THE ROLE OF ZINC IN POULTRY NUTRITION

By

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AN ABSTRACT

Submitted to the School of Advanced Graduate Studies at

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Approved Philip J. Schaible

ABSTRACT

Experiments were conducted on the growing chick with the use of semipurified diets and a relatilvey zinc free environment to determine (1) if the chick requires zinc, (2) the quantitative requirement of zinc for growth (3) the relative availability for the chick of zinc from the chloride, sulfate, carbonate and oxide compounds, and (4) the effect of excessive amounts of calcium and phosphorus on the amount of zinc required. In experiments with feeds composed of natural feedstuffs, studies were made on (5) the effect of high levels of zinc on growth and feed efficiency in young chicks, (6) the effect of supplemental zinc and calcium, alont or in combination, on egg production, hatchability, laying-house mortality, egg weight, Haugh score, and eggshell thickness in the laying hen.

Zinc was found to be an essential nutrient for the young chick for growth, feather development, efficient feed utilization, bone growth, and maintenance of healthy skin. A deficiency of zinc caused retarded growth rate, poor feather development, retention of body down, poor feed utilization, enlarged hocks, and dermatitis of tops of feet and foot pads.

The zinc requirement was twenty ppm of available zinc or less. Zinc in the form of the chloride, sulfate, oxide, and carbonate was equally utilized by the chick. Excessive amounts of calcium in the diet aggravated the zinc-deficiency symptoms and increased the zinc requirement.

With the use of practical diets, zinc levels of 1,000 ppm or less, as the sulfate, oxide,and carbonate, were well tolerated. Above this level the compounds depressed growth and feed efficiency in the

following increasing order: oxide, sulfate, and carbonate.

The addition of zinc and calcium, alone or in combination, to a laying ration, did not affect egg production, hatchability, laying-house mortality, egg weight, and Haugh score. Eggshell thickness was increased in cool but not warm weather by the combination of zinc and calcium, but not by calcium or zinc alone.

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Introduction

For many years poultrymen have been concerned with the proper feeding of farm poultry. As the science of poultry nutrition has advanced, the demand for more qualitative knowledge for all the nutrients has developed and assumed great importance.

Probably in no area of animal nutrition has the qualitative and quantitative determination of essential nutrients received greater impetus than in the growing chick. In recent years, the nutrient requirements of the laying hen have also been intensively investigated.

In view of the report of Tucker and Salmon (1955) that swine fed practical rations required additional zinc, it seemed desirable to determine if supplemental zinc is needed in the ration of chickens.

The research described in this thesis is concerned with the study of zinc in the nutrition of poultry. Qualitative and quantitative studies were made to determine if the growing chick requires zinc. Toxicity studies were undertaken to determine the tolerance level of zinc for the growing chick and the interrelationships of zinc with calcium and phosphorus were also studied. With the laying hen, the effect of zinc on egg production, egg weight, egg shell thickness, and embryonic development was studied.

REVIEW OF LITERATURE

Zinc, an essential nutrient

The presence of zinc in plant and animal tissues (Lechartier, 1877) and in the muscle and liver of man (Raoult, 1877) was first established in 1877. This followed by several years the work of Raulin (1869), a pupil of Pasteur, who determined that zinc was needed for growth of the mold Aspergilus niger.

Much later Tsui (1948) provided a possible explanation for the role of zinc in plants. Zinc is an essential nutrient for growth because the tryptophane content is lowered in zinc-deficient plants. Since indoleacetic acid, a plant growth hormone, is produced from tryptophane, a deficiency of this amino acid causes a deficiency of this hormone and results in a lowered growth rate.

During the 1920's, several investigators (McHargue, 1926; Hubbell and Mendel, 1927) made attempts to determine whether zinc performed an essential function in animal nutrition but met with limited success. The diets employed were so deficient in essential nutrients, especially vitamins, that even with added zinc the experimental rats and mice survived only a short time.

Todd, Elvehjem and Hart (1934) were more successful - by using purified diets they demonstrated that zinc is essential for the growth of rats. Diets adequate in the other nutrients but low in zinc were subsequently used by the Wisconsin workers and others. These studies disclosed unquestionably the importance of zinc in the nutrition of mice as well as rats (Day and Skidmore, 1947; Edwards _et al_., 1958).

Nishimura (1952) found that newborn mice, which were removed from their mother before receiving colostrum and placed with foster mothers in later stages of lactation, developed disorders similar to those reported for zinc deficiency. The administration of colostrum or zinc salts at birth prevented the development of the condition.

In early attempts to investigate the essentiality of zinc for rats, exhaustive purifications of the casein in the diet was necessary to produce a deficiency. Recently Edwards et al. (1958) easily produced a zinc deficiency in rats by the use of isolated soybean protein.

Tucker and Salmon (1955) and Luecke et al. (1956) found that zinc prevented parakeratosis and thus was an essential mineral for the pig.

Titus and Ginn (1931) noted that rice bran was very effective in the prevention of perosis in chicks. In the following year, the zinc content of rice bran was found to be higher than in most feedstuffs and an experiment was initiated to determine if this mineral had any effect on perosis. The perosis preventative effect of rice bran was not found to be due to its zinc content. This constituted the first investigation to determine if the chick benefits from zinc. Insko et al. (1938) also reported that zinc was ineffective in preventing perosis. However, Wilgus et al. (1937) concluded that zinc decreased the incidence of perosis but was less effective than manganese.

O'Dell and Savage (1957) reported that the addition of 6.6 ppm and 56 ppm of zinc, or five percent of distillers solubles promoted the growth of chicks. The chicks were maintained in galvanized (zinc-coated) batteries, and fed a Drackett Assay C-1 P rotein - Cerelose type diet which contained 1.72 percent calcium, 0.6 percent phosphorus and 52 ppm of zinc. Roberson and Schaible (1958) used plastic-coated or glass equipment, purified rations that contained exceedingly low contents of zinc and distilled drinking water and thus proved that the chick requires zinc for growth, feather development and maintenance of normal skin condition. Edwards et al. (1958) produced a zinc deficiency in the chick using plastic equipment or epoxy resin-coated batteries; they did not produce the condition in regular galvanized batteries. Norris et al. (1958) and Morrison and Sarett (1958) also reported that the growing chick requires zinc.

Supplee et al. (1958) reported that the growing turkey requires zinc for rapid normal growth, feather development and prevention of a non-rachitic hock disorder which denoted abnormal bone development.

Zinc deficiency symptoms

Follis et al.(1941) described zinc deficiency symptoms in the rat. They were extreme parakeratosis of the esophagus with a thick layer of partially keratinized cells, lesions of the buccal cavity and in some cases, corneal changes of the eyes. The skin showed hyperkeratinization with loss of hair follicles and hair.

Kernkamp and Ferrin (1953) described a condition in swine, which they termed parakeratosis, characterized by retarded growth, diarrhea, vomiting, anorexia, severe dermatitis and finally death in acute cases.

Tucker and Salmon (1955) obtained parakeratosis in practical swine rations containing 34 - 44 ppm zinc and found that high levels of calcium supplements aggravated the condition. Symptoms similar to those reported by Kernkamp and Ferrin (1953) were observed.

O'Dell and Savage (1957) described symptoms caused by a slight deficiency of zinc in the chick as depressed growth and impaired bone development. According to Roberson and Scheible (1958a, 1958b) conditions causing an acute zinc deficiency in the growing chick produced depressed growth, poor utilization of feed, failure to feather normally with retention of body down and a ragged condition of feathers and dermatitis of the tops of feet and on footpads which caused a high-stepping walk. Norris et al. (1958) observed similar deficiency symptoms and, in addition, found a decrease in bone ash. Symptoms were produced when plastic-coated equipment was used but were not produced when chicks were maintained in galvanized batteries.

In turkey poults, Supplee et al. (1958) noted that a zinc deficiency caused depressed growth, poor feathering, and impaired bone development which resulted in a high incidence of hock disorders.

Symptoms attributable to a zinc deficiency have not been reported for cattle, sheep, dogs, cats, ducks, geese, pheasants or other poultry. However, in view of the essentiality of zinc for the rat, pig, chick, and turkey, it seems likely that zinc is an essential nutrient for other farm animals and pets.

Environmental effects on the development of a zinc deficiency in the chick

Since the zinc coating has been noted to erode quite rapidly on starting batteries, especially the waterers and feeders, only materials preventing the exposure of chicks to extraneous zinc were employed when the research reported in this thesis was initiated. Mehring (1956) observed that enough zinc is dissolved from the galvanized water pans to add 3ppm to the drinking water. If it is assumed that the bird consumes two to three times as much water as feed, an additional 6 to 19 ppm would be added to the level provided in the feed.

In the preliminary experiments, plastic cages, glass feeders and waterers, and reagent grade minerals were used. Subsequent experiments were conducted in Jamesway Batteries with all exposed galvanized parts coated with epoxy resin. The galvanized waterers were replaced with one-gallon glass baby chick founts with plastic bases.

Previous research on unidentified minerals, reported by Morrison et al. (1955, 1956) was conducted in galvanized Petersime Batteries. In the early experiments, the galvanized waterers were not coated; in later work, they were sprayed with plastic varnish. In proving the adequacy of their diet, graded levels of zinc (5 to 100 mg/kg) were added to the basal diet which contained 4.8 ppm added zinc without producing a significant growth response in chicks reared in zinc-coated batteries with zinc-coated waterers.

O'Dell and Savage (1957) obtained a chick growth response when a semi-purified diet was supplemented with graded levels of zinc, but as stated previously the work was conducted in galvanized batteries. The basal diet contained 1.75 percent calcium and 0.6 percent phosphorus. In view of the experimental work reviewed on the calcium-zinc interrelationship, the zinc deficiency they obtained could have resulted from the high level of calcium. Deficiency symptoms were not nearly as severe as those on extremely low levels of zinc and normal levels of calcium reported by Roberson and Schaible (1958). In other laboratories in which plastic-coated equipment was used, researchers (Norris et al., 1958; Pensack and Klussendorf, 1958) substantiated this interpretation.

Edwards et al. (1958) also studied the effect of enviroment on zinc deficiency in the chick. Chicks housed in galvanized batteries with galvanized waterers did not respond to supplemental zinc added to diets apparently deficient in zinc. Chicks, however, developed severe zinc deficiency symptoms when housed in plastic and stainless steel cages and were given distilled water in glass waterers. A less severe, but quite variable, zinc deficiency was obtained with chicks housed in galvanized batteries and given distilled drinking water. When chicks were fed a zinc-deficient diet, in open wooden troughs, growth was increased, but when the troughs were covered to prevent fecal contamination, no growth increase occurred. When the galvanized batteries were coated with epoxy resin, a very severe zinc deficiency, similar to that in plastic cages occurred.

Morrison and Sarett (1958) obtained a growth response in chicks from graded levels of zinc (0,5,25, 100 ppm) added to a semi-purified diet (Morrison et al.1956). The birds were maintained in galvanized batteries and given distilled water in glass jars. A zinc deficiency in this study consisted of a growth depression, and an enlargement and elongation of the tibiotarsal-tarsometatarsal (hock) joint. Bone ash was not affected. The deficiency symptoms were much less severe than those in which a similar diet was used but the birds were reared in plastic-coated batteries.

Water contaminated with zinc has been another source of experimental error in nutritional research. Edwards et al. (1958) found that the tap water he used contained 1.0 ppm zinc and that this gave a chick growth response over distilled water when a semi-purified diet was used.

Romanoff and Romanoff (1949) state that almost all the zinc in the egg is in the yolk. In work reported in this thesis, it is concluded that the quantity of zinc in the yolk sac of day-old chicks is too small to cause significant experimental error in growth experiments. In agreement with this conclusion, deutectomized chicks grow at the same rate as normal chicks when fed a zinc-deficient, semi-purified diet (Edwards et al. 1958).

The source of protein in experimental rations has also affected the results obtained in zinc studies. O'Dell and Savage (1957) obtained a growth response in chicks when supplemental zinc was added to a semi-purified diet containing Drackett Assay C-1 Protein but failed when casein or alpha-protein replaced the soybean protein. The soybean protein, casein and alpha-protein contain similar quantities of zinc. Norris et al. (1958) found that a total zinc content of 20 mg/kg was required by the chick when a casein-type diet containing 5.2 mg/kg was fed. On the other hand, when a Drackett Assay C-1 Protein diet containing nine ppm zinc was used a total of thirty ppm zinc was required. They concluded that the zinc in the soybean protein was not utilizable by the chick.

In studies with poults, Supplee et al. (1958) rarely observed abnormal hocks and poor feathering when an autoclaved soybean protein-starch type diet was fed; whereas, a high incidence of hock disorder and poor feathering was observed on a soybean protein-sucrose-type diet. Possibly the improvement was due to the release of the zinc from the soybean protein during autoclaving so that it became more available to the poults. However, it must not be overlooked that corn starch contains 73 ppm zinc; whereas, sucrose contains very little zinc. Consequently, starch, when used as a primary energy source in a diet could contribute a considerable quantity of zinc.

Edwards et al. (1958) produced without great difficulty a zinc deficiency in rats when soybean protein was used. This is in contrast with early attempts to produce a zinc deficiency in rats with a casein-type diet. Exhaustive purification of the casein was necessary to produce a zinc deficiency in the rats.

Although Morrison and Sarett (1958) obtained a substantial growth response from zinc when soybean protein was used, they obtained only a slight response when casein or gelatin was used as the protein source. Both type diets contained approximately 30 ppm zinc. The addition of excess calcium to the soybean protein diet depressed weight gains and feed efficiency. Supplemental zinc counteracted these effects. The addition of excess calcium to the casein-and gelatin-type diet failed to depress growth or produce other symptoms indicative of a zinc deficiency.

Nishimura (1952) pointed out that the level of zinc in mouse colostrum was great enough to protect newborn mice from a zinc deficiency; whereas, the zinc content of mouse milk was inadequate for normal performance of baby mice.

The physiological role of zinc

Zinc and Hormones

The crystallization of insulin as a zinc salt by Fisher and Scott (1935) led to the misconception that zinc was an integral part of the insulin molecule. This thought persisted in spite of the fact that other metals, namely nickel, cobalt, and cadmium, can be used in the crystallization. Amorphous insulin is as active physiologically as crystallin insulin, and there is little convincing evidence that zinc and insulin must combine *in vivo* to form an active compound (Vallee, 1957).

There is far more zinc in the pancreas than would be necessary for insulin activation and some of it is now accounted for by its presence in carboxypeptidase (Vallee and Neuroth, 1955).

Zinc and Porphyrins

It appears certain that a zinc uroporphyrin exists in higher animals as well as in mollusk (snails, etc.). The zinc complex of the Waldenstrom uroporphyrin (1937) has been shown to be a constant constituent of the urine and feces in cases of intermittent, acute porphyria. In congenital cases of this condition, the porphyrin is excreted in the free state and zinc uroporphyrin is found in the liver. A zinc coproporphyrin has been found in cases of lead poisoning and in the urine of victims of acute rheumatic fever (Vallee, 1957).

Zinc-Containing Enzymes

In contrast to the highly-colored iron and copper proteins and enzymes, the zinc proteins and enzymes are colorless. This fact has probably been responsible for the slow recognition of the importance of zinc in enzymes.

Keilin and Mann (1940) first observed that the carbonic anhydrase (an enzyme which catalyzes the reaction $H_2O + CO_2 \rightleftharpoons H_2CO_3$) of the blood cells of oxen contained 0.33 percent zinc which does not exchange freely with ionic zinc. Zinc is an integral part of the molecule of carbonic anhydrase and the removal of the metal results in irreversible inactivation. Carbonic anhydrase is found in almost all tissue but erythrocytes contain the greatest quantity. Practically all the zinc of erythrocytes is present in their carbonic anhydrase (Vallee, 1957). Wachtel _et al._ (1941) studied the carbonic anhydrase activity in rats deficient in zinc. Although a slight anemia was observed, there was no lowering of the carbonic anhydrase activity of the erythrocytes.

Carboxypeptidase of bovine pancreatic juice was shown by Vallee and Neurath (1955) to be a zinc-containing enzyme. This exopeptidase splits terminal amino acids from peptides having a free alpha carboxyl group adjacent to the peptide bond. The enzyme contains one atom of zinc per molecule and the zinc is indispensible for its enzymatic activity.

A number of zinc-containing dehydrogenases (enzymes which remove hydrogen from a substrate in biological oxidation) have been reported and zinc is necessary for their activity. All of the enzymes are dependent upon diphosphopyridine nucleotide (DPN) for their activity. For example, Vallee and Hoch (1955) stated that yeast alcohol dehydrogenase contained, as an integral part, four atoms of zinc per molecule of enzyme. Theorell et al. (1956) found that horse liver alcohol dehydrogenase contained two atoms of zinc per molecule of enzyme and that zinc is essential for enzymatic activity. Glutamic dehydrogenase of beef liver and lactic dehydrogenase of rabbit skeletal muscle were found to contain zinc as an integral part of the enzyme system (Vallee et al. (1955, 1956). The zinc in these enzymes explains the high content of this mineral in the liver and retina of the eye.

The metabolic role of zinc in the development of bone, skin and feathers is unknown at the present time. O'Dell and Savage (1957) obtained impaired bone development in zinc-deficient chicks while Norris et al. (1958) found a decrease in bone ash. Roberson and Schaible (1958) described dermatitis of the feet and poor feather development in zinc-deficient chicks. As yet, no specific role of zinc in metabolism of these tissues has been reported.

Mawson and Fisher (1951) found high concentrations of zinc in the prostate of the rat, and Mawson (1952) noted a similar condition in the rabbit and man. Gunn et al. (1956) reported that Zn^{65} was selectively taken up by the dorsolateral prostate of the rat-specifically by the cells of the lateral acini (Gunn and Gould, 1956, 1957) and elaborated in the glandular secretion (Gunn and Gould, 1956). The Zn^{65} was found throughout the uterine tract after ejaculation. However, the removal of large amounts of the zinc from the ejaculate did not alter fertility or fecundity (Gunn and Gould, 1958). Thus, the zinc in the prostate gland does not appear to play any particular role in reproduction. Zinc is, however, found in the sperm (Mawson, 1953) and doubtlessly plays an indispensable part in its production or metabolism.

Serum inorganic phosphorus levels were depressed in pigs with parakeratosis but serum calcium, magnesium and alkaline phosphatase were not appreciably affected (Stevenson and Earle, 1956). Significant increases in the zinc concentration of blood plasma, liver and kidney followed supplementation of a ration with 50 ppm zinc (Hoekstra et al. 1956). The zinc concentration of muscle, spleen, lungs and skin were not affected by zinc supplementation. The addition of two percent bone meal to a non-supplemented diet did not affect the zinc concentration of organs. However, the addition of the two percent bone meal to a diet supplemented with fifty ppm zinc resulted in decreased zinc concentration of the liver and kidneys but did not affect that in the other organs.

Morrison and Sarett (1958) reported that zinc deficiency in the chick has no effect on blood-sugar, serum phosphatase, lactic acid and alkaline phosphatase levels, but results in depressed intestinal alkaline phosphatase. Zinc deficiency did not affect the percentage of ash in the tibiotarsal bones or on the composition of the liver or carcass.

The interrelationship of zinc with other minerals

Calcium and phosphorus

Tucker and Salmon (1955) reported that the level of calcium and/or phosphorus affected the incidence and severity of parakeratosis. Increasing the calcium level of the ration decreased weight gains and hastened the onset of parakeratosis (Lewis et al. , 1956). Increasing the phosphorus level of the diet by the addition of monosodium phosphate had no effect on weight gains but decreased the skin lesions when compared with the results obtained with a basal diet containing 0.47 percent phosphorus and 0.82 percent calcium. One hundred ppm supplemental zinc were necessary to completely prevent parakeratosis.

Luecke et al. (1956) produced a 100 percent incidence of parakeratosis in swine by feeding 1.50 percent calcium and 0.80 percent phosphorus in a ration containing 31 ppm zinc. Twenty ppm supplemental zinc prevented parakeratosis in nine of ten pigs. When the calcium level was reduced to 0.98 percent of the diet and phosphorus to 0.70 percent, the incidence of parakeratosis was lowered to three of ten pigs. Supplementation of this ration with twenty ppm zinc completely prevented parakeratosis.

The experience of many workers suggests that the interaction between calcium and zinc may be within the intestinal tract. However, the results obtained at the hands of different investigators suggest that other factors may be involved in this interaction. For example, recently Hoefer et al. (1958) reported that copper would prevent parakeratosis.

Copper

Smith and Larson (1946) depressed growth and produced anemia in rats by feeding a diet excessively high in zinc. The addition of copper to the diet corrected the anemia but did not restore normal growth. Similarly, Gray and Ellis (1950) produced anemia in rats by feeding a high level of zinc but growth was not depressed. Supplemental copper likewise corrected the anemia.

The possible relationship between zinc and unidentified factors

Accoring to Dannenburg et al. (1955) the ash of distillers dried solubles produced a growth stimulation which was equivalent to approximately one-half that obtained from the unashed material. The addition of 0.5 percent calcium to the basal diet, which already contained 1.08 percent calcium, depressed growth. Addition of three percent distillers dried solubles restored normal growth. O'Dell and Savage (1957) ascertained that zinc would replace part, if not all, of the unidentified minerals supplied by distillers dried solubles.

Morrison and Sarett (1958) found that the addition of 2.5 percent of dried fish solubles to an isolated soybean-protein-cerelose type diet (protein level kept constant) produced a ten percent growth response; whereas, no growth response was obtained in the presence of ten ppm of added zinc. Furthermore, fish solubles corrected the depression in growth brought about by the addition of 0.5 percent excess calcium.

Kratzer et al. (1958) produced a hock disorder and depressed growth in turkeys by feeding an isolated soybean protein basal diet containing 1.88 percent calcium, 0.75 percent phosphorus and 25 ppm zinc. The addition of 38 ppm of supplemental zinc to the diet or replacement of the isolated soybean meal with regular soybean meal containing 66 ppm of zinc was required to give optimum growth and have a maxium effect in reducing the incidence of enlarged hock. He concluded that the zinc in the soybean meal corrected the depressed growth and enlarged hock. Soybean meal is a source of an unidentified factor.

GENERAL EXPERIMENTAL PROCEDURE

Similar procedures for the different experiments are outlined in this section; departures therefrom, are described under the individual experiments.

The randomization of chicks to pens or replicates was made in the following manner: one-day-old chicks received from a commercial hatchery or hatched at Michigan State University were weighed and distributed into consecutive weight groups, such as 36-38, 39-41, etc. each having a weight range of three grams. The groups with the lowest and highest weights were discarded. An equal number of chicks from each weight group was randomized to each pen. Thus, this method minimized the effect of differences in initial weights of the chicks on the final results.

Lots were randomly assigned to replicates. In experiments with practical diets, each lot appeared in each battery, therefore, each battery held one replicate of all treatments. This method was used to prevent differences due to position of the batteries in the room.

Birds were maintained in electrically-heated, starting batteries with raised wire floors. Where purified diets were used, all battery parts were coated with epoxy-resin, except the wire floors and dropping pans which were coated with shellac or clear metal primer. The galvanized (zinc plated) water troughs were replaced with one-gallon, glass, baby chick founts with plastic bases. The batteries were kept in a special room which was relatively free of dust. Thus, precautions were taken not only to reduce the amount of zinc in the test ration but also to prevent accidental zinc contamination from equipment and environment. Where practical diets were used, galvanized batteries (uncoated) were utilized.

Group weights of the birds were recorded at the end of two weeks and individual weights at the end of the experiment. Feed consumption was obtained by lot in experiments with purified diets and by replicate with practical diets.

In preparation for an experiment, the heaters on the batteries were turned on and regulated at least one day prior to the arrival of chicks. Feed was placed in the troughs and, at the start, on paper which was laid on the wire floors.

Experimental diets and water were supplied ad libitum. In experiments with purified diets, distilled water was used; with practical diets, tap water was employed. Hardware cloth (uncoated) was placed on the feed in the troughs to minimize feed wastage when practical diets were utilized. With purified diets, this was not practical because of the type of battery used.

Purified diets were mixed in a plastic, twin-shell blender or in a small, steel concrete mixer. Other diets were mixed in a one-half ton upright mixer or in a small concrete mixer. Feeds prior to feeding were handled as follows: Purified diets were placed in plastic or paper bags and stored in a refrigerator at 35° F. Practical diets were stored in galvanized cans in the starting battery room at room temperature (approximately 60° F).

Experiment I

In an exploratory experiment, two groups of five White Leghorn cockerels, hatched at Michigan State University, were allocated to two pens having plastic mesh sides and rubber mesh floors.

One group of chicks was fed a basal diet which was a slight modification of that used by Morrison _et al._ (1955) Table 1, but not including the supplemental zinc. The basal diet contained 16 ppm of zinc. The second group was supplied the basal diet supplemented with 100 ppm of zinc as the sulfate. Analytical reagent grade minerals were added to the diets. Distilled water was supplied for drinking and both feed and water were provided _ad libitum_ in glass feeders and waterers. The experiment was terminated when the birds were four weeks of age.

Results and Discussion:

The results are shown in Table 2. The group receiving the basal diet grew poorly, developed dermatitis of the feet, failed to feather properly and developed a high-stepping walk. Symptoms began to appear about the 14th and were severe by the 21st day. The group fed the zinc-supplemented diet grew satisfactorily during the four-week test period and did not exhibit any of the symptoms noted in the zinc-deficient group.

TABLE 1

COMPOSITION OF BASAL DIET USED IN EXPERIMENT I

Ingredients	Amt./100 gms
Glucose[1]	61.55 gm
Isolated soybean protein[2]	25.57 "
Corn oil	3.00 "
Ground cellulose[3]	3.00 "
DL-Methionine	0.70 "
Glycine	0.30 "
$CaHPO_4$	2.151 "
$CaCO_3$	1.492 "
KH_2PO_4	0.867 "
NaCl	0.600 "
$MgSO_4$	0.25 "
$FeSO_4 \cdot 7H_2O$	0.0333 "
$MnSO_4 \cdot H_2O$	0.0333 "
KI	0.26 mg
$CuSO_4 \cdot 5H_2O$	1.67 mg
$CoCl_2 \cdot 6H_2O$	0.17 mg
$Na_2MoO_4 \cdot 2H_2O$	0.83 mg
Choline chloride	150.00 mg
Inositol	25.00 mg
Niacin	5.00 mg
Ca Pantothenate	2.00 mg
a-Tocopherol acetate	2.00 mg
Thiamin HCl	1.00 mg
Riboflavin	1.00 mg
Pyridoxine HCl	0.45 mg
Folic acid	0.40 mg
Menadione	0.05 mg
Biotin	0.02 mg
Vitamin A	500 IU
Vitamin D_3	37.5 ICU
Vitamin B_{12}	2.00 mcg

1- Cerelose, Corn Products Sales Co., 440 New Center Bldg., Detroit, Michigan
2- Drackett Assay Protein C-1, The Drackett Products Co., Cincinnati 32, Ohio
3- Solka Floc, The Brown Co., Berlin, New Hampshire

TABLE 2

THE EFFECT OF SUPPLEMENTAL ZINC ON CHICK GROWTH TO FOUR WEEKS OF AGE

Lot	Zinc Additions to the basal (ppm)	Chick weights at 4 weeks (gm)
1	None	100
2	100	226

Experiment II

In view of the surprising results obtained in the preliminary experiment, a more comprehensive experiment was designed to substantiate the preliminary experimental findings.

In this experiment the isolated soybean protein was washed with an HCl solution at pH 4.6 in an attempt to remove its residual zinc. The protein was dried for 36 hours at a temperature of approximately 100° C. Two basal diets were used, one contained unwashed isolated soybean protein and the other washed isolated soybean protein. They contained 19 and 7 ppm zinc, respectively, and were derived from the formula show in Table 1. To each basal ration was added 100 ppm of zinc.

White Meateor X White Rock, one-day-old, sexed chicks were allotted as previously described into 12 pens of five males and five females each. Each treatment was applied to three replicates of birds. The chicks were maintained in a special laboratory which was kept relatively free from dust and at a temperature of 75° F.

Feed consumption was recorded for each treatment.

Results and Discussion:

The results are presented in Table 3. An analysis of variance of the data (Table 4) revealed a significant difference (P .01) among lots and a significant difference (P .05) between the responses of the two sexes. Comparisons between treatments were made by Duncan's method (1955).

With both basal rations, the addition of zinc to the diets increased growth significantly (P .01). Extracting the protein in the diet depressed growth significantly (P .01) in the absence of supplemental zinc and ($P < .05$) in the presence of supplemental zinc. Extracted protein in the presence of supplemental zinc did not depress growth significantly at the one percent level of probability.

As in Experiment I, the unsupplemented basal diet produced poor growth, retention of body down with poor feather growth, ragged feathers, enlarged hocks, a severe foot dermatitis and a high-stepping walk. The birds huddled near the source of heat, were inactive, but never emaciated. Retardation of growth was apparent at the end of the first week. Dermatitis began to appear at the second week and was extremely severe by the end of the third week.

The addition of zinc to the diet produced normal growth and prevented all the deficiency symptoms. The livability of chicks receiving supplemental zinc was better than that of chicks receiving no supplemental zinc. Usually, chicks with the more severe symptoms died first.

Feed utilization was improved by supplemental zinc both in the presence of washed and unwashed protein. Washing the protein decreased the efficiency of feed utilization.

It is obvious that extracting the isolated soybean protein is unnecessary to produce a zinc deficiency if other possible zinc contaminants are avoided. The zinc content of the natural protein is low enough, or perhaps not available so that an acute zinc deficiency can be produced in the chick.

TABLE 3

THE EFFECT OF SUPPLEMENTAL ZINC ON CHICK GROWTH, LIVABILITY AND FEED EFFICIENCY TO FOUR WEEKS OF AGE

Lot	Treatment	Average chick at 4 wks. (gm)	Surviving chicks of 30 started	Gain/feed
1	Basal I (unwashed protein)	183	27	0.45
2	Basal I + 100 ppm zinc	363	29	0.69
3	Basal II (washed protein)	101	28	0.35
4	Basal II + 100 ppm zinc	333	29	0.62

TABLE 4

ANALYSIS OF VARIANCE OF CHICK WEIGHTS AT FOUR WEEKS
(Experiment II)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	112				
Subclass	23	56,609	30.09**		2.11
Lot	3	400,752	213.39**		4.04
Replicate	2	3,334	1.77	3.10	
Sex	1	44,758	23.80**		6.93
T X R	6	615	0.32	2.21	
T X S	3	4,412	2.34	2.71	
R X S	2	5,634	3.00	3.10	
T X R X S	6	3,354	1.78	2.20	
Error	89	1,878			

** Significant ($P < .01$)

Comparisons among lots

at 5 percent level of probability

Lot No.	3	1	4	2
4 wk. wt.	101	183	333	363

At 1 percent level of probability

Lot No.	3	1	4	2
4 wk. wt.	101	183	333	363

Zinc deficient chicks are compared with normal chicks in the following figures. FigureI shows the stooped posture typical of zinc deficiency. Wing feathers grew to a limited extent but became rough due to the absence of barbules. The weight of the normal chick was 363 grams as compared to 183 grams for the zinc-deficient chick, thus growth is severely impaired in a zinc deficiency. Figure II shows the dermatitis which is concentrated mainly between the toes and on the skin covering the junction of the Tarsometatarsus with the phalanges. The footpads of a normal and a zinc-deficient chick are shown in Figure III. The dermatitis of the footpads becomes extremely severe, necrotic, and often bleeds. These conditions cause the high-stepping walk characteristic of a deficiency. In Figure IV the hocks of normal and zinc-deficient chicks are shown. The enlarged hocks resemble those of a manganese deficiency but there is no slipping of the tendon. As mentioned before, these symptoms can be overcome by supplementation of the ration with zinc.

Other researchers have also described zinc deficiency symptoms. O'Dell and Savage (1957) and Morrison and Sarett (1958) reported that symptoms of a zinc deficiency in chicks included depressed growth, shorter and thicker long bones and decreased ash content of the leg bones. Since their experiments were conducted in galvanized batteries with substantial amounts of zinc in the basal diet, it is considered that the symptoms given represent a mild, rather than a severe, zinc deficiency.

Pensack and Klussendorf (1957) and Norris et al. (1958) found zinc deficiency symptoms exactly as described by this author. Norris et al (1958) also made histological examinations of the enlarged bone, which revealed a derangement of the cartilage cells in the zone of calcification similar to that found in manganese and choline deficiencies. These symptoms were also reported by Edwards et al. (1958).

Zinc deficiency symptoms of the growing chick as noted by this author, and other workers are depressed growth, impaired bone development causing enlarged hocks and shortened long bones, failure to feather properly, severe dermatitis of tops of feet and between toes and on the footpads, and poor utilization of feed. Zinc additions to the diet prevents all the deficiency symptoms.

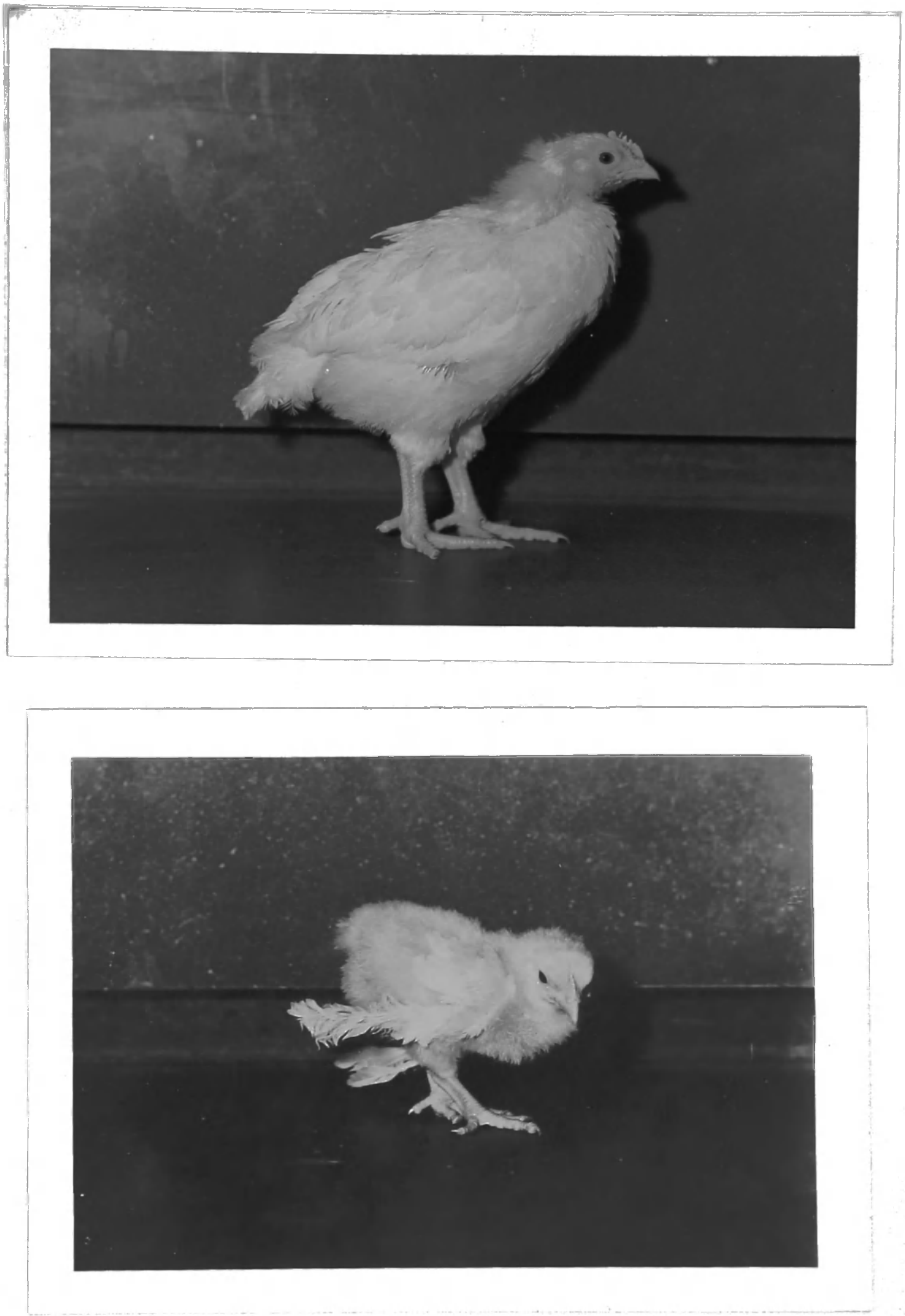

FIGURE I. THE NORMAL CHICKEN (TOP) VS THE ZINC-DEFICIENT CHICKEN (BOTTOM)

FIGURE 2. THE FOOT OF A NORMAL CHICKEN (TOP) VS THE FOOT OF A ZINC DEFICIENT CHICKEN (BOTTOM)

FIGURE 3. THE FOOTPAD OF A NORMAL CHICKEN (TOP) VS THE NECROTIC FOOTPAD OF A ZINC-DEFICIENT CHICKEN (BOTTOM)

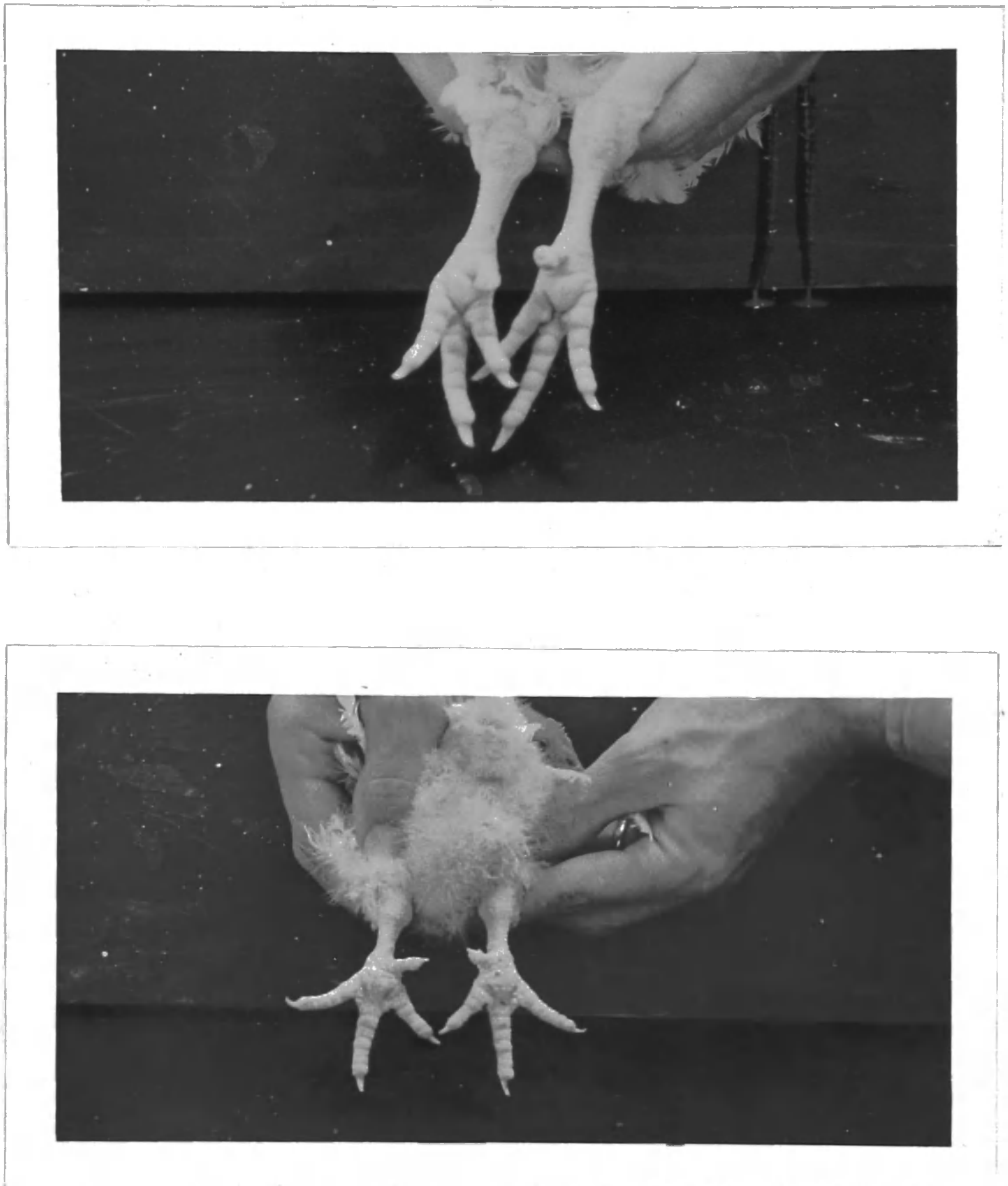

FIGURE 4. THE HOCK OF A NORMAL CHICKEN (TOP) VS THE ENLARGED HOCK OF A ZINC-DEFICIENT CHICKEN (BOTTOM).

EXPERIMENT III

This work was conducted to determine if the yolk sac of day-old chicks contained enough zinc to induce a sizable error into experimental growth data.

The yolk-sacs from one-day-old, White Leghorn, male chicks were carefully removed, weighed, and placed in separate 200 ml Erlenmeyer flasks. To each flask was added 25 ml of concentrated HNO_3. The contents were digested overnight on a steam bath. To each flask containing the clear digested liquid was added eight ml of perchloric acid. The contents in the flasks were heated on a hot plate until dry then taken up in fifty ml of 0.1 N HCL. Aliquots in duplicate from each flask were analyzed for zinc (Benne, 1955). The results were combined into four lots (Table 5) according to the yolk sac weights.

Results and Discussion

The zinc content of the yolk sac seems to be quite small. Only two grams of feed containing twenty ppm of zinc would equal the zinc content of the entire yolk sac. Consequently, differences in zinc content of retained yolk sacs would not introduce experimental error into the results previously obtained. This conclusion was substantiated by Edwards *et al.* (1958) who found that chicks with the yolk sacs removed did not respond differently than normal chicks to zinc deficient diets.

TABLE 5

THE ZINC CONTENT OF YOLK-SACS OF ONE-DAY-OLD WHITE LEGHORN MALE CHICKS

Lot	No. of chicks	Av. wt. of chicks (gm)	Chick wt. minus yolk-sac (gm)	Av. wt. yolk-sacs (gm)	Yolk sac as percent of body wt. (%)	Av. zinc content of yolk-sacs (ppm)	Av. total zinc content of yolk-sacs (mcg)
1	6	33	31	1.7	4.9	22.7	35
2	13	34	32	2.4	7.2	18.9	46
3	4	36	33	3.3	9.1	15.7	53
4	6	36	31	4.8	13.6	11.0	47

EXPERIMENT IV

Since the previous experiments proved that zinc is an essential mineral for chick growth, an experiment was designed to determine its requirement.

One-day-old White Meateor X White Rock, chicks, obtained from a commercial hatchery, were allotted as previously described into five lots, each containing three replicates of ten chicks each. The birds were reared in a plastic-coated battery in a room relatively free of dust at a temperature approximately 75° F.

The basal diet was that shown in Table 1, except that Amisoy, another isolated soybean protein, was used in place of Drackett protein. Added minerals were analytical reagent grade, except the calcium carbonate and dicalcium phosphate which were USP grade. The basal diet analyzed ten ppm zinc (Benne, 1955). To the basal diet 0, 10, 20, 40 and 80 ppm of zinc, respectively, were added as the sulfate.

Experimental diets were fed ad libitum and distilled water was furnished in glass, baby chick founts with plastic bases, growth rate, feed consumption, mortality, and deficiency symptoms were observed and recorded.

Results and Discussion

The experimental design and results are shown in Table 6. An analysis of variance of the data is given in Table 7.

The addition of zinc at all levels improved growth over that of the control, Lot 1. The addition of ten ppm gave significant (P .01) increased growth over the basal diet as did twenty ppm over the basal diet and the basal plus ten ppm zinc. The growth of chicks receiving supplemental levels of 20, 40 and 80 ppm were not significantly different but all were better (P .01) than the 0 and 10 ppm levels. Feed utilization by the chick was improved at all levels of supplemental zinc. However, levels above ten ppm made no further improvement.

Other research concerning the quantitative requirement of zinc by the chick has been reported since the completion of that conducted by the author. Norris et al. (1958) stated that the chick required no more than a total of twenty ppm of zinc when a diet containing casein was used; whereas, a total of 27 ppm (20 ppm of added zinc) was needed when isolated-soybean protein was used. Since the zinc in the isolated soybean protein was found previously to be unavailable to the chick, they concluded that the chick's requirement for this mineral was no more than twenty ppm. Young et al. (1958) added graded levels of 0, 10, 20, 40, 60 and 80 ppm to a diet containing isolated soybean protein and found that the chick required no more than a 40 ppm supplement. When the data were plotted on semi-log paper, the intersection of the sub-maximal and maximal growth lines indicated a zinc requirement of 25 to 30 ppm. Moeller and Scott (1958) reported that the chick required 20 ppm supplemental zinc when isolated soybean protein was used in a diet containing 13 ppm zinc.

These recent reports confirm the findings of the author that the growing chick requires no more than twenty ppm of available zinc. If isolated soybean protein is used in the diet, the total zinc needed is about thirty ppm because the zinc in this product is bound so that it is not utilizable by the chick.

TABLE 6

CHICK GROWTH RESPONSE TO GRADED LEVELS OF ZINC, AS THE SULFATE IN THE DIET TO FOUR WEEKS OF AGE

Lot	Zinc additions to the basal (ppm)	Zinc by analysis (ppm)	Average chick weights at 4 weeks (gm)	Surviving chicks of 30 started	Gain/feed
1	None	10.0	126	29	0.41
2	10	17.6	327	29	0.63
3	20	30.4	390	28	0.64
4	40	52.5	407	27	0.67
5	80	107.6	396	28	0.65

TABLE 7

ANALYSIS OF VARIANCE OF BODY WEIGHTS OF FOUR-WEEK OLD CHICKS
(Experiment IV)

Source of variation	Degrees of freedom	Mean square	F Values Calculated	P = .05	P = .01
Total	140				
Subclass	14	114,776	57.64**		2.23
Lot	4	397,463	119.62**		3.47
Replicate	2	1,029	0.51	3.07	
L X R	8	1,869	0.83	2.01	
Error	126	1,991			

** Significant at $P < .01$

Comparisons among lots

at 1 percent level of probability

Lot	1	2	3	5	4
4 wk. wt.	126	327	390	396	407

at 5 percent level of probability

Lot	1	2	3	5	4
4 wk. wt.	126	327	390	396	407

THE TOLERANCE OF THE GROWING CHICK FOR ZINC.

As information was being acquired concerning the chick's specific need for zinc, it was felt desirable to determine if higher levels were well tolerated or toxic. This knowledge is important because practical supplementation of rations requires a reasonable margin of safety. Furthermore, if a mistake were made in the amount of zinc added to practical rations, then the toxic symptoms and the level at which they occur would be important. Hence, experiments were conducted to determine the zinc tolerance level of growing chicks.

EXPERIMENT V

Male chicks, hatched at Michigan State University from White Leghorn males X strain-cross White Leghorn females were reared on a basal diet (Table 7) until one week of age. At this time they were randomized on the basis of weight into fifty pens of ten chicks each. Five replicates were run on each of nine high levels of zinc oxide in addition to the control lot (Table 9). Ten pens in each of five batteries were used for the experiment in such a way that a portion of each lot appeared once in each battery; thus, a battery constituted a replicate. Feed and tap water were supplied to the chicks ad libitum throughout the experimental period and feed consumption was recorded.

When the birds were five weeks of age, group weights were taken.

Results and Discussion

The results and an analysis of variance of the data are shown in Table 9. Zinc was tolerated by the chicks over the entire range of levels tested without significantly depressing growth. However, significant differences (P .05) occurred among replicates due to battery position. Since all lots were allotted to each battery, this did not invalidate the effect of the different levels of zinc. Feed conversion was also not affected by the addition of the high levels of zinc to the ration.

TABLE 8

COMPOSITION OF BASAL DIET USED IN EXPERIMENT V

Ingredients	Percent
Corn, grd. yellow	45.0
Oats, ground	5.0
Wheat middlings, flour	5.0
Wheat bran	5.0
Alfalfa meal, dehyd. 17% prot.	5.0
Meat and bone meal, 50% prot.	5.0
Soybean meal, 44% prot. solvent extracted	20.0
Fish meal, red	2.5
Whey, dried cheese	2.5
Yeast, dried brewers	2.5
Limestone, grd.	1.5
Bone meal, steamed	1.0
Salt, iodized	0.30
Vitamin A and D feeding oil (2250A, 300 D_3)	0.25
Manganese sulfate, 70% feeding grade	0.02
Choline chloride, 25% dry form	0.15
Pro-pen with B_{12}*	0.05

* Contains: 2 gm procaine penicillin and 3 mg vitamin B_{12} per pound.

TABLE 9

THE EFFECT OF VERY HIGH LEVELS OF ZINC ON THE GROWTH RATE AND FEED CONVERSION OF CROSSBRED WHITE LEGHORN COCKERELS TO FIVE WEEKS OF AGE

Lot	Zinc additions to the basal as the oxide (ppm)	Average chick wts. at 5 wks (gm)	Gain/feed
1	None	485	0.35
2	200	492	0.35
3	300	496	0.35
4	400	476	0.34
5	500	494	0.36
6	600	488	0.35
7	700	471	0.35
8	800	474	0.34
9	900	478	0.35
10	1,000	473	0.34

Analysis of variance of 5-week weights: Experiment V

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	48				
Lot	9	354	1.55	2.16	
Replicate	4	685	2.99*	2.64	3.91
Error	35	229			

* Significant ($P < .05$)

EXPERIMENT VI

The preceding experiment proved that Leghorn chickens tolerate zinc oxide at very high levels in the ration. It was thought desirable to extend this study to broiler-type chickens and zinc in the form of the sulfate, carbonate, as well as the oxide. The latter two compounds are inexpensive forms of zinc and are used in the supplementation of practical swine rations.

One-day-old Cobb's strain of White Rock, male chicks obtained from a commercial hatchery were randomized into thirty pens of ten chicks each in the manner previously described so as to minimize differences in initial weights. The various lots were assigned to three batteries in such a manner that each one appeared once in each battery; thus, each battery had a complete series of lots.

The basal diet of practical ingredients is given in Table 8 and the experimental outline is in Table 9. Three high levels of zinc (1,000, 2,000, and 3,000 ppm) in the forms of the oxide, sulfate and carbonate were added to the basal diet at the expense of corn. The birds were reared as previously described. Group weights were taken at the end of two weeks, individual weights at the end of four weeks of age.

Experimental diets and tap water were supplied ad libitum throughout the experimental period. Feed consumption and mortality were recorded.

Results and Discussion

The results are given in Table 9 and the data are evaluated statistically in Table 9.

Zinc at 1,000 ppm in the forms of oxide sulfate, or carbonate were equally well tolerated and did not depress growth as compared to the basal diet. There was no significant difference among the average weights of lots of birds fed 2,000 ppm of the three compounds, however, when compared with the basal lot growth was depressed at this level of the sulfate and carbonate but not the oxide. Three thousand ppm of the three compounds depressed growth as compared to both the lower levels and the basal.

The efficiency of feed utilization was lowered with each increase in the zinc level. This was true with all three of the compounds. Only 3,000 ppm of zinc, as the carbonate, caused rather severe mortality. The other forms of zinc at this level or below did not adversely affect livability.

At higher levels the zinc compounds were tolerated in the following decreasing order: zinc oxide, zinc sulfate, and zinc carbonate.

TABLE 10

COMPOSITION OF BASAL DIET USED IN EXPERIMENT VI

Ingredients	Amount in 100 lbs. of feed
Corn, Grd. yellow	50.8 lb
Soybean oil meal, 50% prot.	32.7 "
Menhaden fish meal, 60% prot.	2.0 "
Meat and bone scraps, 50% prot.	2.0 "
Alfalfa leaf meal, 20% prot.	2.0 "
Whey product, delactosed	0.5 "
Yeast, dried brewers	0.5 "
Yellow grease, stabilized	6.5 "
Dicalcium phosphate	1.0 "
Limestone, grd.	1.0 "
Salt, iodized	0.5 "
DL-methionine	45.4 gm
Trace mineral mix[1]	45.4 "
Vitamin Suppl. 249C[2]	34.0 "
Vitamin A (10,000 IU/gm)	19.0 "
Vitamin D_3 (1,500 ICU/gm)	10.0 "
Choline chloride, 25% dry form	30.0 "
Vitamin B_{12} suppl. (6 mg/lb)	45.0 "

1- Delamix: at 0.1 percent of diet it supplies in mg per lb. of feed: manganese 27, iodine 0.54, iron 9, copper 0.9 and cobalt 0.09.

2 Contains per lb: 2 grams riboflavin, 4 grams pantothenic acid, 9 grams niacin and 10 grams choline

TABLE 11

EFFECT OF THREE LEVELS OF DIFFERENT SALTS OF ZINC UPON GROWTH, LIVABILITY AND FEED EFFICIENCY OF THE CHICK TO 4 WEEKS OF AGE

Lot	Zinc additions to the basal (ppm)	Source of zinc	Av. chick wts. at 4 wks. (gm)	Surviving chicks of 30 started	Gain/feed
1	None		493	29	0.54
2	1,000	ZnO	472	29	0.58
3	2,000	"	441	30	0.53
4	3,000	"	337	29	0.44
5	1,000	$ZnSO_4$	464	30	0.59
6	2,000	"	419	28	0.52
7	3,000	"	282	29	0.41
8	1,000	$ZnCO_3$	483	30	0.58
9	2,000	"	407	29	0.48
10	3,000	"	214	23	0.29

TABLE 12

ANALYSIS OF VARIANCE OF CHICK WEIGHTS (Experiment VI)

Source of variation	Degrees of freedom	Mean square	F values Calculated	P = .05	P = .01
Total	285				
Subclass	29	77,246	17.40**		1.79
Lot	9	233,499	54.90**		2.50
Replicate	2	9,557	2.24	3.02	
L X R	18	6,807	1.59	1.65	
Error	266	4,256			

** Significant ($P < .01$)

Comparisons among lots

at 5 percent level of probability

Lot	10	7	4	9	6	3	5	1	8	2
4 wk. wt.	214	282	337	407	419	441	464	476	483	488

at 1 percent level of probability

Lot	10	7	4	9	6	3	5	1	8	2
4 wk. wt.	214	282	337	407	419	441	464	476	483	488

EXPERIMENT VII

This experiment was designed to study further the tolerance of the growing chick for high levels of zinc.

Cobb's strain, White Rock, male chicks obtained from a commercial hatchery were randomized into 32 pens of ten chicks each, as described in the previous experiment. Four lots, each consisting of four replicates, were used as shown in Table 11. The basal diet was the same as that used in Experiment VI.

Results and Discussion

The results are reported in Table 12 and the data are evaluated in Table 12. The addition of fifty ppm of zinc as the sulfate and 1,000 ppm in the form of sulfate, oxide, or carbonate did not affect growth but 1,500 ppm depressed growth significantly ($P < 0.01$). At this higher level zinc oxide was less toxic than zinc sulfate or carbonate.

The efficiency of feed utilization was not affected by the lower levels of zinc but the 1,500 ppm level of all forms affected it adversely.

The livability of chicks was good in all lots.

Certain research reports appearing recently have also dealt with this problems with chicks. Mehring _et al_. (1956) found that zinc levels up to 814 ppm did not adversely affect growth and feed efficiency. Klussendorf and Pensack (1957 observed that a level of 1,367 ppm of zinc, as the proteinate, did not depress growth; whereas, 1,823 ppm did depress growth, the chloride being more drastic. They found zinc proteinate, carbonate or acetate at the 2,000 ppm level equal in this regard, but compared to the non-supplemented basal diet these levels depressed growth. The difference between them was not significant but a trend was apparent. They concluded that zinc in excess of 1,000 ppm may be detrimental.

Norris et al. (1958) observed that 1,000 ppm zinc caused poorer growth when a semi-purified, isolated soybean-protein-type diet was used but a level of 500 ppm did not. The form in which the zinc was added was not disclosed.

These recent reports confirm the results of the present study that the growing chick can tolerate 1,000 ppm or less of zinc in the ration and that the oxide form is tolerated to a greater degree than the sulfate and chloride. Since the requirement of the growing chick is not greater than twenty ppm of available zinc, reasonable levels of supplementation of practical rations can be made with assurance that they will be well tolerated.

TABLE 13

THE EFFECT OF AN EXCESS OF ZINC ON CHICK PERFORMANCE TO 4 WEEKS OF AGE

Lot	Zinc additions to the basal (ppm)	Source of zinc	Av. chick wts. at 4 wks. (gm)	Surviving chicks of 30 started	Gain/feed
1	None		491	38	0.62
2	50	$ZnSO_4$	500	40	0.57
3	1,000	ZnO	497	38	0.60
4	1,000	$ZnSO_4$	460	39	0.59
5	1,000	$ZnCO_3$	490	40	0.56
6	1,500	ZnO	455	38	0.54
7	1,500	$ZnSO_4$	402	39	0.51
8	1,500	$ZnCO_3$	390	40	0.50

TABLE 14

ANALYSIS OF VARIANCE OF FOUR-WEEK WEIGHTS
(Experiment VII)

Source of variation	Degrees of freedom	Mean square	F values Calculated	P = .05	P = .01
Total	311				
Subclass	31	19,992	6.08**		1.79
Lot	7	73,778	21.53**		2.73
Replicate	3	7,990	2.43	2.62	
L X R	21	3,777	1.14	1.60	
Error	280	3,287			

** Significant (P < .01)

Comparisons among lots

at 1 percent level of probability

Lot	8	7	6	4	5	1	3	2
4 wk. wt	390	402	455	470	490	491	497	500

at 5 percent of probability

Lot	8	7	6	4	5	1	3	2
4 wk. wt.	390	402	455	470	490	491	497	500

THE AVAILABILITY TO THE CHICK OF ZINC FROM DIFFERENT COMPOUNDS

A group of experiments was conducted to study the availability of zinc in different zinc compounds.

EXPERIMENT VIII

This experiment compared the availability of zinc as the sulfate and chloride with respect to rate of chick growth and feed efficiency.

White Meateor X White Rock, male chicks from a commercial hatchery were allotted to six pens of nine chicks each. Duplicate lots were fed the basal ration, the basal containing zinc sulfate and the basal with zinc chloride (Table 15).

The birds were maintained in a manner previously described for purified diet studies.

Results and Discussion

Zinc in the form of both the sulfate and the chloride at the 100 ppm level produced increased growth as compared to the basal lot. There was no difference in performance of chicks between the two compounds.

Thus, the zinc in the compounds was available and adequate for normal growth at the level used.

Feed utilization was improved at a comparable rate by both the zinc compounds. Livability of the chicks was good in all lots.

TABLE 15

THE AVAILABILITY OF ZINC FROM TWO COMPOUNDS TO THE CHICK TO FOUR WEEKS OF AGE

Lot	Zinc additions to the basal (ppm)	Zinc compound	Average chick wts. at 4 weeks (gm)	Surviving chicks of 30 started	Gain/feed
1	None	None	112	18	0.33
2	100	$ZnCl_2$	398	18	0.65
3	100	$ZnSO_4$	400	17	0.62

TABLE 16

ANALYSIS OF VARIANCE OF THE WEIGHT OF FOUR-WEEK-OLD CHICKS
(Experiment VIII)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	51				
Subclass	5	189,512	145.11**	3.44	
Lot	2	468,795	358.95**	5.10	
Replicate	1	2,646	2.03		4.05
L X R	2	3,663	2.80		3.20
Error	46	1,306			

** Significant ($P < .01$)

Comparisons among lots

at 1 percent level of probability

Lot	1	2	3
4 wk. wt.	112	398	400

at 5 percent level of probability

Lot	1	2	3
4 wk. wt.	112	398	400

EXPERIMENT IX

In this experiment, zinc in the form of the oxide and carbonate was compared with the sulfate. The three compounds were included at levels of ten and twenty ppm of zinc. Since the zinc requirement had been established at twenty ppm of available zinc, the lower level provided a more critical evaluation of the availability of the zinc.

White Rock, male chicks from a commercial hatchery were randomized to three replicates of ten birds for each lot and reared as previously described for purified diet studies. The experimental design is given in Table 17.

Results and Discussion

The results are given in Table 17 and the data statistically evaluated in Table 18. The addition of both levels of supplemental zinc increased growth in every case, but deficiency symptoms were prevented only at the higher level. At each level of zinc, the sulfate, oxide, and the carbonate proved to be about equal, but growth was greater at the higher level.

Comparison of twenty ppm of the sulfate and ten ppm of the oxide at the five percent level of probablility revealed a significant weight decrease with this level of oxide, but no difference at the one percent level.

The efficiency of feed utilization was increased in each instance in which supplemental zinc was added to the diet. The higher level of zinc improved feed efficiency to a greater extent than did the lower.

Thus, the zinc in the three compounds is equally available to the chick and can be used interchangeably in supplementation of practical poultry rations.

This supports the conclusion of Pensack *et al*. (1958) who showed that zinc from the carbonate, oxide, chloride and proteinate was equally available when 6, 20 and 40 ppm of each compound were added to a glucose-casein, gelatin-type diet.

TABLE 17

THE AVAILABILITY OF ZINC FROM THREE COMPOUNDS TO THE CHICK TO FOUR WEEKS OF AGE

Lot	Zinc addition to basal (ppm)	Zinc compound	Average chick weights at 4 weeks (gm)	Gain/feed
1	None	None	163	0.36
2	10	$ZnSO_4$	293	0.50
3	20	$ZnSO_4$	343	0.53
4	10	ZnO	310	0.51
5	20	ZnO	352	0.54
6	10	$ZnCO_3$	299	0.51
7	20	$ZnCO_3$	359	0.55

TABLE 18

ANALYSIS OF VARIANCE OF FOUR-WEEK-OLD CHICK WEIGHTS
(Experiment IX)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	192				
Subclass	20	39,329	15.10**		2.00
Lot	6	125,443	48.30**		2.92
Replicate	2	4,844	1.80	3.06	
L X R	12	2,020	0.78	1.82	
Error	172	2,598			

** Significant ($P < .01$)

Comparisons among lots

at 1 percent level of probability

Lot	1	2	6	4	3	5	7
4 wk. wt.	163	293	299	310	343	352	359

at 5 percent level of probability

Lot	1	2	6	4	3	5	7
4 wk. wt.	163	293	299	310	343	352	359

THE INTERRELATIONSHIP OF ZINC WITH CALCIUM AND PHOSPHORUS

Since high levels of calcium in the ration aggravated parakeratosis, or zinc deficiency in swine, a series of experiments was conducted to study interrelationships between zinc and calcium or phosphorus.

EXPERIMENT X

Cobb's strain, White Rock, male chicks received from a commercial hatchery were randomized to 15 pens of ten chicks each. Each lot was replicated three times.

The chicks were maintained in a plastic-coated battery under conditions previously described for purified diet studies.

The basal diet contained 1.23 percent calcium. A supplemental zinc level of eighty ppm was added to this diet in the absence and presence of one percent supplemental calcium (Table 19). Also, two levels of calcium (0.5 and 1.0 percent) were added to the basal diet in the absence of additional zinc.

Results and Discussion

The chicks receiving the unsupplemented basal diet exhibited zinc deficiency symptoms and grew poorly. The addition of 0.5 percent calcium depressed growth only slightly but caused the incidence and severity of the symptoms to increase. The additions of one percent calcium definitely retarded growth, increased the incidence of symptoms, and made them more acute. Supplementation of the basal diet with zinc alone, or one percent extra calcium plus eighty ppm of zinc, produced normal growth and prevented all deficiency symptoms.

At least, a part of the zinc in the basal diet was available because the elevated calcium levels aggravated the deficiency condition. Since isolated soybean protein was the major source of zinc in the basal ration, part of its zinc must have been available to the chick. Thus it is quite evident that the adverse effect of calcium on growth and deficiency symptoms was produced through its antagonism with zinc.

TABLE 19

THE EFFECT OF ELEVATED LEVELS OF DIETARY CALCIUM ON THE PERFORMANCE OF CHICKS TO FOUR WEEKS OF AGE

Lot	Additions to the basal		Average chick weights at 4 weeks (gm)	Surviving chicks of 30 started	Gain/feed
	Calcium (%)	Zinc (ppm)			
1	--	--	145	29	0.37
2	--	80	356	29	0.55
3	0.5	--	140	29	0.38
4	1.0	--	116	27	0.31
5	1.0	80	343	28	0.54

TABLE 20

ANALYSIS OF VARIANCE OF CHICK WEIGHTS AT FOUR WEEKS
(Experiment X)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	141				
Subclass	14	115,441	62.37**		5.56
Lot	4	400,267	216.25**		6.63
Replicate	2	152	0.08	19.37	
L X R	8	1,851*			
Error	127	865			

* Significant ($P < .05$) interaction with which subclass, lot and replicate mean squares were compared.
** Significant ($P < .01$)

Comparisons among lots

at 1 percent level of probability

Lot	4	3	1	5	2
4 wk. wt.	116	140	145	343	356

at 5 percent level of probability

Lot	4	3	1	5	2
4 wk. wt.	116	140	145	343	353

EXPERIMENT XI

In the previous experiment the retarded growth produced by elevated calcium levels was overcome by zinc levels in excess of the actual requirement. This experiment was conducted to find out if the zinc requirement, as determined with a diet containing a normal level of calcium (1.23 percent), was increased if higher levels of calcium were used.

White Rock, male chicks from a commercial hatchery were allotted as previously described to 21 pens of ten birds each. The lots were replicated three times. The birds were maintained in a plastic-coated battery in the special laboratory under conditions as previously described for purified diet studies.

Two levels of calcium (0.5 and 1.0 percent) were added to the basal diet containing both twenty and eighty ppm of zinc (Table 21). The unsupplemented basal diet contained 1.23 percent calcium.

Results and Discussion

The chicks receiving the basal diet grew poorly and exhibited zinc deficiency symptoms similar to those previously described. The addition of 0.5 percent calcium to the basal diet either in the presence of twenty or eighty ppm zinc produced normal growth and no deficiency symptoms. Similarly, normal performance was obtained following the addition of 1.0 percent calcium in the presence of eighty ppm of zinc. However, one percent of calcium in the presence of twenty ppm retarded growth but did not produce deficiency symptoms typical of acute cases.

The efficiency of feed utilization was reduced in the lots receiving the basal diet and basal diet supplemented with 1.0 percent calcium 20 ppm zinc but was normal in the remaining lots. Livability was good in all lots.

These results indicate that the requirement of the chick for twenty ppm of zinc is not increased if a slight (0.5 percent) excess of calcium is added to the diet (1.73 percent total). However, twenty ppm of supplemental zinc is not sufficient if one percent calcium is added (2.23 percent total). Eighty ppm of zinc neutralizes the effect of one percent additional calcium. Therefore, 60 ppm of zinc will overcome the adverse effects of one percent of calcium. Thus, the zinc requirement is elevated with rather high levels of dietary calcium but not to the extent as in swine.

Several other research workers have mentioned a zinc-calcium interrelationship. Morrison and Sarett (1958) found that the addition of 0.5 and 1.0 percent calcium to an isolated-soybean-protein diet reduced 14-day gains and increased the incidence and severity of the hock disorder; bone ash percentage was not affected. Supplementation of the diets with five ppm zinc only partially prevented the growth inhibition brought about by the extra calcium; whereas 25 ppm completely overcame the depression. Feed efficiency paralleled growth in each case. Norris _et al._ (1958) reported that the addition of one percent calcium to a diet containing 1.23 percent calcium caused deficiency symptoms to become more severe. Fifty ppm of zinc restored normal growth and alleviated all symptoms. Conversely, Pensack _et al._ (1958) observed that growth was not depressed with calcium-phosphorus ratios of 1.0/0.6, 2.0/0.6 and 1.0/1.2 in the presence of 6, 20, and 40 ppm of supplemental zinc. Casein and gelatin, however, were employed in their diets.

TABLE 21

THE EFFECT OF ELEVATED CALCIUM LEVELS ON THE ZINC REQUIREMENT OF THE GROWING CHICK TO FOUR WEEKS OF AGE

Lot	Additions to the basal Calcium (%)	Additions to the basal Zinc (ppm)	Average chick weights at 4 weeks (gm)	Surviving chicks 30 started	Gain/feed
1	None	None	153	30	0.39
2	0.5	20	377	30	0.58
3	0.5	80	368	28	0.56
4	1.0	20	310	29	0.50
5	1.0	80	382	28	0.57

TABLE 22

ANALYSIS OF VARIANCE OF FOUR WEEK WEIGHTS
(Experiment XI)

Source of variation	Degrees of freedom	Mean square	F values Calculated	P = .05	P = .01
Total	144				
Subclass	14	80,757	43.00**		2.23
Lot	4	280,618	150.00**		3.47
Replicate	2	701	0.30	3.07	
L X R	8	841	0.40	2.01	
Error	130	1,871			

** Significant ($P < .01$)

Comparisons among lots

at 1 percent of probability

Lot	1	4	3	2	5
4 wk. wt.	153	310	368	377	382

at 5 percent of probability

Lot	1	4	3	2	5
4 wk. wt.	153	310	368	377	382

EXPERIMENT XII

The purpose of this experiment was to find out if a phosphorus level in excess of the requirement would affect the chicks quantitative need for zinc.

White Rock, male chicks from a commercial hatchery were randomized to nine pens of nine chicks each. Each lot was replicated three times. The chicks were maintained under special conditions as previously described for purified diet studies. Sodium phosphate, at a level of 0.5 percent phosphorus was added to the basal diet, in the absence and presence of one hundred ppm of zinc (Table 23). The basal diet contained twenty ppm supplemental zinc and 0.69 percent phosphorus.

Results and Discussion

The addition of 0.5 percent phosphorus to the basal diet did not increase the chick's zinc requirement. Feed efficiency, growth and feather condition were comparable regardless of the level of phosphorus or zinc.

TABLE 23

THE EFFECT OF A HIGH LEVEL OF DIETARY PHOSPHORUS ON THE CHICK REQUIREMENT FOR ZINC TO FOUR WEEKS OF AGE

Lot	Additions to the basal	Av. chick wt. at 4 weeks (gm)	Chicks surviving 27 started	Gain/feed
1	None	389	27	0.57
2	0.5% Phosphorus	371	26	0.58
3	0.5% Phosphorus + 100 ppm zinc	384	26	0.56

TABLE 24

ANALYSIS OF VARIANCE OF CHICK WEIGHTS AT FOUR WEEKS OF AGE (Experiment XII)

Source of variation	Degrees of freedom	Mean square	F values	
			Calculated	P = .05
Total	78			
Subclass	8	6,753	0.78	3.84
Lot	2	4,953	0.57	19.25
Replicate	2	4,695	0.54	19.25
L X R	4	8,681**		
Error	70	2,155		

** Significant interaction ($P < .01$) used as basis for comparisons with subclass, lot and replicate mean square.

EXPERIMENT XIII

This experiment was conducted in vitro to find out if calcium and phosphorus supplements would remove zinc from solution.

One gram samples of ground oyster shells, feed grade dicalcium phosphate, and bone meal were weighed separately into duplicate flasks. To one flask of each mineral and a blank were added forty ml of a solution containing fifty ppm of zinc, as the sulfate. To the other flasks were added forty ml of a solution containing one hundred ppm zinc, as the sulfate. The flasks were mechanically shaken at a temperature of 37° C for 1.5 hours. In each flask a quantity of the mineral supplement remained in the solid phase. The contents of each flask were filtered and analyzed for zinc (Benne, 1955).

Results and Discussion

The results are given in Table 25. Ground oyster shells, dicalcium phosphate and bone meal removed practically all of the zinc from solution at both levels of zinc. Ground oyster shells and bone meal were more effective in this respect than dicalcium phosphate. These results indicate the manner by which high, or excess levels of mineral act in making zinc unutilizable to the chick.

TABLE 25

THE *IN VITRO* REMOVAL OF ZINC FROM SOLUTION BY MINERAL SUPPLEMENTS

Lot	Mineral	Amount of mineral added (gm)	ppm Zinc in 40 ml of zinc sulfate solution	Zinc solution after equilibrium (ppm)
1	None	--	50	45
2	Ground oyster shells	1	50	<1
3	Dicalcium phosphate	1	50	5
4	Bone meal	1	50	<1
5	None	--	100	109
6	Ground oyster shells	1	100	<1
7	Dicalcium phosphate	1	100	4
8	Bone meal	1	100	<1

EXPERIMENT XIV

This experiment was conducted with laying hens to determine if supplemental zinc, in the presence or absence of one percent of additional calcium (as $CaCO_3$), affected egg production, hatchability and egg shell thickness.

Spring-hatched, hybrid pullets from a commercial hatchery were reared in confinement on wood shavings for litter. A starting ration (Table 26) containing approximately 35 ppm of zinc was fed for the first eight weeks and a growing ration (Table 26) containing approximately 36 ppm of zinc was fed from eight to twenty weeks of age. The management practices employed were comparable to those used under practical conditions. Tap water and feed were given ad libitum .

The pullets were housed at twenty weeks of age in floor pens with wood shavings for litter. Each pen contained 85 square feet of floor space, five trapnests, one hanging feeder, one automatic waterer, and a 4' X 4' dropping pit. Ventilation was natural or forced draft depending on weather conditions but no supplemental heat was used.

At 24 weeks of age the pullets were randomized on the basis of body weight, sexual maturity, and one-month's production into 12 pens of 21 pullets each. Each lot was replicated three times

The basal laying ration in Table 27 contained two percent calcium. One hundred ppm of supplemental zinc as the sulfate was added to the basal diet, in the absence and presence of one percent calcium (Table 28).

The hens were trapnested five days per week for 32 weeks beginning the first of November and continuing into June. In

February and June, three eggs per hen were collected to determine egg weight, Haugh score and shell thickness. Eggs for February were collected during week days, stored in a refrigerator at 48° F, and measurements were made during the morning following the day the eggs were laid.

Egg weights were taken in grams, albumen heights in millimeters, and eggshell thickness in thousandth's of an inch. From the egg weight and albumen height, the Haugh score was derived (Haugh, 1937).

For hatchability data, one week's eggs of each hen were set for the months of February through June. The hatch during May was not included with final results because of incubator failure.

Results and Discussion

The results are given in Table 28 and the analyses of the data in Tables through 32. Egg production and hatchability were not affected by the addition of zinc to the basal ration, or the basal to which extra calcium was added. Also, extra calcium alone did not affect egg production and hatchability. There was no difference in egg weight or Haugh score among the treatments during either February or June.

In February, eggshell thickness was increased ($P < .05$) by the addition of calcium and zinc to the ration compared to zinc alone. However, there was no increase in eggshell thickness when zinc or calcium alone were compared to zinc plus calcium. At the five percent level of probability, the addition of neither calcium nor zinc altered eggshell thickness as compared to the basal but the addition of calcium improved eggshell thickness compared to zinc alone. The addition of calcium plus zinc increased shell thickness when compared to all other treatments.

These results indicated that neither calcium nor zinc alone had any significant effect on eggshell thickness, but when both were added, the egg shell thickness was increased in cool weather. This combination of calcium and zinc did not increase eggshell thickness during warm weather. Apparently the eggshell thickness decreased with age of hen and environmental temperature, regardless of level of calcium or zinc used.

TABLE 26

DIETS FOR REARING PULLETS USED IN EXPERIMENT XII

Ingredients	Amt. per 100 pounds Starter (lbs)	Grower (lbs)
Corn, ground yellow	45.00	45.00
Oats, ground heavy	5.00	15.00
Wheat, ground	--	10.00
Wheat bran	5.00	5.00
Wheat, standard middlings	5.00	5.00
Alfalfa leaf meal, dehyd. 17% prot.	5.00	5.00
Soybean oilmeal, solv. ext. 44% prot.	20.00	15.00
Meat and bone scraps	5.00	2.50
Fish meal, red	2.50	1.25
Whey, dried cheese	2.50	1.25
Yeast, dried brewers	2.50	--
Limestone, ground	1.50	1.25
Bone meal, steamed	1.00	1.50
Salt, iodized	0.30	0.50
Manganese sulfate, 70% feed grade	0.02	0.013
Vitamin A and D feeding oil (2250A, 300D)	0.25	0.150
Choline chloride, 25% dry mix	0.15	--
Pro-pen[1]	0.005	0.10
Calcium pantothenate	--	200 mg

1 An antibiotic supplement containing two grams of procaine penicillin and three grams of vitamin B_{12} per pound.

TABLE 27

BASAL DIET FOR EXPERIMENT XIV

Ingredients	Amt. per 100 lbs.	
Corn, yellow ground	59.53 lbs	
Soybean meal, 50% solv. extracted	17.00 "	
Wheat, flour middlings	5.00 "	
Meat and bone scraps, 50% prot.	3.00 "	
Fish meal	2.00 "	
Whey product, delactosed	1.5 "	
Alfalfa meal, 20% prot.	1.5 "	
Fat, No. 2 yellow grease	5.0 "	
Limestone, ground	2.0 "	
Dicalcium phosphate	2.6 "	
Salt	0.5 "	
Trace mineral mix*	0.1 "	
Choline chloride, 25% dry mixture	0.2 "	
Vitamin B_{12} (6 mg/lb)		23 grams
Vitamin D_3 (3,000 ICU/gm)		15 "
Vitamin A (10,000 IU/gm)		10 "
Calcium pantothenate (32 mg/lb)		2 "
Niacin, 100%		0.5 "

* Delamix - added at 0.1 percent level it provides in mg per pound of feed: manganese 25, iodine 0.54, iron 9, copper 0.9 and cobalt 0.09.

Calculated analysis: Ca 2.0 percent, P 1.0 percent, Zn 34 ppm

TABLE 28

THE EFFECT OF ZINC IN THE PRESENCE AND ABSENCE OF ADDITIONAL CALCIUM ON THE PERFORMANCE OF THE LAYING HEN

Lot	Additions to basal		Egg production 5-day trap survivors	Egg weight (gm)		Haugh units		Egg shell thickness (.001 in)		Laying house mortality (%)	Hatchability (%)	Lbs. feed/doz. eggs
	Ca %	Zn ppm	%	Feb.	June	Feb.	June	Feb.	June			
1	--	--	69.5	59	65	73	78	13.33	12.58	12.6	96.7	4.74
2	--	100	69.0	58	62	74	77	13.10	12.43	11.1	94.8	4.65
3	1.0	--	67.5	58	62	76	77	13.53	12.47	9.5	95.8	4.76
4	1.0	100	66.1	58	63	75	74	13.91	12.53	9.5	97.6	4.72

TABLE 29

ANALYSIS OF VARIANCE OF EGG PRODUCTION
(Experiment XIV)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	223				
Subclass	11	751	0.81	1.83	2.34
Lot	3	161	0.17	2.65	3.88
Replicate	2	1,006	1.08	3.04	4.71
L X R	6	960	1.04	2.14	2.90
Error	212	923			

TABLE 30

ANALYSIS OF VARIANCE OF EGG WEIGHT (February)
(Experiment XIV)

Source of variation	Degrees of freedom	Mean square	F Values		
			Calculated	P = .05	P = .01
Total	219				
Subclass	11	19.9	1.04	1.85	2.34
Lot	3	21.0	1.10	2.65	3.88
Replicate	2	0.5	0.03	3.04	4.71
L X R	6	25.8	1.65	2.14	2.90
Error	208	19.0			

ANALYSIS OF VARIANCE OF EGG WEIGHT (June)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	201				
Subclass	11	34.6	1.38	1.85	2.34
Lot	3	72.30	2.90	2.65	3.88
Replicate	2	34.00	1.36	3.04	4.71
L X R	6	14.1	0.56	2.14	2.90
Error	190	24.9			

TABLE 31

ANALYSIS OF VARIANCE OF EGGSHELL THICKNESS (February)
(Experiment XIV)

Source of variance	Degrees of freedom	Mean square	F values Calculated	P = .05	P = .01
Total	219				
Subclass	11	2.88	2.82**		2.34
Lot	3	6.25	6.12**		3.88
Replicate	2	1.59	1.55	3.04	
L X R	6	1.64	1.60	2.14	
Error	208	1.02			

** Significant ($P < .01$)

Comparison among lots

at 1 percent level of probability

Lot	2	1	3	4
Egg shell thickness	13.10	13.33	13.53	13.91

at 5 percent level of probability

Lot	2	1	3	4
Egg shell thickness	13.10	13.33	13.53	13.91

TABLE 31 (cont'd.)

ANALYSIS OF VARIANCE OF EGGSHELL THICKNESS
(June)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	201				
Subclass	11	1.21	0.90	1.83	--
Lot	3	.27	0.16	2.65	--
Replicate	2	.87	0.65	3.04	--
L X R	6	1.85	1.35	2.14	--
Error	190	1.34			

TABLE 32

ANALYSIS OF VARIANCE OF HAUGH SCORE (February)
(Experiment XIV)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	219				
Subclass	11	57	1.24	1.83	2.34
Lot	3	114	2.49	2.65	
Replicate	2	42.5	0.93	3.04	4.71
L X R	6	33.3	0.73	2.14	2.90
Error	208	45.7			

ANALYSIS OF VARIANCE OF HAUGH SCORE (June)

Source of variation	Degrees of freedom	Mean square	F values		
			Calculated	P = .05	P = .01
Total	201				
Subclass	11	170.4	1.77	1.85	2.34
Lot	3	132.6	1.38	2.65	3.88
Replicate	2	26.5	0.28	3.04	4.71
L X R	6	237.3	2.47*	2.14	2.90
Error	190	96.1			

* Significant ($P < .05$)

GENERAL DISCUSSION

The zinc of the body is widely distributed though concentrated to a greater extent in the red blood cells, skin, hair, liver, spleen, bone, pigments of the eye, and the uterus in the case of the laying hen. Zinc is a trace mineral, and its principal action in the tissue is probably of a catalytic nature rather than a structural one, as are the major minerals calcium and phosphorus. Zinc has been implicated as an essential part of certain enzyme systems. For example, carbonic anhydrase is a zinc-containing enzyme, distributed throughout the body but concentrated in the same areas as those mentioned for zinc (Keilin and Mann, 1940). This enzyme catalyzes the reaction of carbon dioxide with water to produce carbonic acid ($H_2O + CO_2 \rightleftarrows H_2CO_3$). This reaction is necessary for the removal of CO_2 from metabolizing tissue, and its transportation to the lungs for disposal. A number of dehydrogenases (alcohol dehydrogenase of yeast, alcohol dehydrogenase of horse liver, glutamic dehydrogenase of beef liver, and lactic acid dehydrogenase of rabbit skeletal muscle) contain zinc as an indespensible part, and each requires diphosphopyridine nucleotide, a niacin containing compound, as the cofactor (Vallee, 1957). These enzymes remove hydrogen from a substrate in biological oxidation; therefore, they are concerned with energy metabolism. Removal of the zinc from these enzymes leads to irreversible inhibition. Likewise zinc-binding compounds inhibit the enzymes. Carboxypeptidase of the pancreatic juice has been shown to be a zinc-containing enzyme (Vallee, 1955). This enzyme is necessary for hydrolysis of amino acids having a free carboxy group from peptides. Thus, it functions in proteolysis in the small intestine.

Zinc has also been implicated in the enzymatic action of uricase, intestinal phosphatase, and catalase but here its function is not of an indespensible nature because it can be removed or replaced without irreversible inhibition. Here it appears to act as a loosely bound ion.

Growth is drastically depressed in a zinc deficiency, but the zinc content and the enzymatic activity appear to remain normal. It would appear, then that the chick grows only to the extent allowed by the zinc present in the tissue enzymes. The growth is not normal- the long bones are shortened, the hock joint enlarged, the feathers do not develop normally and the healthy condition of the skin cannot be maintained.

Impaired bone growth has been a problem ever since man first began to raise poultry in confinement. A number of nutrients (vitamin D, calcium, phosphorus, niacin, folic acid, choline, biotin, tryptophane, and manganese) have been shown to be required for normal bone development. As can be seen, some of these are necessary for the growth of cartilage while others are necessary in the ossification process. Now, the trace element zinc has been added to this list of minerals necessary for bone growth in chickens.

It would appear that zinc is necessary for the deposition of cartilage since derangement of cartilage cells appears in a zinc deficiency in the chick (Norris, 1958). The enzymes previously discussed are involved in energy metabolism, which in turn is necessary for protein metabolism and thus cartilage synthesis.

Zinc also has been shown to be necessary for calcification. In the growing bone carbonic anhydrase probably functions in making carbonate available from carbonic acid, to react with calcium, from the blood, to form calcium carbonate in the bone or eggshell. Common, (1941) found that the uterus of the laying hen contained more carbonic anhydrase enzyme than other oviducal tissue and Gutowska and Mitchell (1945) noted more of this enzyme in the oviduct of hens laying sound shell eggs than in poor layers or non-laying hens. Sulfonilamide, which inhibits carbonic anhydrase also causes soft shell eggs.

It would then appear that the eggshell (largely $CaCO_3$) is formed by the action of carbonic anhydrase on carbonic acid to produce carbonate which, in turn, reacts with calcium ions from the blood. In this study zinc plus calcium actually increased shell thickness in cool weather but not in warm weather. Apparently both were deficient since neither mineral alone affected the shell thickness. It should be pointed out also that eggshells become thin in warm weather and with advanced age of the hen even in the presence of calcium and zinc supplementation. Thus, it would appear that factors other than this enzyme and theminerals are functioning in eggshell formation. This thinning of eggshells cause great loss in warm months at the close of the laying year and should demand more attention.

The skin of the feet of chickens appears to be the most susceptible area to dermatitis. A deficiency of several nutrients, including biotin, and pantothenic acid causes this condition. Now a zinc deficiency has also been shown to cause a dermatitis of the feet. All these nutrients function in metabolic enzymes; therefore, it would seem that the skin breakdown is the result of generally impaired metabolism.

Zinc apparently plays an important role in the synthetic processes within the feather follicle. In extreme zinc deficiencies in chickens only those feathers showing in the wings at hatching time make any appreciable growth. In other areas the chick retains the body down. A high concentration of the enzyme carbonic anhydrase normally occurs in the hair follicle,and we might also expect this to be true in the feather follicle. Therefore, a deficiency of this enzyme and the dehydrogenases would certainly prevent feather growth.

In the growing chick, zinc in the form of the chloride, sulfate carbonate and oxide is equally and completely available to the chick, when very low levels are used. The chloride and sulfate are highly water-soluble, and the carbonate is water soluble to a lesser extent. The oxide is only slightly water-soluble but more so in dilute acids. Therefore, at the pH of the intestine (pH 6), the solubility of the oxide is increased. This, in addition to the fact that only traces of zinc are needed, may explain the fact that all of the compounds are equally available. The carbonates and oxides of zinc, which are readily available on the market, at least can be used as zinc supplements for chicken rations.

The zinc must of necessity be absorbed from the intestinal tract by the chicken before it can be utilized within the body. An excess of calcium in the diet appears to remove from solution trace elements, such as manganese and zinc and prevents their absorption from the intestine. The excess of insoluble minerals containing the trace elements are then excreted in the feces.

In vitro, calcium and phosphorus supplements remove manganese from solution. This removal process is increased as the pH increases, but the manganese is absorbed on the solid mineral material rather than precipitated as insoluble manganese phosphate or hydroxide

(Schaible and Bandemer, 1942). Likewise, zinc is removed from solution *in vitro* by adsorption on precipitated calcium phosphate. No zinc is removed from solution and no precipitation of calcium phosphate is evident until a pH of three or higher is reached. As the pH is increased, the amount of zinc removed is also increased (Lewis *et al.* 1957).

Soluble zinc leaving the gizzard (pH 2.6) of the chicken would be removed from solution by insoluble calcium phosphate as the chyme enters and passes down the intestine which ranges from pH 5.7 in the duodenum to pH 6.2 in the rectum. The intestinal pH is satisfactory for the removal of soluble zinc from solution provided the insoluble mineral material is present. This condition is made possible by an excess of calcium in the diet. Excess calcium then increases the dietary zinc requirement but not necessarily the tissue requirement.

It may at first appear that high levels of calcium carbonate would raise the pH of the gut and further increase the removal of zinc; however, the chicken is physiologically able to maintain an optimum pH in the intestine while receiving large quantities of basic materials in the feed.

At very high levels of zinc, the oxide was better tolerated by the chicken than the sulfate and carbonate. Zinc oxide is the most insoluble form, even at the pH of the gut. At the high levels of zinc used, this difference in solubility could account, in part, for poorer absorption and, thus, increased growth and feed efficiency.

Since zinc and copper are interrelated, an excess of either would cause an apparent deficiency of the other. This appears to be the mechanism by which an excess of zinc espresses itself. Chickens on high levels of zinc are anemic (Norris, 1958). Since copper and iron are necessary for hematopoiesis and no interrelationship between iron and zinc is know, this antagonism between copper and zinc would be expected.

Practical broiler and laying rations contain approximately thirty to forty ppm of zinc. This level is not too much in excess of the required amount of available zinc (20 ppm). Should a portion of the mineral in practical rations be in a bound form and unavailable to the chicken, a border line deficiency could very well develop. This type, unlike the acute type produced in the laboratory by use of special zinc-low environment, would be expected in the field. Slower growth and slightly rough feathers would be expected. As yet, no reports of a zinc deficiency have been reported under field conditions; however, with the changing patterns of ingredients in the diet, the zinc content of rations should be watched. Zinc supplementation of practical rations may be warranted as a safety factor; however, the entire mineral picture should be kept in mind in order to prevent a mineral imbalance.

CONCLUSIONS

1. By formulating semi-synthetic diets very low in zinc content, coating equipment with plastic, using distilled water in glass waterers, and maintaining chicks in relatively dust-free environment, it has been shown that zinc is an essential nutrient for the young chick for growth, feather development, maintenance of a healthy skin and efficient utilization of feed.
2. A deficiency of zinc in the ration caused reduced body growth, shortened long bones, enlarged hocks, poor and ragged feathering, dermatitis of tops of feet and footpads, a high stepping walk, and poor feed utilization.
3. The zinc requirement of the growing chick is not more than twenty ppm of the ration if it is in an available form.
4. The zinc of the oxide, sulfate,chloride, and carbonate forms are equally available to the chick, at or below the level which meets the chicks actual dietary zinc requirement.
5. Dietary calcium levels in excess of 1.73 percent aggravated zinc deficiency symptoms in the chick and levels of 2.23 percent increased the zinc requirement of the chick, as determined at normal calcium levels (1.23 percent).
6. Supplementation of a practical, laying-hen ration with calcium and zinc increased eggshell thickness of chicken eggs during cold, but not during warm, weather. Additional zinc or calcium alone did not affect eggshell thickness. Egg production, hatchability, egg weight, interior egg quality and laying house mortality were not affected by additional zinc or calcium, alone or in combinations.

7. A zinc level of 1,000 ppm or less was well tolerated by the chick. Higher levels were not tolerated too well and reduced growth rate and feed efficiency in the following increasing order: oxide, sulfate and carbonate.

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APPENDIX EXPERIMENT I

In a preliminary experiment a semi-purified diet was used to determine the effect of supplemental zinc on egg production, egg weight, Haugh score and eggshell thickness of eggs from laying hens.

Twenty-eight White Leghorn hens (strain cross), which had been in production for seven months, were divided into two pens of 14 hens each. The hens had previously received a corn-soy type diet containing approximately 34 ppm of zinc.

The birds were housed in pens containing 74 sq. ft. of floor space, a wooden slat floor, one wooden feeder, and two one-gallon baby chick founts for water. The galvanized metal walls of the pens were covered with tarpaper. All these precautions were taken to prevent the birds from obtaining extraneous zinc.

The hens were placed on experimental diets on May 2, 1958, and trapnested for 43 days beginning May 5. Eggs were collected the last nine days of the period to determine eggshell thickness, Haugh score and egg weight.

The composition of the basal diet is in Appendix Table II. Feed and distilled water were supplied ad libitum.

Results and Discussion

The results of the experiment are presented in Appendix Table I. There was no difference in egg production and egg weight between the two lots. The Haugh score of Lot 1 was significantly higher than Lot 2. The eggshell thickness of Lot 2 receiving the supplemental zinc was significantly thicker than Lot 1; however, the eggshell thickness was extremely poor in both lots. In this experiment the effects of warm weather, age of hens and treatments are confounded and the differences noted are not necessarily due to the addition of zinc.

APPENDIX TABLE I

THE EFFECT OF ZINC SUPPLEMENTATION OF A SEMI-PURIFIED DIET ON EGG PRODUCTION, EGG WEIGHT, HAUGH SCORE AND EGGSHELL THICKNESS OF EGGS FROM LAYING HENS

Lot	Lot additions (ppm)	Egg production (%)	Av. egg weight (gm)	Haugh score	Eggshell thickness (.001")
1	None	67	64	83**	11.1
2	50	61	64	79	11.5**

** Significant ($P < .01$)

APPENDIX TABLE II

BASAL DIET USED IN APPENDIX EXPERIMENT I

Ingredients	Percent
Glucose[1]	59.72
Isolated soybean protein[2]	18.00
Corn oil	10.00
Cellulose	5.00
Butylated hydroxy toluene	.02
DL-Methionine	.24
$CaHPO_4$	1.77
$CaCO_3$	3.88
K_2HPO_4	.50
NaCl	.60
$MgSO_4$	.25
Trace mineral mix[3]	.0695
Vitamin mix[3]	.1934

1. Cerelose, Corn Products Sales Co., 440 New Center Bldg., Detroit, Michigan

2. Amisoy, The Glidden Company, Chicago, Illinois

3. Morrison et al. (1955)

Calculated analyses:		
	Protein	16.00 percent
	Calcium	2.26 percent
	Phosphorus	0.66 percent
	Zinc	10 ppm