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The purpose of this study was to investigate the copper-zinc-iron interrelationship at low to normal levels of dietary zinc intake. The effects of supplements of copper, iron, and zinc on growth and mineral metabolism of young rats fed zinc deficient diets were observed over a period of four weeks.

Results of the experiments indicated that a ten ppm zinc supplement prevented the depressed growth and high hemoglobin levels of the animals receiving zinc deficient diets. Zinc supplements were associated with decreased liver copper and iron levels, and copper supplements were associated with decreased liver zinc levels. There was some indication that supplemental copper could cause a zinc deficiency in animals receiving marginal zinc diets. The relative proportions of copper, zinc, and iron seem to be an important factor in studying the complex interrelationship between copper, zinc, and iron.

# THE EFFECTS OF ZINC NUTRITION ON THE COPPER AND IRON STATUS OF THE YOUNG RAT

by

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# CHAPTER I

# INTRODUCTION

The balance of nutrients in animal and human nutrition is recognized as being essential for proper metabolism of the various nutrients. It has been shown that an excess of some nutrients has an antagonistic effect on other nutrients. Zinc is of interest since toxic levels of zinc have been shown to interfere with the absorption and/or metabolism of calcium, copper, iron, magnezium, and phosphorus.

Several studies have shown an antagonism between copper and zinc in both copper toxicosis and zinc toxicosis. The site of this antagonism is believed to be in the intestine during absorption. It is not known what effects copper and zinc have on each other when zinc is present in normal or marginal levels in the diet. Toxic levels of zinc have also been shown to effect iron metabolism, but the site of this interaction has not been determined. The present investigation was designed to study the zinc-copper-iron interrelationship when the dietary zinc levels are below the minimal level, marginal, and normal in order to gain further information about the extent of this interrelationship.

# CHAPTER II

## REVIEW OF LITERATURE

The presence of zinc in living organisms and its role as an essential nutrient for plants and animals, including man, is well recognized. It was not until 1944 (1), when zinc was found to be an essential part of carbonic anhydrase, that a mode of action of this element could be explained. Since then, zinc has been found as a constituent of many metalloenzymes, including pancreatic carboxypeptidase, alkaline phosphatase, several dehydrogenases, and as a cofactor in several enzyme systems.

Relatively large amounts of zinc can be tolerated in the diet of most animals without causing harmful effects. Rats can tolerate up to 5000 ppm of zinc in the diet, an amount which is about 500 times their minimal requirement, before the signs of zinc toxicity appear (2,3). Zinc toxicity causes growth depression, severe hypochromic microcytic anemia, inhibition of some enzyme systems, and interference with copper and iron metabolism in the young rat (4-6).

Zinc toxicity has been shown to cause a copper deficiency. Davis (7) reported that an increase of dietary zinc caused a decrease in liver copper when the copper levels of the diet were borderline to normal. Supplementing copper to the diets of animals suffering from zinc toxicity raised the liver copper levels, increased hemoglobin levels, and helped to overcome depressed activities of catalase and cytochrome oxidase (6, 8, 9, 10).

Cox and Harris (5) reported a marked copper deficiency with 4000 ppm of zinc. Copper supplements increased the liver copper but further decreased the liver iron, which was lowered by the copper deficiency. Iron supplements increased the liver iron levels and prevented some loss of copper from the liver. Magee and Matrone (6) also showed that zinc interferes with iron metabolism and that copper supplements further decrease liver iron levels, but reported that iron supplements had no effect on liver copper. Magee and Matrone also suggested that zinc had an effect on copper metabolism and one on iron metabolism separately. Cox and Hale (11) reported no decrease in liver iron concentrations when pigs were fed zinc toxic diets.

Reciprocal effects between copper and zinc have been demonstrated in the pig (12-15) in that the development of copper poisoning can be alleviated by dietary supplements of zinc. O'Hara et al. (12) concluded that copper was affecting zinc storage and/or utilization, thus causing a zinc-deficient parakeratosis in the animals. Suttle and Mills (13) showed that 500 ppm of zinc or 750 ppm of iron lowers the serum levels of copper to normal. However, only supplements of iron prevented anemia developed under these conditions.

Zinc deficiency is becoming more of a concern since naturally occurring zinc deficiencies have been found in pigs, cattle, and man (15-17). The requirement for zinc is dependent on several factors, one of which is the dietary protein source. The dietary requirement of zinc for the growth of rats was found to be 18 ppm when the dietary protein source was soy protein, 12 ppm with casein, and 12 ppm with egg white (3). However, as much as 60 ppm may be needed for normal reproduction and 100 ppm if the diet is soybean protein (18). Zinc deficiency causes a marked retardation of growth, skin lesions, changes in bone formation, impaired reproductive development and function, and impaired DNA synthesis (19, 20). Prasad (17) has described zinc deficiency symptoms found in villagers in Egypt and Iran. The men studied had severe retardation of growth, hypogonadism, a decrease in serum iron, increased iron binding capacity, and an increased serum copper level. Zinc supplementation to the diets resulted in growth, genital development, and appearance of secondary sex characteristics. The anemia was partially overcome by zinc supplementation and completely overcome when iron was given along with the zinc.

In studying the mechanism of the zinc-copper interrelationship, zinc was shown to interfere with $^{64}Cu$

absorption from an intestinal segment of the rat (21). A significant depression in $^{64}Cu$ uptake was shown with a zinc to copper ratio of 500 to 1. The effect of zinc on copper was thought to be mediated through a direct effect of zinc on or in the intestine rather than an indirect effect of zinc levels in non-intestinal tissues or serum zinc levels (22). In studies on the mode of copper absorption from the gastrointestinal tract of chickens, Starcher (23) found that copper binds to a duodenal protein in the process of absorption. Zinc also binds to this protein, thus inhibiting copper absorption. Hill and Matrone (24) suggest that the basis for the zinc-copper interaction is the similarity of the electronic structures of their ions.

From these studies it is clear that there is an antagonistic effect between zinc and copper. These effects have been shown in conditions of zinc toxicity and copper toxicity. High levels of zinc decrease copper absorption, and high levels of copper decrease zinc absorption. Further investigation of this antagonism at less than toxic levels of copper and zinc would be helpful in defining the extent of the antagonism. Since zinc toxicity has also shown effects on iron stores, a study of the effects of iron supplements on lower levels of dietary zinc may provide useful information about this interrelationship.

# CHAPTER III

## EXPERIMENTAL PROCEDURES

The purpose of this study was to investigate the zinc-copper-iron interrelationship in animal nutrition. Criteria to be used for indications of this interrelationship were weight gains, hemoglobin levels, and liver levels of zinc, copper,and iron of rats on experimental diets.

The study consisted of 3 experiments. Experiment 1 was designed to show the effects of various dietary levels of zinc on the parameters to be observed in the experimental animals. Experimental diets consisted of a commercially prepared zinc deficient basal diet and the basal diet plus 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm of zinc. Experiments 2 and 3 were designed to show the effects of copper and iron supplementation to diets with differing levels of zinc. A 3 X 2 X 2 experimental design was used for these experiments. In experiment 2 the levels of zinc added to the basal diet were 500 ppm and 1000 ppm. The copper supplement was 200 ppm, and the iron supplement was 400 ppm. In experiment 3 the levels of zinc added to the basal diet were 10 ppm and 500 ppm; copper was supplemented at the 50 ppm level, and iron was supplemented at the 100 ppm level.

Weanling male albino rats[1] were used for all phases of the study. The animals were randomized into replications by initial body weight. Animals and test treatments within a replication were assigned at random to individual wire-bottom stainless steel cages. Food and distilled water were given ad libitum. Each experiment lasted 4 weeks. Weekly weight records were kept for all animals.

Two basal diets were used. A soy protein diet[2] (Appendix A, Table 1) was used for experiments 1 and 2, and an egg albumin diet[3] (Appendix A, Table 2) was used for experiment 3. The soy protein diet was calculated to contain 15.24 ppm of copper, 80 ppm of iron, and 7 ppm of zinc. Information supplied by the manufacturer indicated that the egg albumin diet contained 2.53 ppm of copper, 97.02 ppm of iron, and 4 ppm of zinc. Copper supplements were given as cupric sulfate, iron as ferric sulfate, and zinc as zinc carbonate. All supplements were mixed with the diet.

At the end of each experiment, blood samples were taken from the tail of each animal and hemoglobin determinations were made by the method of Shenk et al. (25). Rats from

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[1]Sprague-Dawley rats purchased from Holtzman Company, Madison, Wisconsin.

[2]Zinc deficient soy protein diet, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[3]Zinc deficient egg albumin diet, General Biochemicals, Chagrin Falls, Ohio.

4 randomly selected replications were sacrificed, and the liver of each was removed and weighed. The whole livers in experiments 1 and 2 and a small portion of each liver in experiment 3 were dried at 35° in order to provide dry weight data. The livers were prepared for mineral analyses by ashing with nitric and perchloric acids on a hot plate. The ash of each sample was dissolved in 3 ml. of 0.6N HCl and brought to a volume of 25 ml. with redistilled water. Zinc determinations were made by the method of McCall, et al. (26). Copper and iron determinations were done by the methods of Parks, et al. (27) and Kitzes, et al. (28), respectively, as modified by Matrone et al. (29).

# CHAPTER IV

# RESULTS AND DISCUSSION

## Effects of Various Levels of Dietary Zinc

Detailed data showing the effects of zinc deficiency, 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm of supplemental zinc on the weight gains, hemoglobin levels, and liver copper, iron, and zinc levels are given in Appendix B, Tables 1 and 2. Mean values of these criteria are presented in Table 1.

All the animals on all soy protein diets had very poor growth. The ones showing the greatest weight gain, a mean of 45 gm. for 4 weeks, were on the zinc deficient basal diet. The addition of zinc produced a significant decrease ($p \leq 0.01$) in weight gain. It was expected that the animals on the zinc supplemented diets would gain weight at a normal rate since none of the levels of zinc used were toxic. The reason the animals gained little weight is not clear. A rancid odor, however, was detected in the soybean diet which would suggest that this feed had deteriorated on storage and had become unpalatable to the animals.

There was no significant difference in the hemoglobin levels. After an initial drop in liver zinc levels, there was a significant increase ($p \leq 0.01$) in the liver zinc at higher levels of zinc supplementation. The addition of zinc had no significant effect on the copper levels. The liver iron was significantly decreased ($p \leq 0.01$) with the addition of zinc to the diet. However, the data indicated that the iron accumulation reached a low point with 1000 ppm of zinc and showed an increase when 2000 ppm of zinc was added.

TABLE 1

EFFECT OF ZINC ON GROWTH, HEMOGLOBIN, AND LIVER COPPER, IRON, AND ZINC LEVELS

| Level of dietary zinc | Weight gain[a] at 4 weeks | Hemoglobin[a] | Liver constituents[b] | | |
|---|---|---|---|---|---|
| | | | Cu | Fe | Zn |
| ppm | gms. | gm./100 ml. | mcg./gm. dry weight | | |
| None | 45[b] | 14.48[c] | 25.27 | 727.25 | 36.24 |
| 100 | 24[d] | 16.95[d] | 22.91 | 675.69 | 18.57 |
| 500 | 9 | 15.30 | 22.51 | 591.68 | 85.08 |
| 1,000 | 36[b] | 14.78[b] | 23.34 | 464.63 | 102.67 |
| 2,000 | 27 | 15.15[c] | 26.77 | 549.14 | 113.02 |

[a]Each figure is the mean of 6 animals unless otherwise indicated.

[b]Mean of 4 animals.

[c]Mean of 3 animals.

[d]Mean of 5 animals

Copper and Iron Supplementation to Soy Protein Diets Containing Various Levels of Zinc

Detailed data showing the effects of copper and iron supplements on weight gains, hemoglobin levels, and liver copper, iron, and zinc levels in animals fed either zinc deficient diets or diets containing 500 or 1000 ppm of zinc are given in Appendix B, Tables 3 and 4. Mean values of these criteria are given in Table 2.

Again, the animals on the zinc deficient diet gained the most weight. All supplements to the basal diet significantly lowered ($p \leq 0.01$) the weight. All the animals receiving diets supplemented with iron alone died. However, when iron and 500 ppm of zinc were given, no deaths occurred, but the animals failed to maintain their initial weights. The animals receiving 1000 ppm of zinc along with the iron supplements gained an average of 21 gms. All the animals did poorly in the presence of both copper and iron.

The analysis of the data indicated that the addition of zinc had little effect on hemoglobin levels. Copper and iron tended to increase the hemoglobin levels, although copper alone had no effect in the presence of zinc.

The copper accumulation in the livers was not affected significantly by the addition of zinc and only slightly raised when iron was added to the diet. Copper supplements raised the liver copper levels greatly, as was expected. These elevated levels were reduced progressively by the addition of increasing amounts of zinc and also by the addition of iron.

The liver iron levels showed no pattern. All the animals receiving iron supplementation to a zinc deficient

TABLE 2

RESPONSE OF RATS TO ZINC DEFICIENT DIETS[a] SUPPLEMENTED WITH ZINC, COPPER AND IRON (EXPERIMENT 2)

| Supplement to basal diet[b] | Weight gain at 4 weeks[c] gm | Hemoglobin[c] | Liver constituents[d] Cu | Fe | Zn |
|---|---|---|---|---|---|
| | gms. | gm./100 ml. | mcg./gm. dry weight | | |
| None | 41[e] | 14.40[e] | 20.97 | 505.64 | 14.20 |
| Cu | 10 | 16.05 | 655.84 | 511.43 | 24.70 |
| Fe | -- | -- | -- | -- | -- |
| Cu + Fe | -11[f] | 16.54[f] | 24.98[f] | 596.09[f] | 46.70[f] |
| 500 ppm Zn | 5[e] | 15.21[e] | 19.06 | 571.15 | 21.59 |
| 500 ppm Zn + Cu | 12 | 14.52 | 546.49 | 481.88 | 34.89 |
| 500 ppm Zn + Fe | -9[e] | 17.43[d] | 22.89 | 630.87 | 36.34 |
| 500 ppm Zn + Cu + Fe | -5[f] | 18.00[f] | 446.48[f] | 495.63[f] | 41.92[f] |
| 1000 ppm Zn | 29[e] | 13.77[e] | 20.07 | 418.70 | 12.66 |
| 1000 ppm Zn + Cu | 24 | 13.97 | 400.79 | 433.41 | 21.11 |
| 1000 ppm Zn + Fe | 21 | 14.91 | 24.34 | 496.41 | 30.33 |
| 1000 ppm Zn + Cu + Fe | -16[g] | 15.84[g] | 283.61[g] | 632.80[g] | 50.72[g] |

[a]Zinc deficient soy protein diet.

[b]Supplements were 200 ppm of copper and 400 ppm of iron.

[c]Each figure is the mean of 6 animals unless otherwise indicated.

[d]Mean of 4 animals.

[e]Mean of 5 animals.

[f]Mean of 2 animals.

[g]Mean of 3 animals.

diet died. Copper may have helped to overcome some of the adverse effects of the iron on the animals because 2 of the 6 animals receiving copper and iron supplements on the zinc deficient diet lived. The supplements of 500 ppm of zinc slightly raised the liver iron levels, and the addition of iron to this diet produced higher iron levels. Increasing the zinc supplement from 500 to 1000 ppm then lowered the liver iron in the iron supplemented animals. Copper tended to lower the iron levels except at the 1000 ppm of zinc diet to which iron had also been added.

The accumulation of zinc was significantly increased ($p \leq 0.01$) with supplements of copper and iron, with the greatest increase occurring when both copper and iron were added. The addition of 500 ppm of zinc raised the liver zinc levels above those of the basal diet, but 1000 ppm of zinc caused no change.

Because such poor results were obtained on the soy protein diets and the levels of supplements of iron increased the mortality of the animals, a third experiment was planned. The third experiment was designed like experiment 2, but a zinc deficient egg albumin diet was used. Ten ppm or 500 ppm of zinc was added to the diet, and copper and iron were supplemented at the 50 and 100 ppm levels, respectively.

## Copper and Iron Supplementation to Egg Albumin Diets Containing Various Levels of Zinc

Detailed data showing the effects of copper and iron supplementation to the zinc deficient, 10 ppm, and 500 ppm of zinc diets are given in Appendix B, Tables 5 and 6. Mean values of these criteria are given in Table 3.

The weight gain of the animals was significantly increased ($p \leq 0.01$) when zinc was added to the diet, although no additional improvement was observed above the 10 ppm level. Copper seemed to antagonize the effect of added zinc, producing slightly reduced weight gains.

Supplementing the diets with 10 ppm of zinc significantly increased ($p \leq 0.05$) liver zinc levels, while a supplement of 500 ppm of zinc was associated with a highly significant increase ($p \leq 0.01$) in liver zinc levels. Zinc supplements significantly decreased ($p \leq 0.01$) hemoglobin, liver copper, and liver iron levels.

The addition of copper to the diet was associated with a rise in copper accumulation in the liver in all cases and a lowering ($p \leq 0.05$) of the zinc accumulation in the liver. Copper supplementation appeared to significantly reduce ($p \leq 0.01$) liver iron levels in the animals receiving 500 ppm of zinc, but was associated with slight increases in iron levels in the animals receiving 10 ppm of zinc.

The addition of iron to the diet lowered the liver zinc levels on the zinc deficient diet, but significantly

TABLE 3

RESPONSE OF RATS TO ZINC DEFICIENT DIETS[a] SUPPLEMENTED WITH ZINC, COPPER, AND IRON (EXPERIMENT 3)

| Supplement to basal diet[b] | Weight gain at 4 weeks[c] | Hemoglobin[c] | Liver constituents[d] | | |
|---|---|---|---|---|---|
| | | | Cu | Fe | Zn |
| | gms. | mg./100 ml. | mcg./gm. dry weight | | |
| None | 64 | 15.79 | 21.29 | 556.49 | 47.58 |
| Cu | 69 | 15.59[e] | 259.00 | 560.30 | 26.73 |
| Fe | 71 | 15.79[f] | 11.56 | 699.57 | 29.14 |
| Cu + Fe | 71 | 16.01[e] | 598.49 | 1192.19 | 41.74 |
| 10 ppm Zn | 162 | 13.12 | 11.97 | 312.39 | 49.80 |
| 10 ppm Zn + Cu | 130 | 12.47[e] | 182.20 | 388.23 | 41.30 |
| 10 ppm Zn + Fe | 167 | 12.78[f] | 12.67 | 538.82 | 58.31 |
| 10 ppm Zn + Cu + Fe | 150 | 13.65 | 189.26 | 407.23 | 47.08 |
| 500 ppm Zn | 161 | 12.64 | 16.42 | 311.34 | 76.80 |
| 500 ppm Zn + Cu | 134[e] | 12.66[e] | 89.83 | 191.46 | 45.16 |
| 500 ppm Zn + Fe | 161 | 13.39 | 10.52 | 317.45 | 103.56 |
| 500 ppm Zn + Cu + Fe | 156[e] | 12.72 | 49.43 | 209.42 | 54.44 |

[a]Zinc deficient egg albumin diet.

[b]Supplements were 50 ppm of copper and 100 ppm of iron.

[c]Each figure is the mean of 7 animals unless otherwise indicated.

[d]Mean of 4 animals.

[e]Mean of 6 animals.

[f]Mean of 5 animals.

increased ($p \leq 0.01$) the level on the 500 ppm diet. Iron significantly decreased ($p \leq 0.05$) the copper level on the zinc deficient diet but had no effect on the 10 ppm of zinc diet. Iron significantly increased ($p \leq 0.01$) the iron levels in all cases.

Regardless of the level of zinc in the diet, the presence of both copper and iron supplements resulted in decreases in liver zinc deposition. Interestingly, on the zinc deficient diet, the addition of copper and iron together increased both the copper and iron levels of the liver to a greater extent than when each one was added separately. This did not occur on the diets containing zinc. On the zinc supplemented diets, copper caused a decrease in iron accumulation, and iron caused a decrease in the copper levels.

# CHAPTER V

## GENERAL DISCUSSION

Results of the present investigation give additional information about the copper-iron-zinc interrelationship at low to normal levels of dietary intake of zinc. A 10 ppm supplement of zinc alleviated the growth depression and high hemoglobin levels associated with zinc deficiency in the rat. Above this level of supplementation, there was no further improvement in growth, which is consistent with the results of Williams and Mills (30). Zinc supplements were also associated with decreased levels of copper and iron in the liver, and a slight increase in the liver zinc levels. Animals receiving supplemental copper showed decreased liver zinc levels. Van Campen, et al. (22, 31), suggest that the effects of copper on lowering liver zinc levels, and the effect of zinc on lowering liver copper levels is due to mutual antagonism during the process of absorption. The results of the present study tend to support this theory since increasing the dietary levels of zinc does not raise the liver zinc levels very much but has great effect on the liver copper and iron levels. In addition to this apparent antagonism, zinc, copper, and iron may be interrelated at sites other than absorption.

Evaluation of the data in this study suggests that both copper and iron adversely affect liver zinc levels when animals were receiving zinc deficient diets. There is the possibility that copper and iron may have caused an increased mobilization of zinc from the liver of the animals on the zinc deficient diet since the liver zinc levels of those animals receiving copper and iron supplements were lower than the animals receiving the zinc deficient diet. This would indicate that the interrelationship between these metals may be more than just an antagonism during absorption.

Animals receiving supplements of copper and iron on the zinc deficient diet had greatly increased liver copper and iron levels as compared to the animals receiving either copper or iron supplements. It would appear that copper enhanced the accumulation of iron in the liver and that iron enhanced the accumulation of liver copper. However, animals receiving supplements of both copper and iron on the marginal zinc diets had liver copper accumulation about the same as the animals receiving just copper supplements, but the animals had lower liver iron levels than those receiving only iron supplements. Furthermore, on the 500 ppm of zinc diets, the animals receiving both copper and iron supplements had less liver copper and iron accumulation than the animals receiving either copper or iron supplements. This suggests a complex interrelationship among these metals which may depend on their relative proportions in the diets of the animals. This explanation may be why Magee and Matrone (6), and Cox and Harris (5) failed to observe the zinc depressing effect of copper in their zinc toxicity studies. With a high zinc to copper ratio, this effect would be small. In the present study, the ratios of zinc and copper were small, and their reciprocal effects were observed. The ratio of iron to the copper and zinc levels also needs to be considered in studying the extent of this interrelationship. In this study, zinc lowered liver iron levels, but iron supplements appeared to raise the liver zinc levels in the presence of zinc. Additional information is necessary to see if this trend holds true.

Possibly, if the experiment had been extended for a longer period of time, a zinc deficiency would have been produced in the animals receiving copper supplements to the marginal zinc diets. The liver zinc levels of the animals receiving 10 ppm of zinc plus copper supplements were below the levels of the animals on the zinc deficient diet. Their weekly weight gains and final weight gains were less than the animals on the 10 ppm of zinc supplemented diets without additional copper. It would appear that copper was lessening the beneficial effects of the added zinc.

# CHAPTER VI

# SUMMARY AND RECOMMENDATIONS

## Summary

Young rats were fed diets containing various levels of zinc in the presence or absence of copper and iron. Under these conditions, the effects of zinc, copper, and iron supplements on the growth, hemoglobin, and liver copper, iron, and zinc levels were observed.

Results of the study showed that the depressed growth and high hemoglobin levels of animals receiving zinc deficient diets could be prevented with supplements of zinc. Supplements of zinc were associated with decreased liver copper and iron levels, and supplements of copper were associated with decreased liver zinc levels. There was some indication that supplemental copper could cause a zinc deficiency state in animals receiving marginal zinc diets over a period of time longer than 4 weeks.

These data tend to support the theory that the reciprocal interrelationship between copper and zinc may take place at the site of absorption. However, copper and iron lowered liver zinc levels in the absence of dietary zinc which would indicate an interrelation in addition to that of absorption. The relative ratios of copper, zinc,

and iron seem to be important in studying their interrelationship.

## Recommendations for Additional Investigation

Since the results of this study indicate that supplemental copper may cause a zinc deficiency in animals on marginal levels of zinc, a study for an extended period of time will be necessary to see if this would actually occur. Studies concerned with long range effects of copper supplements to a marginal zinc diet are necessary to the understanding of the effects of added stresses to animals receiving diets with marginal levels of essential nutrients.

Because the ratios of zinc, iron, and copper are important in the effects they have on each other, further studies need to be done involving variations in the ratios of one metal to the others in order to help to determine the extent of the interrelationship of these metals. The effects of iron, in particular, need further study.

Also, studies should be devised that measure parameters other than those measured in this study, such as blood levels of zinc, copper, and iron, and enzyme levels during various dietary levels of copper, iron, and zinc in order to help determine where these metals are interfering with the metabolism of each other. Radio isotope studies would be beneficial in future studies.

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# APPENDIX A

## COMPOSITIONS OF BASAL DIETS

TABLE 1

COMPOSITION OF THE SOY PROTEIN BASAL DIET

| Constituents | gm./kg. |
|---|---|
| Soy Protein | 150 |
| Glucose | 628 |
| Oil, Corn | 110 |
| Alphacel | 30 |
| Salt Mix | 37 |
| Calcium phosphate, monobasic | 26.196 |
| Cupric sulfate, pentahydrate | 0.060 |
| Cobalt chloride, hexahydrate | 0.040 |
| Ferrous sulfate, heptahydrate | 0.400 |
| Magnesium carbonate | 1.384 |
| Manganese sulfate, monohydrate | 0.121 |
| Potassium carbonate | 5.043 |
| Potassium iodide | 0.004 |
| Sodium chloride | 3.737 |
| Sodium fluoride | 0.008 |
| Vitamin Diet Fortification Mixture | 55 |
| | mg./kg. |
| Vitamin A concentrate | 99.2 |
| Vitamin D concentrate | 5.5 |
| Alpha tocopherol | 110.2 |
| Ascorbic acid | 992.1 |
| Inositol | 110.2 |
| Choline cloride | 1653.5 |
| Menadione | 49.6 |
| p-Aminobenzoic acid | 110.2 |
| Niacin | 99.2 |
| Riboflavin | 22.0 |
| Perodoxine hydrocloride | 22.0 |
| Thiamin hydrocloride | 22.0 |
| Calcium pantothenate | 66.1 |
| Biotin | 0.44 |
| Folic acid | 1.98 |
| Vitamin $B_{12}$ | 0.03 |

TABLE 2

COMPOSITION OF THE EGG ALBUMIN BASAL DIET

| Constituents | g/kg |
|---|---|
| Egg white solids, spray dried | 200.00 |
| Dextrose, hydrate, technical | 631.053 |
| Fiber, non-nutritive | 30.00 |
| Oil, corn | 100.00 |
| Salt mix | |
| Calcium carbonate | 9.94405 |
| Calcium phosphate, dibasic, dihydrate | 3.1489 |
| Cobalt chloride, hexahydrate | 0.00185 |
| Cupric sulfate, pentahydrate | 0.009945 |
| Ferric citrate, pentahydrate | 0.911542 |
| Magnesium sulfate, heptahydrate | 3.38106 |
| Manganese sulfate, monohydrate | 0.008791 |
| Potassium iodide | 0.026518 |
| Potassium phosphate, dibasic, trihydrate | 14.0044 |
| Sodium chloride | 5.55198 |
| Vitamin mix | |
| Biotin | 0.004 |
| $B_{12}$ | 0.020 |
| Calcium pantothenate | 0.016 |
| Choline chloride | 1.5 |
| Chlortetracycline | 0.25 |
| Folic acid | 0.0005 |
| Menadione | 0.00033 |
| Niacin | 0.025 |
| PyridoxineHCl | 0.004 |
| Riboflavin | 0.006 |
| Thiamin HCl | 0.01 |
| | IU/kg |
| Vitamin A palmitate | 10,000.000 |
| Vitamin $D_2$ | 1,250.000 |
| Vitamin E acetate | 110.000 |

# APPENDIX B

# GROWTH, HEMOGLOBIN, AND LIVER MINERAL DATA

TABLE 1

GROWTH AND HEMOGLOBIN DATA FOR EXPERIMENT 1

Treatments

1 - Basal diet
2 - Basal + 100 ppm Zn
3 - Basal + 500 ppm Zn
4 - Basal + 1000 ppm Zn
5 - Basal + 2000 ppm Zn

| Treatments | Replications 1 | 2 | 3 | 4 | 5 | 6 | Mean |
|---|---|---|---|---|---|---|---|
| | | | Growth 4 weeks weight gain (gms.) | | | | |
| 1 | 40 | -- | 59 | 41 | -- | 38 | 44 |
| 2 | 28 | -- | 34 | 28 | 19 | 20 | 26 |
| 3 | 14 | 11 | 3 | 0 | 19 | 9 | 9 |
| 4 | -- | 17 | 49 | 35 | -- | 49 | 42 |
| 5 | 2 | 35 | 34 | 32 | 33 | 29 | 28 |
| | | | Hemoglobin mg./100 ml. blood | | | | |
| 1 | -- | -- | 14.23 | -- | 13.40 | 15.80 | 14.48 |
| 2 | 19.37 | -- | 15.57 | 15.37 | 18.66 | 15.80 | 16.96 |
| 3 | 16.14 | 15.26 | 14.94 | 17.20 | 13.03 | 15.26 | 15.30 |
| 4 | -- | 16.83 | 15.06 | 13.20 | -- | 14.03 | 14.83 |
| 5 | -- | 14.34 | 14.04 | 16.03 | 15.69 | -- | 15.15 |

TABLE 2

LIVER COPPER, IRON, AND ZINC DATA FOR EXPERIMENT 1

Treatments

1 - Basal diet
2 - Basal + 100 ppm Zn
3 - Basal + 500 ppm Zn
4 - Basal + 1000 ppm Zn
5 - Basal + 2000 ppm Zn

| Treatments | Replications 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| COPPER | | | | | |
| 1 | 24.39 | 32.48 | 21.75 | 22.44 | 25.27 |
| 2 | 25.31 | 20.15 | 20.64 | 25.52 | 22.91 |
| 3 | 24.50 | 24.89 | 19.75 | 20.90 | 22.51 |
| 4 | 26.98 | 24.37 | 18.56 | 23.46 | 23.34 |
| 5 | 32.11 | 29.32 | 21.62 | 24.03 | 26.77 |
| IRON | | | | | |
| 1 | 579.78 | 812.61 | 685.77 | 831.17 | 727.25 |
| 2 | 688.66 | 659.29 | 636.94 | 718.38 | 675.69 |
| 3 | 592.02 | 536.16 | 609.59 | 628.95 | 591.68 |
| 4 | 534.02 | 466.95 | 415.27 | 442.29 | 464.63 |
| 5 | 648.46 | 511.06 | 548.31 | 488.73 | 549.14 |
| ZINC | | | | | |
| 1 | 37.43 | 49.06 | 29.72 | 28.74 | 36.24 |
| 2 | 21.12 | 15.09 | 19.49 | 18.58 | 18.57 |
| 3 | 96.54 | 46.73 | 106.20 | 90.85 | 85.08 |
| 4 | 150.71 | 77.20 | 98.64 | 84.12 | 102.67 |
| 5 | 192.44 | 100.38 | 100.38 | 58.88 | 113.02 |

TABLE 3

GROWTH AND HEMOGLOBIN DATA FOR EXPERIMENT 2

Treatments

1 - Basal diet
2 - Basal + Cu
3 - Basal + Fe
4 - Basal + Cu + Fe
5 - Basal + 500 ppm Zn
6 - Basal + 500 ppm Zn + Cu
7 - Basal + 500 ppm Zn + Fe
8 - Basal + 500 ppm Zn + Cu + Fe
9 - Basal + 1000 ppm Zn
10 - Basal + 1000 ppm Zn + Cu
11 - Basal + 1000 ppm Zn + Fe
12 - Basal + 1000 ppm Zn + Cu + Fe

| Treatment | Replications 1 | 2 | 3 | 4 | 5 | 6 | Mean |
|---|---|---|---|---|---|---|---|
| Growth 4 weeks weight gain (gms.) | | | | | | | |
| 1 | 31 | 50 | 45 | 52 | 29 | -- | 41 |
| 2 | 8 | 14 | 7 | 15 | 11 | 3 | 10 |
| 3 | -- | -- | -- | -- | -- | -- | -- |
| 4 | -- | -11 | -- | -11 | -- | -- | -11 |
| 5 | 4 | -2 | 0 | 9 | -- | 12 | 5 |
| 6 | 15 | 8 | 18 | 5 | 17 | 7 | 12 |
| 7 | -6 | -4 | -15 | -10 | -- | -- | -9 |
| 8 | -- | -2 | -2 | -- | -8 | -- | -5 |
| 9 | 23 | 25 | 40 | 32 | 24 | -- | 29 |
| 10 | 21 | 27 | 22 | 31 | 22 | 23 | 24 |
| 11 | 31 | 11 | 30 | 23 | 25 | 4 | 21 |
| 12 | -- | -23 | -9 | -- | -17 | -- | -16 |

TABLE 3--Continued

| Treatment | Replications 1 | 2 | 3 | 4 | 5 | 6 | Mean |
|---|---|---|---|---|---|---|---|
| | Hemoglobin mg./100 ml. blood | | | | | | |
| 1 | 13.86 | 14.23 | 15.14 | 13.40 | 15.37 | -- | 14.40 |
| 2 | 17.09 | 12.00 | 17.20 | 18.23 | 15.80 | 16.03 | 16.05 |
| 3 | -- | -- | -- | -- | -- | -- | -- |
| 4 | -- | 17.72 | -- | 15.37 | -- | -- | 16.54 |
| 5 | 17.32 | 15.06 | 15.49 | 11.46 | -- | 16.72 | 15.21 |
| 6 | 13.03 | 15.37 | 13.86 | 17.20 | 14.34 | 13.29 | 14.52 |
| 7 | 14.86 | 17.20 | 19.86 | 17.83 | -- | -- | 17.43 |
| 8 | -- | 18.80 | -- | -- | 17.20 | -- | 18.00 |
| 9 | 12.69 | 13.77 | 14.94 | 13.12 | 14.34 | -- | 13.77 |
| 10 | 13.40 | 13.57 | 14.34 | 13.12 | 15.49 | 13.94 | 13.97 |
| 11 | 13.40 | 14.43 | 17.09 | 14.63 | 15.57 | 14.34 | 14.91 |
| 12 | -- | 12.86 | 17.09 | -- | 17.57 | -- | 15.84 |

TABLE 4

LIVER COPPER, IRON, AND ZINC DATA FOR EXPERIMENT 2

Treatments

| | |
|---|---|
| 1 - Basal diet | 7 - Basal + 500 ppm Zn + Fe |
| 2 - Basal + Cu | 8 - Basal + 500 ppm Zn + Cu + Fe |
| 3 - Basal + Fe | 9 - Basal + 1000 ppm Zn |
| 4 - Basal + Cu + Fe | 10 - Basal + 1000 ppm Zn + Cu |
| 5 - Basal + 500 ppm Zn | 11 - Basal + 1000 ppm Zn + Fe |
| 6 - Basal + 500 ppm Zn + Cu | 12 - Basal + 1000 ppm Zn + Cu + Fe |

| Treatments | Replications 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| | | COPPER[a] | | | |
| 1 | 23.17 | 20.80 | 19.89 | 20.04 | 20.97 |
| 2 | 718.15 | 451.63 | 928.19 | 525.40 | 655.84 |
| 3 | -- | -- | -- | -- | -- |
| 4 | -- | 681.33 | -- | 321.64 | 501.48 |
| 5 | 22.41 | 18.79 | 17.44 | 17.62 | 19.06 |
| 6 | 298.76 | 813.08 | 737.19 | 336.92 | 546.49 |
| 7 | 23.31 | 23.21 | 20.30 | 24.73 | 22.89 |
| 8 | -- | 381.18 | -- | 509.78 | 446.48 |
| 9 | 16.06 | 18.48 | 20.63 | 25.10 | 20.07 |
| 10 | 373.44 | 586.38 | 327.80 | 315.57 | 400.79 |
| 11 | 24.72 | 28.43 | 21.75 | 22.47 | 24.34 |
| 12 | 357.42 | -- | 257.97 | 235.43 | 283.61 |
| | | IRON[a] | | | |
| 1 | 459.38 | 574.91 | 487.95 | 500.09 | 505.64 |
| 2 | 508.06 | 499.18 | 505.39 | 533.09 | 511.43 |
| 3 | -- | -- | -- | -- | -- |
| 4 | -- | 511.76 | -- | 680.43 | 596.09 |
| 5 | 545.71 | 616.95 | 525.69 | 596.24 | 571.15 |
| 6 | 516.70 | 461.80 | 401.68 | 547.36 | 481.88 |
| 7 | 649.54 | 600.17 | 573.36 | 700.41 | 630.87 |
| 8 | -- | 567.20 | -- | 424.07 | 495.63 |
| 9 | 385.77 | 408.51 | 417.80 | 462.74 | 418.70 |
| 10 | 455.17 | 463.71 | 463.15 | 351.60 | 433.41 |
| 11 | 450.60 | 596.08 | 400.01 | 538.97 | 496.41 |
| 12 | 711.10 | -- | 507.65 | 679.65 | 632.80 |

TABLE 4--Continued

| Treatments | Replications 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| | | | ZINC[a] | | |
| 1 | 23.32 | 9.33 | 18.25 | 5.91 | 14.20 |
| 2 | 33.68 | 11.61 | 45.86 | 7.66 | 24.70 |
| 3 | -- | -- | -- | -- | -- |
| 4 | -- | 56.85 | -- | 36.58 | 46.70 |
| 5 | 14.21 | 26.42 | 17.65 | 28.10 | 21.59 |
| 6 | 38.35 | 39.35 | 43.65 | 18.22 | 34.89 |
| 7 | 44.07 | 35.35 | 40.52 | 25.43 | 36.34 |
| 8 | -- | 35.42 | -- | 48.43 | 41.92 |
| 9 | 11.06 | 18.76 | 12.92 | 7.89 | 12.66 |
| 10 | 25.26 | 25.68 | 11.14 | 22.35 | 21.11 |
| 11 | 21.02 | 35.42 | 37.43 | 27.44 | 30.33 |
| 12 | 31.96 | -- | 53.73 | 66.47 | 50.72 |

[a]Expressed as mcg./gm. dry weight.

TABLE 5

GROWTH AND HEMOGLOBIN DATA FOR EXPERIMENT 3

Treatments

1 - Basal diet
2 - Basal + Cu
3 - Basal + Fe
4 - Basal + Cu + Fe
5 - Basal + 10 ppm Zn
6 - Basal + 10 ppm Zn + Cu
7 - Basal + 10 ppm Zn + Fe
8 - Basal + 10 ppm Zn + Cu + Fe
9 - Basal + 500 ppm Zn
10 - Basal + 500 ppm Zn + Cu
11 - Basal + 500 ppm Zn + Fe
12 - Basal + 500 ppm Zn + Cu + Fe

| Treatment | Replications 1 | 2 | 3 | 4 | 5 | 6 | 7 | Mean |
|---|---|---|---|---|---|---|---|---|
| | Growth<br>4 weeks weight gain (gms.) | | | | | | | |
| 1 | 56 | 70 | 48 | 50 | 75 | 77 | 71 | 64 |
| 2 | -- | 59 | 38 | 68 | 80 | 64 | 105 | 69 |
| 3 | -- | 66 | -- | 70 | 77 | 68 | 74 | 71 |
| 4 | 95 | 86 | 67 | 67 | 68 | -- | 44 | 71 |
| 5 | 184 | 164 | 155 | 164 | 162 | 132 | 171 | 162 |
| 6 | 184 | 134 | 178 | 161 | 73 | 134 | 45 | 130 |
| 7 | 178 | -- | 104 | -- | 180 | 184 | 191 | 167 |
| 8 | 69 | 206 | 175 | 159 | 131 | 146 | 161 | 150 |
| 9 | 168 | 191 | 160 | 146 | 172 | 144 | 144 | 161 |
| 10 | 147 | 109 | 118 | -- | 144 | 128 | 160 | 134 |
| 11 | 161 | 165 | 186 | 191 | 114 | 157 | 152 | 161 |
| 12 | 183 | 182 | 122 | 168 | 129 | 149 | -- | 156 |

TABLE 5--Continued

| Treatment | Replications 1 | 2 | 3 | 4 | 5 | 6 | 7 | Mean |
|---|---|---|---|---|---|---|---|---|
| | Hemoglobin mg./100 ml. blood | | | | | | | |
| 1 | 14.86 | 16.03 | 16.72 | 17.97 | 16. 6 | 13.77 | 14.94 | 15.79 |
| 2 | -- | 16.49 | 17.09 | 15.37 | 15.80 | 17.09 | 13.86 | 15.95 |
| 3 | -- | 14.74 | -- | 17.09 | 16.37 | 14.03 | 16.72 | 15.79 |
| 4 | 15.26 | 17.57 | 16.26 | 13.86 | 16.60 | -- | 16.49 | 16.01 |
| 5 | 12.52 | 13.28 | 13.20 | 12.86 | 13.12 | 12.94 | 13.94 | 13.12 |
| 6 | 13.57 | 11.46 | 12.52 | 10.77 | 13.12 | 13.40 | -- | 12.47 |
| 7 | 13.49 | -- | 12.94 | -- | 11.46 | 12.60 | 13.40 | 12.78 |
| 8 | 13.49 | 14.43 | 14.43 | 12.34 | 14.03 | 14.34 | 13.49 | 13.65 |
| 9 | 11.16 | 12.86 | 12.94 | 12.94 | 13.20 | 12.43 | 12.94 | 12.64 |
| 10 | 12.26 | 13.12 | 12.00 | -- | 13.66 | 12.94 | 12.00 | 12.66 |
| 11 | 13.40 | 11.92 | 15.69 | 12.09 | 14.14 | 12.34 | 14.14 | 13.39 |
| 12 | 13.03 | 13.86 | 13.12 | 13.12 | 12.43 | 10.77 | -- | 12.72 |

TABLE 6

LIVER COPPER, IRON, AND ZINC DATA FOR EXPERIMENT 3

Treatments

| | |
|---|---|
| 1 - Basal diet | 7 - Basal + 10 ppm Zn + Fe |
| 2 - Basal + Cu | 8 - Basal + 10 ppm Zn + Cu + Fe |
| 3 - Basal + Fe | 9 - Basal + 500 ppm Zn |
| 4 - Basal + Cu + Fe | 10 - Basal + 500 ppm Zn + Cu |
| 5 - Basal + 10 ppm Zn | 11 - Basal + 500 ppm Zn + Fe |
| 6 - Basal + 10 ppm Zn + Cu | 12 - Basal + 500 ppm Zn + Cu + Fe |

| Treatments | Replications 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| | | | COPPER[a] | | |
| 1 | 12.40 | 25.73 | 32.51 | 14.52 | 21.29 |
| 2 | 177.19 | 284.24 | 315.57 | 258.99 | 259.00 |
| 3 | 10.30 | 9.27 | 13.56 | 13.10 | 11.56 |
| 4 | 538.27 | 437.38 | 947.56 | 470.65 | 5 8.49 |
| 5 | 12.16 | 9.88 | 10.93 | 14.93 | 11.97 |
| 6 | 137.29 | 234.66 | 131.90 | 224.94 | 182.20 |
| 7 | 10.06 | 13.52 | 18.10 | 9.00 | 12.67 |
| 8 | 227.51 | 125.79 | 151.75 | 251.98 | 189.26 |
| 9 | 12.83 | 14.31 | 9.87 | 29.11 | 16.42 |
| 10 | 67.32 | 41.04 | 123.44 | 127.53 | 89.83 |
| 11 | 14.00 | 6.47 | 11.32 | 10.30 | 10.52 |
| 12 | 71.89 | 59.49 | 31.87 | 34.37 | 49.43 |
| | | | IRON[a] | | |
| 1 | 590.72 | 472.67 | 679.80 | 482.79 | 556.49 |
| 2 | 389.71 | 591.47 | 737.23 | 522.79 | 560.30 |
| 3 | 814.86 | 527.19 | 713.48 | 742.76 | 699.57 |
| 4 | 1537.96 | 725.49 | 1679.98 | 825.34 | 1192.19 |
| 5 | 259.09 | 326.79 | 373.34 | 290.35 | 312.39 |
| 6 | 434.39 | 448.31 | 262.93 | 407.40 | 388.23 |
| 7 | 701.71 | 276.49 | 554.72 | 622.38 | 538.82 |
| 8 | 506.06 | 322.38 | 307.36 | 493.13 | 407.23 |
| 9 | 261.20 | 344.22 | 195.49 | 444.46 | 311.34 |
| 10 | 168.35 | 136.80 | 188.25 | 272.45 | 191.46 |
| 11 | 333.65 | 580.77 | 206.91 | 148.47 | 317.45 |
| 12 | 267.90 | 185.39 | 199.75 | 185.38 | 209.60 |

TABLE 6--Continued

| Treatments | Replications 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| | | | ZINC[a] | | |
| 1 | 21.51 | 28.08 | 79.68 | 75.03 | 47.58 |
| 2 | 15.60 | 24.68 | 28.25 | 38.37 | 26.73 |
| 3 | 6.93 | 40.88 | 35.63 | 33.12 | 29.14 |
| 4 | 20.12 | 19.09 | 46.50 | 81.24 | 41.74 |
| 5 | 53.00 | 58.55 | 50.51 | 37.12 | 49.80 |
| 6 | 20.43 | 42.68 | 55.10 | 46.98 | 41.30 |
| 7 | 20.90 | 56.98 | 94.77 | 60.66 | 58.31 |
| 8 | 14.48 | 47.84 | 50.19 | 75.80 | 47.08 |
| 9 | 48.77 | 120.98 | 91.13 | 46.33 | 76.80 |
| 10 | 15.52 | 43.90 | 85.37 | 35.84 | 45.16 |
| 11 | 108.46 | 81.73 | 151.32 | 72.75 | 103.56 |
| 12 | 36.84 | 68.50 | 76.10 | 76.32 | 54.44 |

[a]Expressed as mcg./gm. dry weight.

APPENDIX C

ANALYSES OF VARIANCE

TABLE 1

ANALYSES OF VARIANCE OF WEIGHT GAIN, HEMOGLOBIN, AND LIVER COPPER, IRON, AND ZINC DATA FOR EXPERIMENT 1

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| | Weight gain | | |
| Total | 24 | 6271 | |
| Replications | 5 | 398 | 79 |
| Treatments | 4 | 4184 | 1046** |
| Error | 15 | 1689 | 112 |
| | Hemoglobin | | |
| Total | 21 | 59 | |
| Replications | 5 | 4 | 0 |
| Treatments | 4 | 23 | 5.8 |
| Error | 12 | 32 | 2.7 |
| | Liver Copper | | |
| Total | 19 | 267 | |
| Replications | 3 | 125 | 41 |
| Treatments | 4 | 52 | 13 |
| Error | 12 | 99 | 8 |
| | Liver Iron | | |
| Total | 19 | 244537 | |
| Replications | 3 | 4916 | 1638 |
| Treatments | 4 | 171710 | 42928** |
| Error | 12 | 67911 | 2326 |
| | Liver Zinc | | |
| Total | 19 | 42982 | |
| Replications | 3 | 6136 | 2045 |
| Treatments | 4 | 27737 | 6934** |
| Error | 12 | 9109 | 759 |

**Highly significant ($p \leq 0.01$).

TABLE 2

ANALYSES OF VARIANCE OF WEIGHT GAIN, HEMOGLOBIN, AND LIVER COPPER, IRON, AND ZINC DATA FOR EXPERIMENT 2

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| | Weight gain | | |
| Total | 49 | 23137 | |
| Replications | 5 | 264 | 53 |
| Treatments | 10 | 20948 | 2095** |
| Error | 34 | 1925 | 57 |
| | Hemoglobin | | |
| Total | 49 | 221 | |
| Replications | 5 | 14 | 3 |
| Treatments | 10 | 116 | 12** |
| Error | 34 | 91 | 3 |
| | Liver Copper | | |
| Total | 38 | 3022576 | |
| Replications | 3 | 56753 | 18918 |
| Treatments | 10 | 2543805 | 254380** |
| Error | 25 | 422018 | 16881 |
| | Liver Iron | | |
| Total | 38 | 334136 | |
| Replications | 3 | 22186 | 7395 |
| Treatments | 10 | 215288 | 21523** |
| Error | 25 | 96662 | 3866 |
| | Liver Zinc | | |
| Total | 38 | 9435 | |
| Replications | 3 | 252 | 84 |
| Treatments | 10 | 6544 | 654** |
| Error | 25 | 2639 | 106 |

**Highly significant ($p \leq 0.01$).

TABLE 3

ANALYSES OF VARIANCE OF WEIGHT GAIN, HEMOGLOBIN, AND LIVER COPPER, IRON, AND ZINC DATA FOR EXPERIMENT 3

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Weight gain, Diets 1-8 | | | |
| Total | 49 | 142568 | |
| Replications | 6 | 1236 | 206 |
| Treatments | 7 | 99883 | 14269** |
| Zn | 1 | 97278 | 97278** |
| Cu | 1 | 311 | 311 |
| Fe | 1 | 1046 | 1046 |
| ZnCu | 1 | 758 | 758 |
| ZnFe | 1 | 224 | 224 |
| CuFe | 1 | 323 | 232 |
| ZnCuFe | 1 | 34 | 34 |
| Error | 36 | 41449 | 1151 |
| Weight gain, Diets 1-4, 9-12 | | | |
| Total | 49 | 118753 | |
| Replications | 6 | 1702 | 284 |
| Treatments | 7 | 102626 | 14661** |
| Zn | 1 | 99036 | 99036** |
| Cu | 1 | 611 | 611 |
| Fe | 1 | 825 | 825 |
| ZnCu | 1 | 1198 | 1198 |
| ZnFe | 1 | 129 | 129 |
| CuFe | 1 | 228 | 228 |
| ZnCuFe | 1 | 598 | 598 |
| Error | 36 | 14407 | 400 |

TABLE 3--Continued

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Weight gain, Diets 5-12 | | | |
| Total | 51 | 54418 | |
| Replications | 6 | 4356 | 726 |
| Treatments | 7 | 5716 | 817 |
| Zn | 1 | 8 | 8 |
| Cu | 1 | 2730 | 2730 |
| Fe | 1 | 1909 | 1909 |
| ZnCu | 1 | 50 | 50 |
| ZnFe | 1 | 13 | 13 |
| CuFe | 1 | 85 | 85 |
| ZnCuFe | 1 | 920 | 920 |
| Error | 38 | 44346 | 1167 |
| Hemoglobin, Diets 1-8 | | | |
| Total | 48 | 168 | |
| Replications | 6 | 3 | 0 |
| Treatments | 7 | 120 | 17** |
| Zn | 1 | 114 | 114** |
| Cu | 1 | 0 | 0 |
| Fe | 1 | 1 | 1 |
| ZnCu | 1 | 0 | 0 |
| ZnFe | 1 | 1 | 1 |
| CuFe | 1 | 2 | 2 |
| ZnCuFe | 1 | 2 | 2 |
| Error | 35 | 54 | 2 |
| Hemoglobin, Diets 1-4, 9-12 | | | |
| Total | 40 | 188 | |
| Replications | 6 | 10 | 1 |
| Treatments | 7 | 132 | 19** |
| Zn | 1 | 129 | 129** |
| Cu | 1 | 0 | 0 |
| Fe | 1 | 1 | 1 |
| ZnCu | 1 | 1 | 1 |
| ZnFe | 1 | 0 | 0 |
| CuFe | 1 | 0 | 0 |
| ZnCuFe | 1 | 1 | 1 |
| Error | 36 | 46 | 1 |

TABLE 3--Continued

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| **Hemoglobin, Diets 5-12** | | | |
| Total | 50 | 46 | |
| Replications | 6 | 6 | 1 |
| Treatments | 7 | 10 | 1 |
| Zn | 1 | 0 | 0 |
| Cu | 1 | 0 | 0 |
| Fe | 1 | 2 | 2 |
| ZnCu | 1 | 1 | 1 |
| ZnFe | 1 | 0 | 0 |
| CuFe | 1 | 1 | 1 |
| ZnCuFe | 1 | 5 | 5 |
| Error | 37 | 30 | 1 |
| **Liver Copper, Diets 1-8** | | | |
| Total | 31 | 1351714 | |
| Replications | 3 | 20080 | 6693 |
| Treatments | 7 | 1153158 | 164737** |
| Zn | 1 | 122142 | 122142** |
| Cu | 1 | 685913 | 685913** |
| Fe | 1 | 56953 | 56953** |
| ZnCu | 1 | 114222 | 114222** |
| ZnFe | 1 | 51762 | 51762* |
| CuFe | 1 | 63390 | 63390* |
| ZnCuFe | 1 | 58739 | 58739* |
| Error | 21 | 178476 | 8499 |
| **Liver Copper, Diets 1-4, 9-12** | | | |
| Total | 31 | 1374213 | |
| Replications | 3 | 31127 | 10376 |
| Treatments | 7 | 1188791 | 169827** |
| Zn | 1 | 262188 | 262188** |
| Cu | 1 | 438850 | 438850** |
| Fe | 1 | 40186 | 40186 |
| ZnCu | 1 | 253700 | 253700** |
| ZnFe | 1 | 70688 | 70688** |
| CuFe | 1 | 49544 | 49544* |
| ZnCuFe | 1 | 73632 | 73632** |
| Error | 21 | 154295 | 7347 |

TABLE 3--Continued

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| **Liver Copper, Diets 5-12** | | | |
| Total | 31 | 189617 | |
| Replications | 3 | 3533 | 1178 |
| Treatments | 7 | 162762 | 23252** |
| Zn | 1 | 26450 | 26450** |
| Cu | 1 | 105340 | 105340** |
| Fe | 1 | 741 | 741 |
| ZnCu | 1 | 27495 | 27495** |
| ZnFe | 1 | 1458 | 1458 |
| CuFe | 1 | 399 | 399 |
| ZnCuFe | 1 | 840 | 840 |
| Error | 21 | 23322 | 1111 |
| **Liver Iron, Diets 1-8** | | | |
| Total | 31 | 3132176 | |
| Replications | 3 | 220610 | 73537 |
| Treatments | 7 | 2119646 | 302807** |
| Zn | 1 | 927132 | 927132** |
| Cu | 1 | 97130 | 97130 |
| Fe | 1 | 520710 | 520710** |
| ZnCu | 1 | 152490 | 152490 |
| ZnFe | 1 | 140185 | 140185 |
| CuFe | 1 | 39451 | 39451 |
| ZnCuFe | 1 | 242556 | 242556** |
| Error | 21 | 791920 | 37710 |
| **Liver Iron, Diets 1-4, 9-12** | | | |
| Total | 31 | 3847323 | |
| Replications | 3 | 102382 | 34127 |
| Treatments | 7 | 3096175 | 442311** |
| Zn | 1 | 1957617 | 1957617** |
| Cu | 1 | 36993 | 36993 |
| Fe | 1 | 319380 | 319380** |
| ZnCu | 1 | 262197 | 262197** |
| ZnFe | 1 | 281794 | 281794** |
| CuFe | 1 | 124525 | 124525** |
| ZnCuFe | 1 | 113669 | 113669** |
| Error | 21 | 648765 | 30894 |

TABLE 3--Continued

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| | Liver Iron, Diets 9-12 | | |
| Total | 31 | 675043 | |
| Replications | 3 | 31754 | 10585 |
| Treatments | 7 | 349211 | 49887* |
| Zn | 1 | 190190 | 190190** |
| Cu | 1 | 40186 | 40186 |
| Fe | 1 | 36430 | 36430 |
| ZnCu | 1 | 14782 | 14782 |
| ZnFe | 1 | 24466 | 24466 |
| CuFe | 1 | 19110 | 19110 |
| ZnCuFe | 1 | 24070 | 24070 |
| Error | 21 | 294078 | 14004 |
| | Liver Zinc, Diets 1-8 | | |
| Total | 31 | 14318 | |
| Replications | 3 | 5947 | 1982 |
| Treatments | 7 | 3166 | 445 |
| Zn | 1 | 1313 | 1313* |
| Cu | 1 | 392 | 392 |
| Fe | 1 | 60 | 60 |
| ZnCu | 1 | 66 | 66 |
| ZnFe | 1 | 158 | 158 |
| CuFe | 1 | 473 | 473 |
| ZnCuFe | 1 | 657 | 657 |
| Error | 21 | 5255 | 250 |
| | Liver Zinc, Diets 1-4, 9-12 | | |
| Total | 31 | 35764 | |
| Replications | 3 | 5955 | 1985 |
| Treatments | 7 | 18864 | 2695** |
| Zn | 1 | 10477 | 10477** |
| Cu | 1 | 3121 | 3121* |
| Fe | 1 | 905 | 905 |
| ZnCu | 1 | 1954 | 1954 |
| ZnFe | 1 | 1226 | 1226 |
| CuFe | 1 | 340 | 340 |
| ZnCuFe | 1 | 841 | 841 |
| Error | 21 | 10945 | 521 |

TABLE 3--Continued

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Liver Zinc, Diets 5-12 | | | |
| Total | 31 | 28939 | |
| Replications | 3 | 7360 | 2453 |
| Treatments | 7 | 12155 | 1736** |
| Zn | 1 | 4371 | 4371** |
| Cu | 1 | 4095 | 4095** |
| Fe | 1 | 1815 | 1815 |
| ZnCu | 1 | 1300 | 1300 |
| ZnFe | 1 | 504 | 504 |
| CuFe | 1 | 52 | 52 |
| ZnCuFe | 1 | 11 | 11 |
| Error | 21 | 9429 | 449 |

*Significant ($p \leq 0.05$).

**Highly significant ($p \leq 0.01$).