AVAILABILITY OF COMPLEXED ZINC TO PIGS AND CHICKS

by

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INTRODUCTION

Research data reported during the past five years has clearly indicated that the dietary zinc requirement of pigs and chicks fed milk-protein sources is lower than for those fed vegetable sources of protein. Further experimental data indicated that the naturally occurring phytic acid of vegetable-protein sources is involved, in some manner, in zinc availability when these sources are incorporated into otherwise zinc-deficient diets. Phytic acid is known to have a high affinity for metal ions and interferes with calcium absorption in non-ruminant animals. High levels of dietary calcium interfere, in some manner, with zinc availability to pigs and chicks. This suggests an interference by both phytic acid and calcium with the availability of zinc to animals fed vegetable-protein diets. Supplemental zinc added to diets containing natural or added phytic acid appears to be adequately available to the pig and chick. This may indicate that the zinc contained in corn and soybean meal is complexed by factors in addition to phytic acid. Since it is highly probable that the zinc contained in milk-protein sources is protein-bound, addition of phytic acid may actually produce a phytic acid-protein-zinc complex. Phytic acid-protein complexes have been isolated from both corn and soybean meal.

The synthetic chelating agent ethylenediaminetetraacetic acid (EDTA) appears to render zinc available in diets containing both natural and added phytic acid.

The purpose of the experiments reported in this dissertation was to determine whether phytic acid, alone or in combination with calcium ions, interferes with the absorption or metabolism of dietary zinc. The role of EDTA and source of calcium was also studied with respect to availability of zinc in soybean meal-protein diets.

REVIEW OF LITERATURE

Zinc Availability

Early investigations

Tucker and Salmon (1955) showed that the skin condition
of swine, called parakeratosis and described by Kernkamp and
Ferrin (1953), was due to a dietary zinc deficiency. Work to
determine the zinc requirements of pigs and chicks demon-
strated that the dietary zinc requirement depended upon the
type of diet fed and the level of certain nutrients contained
therein. O'Dell and Savage (1957) and Supplee et al. (1957)
demonstrated that zinc was necessary for satisfactory gains,
normal feathering and proper bone formation in chicks and
poults, respectively, when soybean-protein semi-purified diets
were fed. Early workers with both species noted that zinc
deficiencies were more severe when high levels of calcium
were used in the diets.

Protein source and zinc requirement

O'Dell and Savage (1957) reported that the zinc require-
ment of chicks fed casein-protein diets appeared to be much
lower than for chicks fed soybean-protein diets. Similar
observations were reported by Morrison and Sarett (1958) and
again by O'Dell and Savage (1960). Zeigler et al. (1961)

reported a requirement of 12 - 14 parts per million (ppm) zinc for chicks fed casein diets compared to a requirement of 27 - 29 ppm zinc for chicks fed an isolated soybean-protein diet.

In working to determine the quantitative zinc requirement of pigs fed isolated soybean-protein diets, Smith et al. (1961) observed a greater response when 10 ppm zinc was added to a casein-protein diet than when the same quantity of zinc was added to the soybean-protein diet. Pigs receiving the zinc-supplemented soybean-protein diet actually lost weight while those receiving the zinc-supplemented casein diet gained at a rate of 2.08 pounds daily. Further studies by Smith et al. (1962) demonstrated no increase in gain from supplementing 50 ppm zinc to diets containing vitamin-free casein, commercial casein or dried skimmed milk as protein sources. The same quantity of zinc added to soybean meal or isolated soybean-protein diets resulted in significant in-creases in pig gains. Pigs fed the two soybean-protein diets without supplemental zinc failed to grow while those fed milk-protein basal diets gained faster than when supplemented with zinc.

Zinc toxicity

Brink et al. (1958) found that the pig could tolerate 2000 ppm zinc in a corn-soybean meal diet, but levels higher

than this caused depressed gain and poor appetite as well as gastritis and hemorrhages in many organs of the body. Death was frequently caused within 21 days when the higher levels of zinc were fed. Johnson et al. (1962) found similar results for the chick. Chick gains were reduced only slightly when 2000 ppm zinc was fed in practical corn-soybean meal diets, whereas higher levels of zinc reduced growth and decreased copper concentration in chick livers. Cox and Harris (1960) demonstrated that addition of 4000 ppm zinc to rat diets decreased blood hemoglobin levels as well as liver storage of copper and iron. Magee and Matrone (1960) fed excess zinc to rats and concluded that zinc interferes with the utilization of copper and iron but not with the absorption of the two elements. Zinc toxicity reduced hemoglobin levels of rats unless copper and/or iron were fed in greater than normal amounts. McCall et al. (1961) reported data showing that soybean-protein diets of either 20 or 30 percent protein content protected the rat against zinc toxicosis compared to casein diets of equal protein level and zinc content. With soybean-protein diets hemoglobin values were not lowered when 5000 ppm zinc was fed at either protein level, but were lowered at both protein levels when this amount of zinc was fed with casein diets. The feeding of excess zinc reduced liver copper and iron to a greater extent in rats fed casein diets than those fed soybean meal diets. These effects are in agreement

with studies mentioned earlier which indicate an interference
with zinc utilization by some component of soybean-protein
sources.

Phytic acid and zinc utilization

A large proportion of the phosphorus of cereal grains
and oilseeds exists organically bound as phytic acid. Chem-
ically, phytic acid is the hexaphosphoric acid ester of
inositol and exists in grains and oilseeds primarily as the
calcium-magnesium salt, commonly called phytin. It is known
to have a high affinity for metal ions and is used to some
extent in the clarification of wines and vinegar and to remove
heavy metals from edible oils.

Soybean meal contains approximately 0.6 percent phos-
phorus, of which 70 - 90 percent exists as phytin phosphorus.
A similar proportion of the 0.25 percent phosphorus content
of corn exists as phytin phosphorus. The phosphorus of phy-
tic acid has been shown to be poorly available to the pig and
chick and to exert a detrimental effect on calcium absorption
in both species. Presumably, because of the rumen microbial
fermentation, the ruminant is able to utilize phytin phos-
phorus as efficiently as inorganic sources.

O'Dell and Savage (1960) studied the effects of adding
commercial phytic acid to casein diets fed to chicks and ob-
tained results similar to those obtained with soybean-protein

diets not supplemented with zinc. Addition of phytic acid to soybean-protein basal diets further depressed chick gains and increased the apparent zinc requirement. Additions of calcium phytate to the basal diet caused no apparent effect on growth of chicks. The data suggest that phytic acid combines with the protein, in some manner, to interfere with zinc availability to the chick. Such a phytic acid-protein complex has been precipitated from water extracts of soybean meal by Smith and Rackis (1957), but has been shown by Rackis et al. (1961) to constitute only 0.3 percent of the nitrogen of the water soluble protein.

Plumlee et al. (1960) demonstrated that the addition of phytic acid to casein diets of pigs caused decreased growth, low feed efficiency and severe parakeratosis. Addition of zinc to the above diet prevented parakeratosis and produced normal growth. Oberleas et al. (1962a) found that phytic acid decreased growth when added to either casein or soybean-protein diets. In this study phytic acid exerted a greater depression on growth when fed in casein rations containing 1.5 percent calcium than in rations containing only 0.8 percent calcium. Without phytic acid, the higher calcium level in casein diets produced no marked adverse effect on gains. This indicates that the adverse effect of high calcium on zinc availability may become manifest through a calcium-phytic acid reaction.

Ethylenediaminetetraacetic acid (EDTA) and zinc availability

Kratzer et al. (1959) found that the addition of ethyl-
enediaminetetraacetic acid (EDTA) to a zinc-deficient semi-
purified diet reduced the requirement of poults for dietary
zinc. The addition of EDTA to the basal diet was equally as
effective as zinc in increasing the length:width ratio of the
tarsometatarsus of poults. Lease et al. (1960) fed purified
diets with sesame meal as the only source of protein. Al-
though it contained 52 ppm zinc by analysis, chicks consuming
this diet grew at a normal rate only when supplemented with
zinc or EDTA. EDTA at 430 ppm in the diet was equivalent to
120 ppm zinc in stimulating chick growth. Plumlee et al.
(1960) demonstrated that supplementing pig diets with 450 ppm
EDTA was equivalent to supplementing with 100 ppm zinc for
correcting parakeratosis in pigs fed casein diets with phytic
acid added. Smith et al. (1962) found EDTA supplementation as
effective as zinc in stimulating growth and healing pigs with
parakeratosis. Forbes (1961) observed that EDTA added to
zinc-deficient rat diets stimulated gains and increased zinc
absorption and retention. It is possible that EDTA is able
to chelate zinc and prevent it from being bound by complexes
involving phytic acid, protein and calcium, thus making the
zinc more available for absorption.

EXPERIMENTAL

General

Eight experiments, six involving early-weaned pigs and two involving day-old broiler type chicks, were conducted during the course of this study. These experiments are designated, by the Swine Nutrition Section, Animal Science Department, Iowa State University, as Experiments 1139, 1157, 1176, 6314, 6322, 6332, 6407 and 6408. Complete details of each experiment are filed at that office.

Pig experiments were conducted at the Swine Nutrition Farm in pens 1 - 18 of Unit C. These pens had concrete floors, wooden partitions, automatic watering cups, plastic coated self feeders and infrared heat lamps. Pigs were bedded with wood shavings and pens were cleaned regularly and rebedded. Pigs had free access to the experimental diets in self feeders and to deionized water in the automatic watering cups.

Chick experiments were conducted in a battery of chick pens housed in the building designated as the number 10 house of Unit G at the Swine Nutrition Farm. The chick pens were plastic coated and had wire floors and conventional glass waterers. Temperature within the building was maintained near 65° F and that of individual pen brooders was started at

95° F and lowered by 5° increments each week of the feeding periods. Chicks had free access to experimental diets on each side of the pen and to glass-distilled water in the waterers.

Diets were mixed in a standard concrete mixer in 100 and 200 pound lots. Premixes were prepared using a Hobart mixer of 50 pound capacity. Additions of zinc, zinc complexes, complexing agents and special preparations were made as a plus value in addition to the natural constituents of the diet, except when an experimental compound was added to maintain the normal calcium and/or phosphorus level of the diet. Compounds of the latter type were incorporated into the diet by altering the corn starch or ground corn content of the basal. Diets were stored until used, ordinarily within 10 days, in pressed cardboard kegs with metal lids.

Crossbred pigs of mixed sexes, obtained from the farrowing houses of the Swine Nutrition Farm, had previously: been ear notched for individual pig identification, had their needle teeth clipped, and received iron dextran shots for prevention of anemia. Also, male pigs had been castrated. Sexed chicks were obtained from the hatchery of the Poultry Department and were wing banded for individual chick identification. Pigs were randomly allotted from outcome groups of individual weight within litter and chicks were randomly assigned to pens by series of wing band numbers. In both chick

and pig experiments the pen was considered the experimental unit. Individual pig and chick weights and pen feed consumption were recorded each week. Pig experiments were conducted for 42 or 49 day periods and chick experiments were of 28 days duration. Upon termination of experiments the data were subjected to analysis of variance for identifying significant treatment effects. Repletion trials with emaciated or parakeratotic pigs were conducted for 14 to 21 days.

Chick tibiae were removed and length and width were measured to the nearest 0.01 cm with standard calipers. Bone ash determinations were made by drying the fresh bones overnight at 90° C, extracting for 5 hours in ether, drying, weighing and ashing for 12 hours at 650° C. Zinc and copper analyses were conducted by the zincon colormetric method described by McCall et al. (1958). Chick livers were moisture free and pig hair was washed and air-dried before zinc and copper analysis. Hemoglobin determinations for pigs were made by the acid hematin method using an electro-hemometer.

Composition of the basal diets and the analyzed zinc concentrations of some of the major feed ingredients are shown in Tables 1 to 5 in the Appendix. Figure illustrations and photographic plates appear in the text of this dissertation and all tables are contained in the Appendix.

Specific Experiments

Experiment 1139 - Availability of injected zinc complexes to the pig

Objectives   This experiment was designed to study the apparent availability of zinc in the zinc complexes, zinc-phytate and zinc-EDTA, after absorption and to measure the effect of absorbed complexing agents on the availability of zinc.

Experimental   Seventy-two pigs averaging 11.9 pounds in weight and 15.3 days of age were allotted, four per pen, to six treatment groups for a 49 day feeding period during February, March and April of 1962. Five of the treatment groups were fed basal diet 1 of Table 1 and the sixth received the basal supplemented with 20 ppm zinc as zinc oxide. This sixth group plus one group receiving the basal diet received injections, once each week, of physiological (0.9 % NaCl) saline. The other four groups received injections of calcium-EDTA, sodium phytate, zinc-EDTA or zinc phytate, in physiological saline.

Zinc-phytate and calcium-EDTA complexes, for injection, were prepared by combining zinc chloride and calcium carbonate in a 1 : 1 molecular ratio with phytic acid and ethylene-diaminetetraacetic acid (disodium salt), respectively. Sodium phytate was prepared by neutralization of phytic acid

with sodium hydroxide. To prepare the zinc-EDTA solution for injection a zinc-versenol chelate, zinc-sodium-ethanolethyl-enediaminetriacetic acid, was dissolved in physiological saline in amounts to give each pig 10.5 mg of zinc per weekly injection. All other injection doses contained this level of zinc or the complexing equivalent as EDTA or phytic acid. Each of the preparations were dissolved or mixed in physiological saline, neutralized and diluted in order that each pig received a 7.0 ml intraperitoneal dose. Zinc phytate precipitated at the neutral pH to give a milky appearing solution.

Pig weights, feed data and incidence of parakeratosis (PK) were recorded each week. At the end of the 49 day feeding period, 2 to 3 grams of hair was clipped from the back of each pig for zinc and copper analysis.

Results   Figure 1 and Table 6 present summaries of the effect of injections on gain, feed conversion and incidence of parakeratosis in pigs, and in Figure 2 and Table 7 the concentration of zinc and copper in pig hair is given. Analysis of variance for gain, feed conversion and concentration of zinc and copper in hair is shown in Table 8.

Pigs receiving zinc, dietary or injected, had significantly ($P \leq .01$) higher gains on significantly ($P \leq .01$) less feed per pound of gain than pigs receiving no zinc. There

Figure 1.   Experiment 1139 - Comparison of gain, feed/gain and incidence of
parakeratosis for pigs receiving supplemental dietary zinc with
those receiving zinc complexes or complexing agents by injection

Figure 2. Experiment 1139 - Comparison of zinc and copper concentrations in hair of pigs receiving supplemental dietary zinc with those receiving zinc complexes or complexing agents by injection

Zn (ppm)

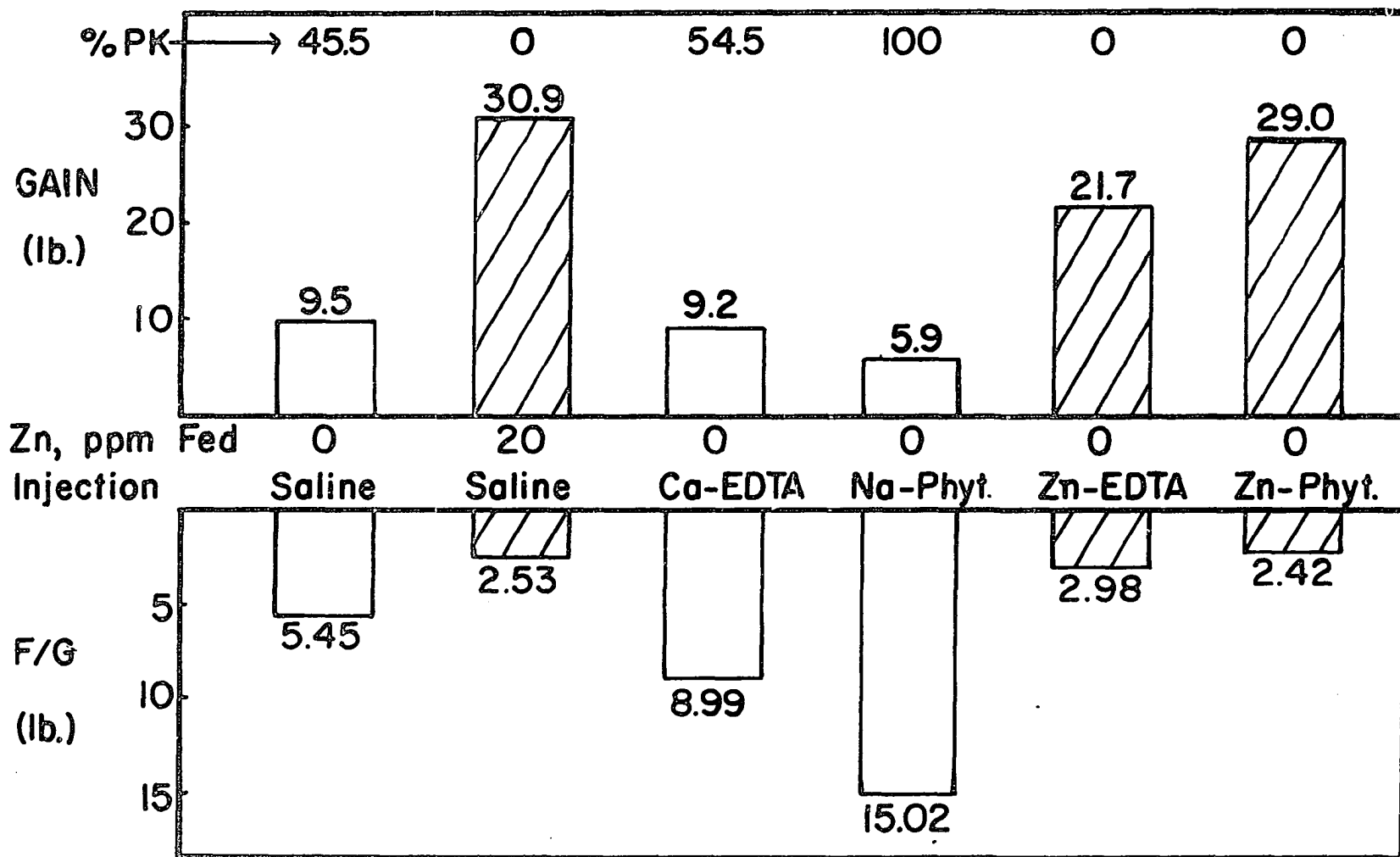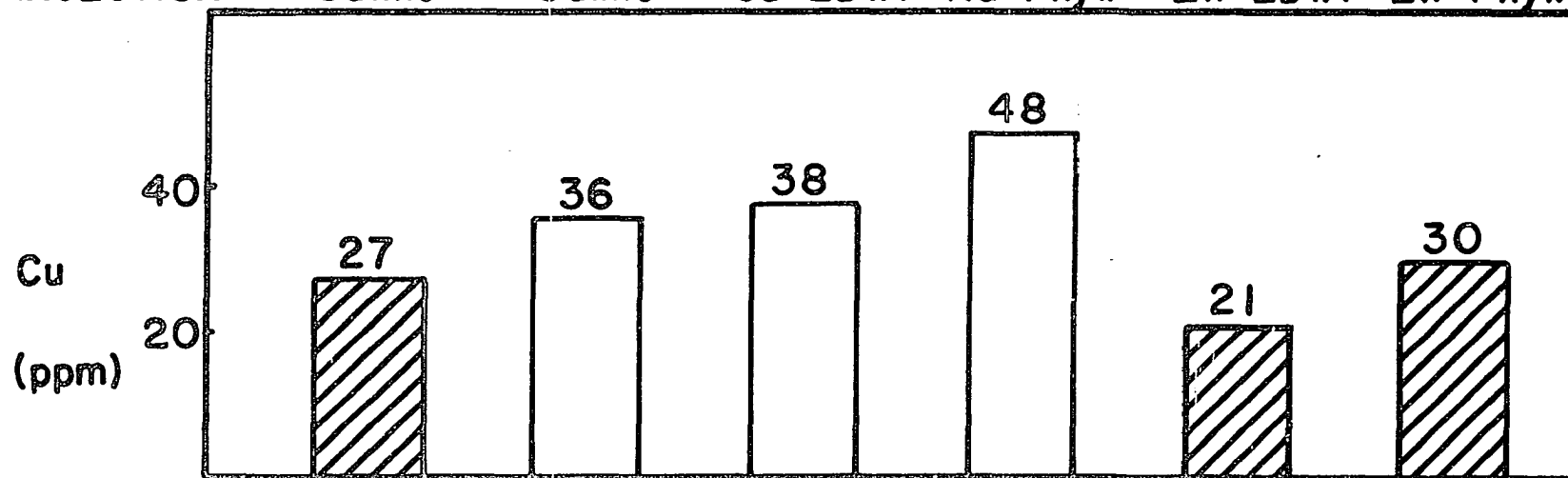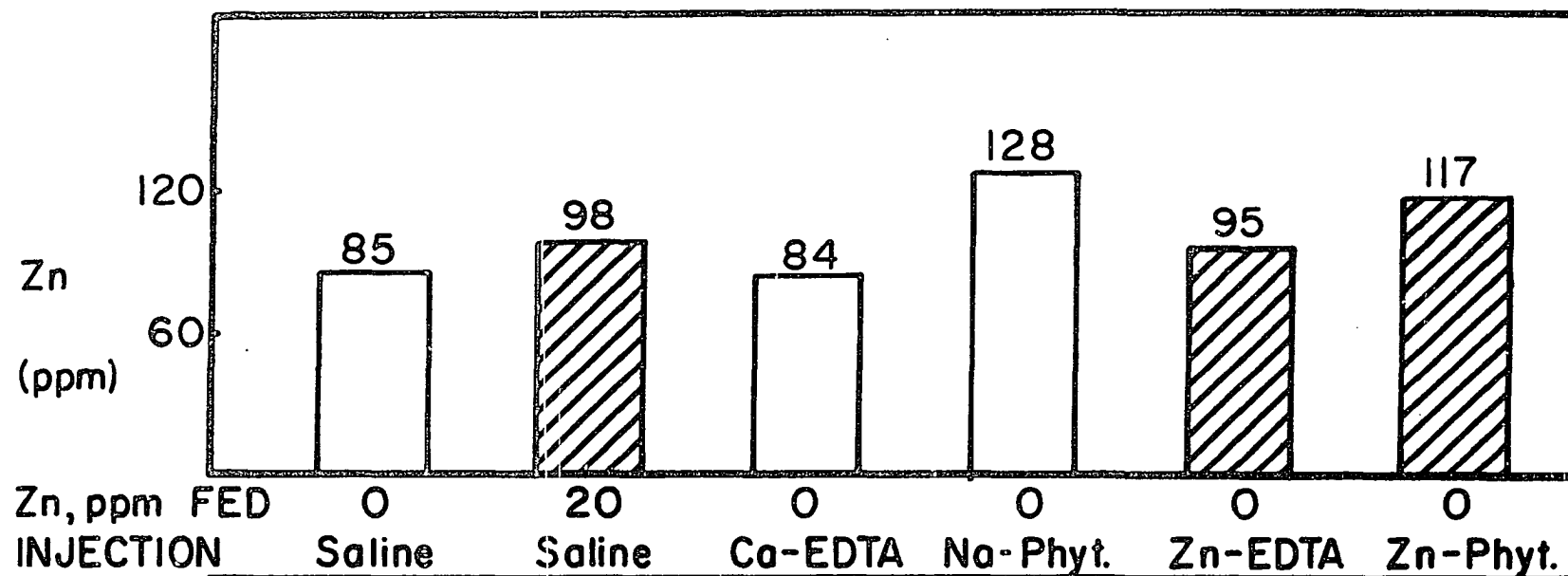|					
85	98	84	128	95	117

Zn, ppm FED: 0, 20, 0, 0, 0, 0

INJECTION: Saline, Saline, Ca-EDTA, Na-Phyt., Zn-EDTA, Zn-Phyt.

Cu (ppm)

|					
27	36	38	48	21	30

were neither significant differences in gain and feed conversion among pigs receiving different forms of zinc, nor significant differences in zinc and copper concentration of hair due to dietary or injected zinc. Parakeratosis occurred in five of 11 pigs receiving the basal diet and saline injection, six of 11 pigs receiving basal diet and calcium-EDTA injection and all 11 surviving pigs receiving basal diet and sodium phytate injection. One pig died in each of these treatments, although death was not attributed to experimental treatment.

Repletion   During a 21-day repletion trial, treatments were reversed, in which pigs that had previously received injections of calcium-EDTA and sodium phytate were injected with zinc-EDTA and zinc phytate, respectively, while those which had received basal diet and saline injection were fed basal plus 20 ppm zinc and continued receiving the saline injections. This reversal of treatments led to correction of parakeratosis and increased gain with less feed required per pound of gain than shown for these pigs during the depletion phase. Figure 3 and Table 9 are summaries of this repletion trial.

Experiment 1157 - Effect of dietary zinc oxide, zinc phytate and EDTA on pig performance

Objectives   The results of Experiment 1139 demonstrated that zinc phytate, if absorbed, was available to the

Figure 3. Experiment 1139 - Effect of dietary zinc or zinc complexes by injection on gain, feed/gain and correction of parakeratosis, during repletion (R), of pigs which received zinc in neither manner during depletion (D)
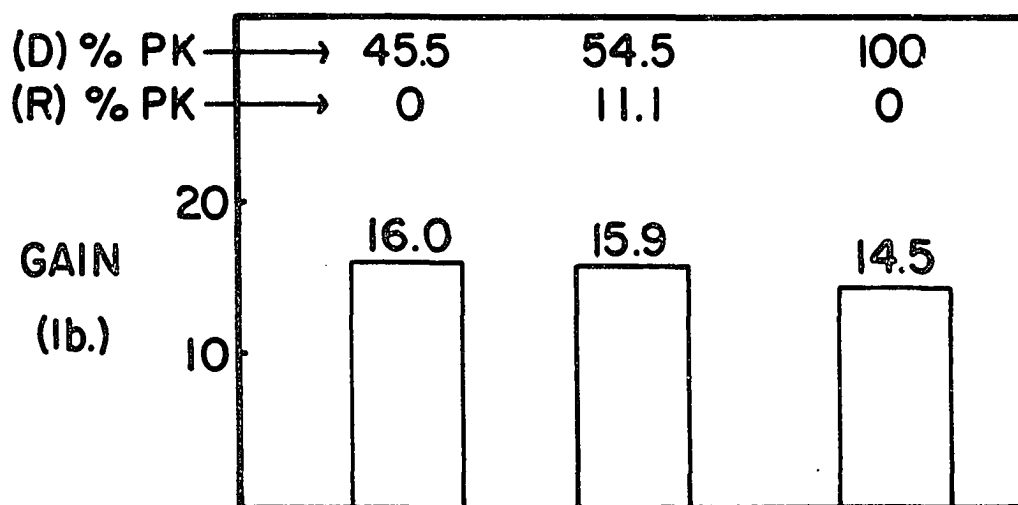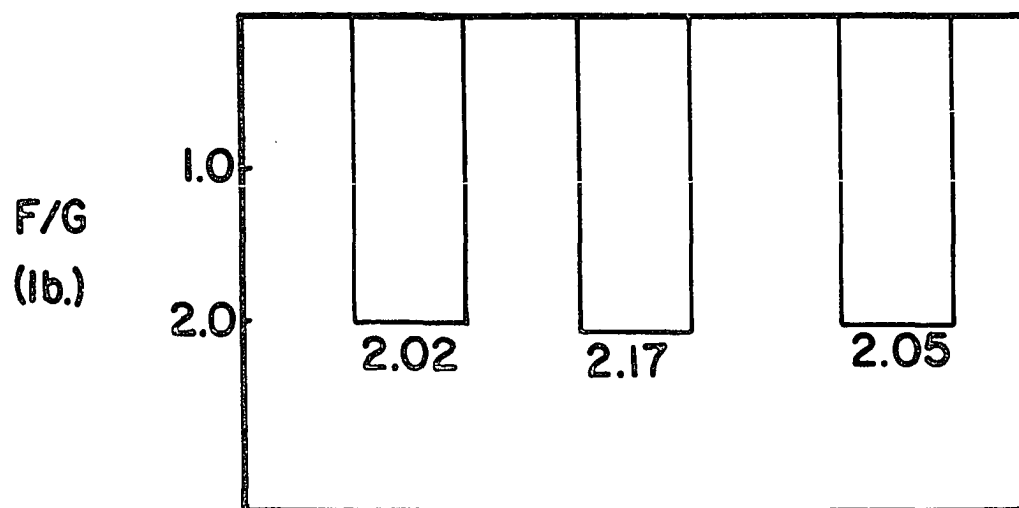
pig and that EDTA alone, if absorbed, did not exert any
beneficial effect. The present experiment was conducted to
study the apparent availability of zinc from dietary zinc
phytate and the effect of EDTA on this availability. A
pilot trial indicated that a diet composed of corn, soybean
meal and purified energy sources could be used in zinc studies.
This led to a change from the soybean meal semi-purified diet
1 of Table 1, to a more free-flowing diet, diet 2 of Table 1,
for all succeeding pig experiments.

Experimental   Seventy-two pigs averaging 12.2 pounds
in weight and 17.8 days of age were allotted, four per pen,
to six dietary treatments for a 42 day feeding period during
May, June and July of 1962. The six treatments consisted of:
basal diet, basal diet plus 40 ppm zinc as zinc oxide, and
basal plus 40 ppm zinc as zinc phytate, all fed with 0 or
450 ppm EDTA (disodium salt). Pig weights, feed data and in-
cidence of parakeratosis were recorded weekly. Zinc phytate
was prepared by combining zinc oxide in a 1:1 molecular ratio
with phytic acid in dilute hydrochloric acid and neutralizing
with sodium hydroxide after the zinc oxide was completely
dissolved. Zinc phytate precipitates in neutral solution,
but was added to the diet as the wet precipitate.

Results   The results of this experiment are summarized
in Figure 4 and Table 10. Analysis of variance for gain and
feed conversion appears in Table 11. Addition of EDTA, zinc

Figure 4. Experiment 1157 - Gain, feed/gain and icidence of parakeratosis in pigs fed zinc oxide or zinc phytate and EDTA

% PK: 75.0 | 0 | 0 | 0 | 0 | 0

GAIN (lb.): 18.2 | 30.2 | 30.2 | 32.8 | 33.0 | 29.8

(y-axis GAIN (lb.): 30, 20, 10)

Zn, ppm	0	40	0	0	40	0
Zn-Phyt. ppm	0	0	40	0	0	40
EDTA, ppm	0	0	0	450	450	450

F/G (lb.): 2.15 | 1.85 | 1.97 | 1.89 | 1.83 | 2.00

(y-axis F/G (lb.): 1.0, 2.0, 3.0)

23
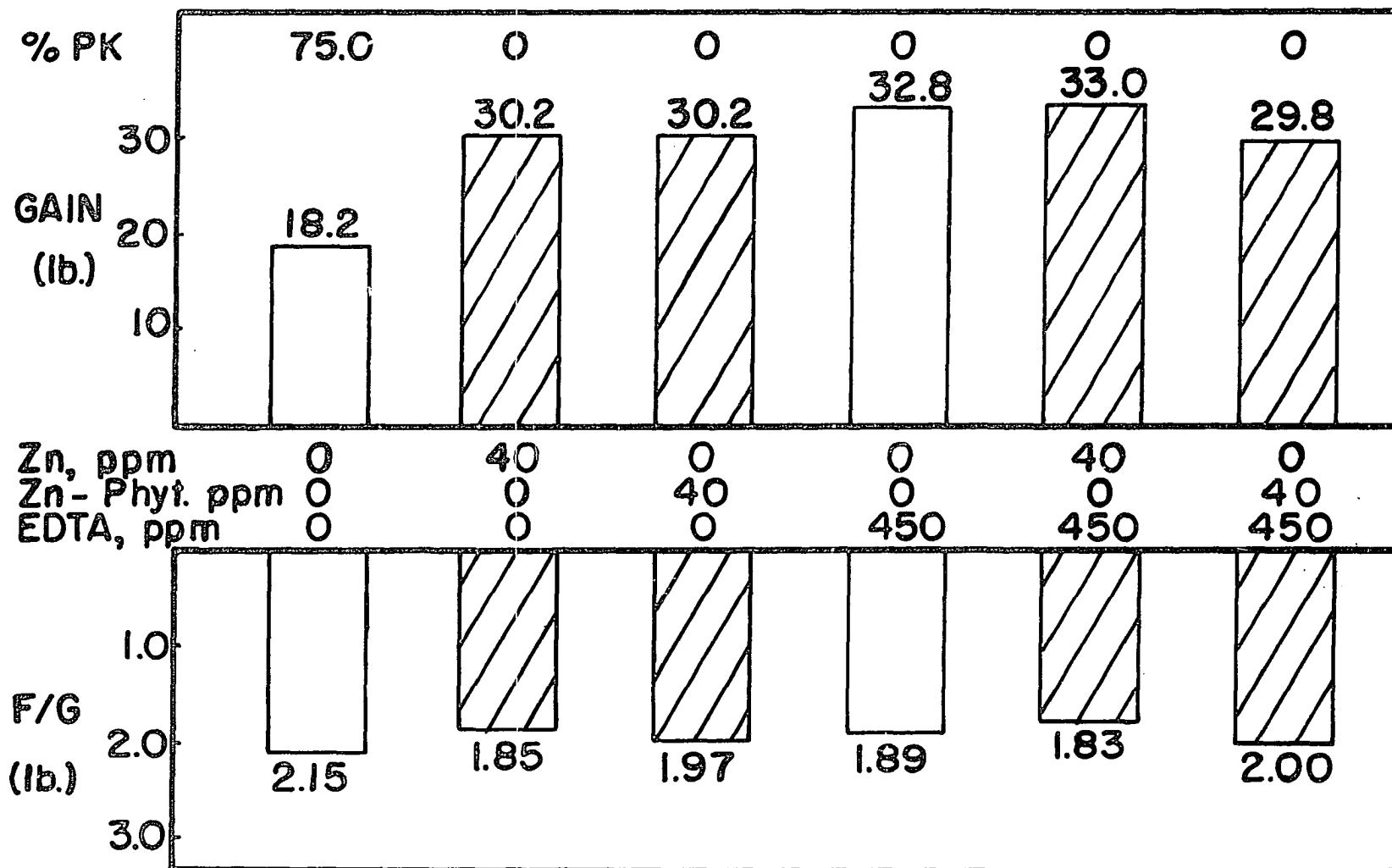
oxide or zinc phytate to the basal diet produced significantly
(P≤ .01) higher gains on less feed per pound of gain than pigs
receiving the basal diet. The EDTA-zinc oxide interaction was
significant (P≤ .01) for gain and (P≤ .05) for feed conversion
while the interaction for zinc phytate and EDTA was signifi-
cant (P≤ .01) for both gain and feed conversion. Nine of 12
pigs receiving the basal diet showed symptoms of parakeratosis.
Comparisons of appearance of pigs receiving the basal diet,
basal plus 40 ppm zinc as zinc phytate and basal plus 450 ppm
EDTA appear in Plates 1 and 2.

Repletion   The feeding of 450 ppm EDTA or 40 ppm zinc
as zinc phytate was effective in stimulating gains and correct-
ing parakeratosis of pigs from the basal diet treatment.
Table 12 is a summary of this 14-day repletion phase and
Plates 3 and 4 demonstrate the effect of EDTA in correcting
parakeratosis and stimulating pig gains.

Experiment 1176 - Effect of EDTA and phytic acid on zinc
toxicity to the pig

Objectives   It has been shown that EDTA increases and
phytic acid decreases the apparent availability of natural
dietary zinc to pigs and chicks. This experiment was de-
signed to study the effect of the two complexing agents on
pigs receiving a near-toxic level of zinc.

Plate 1. Experiment 1157 - Comparison of condition of pigs
receiving the basal corn-soybean meal diet (left)
with that of pigs receiving the same ration plus
40 ppm zinc as the Zn-phytate precipitate (right)

Plate 2. Experiment 1157 - Comparison of condition of pigs
receiving 450 ppm EDTA in the diet (left) with
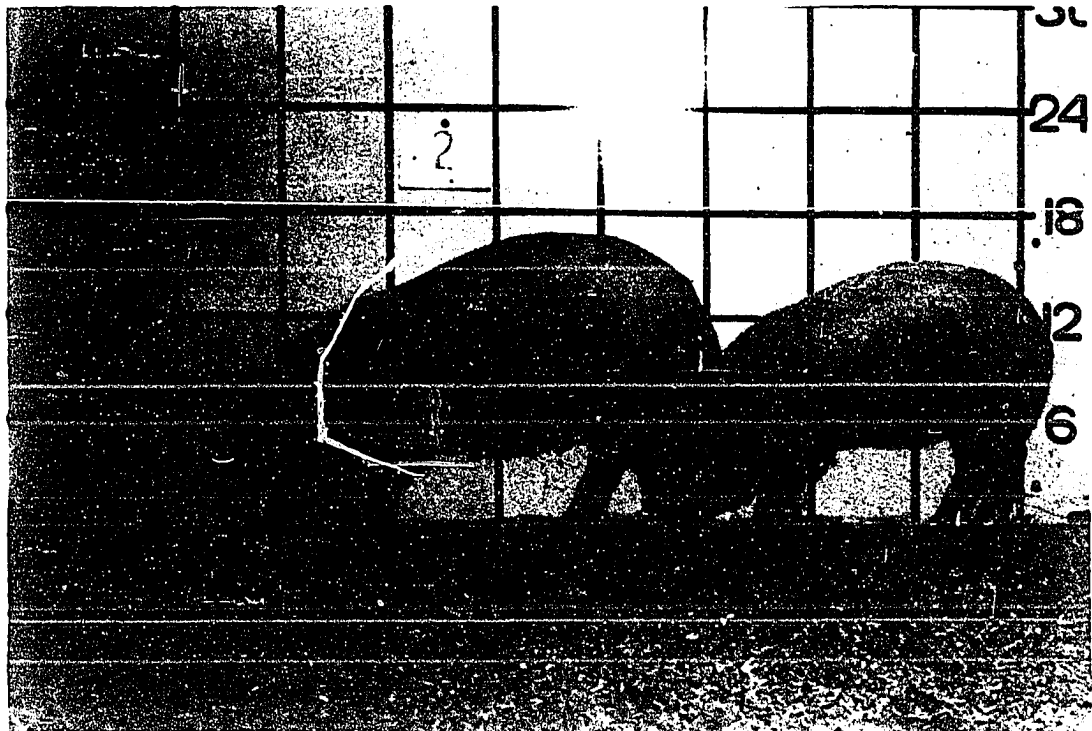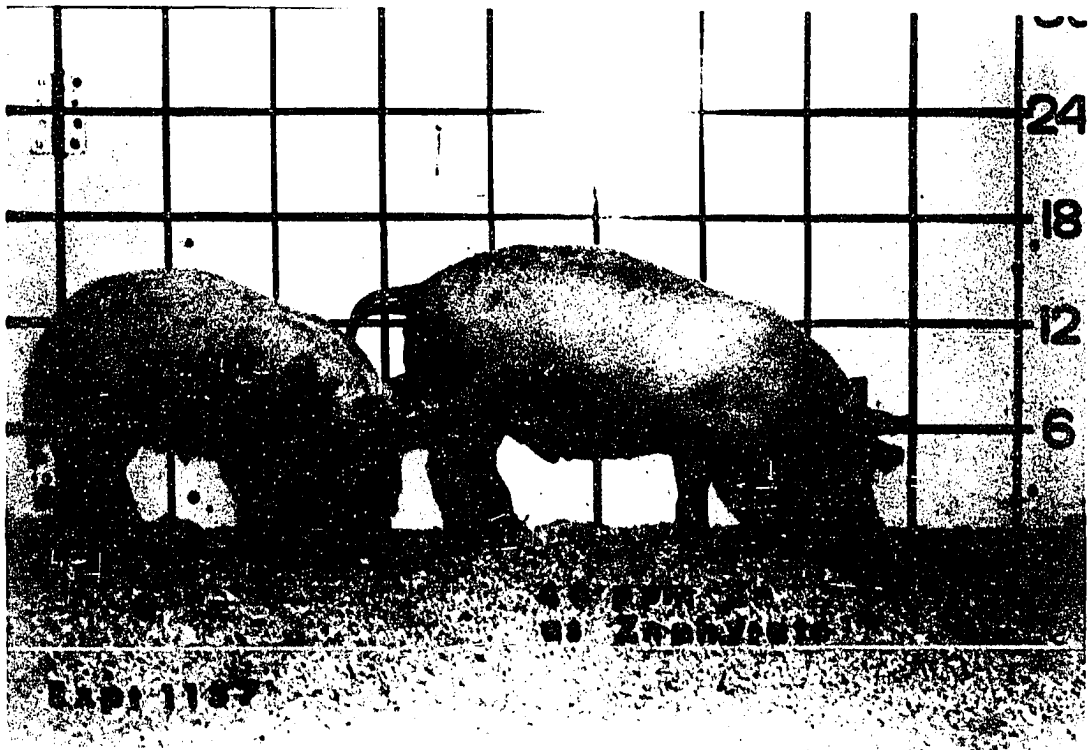pigs receiving the basal diet without EDTA (right)

Plate 3. Experiment 1157 - Pig with parakeratosis at the end of the depletion phase

Plate 4. Experiment 1157 - Same pig after being fed the basal ration plus 450 ppm EDTA for 17 days

450 ppm EDTA

Day 17

PK

Day 0
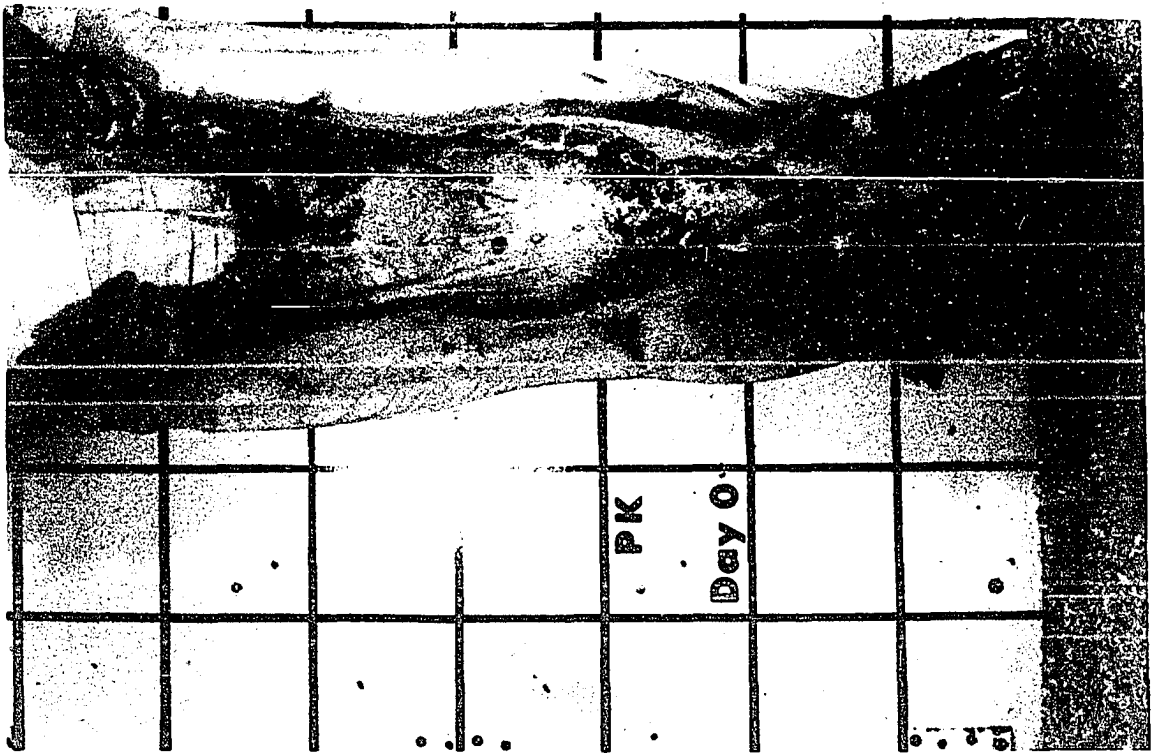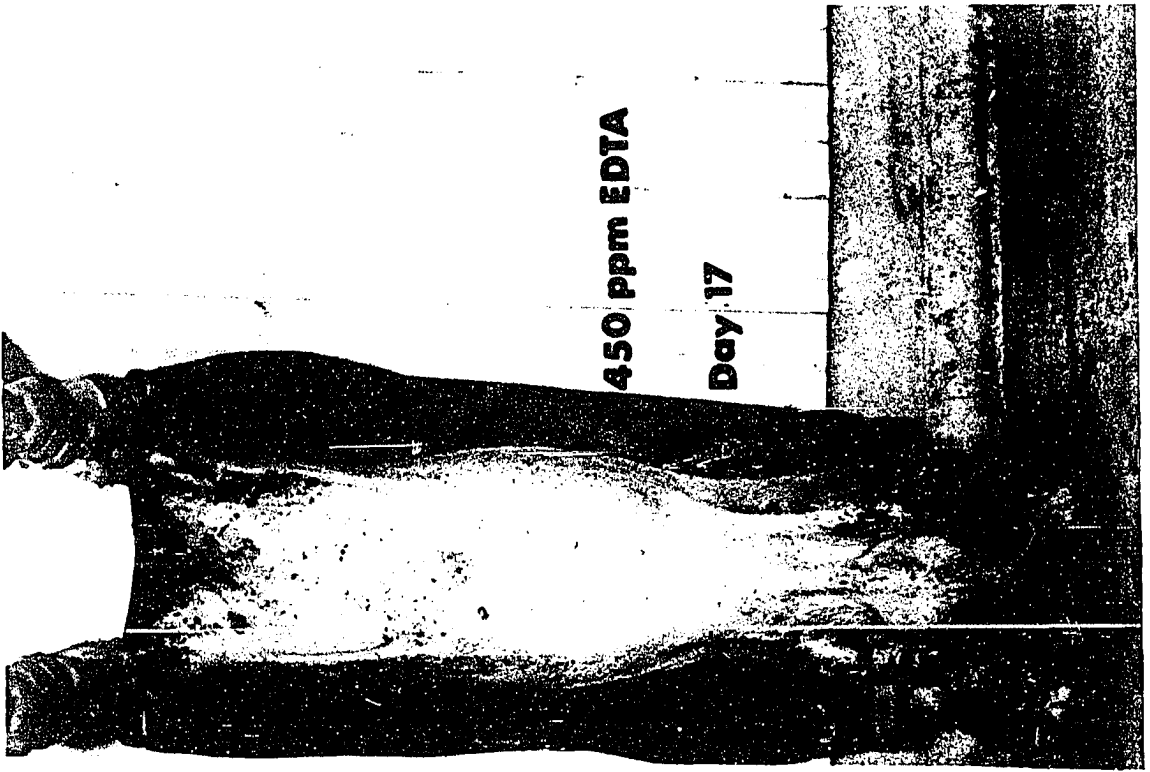
Experimental   Seventy-two pigs averaging 10.7 pounds in weight and 14.5 days of age were allotted, four per pen, to six treatments for a 42-day feeding period during October and November of 1962. The six dietary treatments consisted of basal diet and basal diet plus 3000 ppm zinc as zinc oxide, both alone and with EDTA or 0.6 percent phytic acid added. However, the EDTA level fed with the basal diet was 450 ppm whereas with the basal plus 3000 ppm zinc the EDTA addition was increased to 6700 ppm in order to have chelating capacity for at least one-half of the excess zinc. The basal was diet 2 of Table 1. Pig weights, feed data and incidence of parakeratosis were recorded each week. Blood was collected by ear puncture for 26- and 42- day hemoglobin determinations.

Results   The addition of 3000 ppm zinc to the basal diet produced no obvious zinc toxicosis in pigs and phytic acid or EDTA had no apparent effect on decreasing or increasing the availability of this level of zinc. There was no reduction of hemoglobin in pigs fed excess zinc. Pigs receiving 3000 ppm zinc gained significantly ($P \leq$ .01) more on significantly ($P \leq$ .05) less feed per pound of gain than pigs receiving no supplemental zinc. At 26 days pigs receiving EDTA had significantly ($P \leq$ .05) lower hemoglobin values than those not receiving EDTA. However, at 42 days, pigs receiving 3000 ppm zinc had significantly ($P \leq$ .01) higher hemoglobin values than those receiving no supplemental zinc. Pigs re-

ceiving 6700 ppm EDTA had significantly ($P \leq$ .05) higher hemo-globin values than those receiving 450 ppm EDTA at 42 days. Parakeratosis occurred in three of 12 pigs fed the basal diet and in nine of 12 pigs fed the basal plus 0.6 percent phytic acid. Pigs receiving the basal plus 0.6 percent phytic acid gained less and required more feed per pound of gain than pigs receiving the basal alone. Figure 5 and Table 13 are summaries of gain, feed conversion and incidence of parakeratosis. In Figure 6 and Table 14 are shown the 42-day hemo-globin values and Table 15 contains analysis of variance for gain, feed conversion and both 26- and 42- day hemoglobin values.

Repletion   Pigs which had been fed the basal diet or the basal plus 0.6 percent phytic acid were allotted to two repletion trials, one of 14 days and one of 16 days duration. In the 14-day repletion trial fifteen 30-pound pigs, five per pen, were fed either 40 ppm zinc as zinc oxide, 10 ppm zinc as the zinc-cysteine complex or 10 ppm zinc as the zinc-histidine complex. The complexes were prepared by combining zinc oxide with the respective amino acid in a 1:2 zinc to amino acid ratio in dilute hydrochloric acid and neutralizing with sodium hydroxide. As shown in Table 16, all three treatments were effective in correcting parakeratosis and stimulating pig gains.

Figure 5.   Experiment 1176 – Effect of EDTA and phytic acid fed in diets con-
           taining 0 or 3000 ppm supplemental zinc on gain, feed/gain and
           incidence of parakeratosis in pigs
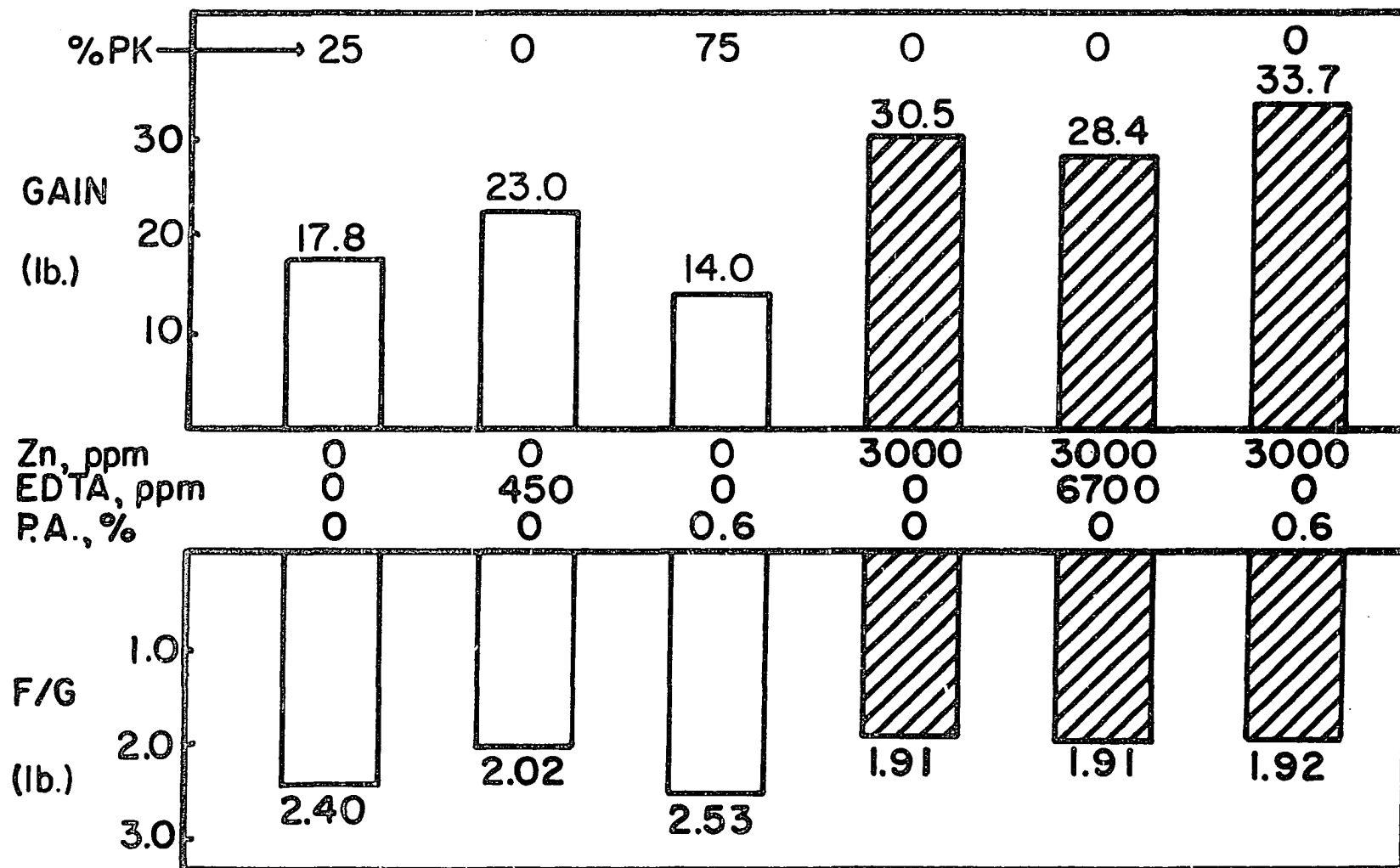
Figure 6. Experiment 1176 - Effect of EDTA and phytic acid fed in diets containing 0 or 3000 ppm supplemental zinc on 42-day hemoglobin values of pigs

	10.4	10.1	10.6	12.1	11.2	10.9

HB. (gm. %)

12.0
8.0
4.0

Zn , ppm	0	0	0	3000	3000	3000
EDTA, ppm	0	450	0	0	6700	0
P.A., %	0	0	0.6	0	0	0.6

34
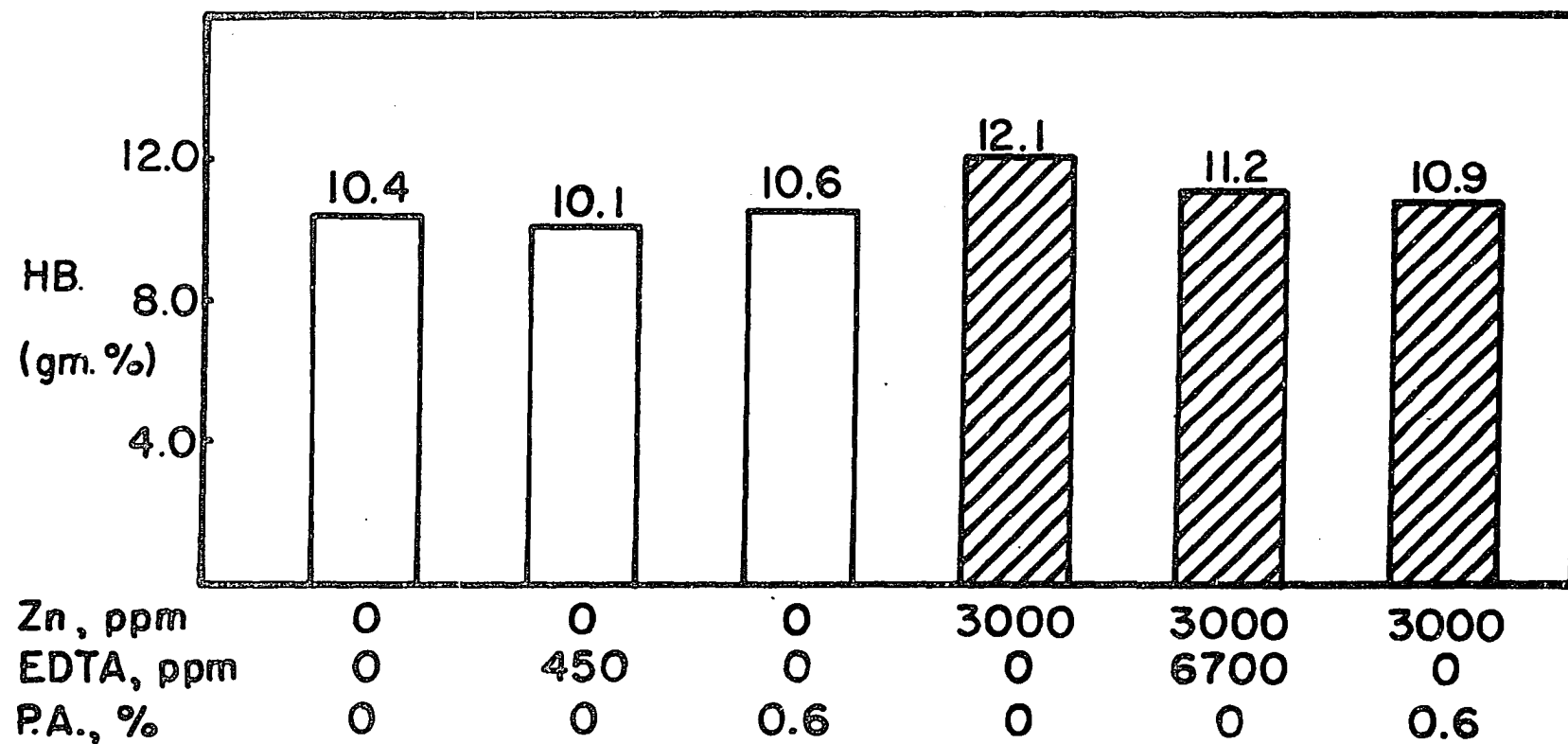
In the 16-day repletion trial nine 17-pound pigs, three per pen, were fed 40 ppm zinc as either zinc phytate, zinc-phytate-cysteine complex or zinc-(calcium)$_2$-phytate complex. All three treatments were effective in correcting parakeratosis and stimulating pig gains as shown in Table 17. Plates 5 and 6 illustrate the effect of zinc phytate in correcting parakeratosis during the trial.

Experiment 6314A - Zinc studies with chicks

Objectives   Studies with chicks were initiated to determine the effects of complexing agents on zinc and copper concentration in liver and upon bone development. The present experiment was conducted to determine the type of diet, practical or semi-purified, to use in zinc studies with chicks. The corn-soybean meal diet, diet 3 of Table 1, actually contains less zinc and more phytic acid than the dextrose-soybean meal diet, diet 4 of Table 1.

Experimental   One hundred and thirty day-old female broiler chicks were allotted, 13 per pen, to six dietary treatments for a 28-day feeding period during April and May of 1963. Dietary treatments were: 0, 10, and 20 ppm zinc, as zinc oxide, in either the corn-soybean meal or the dextrose-soybean meal diets. Because the battery contained only 10 pens the 20 ppm level of zinc supplementation was not

Plate 5. Experiment 1176 – Pigs showing parakeratosis
          and emaciation at the end of the depletion
          phase

Plate 6. Experiment 1176 – Same three pigs after receiving
          the basal diet supplemented with 40 ppm zinc as
          the Zn-phytate precipitate for 16 days
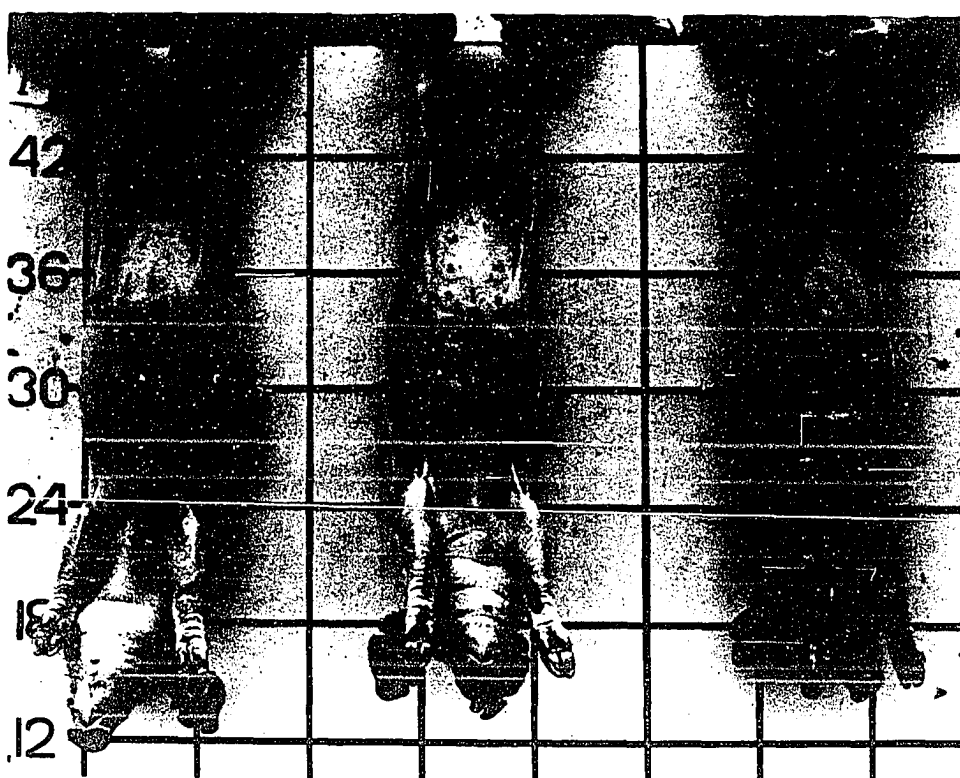
replicated with either diet. Chick weights and feed data
were recorded each week. Upon termination, five chicks from
each pen were sacrificed to obtain livers for zinc and copper
analysis and tibiae for length measurement.

Results   Chicks fed the corn-soybean meal diet gained
significantly (P≤ .05) more on significantly (P≤ .01) less
feed per gram of gain than chicks fed the dextrose-soybean
meal diet. Chicks fed zinc-supplemented diets gained more,
approaching significance (P≤ .05), on less feed than chicks
fed the basal diets. In Figure 7 and Table 18 are presented
summaries of gain and feed data. There was no difference in
tibia length either with respect to type of diet or zinc
supplementation. There was no difference in zinc concentra-
tion of livers with respect to diet or zinc supplementation,
but the livers of chicks fed the corn-soybean meal diet con-
tained significantly (P≤ .01) more copper than those of chicks
fed the dextrose-soybean meal diet. Figure 8 is a summary of
the zinc and copper data and in Table 19 there is a summary
of tibia length and the concentration of zinc and copper in
livers. Analysis of variance for all the above criteria ap-
pear in Table 20.

Experiment 6314B - Second 4-week feeding phase of zinc studies
with chicks

Objectives   This second 4-week feeding phase was con-
ducted to determine the effects of high calcium, EDTA and

Figure 7. Experiment 6314A - Effect of supplemental zinc in practical and semi-purified diets on gain and feed/gain of chicks

GAIN (gm.)

	CORN-SBM			DEXTROSE-SBM		
	388	423	416	355	393	365

Zn, ppm: 0 10 20 | 0 10 20

F/G (gm.)

| 2.00 | 1.90 | 1.94 | 2.24 | 2.14 | 2.15 |
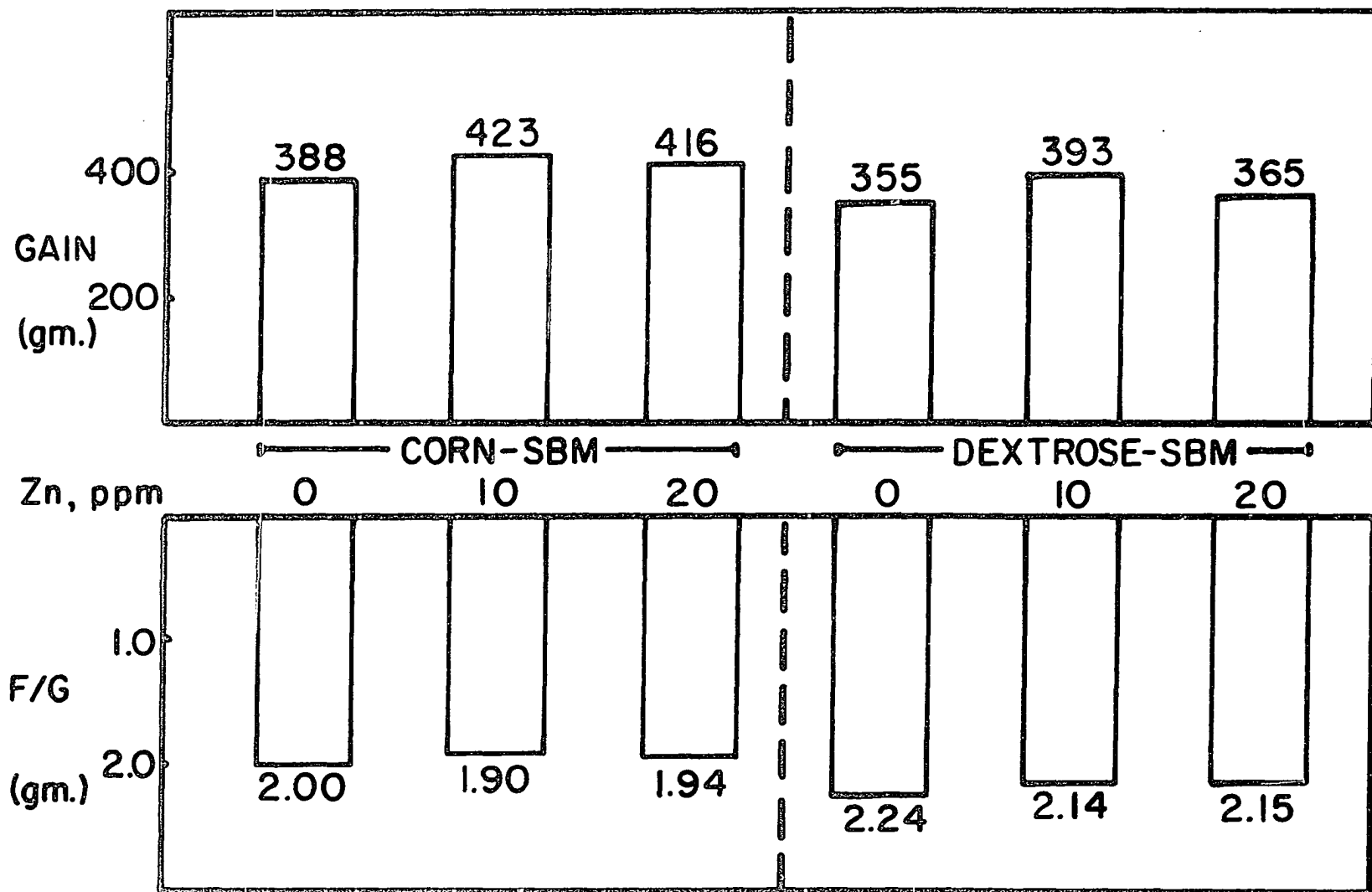
Figure 8.   Experiment 6314A - Effect of supplemental zinc in practical
            and semi-purified diets on concentration of zinc and copper
            in chick livers
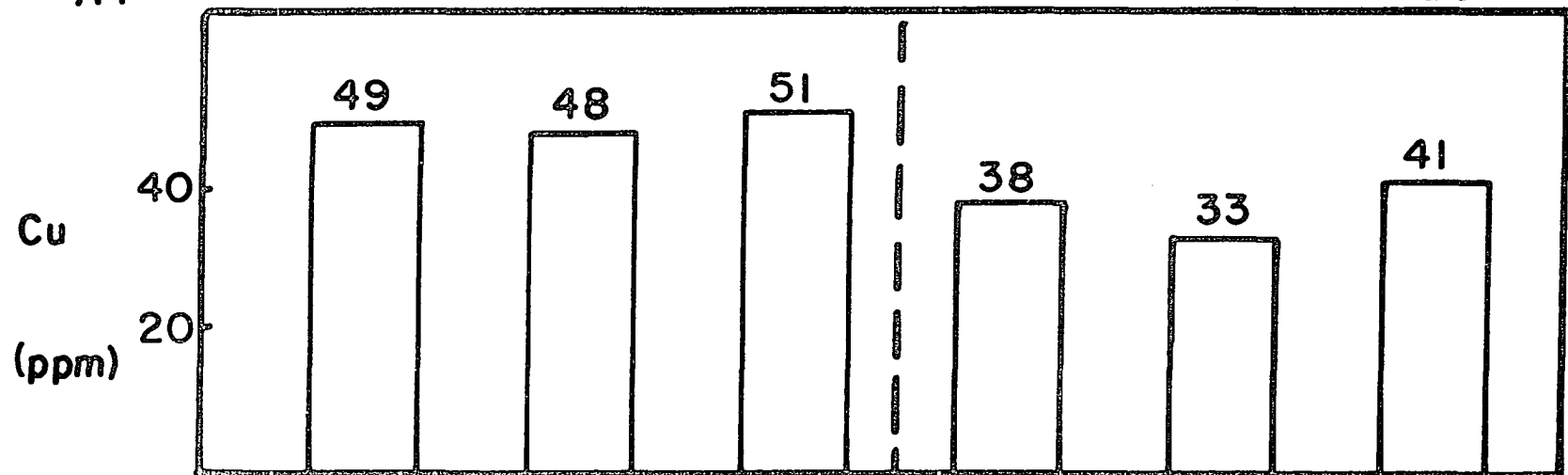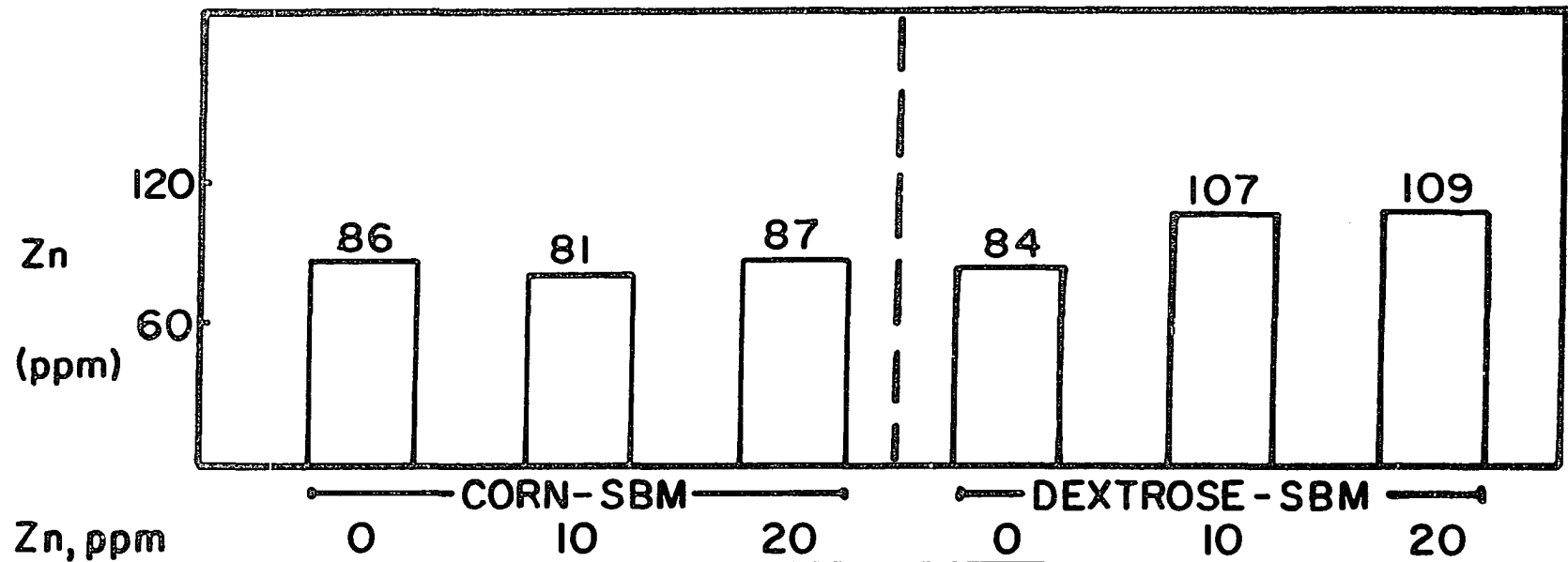
phytic acid on growth, feed conversion and tibia development of chicks fed the two types of diet.

Experimental   The 16 remaining birds of the two basal-fed groups and four of the remaining birds from each group fed the basals plus 10 ppm zinc were reallotted, four per pen, to 10 dietary treatments. Each type of diet was fed to a pen of birds as either basal, basal plus 1.0 percent calcium, basal plus 1.0 percent calcium and 450 ppm EDTA, basal plus 0.5 percent phytic acid or basal plus 10 ppm zinc. Birds receiving the latter treatment had received the same treatment during the first 4-week feeding phase. Upon termination of this feeding period, all chicks were sacrificed for collection of livers for zinc and copper analysis and tibiae for length and diameter measurements. Fat-free, dry, bone ash determinations were made on the tibiae after linear measurements were made.

Results   Among chicks fed the corn-soybean meal diet, elevating the calcium level from 1.0 to 2.0 percent decreased chick gains and increased feed required per gram of gain as well as decreased tibia length:width ratio. Supplementation of the high calcium diet with 450 ppm EDTA (disodium salt) produced gains, feed conversion and tibia length:width ratio equal to that of the basal-diet. Chicks receiving 0.5 percent phytic acid in either diet grew well but had shorter

and thicker tibiae than those chicks fed the diets supplemented with 10 ppm zinc or the unsupplemented basal diets.

Growth and feed conversion for chicks fed the dextrose-soybean meal diet were not affected in the same manner by treatment as for chicks receiving the corn-soybean meal diet. Chicks fed the dextrose-soybean meal diet exhibited symptoms of exudative diathesis throughout the first three weeks of the trial. These symptoms were cleared up during the fourth week by dietary vitamin E therapy.

No definite differences were observed, due to treatment, with respect to bone ash or liver zinc and copper concentration. A summary of gain, feed conversion, tibia data and zinc and copper concentration of chick livers is presented in Table 21.

Experiment 6322 - Zinc fed as zinc chelates to pigs

Objectives   Prior to conducting this experiment, the results of a pilot trial indicated that 4 ppm zinc as zinc-histidine chelate was as effective in supporting pig gains and preventing parakeratosis as was 40 ppm zinc as zinc oxide. A summary of this pilot trial is given in Table 22. However, only two pigs per treatment were used in this trial. It should be recalled that 10 ppm zinc, as zinc-cysteine or zinc-histidine, was equally as effective as 40 ppm zinc as zinc oxide in correcting parakeratosis during the repletion

phase of Experiment 1176 (Table 16). The present experiment was conducted to study the effects of 5 ppm chelated zinc in the diet of baby pigs. It is quite possible that the natural zinc of animal protein sources is bound as chelates with amino acids or peptides similar to the zinc chelates used in this experiment.

Experimental   Seventy-two pigs averaging 10.9 pounds in weight and 15.8 days of age were allotted, four per pen, to six dietary treatments for a 42-day feeding period during May and June of 1963. Additions to basal diet 2 to make up the six treatments were: 5 ppm zinc as zinc oxide, 30 ppm zinc as zinc oxide, 5 ppm zinc as zinc-histidine, 5 ppm zinc as zinc-EDTA, 5 ppm zinc as zinc-histidine plus 30 ppm EDTA (disodium salt), and 5 ppm zinc as zinc-EDTA plus 30 ppm histidine monochloride. The zinc-histidine chelate was prepared by combining zinc oxide and histidine monochloride in a 1:1 molecular ratio with EDTA (disodium salt). The combinations above were dissolved in dilute hydrochloric acid and the pH was then adjusted to 6.5. Solutions were then mixed directly into the feed. Pig weights and feed data were recorded each week and pigs were examined on weigh days for parakeratosis.

Results   The results of this experiment are summarized in Figure 9 and Table 23. As indicated in Table 24, there were no significant differences in gains and feed conversion among the six treatments. Even the supplementation of 30 ppm

Figure 9. Experiment 6322 - Effect of zinc-EDTA or zinc-histidine chelates
fed at low zinc levels on gain and feed/gain of pigs

GAIN (lb.)	18.3	23.4	21.3	20.0	19.6	22.3

ZnO, ppm Zn	5	30	0	0	0	0
Zn-His., ppm Zn	0	0	5	0	5	0
Zn-EDTA, ppm Zn	0	0	0	5	0	5
EDTA(Na)$_2$, ppm	0	0	0	0	30	0
His·HCl, ppm	0	0	0	0	0	30

F/G (lb.)	2.22	2.04	2.16	2.08	2.25	1.98

47
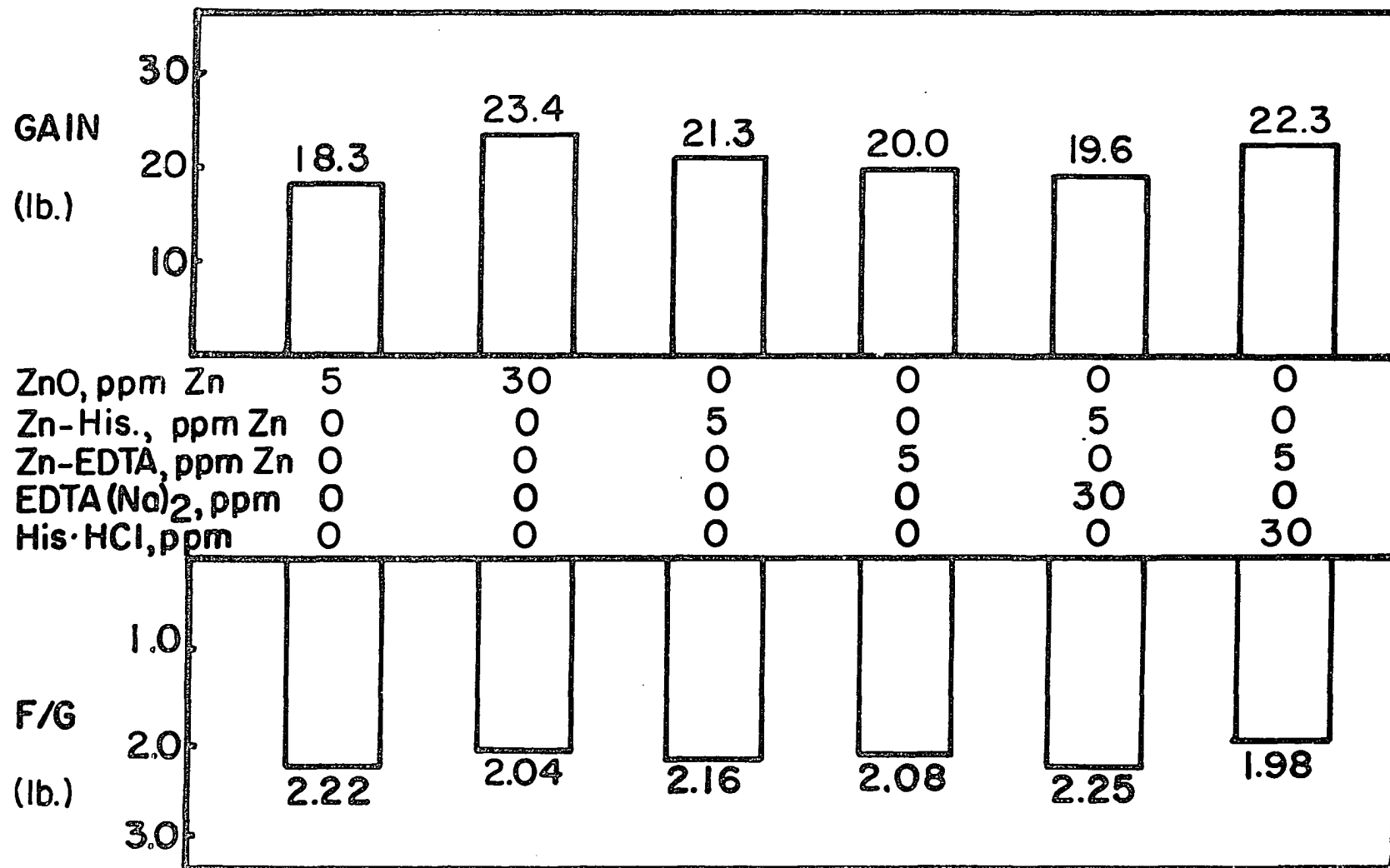
zinc as zinc oxide failed to produce gains noticeably higher than other treatments. Parakeratosis was absent in all treatment groups.

Experiment 6332 - Influence of source of dietary calcium on zinc availability to pigs

Objectives   Widening the calcium:phosphorus ratio of the diet has been shown to decrease the apparent availability of zinc to pigs and chicks when plant-protein sources are used in the diet. The calcium:phosphorus ratio is most commonly widened by increasing the level of calcium carbonate in the diet. It was hypothesized that the calcium carbonate becomes more highly dissociated in the intestine than dicalcium phosphate, thus furnishing a more readily available supply of calcium ions to precipitate with phytic acid and interfere with availability of zinc. This experiment was designed to study the apparent availability of zinc affected by supplying none, part or all the supplemental dietary calcium as calcium carbonate, yet maintaining a near 1:1 calcium:phosphorus ratio.

Experimental   Ninety-six pigs averaging 12.1 pounds in weight and 20.4 days of age were allotted, four per pen, to six dietary treatments, of 42-day feeding periods during September, October, November and December of 1963. Basal diet of 2 of Table 1 was altered to contain 0, 0.10 or 0.52

percent calcium as calcium carbonate. Calcium level was adjusted in the first two diets by using 0.52 and 0.45 percent calcium, respectively, as dicalcium phosphate. The dicalcium phosphate served as the supplemental phosphorus source for the first two diets whereas potassium monophosphate was the source of supplementary phosphorus in the diet containing 0.52 percent calcium as calcium carbonate. Potassium content was equalized in the three diets by including potassium acetate in the first two diets. Although the calcium and phosphorus content of the three diets could not be equalized the calculated calcium content ranged only from 0.59 to 0.62 percent while the phosphorus content ranged from 0.62 to 0.67 percent of the diet. Each of the three diets were fed with 0 to 50 ppm zinc supplementation. Pig weights, feed data and incidence of parakeratosis were recorded weekly.

Results   Pigs receiving 50 ppm supplemental zinc gained significantly ($P \leq .01$) more on less feed per pound of gain than pigs receiving no supplemental zinc. There was a significant ($P \leq .01$) calcium effect on gain and feed efficiency favoring pigs receiving all dicalcium phosphate compared to pigs receiving only 0.45 percent calcium as dicalcium phosphate. Effects on gain and feed conversion, summarized in Figure 10 and Table 25, seem to be entirely due to source of calcium rather than availability of zinc. However, 13 of 16 pigs receiving the diet containing 0.10 percent

Figure 10. Experiment 6332 - Effect of source of calcium and supplemental
            zinc on gain, feed/gain and incidence of parakeratosis in pigs
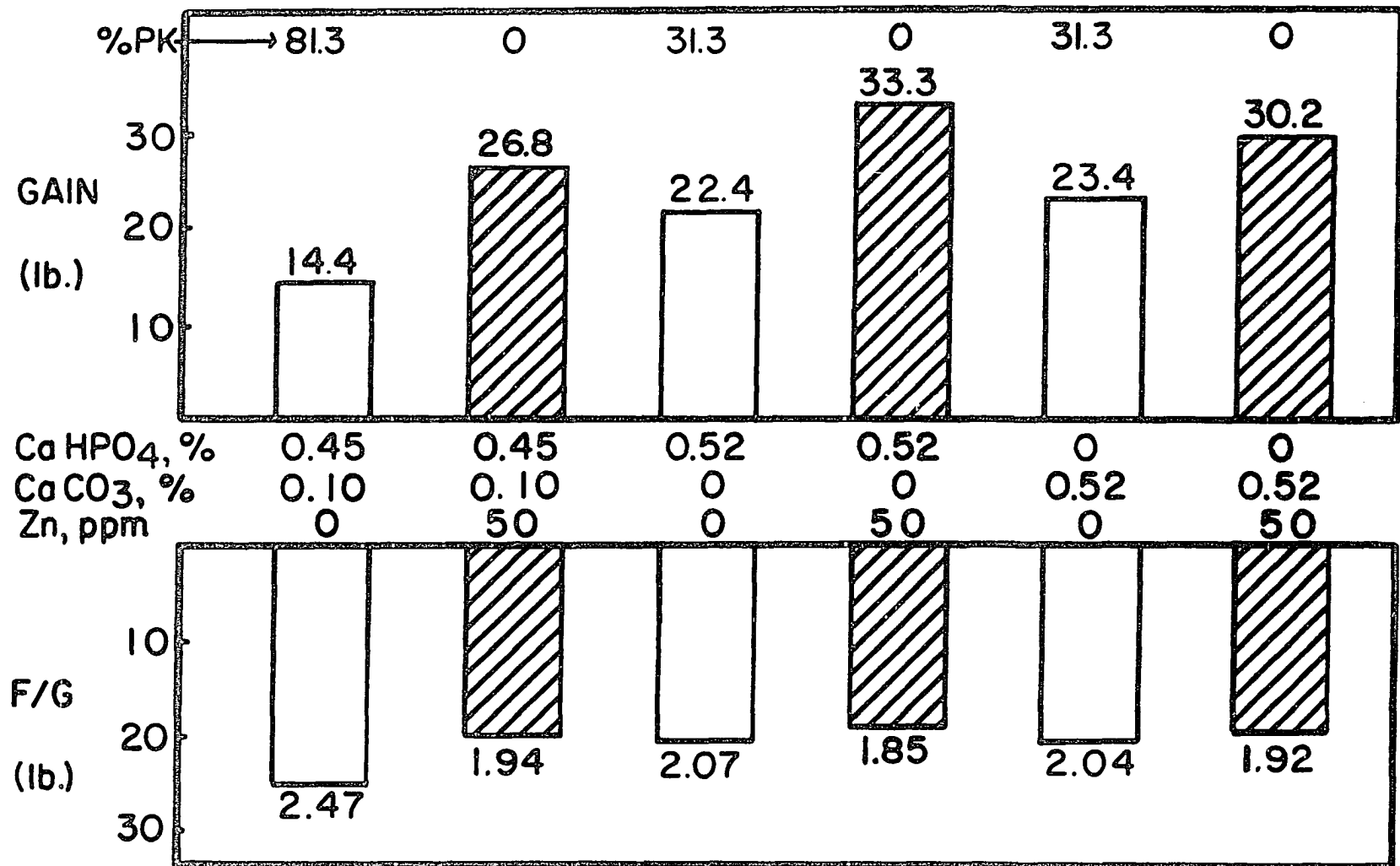
calcium as calcium carbonate developed parakeratosis compared to only five of 16 pigs receiving the other two diets not supplemented with zinc. Analysis of variance for gain and feed conversion appears in Table 26.

Repletion   Four pigs with parakeratosis were fed the basal diet plus 12 ppm zinc mixed with phytic acid at 0.3 percent of the diet, and five pigs with parakeratosis were fed 12 ppm zinc mixed with phytic acid at 0.3 percent of the diet and vitamin-free casein at 0.5 percent of the diet. The latter combination was mixed in a slurry with water and evaporated until "crumbly" in the drying oven at 90° C before being mixed into the diet. The former was mixed directly into the diet after the zinc oxide was thoroughly dissolved in the phytic acid.

Both treatments were effective in curing parakeratosis and producing rapid gains during this 21-day feeding period. The results can be seen in Table 27.

Experiment 6407 - Effect of source of water upon pigs receiving a zinc-deficient diet

Objective   This experiment was conducted to compare the performance of pigs drinking deionized water or untreated tap water while consuming a zinc-deficient diet.

Experimental   Twenty-four pigs averaging 13.1 pounds in weight and 21.2 days of age were allotted, four per pen, to

groups receiving either deionized or untreated tap water for
a 42-day feeding period during February and March of 1964.
Basal ration 2 of Table 1 was fed to pigs receiving either
source of drinking water. Pig weights, feed data and in-
cidence of parakeratosis were recorded weekly.

Results   All pigs gained poorly, but surprisingly the
pigs receiving untreated tap water gained more slowly than
those receiving deionized water. These differences were not
significant. Three of 12 pigs receiving deionized water show-
ed lesions of parakeratosis, upon termination of the experi-
ment, compared to five of 12 pigs receiving the untreated
tap water. Pigs receiving deionized water required less feed
per pound of gain than those receiving untreated tap water.
These results and the analysis of variance are shown in
Tables 28 and 29, respectively.

Repletion   Nine pigs showing emaciation and parakera-
tosis were allotted, three per pen, to treatments of 10 ppm
zinc as zinc oxide, 10 ppm zinc precipitated with phytin
$\left[Ca_5 Mg(C_6 H_{12} O_{24} P_6 \cdot 3H_2 0)_2 \right]$ in a 1:1 ratio, and 10 ppm zinc
as zinc oxide in the diet altered to contain all its supple-
mental phosphorus as phytin. The level of zinc treatment was
doubled after 2 weeks due to slow recovery of the pigs from
parakeratosis. The zinc-phytin precipitate was prepared by
dissolving phytin and zinc oxide in a 1:1 molecular ratio in

dilute hydrochloric acid and neutralizing with sodium hydroxide. The wet precipitate was mixed into the diet.

All three treatments were effective in correcting parakeratosis, but pigs consuming the diet which contained phytin as the source of supplemental phosphorus showed low feed consumption and required somewhat more feed per pound of gain. In Table 30 there is given a summary of this 21-day repletion trial.

Experiment 6408 - Effects of source of calcium on apparent availability of zinc to chicks receiving dietary phytic acid or phytin phosphorus

Objectives   This experiment was conducted to determine the effects of furnishing a portion of the calcium as calcium lactate or calcium phytate (phytin) in addition to calcium carbonate in rations which contained only phytic acid or phytin as the source of supplemental phosphorus. The effect of adding EDTA to diets containing these sources of calcium was also of interest. Lactic acid has been shown to increase calcium utilization of pigs receiving phytin phosphorus, and calcium lactate improved egg production and shell quality for hens receiving a diet with predominately organic sources of phosphorous. Feeding calcium as calcium lactate may prevent calcium from combining with phytic acid and decreasing zinc availability.

Experimental   One hundred and twenty day-old male broiler type chicks were allotted, 12 per pen, to six treatments for a 28-day feeding period during February and March of 1964. In the six treatments, basal diet 3 of Table 1 was altered to contain 1.00, 0.60 or 0.85 percent calcium as calcium carbonate with calcium lactate and phytin contributing 0.40 and 0.15 percent calcium, respectively, to the latter two diets in order that all three diets contained 1.00 percent supplemental calcium. Phytic acid was the only source of supplemental phosphorus in the first two diets and the phosphorus contained in phytin supplemented the third diet. Each of the three diets were fed with 0 or 1000 ppm EDTA (disodium salt). Because the battery contained only 10 pens, treatments with chicks fed phytin were not replicated. Chick weights and feed data were recorded weekly. At termination, eight chicks from each pen were sacrificed and livers were taken for zinc and copper analyses, and tibae were removed for determination of length:width ratio and bone ash determination.

Results   Gains of chicks receiving 1.00 percent calcium from calcium carbonate were similar to those of chicks receiving 0.60 percent calcium from calcium carbonate and 0.40 percent from calcium lactate, however, significantly (P≤ .01) less feed was required per gram of gain by chicks receiving only calcium carbonate. The addition of 1000 ppm EDTA to

these diets produced significantly (P≤ .05) higher gains on less feed per gram of gain than chicks fed diets without EDTA. The tibia length:width ratio for chicks receiving the four diets above did not differ significantly, but chicks receiving calcium lactate had significantly (P≤ .05) higher bone ash values than chicks receiving all calcium carbonate. Chicks receiving calcium phytate (phytin) grew poorly and required more feed per gram of gain than chicks receiving the other diets. Bones of the chicks fed phytin were too fragile to remove intact and could not be measured accurately to determine tibia length:width ratio. These chicks gained significantly (P≤ .01) less on more feed per gram of gain, were rachitic, and had lower bone ash values than chicks receiving calcium carbonate, or calcium carbonate and calcium lactate. Because chick groups receiving phytin were not replicated the error term calculated for the replicated treatments was used in making the comparison above. The addition of EDTA to the diets containing phytin failed to stimulate gains and bone development. There were no differences in liver zinc concentration among the treatments, but EDTA lowered copper concentration significantly (P≤ .01) when fed in the diet. Typical comparisons of chick appearance for the six treatments and the poor condition of chicks receiving phytin can be seen in Plates 7, 8 and 9. Gain and feed con-

version data are summarized in Figure 11 and Table 31, tibia

data and zinc and copper concentrations of chick livers in

Figures 12 and 13 and Table 32. The analysis of variance

for all the above data appears in Table 33.

Figure 11.   Experiment 6408 – Effect of EDTA and source of calcium on gain and
             feed/gain of chicks fed phytic acid or phytin phosphorus
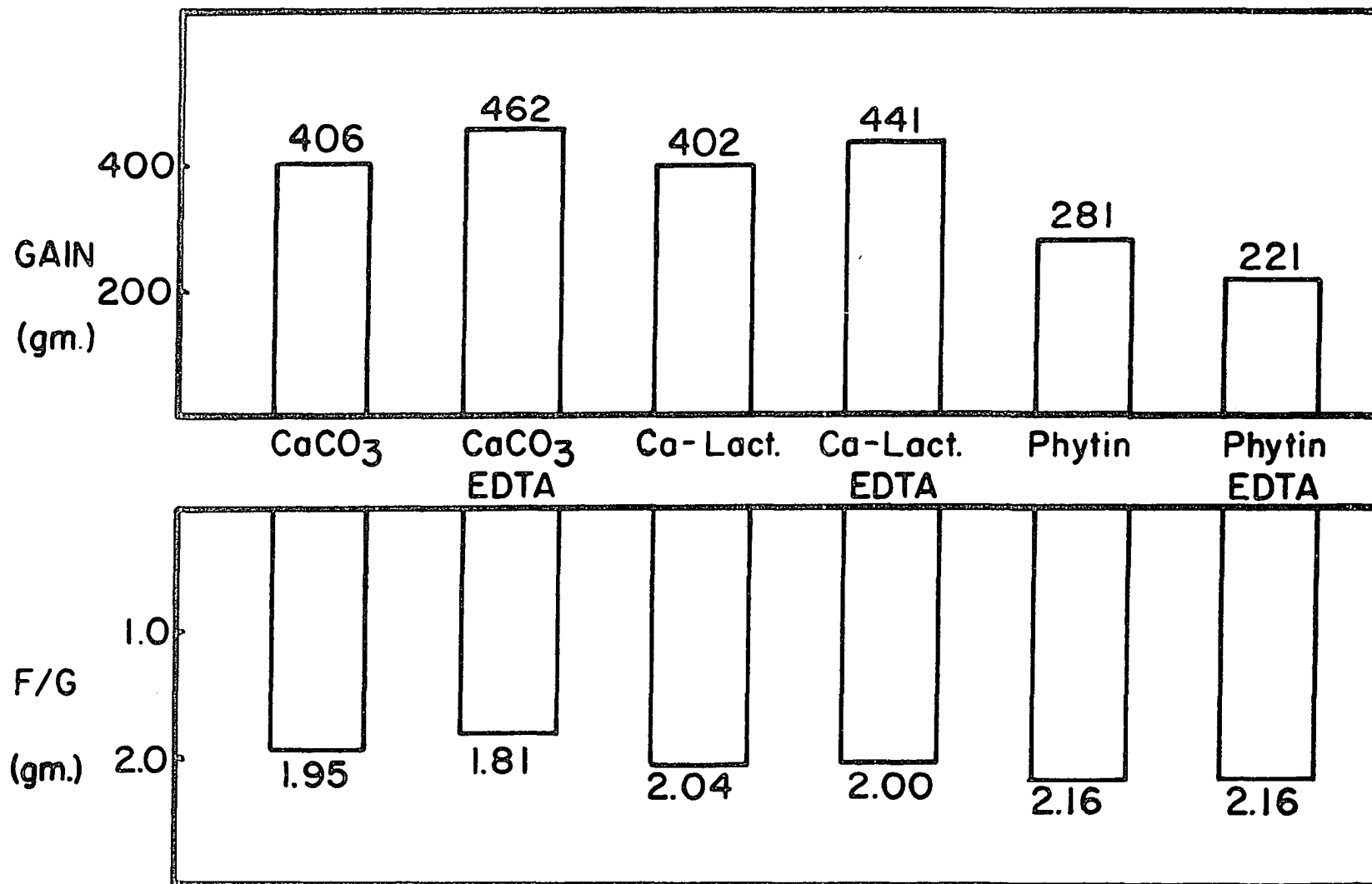
Figure 12.   Experiment 6408 - Effect of EDTA and source of calcium fed with
             phytic acid or phytin phosphorus on bone (tibia) ash of chicks

BONE

ASH

(%

| 53.4 | 53.7 | 54.6 | 54.7 | 32.9 | 31.8 |

50

25

CaCO$_3$ CaCO$_3$ Ca-Lact.   Ca-Lact.   Phytin   Phytin
          EDTA   EDTA   EDTA

57b
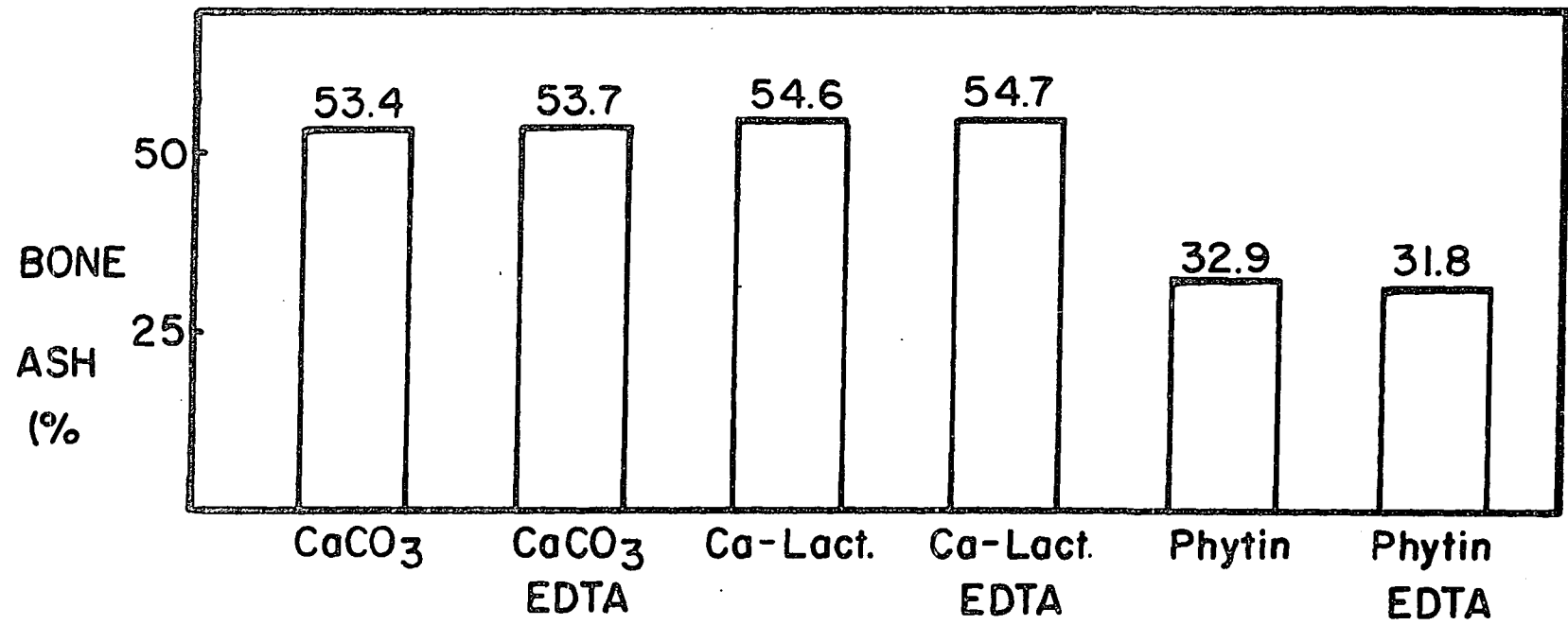
Figure 13. Experiment 6408 – Effect of EDTA and source of calcium on the liver concentration of zinc and copper in chicks fed phytic acid or phytin phosphorus

58b
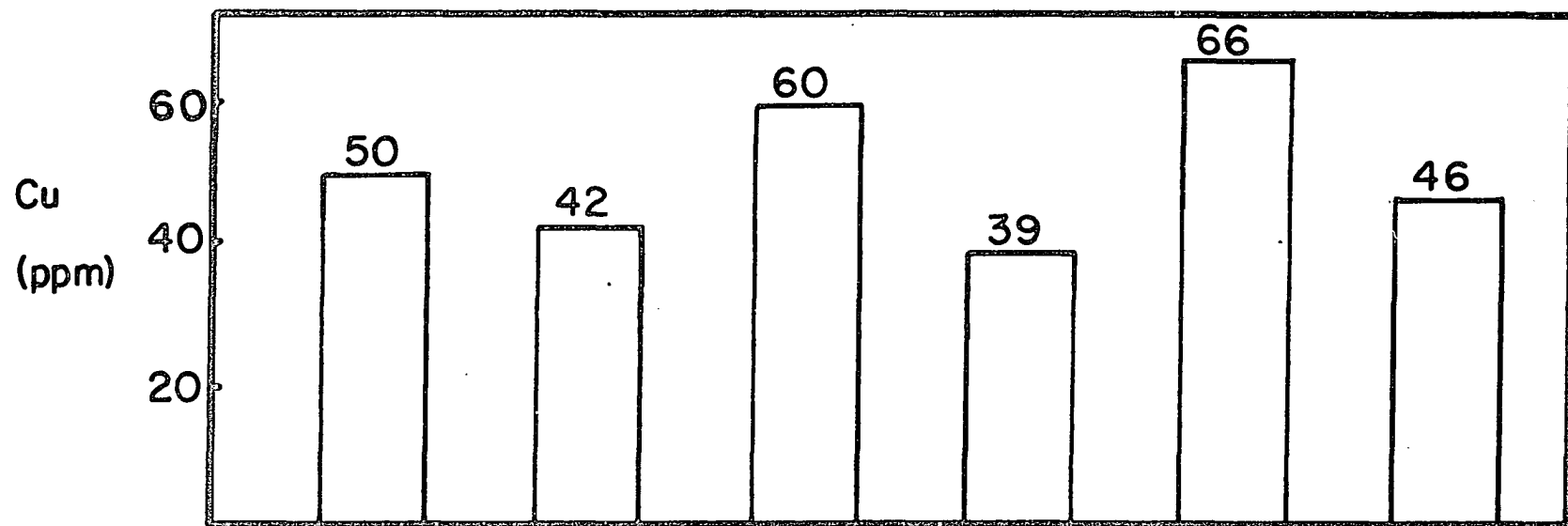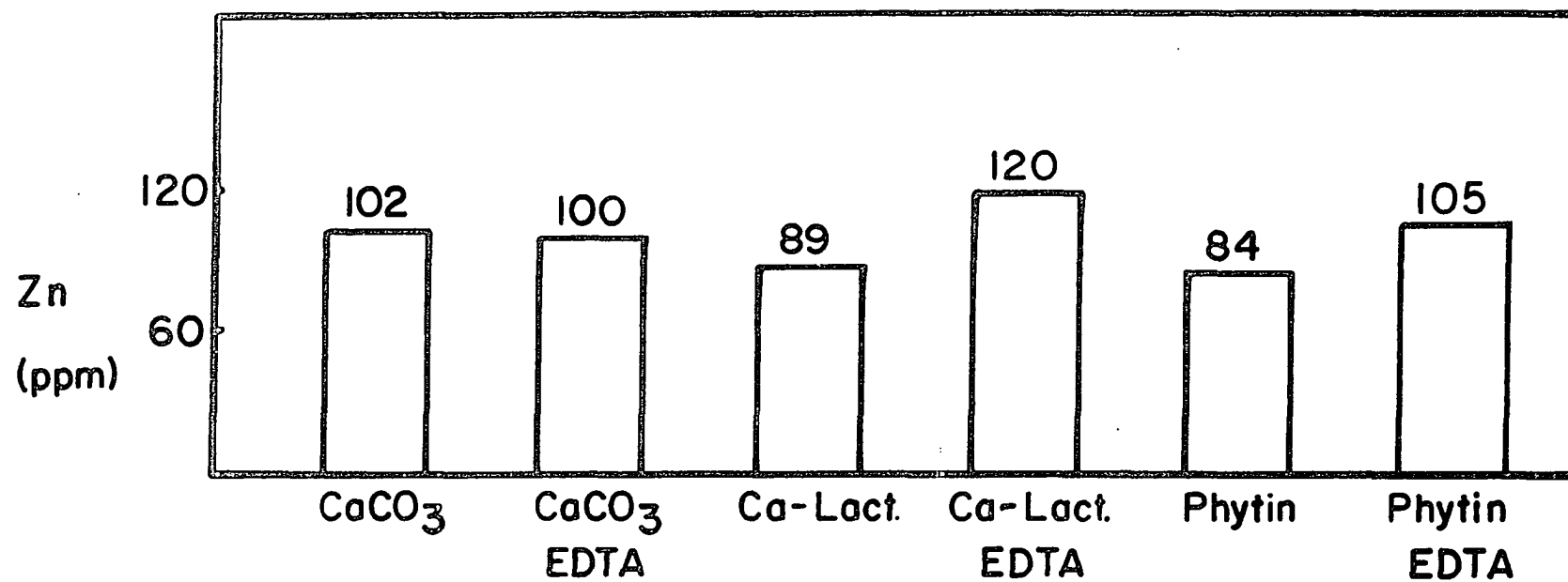
Plate 7.   Experiment 6408 - Comparison of chicks fed 1000
           ppm EDTA in diets containing calcium carbonate,
           calcium lactate and phytin, respectively, from
           left to right

Plate 8.   Experiment 6408 - Comparison of chicks fed diets
           containing calcium carbonate, calcium lactate
           and phytin, respectively, from left to right

Plate 9.   Experiment 6408 - Chicks fed phytin phosphorus
           showing very poor legs and variation in growth
           within this treatment
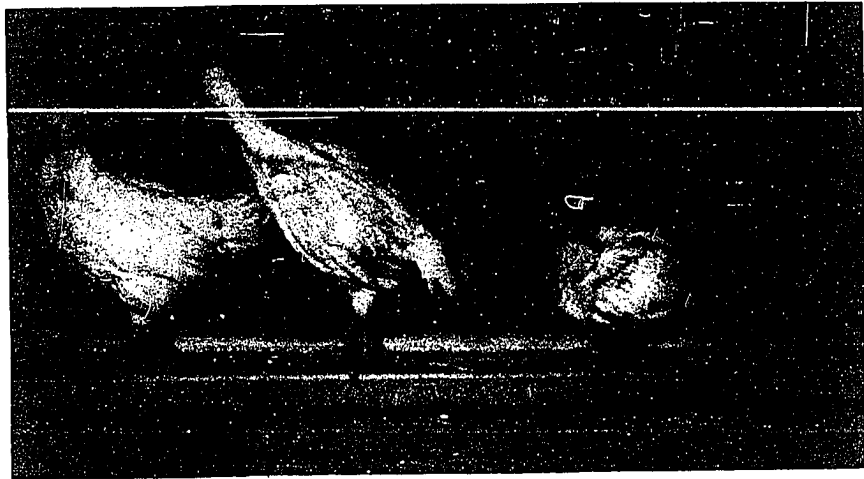
DISCUSSION

The importance of zinc in animal nutrition can be more fully appreciated when the enzymes of which zinc is an integral part are considered. Carbonic anhydrase, pancreatic carboxypeptidase and alcohol dehydrogenase are known to contain zinc in their natural structure. It has not been established that a malfunction of one of these enzyme systems is the cause of parakeratosis and poor growth in pigs, and the delayed feather development, dermatitis of feet and legs, and abnormal bone development of chicks. Regardless of the enzyme systems involved a better understanding of the absorption and metabolism of zinc is of economic, as well as academic importance.

Following reports by O'Dell and Savage (1960), Plumlee et al. (1960) and Oberleas et al. (1962a), which indicated that phytic acid interferes with zinc metabolism, interest increased in whether phytic acid exerts its detrimental effect at the site of absorption or at the cellular level. Data taken from Experiment 1139 of this study indicated that zinc-phytate complexes were metabolized adequately to render zinc available for essential processes, after it was absorbed in the complexed form. However, data from Experiment 1157 indicated that the zinc-phytate complex is also readily available

when fed in the diet. Probably the complex injected or fed in these experiments is not in the same form as the complex contained in plant-protein diets or in casein diets to which phytic acid had been added, otherwise it would not have been available when fed in the diet. During the preparation of zinc phytate for these two experiments, it was noted that the zinc-phytate precipitate began to appear near pH 3.0 and increased as the solution approached neutrality. This led to the idea that the similar pH change of the food mass, upon leaving the stomach and traversing the small intestine, could cause precipitation of dietary zinc by the phytic acid contained in plant-protein sources. Such a belief was, in part, prompted by the report of Mollgaard et al. (1946) who stated that the pH range in which pentacalcium phytate precipitated from a solution of calcium chloride and sodium phytate was similar to the pH change of food moving from the stomach through the small intestine. In the studies of this dissertation, it was found, by precipitation and centrifugation procedure, that the precipitation of zinc phytate occurred in the same pH range as the precipitation of calcium phytate, and that as many as three zinc ions were precipitated per molecule of phytic acid. It was concluded that the availability of zinc from dietary zinc-phytate in Experiment 1157 may have been due to a slight solubility of the precipitate in the in-

testine. This led to an attempt to determine, in Experiment 1176, if phytic acid would exert a protective effect on pigs fed diets containing 3000 ppm zinc as zinc oxide. This level was thought to be toxic to the pig, but the data from this experiment show that this level of zinc was not toxic when fed in the basal diet. This level of zinc did not reduce hemoglobin levels, typical of zinc toxicities with rats shown by Cox and Harris (1960), Magee and Matrone (1960), and McCall et al. (1961). Therefore, the effect of dietary phytic acid on pigs receiving toxic levels of zinc could not be determined in this experiment. Cox and Hale (1962) reported that as much as 4000 ppm zinc, as zinc oxide, was not toxic to weanling pigs fed a corn-soybean meal diet. The hemoglobin levels for pigs receiving 4000 ppm zinc were higher than for the basal ration just as those for pigs receiving 3000 ppm zinc were higher than controls in Experiment 1176.

Addition of phytic acid to the basal diet in Experiment 1176 led to a further depression of gains and feed conversion and a greater and more severe incidence of parakeratosis than seen in pigs fed the basal diet. This indicates an additional interference with zinc availability when an amount of phytic acid is added equivalent to the amount naturally present in the diet. Feeding such a "double dose" of phytic acid was shown by Green (1961) and Oberleas et al. (1962a) to reduce

gains of pigs fed a soybean-protein diet by more than 50 percent compared to the basal diet.

The addition of phytic acid to chick diets as the only source of supplemental phosphorus did not produce symptoms of zinc deficiency in chicks. And, surprisingly, the phosphorus of phytic acid was readily available to the chick for bone development as evidenced by bone ash values.

The phenomenon whereby ethylenediaminetetraacetic acid (EDTA) apparently increases zinc availability in plant protein diets has been equally as interesting and as "baffling" as the role of phytic acid in reducing zinc availability. The addition of EDTA to the basal diet increased gains and prevented parakeratosis in pigs during Experiments 1157 and 1176. The data of Green (1961) indicate that this same level of EDTA (450 ppm) was equally as effective for stimulating gains and preventing parakeratosis of pigs receiving the basal diet plus 0.6 percent phytic acid as for pigs receiving the basal diet. If the effect of EDTA is actually one of increasing absorption and utilization of dietary zinc, then EDTA must have a greater affinity for zinc than the complex of plant proteins which reduces zinc availability. An interesting finding in the present study was that a solution of EDTA would "dissolve" the zinc-phytate precipitate without depressing the pH nearly to the point of solubility for zinc phytate alone. Davis et al. (1962a) found that washing isolated soybean protein with water reduced the zinc content only from 52 to 31 ppm, whereas two washings with aqueous EDTA solutions reduced the zinc content to 2 ppm. The same workers, Davis et al. (1962b) reported

that isolated soybean protein contains a component which combines with zinc, manganese and copper, lowering their availability to the chick. The addition of EDTA to diets individually deficient in any one of these elements reduced the chicks' requirement for that particular element.

Several of the amino acids as well as peptides have relatively high stability constants when complexed with copper, iron, manganese, and zinc. The stability constant for the zinc-EDTA chelate is higher than that shown for any zinc-amino acid or zinc-peptide complex according to Chaberek and Martell (1959). Higher stability constants for copper and iron with some amino acids than with EDTA may account for the inability of Davis et al. (1962a) to remove any sizeable portion of the copper and iron from isolated soybean protein with EDTA solutions. Also, EDTA was ineffective in reducing the iron requirement of chicks fed the iron deficient diet by Davis et al. (1962b).

The data from the repletion phase of Experiment 1176 and from Experiment 6322 of this study indicate that prepared zinc-amino acid chelates, fed at 4 to 10 ppm zinc, may be equally as effective as 30 to 40 ppm zinc, as zinc oxide, for supporting pig gains and preventing parakeratosis. However, it should be pointed out that a very small quantity of the supplemental zinc needs to be absorbed to support pig gains and prevent parakeratosis, as evidenced by zinc injections in

Experiment 1139 in which the quantity of zinc injected represented only 3 to 4 ppm if it had been incorporated uniformly into the feed consumed. Chaberek and Martell (1959) report stability constants of zinc with EDTA, histitine and cysteine to be 16.50, 12.88, and 9.86, respectively. Vohra and Kratzer (1964), who studied the effects of adding several different chelating agents to zinc-deficient diets, stated that chelating agents with stability constants for zinc between 13 and 17 were most satisfactory in stimulating chick gains. Scott and Zeigler (1963) reported evidence for natural chelates in several sources of unidentified growth factors which aid in the utilization of zinc in a manner apparently similar to EDTA.

The mechanism whereby increased dietary calcium levels interfere with zinc availability has not been determined. However, it is of interest that high calcium levels depressed gains significantly and caused parakeratosis in pigs fed casein diets only when phytic acid was added to the diet, according to the studies of Oberleas et al. (1962a). Oberleas et al. (1962b) also found that the addition of calcium to solutions containing zinc and phytic acid produced more than an additive amount of precipitate when the pH of the solution was increased from 3.0 to 6.0.

Many studies have been conducted on the effect of the dietary calcium:phosphorus ratio with respect to zinc avail-

ability, but little interest has been shown in the effect of source of calcium on availability of zinc. The report of Mollgaard et al. (1946) indicates that some cereal grains, notably wheat and barley, contain a natural enzyme, phytase, which will cleave inorganic phosphorus from phytic acid when these grains are suspended in a solution buffered at pH 5.0 at 40° C for 2 hours. The presence of calcium ions in the suspension reduced the degradation of phytic acid by precipitating calcium phytate. They found that lactic acid added to the suspension would form the calcium-lactate complex and prevent the precipitation of calcium phytate, which then allowed the phytase to cleave phytic acid. Addition of lactic acid to pig diets increased the absorption of calcium and the digestability and absorption of the phytin phosphorus of the diet. The data from Experiment 6332 gave little indication that source of calcium was especially important in zinc utilization, assuming that 50 ppm supplemental zinc is adequate for this diet. However, the definite difference in performance of pigs due to source of calcium was interesting. According to analysis, differences in gain of pigs were strictly due to source of calcium, but parakeratosis was more severe and gains were less in pigs receiving calcium carbonate and dicalcium phosphate than among pigs receiving calcium only as dicalcium phosphate or calcium carbonate. This indicated, at least, an aggravation of parakeratosis due to source of dietary calcium

and that the form of accompanying phosphate ion may be important in studies of calcium and zinc relationships.

In the chick experiments of this study, there were no occurrences of severe zinc deficiencies in chicks as described by Klussendorf and Pensack (1958), O'Dell et al. (1958), and Zeigler et al. (1962). Chick diets supplemented with zinc or EDTA did produce sizeable increases in chick gains and reduced feed required per gram of gain.

High calcium diets supplemented with EDTA prevented the depression in gains seen with the unsupplemented diet. Addition of phytic acid to chick diets resulted in shorter and thicker tibiae but did not depress chick gains in the second (B) phase of Experiment 6314.

It was assumed, for Experiment 6408, that the combination of phytic acid and calcium carbonate in a diet caused a depression in both bone ash and tibia length:width ratio. However, bone ash values were as high as with chicks, in Experiment 6314, fed calcium carbonate and dicalcium phosphate. The only evidence of zinc deficiency with chicks fed all calcium carbonate or part calcium lactate was the response to EDTA supplementation. This response to EDTA was greater than the response obtained with 10 ppm supplemental zinc in Experiment 6314.

It is puzzling that phytin phosphorus was so poorly utilized in Experiment 6408, since phytic acid and calcium

68

carbonate would be expected to precipitate in similar pro-
portions to that contained in phytin. There was no apparent
interference with zinc utilization by phytin, since EDTA
supplementation of these diets failed to produce a growth
response. Also, dissolving phytin and reprecipitating with
zinc failed to interfere with the utilization of zinc in the
precipitate by pigs. Perhaps the magnesium ion in the phytin
molecule contributes to a greater insolubility of the material
in the intestine.

Attempts to detect availability of small quantities of
dietary zinc as reflections of zinc content in pig hair or
chick livers were unsuccessful. Higher levels of dietary
zinc could probably be detected by these methods.

The data from Experiment 6407 showed that the results
with the basal diets employed would have been just as great
for zinc deficiencies in pigs had tap water been used instead
of deionized water.

SUMMARY

Six experiments involving 420 pigs and two experiments involving 250 chicks were conducted to study the effects of zinc complexes and complexing agents on growth, feed conversion, and appearance of zinc deficiency or toxicity symptoms. In addition, the effects of source of dietary calcium and treatment of drinking water on zinc availability were studied.

The results of the experiments indicated that phytic acid was involved in reducing the availability of zinc to pigs and chicks, although not merely by means of zinc-phytate precipitation. It was equally apparent that 3000 ppm zinc as zinc oxide was not toxic when fed to pigs in a diet with corn and soybean meal as sources of protein, and that EDTA will not enhance toxicity in pigs fed this level of zinc. However, data from these experiments further indicated that EDTA enhanced the availability of zinc in unsupplemented diets of this type for pigs and chicks.

The source of calcium and the form of accompanying phosphorus may affect the degree of severity of zinc deficiencies in swine, but this appeared to be an indirect effect rather than one of source of calcium ion on zinc availability. Two different combinations of zinc, calcium, and phytic acid were effective in correcting parakeratosis of pigs. Also, calcium

carbonate and phytic acid fed together failed to cause outward zinc deficiency symptoms of chicks.

Zinc supplemented in a chelated form with the amino acids, cysteine and histidine, was little more effective than the same levels of zinc from zinc oxide. It is highly probable that amino acids and peptides are involved in the binding of zinc in both animal and plant sources of protein. In animal sources of protein the zinc-amino acid complexes may be readily absorbed while in plant protein the complex could involve amino acids, zinc and phytic acid all in a complex which is rendered more insoluble in the intestine by precipitation aggravated by high levels of dietary calcium. The structure of phytic acid, a sample of amino acid-zinc chelates, and the possible manner of zinc binding are shown in Figure 14.

Phytic acid phosphorus was utilized by the chick as evidenced by bone ash values. Supplementing a portion of the calcium as calcium lactate produced higher bone ash values but lower gains and feed conversion than chicks receiving all calcium carbonate. Supplemental calcium phytate (phytin) phosphorus was evidently not utilized by the chick.

Zinc or EDTA in practical chick diets increased gains and feed conversion. Supplemental EDTA did not overcome the adverse effects of feeding phytin phosphorus to chicks.

Figure 14. Basic phytic acid structure, amino acid
            chelation of zinc and possible phytic acid-
            protein binding of zinc

PHYTIC ACID

$$R - \left(\text{cyclohexane ring} \right) - O - P = O \begin{array}{c} OH \\ OH \end{array}$$

$$\left(-O - P = O \begin{array}{c} OH \\ OH \end{array} \right) = R$$

Zn - CYSTEINE CHELATE

$$Zn \begin{array}{c} S - CH_2 \\ NH_2 - CH - COO^- \end{array}$$

$$Zn \begin{array}{c} S - CH_2 \\ O - C = O \end{array} \begin{array}{c} H \\ C \\ NH_2 \end{array}$$

$$Zn \begin{array}{c} S- \\ CH_2 \\ NH_2 - CH \\ O - C = O \end{array}$$

COMBINED ZINC BINDING - ?

$$-O - P = O \begin{array}{c} OH \\ O - Zn - S - \end{array}$$

$$-O - P = O \begin{array}{c} OH \\ O - Zn - NH_2 \end{array}$$

$$-O - P = O \begin{array}{c} OH \\ O - Zn - O - \end{array}$$
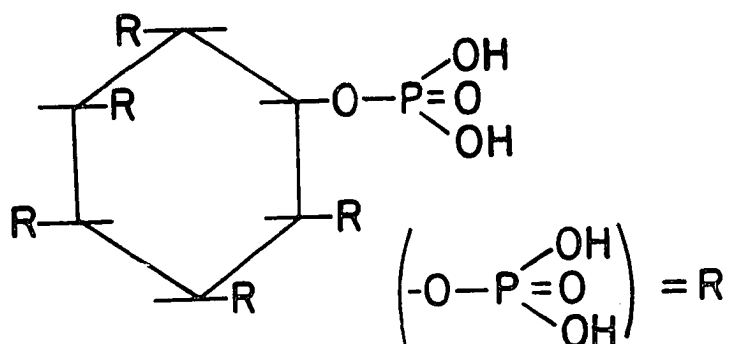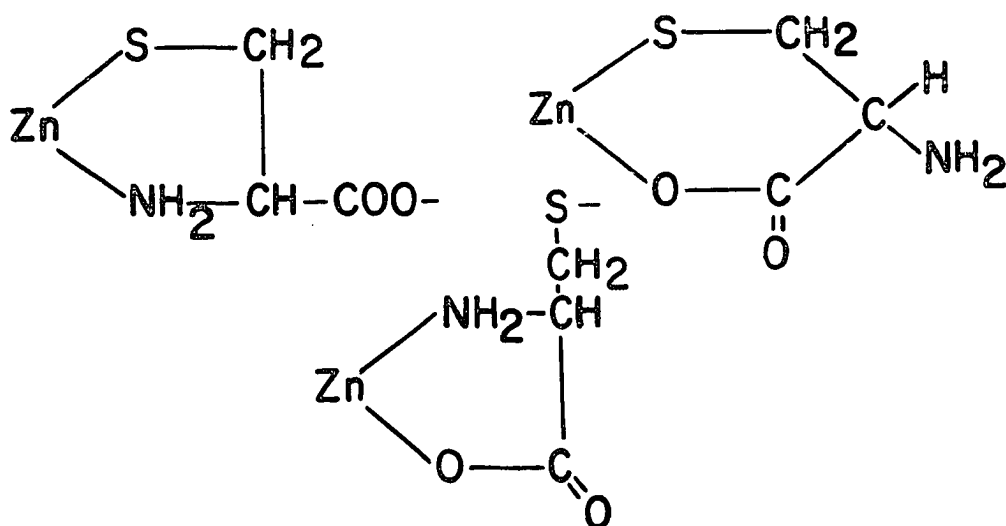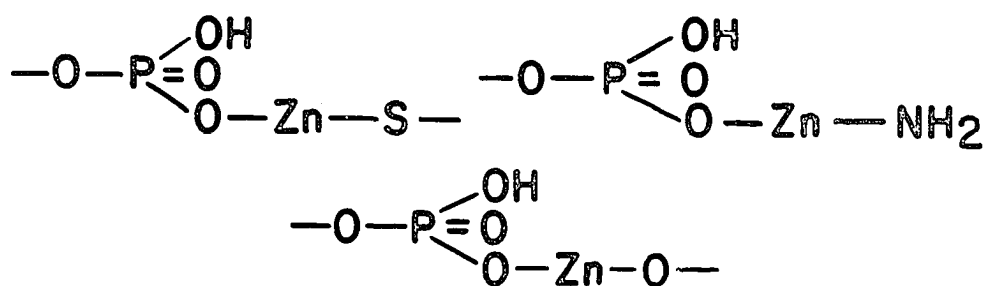
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APPENDIX

Table 1. Composition of basal diets

Ingredient	Diet			
	1[a]	2[b]	3[c]	4[d]
Ground yellow corn	--	30.00	65.00	--
Solvent soybean meal (50% prot.)	40.00	35.00	29.00	40.00
Cerelose (dextrose)	10.00	12.00	--	53.00
Sucrose	15.00	12.00	--	--
Corn starch	25.35	5.05	--	--
Solka floc (alpha cellulose)	3.00	--	--	--
Stabilized lard	2.00	2.00	1.00	2.00
Iodized salt	0.50	0.50	0.50	0.50
Calcium carbonate	1.20	0.50	1.25	1.00
Dicalcium phosphate	1.75	1.75	1.75	2.00
Vitamin premix[e]	1.00	1.00	1.00	1.00
Trace mineral premix[f]	0.20	0.20	0.50	0.50
Total	100.00	100.00	100.00	100.00

[a]Basal diet Experiment 1139.

[b]Basal diet Experiments 1157, 1176, 6322, 6332, and 6407.

[c]Basal diet Experiments 6314 and 6408.

[d]Basal diet Experiment 6314.

[e]Contribution of vitamins to each diet shown in Table 2.

[f]Contribution of trace elements to each diet shown in Table 3.

Table 2. Vitamins added per pound of diet

Vitamin		Diet 1	2	3	4
Vitamin A	IU	1000	1000	3000	3000
Vitamin D_2	IU	100	100[a]	---	---
Vitamin D_3	ICU	---	---	400	400
Riboflavin	mg	2.0	2.0	2.0	2.0
Pantothenic acid	mg	3.0	3.0	4.0	4.0
Niacin	mg	6.00	6.0	12.0	12.0
Choline	mg	---	---	200	200
Vitamin B_{12}	mcg	10.0	10.0	5.0	5.0
Vitamin E	mg	10.0	10.0	4.0[b]	---
Vitamin K	mg	1.0	1.0	1.0	1.0
Thiamine	mg	2.0	2.0	---	---
Pyridoxine	mg	2.0	2.0	---	---
Inositol	mg	25.0	25.0	---	---
Folic acid	mcg	1500	1500	---	---
Biotin	mcg	100	100	---	---

[a]Vitamin D_2 level was raised to 300 IU/lb for Experiments 1176, 6322, 6332 and 6407.

[b]This level of vitamin E was used only in Experiment 6408.

Table 3. Trace elements, in parts per million, added to the
basal diet by the trace mineral premix

Element	Source	Diet			
		1	2	3	4
		ppm			
Mg	$MgCO_3 \cdot H_2O$ (24% Mg)	400	400[a]	---	---
Mn	$MnSO_4 \cdot H_2O$ (32.5% Mn)	39	39	110	110
Fe	$FeSO_4 \cdot 2H_2O$ (29.7% Fe)	72	72	44	44
Cu	$CuSO_4$ (39.8% Cu)	6.6	6.6	4.4	4.4

[a]Corn starch replaced $MgCO_3 \cdot H_2O$ in the premixes for
Experiments 6332 and 6407.

Table 4. Analyzed zinc content of major feed ingredients

Ingredient	Date sampled	Zinc (ppm)
Solvent soybean meal (50%)	January 1962	49.0
Solvent soybean meal (50%)	April 1963	61.0
Solvent soybean meal (50%)	March 1964	20.0
Solvent soybean meal (50%)	March 1964	22.0
Yellow corn	April 1962	12.0
Yellow corn	April 1963	5.0
Yellow corn	March 1964	14.0
Yellow corn	March 1964	17.0
Sucrose	March 1964	2.6
Sucrose	March 1964	2.3
Dextrose	March 1964	2.8
Dextrose	March 1964	6.0
Corn starch	March 1964	4.8

Table 5. Calculated analyses of basal diets

Component	% or /lb.	Diet			
		1	2	3	4
Protein	percent	20.00	20.08	20.10	20.00
Fat	percent	2.20	3.32	3.61	2.20
Fiber	percent	5.80	3.20	3.66	2.80
Calcium	percent	0.99	0.70	1.00	1.00
Phosphorus	percent	0.55	0.60	0.63	0.60
Vitamin A	IU	1000	1000	3000	3000
Vitamin D_2	IU	100	100[a]	---	---
Vitamin D_3	ICU	---	---	400	400
Riboflavin	mg	2.53	2.60	2.70	2.52
Pantothenic acid	mg	5.40	5.76	7.17	6.40
Niacin	mg	10.80	13.10	21.78	16.80
Choline	mg	480	480	678	680
Vitamin B_{12}	mcg	10.00	10.00	5.00	5.00
Vitamin E	mg	10.00	10.00	5.08[b]	0.14
Vitamin K	mg	1.00	1.00	1.00	1.00
Thiamine	mg	3.60	3.20	---	---
Pyridoxine	mg	2.00	2.00	---	---
Inositol	mg	25.00	25.00	---	---
Folic acid	mcg	1500	1500	---	---
Biotin	mcg	100	100	---	---

[a]Vitamin D_2 level was raised to 300 IU/lb. for Experiments 1176, 6322, 6332 and 6407.

[b]Includes the addition of 4.0 mg used only in Experiment 6408.

Table 6. Experiment 1139 – Summary of gain, feed required per pound of gain and incidence of parakeratosis in pigs

Zn, ppm fed Injection[a]	0 Saline	20 Saline	0 Ca-EDTA	0 Na-phytate	0 Zn-EDTA[b]	0 Zn-phytate
			Treatment			
Replication			Gain, (lb.)			
1	4.20	34.00	17.93	12.53	23.45	24.32
2	8.53	27.75	1.48	1.10	19.78	25.38
3	15.88	30.92	8.28	4.05	21.82	37.37
Average	9.54	30.89	9.23	5.89	21.68	29.02
			Feed/gain (lb.)			
1	9.02	2.38	3.22	4.31	3.10	2.67
2	4.30	2.84	19.92	32.95	3.03	2.30
3	3.02	2.36	3.82	7.81	2.82	2.30
Average	5.45	2.53	8.99	15.02	2.98	2.42
			No. of pigs with parakeratosis			
Total	5	none	6	11	none	none

[a]Injections were performed once each week and contained 10.5 mg of complexed zinc or the equivalent of complexing agent per pig for the first two weeks, then the level of injection was doubled. Control animals received injections of physiological saline.

[b]Zinc-versenol chelate, zinc-sodium-ethanolethylenediaminetriacetic acid.

Table 7. Experiment 1139 - Summary of zinc and copper content of pig hair

Zn, ppm fed Injection[a]	Treatment					
	0 Saline	20 Saline	0 Ca-EDTA	0 Na-phytate	0 Zn-EDTA[b]	0 Zn-phytate
Replication				Zinc (ppm)		
1	104.7	100.3	90.0	142.5	92.3	88.8
2	68.3	93.2	77.1	120.0	132.6	117.2
3	80.6	100.2	85.1	119.9	60.6	146.0
Average	84.5	97.9	84.0	127.5	95.2	117.3
				Copper (ppm)		
1	23.0	29.5	35.6	45.5	25.0	25.0
2	19.2	45.5	21.2	38.7	8.9	21.4
3	38.5	33.6	57.6	61.1	30.0	44.6
Average	26.9	36.2	38.1	48.4	21.3	30.3

[a]See footnote of Table 6.

[b]Zinc-versenol chelate, zinc-sodium-ethanolethylenediaminetriacetic acid.

Table 8. Experiment 1139 - Analysis of variance for gain, feed required per pound of gain, and zinc and copper concentration in pig hair

Source of variation	d. f.	Mean squares			
		Gain	Feed/gain	Zinc	Copper
Replication	2	61.9575	97.9256	29.07	547.92[a]
Treatment	5	357.5032[b]	74.5691	941.25	272.51[a]
Zinc vs. no zinc	1	1620.8920[b]	231.6259[a]	102.72	328.53
Zinc fed vs. zinc injection	1	61.3094	.0624	139.44	215.63
Zn-EDTA vs. Zn-phytate	1	80.8134	.4704	737.04	122.40
Basal vs chelating agent injection	1	7.8012	86.0235	901.71	536.83[a]
Ca-EDTA vs. Na-phytate	1	16.7000	54.6620	2825.34[b]	159.14
Error	10	28.4248	49.2062	532.48	74.20
Totals	17	129.1576	62.3976	593.48	188.27

[a]Significant at P = .05 or less.

[b]Significant at P = .01 or less.

Table 9. Experiment 1139 - Repletion phase - Summary of gain, feed required per pound of gain and correction of parakeratosis

Zn, ppm fed (R)	Treatment		
	20	0	0
Injection[a] (R)[b]	Saline	Zn-EDTA	Zn-phytate
Zn, ppm fed (D)[c]	0	0	0
Injection (D)	Saline	Ca-EDTA	Na-phytate
Replication	Gain (lb.)		
1	18.88	12.00	14.25
2	16.67	18.33	14.88
3	12.50	17.25	14.38
Average	16.01	15.86	14.50
	Feed/gain (lb.)		
1	1.83	2.77	2.00
2	1.86	1.74	2.09
3	2.38	1.99	2.07
Average	2.02	2.17	2.05
	No. of pigs with parakeratosis (total)		
Initial[d]	7	6	8
Final	none	1	none

[a]Injections were performed once each week and zinc-complex injections contained 21.0 mg of zinc per pig.

[b]Repletion.

[c]Depletion.

[d]Number is not necessarily the same as in Table 6 due to initiation of repletion only after all replicates of depletion were completed, and some heavy pigs were removed.

Table 10. Experiment 1157 - Summary of gain, feed required
per pound of gain and incidence of parakeratosis

	Treatment					
Zn, ppm	0	40	0	0	40	0
Zn-phytate, ppm	0	0	40	0	0	40
EDTA, ppm	0	0	0	450	450	450
Replication	Gain (lb.)					
1	21.32	32.18	30.68	34.38	31.92	29.15
2	17.10	33.48	31.82	32.42	32.80	33.10
3	16.28	25.00	28.12	31.55	34.38	27.20
Average	18.23	30.22	30.21	32.78	33.03	29.82
	Feed/gain (lb.)					
1	2.26	1.88	2.12	1.83	1.68	1.93
2	2.11	1.78	1.80	1.88	1.92	1.85
3	2.08	1.90	1.98	1.95	1.88	2.21
Average	2.15	1.85	1.97	1.89	1.83	2.00
	No. of pigs with parakeratosis					
Total	9	none	none	none	none	none

Table 11. Experiment 1157 - Analysis of variance for gain and feed/gain

Source of variation	d. f.	Mean squares Gain	Feed/gain
Blocks	2	17.3465	.0182
Treatments	5	90.0597^a	.0426
Zinc oxide & EDTA treatment	3^c	441.7280^a	$.2005^a$
Zinc	1	336.9060^a	$.2862^a$
EDTA	1	678.3420^a	$.1892^a$
Interaction	1	309.9361^a	$.1261^b$
Zinc phytate & EDTA treatment	3^c	378.6219^a	$.1094^a$
Zinc phytate	1	182.5201^a	.0121
EDTA	1	451.1376^a	$.1225^b$
Interaction	1	502.2081^a	$.1936^a$
Error	10	5.4215	.0154
Totals	17	31.7180	.0237

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

[c]Treatment comparisons were made by considering two 4-treatment factorial experiments. Thus, the estimate of treatment components may be slightly biased since certain treatments were used twice in the treatment comparisons.

Table 12. Experiment 1157 - Repletion phase - Summary of gain, feed required per pound of gain and correction of parakeratosis

	Treatment	
	40 ppm zinc as Zn-phytate	450 ppm EDTA
Gain (lb.)	21.44	17.25
Feed/gain (lb.)	1.88	1.70
No. of pigs with parakeratosis:		
Initial	5	4
Final	none	none

Table 13. Experiment 1176 - Summary of gain, feed required per pound of gain and incidence of parakeratosis in pigs

	Treatment					
Zn, ppm	0	0	0	3000	3000	3000
EDTA, ppm	0	450	0	0	6700	0
Phytic acid,%	0	0	0.6	0	0	0.6

Replication	Gain (lb.)					
1	18.02	19.20	11.22	28.48	29.15	31.38
2	9.72	23.20	19.02	26.28	27.60	31.85
3	25.60	26.72	11.75	36.70	28.40	37.95
Average	17.78	23.04	14.00	30.48	28.38	33.73

	Feed/gain (lb.)					
1	1.96	2.14	2.46	1.80	1.83	1.87
2	3.17	1.92	2.19	2.06	1.91	1.91
3	2.08	2.01	2.93	1.88	2.00	1.97
Average	2.40	2.02	2.53	1.91	1.91	1.92

	No. of pigs with parakeratosis					
Total	3	none	9	none	none	none

Table 14. Experiment 1176 – Summary of 26-and 42-day
          hemoglobin levels of pigs

	Treatment					
Zn, ppm	0	0	0	3000	3000	3000
EDTA, ppm	0	450	0	0	6700	0
Phytic acid,%	0	0	0.6	0	0	0.6
Replication	26-day hemoglobin (gm/100 ml)					
1	10.4	9.7	10.4	10.0	9.4	10.8
2	10.0	9.1	10.9	9.4	8.9	10.6
3	9.0	9.6	10.0	10.6	9.9	10.1
Average	9.8	9.5	10.4	10.0	9.4	10.5
	42-day hemoglobin (gm/100 ml)					
1	10.1	10.0	11.1	11.9	11.9	10.6
2	10.1	9.6	10.4	12.2	10.1	10.2
3	10.9	10.6	10.3	12.3	11.6	11.8
Average	10.4	10.1	10.6	12.1	11.2	10.9

Table 15. Experiment 1176 - Analysis of variance for gain, feed/gain and 26-and 42-day hemoglobin values

Source of variation	d. f.	Mean squares			
		Gain	Feed/gain	26-day Hb.	42-day Hb.
Replication	2	48.5461	.0542	.1550	1.0172
Treatment	5	176.1800[a]	.2290	.6573	1.6059[a]
Treatments excluding EDTA	3	275.4399[a]	.3101	.3433	1.8631[a]
3000 ppm vs. 0 ppm zinc	1	789.1030[a]	.9075[b]	.0533	3.1008[a]
0.6% phytic acid vs. none	1	.2214	.0120	.9633	.8008
Interaction	1	36.9954	.0108	.0134	1.6876[b]
EDTA vs. others	1	11.7534	.1965	2.2500[b]	.5136
450 ppm vs. 6700 ppm EDTA	1	42.8268	.0182	.0067	1.9267[b]
Error	10	18.3782	.1140	.2823	.2612
Totals	17	68.3396	.1408	.3776	.7456

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 16. Experiment 1176 - 14-day repletion phase - Summary of gain, feed required per pound of gain and correction of parakeratosis

Zn, ppm as:	Treatment		
	40 ZnO	10 Zn-histidine	10 Zn-cysteine
Gain, lb.	16.60	17.40	16.30
Feed/gain, lb.	1.96	2.18	1.76
No. of pigs with parakeratosis:			
Initial	1	2	3
Final	none	none	none

Table 17. Experiment 1176 - 16-day repletion phase - Summary of gain, feed required per pound of gain and correction of parakeratosis

40 ppm Zn as:	Treatment		
	Zn-phytate	Zn-phytate-cysteine	$Zn(Ca)_2$-phytate
Gain, lb.	15.33	14.17	14.67
Feed/gain, lb.	1.72	1.81	1.84
No. of pigs with parakeratosis:			
Initial	2	2	3
Final	none	none	none

Table 18. Experiment 6314A - Summary of chick gain and feed
required per gram of gain

Type of diet Zn, ppm	Corn-soybean meal			Dextrose-soybean meal		
	0	10	20	0	10	20
Replication			Gain (gm)			
1	373	407	416	351	392	365
2	403	438	--	359	394	--
Average	388	423	416	355	393	365
			Feed/gain (gm)			
1	2.06	1.95	1.94	2.30	2.16	2.15
2	1.95	1.85	--	2.19	2.11	--
Average	2.00	1.90	1.94	2.24	2.14	2.15

Table 19. Experiment 6314A - Summary of tibia length and concentration of zinc and copper in chick liver

Type of diet	Treatment					
	Corn-soybean meal			Dextrose-soybean meal		
Zn, ppm	0	10	20	0	10	20
Replication	Tibia length (cm)					
1	6.49	6.71	6.64	6.76	6.59	6.64
2	6.72	6.76	--	6.73	6.71	--
Average	6.60	6.74	6.64	6.74	6.65	6.64
	Zinc (ppm dry wt.)					
1	89	81	87	66	105	109
2	82	80	--	101	108	--
Average	86	81	87	84	107	109
	Copper (ppm dry wt.)					
1	48	47	51	39	31	41
2	49	49	--	37	35	--
Average	49	48	51	38	33	41

Table 20. Experiment 6314A - Analysis of variance for gain, feed/gain, tibia length, and zinc and copper concentration in chick livers

Source of variation	d. f.	Mean squares				
		Gain	Feed/gain	Tibia length	Zinc	Copper
Treatments	5	1178.0	.0318[a]	.0061	243.0	85.0[b]
Corn-SBM vs. dextrose-SBM	1	3098.0[a]	.1345[b]	.0013	490.0	372.0[b]
Levels of zinc (0-10-20 ppm)	2	1314.0	.0120	.0018	146.0	21.0
Interaction	2	81.0	.0001	.0127	216.0	6.0
Error	4	241.0	.0046	.0089	160.0	3.0
Totals	9	762.0	.0197	.0073	206.0	49.0

[a]Significant at P = .05 or less.

[b]Significant at P = .01 or less.

Table 21. Experiment 6314B - Summary of gain, feed/gain, tibia length, tibia length/width ratio, tibia ash, and zinc and copper concentration in chick liver

	Treatment									
	Corn-soybean meal					Dextrose-soybean meal				
Ca, %	1.0	2.0	2.0	1.0	1.0	1.0	2.0	2.0	1.0	1.0
EDTA, ppm	0	0	450	0	0	0	0	450	0	0
Phytic acid, %	0	0	0	0.5	0	0	0	0	0.5	0
Zn, ppm	0	0	0	0	10	0	0	0	0	10
Gain, gm	657	583	655	682	675	569	541	541	581	530
Feed/gain, gm	2.80	2.97	2.82	2.81	2.65	2.86	3.13	2.94	3.28	2.71
Tibia length, cm	9.68	9.14	9.78	9.65	10.04	9.28	8.87	9.52	9.13	9.31
Tibia length/width	15.07	14.01	15.58	14.63	16.88	15.59	13.94	16.13	14.55	16.06
Tibia ash, %	56.9	52.5	54.2	52.6	56.3	55.7	55.1	53.3	53.5	55.0
Liver Zn, ppm dry wt.	102	102	84	77	98	98	73	83	122	114
Liver Cu, ppm dry wt.	58	75	86	79	61	73	53	77	61	56

Table 22. Experiment 6322 - Pilot trial - Summary of gain, feed required per pound of gain and incidence of parakeratosis in pigs

	Treatment					
Zn, ppm as ZnO	0	4	40	0	0	0
Zn, ppm as Zn-histidine	0	0	0	4	0	0
Zn, ppm as Zn-cysteine	0	0	0	0	4	0
Zn, ppm as Zn-versenol[a]	0	0	0	0	0	4
Gain, lb.	8.0	19.0	21.3	22.0	17.8	16.8
Feed/gain, lb.	3.31	2.12	1.94	2.06	2.34	2.37
No. of pigs with parakeratosis	1	1	none	none	none	1

[a]Zinc chelate of sodium ethanolethylenediaminetriacetic acid (10% Zn).

Table 23. Experiment 6322 - Summary of gain, feed required per pound of gain and incidence of parakeratosis in pigs

	Treatment					
Zn, ppm as ZnO	5	30	0	0	0	0
Zn, ppm as Zn-histidine	0	0	5	0	5	0
Zn, ppm as Zn-EDTA	0	0	0	5	0	5
EDTA(Na)$_2$, ppm	0	0	0	0	30	0
Histidine.HCl, ppm	0	0	0	0	0	30

Replication	Gain (lb.)					
1	19.60	20.48	23.50	16.80	20.92	21.22
2	19.60	24.62	22.50	24.92	20.60	22.58
3	15.72	25.02	17.78	18.28	17.38	23.15
Average	18.31	23.37	21.26	20.00	19.63	22.32

	Feed/gain (lb.)					
1	2.06	2.12	1.84	2.12	2.23	1.83
2	2.12	1.97	2.15	1.86	2.21	2.07
3	2.47	2.02	2.49	2.25	2.30	2.05
Average	2.22	2.04	2.16	2.08	2.25	1.98

	No. of pigs with parakeratosis					
Total	none	none	none	none	none	none

Table 24. Experiment 6322 – Analysis of variance for gain and feed/gain.

| Source of variation | d. f. | | Mean squares | |
			Gain	Feed/gain
Replication	2		13.4480	.0938
Treatment	5		10.4102	.0327
30 ppm Zn vs. others		1	23.5622	.0250
Treatments with 5 ppm Zn		4	7.1222	.0346
5 ppm Zn vs. others		1	14.9500	.0240
Zn-histidine vs. Zn-EDTA		1	1.5194	.0901
Free chelate components		1	.3571	.0000
Zn-chelate x free chelate interaction		1	11.6624	.0243
Error	10		6.1484	.0252
Totals	17		8.2605	.0355

Table 25. Experiment 6332 – Summary of gain, feed required per pound of gain and incidence of parakeratosis in pigs

	Treatment					
Ca as $CaHOP_4$, %	0.45	0.45	0.52	0.52	0	0
Ca as $CaCO_3$, %	0.10	0.10	0	0	0.52	0.52
Zn, ppm	0	50	0	50	0	50
Replication	Gain (lb.)					
1	16.50	31.52	28.12	39.75	25.82	31.10
2	13.72	20.10	12.98	24.62	12.08	21.20
3	14.60	24.65	20.20	32.55	26.25	29.08
4	12.78	30.98	28.48	36.35	29.58	39.50
Average	14.40	26.81	22.44	33.32	23.43	30.22
	Feed/gain (lb.)					
1	2.39	1.92	2.12	1.93	2.06	1.90
2	2.49	2.13	2.32	1.92	2.45	2.12
3	2.17	1.85	2.02	1.75	1.82	1.83
4	2.82	1.86	1.83	1.81	1.83	1.84
Average	2.47	1.94	2.07	1.85	2.04	1.92
	No. of pigs with parakeratosis					
Total	13	none	5	none	5	none

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Table 26. Experiment 6332 - Analysis of variance for gain
and feed/gain

| Source of variation | d. f. | Mean squares | |
		Gain	Feed/gain
Replication	3	192.4205[a]	.1174[b]
Treatment	5	176.7951[a]	.1938[a]
Zn vs. no Zn	1	602.9035[a]	.4988[a]
Source of calcium	2	123.6360[a]	.1441[b]
Basal and dical. only vs. calcium carbonate only	1	35.5696	.0554
Basal vs. dical. only	1	211.7025[a]	.2328[a]
Source of calcium x Zn interaction	2	16.8998	.0910
Error	15	11.9641	.0255
Totals	23	70.4652	.0740

[a]Significant at $P = .01$ or less.

[b]Significant at $P = .05$ or less.

Table 27. Experiment 6332 - Repletion phase - Summary of
gain, feed required per pound of gain and cor-
rection of parakeratosis

Combination fed	12 ppm Zn as ZnO in phytic acid, 0.3% of diet	12 ppm Zn as ZnO in phytic acid, 0.3% of diet + casein, 0.5% of diet
Gain, lb.	25.00	22.30
Feed/gain, lb.	1.98	1.82
No. of pigs with parakeratosis:		
Initial	4	5
Final	none	none

Table 28. Experiment 6407 - Summary of gain, feed required per pound of gain and incidence of parakeratosis in pigs

Source of drinking water	Deionized	Untreated[a]
Replication		Gain (lb.)
1	19.08	9.62
2	18.12	10.42
3	17.45	20.98
Average	18.22	13.67
		Feed/gain (lb.)
1	2.35	3.43
2	2.50	3.62
3	2.61	2.26
Average	2.49	3.10
		No. of pigs with parakeratosis
Total	3	5

[a]Ames city water system.

Table 29. Experiment 6407 - Analysis of variance for gain and feed/gain

Source of variation	d. f.	Mean squares Gain	Feed/gain
Replication	2	16.0426	.2088
Treatment	1	30.9629	.5704
Error	2	24.8292	.3506
Totals	5	22.5413	.3379

Table 30. Experiment 6407 - Repletion phase - Summary of gain, feed required per pound of gain and correction of parakeratosis

	Treatment[a]		
Zn, ppm as ZnO	10	0	10
Zn, ppm as Zn-phytin	0	10	0
Source of phosphorus	$CaHPO_4$	$CaHPO_4$	Phytin[b]
Gain, lb.	14.17	18.83	10.50
Feed/gain, lb.	2.20	1.99	2.31
No. of pigs with parakeratosis:			
Initial	2	3	2
Final	none	none	none

[a]After two weeks the zinc level was raised to 20 ppm for each treatment.

[b]$Ca_5Mg(C_6H_{12}O_{24}P_6 \cdot 3H_2O)_2$.
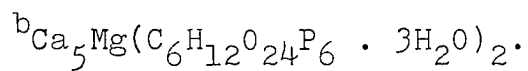
Table 31. Experiment 6408 - Summary of gain and feed required per gram of gain for chicks

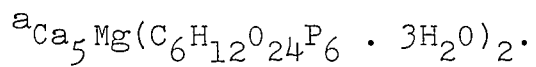|	Treatment					
Ca as CaCO$_3$, %	1.00	1.00	0.60	0.60	0.85	0.85
Ca as Ca-lactate, %	0	0	0.40	0.40	0	0
Ca as phytin[a]	0	0	0	0	0.15	0.15
EDTA, ppm	0	1000	0	1000	0	1000
Replication	Gain (gm)					
1	419	470	396	452	281	221
2	393	454	407	430		
Average	406	462	402	441	281	221
	Feed/gain (gm)					
1	1.90	1.78	2.01	1.92	2.16	2.16
2	2.00	1.84	2.08	2.07		
Average	1.95	1.81	2.04	2.00	2.16	2.16

[a] $Ca_5Mg(C_6H_{12}O_{24}P_6 \cdot 3H_2O)_2$.

Table 32. Experiment 6408 - Summary of tibia length/width ratio, tibia ash and concentration of zinc and copper in chick liver

	Treatment					
Ca as $CaCO_3$, %	1.00	1.00	0.60	0.60	0.85	0.85
Ca as Ca-lactate, %	0	0	0.40	0.40	0	0
Ca as phytin, %	0	0	0	0	0.15	0.15
EDTA, ppm	0	1000	0	1000	0	1000
Replication	Tibia length/width					
1	11.79	11.45	12.00	11.77	[a]	[a]
2	11.65	11.60	11.57	11.41		
Average	11.72	11.52	11.79	11.59		
	Tibia ash (%)					
1	53.1	53.8	54.5	54.9	32.9	31.8
2	53.7	53.6	54.6	54.4		
Average	53.4	53.7	54.6	54.7	32.9	31.8
	Zinc (ppm dry wt.)					
1	80	106	75	115	84	105
2	124	94	103	126		
Average	102	100	89	120	84	105
	Copper (ppm dry wt.)					
1	48	38	53	39	66	46
2	53	45	68	39		
Average	50	42	60	39	66	46

[a]Bones too fragile to remove intact and get correct measurement.

Table 33. Experiment 6408 - Analysis of variance for gain, feed/gain, tibia length/width, bone ash and zinc and copper concentration in chick livers

Source of variation	d. f.	Gain	Feed/ gain	Tibia length/width	Bone ash	Zinc	Copper
				Mean squares			
Replication	1	351.0	.0180[a]	.0760	.0000	630.0	91.0
Treatment	3	1674.0[a]	.0204[a]	.0282	.7700	341.0	191.0[a]
Ca-lactate vs. $CaCO_3$	1	325.0	.0392[b]	.0084	2.2100[a]	28.0	28.0
EDTA vs. no EDTA	1	4560.0[a]	.0180[a]	.0760	.0800	435.0	354.0[a]
Interaction	1	136.0	.0041	.0001	.0200	561.0	190.0
Error	3	139.0	.0008	.0341	.1100	288.0	19.0
Totals	7	827.0	.0117	.0375	.3800	360.0	103.0

[a]Significant at P = .05 or less.

[b]Significant at P = .01 or less.