EFFECTS OF FEED ADDITIVES AND ZINC ON TISSUE CONSTITUENTS OF RATS AND CHICKENS FED ZINC-DEFICIENT DIETS

by

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INTRODUCTION

Livestock production, which is a dynamic business in the U. S. A., is destined for greater efficiency. Research workers, men from the feed industry, and livestock farmers are trying to develop new techniques and methods to cut the feed cost and to get the fastest weight gains in production. Recent nutrition research discoveries indicate that feed additives may play an important role in attaining these objectives. The widespread and successful use of diethylstilbestrol as a feed additive for the past five to seven years has stimulated many attempts to develop other feed additives. Antibiotics, diethylstilbestrol and tranquilizers have been widely tested as growth stimulants. Simultaneously, widespread and promiscuous use of these feed additives has created a fear in man as to possible residues occurring in meat, milk and eggs which are harmful when consumed.

The reproductive disturbances and cancer producing possibilities of diethylstilbestrol and other feed additives are receiving attention and close scrutiny by the Food and Drug Administration. The beneficial effects of estrogens in atherosclerosis has been investigated by some workers. Efforts are now directed towards the etiology of biochemical lesions that may result in hardening of arteries, diabetes and cancer. Dietary factors affecting blood cholesterol level of different species have been extensively investigated. This keen interest

in blood cholesterol level is due to the suggested relationship between the occurrence of atherosclerosis and the level of cholesterol in the blood.

The appearance of parakeratosis in swine, which is cited as being caused by a high calcium and low zinc ration, has refocused the attention of animal nutritionists and physiologists to the importance of interrelationships among nutrients. The interrelationship between blood cholesterol level and essential fatty acids has been reported on numerous occasions. The effects of feed additives and zinc deficiency on erythrocytes are of much interest to physiologists.

The objectives of this investigation were: (a) to compare the effects of additional dietary zinc, bacitracin, zinc bacitracin, corn oil and tranquilizers on body weight gains, blood cholesterol, hemoglobin, and hematocrit of rats fed a zinc-deficient diet and (b) to study the effects of additional dietary zinc, bacitracin, zinc bacitracin and diethylstilbestrol (hereafter called stilbestrol) on the weight gains, blood cholesterol, hemoglobin and hematocrit of day old chicks fed a zinc-deficient diet for twelve weeks. Data are also presented on weights of some organs taken from rats and chickens at the end of the experiment.

REVIEW OF LITERATURE

General Effects of Zinc Deficiency

Todd and his associates (1934) were the first to show evidence of a need for zinc in the diet of animals. They found that the addition of zinc salts to a synthetic diet improved the growth rate of rats.

Forbes and his associate (1960) found in their zinc requirement and balance studies that the zinc requirement of young albino rats was 18 ppm. They have reported that the increase of calcium from 0.8 to 1.6% level of the diet caused reduction in weight gains at all levels of zinc.

In reviewing the biochemistry, physiology and pathology of zinc, Bert (1959) stated that the growth of zinc-deficient rats proceeds at one-third the normal rate. A zinc deficiency could be produced by giving a diet which provides 22μ gm. per day and 30μ gm. per day caused marked improvements. He is of the opinion that high calcium content in the diet is a trigger factor in producing zinc deficiency. According to him there may be some unidentified factors in the diet other than calcium which are involved in producing zinc deficiency. He has also listed a number of metal enzyme complexes activated by zinc. He has mentioned that erythrocytes and bone were found to accumulate zinc, while a high zinc content has been noticed in the male genital tract.

While studying the effects of zinc deficiency on the reproductive system of male rats Millar et al. (1958) found that zinc deficient rats showed slow body growth, depressed growth and development of testes, accessory sex organs and, in some, severe atrophy of testicular germinal epithelium.

Mehring et al. (1956) made a comparison of the growth of chicks fed diets containing different quantities of zinc. From hatching to nine weeks of age, groups of chickens were given a basal ration and water from which the total intake of zinc was 42 to 45 ppm. Supplements of zinc were given to nine experimental groups so that the intake was from 57 to 823 ppm. Growth and feed efficiency were not affected by zinc intake.

Roberson and Schiable (1958) have found that a purified ration containing Drackett protein and 19 ppm. zinc when given to day old chicks caused dermatitis of the feet, abnormal gait and abnormal feathering. When they supplemented the ration with 100 ppm. zinc, growth was significantly improved and the efficiency of feed conversion increased. In another study the same authors did not notice any leg abnormalities in chicks fed a basal diet containing 10 ppm. zinc but severe dermatitis was present. In their opinion 20 ppm. supplemental zinc was the minimum requirement for satisfactory growth.

Edwards et al. (1959) found that addition of zinc to a corn soya basal diet containing either 37 or 41 ppm. of zinc

improved growth and feed efficiency of chicks. The improvement resulting from 20 ppm. of supplemental zinc in the basal diet containing 37 ppm. of zinc was found to be statistically significant at the 1% level. There was a growth response from the addition of zinc to the diet containing 41 ppm., but this was not statistically significant at the 5% level.

Moeller and Scott (1958) observed marked deficiency symptoms in chicks fed a basal diet containing 13 ppm. zinc. According to them maximum growth was obtained with 20 ppm. of additional zinc.

Young and his associates (1958) noticed short stocky legs, enlarged and stiff joints as the symptoms of zinc deficiency. They found that an additional 40 ppm. of zinc were required for chicks fed a diet containing 15 ppm.

Supplee and his associates (1958b) observed abnormal hock development in battery reared chicks on a purified diet when zinc was not supplemented. Zinc supplementation relieved this condition and improved growth and normal hocks resulted.

Supplee et al. (1958a) have also reported that white poults fed a ration containing 24 ppm. zinc in a basal ration showed a relatively high incidence of hock disorders in all pens except those receiving 0.01% zinc chloride.

Slinger et al. (1956) found that the addition of 50 ppm.

of zinc to the basal diet of chicks which contained by analysis 58 ppm. resulted in a significant increase in the four weeks' weight of males and tended to depress the weight of females.

O'Dell and Savage (1956) gave chicks a purified basal ration containing 6% of a mineral mixture with zinc chloride 0.023% and an additional 1.25% calcium carbonate, and showed that supplementation of zinc stimulated growth. Kratzer and his associates (1958) could produce perosis in turkey poults by feeding them a purified ration containing an isolated soybean protein. These authors have reported that the addition of 40 ppm. of zinc to the basal ration containing 26 ppm. zinc, improved growth and markedly reduced the severity of perosis.

Pensack and his associates (1958) found that chicks grew poorly on a ration containing 6 ppm. zinc and exhibited abnormal feathering and a stiff-legged gait. According to them a purified casein ration containing 20 ppm. zinc meets the requirement of the growing chick.

There are several reports about the incidence of parakeratosis in swine related to zinc deficiency. According to Klussendorf (1956) parakeratosis has been observed in nearly all regions of the United States, and several workers have produced the condition almost at will and corrected the

condition by varying the amount of zinc and calcium in the ration.

Hoefer et al. (1959) found that parakeratosis occurred in all growing swine not receiving a zinc supplement. Zinc supplementation improved the performance at all calcium levels even though in each case pig performance on the lower calcium level was better.

Smith et al. (1959) observed that a basal diet of corn and soybean meal containing 33 ppm. zinc produced parakeratosis from 12 to 16 weeks.

Febrando and Seller (1957) reported a summary of experiments on several farms, where groups of pigs with parakeratosis were given choline, methionine and sodium sulfate or 1,000,000 I.U. of vitamin A daily or 22.5 mg. zinc carbonate daily per 100 kg. diet. According to them the first treatment restored appetite but had no further effect. The second treatment caused some improvement and the third brought about complete cure. The zinc salt was less effective if the diet was high in calcium and phosphorous.

Hoefer and his associates (1960) found that although less effective than zinc, copper reduced the incidence and severity of parakeratosis in swine.

Effects of Feed Additives on Growth Rate

Antibiotics

The effect of antibiotics on growth rate of different species has been reported by several workers. Maeda (1958) has reported that aureomycin (chlortetracycline) improved growth of chicks by 17% and terramycin (oxytetracycline) by 2.6% compared with rations not containing antibiotics. Braham et al. (1959) have found an increase in weight of chickens and rats when zinc bacitracin was added to the diet in which soybean meal was the only source of protein. Dixon and Thayer (1951), Henser and Norris (1952), McGinnis et al. (1950) are other workers who have reported beneficial effects of antibiotics on chicks. Revat and associates (1957) have shown that antibiotics, erythromycin or soframycin in the diet increased the weight gains of rats.

Bird and associates (1952) found that chicks fed aureomycin as a feed supplement showed growth responses only after three to four weeks of age in a new environment while the response to the same antibiotic was evident after one week in an old environment.

Day and associates (1958) have reported that the addition of bacitracin to a broiler ration containing 19.4% protein produced 12.1% weight increase over the basal. Lewis and Sanford (1953) have shown that the supplemental bacitracin to either the soybean

or the cottonseed basal diet caused significant growth stimulation. According to them the combination of the antibiotic supplements Aurofac and bacitracin appeared to be the most consistent of all antibiotic supplements used by them.

Fletcher and Barrentine (1954) have shown that increases in rate of gain produced in pigs by aureomycin or penicillin supplementation of a corn cottonseed meal ration were near but did not reach levels necessary for statistical significance.

Beeson and associates (1954) implanted bacitracin pellets in suckling lambs and have shown that these antibiotic pellets had no significant effect on the growth rate.

Bridges and associates (1954) have described two experiments involving 84 weanling pigs and they concluded that the addition of bacitracin and penicillin to a milo-soybean oil meal ration did not significantly increase the growth rate above the control.

Hanson et al. (1956) have reported that under the conditions of their experiment the implantation of bacitracin pellets did not affect weaning weights or survival of the pigs to 8 weeks of age. Swenson et al. (1958) reported that bacitracin pellets implanted subcutaneously did not alter the growth rate, hemoglobin, and hematocrit. They did not find any histological changes caused by bacitracin in liver, heart, kidneys, spleen, ovaries or testicles of pigs at 8 weeks of age.

Waibel et al. (1954) suggested that continuous use of antibiotics over a long period produced an environment with a lowered germ load or disease potential.

Sherman and Donovan (1958) have observed that a decreased gain in weight obtained in some laboratories is a relative change inasmuch as performance of control birds had improved, presumably because continued antibiotic feeding reduced the environmental disease level.

Tranquilizers

There are some reports in the literature emphasizing tranquilizers as growth stimulants when used as feed additives in cattle rations. No information, however, could be obtained regarding the action of trifluomeprazine on rats in this respect.

Jordan and Hanke (1960) studied the effect of various tranquilizers on growth and fattening of lambs. They observed that trifluomeprazine had no significant effect on average daily gains, feed consumption and feed efficiency regardless of the level at which it was fed.

Burroughs and associates (1959) fed 5 to 20 mg. trifluomeprazine per day to fattening steers, and obtained little response at these levels. However, when they fed 10 to 20 mg. per day to open heifers, they obtained additional daily gain.

Perry et al. (1960) demonstrated that feeding

trifluomeprazine at 2.5, 5 or 10 mg. per day to beef steers either with implanted stilbestrol or with oral antibiotic did not result in increased rate of gain or improved feed efficiency.

Corn oil

The necessity of essential fatty acids in the diet of animals and man has received considerable attention by nutritionists. The use of corn oil for curing zinc deficiency or promoting growth gains has not been tried, particularly in rats.

Lewis and associates (1955) have reported that birds getting additional 2% maize oil in their diet were heavier than those getting the basal ration. Donaldson and associates (1957) could improve the growth of chicks by adding 28.7% maize oil to a semi-synthetic diet containing 2% fat. Significant increases in live weight of pullets due to the addition of 8% corn oil were reported by Heywant (1943). According to Carver and Johnson (1953) crude and refined corn oils contain some factors which stimulated chick growth. By comparing rape seed oil with corn oil Beare and associates (1959) found that rats fed corn oil had greater weight gains.

Holman (1957) has reported that parakeratosis can be treated by administration of unsaturated oils. He has also induced parakeratosis in swine by the feeding of a diet low in essential fatty acids. It thus appears that the disease

may be an EFA deficiency which is complicated by imbalances in dietary minerals. In the experiment conducted by Holman, the onset of EFA deficiency as judged by dermal symptoms was most rapid in the group fed high calcium and no zinc. It was least rapid in the group fed zinc and low calcium. The results suggest that the dermal symptoms seen in the rats and parakeratosis of swine induced by dietary inadequate EFA are influenced by imbalance in dietary minerals.

Diethylstilbestrol

There are several reports about the effects of stilbestrol on the growth rate of different species. Chemical caponization by hormone implantation has been very popular until recently in the United States.

Geneva and Meyer (1956) have shown that stilbestrol injection in cockerels improved the rate of gain, but did not affect feed efficiency.

Data obtained by Camp et al. (1957) indicate that the greatest growth response from this hormone injection was obtained in protein levels of 14% and 16%. They could not obtain any growth response when the birds were fed a 21% protein ration after the injection.

Lorenz (1945) attempted to produce the tenderizing of the flesh and fattening by feeding stilbestrol orally at levels

up to 64 mg. per Kg. of feed. According to him his attempts were largely unsuccessful.

According to Jaap (1945) and Munro and Kosin (1946) stilbestrol has a relatively low oral estrogenic potency for the chicken. Thayer and associates (1944) concluded that large oral doses of estrogens are required for fattening chickens. Mountney et al. (1957) studied the influence of stilbestrol on the growth and quality of turkeys and found that stilbestrol fed at the level of 20 mg. per pound of feed gave the best over all results. Carter and associates (1958) have shown that the addition of 16 and 32 mg. stilbestrol in 100 pounds of feed when fed to white turkeys from 9 to 12 weeks of age significantly improved weight gains.

Serum Cholesterol

Effects of dietary fat

Among the dietary factors affecting serum cholesterol level, essential fatty acids, saturated and unsaturated fat have been studied in several species by a number of workers. Avigan and Steinsberg (1958) have shown that rats fed a diet containing 20% corn oil have a significantly lower concentration of serum cholesterol than those fed equal amounts of cocoanut oil, but both high fat diets lead to elevation of serum cholesterol above that seen on control diets.

In a review of dietary regulation of serum cholesterol level, Portman and his associates (1959) have listed the different dietary factors regulating the serum cholesterol levels of experimental animals. The authors have discussed in detail the effects of dietary fats on blood cholesterol level.

By feeding adult male albino rats diets containing fats of varying degrees of saturation and essential fatty acid content Coleman and his associates (1959) found that serum cholesterol is elevated by increasing saturation and that dietary cholesterol augments this effect for the more saturated oils. According to them, elevation of serum cholesterol appears transient in less saturated oil but is more sustained for highly saturated fats.

Grunbaum et al. (1957) found an increase in serum cholesterol of rats subsisting on Purina Chow diet when supplemented with butter fat or sunflower seed oil. Cuthbertson, et al. (1959) reported a drop in rat serum cholesterol level when hydrogenated arachis oil in diet was replaced by arachis oil or maize oil. According to Olson et al. (1958) the degree of unsaturation of the dietary fat fed to rats did not seem to affect blood cholesterol concentration. Lambert and his associates (1958) showed that rabbits fed cholesterol supplemented purified diets containing 20% hydrogenated shortening or safflower oil had lower plasma cholesterol levels on safflower oil diets. Similar studies with cholesterol free diets showed

that rabbits on hydrogenated cocoanut oil are much more prone to hypercholesteremia than rabbits on safflower oil diets. Reports about the serum cholesterol lowering effect of unsaturated fat in man prompted Kritchevsky et al. (1959) to investigate the effects of these fats on cholesterol metabolism. They found that the mitochondrial oxidation of the terminal carbon atoms of cholesterol is much greater in rats fed large quantities of saturated fat than in rats fed equivalent amounts of unsaturated fat.

The relation between dietary fat and serum cholesterol level in humans has been extensively investigated because of the suggested relationship that exists between serum cholesterol level and atherosclerosis. Swell et al. (1955) found that the levels of serum cholesterol in rats were highest when unsaturated fats were fed. Grande et al. (1958) have concluded that unsaponifiable matter of 100 gms. of corn oil lowers serum cholesterol between 6 and 12 mg. per 100 ml. of human blood serum. In studying the effects of unsaturated fatty acids on α and β lipoproteins and serum cholesterol levels Labacki and associates (1958) found that 20% of cases showed lower total serum cholesterol levels. Katz and his associates (1958) have reported that isocaloric substitution of unsaturated vegetable oil for saturated animal lipids in the diet resulted in a significant fall in plasma cholesterol levels of man and animals. Cook (1958) has observed that plasma cholesterol levels of vegetarians

is lower than that of those who take vegetable, eggs and milk, while both these groups had lower cholesterol level than the nonvegetarians. In studying the effect of consuming eggs with increased content of unsaturated lipids Gordon et al. (1958) found that blood cholesterol level was increased after consumption of such eggs. Kinsel et al. (1953) found reduced serum cholesterol levels after the administration by stomach tube of emulsions of cotton seed oil, alone or mixed with soybean oil. Beveridge et al. (1956) could produce a significant reduction in plasma cholesterol level by substituting corn oil and margarine for butter in diet. They have concluded that corn oil contains a factor that helps in the reduction of plasma cholesterol level.

Anderson et al. (1957), Tobian and Tuna (1957) and Sinclair (1956) are other workers who have confirmed the finding that corn oil causes a depression of serum cholesterol level.

Contrary to the above findings Pomeranze and his associates (1958) found that serum cholesterol in three infants rose when a daily supplement of 50 gm. maize oil was added to a diet of evaporated milk and fell when it was omitted.

There are also reports about the influence of corn oil, nature of the fat, and composition of the diet on blood cholesterol concentration in the chick. Svacha et al. (1958)

observed that corn oil, animal tallow, butter and oleomargarine are different in their effect on serum cholesterol in the chick. Daghir (1960) found that 12% soybean oil has a significant depressing effect on serum cholesterol concentration when compared with 12% grease or with a normal laying mash containing no added fat.

Effects of metallic elements

In the search for finding out therapeutic agents for lowering blood cholesterol level, the effects of mineral deficiency, administration of metallic elements, and the interrelationship among minerals and cholesterol have also been extensively studied.

Curran (1954) has investigated the effect of several of the transient group elements on the synthesis of cholesterol by rat liver homogenates and found that vanadium and iron salts significantly reduced synthesis of cholesterol while titanium, nickel, copper and zinc had no effect.

Tennent et al. (1958) found that chickens fed as much as 0.05% of the diet as cobaltous chloride have significantly reduced serum cholesterol concentrations. They have concluded that this effect is by a local action in the intestinal tract.

Day and Peters (1958) found that in 10 days after injection of uranium acetate into normally fed rabbits cholesterol levels

rose.

Boyd and Maclean (1959) found that intravenous injections of cobalt raised the plasma cholesterol in rabbits.

Vitale and his associates (1957) found that additions of cholesterol and cholic acid to diets of young rats containing 24 mg. per cent magnesium induced magnesium deficiency. Later Vitale and his associates (1959) while studying the interrelationship between dietary calcium and magnesium in atherogenesis found that the level of magnesium in the diet of young rats had no effect on serum cholesterol, but they found unexpected high serum cholesterol values in rats on low calcium diets without addition of cholesterol.

Mountain et al. (1956) have shown that elevation of free and total cholesterol in plasma was significantly restricted by the addition of 50 ppm. vanadium to the diet.

Siperstein and associates (1953) have reported that feeding ferric chloride largely depressed the hypercholesteremia associated with feeding diets containing cholesterol or cholesterol and bile concentration. They believed that this is as a result of precipitation of bile salts by ferric chloride.

Beher et al. (1957) studied the effect of ferric chloride on mobilization of accumulated liver cholesterol in mice. They came to the conclusion that ferric ions had no effect.

Effects of tranquilizers

Wertlake and his associates (1958) have shown that significant increases in mean value for serum cholesterol accompanies mental and emotional stress. The effect of tranquilizers on blood cholesterol level has not been known to have received much attention, even though tranquilizers are being used in atherosclerosis with the idea of decreasing stress conditions. Kritchevsky (1958) has quoted several workers who found sizable reduction of blood cholesterol level in clinical trials with 2 - phenylbutyric acid.

Effects of antibiotics

Literature related to the action of antibiotics on serum cholesterol is scarce. Samuel and Steiner (1959) found that the oral administration of neomycin was associated with a significant decrease in serum cholesterol concentration of all 10 human patients treated and the fall remained until the end of the experiment. They have reported that further studies using antibiotics other than neomycin are in progress. Later Samuel (1959) reported that oral administration of neomycin decreased blood cholesterol level significantly in 18 patients. When phthalylsulfathiazole, isoniazid, dihydrostreptomycin, oxytetracycline, polymixin B sulfate, bacitracin and novobiocin were given orally, no appreciable changes in serum cholesterol resulted. According to Nelson and his associates (1953) blood

cholesterol level was increased when aureomycin was given with cholesterol orally to rabbits.

Effects of diethylstilbestrol

It has been demonstrated by Pick et al. (1952), Muskowitz et al. (1956), and other workers that estrogens are able to prevent atherosclerosis in several species and exert a beneficial effect even in man. Malinow et al. (1958) have shown that intravenous administration of estradiol benzoate prevented aortic atherosclerosis in cholesterol fed rabbits.

Forbes and Petterson (1951) have reported that subcutaneous administration of stilbestrol to three month old cockerels increased greatly the concentration of readily extractable cholesterol, total cholesterol and neutral fat in plasma. Baum and Meyer (1956) have reported that blood lipids are elevated and depot fat increased in intact birds by both a regular and fat free diet after injection of stilbestrol.

Clyde and Suydam (1959) have produced atheromatosis and minor changes in serum cholesterol levels in chickens by subcutaneous implantation of stilbestrol. The administration of estrogens to chickens has been shown by Fleischmann and Fried (1945) to increase the total cholesterol of blood plasma. Thus as shown above some workers have obtained increase in blood cholesterol level, while others have shown that estrogens

are able to prevent atherosclerosis.

General Effects of Feed Additives on Organs and Tissues

The effects of stilbestrol on organs and tissues have been studied extensively, but there is very little information in the literature concerning the other feed additives.

Moreng and his associates (1956) have demonstrated that a minimum dose of 12 mg. stilbestrol implanted subcutaneously resulted in a decreased testicular size. According to them, response to treatment varied with breeds and younger birds responded more readily.

Data obtained by Eaton et al. (1955) indicate that cockerels 10 or 11 weeks of age implanted with stilbestrol were slightly less fertile than control group. The semen volume was reduced but their testes size at 32 weeks was not affected.

Fraps and associates (1956) tried to ascertain the effects of excessive dosages of this hormone on subsequent reproductive performance of chicks by implanting 2, 4 or 8 pellets containing 15 mg. stilbestrol in each pellet. The onset of semen production in treated birds was delayed and subsequently semen volume was considerably lower than in controls. The adverse effect increased with increasing dosage. The hatchability was little affected by single pellets but was appreciably reduced

with higher dosage.

In the group of 8 to 16 week old chickens fed synthetic estrogens, Kumar and Turner (1949) observed that the testicular growth was greatly inhibited, but not completely. According to them histological examination of the testes revealed that spermatogenesis had not progressed beyond spermatogonial stage. They believe that estrogens depress the secretion of F.S.H. They also observed the depression in the growth of the comb. In their experiment they found that the body and pancreas weights were higher in experimental birds than controls. According to Sturkie (1954) almost all investigators have reported that estrogens increase the amount of fat deposited in the tissues.

Campbell (1959) found that administration of estrogens increased blood volume and fall in hemoglobin concentration, probably due to a direct effect of increased blood volume. Campbell (1960) could alter hemoglobin concentration in immature pullets by administration of (a) estrogen and thyroxine and (b) estrogen and sulphamethazine.

In studying the effects of aureomycin on the liver of rats Murray and Campbell (1955) found that the livers of females given aureomycin were significantly smaller than those livers of females not given aureomycin, but in males there was no significant difference in weights.

Investigating the effects of feeding zinc on the livers of rats Sadasivan (1951) found that addition of 0.5% and 1% of zinc to a rat stock diet resulted in lowering gains and smaller livers.

MATERIALS AND METHODS

Sixty male and 60 female rats aged 24 days were used in the first experiment. Five rats from each group were selected at random and allotted to each cage. The rats were numbered by cutting notches in their ears. Treatments were assigned to the 12 cages containing male rats and 12 additional cages containing female rats from a table of random numbers as given by Snedecor (1957). The rats were of the Sprague Dawley strain.

In experiment II, 50 one-day old cross-bred White Cornish male chickens were used. They were selected at random and divided into two equal groups and assigned to each compartment of a battery brooder after they were wingbanded.

In the 3rd experiment 100 White Cornish cross-bred broiler female chickens were used. Twenty-five chickens were selected at random and allotted to each compartment of a battery brooder and wing banded. Treatments were assigned randomly to these groups.

Special precautions were taken to prevent the rats and chickens used in the experiments from obtaining supplemental amounts of zinc from water or from contact with cages, brooders and growing batteries. These precautions consisted of coating cages, brooder and growing batteries with two coats of plastic paint. The rats were given feed in acid washed porcelain feeders and water in acid washed glass fountains. The chicks

were fed and watered in plastic coated feed and water troughs. All the rations were fed ad libitum throughout the test period. Fifty pounds of feed for each lot were mixed in a large mixer and stored in paper bags. The feed additives were first mixed in 1 lb. of soybean meal in a mortar and pestle and then this quantity was added to the feed at the time of mixing. First 13 chicks from each treatment were removed to a new growing battery when they were one month old and the remaining twelve of each group were removed to another growing battery when they were one and one-half months old.

In the first experiment the basal diet shown in Table 1 was used. Care was taken to see that the diets adequately supplied the nutritive requirements of the young growing rats (Spector, 1956, Brown and Sturtevant, 1949 and Rose, 1938). According to calculation the basal diet contained only 22.3 p. p. m. of zinc. Zinc, bacitracin, zinc bacitracin, corn oil and trifluomeprazine were the feed additives added to the different treatments.

In the second experiment the basal diet shown in Table II was used for one group and stilbestrol was added to the basal diet for the other group. This ration was prepared so as to supply all the established nutritive requirements for the young growing chicken (Bird et al., 1954). According to calculation this diet contained only 16.9 p. p. m. zinc.

Table I. Basal diet of rats used in experiment I.

Ingredients	Amount %
Corn dent yellow	47.5
Soybean meal (solvent extracted)	20.0
Alfalfa meal (dehydrated)	7.5
Fishmeal (menhaden)	5.0
Brewer's yeast dried	7.5
Dried skim milk	10.0
Iodized salt	0.5
*Mineral mix (salt mix)	2.0

*Salt mix - prepared according to the formula of Hubbel, Mendel and Wakeman, (1937) by General Biochemicals Inc., Chagrin Falls, Ohio was composed of the following ingredients:

	%
Aluminum potassium sulfate	0.017
Calcium carbonate	54.300
Ferric phosphate	2.050
Copper sulfate	0.090
Magnesium carbonate	2.500
Magnesium sulfate	1.600
Manganese sulfate	0.039
Potassium chloride	11.200
Potassium iodide (stabilized)	0.011
Potassium phosphate (monobasic)	21.200
Sodium chloride	6.900
Sodium fluoride	0.100

In the third experiment the basal diet was the same as used for the second experiment. Bacitracin, zinc bacitracin and zinc were the feed additives used in the different rations. One group was fed the basal diet. Tables III and IV give the calculated analysis of the basal diets.

Table II. Basal diet for chickens used in experiment II.

Ingredients	Amount %
Corn dent yellow	65
Soybean meal (solvent extracted)	20
Alfalfa meal (dehydrated)	5
Fishmeal (menhaden)	5
Dried skim milk	2
Calcium carbonate	1
Dicalcium phosphate	0.5
Iodized salt	0.5
*Vitamin premix (I.S.U.)	1.0
Manganese sulfate	3 gm. /100 lbs.

*Vitamin premix, supplied by the Poultry Husbandry Department of Iowa State University, provides when added at 1% in a lb. of ration of the following:

Vitamin A	3000 I. U.
Vitamin D	500 I.C.U.
Vitamin K	1 mg.
Riboflavin	2 mg.
Panthothenic acid	4 mg.
Choline	200 mg.
Vitamin B_{12}	7.5 mcg
Methionine	0.05%
Protein	0.2 %
Niacin	13 mg.

Table V shows the quantities of feed additives included in the different rations of the experiments. The rat and chicken rations were added to the feeders twice a day for 4

weeks. After 4 weeks the rations were added to the feeders only once daily. Distilled water was provided ad libitum and changed daily.

Table III. Calculated analyses of basal diets of rats in experiment I.

Protein	24.88%
Fat	2.90%
Fiber	4.22%
T. D. N.	73.85%
Calcium	1.17%
Phosphorous	0.73%
Zinc	22.3 p.p.m.
Vitamin A	333 mg./100 lbs.

Table IV. Calculated analyses of basal diets of chickens in experiments I and II.

Protein	19.70%
Fat	3.40%
Fiber	3.74%
T. D. N.	72.13%
Calcium	0.994%
Phosphorous	0.608%
Zinc	16.9 p.p.m.
Vitamin A	7300 IU/lb.

Table V. Quantities of Feed additives added.

Experiment No.	Treatment	Diet and feed additives used
I.	1	Basal diet (Table I)
	2	Basal diet (Table I) + zinc carbonate 3.508 gms/100 lbs. (40 ppm)
	3	Basal diet (Table I) + bacitracin* 5 gms/100 lbs.
	4	Basal diet (Table I) + zinc bacitracin* 5 gms/100 lbs.
	5	Basal diet (Table I) + corn oil** 2 lbs/100 lbs.
	6	Basal diet (Table I) + trifluomeprazine*** 227 mgm/100 lbs.
II.	1	Basal diet (Table II)
	2	Basal diet (Table II) + stilbestrol**** 7.5 gms/100 lbs.
III.	1	Basal diet (Table II)
	2	Basal diet (Table II) + bacitracin 5 gms/100 lbs.
	3	Basal diet (Table II) + zinc bacitracin 5 gms/100 lbs.
	4	Basal diet (Table II) + zinc carbonate 3.508 gms/100 lbs. or 40 ppm.

*Bacitracin and zinc bacitracin used in the experiments were supplied by Commercial Solvents Corporation, Terre Haute, Indiana.

**Corn oil was manufacutred by Corn Products Company, New York.

***Trifluomeprazine was the product of Research Laboratories, Smith Kline and French Labs., Philadelphia.

****Stilbestrol was provided by Eli Lilly Laboratories.

The rats in experiment I were weighed individually at the beginning of the experiment and then once a week for 11 weeks. The rats were examined regularly for symptoms of dermatitis. At the end of 11 weeks one rat from each cage was sacrificed every day. The rats were stunned by a blow on the head and the axilla was opened by a sharp knife. Two samples of blood were taken, 2 ml. in a clean, dry test tube and 3 ml. in a test tube containing a dry anticoagulant recommended by Heller and Paul (1934).

The heart, liver and kidneys were removed, stripped of extraneous tissue and weighed. In the male rats the testicles were also weighed.

The chickens in experiments II and III were weighed individually when one day old, then once a week for 12 weeks. The birds were examined regularly for bone deformities, abnormal gait, abnormal feathering or dermatitis of the feet. At the end of 12 weeks two birds from each growing battery were bled by heart puncture using a 5 ml. glass syringe and 20 gauge needle. The method used in obtaining the blood is the method described by Hofstad (1950). Two samples of blood were taken, 2 ml. in a clean dry test tube and 3 ml. in an oxalated tube as in the previous experiment.

The birds were then sacrificed by dislocating the neck at atlanto-occipital joint and their liver, pancreas, spleen,

ovary or testicles removed and weighed. The testicles from the chickens of experiment II were preserved in formalin solution for histological examination.

Immediately after collection the blood was stored in a refrigerator for a period not exceeding 36 hours. The coagulated blood was centrifuged and the serum used for estimation of total cholesterol.

The method used was that described by Zlatkis et al. (1953) using a Beckman B model spectrophotometer. A description of the procedure is given in the Appendix. Rosenthal and associates (1957) have mentioned that stock ferric chloride solutions as well as the color reagent prepared from the stock solution used in this method precipitates on standing. To prevent this, freshly prepared color reagent was used and when kept for a couple of days the color reagent was kept in the refrigerator.

Immediately after bleeding, capillary tubes were filled with oxalated blood, sealed, and centrifuged in the International microcapillary centrifuge for 5 minutes. Hematocrit values were read in an Adam's microhematocrit reader.

The hemoglobin content of the rat blood was determined by the acid hematin method described by Cohen and Smith (1919).

The hemoglobin in the chicken blood was determined in the spectrophotometer by a method described by Swenson (1951). A summary of the method is given in the Appendix.

EXPERIMENTAL RESULTS

General Symptoms of Zinc Deficiency

The symptoms of zinc deficiency could not be observed in the rats used in experiment I. One month after the beginning of the treatments, the rats fed the basal ration continuously gnawed at the cage and on examination of the cage it was found that most of the plastic paint was removed and the metal was exposed.

Among the chickens the male birds fed the basal ration showed pronounced symptoms of zinc deficiency. Enlargement of the hock, crooked keel or both were noticed in six out of eleven birds in one growing battery fed the basal ration and three out of thirteen birds in another growing battery fed the basal ration. These birds with bone abnormalities showed abnormal feathering also. No severe symptoms of dermatitis of the feet could be observed in the experiment. Only two stilbestrol fed chickens had bone abnormalities.

In experiment III the symptoms of zinc deficiency were not evident.

Effects of Feed Additives on Weight Gains of Rats and Chickens

The weekly weights of rats in grams are given in Table VI. The details of analysis of variance in the weights of the male

and female rats among different treatments at the end of the treatment are presented in the Appendix. In the male rats this analysis did not reveal any significant differences among treatments. In female rats the differences in weights at 11 weeks were significant at .05 level among treatments. The group of rats fed corn oil showed the greatest gain in weight. The bacitracin group had the lowest gain in weight. The difference (15.4 gm) between the corn oil group and those fed bacitracin was significant at the .05 level. This was also true for the difference (13.0 gm.) between the corn oil and zinc groups. The difference (14.6 gm.) between the trifluomeprazine group and rats fed bacitracin was significant at the .05 level as was also true for the difference (12.2 gm.) between the trifluomeprazine and zinc groups. The difference (116.4 gm.) between chickens fed zinc bacitracin and zinc and those fed bacitracin in their rations is significant at .05 level. The difference of 66.8 grams between the basal group and those supplemented with zinc and with zinc bacitracin reached the .05 level of significance. Among treatment comparisons are also presented in the Appendix.

The stilbestrol fed chickens in experiment II showed a significant increase in weights at .01 level at the end of the experimental period. The weekly weights of chickens are shown in Table VII.. Analysis of variance is given in the appendix.

Table VI. Experiment I. Average weekly weights of rats in grams

Weeks	Treatments											
	Basal		Zinc		ZN Bacitracin		Bacitracin		Corn oil		Trifluomeprazine	
	F	M	F	M	F	M	F	M	F	M	F	M
0	48.0	60.4	50.4	58.6	52.0	57.3	51.0	60.5	51.9	60.55	52.2	57.7
1	78.1	99.2	83.3	93.4	83.6	90.7	80.8	97.3	82.5	95.5	86.5	95.2
2	103.9	136.8	114.4	133.1	119.1	133.0	110.8	139.0	117.0	130.2	116.4	132.6
3	130.4	172.6	132.5	164.3	138.4	167.2	126.4	173.3	136.6	160.3	138.2	160.5
4	146.5	203.0	193.0	197.3	157.2	203.7	144.2	213.71	153.8	192.6	157.6	193.9
5	158.3	236.4	158.4	232.2	170.4	241.6	154.6	251.3	167.3	225.3	165.2	226.9
6	176.8	270.5	174.2	260.1	183.4	273.2	172.2	283.8	181.7	264.1	185.0	263.5
7	188.6	292.6	184.0	283.0	191.9	303.0	181.1	308.3	193.7	286.6	194.1	283.7
8	203.5	318.2	185.9	290.9	204.7	323.0	189.5	325.6	202.8	305.3	202.9	304.0
9	206.6	336.8	200.5	319.8	210.6	342.0	198.4	341.7	210.7	329.6	209.0	325.3
10	208.0	346.8	206.4	334.2	213.8	351.3	203.4	351.8	215.8	344.7	214.4	340.80
11	215.0	365.4	211.3	351.7	220.2	370.0	208.9	370.5	224.3	361.7	223.5	355.4

F = females, M = males.

In experiment III analysis of variance also showed (vide appendix) significant differences among different treatments at .01 level at the end of the treatment. Bacitracin group had the lowest gain in weight in this experiment also. The weekly mean weights of chickens are shown in Table VIII. Zinc bacitracin and zinc supplemented groups showed the greatest gain in weight. Among treatment comparisons are also presented in the appendix.

Table VII. Average weekly weights in gm. of male chickens in experiment II

Weeks	Treatments	
	Basal	Stilbestrol
0	44.4	43.3
1	105.4	105.8
2	198.2	202.9
3	327.2	362.1
4	459.4	536.1
5	651.3	771.3
6	908.4	1024.5
7	1059.4	1240.0
8	1292.1	1496.7
9	1556.3	1770.3
10	1794.6	2080.3
11	2025.4	2294.3
12	2264.0	2525.0

Table VIII. Average weekly weights in gm. of female chickens in experiment III

Weeks	Treatments			
	Basal	Bacitracin	ZN Bacitracin	Zinc
0	42.9	42.5	42.5	43.2
1	95.1	98.1	98.7	100.4
2	182.5	180.3	189.0	190.1
3	298.1	290.2	306.8	296.0
4	428.3	408.6	443.1	420.1
5	599.4	570.0	622.0	597.4
6	779.8	754.1	820.7	788.8
7	935.4	923.6	1003.6	967.5
8	1130.3	1122.6	1224.7	1183.4
9	1326.5	1314.1	1416.4	1393.6
10	1519.4	1489.6	1612.1	1591.2
11	1708.3	1678.7	1800.5	1795.3
12	1850.6	1831.0	1947.4	1947.4

Effects on Serum Cholesterol

The blood cholesterol levels in the male and female rats fed the different rations in experiment I are shown in Table IX. The mean cholesterol level of rats fed the basal ration was highest in both males and females. The male rats fed bacitracin had the lowest cholesterol level and the next was

Table IX. Mean (±SE) cholesterol level (mg/100 ml. of blood) of rats in experiment I

Sex	Treatments - Feed additives added					
	1 Basal	2 Zinc	3 Zinc Bacitra-cin	4 Bacitracin	5 Corn Oil	6 Trifluome-prazine
Male	166.4±7.82	158.4±7.82	165.00±7.8	142.3±7.8	164.9±7.8	143.9±7.82
Female	183.00±7.91	170.5±7.91	167.1 ±7.91	181.8±7.91	168.7±7.91	178.80±7.91

the male rats fed trifluomeprazine. These differences did not reach the level of statistical significance. The mean blood cholesterol level of male chickens in experiment II is shown in table X. In stilbestrol fed group of chickens there was an increase in blood cholesterol level which was found to be not significant on statistical analysis. It was suspected that the variance in this might be proportional to the treatment means, hence a logarithmic transformation was applied to the data. On this scale, the cage Mean Square was just significant at the .05 level. In spite of this it was decided that this M. S. and the individual Mean Square should be pooled for the error term in testing the treatment Mean Square. The F value obtained was 17.1 which was highly significant. The F value at the .01 level for 1 and 46 degrees of freedom is 1.21. A similar transformation was applied to the cholesterol data of male rats in experiment I and the results were unchanged statistically.

Blood cholesterol levels of female chickens in experiment III are shown in table XI. There were no significant differences among treatments.

Table X. Mean (±SE) cholesterol level (mg/100 ml. blood) of male chickens in Experiment II.

Basal Diet	Stilbestrol Added
150.8±6.58	188.67±6.58

Table XI. Mean (±SE) cholesterol level (mg/100 ml. blood) of female chickens in Experiment III.

Basal	Bacitracin	Zinc Bacitracin	Zinc
150.7±9.83	153.2±9.83	148.±9.83	148.20±9.83

Effects on Organs and Tissues

The blood hemoglobin levels of rats and chickens used in experiments I, II and III are shown in Tables XII, XIII, and XIV, respectively. No significant differences among treatments in blood hemoglobin level were noticed in experiments I and II. In experiment III there was a significant difference among treatments at .01 level. Those chickens fed the basal ration had the highest hemoglobin concentration (10.93), the zinc bacitracin group was next (10.28), and the bacitracin fed group had the lowest hemoglobin concentration (8.96).

The mean hematocrits of rats and chickens used in the experiments I, II and III are shown in tables XV, XVI and XVII respectively. In experiments I and II there were no significant differences among treatments in these values also. In treatment III there were significant differences among treatments at .05 level. The chickens fed the basal diet had the highest hematocrit value and the zinc bacitracin group had the next highest value. The chickens fed the additional zinc had the lowest value.

Table XII. Mean hemoglobin concentration (gm.%) of rats in Experiment I

	Treatments - Feed additives added	Male	Female
1	Basal	17.35±.50	18.51±.51
2	Zinc	17.05±.50	16.84±.51
3	Zinc Bacitracin	18.36±.50	17.86±.51
4	Bacitracin	18.14±.50	17.6 ±.51
5	Corn Oil	17.66±.50	16.70±.51
6	Trifluomeprazine	17.96±.50	16.80±.51

Table XIII. Mean hemoglobin concentration (gm.%) of chickens in Experiment II. males.

Basal	Stilbestrol
10.19±.45	10.71±.45

Table XIV. Mean hemoglobin concentration (gm.%) of chickens in Experiment III. females.

Treatments - Feed additives added			
1 Basal	2 Bacitracin	3 Zinc Bacitracin	4 Zinc
10.93±.27	8.96±.27	10.28±.27	9.54±.27

Table XV. Mean hematocrit % of rats in Experiment I.

	Treatments - Feed additives added	Male	Female
1	Basal	45.80±.76	44.60±.52
2	Zinc	45.80±.76	44.50±.52
3	Zinc Bacitracin	46.30±.76	44.50±.52
4	Bacitracin	46.30±.76	43.30±.52
5	Corn Oil	45.80±.76	44.60±.52
6	Trifluomeprazine	46.30±.76	44.60±.52

Table XVI. Mean hematocrit % of chickens in Experiment II

Basal	Stilbestrol
29.83±.60	30.05±.60

Table XVII. Mean hematocrit % of chickens in Experiment III

Treatments - Feed additives added			
1 Basal	2 Bacitracin	3 Zinc Bacitracin	4 Zinc
30.48±.53	28.68±.53	29.40±.53	28.04±.53

The mean weights of organs of the rats used in experiment I are shown in table XVIII.

In male rats no significant differences among treatments could be observed regarding the weight of organs. In female rats the weights of the livers had been changed significantly at .01 level by treatments. The rats fed trifluomeprazine had the heaviest liver and thus showed a difference between all other treatments at .05 level. The corn oil fed group was the next and this too had significant differences in weight of liver at .05 level with all other groups. The zinc bacitracin group was next and the livers of the bacitracin fed group of rats had the lowest weight. This group had the lowest body weight also.

The weights of the kidneys of the female rats showed significant differences among treatments at .05 level. As in the case of the livers the group of rats fed the tranquilizer had the heaviest kidneys and the next in order was the group fed corn oil. The kidneys of the group of rats fed additional zinc had the lowest weight.

The differences in weight of the hearts also were significant at .05 level among treatments and these differences followed the same pattern as that of kidney weights.

The mean weights of organs from male chickens of experiment II are shown in table XIX. In this experiment significant

differences between treatments at .01 level were present in the weights of liver, spleen and pancreas. Regarding the weights of testicles between cage variation was significant. Thus the mean square for cages had to be used as the error term for testing treatments and with only two and one degree of freedom respectively for cages and treatment the mean square for treatment was not significant. However, examination of the data shows that for the treatment one, the sum of the testicles weights in cages one and two were 80.29 and 27.04 gm. respectively, which were considerably higher than the cage sums of treatment two, the sums being 8.81 and 8.67. In view of this there is reason to believe that there is a treatment difference.

The mean weights of organs of female chickens used in experiment III are shown in table XX.. No significant differences among treatments could be detected regarding the weight of ovaries and pancreas, but significant differences at .01 level among treatments in the weight of liver and spleen and at .05 level in the weight of heart were present. The group of chickens fed the basal diet had the heaviest liver and the ones fed bacitracin had the lowest mean weight of liver and the differences of the liver weight of the bacitracin fed group was significant at .05 level with all other groups. The differences between other groups were not significant. The mean weight of the spleen was heaviest in the group fed zinc bacitracin and the group fed the basal diet had the lowest weight.

There was a significant difference at .05 level between basal and other groups and also between zinc bacitracin group and other groups. The heart weight followed the pattern of body weight. There was a significant difference at .05 level between the group of chickens fed zinc bacitracin and the basal and also between the zinc and basal. The zinc bacitracin group had the heaviest heart and the bacitracin group had the smallest heart by weight.

The analysis of variance and among treatment comparisons of all the studies are given in the Appendix. The variances of the male and female rats used in the experiment were different, hence a statistical problem arose in combining the male and female data for testing the sex and treatment interaction. In view of this, an approximation test of this interaction was made by use of a simple two way contingency table (sex and treatment) for which the test criterion is distributed approximately as Chi-square. None of these interactions were significant.

The mortality of rats and chickens used in the experiments was very low. In the group fed the tranquilizer one rat died two weeks after treatment and two chicks died in the group of chickens fed stilbestrol. The postmortem did not reveal any morbid changes attributable to the treatment.

After the rats and chickens were sacrificed, they were

examined for pathological changes of internal organs which could be attributed to treatments. No pathological changes attributable to treatments could be observed macroscopically except those described below. All the chickens in the stilbestrol fed group had more yellowish subcutaneous and mesenteric fat than the control group. The testicles of the chickens showing bone abnormalities (deformed hock and crooked keel) were very small compared with the chickens in the same group which were not showing bone abnormalities. The incidence of abnormalities was particularly high in one cage. The chickens in this cage were removed from battery brooder only at one and a half months age.

The testicles of the stilbestrol fed group, the birds in the basal group showing the bone deformity and the apparently normal birds of the same group were examined microscopically. In the apparently normal birds in the basal group histological examination revealed well developed seminiferous tubules and abundant spermatids. The histological examination of the testicles of the birds with abnormal bones revealed undeveloped tubules and very few spermatids. Sections of the testicles of the stilbestrol treated birds on microscopical examination revealed undeveloped tubules and the spermatogenesis was not progressed beyond spermatogonial stage.

The comb growth of the stilbestrol treated birds was much suppressed compared with the controls and the stilbestrol treated birds were less aggressive.

Table XVIII. Mean weight of organs (gm.). of rats in Experiment I

Treatment according to sex		Liver	Heart	Kidney	Testicle
Basal	F	6.62±.243	0.81±.021	1.62±.046	-
	M	13.71±.479	1.29±.057	2.79±.026	3.48±.063
Zinc	F	6.24±.243	0.78±.021	1.45±.046	-
	M	12.64±.479	1.16±.057	2.59±.026	3.37±.063
ZN Bacitracin	F	6.68±.243	0.80±.021	1.59±.046	-
	M	13.12±.479	1.27±.057	2.70±.026	3.50±.063
Bacitracin	F	5.94±.243	0.79±.021	1.53±.046	-
	M	13.77±.479	1.18±.057	2.83±.026	3.49±.063
Corn Oil	F	7.14±.243	0.84±.021	1.67±.046	-
	M	13.35±.479	1.30±.057	2.78±.026	3.31±.063
Trifluomeprazine	F	7.47±.243	0.88±.021	1.68±.046	-
	M	12.57±.479	1.26±.057	2.74±.026	3.57±.063

F = female.

M = male.

Table XIX. Mean weight (±SE) of organs (gm.) of male chickens in Experiment II

Treatment	Liver	Heart	Spleen	Pancreas	Testicle
Basal	41.54±2.520	11.71±.350	3.95±.210	3.46±.161	4.47±.765
Stilbestrol	61.98±2.520	12.25±.350	3.23±.210	4.04±.161	0.73±.765

Table XX. Mean weight (±SE) of organs (gm.) of female chickens in Experiment III

Treatment	Liver	Heart	Spleen	Pancreas	Ovary
Basal	38.02±.891	8.81±.226	3.42±.137	3.28±.112	0.74±.036
Bacitracin	33.33±.891	8.31±.226	3.13±.137	3.18±.112	0.69±.036
ZN Bacitracin	36.01±.891	9.16±.226	3.77±.137	3.16±.112	0.70±.036
Zinc	36.81±.891	9.10±.226	3.26±.137	3.43±.112	0.68±.036

GENERAL DISCUSSION

Under the conditions of this experiment the rats did not show signs of zinc deficiency. Even though precautions were taken to prevent the rats getting extraneous sources of zinc, the group of rats fed the basal diet gnawed the cages and the metal (galvanized iron) of the cages became exposed. The latest balance studies of Forbes and his associate (1960) showed that the zinc requirement of young albino rats was 18 ppm. They have reported that the increase of calcium from 0.8 to 1.6% level of the diet caused reduction in weight gains at all levels of zinc. In this study 22.3 ppm zinc and 1.166% calcium were calculated in the basal ration.

Pronounced symptoms of zinc deficiency observed among male chickens fed the basal ration confirm the findings of Young and his associates (1958), Supplee et al. (1958), Pensack et al. (1958). However, the absence of deficiency symptoms in most of the chickens fed stilbestrol is interesting and, for the most part, unexplainable.

The interrelationship of zinc and calcium has been reported by Klussendorf (1956), Hoefer et al. (1959), and Febrando and Seller (1957). Sturkie (1954) has quoted the findings of several investigators who have shown that estrogens increase the blood calcium level of chickens. It is possible that this interrelationship might be a cause for the

absence of zinc deficiency symptoms in stilbestrol treated cockerels. It is also interesting that none of the female chicks used in the experiment showed pronounced symptoms of zinc deficiency.

It is generally accepted that antibiotics when added to the ration promote growth in several species of animals. Lewis and Sanford (1953), Day and associates (1958) and Braham et al. (1959) have shown that the addition of bacitracin to poultry rations increased the rate of gain. There are also reports by Beeson and associates (1954), Bridges and associates (1954), Hanson et al. (1956) and Swenson et al. (1958) stating that bacitracin had no significant effect on growth rate. In this study, both female rats and female chickens fed bacitracin in diet weighed less than controls at the end of the experiment. In male rats the bacitracin fed group of rats had higher body weights, but this did not reach the level of statistical significance. Zinc bacitracin fed group of female rats showed greater rates of gain in weight than the groups fed basal ration, zinc or bacitracin. In female chickens the zinc bacitracin fed group had the greatest gain in weight which was significant at .05 level with all groups except with the zinc fed group.

According to Carver and Johnson (1953) corn oil contains factors which stimulate chick growth. Beare and associates (1959) have reported that rats fed corn oil had better weight

gains. Holman (1957) has suggested that the dermal lesions in rats and parakeratosis in swine induced by dietary inadequate EFA are influenced by imbalance in dietary minerals. The corn oil fed female rats had the highest gain in weight in this study. The significant growth response obtained in this study may be due to the interrelationship between EFA and mineral imbalance as reported by Holman.

Reports regarding the efficiency of trifluomeprazine as a feed additive are controversial as seen in the review of literature. The results of this study indicate that trifluomeprazine has significant growth promoting properties in female rats fed zinc deficient diet.

Even though chemical caponisation by stilbestrol has been popular, the reports about the oral use of stilbestrol for improving growth and quality of broilers indicate differences of opinion among authors. In this study male chickens fed stilbestrol (3.75 gm/50 lb. feed) for 12 weeks had increased their weights significantly at .01 level.

Most extensive data are available in the field of dietary factors affecting blood cholesterol level and there are many findings of a controversial nature in this field. There are several reports on the effects of essential fatty acids on blood cholesterol levels. Reports by Avigan and Steinsberg (1958), Cuthbertson et al. (1959), Grande et al. (1958),

Beveridge et al. (1956) and others indicate that corn oil causes a depression of serum cholesterol level. Contrary to the above findings Pomeranze and his associates (1958) found that serum cholesterol in 3 infants rose, when a daily supplement of 50 gm. maize oil was added and fell when it was omitted from the diet. In the present study no significant difference was observed due to feeding corn oil.

Metallic elements have been tried by different workers as therapeutic agents for lowering serum cholesterol level with varying results. Curran (1954) found that vanadium and iron salts significantly reduced synthesis of cholesterol, while zinc had no effect. In experiment I the mean cholesterol level of both male and female rats fed a zinc deficient diet was the highest and there was some reduction in cholesterol level in the group which was given zinc supplements; but this difference was not of statistical significance. Hence a physiological relationship between zinc deficiency and blood cholesterol level was not shown conclusively.

The effect of tranquilizers on blood cholesterol level deserves attention. The beneficial effects of tranquilizers on blood cholesterol level may be due to reduction in stress condition. Study in animals kept under uniform environments will be definitely interesting. In experiment one, the trifluomeprazine fed male rats had a low cholesterol level even though the differences among treatments were not of statistical

significance.

The effect of antibiotics on blood cholesterol level has not received much attention. Only limited data were available on this subject. Samuel (1959) has reported that administration of neomycin reduced blood cholesterol level in 18 human patients, while oral administration of streptomycin, oxytetracycline, bacitracin and other antibiotics had no appreciable effects. In experiment I the male rats fed bacitracin had the lowest cholesterol level even though the differences among treatments did not reach the level of statistical significance. Although bacitracin did not reduce blood cholesterol significantly, one should not conclude that antibiotics have no effect on blood cholesterol without further investigation.

The influence of estrogens on atherosclerosis has been emphasized in recent years and there are several reports citing the beneficial effects of estrogens. Forbes and Petterson (1951), Baum and Meyer (1956), Clyde and Suydam (1959) and other workers have shown that administration of synthetic estrogens increase the blood cholesterol level. In experiment II the blood cholesterol level of the stilbestrol treated chickens was 188.7 mg/100 ml. of blood, while that of the controls was only 150.8. In experiment III there was no significant difference in blood cholesterol level among treatments.

The various effects of stilbestrol on organs and tissues

have been receiving close scrutiny at the hands of animal nutritionists as well as public health authorities. The actions of tranquilizers, corn oil and antibiotics on organs and tissues have not been studied extensively and hence there is very little information in the literature concerning this topic.

There were no significant differences in hemoglobin and hematocrit values among treatments in experiment I. There was also no significant difference in these values between stilbestrol treated and control chickens in experiment II. This finding does not support the view of Campbell (1959) that administration of estrogens decreases hemoglobin concentration. In treatment II there were significant differences in hemoglobin and hematocrit values among treatments. Swenson et al. (1958) did not find any change in hemoglobin and hematocrit after subcutaneous implantation of bacitracin pellets in pigs. The chicks in the experiment fed the basal diet had the highest values. These results are interesting but cannot be explained without further study.

In the male rats no significant differences among treatments could be observed regarding the weight of organs. In this connection it is to be remembered that the male rats had no significant differences in body weight. In the female rats the weights of the liver and kidneys were not significantly different among treatments. The rats fed the trifluomeprazine

had the heaviest livers and the corn oil group was next. Regarding body weight the corn oil group was the heaviest and the tranquilizer fed group was next. The livers of the bacitracin fed group of rats had the lowest weight. This group had also the lowest body weight. This finding supports the finding of Murray and Campbell (1955) that the livers of females given aureomycin were significantly smaller than those females not given aureomycin, but in males there was no significant difference in weight. The kidneys of the group fed zinc had the lowest weight.

The differences in weight of the hearts also were significant at .05 level among treatments and these differences followed the same pattern as that of kidney weights. In view of the physiological relationship among these organs these findings are only natural.

In experiment II, stilbestrol treated chickens had significantly larger organs, viz. liver, spleen, and pancreas. Sturkie (1954) has reported that estrogens increase the weight of the liver approximately 80 per cent depending on dosage.

The sums of weights of testicles of the control chickens in cages 1 and 2 were 80.29 and 27.04 gm. respectively which were considerably higher than the cage sums of stilbestrol treated chickens, the sums being 8.81 and 8.67. The smaller testicle weight among the birds fed the basal ration was

particularly noticed in cage 2 and the birds in this cage were removed from battery brooder only at one and one half months age. It may be possible that in the growing batteries which were old ones (even though plastic painted) the chickens were getting some extraneous zinc probably due to the plastic paint dropping off and the galvanized metal becoming exposed. Because of the significant cage variations, the treatment differences were not significant. Still there is reason to believe that there is a treatment difference, and this difference in weight of testicles is in agreement with the findings of Moreng and his associates (1956) and other workers.

In experiment III significant differences among treatments could be noticed regarding the mean weights of liver, spleen and heart. The chickens fed the bacitracin had the smallest livers in this experiment also as in the case of rats. The mean weight of the spleen was heaviest in the group of rats fed zinc bacitracin and the spleen of the group fed the basal diet had the lowest weight.

The zinc bacitracin group had the heaviest hearts and the bacitracin group had the smallest hearts by weight. Very little experimental work has been done regarding this topic and the findings in this study may contribute to the original elaboration of the concept of dietary regulation of weight of organs.

The excess of yellowish subcutaneous fat in the stilbestrol

fed group of chickens confirmed the findings of other investigators as reported by Sturkie (1954). It is also interesting to note that the testicles of birds showing crooked keel and leg abnormalities were abnormally small compared to those of apparently normal birds of the other groups. The findings of Miller et al. (1958) that zinc deficiency depressed growth and development of testes of rats is of interest in this respect. Bert (1959) has also reported that a high zinc content has been noticed in the male genital tract. The histological examination of the testes of these birds showed undeveloped seminiferous tubules and arrested development of spermatids. The histological picture of the testicles of the stilbestrol treated chicken was almost similar. They also revealed undeveloped tubules and the spermatogenesis was not developed beyond spermatogonial stage. This finding is in agreement with the findings of Kumar and Turner (1949). They have also noticed depression of the growth of comb of treated chickens which was true in this study also.

SUMMARY AND CONCLUSIONS

The rats fed a zinc deficient diet of 22.3 ppm did not show symptoms of zinc deficiency. The male chickens fed the basal (zinc deficient) diet in experiment II showed abnormal hocks and crooked keels which were considered as symptoms of zinc deficiency. Only two of the stilbestrol fed chicks showed bone deformities. The female chickens in experiment III did not show such symptoms.

In the male rats in experiment I there were no significant differences in body weights among treatments. The addition of corn oil seems to increase the gain in weight in female rats fed the zinc deficient diet. The addition of trifluomeprazine to the ration also had some beneficial effects on weight gains in female rats in this study. The group of rats fed bacitracin had the lowest gain in weight. In experiment II the stilbestrol fed male chickens had significantly increased their weights at the .01 level as compared with the controls. In experiment III the chickens fed zinc bacitracin showed the greatest gain in weight while the group of chickens fed bacitracin showed the lowest gain in this experiment also. Addition of zinc produced beneficial effects regarding weight gains.

The feed additives used in these experiments had no significant effect on blood cholesterol level, but in experiment II

the blood cholesterol level was definitely higher in stilbestrol treated chickens even though it was not of statistical significance.

There was no significant differences in hemoglobin and hematocrit values among treatments except in treatment III in which the chickens fed the basal diet had the highest values. In experiment I there were significant differences in weight of liver, kidney and heart among treatments in the female rats. The rats fed trifluomeprazine had the heaviest livers and the corn oil group was next. The livers of the bacitracin fed group had the lowest weight. The kidneys of the group of rats fed zinc had the lowest weight. The differences in weight of the heart among treatments followed the same pattern as kidney weights. In experiment II stilbestrol fed chickens had the larger livers, spleens and pancreases. The weights of the testicles of the control chickens were greater than those from the stilbestrol chickens. Even though this did not reach the level of statistical significance there is reason to believe that there is a treatment difference. In experiment III significant differences in mean weights of liver, spleen and heart could be noticed among treatments. The chickens fed bacitracin had the smallest livers in this treatment also. The mean weight of the spleen was heaviest in the group of chickens fed zinc bacitracin and lowest in the group fed basal ration. The zinc bacitracin fed group of chickens had the

heaviest heart and the bacitracin group had the smallest heart by weight.

The stilbestrol treated chickens had more subcutaneous fat than the controls. There is reason to believe that zinc deficiency depresses the growth of testicles in chickens. On histological examination there was very little difference between the testicles of the birds having undeveloped testicles in the basal group and the testicles of the stilbestrol treated birds. In other birds, however, which had well developed testicles in the basal group, histological examination revealed well developed seminiferous tubules and a large number of spermatids.

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APPENDIX

Determination of Total Serum Cholesterol[1]

Method

Reagents I. Standard cholesterol solution (1 mg/ml): Dissolve 100 mg of pure, dry, ash-free cholesterol in 100 ml of 100% glacial acetic acid.

II. Ferric chloride solution: Dissolve 10 gm. of ferric chloride, reagent grade, in 100 ml of 100% glacial acetic acid.

III. Color reagent: Dilute 2.0 ml of the ferric chloride solution to 200 ml with C.P. concentrated sulfuric acid.

Procedure

Preparation of the standard curve Pipette 0.1, 0.2, 0.3, 0.4, and 0.5 ml of the standard cholesterol solution into clean, dry, 30 ml test tubes and dilute each with glacial acetic acid to 3.0 ml. Add 0.1 ml of distilled water to each standard and mix thoroughly. A blank is prepared which contains 3.0 ml of glacial acetic acid and 0.1 ml of distilled water. Pipette in 2.0 ml of the color reagent by carefully allowing it to flow down the side of the test tube, thus producing two layers. Strike the tube sharply while holding it at the top between the thumb and fore-finger to effect mixing

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[1]Zlatkis, A., B. Zak, and A. Boyle, 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41: 486-492.

and even heat distribution. A light brown color first appears which changes to an intense purple within a minute. When the tube has cooled to room temperature, measure the absorbency in a spectrophotometer at 560 mu using a 1 cm cuvette. The standard curve is obtained by plotting absorbency against concentration.

Determination of total serum cholesterol Pipette 0.1 ml of the serum into 3.0 ml of glacial acetic acid in a dry, clean 30 ml test tube. Add the color reagent and mix as described above. Measure the absorbency of the solution after it has come to room temperature and determine the total cholesterol content from the calibration curve.

Determination of Hemoglobin of Chicken Blood*

Method

The hemoglobin content of the blood is determined with a spectrophotometer, measuring the optical density and plotting a standard curve from the readings of a stock standard hematin solution.

Procedure

Place 5 ml. of 0.4 per cent NH_4OH in a 10 ml. test tube.

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*From article by M. J. Swenson which in Am. J. of Vet. Res. 12: 147-151, 1957 entitled "Effect of a vitamin B_{12} concentrate and liver meal on the hematology of chicks fed an all plant protein ration.

Add 0.02 ml. of blood with blood pipette, rinsing three times with the NH_4OH solution.

Stopper test tubes and mix well by inverting three times.

Let stand at least one minute.

Add 0.12 ml. of concentrated HCl, or until pH is 1.1.

Stopper test tubes and mix well by inverting three times.

Let stand forty minutes.

Read unknown against a blank.

Calculate the concentration from the standard curve.

Analyses of Variance[1]

Experiment I. Female rats

Body weight in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	2056.48	411.30*
Cages/treatments	6)		
Rats/cages/treatments	48)	7483.70	138.60
Total	59	9540.18	

Cholesterol in mg./100 ml. blood serum

Source of variation	d.f.	S.S.	M.S.
Treatments	5	2470.28	494.06
Cages/treatments	6	3323.90	553.95
Rats/cages/treatments	44	27976.80	635.84
Total	55	33770.98	

[1]The following symbols are used in the Analyses of Variance:

* denotes significance at .05 level

** denotes significance at .01 level

) (one parenthesis)
denotes the pooling of the cage and rat sum-of-squares to form a pooled error. This was done only if the cage mean square was not significantly different from the rats mean square.

Hematocrit %

Source of variation	d.f.	S.S.	M.S.
Treatments	5	13.3500	2.6700
Cages/treatments	6	15.9000	2.6500
Rats/cages/treatments	45	122.4000	2.7200
Total	56	151.6500	

Hemoglobin gm. %

Source of variation	d.f.	S.S.	M.S.
Treatments	5	26.0995	5.2199
Cages/treatments	6	16.3170	2.7195
Rats/cages/treatments	45	116.5200	2.5893
Total	56	158.9365	

Weight of liver in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	15.8425	3.168**
Cages/treatments Rats/cages/treatments	6) 48)	31.9600	0.592
Total	59	47.8025	

Weight of kidneys in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	0.2850	0.057*
Cages/treatments	6)		
Rats/cages/treatments	48)	1.1565	0.0214
Total	59	1.4415	

Weight of heart in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	0.0716	0.0143*
Cages/treatments	6)		
Rats/cages/treatments	48)	0.2405	0.00445
Total	59	0.3121	

Experiment 1. Male rats

Body weight in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	2963.35	592.67
Cages/treatments	6	2789.50	464.92
Rats/cages/treatments	48	31574.00	657.79
Total	59	37326.85	

Cholesterol in mg./100 ml. blood serum

Source of variation	d.f.	S.S.	M.S.
Treatments	5	6042.28	1208.50
Cages/treatments	6	2481.50	413.55
Rats/cages/treatments	41	26285.20	641.10
Total	52	34808.98	

Hematocrit %

Source of variation	d.f.	S.S.	M.S.
Treatments	5	3.7500	0.7500
Cages/treatments	6	83.1000	13.8500
Rats/cages/treatments	46	214.0000	4.6522
Total	57	300.8500	

Hemoglobin gm. %

Source of variation	d.f.	S.S.	M.S.
Treatments	5	12.2633	2.4527
Cages/treatments	6	14.8580	2.4763
Rats/cages/treatments	46	115.3280	2.5071
Total	57	142.4493	

Weight of liver in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	13.1718	2.6344
Cages/treatments	6	9.2062	1.5344
Rats/cages/treatments	48	114.6029	2.3876
Total	59	136.9809	

Weight of kidneys in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	0.355775	0.071155
Cages/treatments	6	0.372790	0.062132
Rats/cages/treatments	48	3.437320	0.071611
Total	59	4.165885	

Weight of testicles in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	0.459740	0.091948
Cages/treatments	6	0.271020	0.045170
Rats/cages/treatments	48	1.852480	0.038593
Total	59	2.583240	

Weight of heart in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	5	0.173453	0.034691
Cages/treatments	6	0.134580	0.022430
Rats/cages/treatments	48	1.608360	0.033508
Total	59	1.916393	

Experiment II. Male chickens

Body weight in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	1	813021.02	813021.02**
Cages/treatments	2)		
Chickens/cages/treatments	44)	2215367.46	48160.02
Total	47	3028388.48	

Cholesterol in mg./100 ml. blood serum

Source of variation	d.f.	S.S.	M.S.
Treatments	1	17176.33	17176.33
Cages/treatments	2	7574.67	3787.34*
Chickens/cages/treatments	44	40162.00	912.77
Total	47	64913.00	

Hematocrit %

Source of variation	d.f.	S.S.	M.S.
Treatments	1	0.5208	0.5208
Cages/treatments	2	51.2917	25.6458*
Chickens/cages/treatments	41	323.0000	7.8780
Total	44	374.8125	

Hemoglobin gm. %

Source of variation	d.f.	S.S.	M.S.
Treatments	1	3.2552	3.2552
Cages/treatments	2	3.6246	1.8123
Chickens/cages/treatments	41	207.8000	5.0683
Total	44	214.6798	

Weight of liver in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	1	5014.95	5014.95**
Cages/treatments	2)		
Chickens/cages/treatments	44)	7037.03	152.98
Total	47	12051.93	

Weight of testicles in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	1	168.1880	168.1880
Cages/treatments	2	82.4367	41.2184*
Chickens/cages/treatments	44	564.0080	12.8184
Total	47	814.6327	

Weight of heart in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	1	3.5100	3.5100
Cages/treatments	2	9.3042	4.6521
Chickens/cages/treatments	46	125.9365	2.8622
Total	47	138.7507	

Weight of spleen in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	1	6.2496	6.2496**
Cages/treatments	2)		
Chickens/cages/treatments	44)	48.7680	1.06
Total	47	55.0176	

Weight of pancreas in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	1	4.0310	4.0310**
Cages/treatments	2)		
Chickens/cages/treatments	44)	28.2649	0.614
Total	47	32.2959	

Experiment III. Female chicken

Body weight in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	3	289017.3	96339.10**
Cages/treatments	4)		
Chickens/cages/treatments	89)	1759770.8	18922.2
Total	96	2048788.19	

Cholesterol in mg./100 ml. blood serum

Source of variation	d.f.	S.S.	M.S.
Treatments	3	453.96	151.32
Cages/treatments	4	1034.00	258.50
Chickens/cages/treatments	89	21472.00	241.46
Total	96	22959.96	

Hematocrit %

Source of variation	d.f.	S.S.	M.S.
Treatments	3	82.11	27.37*
Cages/treatments	4)		
Chickens/cages/treatments	80)	586.64	6.98
Total	87	668.75	

Hemoglobin gm. %

Source of variation	d.f.	S.S.	M.S.
Treatments	3	55.5651	18.52*
Cages/treatments	4)		
Chickens/cages/treatments	80)	151.9120	1.81
Total	87	207.4771	

Weight of liver in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	3	296.43	98.81**
Cages/treatments	4)		
Chickens/cages/treatments	88)	1825.30	19.84
Total	95	2121.73	

Weight of ovary in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	3	.0592	.0197
Cages/treatments	4	.1361	.0340
Chickens/cages/treatments	88	2.8818	.0357
Total	95	3.0771	

Weight of heart in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	3	10.7010	3.5670*
Cages/treatments	4)	117.5465	1.2780
Chickens/cages/treatments	88)		
Total	95	128.2475	

Weight of spleen in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	3	5.6989	1.8996**
Cages/treatments	4)		
Chickens/cages/treatments	88)	43.0798	.4682
Total	95	48.7787	

Weight of pancreas in gm.

Source of variation	d.f.	S.S.	M.S.
Treatments	3	1.1143	.3714
Cages/treatments	4	1.0830	.2708
Chickens/cages/treatments	88	27.9324	.3174
Total	95	30.1297	

Among treatment comparisons

Experiment I. Female rats. Use Duncan's Multiple Range Test (.05% level)

Body weight in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_4$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_3$	$\bar{x} - \bar{t}_6$
T_5	224.3	15.4*	13.0*	9.3	4.1	0.8
T_6	223.5	14.6*	12.2*	8.5	3.3	
T_3	220.2	11.3	8.9	5.2		
T_1	215.0	6.1	3.7			
T_2	211.3					
T_4	208.3					

$S_{\bar{x}} = \sqrt{\frac{138.6}{10}} = 3.72$, d.f. = 54

Weight of liver in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_4$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_3$	$\bar{x} - \bar{t}_5$
T_6	7.469	1.532*	1.230*	0.849*	0.791*	0.329
T_5	7.140	1.203*	0.901*	0.520	0.471	
T_3	6.678	0.741	0.439	0.058		
T_1	6.620	0.683	0.381			
T_2	6.239	0.302				
T_4	5.937					

$S_{\bar{x}} = \sqrt{\frac{.592}{10}} = .241$ d.f. = 54

Weight of kidneys in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_4$	$\bar{x} - \bar{t}_3$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_5$
T_6	1.683	0.186*	0.157*	0.091	0.059	0.023
T_5	1.660	0.163*	0.134	0.068	0.036	
T_1	1.624	0.127	0.098	0.032		
T_3	1.592	0.095	0.056			
T_4	1.526	0.029				
T_2	1.497					

$S_{\bar{x}} = \sqrt{\frac{.0214}{10}} = 0.463$ d.f. = 54

Weight of heart in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_4$	$\bar{x} - \bar{t}_3$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_5$
T_6	0.877	0.102*	0.092*	0.080*	0.065*	0.040
T_5	0.837	0.062	0.052	0.040	0.025	
T_1	0.812	0.037	0.027	0.015		
T_3	0.797	0.022	0.012			
T_4	0.785	0.010				
T_2	0.775					

$S_{\bar{x}} = \sqrt{\frac{.00445}{10}} = 0.0211$ d.f. = 54

Experiment III. Female chickens

Body weight in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_4$
T_3	1947.44	116.48*	96.84*	.08
T_4	1947.36	116.40*	96.76*	
T_1	1850.6	19.64		
T_2	1830.96			

$S_{\bar{x}} = \sqrt{\frac{18922.2}{25}} = 27.51$ d.f. = 93

Hematocrit %

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_4$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_3$
T_1	30.48	2.44*	1.80*	1.08
T_3	29.40	1.36	.72	
T_2	28.68	.64		
T_4	28.04			

$S_{\bar{x}} = \sqrt{\frac{6.98}{25}} = .528$ d.f. = 84

Hemoglobin gm. %

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_4$	$\bar{x} - \bar{t}_3$
T_1	10.93	1.97*	1.39*	.65
T_3	10.28	1.32*	.74	
T_4	9.54	.58		
T_2	8.96			

$S_{\bar{x}} = \sqrt{\frac{1.81}{25}} = .269$ d.f. = 84

Weight of liver in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_3$	$\bar{x} - \bar{t}_4$
T_1	38.02	4.69*	2.01	1.21
T_4	36.81	3.48*	.80	
T_3	36.01	2.68*		
T_2	33.33			

$S_{\bar{x}} = \sqrt{\frac{19.84}{25}} = .891$ d.f. = 92

Weight of heart in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_4$
T_3	9.17	.86*	.35	.11
T_4	9.06	.75*	.25	
T_1	8.81	.50		
T_2	8.31			

$S_{\bar{x}} = \sqrt{\frac{1.278}{25}} = .226$ d.f. = 92

Weight of spleen in gm.

Treatment	$\bar{x}$	$\bar{x} - \bar{t}_1$	$\bar{x} - \bar{t}_2$	$\bar{x} - \bar{t}_4$
T_3	3.77	2.17*	.64*	.51*
T_4	3.26	1.66*	.13	
T_2	3.13	1.53*		
T_1	1.60			

$S_{\bar{x}} = \sqrt{\frac{.4682}{25}} = .137$ d.f. = 92