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MOTSINGER, BRENDA MCADAMS. *Influence of Zinc Supplementation on Protein Utilization in Young Rats.* (1977) Directed by: Dr. Aden C. Magee. Pp. 57.

Two experiments were conducted to determine the effects of zinc supplementation on the utilization of various qualities of protein for growth and development of rats. A 4x2x3 factorial experimental design was used in which four protein sources (egg white solids, casein, soy protein, and wheat gluten), two protein levels (7.5% and 15.0%), and three levels of zinc supplements (0 ppm, 25 ppm, and 50 ppm) were combined in various ways for a total of twenty-four test diets. Rats were fed the diets for a period of four weeks. Criteria for evaluating animal responses to the test diets included weight gain, hemoglobin concentrations, and iron, copper, and zinc contents of the liver.

Zinc supplementation was associated with increased weight gains at both the 7.5% and 15.0% level of dietary egg white solids, whereas a 15.0% level of dietary casein was needed to obtain increased growth rates with zinc supplementation. In the presence of zinc, increasing the level of dietary protein was associated with increases in weight gains of rats receiving egg white solids and casein diets. Increasing the zinc supplement from 25 ppm to 50 ppm of zinc in casein and egg white solid diets was associated with slight, although not significant, increases in weight gains indicating that 25 ppm of zinc may be marginal for rapidly growing animals receiving these two proteins. The mean weight gains of rats fed zinc-supplemented soy protein and wheat gluten diets were not significantly different from those of rats fed the non-supplemented diets containing these two proteins.

The source of protein in the diet was found to exert the major influence on hemoglobin levels. Egg white solids and soy protein diets were associated with increased hemoglobin levels. Zinc supplements appeared to alter hemoglobin levels depending on the source of dietary protein.

Liver iron and liver copper levels of rats fed egg white solid diets generally decreased when the diets were supplemented with zinc, whereas liver iron and liver copper levels of rats fed soy protein and wheat gluten diets did not appear to be influenced by dietary zinc supplements. Zinc-supplemented casein diets were associated with increased amounts of copper in the liver and no significant differences in liver iron incorporation. The data suggest that physiological levels of zinc may interfere with iron and copper metabolism depending on the source of dietary protein.

The results indicated that physiological levels of dietary zinc may decrease the incorporation of zinc into the liver depending on the source of dietary protein. Zinc-supplemented egg white solid diets were associated with decreased liver zinc levels.

*INFLUENCE OF ZINC SUPPLEMENTATION
ON PROTEIN UTILIZATION
IN YOUNG RATS*

by

Brenda McAdams Motsinger

*A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
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Approved by

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APPROVAL PAGE

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CHAPTER I
INTRODUCTION

The importance of protein in the diet is basically to supply the cells with a source of amino acids to be used for cell metabolism. It has generally been accepted that the amount and ratio of these amino acids, the most limiting amino acid theory, determines to a large degree how well these amino acids will be utilized for protein synthesis. With research supporting the concept that zinc plays a crucial role in the synthesis of protein and nucleic acids, the question has arisen as to whether the importance accorded dietary protein per se has been overemphasized. Speculation exists as to whether the supplementation of protein with dietary zinc may improve the utilization of high quality as well as poor quality proteins.

Various studies have indicated that the utilization of protein may be improved if these proteins are supplemented with zinc. However, in many of these investigations the protein, in addition to receiving zinc supplements, was also supplemented with its limiting amino acid. The present study was designed to determine the effect of zinc supplementation on the utilization of various quality proteins at varying levels of dietary intake without amino acid supplements.

CHAPTER II
REVIEW OF LITERATURE

Todd and his associates (1) first demonstrated the dietary essentiality of zinc for higher animals in 1934 when they observed it was necessary for growth and well being of the rat. Subsequently, other researchers demonstrated the essentiality of zinc in other animals and described a variety of zinc deficiency symptoms and biochemical changes for several animal species (2, 3, 4, 5, 6, 7, 8). The essentiality of zinc for humans was demonstrated in 1940 when it was found to be an essential component of the carbonic anhydrase system (9). A zinc-responsive disease occurring in man, however, was not detected until 1960 when it was reported that a type of dwarfism and sexual infantilism in males and females could be alleviated with dietary zinc (10, 11). A summary report by Hambidge and Walravens (12) described varying degrees of zinc deficiency found in young children in Iran. In addition, zinc deficiency was observed in young children suffering from protein-caloric malnutrition in Egypt, South Africa, and India. Incidents of marginal zinc deficiency in various groups in the United States have also been reported, and there is the possibility that marginal zinc deficiency in the United States' population may be widespread throughout varying geographical regions and economic classes (13, 14).

The availability of zinc for absorption is affected by various dietary

factors. Studies have supported the view that phytate, associated with plant protein, is responsible for the lower availability of zinc in foods prepared from plants (15). O'Dell (16) stated that the higher content of phytate in plant sources of protein is one of the outstanding differences between animal and plant protein. Supplementation of zinc to plant protein has been shown to result in increased growth rates (16, 17). Oberleas et al. (18) demonstrated that the uptake of zinc was progressively decreased as the ratio of calcium to phytate was increased in the diet. They postulated that in the presence of phytate an insoluble zinc-phytate complex is formed. The addition of calcium results in an even more insoluble complex containing zinc, calcium, and phytate.

Subsequent studies have revealed that the fiber content of the diet may significantly affect the availability of zinc (19). Reinhold et al. (20) stated that although the decrease in zinc availability may in part be attributed to the phytate content of the diet, the complexing action of fiber also reduces the availability of zinc. These researchers suggested that previous studies in which only phytate was considered as the complexing agent need to be reinterpreted.

The influence of dietary protein on zinc absorption and metabolism has been the area of interest in several studies. In balance studies with preadolescent girls, the retention of zinc was shown to increase as the level of dietary protein was increased (21). Other investigations supported the observation that the level of dietary protein influences the absorption and

retention of zinc (22, 23). Several studies have demonstrated that zinc from plant protein is absorbed less efficiently than zinc from animal protein (4, 16, 24). Spencer et al. (23) suggested that additional research is needed with regard to the availability of zinc from different foods and with regard to the effect of protein on zinc absorption.

The biological value is often used for the evaluation of the nutritive value of protein (17). Hegsted and Chang (25, 26), however, questioned the use of biological value and its variants for the evaluation of proteins, and proposed the relative growth index as a satisfactory measure of nutritive value. The relative growth index is obtained by the slope-ratio technique using weight gain as the response and nitrogen intake as the measure of dose with the relative growth index being the slope of the regression between dose and response expressed in percentage. They found casein, soy protein, and wheat gluten to have relative growth indexes of 70.0%, 33.7%, and 21.8%, respectively, when compared to lactalbumin with a relative potency of 100%. Oberleas and Prasad (17) speculated that previous values of protein quality may be misleading because zinc was not considered a dietary essential in many studies on protein quality. They found that when plant seed protein was supplemented with zinc these proteins were equal in quality to animal protein, and suggested the need for additional research with regard to zinc supplementation to plant protein.

Poor appetite is one of the characteristic symptoms of zinc deficiency, and Chesters et al. (27, 28) have demonstrated a zinc-protein interrelation-

ship with regard to appetite. They observed that there is a markedly increased day-to-day variation in food intake when diets fed to zinc-deficient rats contained an adequate level of high quality protein. This variation did not occur when the diets did not contain protein, leading Chesters and his associates to postulate that the cyclical pattern of food intake of the zinc-deficient rat results from appetite being regulated by corresponding rises and falls in the concentration of one or more metabolites. After elevated food intakes containing adequate protein, these metabolites accumulate, reaching deleterious concentrations, and appetite is inhibited.

Griffith and Alexander (29) also observed that rats receiving a high protein zinc-deficient diet had an increased variability in daily food intake, whereas rats receiving zinc-deficient low protein diets did not. The plasma level of tyrosine was found to correlate with the daily fluctuation of food consumption, leading Griffith and Alexander to suggest that the plasma level of tyrosine may play a part in appetite control of zinc-deficient rats.

In both young animals and man, retardation of growth has been a characteristic manifestation of zinc deficiency (1, 6, 11, 17, 30, 31). However, the growth retardation observed in zinc-deficient animals does not appear to be simply the result of decreased food intake. In pair-fed studies with pigs, Miller et al. (32) found that growth rates diminished in zinc-deficient pigs before food intake was reduced. Similar evidence has been reported with rats (27).

Although Mill and his associates (33) found that the carboxypeptidase activity, a zinc-dependent enzyme (34), was appreciably lower in zinc deficiency and returned to normal with zinc therapy, they found no evidence to suggest a reduction in the rate of protein digestion due to the reduction in carboxypeptidase activity in the rat. Pallauf and Kirchgessner (35), however, found the digestibility of dry matter and crude protein to be lower in zinc-deficient rats than in pair-fed controls. These differences in digestibility were relatively small, and Kirchgessner et al. (5) stated that these small differences in digestibility cannot account for the profound effect zinc deficiency has on nutrient utilization and growth rates. They suggested that fundamental defects in the biochemical processes of the cells must be responsible.

The inhibiting effect of zinc deficiency on growth rates, along with the observation that reduced intake of food and reduction in digestibility cannot account for the growth retardation seen in zinc deficiency, has led to investigations of the role of zinc in protein and nucleic acid metabolism.

A review by Kirchgessner et al. (5) suggested that zinc deficiency may stimulate both protein catabolism and inhibition of protein synthesis. Studies have revealed that the total protein content of various tissues of zinc-deficient rats was lower than the protein content of tissues from zinc-supplemented rats; that zinc deficiency results in increased urinary excretion of nitrogen and sulfur and is associated with abnormalities in amino acid metabolism (5, 29, 36, 37).

Studies have shown that zinc deficiency is associated with alternations in nucleic acid metabolism and may thereby impair protein synthesis. It has been demonstrated that with zinc deficiency there is a decrease in the DNA and RNA content in certain tissues and a decreased incorporation of labeled thymidine into DNA (38, 39, 40). The DNA and RNA polymerases, enzymes which catalyze the replication of DNA and transcription of RNA, show a decrease in enzymatic activities in zinc-deficient tissues (5). Thymidine kinase activity is also decreased in zinc-deficient tissues (5, 40). However, ribonuclease, the enzyme involved in the catabolism of RNA, shows an increase in catalytic activity in zinc-deficient tissues (5).

Subsequent research has revealed that the DNA and RNA polymerases of Escherichia coli contain zinc essential for catalytic activities (41), and the RNA-dependent DNA polymerase (reverse transcriptase) of the virus avian myeloblastosis is a zinc metalloenzyme (42). Auld and his associates (42) stated that it would seem that the importance of the role of zinc in the formation of DNA and RNA from DNA templates has been established.

Research, having indicated that zinc plays a crucial role in protein metabolism and the interrelationship of zinc and protein in the diet affects appetite as well as the availability of zinc, has led to speculation that zinc supplementation could aid in the utilization of low quality protein. Caldwell and Oberleas (43) suggested that the importance accorded both quantity and quality of dietary protein per se in the development of the

organism needs reappraisal. The following study was designed to investigate the interrelationships of zinc supplementation and quantity and quality of protein on the utilization of dietary protein in the rat.

CHAPTER III
EXPERIMENTAL PROCEDURES

The present study was designed to investigate the effect of zinc supplementation on the utilization of dietary proteins of varying qualities for growth and development of rats. Data were also collected on hemoglobin concentration and the copper, iron, and zinc content of the livers as criteria for evaluating the rats' responses to the various test diets.

Four different sources of protein, ranging from good to poor in quality, were selected on the basis of their biological values (BV) in the growing rat. Spray dried egg white solids¹, with a BV of 97, was considered a protein source of good quality. Soy protein² (BV=75) and casein³ (BV=69) were selected as intermediate qualities of protein, with wheat gluten⁴ (BV=40) being a poor quality protein (44).

The investigation consisted of two experiments. Spray dried egg white solids and casein were used as sources of protein in the first experiment, with wheat gluten and soy protein being the sources of protein in the second experiment.

¹*Purchased from Tekland Mills, Chagrin Falls, Ohio.*

²*Alpha protein, ICN Biochemicals, Cleveland, Ohio.*

³*Purchased from ICN Biochemicals, Cleveland, Ohio.*

⁴*Purchased from ICN Biochemicals, Cleveland, Ohio.*

Other than the sources of protein, the test diets were the same for both experiments. The percentage composition of the test diet consisted of vegetable shortening, 10⁵; mineral mix, 4⁶; vitamin mix, 2; cellulose, 2⁷; protein, 7.5 or 15; with varying amounts of dextrose⁸ added to yield 2 kilograms of diet. Twenty-four drops of oleum percomorphum⁹ were added per kilogram of diet. Diets which received the zinc supplementation were supplemented with either 25 ppm or 50 ppm zinc as zinc carbonate. Table 1 in the text gives the various combinations of test treatments used for Experiments I and II. See Tables 1 and 2, Table 3, and Table 4 in Appendix A for complete information on the composition of diets, mineral mix, and vitamin mix, respectively.

Diets 1, 4, 7, and 10 in Experiment I were not supplemented with zinc and were considered zinc deficient. Analysis of the diets revealed that Diets 1 and 4, Diet 7, and Diet 10 contained approximately 4 ppm, 1 ppm, and 5 ppm of zinc, respectively. In Experiment II, Diets 13, 16, 19 and 22 were not supplemented with zinc and were considered zinc deficient. Analysis of these diets revealed that Diets 13 and 19, Diet 16 and Diet 22 contained approximately 2 ppm, 5 ppm, and 6 ppm of zinc, respectively.

⁵Crisco, Proctor and Gamble Company, Cincinnati, Ohio.

⁶Salt Mixture W, ICN Biochemicals, Cleveland, Ohio.

⁷Alphacel, ICN Biochemicals, Cleveland, Ohio.

⁸Purchased from ICN Biochemicals, Cleveland, Ohio.

⁹Product of Mead Johnson and Company, Evansville, Indiana.

TABLE I
 VARIOUS COMBINATIONS OF TEST DIETS FOR EXPERIMENTS I AND II

<i>Experiment I</i>			
<i>Diet Number</i>	<i>Protein Source</i>	<i>Protein Level</i>	<i>Zinc Supplement</i>
1	<i>Egg White Solids</i>	7.5%	0 ppm
2	<i>Egg White Solids</i>	7.5%	25 ppm
3	<i>Egg White Solids</i>	7.5%	50 ppm
4	<i>Egg White Solids</i>	15%	0 ppm
5	<i>Egg White Solids</i>	15%	25 ppm
6	<i>Egg White Solids</i>	15%	50 ppm
7	<i>Casein</i>	7.5%	0 ppm
8	<i>Casein</i>	7.5%	25 ppm
9	<i>Casein</i>	7.5%	50 ppm
10	<i>Casein</i>	15%	0 ppm
11	<i>Casein</i>	15%	25 ppm
12	<i>Casein</i>	15%	50 ppm
<i>Experiment II</i>			
13	<i>Soy Protein</i>	7.5%	0 ppm
14	<i>Soy Protein</i>	7.5%	25 ppm
15	<i>Soy Protein</i>	7.5%	50 ppm
16	<i>Soy Protein</i>	15%	0 ppm
17	<i>Soy Protein</i>	15%	25 ppm
18	<i>Soy Protein</i>	15%	50 ppm
19	<i>Wheat Gluten</i>	7.5%	0 ppm
20	<i>Wheat Gluten</i>	7.5%	25 ppm
21	<i>Wheat Gluten</i>	7.5%	50 ppm
22	<i>Wheat Gluten</i>	15%	0 ppm
23	<i>Wheat Gluten</i>	15%	25 ppm
24	<i>Wheat Gluten</i>	15%	50 ppm

Young male rats¹⁰, approximately three weeks of age, were used in the experiments and were randomly assigned to replications according to initial body weight. The test diets were randomly assigned to individual animals within each of the six replications. The animals were housed individually in wire-bottom stainless steel cages and allowed free access to food from glass jars. Demineralized water was available from glass bottles with stainless steel nipples.

Growth records were obtained from weekly weighing of the rats for a total of four weeks. At the end of the four week period, blood samples were taken from the tails of the animals for hemoglobin determination by the method of Shenk et al. (45).

Rats from four of the six replications were then killed and the livers removed. A sample was removed from each liver to obtain dry weight measurements, and the remaining portions of the livers were ashed with nitric acid and perchloric acid. The method of Park et al. (46) as modified by Matrone et al. (47) was used for the analysis of the iron and copper content of the liver. Analysis of liver zinc was determined using the method of McCall et al. (48).

All data were analyzed by the statistical method of analysis of variance.

¹⁰Sprague-Dawley rats purchased from Holtzman Company, Madison, Wisconsin.

CHAPTER IV
RESULTS AND DISCUSSION

Experiment I

The various responses of the rats to the experimental treatments used in Experiment I are given in Table 2 in the text. Detailed data obtained from this experiment can be found in Tables 1 through 5 in Appendix B. Table 6 in Appendix B contains the statistical analyses of these data.

Weight Gain

Analysis of the data revealed that the level and source of dietary protein and zinc supplementation significantly influenced weight gains. Weight gains of animals receiving casein diets were generally higher ($p \leq 0.01$) than weight gains of rats receiving egg white solid diets. However, interpretation of a highly significant protein source \times zinc interaction ($p \leq 0.01$) suggested that supplementing the egg white solid diets with zinc was more effective in stimulating weight gains than supplementing dietary casein with zinc. Animals which received the non-zinc-supplemented egg white solid diets gained an average of 34 gm during the four week experiment, whereas animals receiving the non-zinc-supplemented casein diets gained an average of 96 gm. Supplementing the egg white solid diets with zinc resulted in an average increase of 76 gm for a four week period,

TABLE 2
Experiment I
EFFECT OF ZINC SUPPLEMENTS ON RATS FED VARYING
LEVELS OF EGG WHITE SOLIDS AND CASEIN

Protein Source	Protein Level	Zinc Supplement	Wt. Gain at 4 weeks	Hemoglobin	Liver Constituent		
					Cu	Fe	Zn
	percent	ppm	gm ^a	gm/100 ml ^a	mcg/gm dry weight ^b		
Egg white solids	7.5	0	34	14.48	14.49	652.08	27.53
Egg white solids	7.5	25	87 ^c	13.44 ^c	7.52	261.80	10.64
Egg white solids	7.5	50	74 ^c	14.50	7.47	308.37	12.42
Egg white solids	15.0	0	33 ^c	16.41 ^c	12.32	471.01	37.24
Egg white solids	15.0	25	134 ^c	14.20 ^c	12.40	359.82	15.87
Egg white solids	15.0	50	145	14.21	9.71	358.92	12.17
Casein	7.5	0	56	13.48	10.35	328.36	21.00
Casein	7.5	25	54	13.96	10.63	372.53	18.12
Casein	7.5	50	49	13.85	15.49	381.96	22.17
Casein	15.0	0	136	12.25	12.53	268.98	11.34
Casein	15.0	25	161	13.39	9.76	301.48	10.61
Casein	15.0	50	170 ^c	13.44 ^c	10.51	283.86	11.45
L.S.D. 0.05 ^d			22	1.43	5.13	103.82	10.30
L.S.D. 0.01			30	1.90	6.90	139.56	13.85

^aEach figure represents the mean of 6 animals unless indicated otherwise.

^bEach figure represents the mean of 4 animals.

^cFigure represents the mean of 5 animals.

^dLeast significant difference at specified probability levels.

whereas adding zinc supplements to the casein diets only increased the average four week weight gain by 13 gm. In general, supplementing the diets with zinc increased ($p \leq 0.01$) growth rates. Decreasing the protein level from 15.0% to 7.5% was associated with decreases ($p \leq 0.01$) in weight gain. Interpretation of a protein level \times protein source interaction ($p \leq 0.01$) suggested that increasing the level of an intermediate quality protein such as casein resulted in greater increases in weight gain than increasing the level of a high quality protein such as egg white solids. Zinc supplementation was associated with increased growth rates at either the 7.5% or 15.0% level of dietary egg white solids, whereas a 15.0% level of dietary casein was needed to obtain increased growth rates with zinc supplementation. In the presence of zinc, increasing the level of dietary protein was generally associated with highly significant increases ($p \leq 0.01$) in weight gain of rats.

Hemoglobin

The source of protein in the diet appeared to exert the major influence on hemoglobin concentrations. Hemoglobin levels of rats fed diets containing egg white solids were generally higher ($p \leq 0.01$) than those of rats fed casein. Zinc supplementation of dietary casein did not appear to be related to changes in hemoglobin levels, whereas the addition of dietary zinc to the egg white solid diets was associated with decreases ($p \leq 0.05$) in hemoglobin levels.

Liver Iron

The results indicated that the source and level of dietary protein and zinc supplementation of the diets significantly influenced the incorporation of iron into the rats' livers. Animals receiving egg white solid diets generally had higher ($p \leq 0.01$) liver iron levels than animals fed casein diets. Low level protein diets were associated with significant increases ($p \leq 0.05$) in liver iron. Supplementing the diets with zinc generally decreased ($p \leq 0.01$) the incorporation of iron into the liver. However, a highly significant protein source \times zinc interaction ($p \leq 0.01$) indicated that in rats fed egg white solid diets liver iron levels generally decreased when the diets were supplemented with dietary zinc, whereas liver iron levels of rats receiving casein diets did not appear to be influenced by dietary zinc supplements. Interpretation of a protein level \times protein source \times zinc interaction ($p \leq 0.05$) suggested that the level of dietary protein may also influence liver iron incorporation, depending on the source of dietary protein and the level of zinc supplementation. The increase in liver sizes of rats receiving zinc supplements may in part explain the reduction in liver iron levels when liver iron is expressed in mcg/gm dry weight, indicating that to some degree the level of iron incorporation was a reflection of the amount of liver tissue available to incorporate iron. However, the data seemed to indicate that even physiological levels of zinc in some way interfere with iron metabolism, depending on the source of dietary protein. Whether zinc serves as a means of

protection for the animal against iron toxicity or is detrimental to the animal is not apparent from this study.

Liver Copper

Analysis of the data revealed a significant protein source x zinc interaction ($p \leq 0.05$) indicating that zinc supplementation of the egg white solid diets decreased liver copper levels, whereas zinc supplementation of dietary casein generally increased the level of copper in the liver. Interpretation of a protein source x protein level x zinc interaction ($p \leq 0.05$) suggested that increasing the level of dietary egg white solids afforded some protection against the reduction of liver copper levels when zinc supplements were added to the diets, whereas increasing the level of dietary casein appeared to increase the adverse effect of zinc on liver copper incorporation. The results suggested that physiological levels of zinc may interfere with the incorporation of copper into the rats' livers, and that different sources and levels of protein in the diet may inhibit or accentuate the adverse effect of zinc on liver copper incorporation.

Liver Zinc

The data indicated that in general supplementing the diets with physiological levels of dietary zinc significantly lowered ($p \leq 0.01$) the incorporation of zinc into the liver. However, a highly significant protein source x zinc interaction ($p \leq 0.01$) revealed that liver zinc levels of rats receiving dietary egg white solids were generally decreased by the supplementation

of these diets with zinc, although zinc supplementation of the casein diets did not appear to influence the incorporation of zinc into the liver. Increasing the level of dietary egg white solids was associated with increases in liver zinc levels, whereas increasing the level of casein in the diet generally decreased liver zinc levels ($p \leq 0.01$). Although previous studies with zinc toxicity have demonstrated that high levels of dietary zinc increase the level of zinc in the liver (49, 50), an investigation by Grainger (30) indicated that the level of dietary protein appeared to influence the incorporation of zinc into the liver to a greater degree than zinc when supplements of 100 ppm or less were added to diets. The results of the present study suggest that the effects of zinc supplements and level of dietary protein on liver zinc levels are influenced by the source of the dietary protein.

Experiment II

The results of this experiment are shown in Table 3 in the text. For more complete data, see Tables 7 through 11 in Appendix B. The statistical analyses of these data can be found in Appendix B, Table 12.

Weight Gain

The results of Experiment II indicated that the level and source of dietary protein significantly influenced weight gains. Animals fed wheat gluten diets generally had higher ($p \leq 0.01$) weight gains than rats fed soy protein. Increasing the level of protein in the diet from 7.5% to 15.0% was

TABLE 3
Experiment II
EFFECT OF ZINC SUPPLEMENTS ON RATS FED VARYING
LEVELS OF SOY PROTEIN AND WHEAT GLUTEN

Protein Source	Protein Level	Zinc Supplement	Wt. Gain at 4 weeks	Hemoglobin	Liver Constituent		
					Cu	Fe	Zn
	percent	ppm	gm ^a	gm/100 ml ^a	mcg/gm dry weight ^b		
Soy protein	7.5	0	0	14.51 ^b	30.40	548.57	45.88
Soy protein	7.5	25	4	15.29	33.30	560.91	43.69 ^d
Soy protein	7.5	50	4 ^c	16.01 ^c	18.07	534.82	50.75
Soy protein	15.0	0	29	14.22	13.00	390.94	43.25
Soy protein	15.0	25	32	15.46	18.70	414.88	52.08
Soy protein	15.0	50	35 ^b	15.78 ^b	24.71	405.56	41.58
Wheat gluten	7.5	0	20	12.94 ^c	11.51	539.61	32.60
Wheat gluten	7.5	25	17	13.36	18.01	545.50	52.03
Wheat gluten	7.5	50	20 ^c	12.34 ^b	7.24	496.35	34.64
Wheat gluten	15.0	0	77	14.24	10.88	290.41	28.03
Wheat gluten	15.0	25	62 ^c	15.91 ^d	9.80	281.22	40.07 ^d
Wheat gluten	15.0	50	64	13.96	10.90	312.89	36.17
L.S.D. 0.05 ^e			15	1.86	19.02	149.32	25.83
L.S.D. 0.01			21	2.14	25.56	200.73	34.79

^aEach figure represents the mean of 6 animals unless indicated otherwise.

^bEach figure represents the mean of 4 animals.

^cFigure represents the mean of 5 animals.

^dFigure represents the mean of 3 animals.

^eLeast significant difference at specified probability levels.

associated with increased weight gains. A highly significant protein source \times protein level interaction ($p \leq 0.01$) indicated, as in Experiment I, that increasing the level of the lower quality protein, wheat gluten, results in a greater increase in weight gains than increasing the level of the higher quality protein, soy protein. Supplementing the diets with zinc had no apparent effect on weight gains in this experiment. It was observed, however, that rats receiving the soy protein diets, with or without zinc supplements, tended to reject the diets and did not eat well, and it is possible that these diets may have become rancid or for other reasons were unacceptable to the rats.

Hemoglobin

As in Experiment I, the source of protein appeared to exert the major influence on hemoglobin concentrations. Hemoglobin levels of rats receiving dietary soy protein were generally higher ($p \leq 0.01$) than hemoglobin levels of rats fed wheat gluten. Increasing the level of wheat gluten in the diet was associated with increases ($p \leq 0.05$) in hemoglobin levels, whereas increasing the level of dietary soy protein had no apparent effect on hemoglobin levels. A significant protein source \times zinc interaction ($p \leq 0.05$) revealed that zinc supplementation of dietary soy protein increased hemoglobin levels, whereas the addition of dietary zinc supplements to wheat gluten diets had no apparent effect on hemoglobin levels.

Liver Iron

Liver iron levels of rats receiving soy protein diets were generally higher ($p \leq 0.05$) than those of rats receiving wheat gluten diets. Increasing the level of dietary protein was associated with decreases ($p \leq 0.01$) in liver iron levels. The mean liver iron levels of rats receiving zinc supplements were not significantly different from those of rats fed the non-supplemented diets.

Liver Copper

Analysis of the data indicated that the source of protein significantly influenced liver copper incorporation. Liver copper levels of rats fed soy protein showed a highly significant increase ($p \leq 0.01$) in liver copper, whereas wheat gluten diets were associated with decreased liver copper levels. Again, as with iron, zinc supplementation of these proteins did not appear to influence liver copper incorporation.

Liver Zinc

The various experimental treatments of Experiment II were not associated with significant differences in liver zinc levels. As in Experiment I, the data suggested that the source of protein influenced liver zinc incorporation. Soy protein diets were associated with increased liver zinc levels, although the differences were not significant.

CHAPTER V
GENERAL DISCUSSION

Dietary protein supplies the cell with amino acids to be used for cell metabolism. Research has indicated that zinc plays a crucial role in the utilization of these amino acids for protein synthesis (5, 37, 38). The purpose of the present investigation was to determine the effects of zinc supplementation on the utilization of various quality proteins at varying levels of dietary protein intake. A previous study indicated that zinc improves the utilization of high quality protein even when the level of protein in the diet is inadequate (30). Results of the present investigation also indicated that zinc supplements significantly improved the utilization of egg white solids, a high quality protein, at both an adequate (15.0%) and an inadequate (7.5%) level of dietary intake. However, this study revealed that the utilization of casein, an intermediate quality protein, was improved only at an adequate level of dietary protein intake and that zinc supplementation of egg white solids was more effective in stimulating weight gains in rats than supplementing dietary casein with zinc. Zinc supplementation had no apparent effect on weight gains of rats fed soy protein or wheat gluten diets in this investigation.

Previous research demonstrated that the effect of zinc deficiency on appetite may be accentuated or inhibited, depending on the level of dietary

protein intake. Zinc-deficient low protein diets were not associated with fluctuations in appetite as were zinc-deficient high protein diets (27, 28, 29). It is possible that zinc-deficient rats receiving a high quality protein such as egg white solids may experience a greater degree of appetite impairment than zinc-deficient rats receiving an intermediate quality protein such as casein. As the design of the present study did not control for appetite, one may only suggest that part of the decrease in growth rates experienced by rats fed the zinc-deficient egg white solid diets may be due to a greater decrease in food intake than rats receiving the zinc-deficient casein diets.

Research with soy protein has indicated that soy protein may be equal in quality to animal protein if the soy protein diets were supplemented with zinc (17, 51). However, the diets in these studies were supplemented with methionine as well as zinc. As the sulfur-containing amino acids are the limiting amino acids in soy protein, the methionine supplementation would in itself seem to increase the utilization of the protein. Methionine supplements were not added to the diets in the present study and zinc supplementation of the soy protein diets was not associated with increased weight gains. However, the rats receiving the soy protein diets tended to reject the diets and there exists the possibility that these diets may have become rancid. Further investigation of the effect of zinc supplementation on the utilization of soy protein without methionine supplements seems warranted.

Zinc supplementation of the wheat gluten diets was not associated with increased weight gains, indicating that zinc supplements are effective in improving the utilization of protein only if an appropriate ratio of amino acids is present. As protein levels higher than 15.0% were not used in the present investigation, it is possible that zinc supplementation of higher levels of wheat gluten might increase the utilization of this protein. It is also possible that supplements higher than 50 ppm of zinc might increase weight gains of rats receiving the plant proteins, wheat gluten and soy protein. A report by Hurley (51) indicated that 60 to 100 ppm of zinc may be needed for optimal improvement in weight gain of rats receiving soy protein.

In general 25 ppm of zinc was as effective as 50 ppm of zinc in increasing weight gain of rats fed casein or egg white solids. However, there was a slight, although not significant, increase in weight gain of rats when the zinc supplements were increased from 25 to 50 ppm of zinc, indicating that 25 ppm of zinc may be marginal for rapidly growing animals receiving these proteins.

In both experiments, the source of protein in the diet appeared to exert the major influence on hemoglobin levels. The higher quality protein in each experiment, that is egg white solids in Experiment I and soy protein in Experiment II, was associated with increased hemoglobin levels. Zinc supplements appeared to alter hemoglobin levels depending on the source of protein. The addition of dietary zinc to egg white solids and

soy protein was associated with decreased hemoglobin levels, whereas zinc supplementation of casein and wheat gluten had no apparent effect on hemoglobin levels.

Interpretation of the results suggests that the antagonistic inter-relationship of zinc to iron and zinc to copper reported by several researchers (49, 50, 52, 53) may be inhibited or accentuated, depending on the source of dietary protein. Egg white solid diets were associated with decreased amounts of iron and copper in the liver when supplemented with zinc, although zinc supplements had no apparent effect on incorporation of iron and copper into the liver when rats were fed soy protein or wheat gluten. Zinc supplementation of the casein diets was associated with increased amounts of copper in the liver, although there were no significant differences in liver iron levels with zinc supplementation of casein. Increased amounts of iron in the liver with inadequate levels of all sources of dietary protein used in this study may indicate that the rats were unable to utilize the iron due to an inadequate intake of protein, leaving the iron to be stored in the liver.

The results of the present study suggest that physiological levels of dietary zinc may decrease the level of zinc in the liver, depending on the source of protein. Liver zinc levels of rats receiving egg white solid diets were decreased by the supplementation of these diets with zinc, while zinc supplements had no apparent effect on liver zinc levels of rats receiving casein, soy protein, or wheat gluten. The present study also

revealed that zinc supplementation of egg white solid diets was more effective in increasing growth rates than zinc supplementation of the other three proteins. A possible explanation may be that the improved growth rates, observed with rats receiving the zinc-supplemented egg white solid diets, increased the zinc requirement of various cells leaving less zinc available, when only physiological levels of zinc are present, to be incorporated into the liver.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Various qualities of protein (egg white solids, casein, soy protein, and wheat gluten) were supplemented with varying levels of dietary zinc to yield twenty-four experimental diets which were fed to young rats to determine the effect of zinc supplementation on the utilization of proteins for growth and development of rats. Measurements of weight gain, hemoglobin concentrations, and iron, copper, and zinc contents of the liver were used as criteria for evaluating the rats' responses to the diets.

Analysis of the data revealed that zinc supplementation of the egg white solid diets was effective in stimulating weight gains at either an adequate or an inadequate level of dietary intake, whereas an adequate level of dietary casein was needed to obtain increased growth rates with zinc supplementation. Interpretation of a protein source x zinc interaction suggests that zinc supplementation of egg white solids was more effective in stimulating weight gains than supplementation of casein. Zinc supplementation was not associated with increased weight gains of rats fed soy protein or wheat gluten diets.

The source of dietary protein appeared to exert the major influence on hemoglobin levels. Egg white solids and casein diets were associated with increased hemoglobin levels. Zinc supplementation of the egg white

solids and soy protein diets generally decreased hemoglobin levels, whereas zinc supplementation of casein and wheat gluten diets had no apparent effect on hemoglobin levels.

The results suggested that the effect of zinc supplementation on liver iron and liver copper levels varies depending on the source and level of dietary protein. The supplementation of dietary egg white solids with zinc was associated with decreased incorporation of iron and copper into the liver, whereas zinc supplements had no apparent effect on liver iron and liver copper levels of rats receiving soy protein and wheat gluten diets. Zinc-supplemented casein diets were associated with increased amounts of copper in the liver and no significant differences in liver iron levels.

Supplementing egg white solid diets with zinc was associated with decreased incorporation of zinc into the liver. Zinc supplementation of the other three proteins was not found to influence liver zinc levels.

Recommendations for Additional Studies

In the present study, weight gains of rats fed casein diets were generally higher than those of rats fed egg white solid diets. The differences between the means of weight gains of rats fed casein and egg white solid diets were observed to result primarily from restricted weight gains of rats fed zinc-deficient egg white solid diets. As previous research has demonstrated that zinc-deficient rats fed high protein diets experience a greater day-to-day variation in food intake than is experienced by zinc-

deficient rats receiving low protein diets, the researcher of the present study suggests that part of the decrease in weight gain of rats receiving zinc-deficient egg white solid diets may be due to a greater degree of appetite impairment when zinc-deficient rats are fed a high quality protein such as egg white solids as opposed to other qualities of protein. Research to determine the effect of zinc deficiency on appetite of rats fed various qualities of protein seems warranted.

Previous studies have demonstrated that growth rates of rats fed soy protein diets may be increased if zinc supplements are added to the diet. However, many of these diets were supplemented with methionine as well as zinc. Methionine supplements were not added to the diets in the present study, and zinc supplementation of soy protein diets was not associated with increased weight gains. As the rats tended to reject the zinc-supplemented as well as the non-supplement soy protein diets in this study, further investigation of the effect of zinc supplementation on the utilization of soy protein without methionine supplements appears to be needed. Zinc supplements greater than 50 ppm may be needed with plant proteins.

Zinc supplementation of wheat gluten diets was not associated with increased growth rates in the present study, however it is possible that zinc supplements greater than 50 ppm may be effective in stimulating weight gains of rats fed wheat gluten. It is also possible that zinc supplementation of a protein level higher than 15.0% wheat gluten may increase the utilization of this protein. Although the results of this study indicated that zinc

supplementation is effective in improving the utilization of protein only if the proper ratio of amino acids is present in the diet, further research with zinc supplementation of other low quality proteins may be needed.

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TABLE 7

COMPOSITION OF 1959 EGGS BASED ON ESTIMATED DATA

Constituents	Protein %		Water %	Ash %
	1959	1958		
Egg white solids	140	140	10	11.77
Casein	205	205	10	11.77
Styrenole cholesterol	40	40	10	11.77
Mineral medium	40	40	10	11.77
Yeast extract	40	40	10	11.77
Cellulose	40	40	10	11.77
Casein	40	40	10	11.77
Glucose permeant	40	40	10	11.77
Total				
	100	100	100	100

APPENDIX A

Protein %
per zinc carbonate/lb

1 - 2.7% Egg white solids	7	7.74	Casein
2 - 2.5% Egg white solids + .05% zinc carbonate	8	7.74	Casein + .05% zinc carbonate
3 - 2.5% Egg white solids + .15% zinc carbonate	9	7.74	Casein + .15% zinc carbonate
4 - 2.5% Egg white solids	10	7.74	Casein
5 - 2.5% Egg white solids + .05% zinc carbonate	11	7.74	Casein + .05% zinc carbonate
6 - 2.5% Egg white solids + .15% zinc carbonate	12	7.74	Casein + .15% zinc carbonate

TABLE I
COMPOSITION OF TEST DIETS USED IN EXPERIMENT I

<i>Treatments</i>				
<i>gm zinc carbonate/2 kg</i>				
1 - 7.5% Egg white solids				7 - 7.5% Casein
2 - 7.5% Egg white solids + .096 zinc carbonate				8 - 7.5% Casein + .096 zinc carbonate
3 - 7.5% Egg white solids + .192 zinc carbonate				9 - 7.5% Casein + .192 zinc carbonate
4 - 15.0% Egg white solids				10 - 15.0% Casein
5 - 15.0% Egg white solids + .096 zinc carbonate				11 - 15.0% Casein + .096 zinc carbonate
6 - 15.0% Egg white solids + .192 zinc carbonate				12 - 15.0% Casein + .192 zinc carbonate

<i>Constituents</i>	<i>Diets</i> 1, 2 & 3	<i>Diets</i> 4, 5 & 6	<i>Diets</i> 7, 8 & 9	<i>Diets</i> 10, 11 & 12
	<i>gm/2 kg</i>			
Egg white solids	188	375		
Casein			166	331
Vegetable shortening	200	200	200	200
Mineral mixture	80	80	80	80
Vitamin mixture	40	40	40	40
Cellulose	40	40	40	40
Dextrose	1,452	1,265	1,474	1,309
Oleum percomorphum	48 drops/2 kg in all diets			

TABLE 2
COMPOSITION OF TEST DIETS USED IN EXPERIMENT II

<i>Treatments</i>				
<i>gm zinc carbonate/2 kg</i>				
13 - 7.5% Soy protein			19 - 7.5% Wheat gluten	
14 - 7.5% Soy Protein + .096 zinc carbonate			20 - 7.5% Wheat gluten + .096 zinc carbonate	
15 - 7.5% Soy protein + .192 zinc carbonate			21 - 7.5% Wheat gluten + .192 zinc carbonate	
16 - 15.0% Soy protein			22 - 15.0% Wheat gluten	
17 - 15.0% Soy protein + .096 zinc carbonate			23 - 15.0% Wheat gluten + .096 zinc carbonate	
18 - 15.0% Soy protein + .192 zinc carbonate			24 - 15.0% Wheat gluten + .192 zinc carbonate	

<i>Constituents</i>	<i>Diets</i>		<i>Diets</i>	
	<i>13, 14 & 15</i>	<i>16, 17 & 18</i>	<i>19, 20 & 21</i>	<i>22, 23 & 24</i>
	<i>gm/2 kg</i>			
Soy protein	155	310		
Wheat gluten			362	725
Vegetable shortening	200	200	200	200
Mineral mixture	80	80	80	80
Vitamin mixture	40	40	40	40
Cellulose	40	40	40	40
Dextrose	1,485	1,330	1,278	915
Oleum percomorphum	<i>48 drops/2 kg in all diets</i>			

TABLE 3
COMPOSITION OF SALT MIXTURE - W^a

<i>Constituents</i>	<i>Per cent</i>
<i>Calcium carbonate</i>	<i>21.000</i>
<i>Copper sulfate (5 H₂O)</i>	<i>0.039</i>
<i>Ferric phosphate</i>	<i>1.470</i>
<i>Manganous sulfate (anhyd.)</i>	<i>0.020</i>
<i>Magnesium sulfate (anhyd.)</i>	<i>9.000</i>
<i>Potassium aluminum sulfate</i>	<i>0.009</i>
<i>Potassium chloride</i>	<i>12.000</i>
<i>Potassium dihydrogen phosphate</i>	<i>31.000</i>
<i>Potassium iodide</i>	<i>0.005</i>
<i>Sodium chloride</i>	<i>10.500</i>
<i>Sodium fluoride</i>	<i>0.057</i>
<i>Tricalcium phosphate</i>	<i>14.900</i>

^aProduct of ICN Biochemicals, Cleveland, Ohio.

TABLE 4
COMPOSITION OF VITAMIN MIXTURE

<i>Constituents</i>	<i>gm/2 kg mix</i>
<i>Biotin</i>	<i>0.020</i>
<i>Folic acid</i>	<i>0.100</i>
<i>Thiamine HC1</i>	<i>0.500</i>
<i>Pyridoxine HC1</i>	<i>0.500</i>
<i>Menadione (2-methyl-naphthoquinone)</i>	<i>1.000</i>
<i>Riboflavin</i>	<i>1.000</i>
<i>Nicotinic acid</i>	<i>1.000</i>
<i>Ca pantothenate</i>	<i>3.000</i>
<i>p-aminobenzoic acid</i>	<i>10.000</i>
<i>0.1% Vitamin B₁₂ (mannitol trituration)</i>	<i>2.000</i>
<i>Inositol</i>	<i>100.000</i>
<i>Choline chloride</i>	<i>150.000</i>
<i>Corn starch</i>	<i>1732.000</i>

TABLE I
WEIGHT LOSS DATA FROM EXPERIMENT 1

Treatment	Regeneration					Mean
	1	2	3	4	5	
7.5% Egg white solids	86	86	85	86	85	84
7.5% Egg white solids + 25 ppm Zn	92	103	85	78	(127) ^a	87
7.5% Egg white solids + 50 ppm Zn	82	85	85	(74)	81	79
7.5% Egg white solids	77	77	77	75	75	75
7.5% Egg white solids + 25 ppm Zn	73	73	73	73	73	73
7.5% Egg white solids + 50 ppm Zn	70	70	70	70	70	70
7.5% Casein	82	82	82	82	82	82
7.5% Casein + 25 ppm Zn	87	87	87	87	87	87
7.5% Casein + 50 ppm Zn	82	82	82	82	82	82
7.5% Casein	77	77	77	77	77	77
7.5% Casein + 25 ppm Zn	72	72	72	72	72	72
7.5% Casein + 50 ppm Zn	77	77	77	77	77	77

APPENDIX B

^a 7 incubator contained missing data points

TABLE 1
WEIGHT GAIN DATA FROM EXPERIMENT I

Treatments	Replications						Mean
	1	2	3	4	5	6	
	Weight gain at 4 weeks (gm)						
7.5% Egg white solids	16	36	36	36	35	42	34
7.5% Egg white solids + 25 ppm Zn	92	103	85	79	(87) ^a	78	87
7.5% Egg white solids + 50 ppm Zn	83	63	86	(74)	51	86	74
15.0% Egg white solids	39	(33)	21	25	37	45	33
15.0% Egg white solids + 25 ppm Zn	(134)	133	153	126	148	109	134
15.0% Egg white solids + 50 ppm Zn	108	114	154	189	145	161	145
7.5% Casein	52	54	68	56	48	59	56
7.5% Casein + 25 ppm Zn	52	37	60	33	75	67	54
7.5% Casein + 50 ppm Zn	58	57	33	51	44	51	49
15.0% Casein	101	83	181	131	146	171	136
15.0% Casein + 25 ppm Zn	162	162	167	166	163	144	161
15.0% Casein + 50 ppm Zn	148	193	148	(170)	182	181	170

^a() indicates estimated missing plot value.

TABLE 2
HEMOGLOBIN DATA FROM EXPERIMENT I

Treatments	Replications						Mean
	1	2	3	4	5	6	
	<i>gm/100 ml blood</i>						
7.5% Egg white solids	13.94	13.40	15.49	14.43	16.03	13.57	14.48
7.5% Egg white solids + 25 ppm Zn	15.49	12.86	11.96	13.94	(13.44) ^a	12.94	13.44
7.5% Egg white solids + 50 ppm Zn	13.12	13.66	14.63	17.57	14.14	13.86	14.50
15.0% Egg white solids	16.26	(16.41)	15.92	13.40	18.52	17.97	16.41
15.0% Egg white solids + 25 ppm Zn	(14.20)	13.57	14.54	12.52	14.23	16.14	14.20
15.0% Egg white solids + 50 ppm Zn	13.94	15.06	13.66	13.49	13.94	15.14	14.21
7.5% Casein	14.43	13.86	13.12	11.83	13.77	13.86	13.48
7.5% Casein + 25 ppm Zn	13.66	12.94	15.57	15.49	13.77	12.34	13.96
7.5% Casein + 50 ppm Zn	14.23	14.43	13.57	14.43	13.77	12.69	13.85
15.0% Casein	10.54	11.29	12.60	13.77	13.20	12.09	12.25
15.0% Casein + 25 ppm Zn	13.29	13.57	14.03	14.14	12.43	12.86	13.39
15.0% Casein + 50 ppm Zn	12.94	13.77	12.86	(13.44)	14.34	13.29	13.44

^a() indicates estimated missing plot value.

TABLE 3
LIVER IRON DATA FROM EXPERIMENT I

Treatments	Replications				Mean
	1	2	3	4	
	<i>mcg/gm dry weight</i>				
7.5% Egg white solids	584.69	793.15	720.26	510.22	652.08
7.5% Egg white solids + 25 ppm Zn	289.34	287.65	213.17	257.02	261.80
7.5% Egg white solids + 50 ppm Zn	341.88	266.31	318.49	306.78	308.37
15.0% Egg white solids	441.18	476.29	415.43	551.15	471.01
15.0% Egg white solids + 25 ppm Zn	379.44	262.28	260.33	537.21	359.82
15.0% Egg white solids + 50 ppm Zn	338.91	460.17	267.19	369.41	358.92
7.5% Casein	332.35	323.75	319.42	337.90	328.36
7.5% Casein + 25 ppm Zn	322.38	474.78	332.50	360.45	372.53
7.5% Casein + 50 ppm Zn	365.24	401.84	370.31	390.46	381.96
15.0% Casein	340.22	183.55	263.28	288.86	268.98
15.0% Casein + 25 ppm Zn	297.80	264.97	235.96	407.18	301.48
15.0% Casein + 50 ppm Zn	307.49	324.48	276.09	227.37	283.86

TABLE 4
LIVER COPPER DATA FROM EXPERIMENT I

Treatments	Replications				Mean
	1	2	3	4	
		<i>mcg/gm dry weight</i>			
7.5% Egg white solids	24.34	12.45	11.01	10.14	14.49
7.5% Egg white solids + 25 ppm Zn	8.14	8.81	6.49	6.62	7.52
7.5% Egg white solids + 50 ppm Zn	9.66	5.10	7.82	7.29	7.47
15.0% Egg white solids	13.68	10.66	14.04	10.89	12.32
15.0% Egg white solids + 25 ppm Zn	22.27	8.33	7.64	11.35	12.40
15.0% Egg white solids + 50 ppm Zn	15.09	5.30	9.33	9.10	9.71
7.5% Casein	9.23	12.44	9.61	10.10	10.35
7.5% Casein + 25 ppm Zn	10.19	11.59	12.19	8.56	10.63
7.5% Casein + 50 ppm Zn	28.41	14.22	10.15	9.18	15.49
15.0% Casein	14.04	11.10	13.71	11.27	12.53
15.0% Casein + 25 ppm Zn	10.13	5.94	10.00	12.97	9.76
15.0% Casein + 50 ppm Zn	11.10	10.30	10.66	9.98	10.51

TABLE 5
LIVER ZINC DATA FROM EXPERIMENT I

Treatments	Replications				Mean
	1	2	3	4	
	<i>mcg/gm dry weight</i>				
7.5% Egg white solids	44.48	32.87	21.12	11.66	27.53
7.5% Egg white solids + 25 ppm Zn	10.80	13.57	6.03	12.16	10.64
7.5% Egg white solids + 50 ppm Zn	18.97	10.97	3.52	16.23	12.42
15.0% Egg white solids	34.46	38.01	27.86	48.61	37.24
15.0% Egg white solids + 25 ppm Zn	21.84	14.75	3.47	23.41	15.87
15.0% Egg white solids + 50 ppm Zn	13.23	15.92	4.60	14.93	12.17
7.5% Casein	14.87	19.26	13.28	36.60	21.00
7.5% Casein + 25 ppm Zn	19.43	26.71	9.75	16.60	18.12
7.5% Casein + 50 ppm Zn	17.61	43.25	12.00	15.80	22.17
15.0% Casein	14.73	10.41	7.39	12.83	11.34
15.0% Casein + 25 ppm Zn	8.52	11.38	6.09	16.44	10.61
15.0% Casein + 50 ppm Zn	11.72	15.70	8.60	9.78	11.45

TABLE 6
ANALYSES OF VARIANCE OF DATA FROM EXPERIMENT I

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
<i>Weight Gain</i>			
Total	66	193963	
Replications	5	1664	333
Treatments	11	174267	15842**
Protein Level (P)	1	90313	90313**
Protein Source (S)	1	7014	7014**
Linear Zn (L)	1	24255	24255**
Quadratic Zn (Q)	1	7642	7642**
(P) x (S)	1	17987	17987**
(P) x (L)	1	9662	9662**
(P) x (Q)	1	298	298**
(S) x (L)	1	11625	11625**
(S) x (Q)	1	4773	4773**
(P) x (S) x (L)	1	653	653
(P) x (S) x (Q)	1	21	21
Error	50	18032	361
<i>Hemoglobin</i>			
Total	67	144.13	
Replications	5	2.30	0.46
Treatments	11	64.41	5.86**
Protein Level (P)	1	0.02	0.02
Protein Source (S)	1	23.54	23.54**
Linear Zn (L)	1	0.29	0.29
Quadratic Zn (Q)	1	1.74	1.74
(P) x (S)	1	10.70	10.70*
(P) x (L)	1	1.50	1.50
(P) x (Q)	1	0.04	0.03
(S) x (L)	1	10.58	10.58*
(S) x (Q)	1	8.98	8.98*
(P) x (S) x (L)	1	6.95	6.95*
(P) x (S) x (Q)	1	0.10	0.10
Error	51	77.42	1.52

TABLE 6 -- *Continued*

<i>Source of Variation</i>	<i>Degrees of Freedom</i>	<i>Sum of Squares</i>	<i>Mean Square</i>
<i>Liver Iron</i>			
<i>Total</i>	47	703873.16	
<i>Replications</i>	3	16184.26	5394.75
<i>Treatments</i>	11	516094.67	46197.70**
<i>Protein Level (P)</i>	1	22711.35	22711.35*
<i>Protein Source (S)</i>	1	75154.51	75154.51**
<i>Linear Zn (L)</i>	1	75008.39	75008.39**
<i>Quadratic Zn (Q)</i>	1	35620.22	35620.22*
<i>(P) x (S)</i>	1	12807.95	12807.95
<i>(P) x (L)</i>	1	18604.24	18604.24
<i>(P) x (Q)</i>	1	19486.59	19486.59
<i>(S) x (L)</i>	1	137442.62	137442.62**
<i>(S) x (Q)</i>	1	66573.83	66573.83**
<i>(P) x (S) x (L)</i>	1	36544.56	36544.56*
<i>(P) x (S) x (Q)</i>	1	16137.79	16137.79
<i>Error</i>	33	171594.23	5199.83
<i>Liver Copper</i>			
<i>Total</i>	47	894.45	
<i>Replications</i>	3	208.58	69.53
<i>Treatments</i>	11	267.47	24.32
<i>Protein Level (P)</i>	1	0.55	0.55
<i>Protein Source (S)</i>	1	9.65	9.65
<i>Linear Zn (L)</i>	1	21.16	21.16
<i>Quadratic Zn (Q)</i>	1	24.97	24.97
<i>(P) x (S)</i>	1	24.77	24.77
<i>(P) x (L)</i>	1	3.81	3.81
<i>(P) x (Q)</i>	1	19.24	19.24
<i>(S) x (L)</i>	1	81.35	81.35*
<i>(S) x (Q)</i>	1	2.59	2.59
<i>(P) x (S) x (L)</i>	1	66.93	66.93*
<i>(P) x (S) x (Q)</i>	1	12.46	12.46
<i>Error</i>	33	418.40	12.68

TABLE 6 -- *Continued*

<i>Source of Variation</i>	<i>Degrees of Freedom</i>	<i>Sum of Squares</i>	<i>Mean Square</i>
<i>Liver Zinc</i>			
<i>Total</i>	47	5553.98	
<i>Replications</i>	3	860.91	286.97
<i>Treatments</i>	11	3001.93	272.90**
<i>Protein Level (P)</i>	1	58.21	58.21
<i>Protein Source (S)</i>	1	149.53	149.53
<i>Linear Zn (L)</i>	1	756.70	756.70**
<i>Quadratic Zn (Q)</i>	1	335.14	335.14*
<i>(P) x (S)</i>	1	604.07	604.07**
<i>(P) x (L)</i>	1	60.58	60.58
<i>(P) x (Q)</i>	1	6.73	6.73
<i>(S) x (L)</i>	1	858.95	858.95**
<i>(S) x (Q)</i>	1	129.25	129.25
<i>(P) x (S) x (L)</i>	1	39.63	39.63
<i>(P) x (S) x (Q)</i>	1	3.14	3.14
<i>Error</i>	33	1691.14	51.25

*Significant ($p \leq 0.05$).

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

TABLE 7
WEIGHT GAIN DATA FROM EXPERIMENT II

Treatments	Replications						Mean
	1	2	3	4	5	6	
	Weight gain at 4 weeks (gm)						
7.5% Soy protein	3	0	0	3	-1	-6	0
7.5% Soy protein + 25 ppm Zn	2	2	14	7	-10	6	4
7.5% Soy protein + 50 ppm Zn	13	(4) ^a	11	-10	6	-2	4
15.0% Soy protein	31	50	26	1	39	26	29
15.0% Soy protein + 25 ppm Zn	50	27	35	4	37	41	32
15.0% Soy protein + 50 ppm Zn	44	15	48	(35)	33	(35)	35
7.5% Wheat gluten	22	21	12	21	23	23	20
7.5% Wheat gluten + 25 ppm Zn	17	19	18	28	8	14	17
7.5% Wheat gluten + 50 ppm Zn	15	23	25	19	18	(20)	20
15.0% Wheat gluten	62	73	86	77	85	77	77
15.0% Wheat gluten + 25 ppm Zn	32	62	45	102	(62)	68	62
15.0% Wheat gluten + 50 ppm Zn	83	45	68	47	86	57	64

^a () indicates estimated missing plot value.

TABLE 8
HEMOGLOBIN DATA FROM EXPERIMENT II

Treatments	Replications						Mean
	1	2	3	4	5	6	
	gm/100 ml blood						
7.5% Soy protein	15.57	(14.51) ^a	16.03	(14.51)	12.09	14.34	14.51
7.5% Soy protein + 25 ppm Zn	12.60	17.09	18.66	16.97	14.74	11.69	15.29
7.5% Soy protein + 50 ppm Zn	16.49	(16.01)	16.97	18.12	15.37	13.12	16.01
15.0% Soy protein	13.94	14.86	16.26	(14.22)	12.52	13.49	14.22
15.0% Soy protein + 25 ppm Zn	11.52	14.03	17.20	17.72	14.74	17.57	15.46
15.0% Soy protein + 50 ppm Zn	15.26	14.63	16.97	(15.78)	16.26	(15.78)	15.78
7.5% Wheat gluten	11.52	(12.94)	16.14	14.03	11.83	11.20	12.94
7.5% Wheat gluten + 25 ppm Zn	13.29	11.77	13.20	16.37	13.03	12.52	13.36
7.5% Wheat gluten + 50 ppm Zn	9.49	(12.34)	12.60	13.77	13.49	(12.34)	12.34
15.0% Wheat gluten	12.34	15.26	16.26	15.69	13.03	12.86	14.24
15.0% Wheat gluten + 25 ppm Zn	(15.91)	(15.91)	18.12	16.49	(15.91)	13.12	15.91
15.0% Wheat gluten + 50 ppm Zn	13.20	12.52	14.54	12.94	15.49	15.06	13.96

^a() indicates estimated missing plot value.

TABLE 9
LIVER IRON DATA FROM EXPERIMENT II

Treatments	Replications				Mean
	1	2	3	4	
	<i>mcg/gm dry weight</i>				
7.5% Soy protein	534.69	645.37	533.24	480.96	548.57
7.5% Soy protein + 25 ppm Zn	622.28	506.53	399.16	715.67	560.91
7.5% Soy protein + 50 ppm Zn	397.41	667.66	483.22	591.00	534.82
15.0% Soy protein	381.74	362.15	384.75	435.13	390.94
15.0% Soy protein + 25 ppm Zn	331.50	367.46	442.12	518.45	414.88
15.0% Soy protein + 50 ppm Zn	389.71	471.17	387.22	374.15	405.56
7.5% Wheat gluten	456.27	573.06	558.69	570.40	539.61
7.5% Wheat gluten + 25 ppm Zn	648.04	752.45	293.84	487.68	545.50
7.5% Wheat gluten + 50 ppm Zn	484.54	515.05	473.75	512.04	496.35
15.0% Wheat gluten	408.73	351.83	197.92	203.17	290.41
15.0% Wheat gluten + 25 ppm Zn	230.55	204.15	462.36	227.82	281.22
15.0% Wheat gluten + 50 ppm Zn	274.73	320.40	437.37	219.07	312.89

TABLE 10
LIVER COPPER DATA FROM EXPERIMENT II

Treatments	Replications				Mean
	1	2	3	4	
	<i>mcg/gm dry weight</i>				
7.5% Soy protein	61.68	11.01	8.86	40.06	30.40
7.5% Soy protein + 25 ppm Zn	16.75	10.73	30.21	75.52	33.30
7.5% Soy protein + 50 ppm Zn	17.64	30.94	14.84	8.84	18.07
15.0% Soy protein	12.48	2.09	16.01	21.41	13.00
15.0% Soy protein + 25 ppm Zn	25.11	13.40	10.96	25.32	18.70
15.0% Soy protein + 50 ppm Zn	28.39	22.92	23.99	23.52	24.71
7.5% Wheat gluten	8.08	9.23	21.72	7.02	11.51
7.5% Wheat gluten + 25 ppm Zn	46.27	6.43	13.85	5.47	18.01
7.5% Wheat gluten + 50 ppm Zn	9.70	7.99	5.87	5.39	7.24
15.0% Wheat gluten	17.64	9.79	9.28	6.79	10.88
15.0% Wheat gluten + 25 ppm Zn	8.20	11.44	10.01	9.55	9.80
15.0% Wheat gluten + 50 ppm Zn	12.99	13.57	8.31	8.73	10.90

TABLE 11
LIVER ZINC DATA FROM EXPERIMENT II

Treatments	Replications				Mean
	1	2	3	4	
	<i>mcg/gm dry weight</i>				
7.5% Soy protein	31.01	71.69	33.69	47.12	45.88
7.5% Soy protein + 25 ppm Zn	10.26	44.27	76.55	(43.69) ^a	43.69
7.5% Soy protein + 50 ppm Zn	37.56	72.35	47.49	45.58	50.75
15.0% Soy protein	42.91	22.69	61.55	45.84	43.25
15.0% Soy protein + 25 ppm Zn	54.46	24.08	48.82	80.94	52.08
15.0% Soy protein + 50 ppm Zn	14.95	30.67	56.25	64.46	41.58
7.5% Wheat gluten	23.80	27.18	46.39	33.03	32.60
7.5% Wheat gluten + 25 ppm Zn	80.79	30.76	36.61	59.96	52.03
7.5% Wheat gluten + 50 ppm Zn	10.67	53.22	36.91	37.75	34.64
15.0% Wheat gluten	10.14	17.05	49.36	35.55	28.03
15.0% Wheat gluten + 25 ppm Zn	(40.07)	26.98	47.63	45.61	40.07
15.0% Wheat gluten + 50 ppm Zn	41.24	26.34	35.10	42.01	36.17

^a () indicates estimated missing plot value.

TABLE 12
ANALYSES OF VARIANCE OF DATA FROM EXPERIMENT II

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
<i>Weight Gain</i>			
Total	66	51481	
Replications	5	218	44
Treatments	11	42361	3851**
Protein Level (P)	1	27456	27456**
Protein Source (S)	1	12377	12377**
Linear Zn (L)	1	5	5
Quadratic Zn (Q)	1	87	87
(P) x (S)	1	1568	1568**
(P) x (L)	1	70	70
(P) x (Q)	1	51	51
(S) x (L)	1	385	385
(S) x (Q)	1	187	187
(P) x (S) x (L)	1	154	154
(P) x (S) x (Q)	1	20	20
Error	50	8902	178
<i>Hemoglobin</i>			
Total	59	277.90	
Replications	5	69.80	13.96
Treatments	11	97.81	8.89**
Protein Level (P)	1	13.05	13.05*
Protein Source (S)	1	36.28	36.28**
Linear Zn (L)	1	3.58	3.58
Quadratic Zn (Q)	1	9.18	9.18
(P) x (S)	1	16.93	16.93*
(P) x (L)	1	0.11	0.11
(P) x (Q)	1	2.32	2.32
(S) x (L)	1	11.74	11.74*
(S) x (Q)	1	4.15	4.15
(P) x (S) x (L)	1	0.05	0.05
(P) x (S) x (Q)	1	0.43	0.43
Error	43	110.29	2.56

TABLE 12 -- *Continued*

<i>Source of Variation</i>	<i>Degrees of Freedom</i>	<i>Sum of Squares</i>	<i>Mean Square</i>
<i>Liver Iron</i>			
<i>Total</i>	47	887350.00	
<i>Replications</i>	3	22569.99	7523.33
<i>Treatments</i>	11	509784.93	46344.09**
<i>Protein Level (P)</i>	1	425510.93	425510.93**
<i>Protein Source (S)</i>	1	50623.98	50623.98*
<i>Linear Zn (L)</i>	1	198.06	198.06
<i>Quadratic Zn (Q)</i>	1	1229.30	1229.30
<i>(P) x (S)</i>	1	23235.08	23235.08
<i>(P) x (L)</i>	1	4427.64	4427.64
<i>(P) x (Q)</i>	1	1703.28	1703.28
<i>(S) x (L)</i>	1	234.52	234.52
<i>(S) x (Q)</i>	1	551.09	551.09
<i>(P) x (S) x (L)</i>	1	698.54	698.54
<i>(P) x (S) x (Q)</i>	1	1372.52	1372.52
<i>Error</i>	33	354995.08	10757.43
<i>Liver Copper</i>			
<i>Total</i>	47	9585.30	
<i>Replications</i>	3	724.09	241.36
<i>Treatments</i>	11	3109.10	282.65
<i>Protein Level (P)</i>	1	311.10	311.10
<i>Protein Source (S)</i>	1	1625.88	1625.88**
<i>Linear Zn (L)</i>	1	11.91	11.91
<i>Quadratic Zn (Q)</i>	1	180.57	180.57
<i>(P) x (S)</i>	1	135.88	135.88
<i>(P) x (L)</i>	1	401.72	401.72
<i>(P) x (Q)</i>	1	239.15	239.15
<i>(S) x (L)</i>	1	6.55	6.55
<i>(S) x (Q)</i>	1	1.26	1.26
<i>(P) x (S) x (L)</i>	1	194.93	194.93
<i>(P) x (S) x (Q)</i>	1	0.16	0.16
<i>Error</i>	33	5752.11	174.31

TABLE 12 -- *Continued*

<i>Source of Variation</i>	<i>Degrees of Freedom</i>	<i>Sum of Squares</i>	<i>Mean Square</i>
<i>Liver Zinc</i>			
<i>Total</i>	45	14749.88	
<i>Replications</i>	3	2140.65	713.55
<i>Treatments</i>	11	2691.51	244.68
<i>Protein Level (P)</i>	1	112.95	112.95
<i>Protein Source (S)</i>	1	960.60	960.60
<i>Linear Zn (L)</i>	1	89.61	89.61
<i>Quadratic Zn (Q)</i>	1	658.41	658.41
<i>(P) x (S)</i>	1	44.76	44.76
<i>(P) x (L)</i>	1	0.09	0.09
<i>(P) x (Q)</i>	1	9.84	9.84
<i>(S) x (L)</i>	1	24.38	24.38
<i>(S) x (Q)</i>	1	304.10	304.10
<i>(P) x (S) x (L)</i>	1	79.92	79.92
<i>(P) x (S) x (Q)</i>	1	407.26	407.26
<i>Error</i>	31	9917.72	319.93

*Significant ($p \leq 0.05$).

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