body weights of poults up to 8 weeks of age. Turkeys on diets containing 60 ppm Cu did not show any response to this feed additive at 4 weeks of age and their body weights were comparable to poults receiving the control diet. However, addition of this level of Cu significantly ($P<0.05$) increased body weights at 8 weeks of age.

Tables 7 and 8 show the average body weights of turkeys at 12, 16, 20 and 24 weeks of age. At 12 weeks of age, the results for the S-AA treatments were the same as for 8 weeks of age with turkeys on the 100 or 125% S-AA levels being significantly ($P<0.05$) heavier than those on the 75% levels of S-AA. At 16 weeks of age, poults receiving the 100 or 125% S-AA were still heavier than those receiving the 75% of NRC levels of S-AA but the significance level was at $P<0.1$ rather than $P<0.05$. No significant differences in body weights were observed in the 12 to

24 week data due to the addition of any levels of Cu. No interactions between levels of methionine and copper were observed at any age.

Table 6. Average body weights of male turkeys at 4 and 8 weeks of age as affected by levels of Cu and S-AA.

S-AA content as % of NRC	Copper, ppm 0	60	120	240	Average
			BW @ 4 weeks, gm		
75	780	786	736	717	755 [a]
100	821	875	817	796	823 [b]
125	835	833	773	798	810 [b]
Average	812 [b]	825 [b]	775 [a]	770 [a]	
			BW @ 8 weeks, kg		
75	2.86	2.94	2.71	2.66	2.79 [a]
100	3.17	3.24	3.06	3.07	3.14 [b]
125	3.16	3.25	3.11	3.12	3.16 [b]
Average	3.06 [b]	3.15 [c]	2.97 [a]	2.96 [a]	

a,b,c Means with different superscripts are significantly different ($P<0.05$).

Table 7. Average body weights of male turkeys at 12 and 16 weeks of age as affected by levels of Cu and S-AA.

S-AA content as % of NRC	Copper, ppm 0	60	120	240	Average
			BW @ 12 weeks, kg		
75	5.68	5.53	5.49	5.35	5.51[a]
100	5.81	5.93	5.91	5.88	5.88[b]
125	5.92	5.91	5.78	5.79	5.85[b]
Average	5.80	5.79	5.73	5.69	
			BW @ 16 weeks, kg		
75	8.71	8.73	8.70	8.52	8.66*
100	9.06	9.26	8.95	9.21	9.13
125	9.20	9.05	9.04	9.21	9.13
Average	9.00	9.01	8.90	8.98	

a,b Means with different superscripts are significantly different ($P<0.05$). The absence of superscripts on main effect averages indicates no significance.

* $P<0.1$.

Table 8. Average body weights of male turkeys at 20 and 24 weeks of age affected by levels of Cu and S-AA.

S-AA content as % of NRC	Copper, ppm 0	60	120	240	Average
			BW @ 20 weeks, kg		
75	11.53	11.30	11.53	11.44	11.45[a]
100	11.58	11.74	11.30	11.68	11.58
125	11.76	11.71	11.78	11.84	11.79
Average	11.63	11.58	11.54	11.68	
			BW @ 24 weeks, kg		
75	13.47	13.30	13.43	13.60	13.45
100	13.59	13.62	13.15	13.75	13.53
125	13.50	13.64	13.66	13.58	13.59
Average	13.52	13.52	13.42	13.64	

[a] The absence of superscripts on mean effect averages indicates no significant differences.

The results from this experiment which showed growth responses due to the addition of methionine up to 16 weeks of age confirms the applicability of NRC (1977) data for the S-AA requirements by turkeys up to this age. These levels of S-AA are higher than those reported to be required by Potter and Shelton (1974). They obtained maximum body weights of turkeys at 4 and 8 weeks of age by using lower levels of protein and sulfur amino acids. The

disappearance of a response to methionine at higher than 75% of the NRC recommended levels after 16 weeks in this experiment indicate that 75% of the S-AA may be adequate for older turkeys.

The growth depression effect up to 8 weeks of age due to the addition of 120 or 240 ppm copper is in general agreement with other reports. Harms and Eberst (1974) and Carlson et al. (1979) reported growth depressions due to the addition of 120 or 130 ppm Cu. On the other hand, Sullivan and Tordrop (1975) did not obtain any responses from the addition of 125 or 250 ppm Cu to turkey diets. However, Scott and Peter (1965) compared copper sulfate (550 mg/kg) with 13 other antibiotics and antimicrobial agents and reported a growth stimulation from copper sulfate comparable to that from effective antibiotics and other antimicrobial agents. Guenthner et al. (1978) reported a growth stimulation effect in turkeys from 120 or 240 ppm Cu when normal or low protein diets contained 4-nitrophenylarsonic acid (4-nitro) at 0.01875% of the diet. These authors suggested that it is possible that 4-nitro at the recommended level ties up the naturally occurring copper in the feed and causes growth depression in turkeys receiving diets without copper supplementation. They also reported that incidence of aortic rupture was higher among turkeys receiving 4-nitro, with copper supplementation dramatically reducing this incidence in the presence

of 4-nitro. Bowen et al. (1971a, 1971b) demonstrated that cupric sulfate interferes with the prophylactic efficacy of 4-nitro and two other antihistomonal agents against histomonasis (Blackhead disease) in turkeys. Czarnecki and Baker (1984) reported that Roxarsone (3-nitro-4-hydroxyphenylarsonic acid), a commonly used feed additive which has been used to increase chick gain and improve feed efficiency, when used in the treatment of coccidiosis has caused growth depression in the presence of 250 ppm Cu. It should also be noted that although the NRC (1977) recommended level for copper is 4-6 ppm for turkey poults, studies indicate that the requirement may be higher (Scott et al. 1982). The latter authors recommend a 10 ppm copper level for poultry. The total copper content in the rations used by Guenthner et al. (1978) was about 7 ppm. If 4-nitro ties up Cu, a copper deficiency with depressed growth would result. Addition of copper would overcome this deficiency and improve growth. The higher incidence of aortic rupture among turkeys receiving diets without copper supplementation, in the Guenthner et al. (1978) study, can also be explained in that copper is required for incorporation of lysine into desmosine (elastin) in the aorta.

Because many turkeys are now raised in confinement and the risk of blackhead disease is minimal, the antihistomonal agents frequently are not used in turkey diets which may minimize the chance of copper deficiency. In this case copper probably will provide its stimulating effect at lower levels (60 ppm), and higher levels such as 120 or 240 ppm could be harmful to turkey performance perhaps because of its possible interactions with other nutrients such as sulfur or with feed additives.

Copper was shown to interfere with sulfur-containing amino acids utilization in chickens when purified or semi-purified diets were used (Robbins and Baker, 1980a, 1980b). These authors also showed that the addition of 250 ppm copper increased liver and gizzard copper concentration and gizzard erosion score. Poupoulis and Jensen (1976) and Jensen and Maurice (1978) also reported an increase in gizzard erosion score due to copper additions. Methionine supplementation was reported to decrease copper concentration in the liver (Robbins and Baker, 1980a). However, supplemental methionine was reported to be inactive in preventing the copper-induced gizzard erosion (Robbins and Baker, 1980b, Jensen and Maurice, 1978), supporting the idea that copper per se, and not the copper induced S-AA deficiency, was responsible for gizzard erosion.

High levels of copper (500 ppm) have been shown to increase S-AA requirements by 30% (Robbins and Baker,

1980b). Severe methionine deficiency may lead to a decrease in critical sulfur-containing metabolites such as glutathione and result in an increased susceptibility of the gizzard lining to oxidative damage. Similarly, copper is a strong oxidizer and also is readily associated with free SH-groups. Thus, Cu may limit glutathione availability and also increase gizzard susceptibility to oxidation. Glutathione is required for conversion of cystine to cysteine. Although animals can utilize cystine, cysteine, per se, is the form required for protein synthesis. Copper can readily be chelated by compounds with free SH-groups such as cysteine or glutathione (Figure 4). Once the copper-SH bond has formed (with cysteine or glutathione) the product can not be easily dissociated and would be excreted as such. This would limit the cysteine availability. The cystine-Cu chelate also could lower copper toxicity.

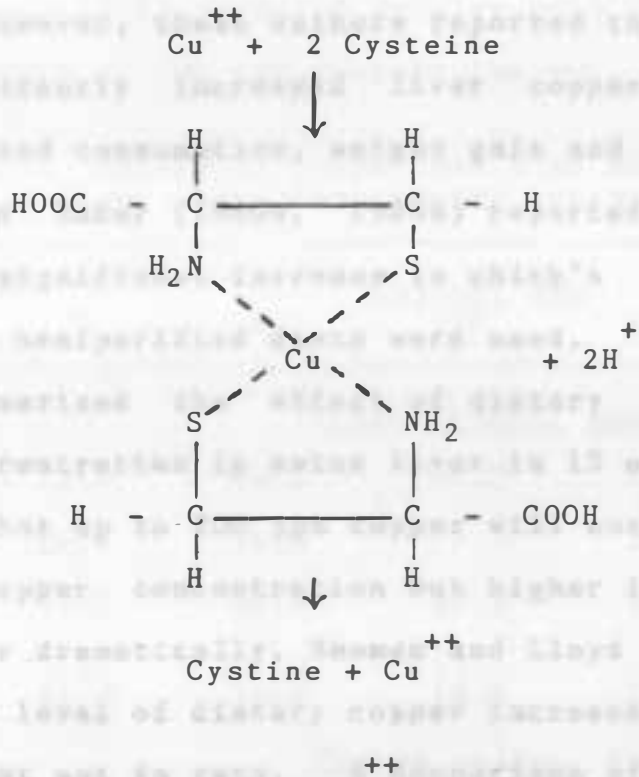


Figure 4. The chelation of Cu^{++} by cysteine and subsequent oxidation to cystine.

As can be seen from Table 9, copper concentration in liver and plasma were not affected significantly by addition of any levels of copper or methionine. These results are consistent with the report of Guenthner et al. (1978) that showed no change in liver copper of male turkeys at 24 weeks of age due to the addition of 240 ppm Cu to the diet. Unchanged plasma copper values in this experiment are also consistent with the Thomas and Goatcher (1976) report which showed no significant increase in the blood copper of laying hens from the addition of 720 ppm dietary

copper. However, these authors reported that 720 ppm copper significantly increased liver copper content, and decreased feed consumption, weight gain and egg production. Robbins and Baker (1980a, 1980b) reported that 250 ppm caused a significant increase in chick's liver Cu when purified or semipurified diets were used. Cromwell et al. (1981) summarized the effect of dietary copper on the copper concentration in swine liver in 15 experiments and reported that up to 200 ppm copper will not significantly increase copper concentration but higher levels increase liver copper dramatically. Beames and Lloyd (1965) reported that a high level of dietary copper increased liver copper in pigs but not in rats. A comparison of these results with the results obtained from this experiment indicates that the metabolism of high dietary copper in turkeys must be different from chickens. The data in this experiment showing no changes in plasma and liver copper contents not support an increased absorption of copper with dietary increases up to 240 ppm. Thus, it could be concluded that the effect of copper on growth is not due to its absorption and metabolism but could be due to its effect on the elimination of certain microorganisms in the intestine or changes in the thickness of the small intestinal wall. King (1972) had reported that the weights of the small intestine of chicks expressed as a percentage of body weights were reduced by feeding 100 ppm copper. In another

report, King (1975) reported smaller ceca in ducklings due to the addition of 100 ppm dietary copper.

Table 9. Effect of copper and S-AA addition on liver and plasma copper content.

S-AA content as % of NRC	Copper, ppm 0	60	120	240	Average
			Liver copper, ppm*		
75	13.9	18.0	18.3	16.1	16.6[a]
100	14.4	16.5	17.7	24.9	18.4
125	17.7	16.6	22.3	15.5	17.5
Average	15.3	16.4	19.4	18.8	
			Plasma copper, ppm		
75	.16	.14	.14	.16	.15
100	.17	.15	.17	.19	.17
125	.15	.16	.19	.15	.16
Average	.16	.15	.17	.17	

* Dry basis.

[a] The absence of superscripts on main effect means indicate no significant differences.

Table 10 shows the overall feed conversion, (units of feed required to produce one unit of gain), obtained in this experiment. Turkeys on 100 or 125% of the S-AA were slightly more efficient (1%) in converting feed than those on 75% S-AA. Addition of copper to the diets did not make

any significant changes in overall feed consumption in this study. However, at 16 weeks, the age of which many turkeys are being marketed, birds on 120 or 240 ppm Cu were 8% more efficient and those on 60 ppm copper were 3% more efficient in converting feed as compared to the control group.

Table 10. Effect of levels of copper and S-AA on the accumulative feed conversion (0-24 weeks).

S-AA content as % of NRC	Copper, ppm 0	60	120	240	Average
75	3.05	3.12	3.07	3.03	3.08[a]
100	3.07	3.05	3.06	2.99	3.04
125	3.08	2.98	3.06	3.03	3.04
Average	3.07	3.05	3.06	3.02	

[a] The absence of superscripts on main effect means indicate no significant differences.

Experiment 2

Table 11 shows the average body weights of turkeys at 4 and 8 weeks of age. At all stages, turkeys receiving the higher protein diet showed significantly ($P<0.05$) heavier body weights compared to those on the low protein diets. Poults on diets with the 125% NRC levels of S-AA grew at a significantly ($P<0.05$) increased rate over those on the 75% S-AA diets at this age. Addition of Neo-Terramycin at 4 weeks of age caused a significant ($P<0.05$) increase in body

weights over that of poults receiving 60 ppm copper or no feed additives at all. No significant differences ($P>0.05$) were observed among poults receiving either Neo-Terramycin or bacitracin in their diets although poults on the Neo-Terramycin containing diet seemed to be heavier than those on bacitracin.

At 8 weeks of age, poults receiving Neo-Terramycin or bacitracin were significantly ($P<0.05$) heavier than those on the control diet or those receiving 60 ppm of copper. Turkeys on Neo-Terramycin were also significantly ($P<0.05$) heavier than those on bacitracin. No significant differences were observed due to the addition of 60 ppm copper at this age. As at 4 weeks of age, poults on the low protein diet with the 125% NRC levels of S-AA were produced significantly ($P<0.05$) heavier than those on the 75% levels of S-AA.

Table 12 shows the average body weights at 12 and 16 weeks of age. Turkeys on the high protein diet still showed significant ($P<0.05$) superiority over those on low protein diets. On low protein diets, turkeys receiving the 125% NRC levels of S-AA had significantly ($P<0.05$) heavier body weights compared to those receiving only 75% of the NRC levels of S-AA. For the 12-week data, addition of Neo-Terramycin caused a significant ($P<0.05$) increase in body weight over that of turkeys receiving either the control diet or the diet with 60 ppm copper. Also, at 12 weeks of

age, turkeys receiving bacitracin were significantly heavier compared with those on the supplemented copper diet. At 16 weeks of age, no significant differences ($P>0.05$) were observed due to the addition of any of the feed additives studied in this experiment, although turkeys receiving bacitracin seemed to do better.

The average body weights of turkeys at 20 and 24 weeks of age are shown in Table 13. At these ages, although the 125% NRC levels of S-AA caused only a slight increase in body weight compared to 75% S-AA on the low protein diets, the differences were not statistically significant ($P>0.05$). None of the feed additives under investigation caused any significant differences in body weights although Terramycin and bacitracin appeared to show some numerical increase in body weights at 24 weeks of age. Copper at 60 ppm caused a slight decrease in body weight. Statistical analyses did not show any interactions between levels of protein and feed additives used in this experiment.

Table 11. Average body weights of male turkeys at 4 and 8 weeks of age as affected by levels of protein, S-AA and feed additives.

Diet	Control	Neo-Terramycin 200 gm/ton	Bacitracin 50 gm/ton	Cu 60 ppm	Average
		BW @ 4 weeks, gm			
Low protein 75% S-AA	775	803	776	754	777[a]
Low protein 125% S-AA	789	875	834	808	822[b]
High protein 125% S-AA	1029	1033	1018	1012	1023[c]
Average	864[a]	898[b]	876[a,b]	858[a]	
		BW @ 8 weeks, kg			
Low protein 75% S-AA	2.75	2.95	2.89	2.79	2.85[a]
Low protein 125% S-AA	3.05	3.29	3.15	3.06	3.14[b]
High protein 125% S-AA	3.90	3.99	3.91	3.84	3.91[c]
Average	3.23[a]	3.41[c]	3.31[b]	3.23[a]	

a,b,c Means with different superscripts are significantly different ($P<0.05$).

Table 12. Average body weights of male turkeys at 12 and 16 weeks of age as affected by levels of protein, S-AA and feed additives.

Diet	Control	Terramycin 200 gm/ton	Bacitracin 50 gm/ton	Cu 60 ppm	Average
		BW @ 12 weeks, kg			
Low protein 75% S-AA	5.79	6.03	5.99	5.67	5.87 [a]
Low protein 125% S-AA	6.26	6.44	6.28	6.16	6.28 [b]
High protein 125% S-AA	7.19	7.40	7.36	7.16	7.28 [c]
Average	6.41 [a,b]	6.62 [c]	6.54 [b,c]	6.32 [a]	
		BW @ 16 weeks, kg			
Low protein 75% S-AA	8.86	8.77	9.07	8.62	8.83 [a]
Low protein 75% S-AA	9.29	9.23	9.41	9.07	9.25 [b]
High protein 125% S-AA	10.45	10.67	10.69	10.74	10.64 [c]
Average	9.53	9.56	9.72	9.48	

[a,b,c] Means with different superscripts are significantly different ($P<0.05$).

Table 13. Average body weights of male turkeys at 20 and 24 weeks of age as affected by protein, S-AA and feed additives.

Diet	Control	Terramycin 80 gm/ton	Bacitracin 25 gm/ton	Cu 60 ppm	Average
		BW @ 20 weeks, kg			
Low protein 75% S-AA	11.56	11.96	11.66	11.42	11.65 [a]
Low protein 125% S-AA	11.83	11.97	12.00	11.67	11.87 [a]
High protein 125% S-AA	13.77	14.18	14.02	14.05	14.00 [b]
Average	12.38	12.70	12.56	12.38	
		BW @ 24 weeks, kg			
Low protein 75% S-AA	13.41	13.91	13.40	13.24	13.49 [a]
Low protein 125% S-AA	13.62	13.96	13.88	13.21	13.67 [a]
High protein 125% S-AA	16.09	16.77	16.37	16.33	16.39 [b]
Average	14.37	14.88	14.55	14.26	

a,b Means with different superscripts are significantly different ($P<0.05$).

The results obtained in this experiment agree in general with the Guenthner (1978) report which showed that turkeys on high protein diets maintained significantly ($P<0.05$) heavier body weights compared with turkeys receiving the low protein diets. Significant ($P<0.05$) differences in body weights up to 16 weeks of age of turkeys on low protein diets receiving 75 or 125% of the NRC (1977) recommended level of sulfur-containing amino acids confirms the results obtained in our first experiment. After 16 weeks, turkeys on the 125% S-AA low protein diet were only slightly heavier than those receiving 75% S-AA. The differences were not significantly different, confirming once again that 75% S-AA must be adequate for the older turkey for optimum growth. However, it should be noted that a normal protein diet which is routinely used in turkey production, contains more soybean meal such that by the time that birds are about 12 weeks of age, their diets provide enough methionine and cysteine. These levels probably are even greater than the 100% NRC recommended levels.

The lack of a copper effect on growth rate disagrees with the results obtained in Experiment 1 which showed significant increases in poult body weights at 8 weeks of age. Guenthner *et al.* (1978) also reported that 60 ppm copper was not adequate for stimulating growth in turkeys. The lack of copper stimulatory effect could be because

growth responses to antibiotics often disappear on continued use in an old environment (Waibel et al., 1954; Libby and Schaible, 1955). Copper is believed to stimulate growth like antibiotics by eliminating harmful microorganisms in the intestine (Cromwell et al., 1981; Stutz, et al., 1983). However, Heth and Bird (1962) reported that the stimulatory effect of antibiotics could reappear in the same old facilities. Recently, Dafwang et al. (1984) reported that several antibiotics are still effective in promoting growth even after they have been used continuously for over 30 years. Some reports indicated that new antibiotics, which have not been used previously, stimulated growth more than older antibiotics (McGinnis et al., 1958; Wiese and Petersen, 1959). We have used copper in our experiments for several years and if the information about disappearance of an antibiotics effect is true, we could be in the situation of losing the copper stimulatory effect. Waible et al. (1954) also reported that in such disappearance cases, the growth rate of the basal group was often improved to the level achieved by antibiotic supplementation. According to growth standards reported by Jensen in 1981, large type male turkeys should weigh 11.57 kg at 20 weeks of age. In our studies, the average weights of turkeys not receiving any medication or 60 ppm copper at 20 weeks of age were 12.38 kg which is considerably higher

than the standard weights. This is relevant, as feed additive supplements usually show greater growth responses when the growth level is suboptimum.

The stimulatory effect of bacitracin and Neo-Terramycin at an early age is in agreement with the reports of several other investigators. Wiegers and Sullivan (1959) and Daghighian and Waibel (1982) reported a significant increase in body weights of turkeys up to 8 weeks of age from the addition of bacitracin. Combs and Bossard (1963) compared different antibiotics with each other and showed male chicks responded significantly to Terramycin at 28 days but not at 47 days of age. They did not find any significant difference in body weight due to addition of bacitracin. Chang and Waible (1970) reported a significant increase in body weights of three week old turkeys due to the addition of bacitracin. Heuser and Norris (1952) reported that bacitracin and Terramycin stimulated growth in chickens and the greatest relative growth stimulation of chicks due to antibiotics was found to occur during the first four weeks. The differences in weight disappeared as the chicks grew older. The same results have also been reported by Potter et al. (1962) who showed that chicks responded significantly to antibiotics at 4 weeks but not at 8 weeks of age. Our results confirm these reports in that the effect of bacitracin disappeared after 8 weeks of age and the Neo-Terramycin effect on body weights was

significant ($P<0.05$) only up to 12 weeks of age. The longer stimulatory effect of Neo-Terramycin on weight gain was probably due to the fact that this antibiotic was first used in our experimental station for this study. Some investigators believe that the magnitude of responses obtained from newly introduced antibiotics is greater than from those that have been in use for many years (McGinnis et al., 1958; Marusich et al., 1973).

Table 14 shows the accumulative feed conversion obtained in this experiment. Turkeys on high protein diets consumed significantly ($P<0.05$) less feed per unit of gain and converted feed 20% more efficiently when compared with those on low protein diets. On low protein diets, turkeys receiving the 125% NRC levels of S-AA were 2.5% more efficient than those on 75% S-AA levels. All feed additives appeared to improve feed conversion (though not statistically significant) with Neo-Terramycin showing the most effect.

The results on feed efficiency in this experiment showing better feed:gain ratio due to feeding high protein diets are similar to those obtained by Waibel (1981) and Guenthner et al. (1978). The feed efficiencies of turkeys on low protein diets receiving the 75% S-AA were poorer than those on 125% S-AA. This is probably due to the fact that these turkeys had to consume more feed to satisfy

their sulfur amino acids need rather than, as generally accepted, their energy need (Carlson, 1984).

Table 14. Accumulative feed conversion of male turkeys as affected by protein, S-AA and feed additives.

Diet	Control	Terramycin	Bacitracin	Cu	Average
Low protein 75% S-AA	3.22	3.08	3.10	3.15	3.14[b]
Low protein 125% S-AA	3.12	3.01	3.08	3.06	3.07[b]
High protein 125% S-AA	2.62	2.55	2.51	2.59	2.57[a]
Average	2.98	2.88	2.90	2.93	

a,b Means with different superscrpits are significantly different ($P<0.05$).

Experiment 3

Tables 15 and 16 show the average body weights obtained due to the addition of Neo-Terramycin (Terramycin after 8 weeks) and a combination of Neo-Terramycin and copper. Inclusion of 20 percent wheat bran in this study resulted in a significant ($P<0.05$) decrease in body weights from 4 to 24 weeks of age. At 4 weeks of age, although poults receiving diets without wheat bran seemed to be heavier, the differences were not statistically significant. The results from this study agree with the results obtained by other investigators. Hollister and Nakaue (1982) and Cannon et al. (1982) observed a decreased growth rate and poorer feed efficiency when the amount of fiber was increased in the diet of chicks. Bayer et al. (1978) reported that the addition of 6% cellulose to a chicks' diet after 3 weeks of age resulted in significantly lower average weights and poorer feed efficiency.

The lower body weight of turkeys on the diet with 20% wheat bran is probably due to the lower energy content of this diet. As mentioned before, the low protein diets without wheat bran for each 4-week interval were isonitrogenous but not isocaloric to the corresponding diet with 20% wheat bran. A comparison of the data in Tables 2 and 4 shows that the metabolizable energy contents of the wheat bran diets were approximately 470 Kcal per kg lower than

for the diets without wheat bran. Wheat milling by-products are relatively low in available energy and high in fiber content and animals would have to eat more of these types of diets to satisfy their energy and amino acid needs. Hill and Dansky (1954) reported that the chick is able to increase its intake of nutrients to compensate for the lower energy of certain diets, but its ability to increase the amount of bulk ingested has physical limitations. However, the chick's capacity to increase the consumption of fibrous feeds is greater for those feeds of high moisture-absorbing capacity such as wheat bran (Bell, 1960).

Wheat bran is not usually used in high energy poultry rations because of its relatively low metabolizable energy content. However, there may be a potential for increasing the ME content of this product. Cave et al. (1965) reported that for growing chicks pelleting wheat bran increased its ME availability content by 30%. Saunders et al. (1968 and 1969) showed that pelleting wheat bran ruptured the cell walls, thus making nutrients more available. Enzymic treatment of wheat bran has also been reported to increase its protein digestibility (Neudoerffer and Smith, 1969; Saunders et al. 1972).

In another study, pelleting of this feed produced turkeys that were significantly heavier than those receiving the diet in the form of mash.

Feed additives did not produce any significant differences in body weights at 4 weeks of age. At 8 weeks of age, poults fed Neo-Terramycin or a combination of Neo-Terramycin and copper were significantly ($P<0.05$) heavier than those receiving the control diets. There were no significant differences in body weights between poults receiving the combination of Neo-Terramycin and copper or Neo-Terramycin alone. However, at this age, those poults which received a combination of the two feed additives, seemed to be slightly heavier indicating that the effect of the combination could be additive. Cromwell et al. (1981) reported that as compared to the effects of single additions a combination of copper and tetracyclines has resulted in a better rate of gain and an improvement in feed efficiency in young pigs. Guenthner et al. (1978) indicated that bacitracin may have enhanced the responses to copper by turkeys. In contrast, Bowen and Sullivan (1971) reported that the addition of copper to the diets containing a low level of antibiotics (22 ppm of penicillin - streptomycin, 1:3) resulted in reduced body weights of turkeys at 4 weeks of age. The results obtained from our first experiment showed that 120 ppm Cu significantly decreased body weights. This level of copper also might have decreased the body weights of turkeys at 8 weeks of age instead of stimulating it. The results might have been different if 60 ppm copper would have been used. However,

more studies should be conducted with different rations to clarify the additive effect of a combination of copper and different antibiotics since Beames and Lloyd (1965) indicated that it appears that ration composition may influence the response to copper. Cromwell et al. (1981) reported that the additive response to copper may be due to (1) copper having a broader spectrum of antibacterial activity, or (2) copper having a different mode of action than the antibiotics.

No significant differences due to the addition of Terramycin or a combination of Terramycin and copper were observed after 8 weeks of age. However, at 24 weeks of age, turkeys receiving only Terramycin in their diet seems to be heavier. Turkeys on the diet containing both Terramycin and copper were slightly smaller than the controls. It should be also noted that the suppressing effect due to copper was apparently greater for birds receiving diets with 20% wheat bran. The interactions between factors under investigation were not significant at any age.

Table 15. Effect of form of diet and feed additives on performance of turkeys (4, 8 and 12 weeks).

Diet	Control	Neo-Terramycin 200 gm/ton	Neo-Terramycin + Cu 200 gm/ton + 120 ppm	Average
		BW @ 4 weeks, gm		
Low protein	896	878	877	884[a]
Low protein (20% wheat bran)	814	881	807	830[a]
Average	855[a]	880[a]	842[a]	
		BW @ 8 weeks, kg		
Low protein	3.18	3.29	3.32	3.26[a]
Low protein (20% wheat bran)	2.77	2.97	3.08	2.94[b]
Average	2.87[a]	3.13[b]	3.20[b]	
		BW @ 12 weeks, kg		
Low protein	6.99	7.33	7.07	7.13[a]
Low protein (20% wheat bran)	6.35	6.52	6.56	6.47[b]
Average	6.67	6.92	6.81	

[a,b] Means with different superscripts are significantly different ($P<0.05$).

Table 16. Effect of form of diet and feed additives on performance of turkeys (16, 20 and 24 weeks).

Diet	Control	Terramycin 80 gm/ton	Terramycin + Cu 80 gm/ton + 120 ppm	Average
		BW @ 16 wks, kg		
Low protein	10.10	10.34	9.97	10.13[a]
Low protein (20% wheat bran)	9.37	9.58	9.40	9.45[b]
Average	9.73	9.96	9.68	
		BW @ 20 wks, kg		
Low protein	12.45	12.83	12.52	12.60[a]
Low protein (20% wheat bran)	11.42	11.37	11.24	11.34[b]
Average	11.93	12.10	11.88	
		BW @ 24 wks, kg		
Low protein	14.69	14.87	14.67	14.75[a]
Low protein (20% wheat bran)	13.41	13.39	13.16	13.32[b]
Average	14.05	14.13	13.81	

a,b Means with different superscripts are significantly different ($P<0.05$).

Table 17 shows the accumulative feed conversion obtained in this study. Turkeys on the diets containing 20% wheat bran converted feed over 11% less efficiently than those not receiving wheat bran. These results indicate that such diets are not applicable for turkey feeding unless the price of wheat bran is low enough to compensate for the reduced rate of growth and poorer feed efficiency. Turkeys on the Terramycin diet alone were apparently less efficient in converting feed as compared to the controls or as compared to the combination of Terramycin and copper.

Table 17. Effect of form of diet and feed additives on accumulative feed efficiency of turkeys (0-24 wks). (Unit feed/Unit gain)

Diet	Control	Terramycin 80 gm/ton	Terramycin ± Cu 80 gm/ton + 120 ppm	Average
Low protein	3.42	3.61	3.36	3.46[a]
Low protein (20% wheat bran)	3.79	3.92	3.89	3.86[b]
Average	3.60	3.76	3.62	

[a,b] Means with different superscripts were significantly different ($P<0.05$).

Experiment 4

Table 18 shows the average body weights and overall feed conversion of each sex obtained in this study from the virginiamycin addition. Virginiamycin supplementation significantly ($P<0.05$) increased body weights of turkeys (both males and females) up to 16 weeks of age. However, after 16 weeks of age, when the females were removed from the experiment, no significant differences were observed.

Feed conversion data in this table do not show significant differences due to virginiamycin supplementation. However, at their usual market age, females on the virginiamycin diet had converted feed 2% better than those on the control diet. Males showed no effect at this time.

Table 18. Effect of virginiamycin on performance of turkeys.

	Males		Females		Averages (♂ and ♀)	
	Control*	Virginia-mycin*	Control	Virginia-mycin	Control	Virginia-mycin
Period, wks			BW, kg			
4 weeks	.927	.974	.809	.868	.868[a]	.921[b]
8 weeks	3.37	3.47	2.73	2.89	3.05[a]	3.18[b]
12 weeks	6.36	6.56	4.94	5.30	5.65[a]	5.93[b]
16 weeks	9.80	10.10	7.14	7.46	8.47[a]	8.78[b]
20 weeks	12.35	12.23	--	--	--	--
F/G 0-20 (16) weeks	3.19	3.17	3.12	3.06		

* All diets contained 0.025% amprolium.

a,b Means with different superscripts were significantly different ($P<0.05$).

Although virginiamycin was isolated by DeSomer and Van Dijck in 1955, its usage in animal feeds has not been studied extensively. In our experiment, virginiamycin was used in the presence of 0.025% amprolium which combination had not yet been approved by the Food and Drug Administration (FDA). Our results are consistent with the results obtained by other researchers. As early as 1962, Yates and Shaible had reported positive responses by chicks and poults to virginiamycin both as to weight gain and feed efficiency. Combs and Bossard (1963) reported that virginiamycin stimulated growth of broiler chickens up to market age. Miles et al. (1981) observed that virginiamycin supplementation of layer diets resulted in significantly better egg production, feed efficiency and egg specific gravity. However, virginiamycin fed hens produced eggs that were 2 grams smaller in this study. These workers in another experiment fed virginiamycin to Leghorn-type pullets from 8 to 20 weeks of age. They observed that the addition of virginiamycin to the low protein diets resulted in heavier body weights at 12, 16 and 20 weeks of age as well as improved feed efficiency during the overall 8 to 20 week period (Douglas et al., 1982). Harms and Miles (1983) reported that virginiamycin significantly increased turkey poult body weights when the diet was deficient in methionine, indicating that virginiamycin could spare the methionine requirements.

Concerns regarding the possible hazards of continuous low level feeding of antibiotics which are important in human and veterinary medicine has stimulated interest in the dietary use of those antibiotics such as virginiamycin which are not generally used for disease control. It has been proposed that meat, milk and eggs from animals fed antibiotics contribute to the incidence of transferable drug resistance in consumers. Virginiamycin is one of the newer antibiotics approved for use in animal feeds. Tissue residues due to the addition of virginiamycin are uncommon because like bacitracin, the activity of this antibiotic is isolated to the intestinal tract. Virginiamycin is known to be active against the gram-positive bacteria of the gut, thus development and transfer of resistance, a phenomenon limited to the gram-negative Enterobacteriaceae is negligible.

Careful studies are needed to determine the source of antibiotic resistance in humans and to what degree that resistance originates in animals and their flora. Further research is needed to determine if chronic oral exposure of humans to antibiotics and low-level, feed additive usage in animals compromises therapy in human or animals enough to warrant discontinuation of their use. Perhaps, it is appropriate to end this dissertation with a statement from Dr. T. H. Jukes, one of the great pioneers in antibiotics

research for agricultural use, which appeared in Federation Proceedings in 1977.

> "In recent years, the Food and Drug Administration has been making strenuous efforts to diminish or ban the use of the leading antibiotics in agriculture. Whether or not such bureaucratic measures are imposed on American agriculture, the finding that small amounts of antibiotics, added to the diet, improved the growth rate and utilization of food in apparently healthy animals, remains as one of the best established and least expected findings in nutrition."

SUMMARY

Four experiments with Large White Nicholas stock were designed to study the effect of four different feed additives on the growth of turkeys when different levels of protein in corn-soy type diets were used. The data obtained in these experiments support the following general conclusions:

1. The NRC (1977) recommended levels of sulfur-containing amino acids for turkeys up to 16 weeks of age were applicable when a low protein dietary series were used.

2. Copper at 60 ppm stimulated the growth rate of turkeys while 120 or 240 ppm Cu depressed growth up to 8 weeks of age.

3. Addition of sulfur-containing amino acids up to 125% of the NRC (1977) recommended level did not overcome the growth suppression shown by the addition of 120 or 240 ppm Cu. This indicates that the depressed growth is not related to the interference of Cu with S-AA metabolism.

4. Addition of Cu did not influence liver or blood copper content.

5. High protein diets produced heavier turkeys and a substantial improvement in feed efficiency as compared to the observations made with low protein diets.

6. Bacitracin stimulated growth up to 8 weeks of age while Neo-Terramycin stimulated growth up to 12 weeks of

age and virginiamycin promoted growth in the presence of amprolium up to 16 weeks of age.

7. The combination of Neo-Terramycin and copper did not show that the effects of these drugs are additive under our conditions.

8. Although feed additives appeared to improve feed efficiency, these differences were not statistically significant.

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