GRAINGER, FRANCES POWE. Protein-Zinc Interrelationship in Young Rats. (1975) Directed by: Dr. Aden C. Magee. Pp. 37.

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Results indicated that all animals receiving the zinc deficient diets exhibited poor growth rates regardless of the level of dietary protein. The addition of zinc to the diets was associated with highly significant increases in weight gain. Maximum growth was obtained with 50 ppm of zinc added to the diet containing 15% protein. In the presence of adequate zinc, increasing levels of dietary protein were associated with highly significant increases in weight gain. Even when zinc was added to the diet containing a sub-optimal level of protein there was significant improvement in weight gain; thus there is indication that zinc improves the utilization of an inadequate level of protein in the diet.

A highly significant zinc-protein interaction was also associated with growth data. Increasing the level of zinc above 50 ppm, however, appeared to have an adverse effect on protein utilization.

Data suggested that there is an antagonistic interrelationship between zinc and copper at levels of zinc not considered toxic to the young rat. There also appeared to be an antagonistic interrelationship between zinc and iron.

PROTEIN-ZINC INTERRELATIONSHIP IN YOUNG RATS

by

Frances Powe Grainger

A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
1975

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ACKNOWLEDGMENTS

The author wishes to express deep appreciation and gratitude to her adviser, Dr. Aden C. Magee, for his guidance throughout this study. Appreciation is also expressed to the members of the advisory committee, Dr. Joan P. Cassilly and Dr. Mildred B. Johnson, for their helpful suggestions. The author wishes to thank Mrs. Wilda P. Wade for her technical assistance and Mrs. B. Swanson Tillery for typing the manuscript.

This thesis is dedicated to Mrs. Susan Gower Smith, who first introduced the author to research in nutrition. Mrs. Smith and her husband, Dr. David T. Smith, were pioneers in vitamin research and were the first to demonstrate the successful treatment of pellagra in humans by means of nicotinic acid.

Special recognition goes to the author's sons, Victor and Stuart Grainger, whose belief in her was a constant source of inspiration during her entire graduate program.

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CHAPTER 1

INTRODUCTION

Since 1934 when evidence was first produced that zinc was necessary for the growth and well-being of the young rat, studies have been conducted with various animals and man to determine the role of zinc. Since that time zinc has been found to be a component of several metalloenzymes and also to activate enzymes important in the metabolism of protein. Zinc participates in the synthesis of nucleic acids and protein. It has been shown that the synthesis of RNA and the utilization of amino acids in the synthesis of protein are decreased in zinc deficiency systems.

Dietary deficiency of zinc can cause extreme retardation of growth. Such deficiency has been found in rats, sheep, pigs, and cattle. In man dietary deficiency of zinc is associated with dwarfism as well as with gonadal hypofunction.

Several studies have shown that the source of protein in the diet has a marked effect upon the utilization of zinc in food. Decreased availability of zinc from diets consisting of plant-seed protein has been reported, and this decreased availability of zinc attributed to phytate, a component of plant-seed proteins.

The utilization of dietary proteins is maximal at low levels of intake. When the intake of protein exceeds the requirement, the efficiency of utilization decreases rapidly. Since dietary protein

has become a critical food item world-wide, investigations of any dietary factor which could increase protein utilization would have potential nutritional and economic value.

The present study was undertaken to investigate the possibility that the utilization of dietary protein may be substantially improved or influenced by the presence of zinc. In addition, the possible effect of zinc on growth stimulation in the young rat fed a diet containing a sub-optimal level of protein was investigated.

CHAPTER II

REVIEW OF LITERATURE

The role of zinc as an important nutrient was first recognized in 1896 when Raulin demonstrated that it was necessary for growth of the mold Aspergillus niger (1). It was not until 1934, however, that Todd, Elvehjem, and Hart (2) produced evidence that zinc was necessary for the growth and well-being of the rat. In 1939 Keilen and Mann (3) showed that zinc was a constituent of the enzyme carbonic anhydrase. In 1955 Tucker and Salmon (4) discovered that zinc cures and prevents parakeratosis in pigs, and in 1958 O'Dell and coworkers (5) showed that zinc was required for growth and various functions in birds. Although zinc deficiency had been produced experimentally in rats, pigs, calves, lambs, dogs, turkey poults, and young Japanese quail, it was not until 1961 that Prasad (6) found evidence of nutritional deficiency of zinc in man.

Keilen and Mann (3) demonstrated that the enzyme carbonic anhydrase contains 0.33% of zinc and showed that the presence of zinc is essential in the elimination and incorporation of CO_2. Many enzymes were found to contain or to be activated by zinc. Zinc metalloenzymes of known metal content include: carboxypeptidase, alcohol dehydrogenase, glutamic acid, muscle lactic dehydrogenase, and kidney alkaline phosphatase (7). In addition, zinc activates a number of enzymes important in the metabolism of protein including: glycylglycine dipeptidase, arginase, dehydropeptidase, tripeptidase,

carnosinase, histidine deaminase, as well as oxaloacetic carboxylase and some lecithinases and enolases. Since such enzymes are present in plants and in animals, zinc is of great importance in agriculture as well as in husbandry.

The whole body of a normal 70 kg man contains 1.4 gm - 2.3 gm of zinc or an overall concentration of 20-30 ppm, which is 10-15 times that of body copper and less than half that of total body iron (1). Similar levels of zinc are found in the whole bodies of other mammals. Although there is little fetal storage of zinc, as with iron and copper, the young mammal can readily obtain zinc requirements with maternal milk, whereas zinc is 4-5 times richer in colostrum than in milk itself.

Although zinc occurs in all living cells in varying concentrations, significantly higher levels are found in portions of the eye, the male sex glands and their secretions, the hair, and the bones than in other tissues and fluids. Relatively high concentrations, ranging from 150-250 ppm, are found in bones and teeth. Considerable amounts are found in the hair, fur, or wool of animals. No less than 38% of the whole body zinc is found in the hair and skin of the rat (8). In tissues zinc occurs largely in combination with protein.

Little is known of the mechanism or of the sites of absorption of zinc. The ability of animals to absorb zinc varies with the chemical form or combination in which it is ingested. Different protein sources vary greatly in their capacity to supply zinc for needs of animals and humans.

Zinc requirements vary with the species, the chemical form or combination in which the metal occurs, the nature of the rest of the diet, and the criteria of adequacy employed (1). Forbes and Yohe (9) found markedly subnormal growth and other typical manifestations of zinc deficiency on basal diets supplying 7 ppm of zinc. When these diets contained casein or egg white as the protein source, a supplement of $ZnCO_3$ providing an additional 5 ppm was adequate. On a soybean basal diet, however, 11 ppm of zinc as $ZnCO_3$ was necessary to prevent symptoms of a zinc deficiency. These findings indicate a minimum zinc requirement for growing rats of 12 ppm on the former diets and 18 ppm on the latter. On the basis of zinc balance studies, Forbes and Yohe found that the minimum requirement in terms of absorbable zinc is 8-9 ppm.

Dietary zinc deficiency has been demonstrated in rats (10), pigs (4), poultry (11), cattle (12), sheep (13) and man (6). Follis et al (14) found that zinc deficiency resulted in retardation or failure of growth and alopecia accompanied by gross epithelial lesions, especially cutaneous lesions in rats and mice. Parakeratosis, a thickening or hyperkeratinization of epithelial cells of the skin and esophagus, together with atropic seminiferous tubules in the male were also observed. In more severely zinc deficient diets in rats, Forbes and Yohe (9) observed markedly subnormal growth, coarseness and loss of hair, dermatitis, scaling and cracking of paws, and erratic appetite. Retarded development of the testes, epididymes, prostate, and pituitary glands, with severe atrophy of the testicular germinal epithelium were also observed in males. Swenerton and Hurley (10)

in studies on zinc deficiency in pregnant rats observed congenital malformations that included cleft palate, short or missing mandible, curvature of the spine, clubbed feet, hydrocephalus, and heart, lung, and other congenital abnormalities.

Dietary deficiency in man as well as in experimental animals can cause extreme retardation of growth and of gonadal development. Prasad (6) found clinical manifestations in male subjects in Iran and Egypt, including short stature, marked hypogonadism, hepatosplenomegaly, and iron-deficiency anemia. The diet of these patients consisted almost exclusively of bread made of wheat flour, and the intake of animal protein was negligible. Growth retardation and gonadal hypofunction in these subjects were found to be related to zinc deficiency.

Several studies have shown that the source of protein in the diet has a marked effect upon the utilization of zinc in food (15). O'Dell and Savage (11) reported that zinc in soybean protein was less available to chickens than that in casein. Forbes and Yohe (9) made similar observations with rats and pigs. O'Dell (15) found that rats fed casein or egg white as the source of protein required approximately 12 ppm of zinc in the diet, while those fed soybean protein required 18 ppm. The apparent absorption of zinc by rats fed casein was 84% compared to 44% by those fed soybean protein. O'Dell and Savage (11) attributed the decreased availability of zinc from plant seed proteins to phytate.

The interrelationship between zinc and protein was investigated by Oberleas and Prasad in 1969 (16). They studied the effect of

supplementation of zinc to plant seed protein diets at several levels of dietary protein in weanling male rats. These studies showed that the addition of zinc to plant seed protein diets resulted in growth rates comparable to those achieved by the use of animal protein in similar amounts. Basic protein requirements for these animals were achieved at approximately a 14% level of dietary protein, and little was gained by feeding extra protein. Though a portion of decreased growth rate in zinc deficiency was attributed to the decreased food intake, highly significant differences were still demonstratable as a result of zinc supplementation. Oberleas and Prasad suggested that zinc supplementation of cereal diets consumed by large segments of the human population in many areas of the world may improve growth and well-being.

The utilization of dietary proteins is maximal at low levels of intake (17). When the intake of protein exceeds the requirement, the efficiency of utilization must decrease rapidly since protein cannot be stored in the body to any appreciable extent. Hegsted and Chang (17) studied protein utilization in growing rats at different levels of intake using lactalbumin, casein, soy protein, and wheat gluten. They concluded that the net nitrogen utilization is essentially constant for each of these proteins at all levels of intake up to those which will support approximately maximal growth.

In methods for biological evaluation of protein quality, growth is frequently one criterion considered (16). Decreased growth is one of the earliest manifestations of zinc inadequacy, and this decreased

growth can be partly accounted for on the basis of anorexia, which zinc deficient rats exhibit. Prasad et al (18) found that zinc deficient rats need more feed to gain an equal amount of weight than rats with sufficient zinc. Optimum utilization of feed and therefore of protein thus contains important economic aspects because of the high cost of protein.

Results of several investigations (19, 20, 21, 22, 23, 24) with microorganisms and experimental animals indicate that zinc participates in the synthesis of nucleic acids and protein since it has been shown that the synthesis of RNA, the incorporation of thymidine into DNA of rapidly growing tissue, and the utilization of amino acids in the synthesis of protein are decreased in zinc deficient systems. Interpretation of some of the available data suggest that the utilization of dietary protein may be substantially improved or influenced by the presence of zinc. Since dietary protein has become a critical food item, investigations of any dietary factor which could increase protein utilization would feasibly have potential economic and nutritional value.

CHAPTER III

EXPERIMENTAL PROCEDURES

The purposes of this study were to determine if the presence of zinc could improve the utilization of dietary protein by young rats and if the response of young rats to zinc supplementation were affected by the level of protein in the diet. Criteria for evaluating animal responses to various test diets used in the study included weight gain, hemoglobin concentrations, and the levels of copper, iron, and zinc in the liver.

Nine test diets, based on combinations of three levels of protein (7.5%, 15.0%, and 30.0%) and three levels of zinc supplements (0 ppm, 50 ppm, and 100 ppm) were used in the study. The following treatments were used to make the test diets:

Diet 1 - 7.5% Protein

Diet 2 - 7.5% Protein + 50 ppm Zn

Diet 3 - 7.5% Protein + 100 ppm Zn

Diet 4 - 15.0% Protein

Diet 5 - 15.0% Protein + 50 ppm Zn

Diet 6 - 15.0% Protein + 100 ppm Zn

Diet 7 - 30.0% Protein

Diet 8 - 30.0% Protein + 50 ppm Zn

Diet 9 - 30.0% Protein + 100 ppm Zn

The source of protein used for the diets was spray dried egg white solids,[1] and zinc carbonate was used as the source of the zinc supplements. In addition to egg white solids, the diets contained 20% of vegetable shortening,[2] 4% of mineral mix,[3] 2% of vitamin mix, 2% of cellulose,[4] and 24 drops of oleum percomorphum[5] per kilogram of diet. Varying amounts of dextrose[6] were used to bring the total composition of each diet to 2 kilogram. The composition of all diets used in the study is given in Appendix Table 1. Compositions of the mineral and vitamin mixtures used are given in Appendix Tables 2 and 3, respectively.

Diets 1, 4, and 7 were considered to be deficient in zinc. Wet weight analysis of each of these diets revealed that Diets 1 and 4 contained approximately 2 ppm of zinc and Diet 7 approximately 1 ppm. These levels were considerably lower than 7 ppm of zinc, the level at which Forbes and Yohe (9) found markedly subnormal growth and other typical manifestations of zinc deficiency.

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[1]Purchased from Teklad Mills, Chagrin Falls, Ohio.

[2]Crisco, Procter and Gamble Company, Cincinnati, Ohio.

[3]Salt Mixture W, ICN Pharmaceuticals, Cleveland, Ohio.

[4]Alphacel, ICN Pharmaceuticals, Cleveland, Ohio.

[5]Product of Mead Johnson and Company, Evansville, Indiana.

[6]Purchased from ICN Pharmaceuticals, Cleveland, Ohio.

Weanling male albino rats[7] were used for all phases of this study. The animals were randomized into replications according to initial body weight. Both the animals and the test treatments within a replication were assigned at random to individual wire-bottom stainless steel cages. The animals were given food ad libitum from glass jars and distilled water from pyrex bottles with stainless steel nipples. Weekly weight records were kept for all animals. The experiment lasted for 4 weeks.

At the end of the experiment, blood samples were taken from the tail of each animal and hemoglobin determinations were made using the method developed by Shenk et al (25). Rats from five randomly selected replications were sacrificed, and the livers removed and weighed. A small portion of each liver was dried at 50°C in a dry oven in order to provide dry weight data.

The livers were prepared for mineral analysis by ashing with nitric and perchloric acids on a hot plate. The ash of each sample was dissolved in 3 ml of 0.6 N HCl and brought to a volume of 25 ml with redistilled water. The method of McCall et al (26) was used to determine the zinc content of the livers. The methods of Parks et al (27) and Kitzes et al (28), as modified by Matrone et al (29), were used respectively to determine the copper and iron content of the livers. All data were subject to an analysis of variance.

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

CHAPTER IV

RESULTS AND DISCUSSION

Detailed data obtained from this study are presented in Appendix Tables 4-8. Analysis of variance of this data is given in Appendix Table 9. The mean values of weight gains, hemoglobin levels and the deposition of copper, iron, and zinc in the livers of young rats fed 7.5, 15.0, and 30.0 percent protein with and without added zinc are presented in tables within the text.

Growth

All animals receiving the zinc deficient diets which were not supplemented with extra zinc exhibited poor growth rates, regardless of the level of protein in the diet (Table 1). The addition of zinc to the diets was associated with highly significant increases ($p \leq 0.01$) in weight gains. The results also indicated that maximum growth was obtained with 50 ppm of zinc added to the diet. In the presence of adequate zinc, increasing levels of dietary protein were associated with highly significant increases ($p \leq 0.01$) in weight gains. The fact that additional growth was not obtained with 30 percent protein was not unexpected since it is well known that a level of 15 - 20 percent protein is considered adequate for optimum growth and that protein utilization tends to decrease as the level of protein is increased beyond 20 percent. A highly significant zinc-protein interaction ($p \leq 0.01$) was also associated with the growth data, and interpretation of the data would suggest the possibility that increasing the level of zinc above 50 ppm possibly has an adverse effect on protein utilization.

TABLE 1

EFFECT OF ZINC SUPPLEMENTS ON WEIGHT GAINS OF RATS[a] FED VARYING LEVELS OF DIETARY PROTEIN

Level of Dietary Protein (percent)	Level of zinc supplement (ppm) 0	50	100	Average of Mean
	Weight gain at 4 weeks (gm)[b]			
7.5	39	85	85	70
15.0	42	150	138	110
30.0	41	125	135	100
Average of Means	41	120	119	

[a]Sprague Dawley rats averaging 56 gm in weight initially.

[b]Each figure represents the mean of 7 animals.

Hemoglobin

Increasing the zinc level in the diets to 100 ppm was generally associated with corresponding decreases in hemoglobin levels which were significant at the 0.01 level of probability (Table 2). The level of dietary protein, however, had no apparent effect on hemoglobin formation.

Liver Copper

The addition of zinc supplements to diets deficient in zinc was associated with highly significant decreases ($p \leq 0.01$) in liver copper deposition (Table 3). Increases in dietary protein levels, however, were associated with highly significant increases ($p \leq 0.01$) in liver copper deposition. The level of protein, however, did not

TABLE 2

EFFECT OF ZINC SUPPLEMENTS ON HEMOGLOBIN LEVELS OF RATS FED VARYING LEVELS OF DIETARY PROTEIN

Level of Dietary Protein (percent)	Level of zinc supplement (ppm)[a] 0	50	100	Average of Mean
	gm/100 ml blood			
7.5	16.30	14.34[b]	13.26[b]	14.63
15.0	15.27	14.13	15.08	14.83
30.0	16.40	14.42	14.99	15.27
Average of Means	15.99	14.30	14.44	

[a]Each value is the mean of 7 animals unless otherwise indicated.

[b]Mean of 6 animals.

TABLE 3

EFFECT OF ZINC SUPPLEMENTS ON LIVER COPPER DEPOSITION OF RATS FED VARYING LEVELS OF DIETARY PROTEIN

Level of Dietary Protein (percent)	Level of zinc supplement (ppm)[a] 0	50	100	Average of Mean
	mcg/gm dry weight			
7.5	6.68	4.45	4.58	5.24
15.0	7.31[b]	4.35	5.81	5.82
30.0	11.65	7.89	7.23	8.92
Average of Means	8.55	5.56	5.87	

[a]Each figure is the mean of 5 animals unless otherwise indicated.

[b]Mean of 4 animals.

prevent the adverse effect of zinc on liver copper deposition. These data support the antagonistic interrelationship of zinc to copper that has been reported by several workers (30, 31, 32, 33, 34, 35) and suggest that this interrelationship may be possible with levels of zinc that are not considered toxic to the young rat.

Liver Iron

A highly significant decrease ($p \leq 0.01$) in liver iron deposition was observed since level of zinc in the diet increased regardless of the level of protein in the diet (Table 4). These data appear to be the first indication of an antagonistic interrelationship of zinc to iron involving physiological levels of zinc supplements. Increasing the dietary level of protein was associated with highly significant increases ($p \leq 0.01$) in liver iron deposition. The influence of protein on iron deposition, however, was markedly reduced when zinc was added to the system. Based on these data, it would appear that if zinc is deficient in the diet, the animal may incorporate extremely high levels of iron into the liver, a condition which could have an adverse effect on the animal system.

Liver Zinc

The level of zinc in the diet did not appear to have as much of an effect on liver zinc deposition as did the level of protein (Table 5). Increasing the protein level from 7.5% to 30.0% resulted in a highly significant increase ($p \leq 0.1$) in liver zinc accumulation. An increase in liver zinc deposition was observed with zinc supplementation only

TABLE 4

EFFECT OF ZINC SUPPLEMENTS ON LIVER IRON DEPOSITION OF RATS FED VARYING LEVELS OF DIETARY PROTEIN

Level of Dietary Protein (percent)	Level of zinc supplement (ppm)[a] 0	50	100	Average of Mean
	mcg/gm dry weight			
7.5	483.56	223.96	248.12	318.55
15.0	755.49[b]	373.68	309.74	479.64
30.0	860.97	339.71	344.39	515.02
Average of means	700.01	312.45	300.75	

[a]Each figure is the mean of 5 animals unless otherwise indicated.

[b]Mean of 4 animals.

TABLE 5

EFFECT OF ZINC SUPPLEMENTS ON LIVER ZINC DEPOSITION OF RATS FED VARYING LEVELS OF DIETARY PROTEIN

Level of Dietary Protein (percent)	Level of zinc supplement (ppm)[a] 0	50	100	Average of Mean
7.5	45.34	29.65	38.66	37.88
15.0	64.71[b]	44.26	53.38	54.12
30.0	59.64	60.67	75.71	65.34
Average of means	56.56	44.86	55.92	

[a]Each figure is the mean of 5 animals unless otherwise indicated.

[b]Mean of 4 animals.

when the diet contained 30.0% of protein. At the two lower levels of dietary protein, the addition of zinc appeared to have an adverse effect on liver zinc deposition.

CHAPTER V

GENERAL DISCUSSION

Although the growth of rats fed diets containing 7.5% protein did not reach a maximum during the length of the experimental period, the significant improvement in weight gain when 50 ppm of zinc was added to a diet containing 7.5% protein suggests that zinc apparently improves the utilization of an inadequate level of protein in the diet. The exact mechanism of how zinc might be involved is not apparent from this study, but the results do suggest that further investigation of this particular phenomenon with other types of protein materials is warranted.

Results of this study also indicate that an optimum level of zinc to be considered for diets of the type used in this study is approximately 50 ppm. It is possible this level could be lower since other researchers have obtained maximum growth in young animals with lower levels of supplemental zinc. A level of 100 ppm, however, could be considered to be toxic in view of the results of this study.

Although 50 ppm of zinc was associated with marked decrease in hemoglobin formation, conditions of anemia apparently did not develop. Since the presence of supplemental zinc was associated with decreased deposition of copper and iron in the liver, there is the possibility that the stimulation in growth resulting from the zinc supplements resulted in a concomitant increase in blood volume. The copper and iron reserves of the system, however, may be marginal to deficient,

and since the diet did not provide extra copper and iron, there is not sufficient copper and iron present for the manufacture of hemoglobin to maintain certain levels in the rapidly growing animal. There is also the possibility that zinc in the system at any level interferes in some way with the synthesis of hemoglobin.

The addition of zinc supplements to diets deficient in zinc was associated with highly significant decreases in liver copper deposition, while increases in dietary protein were associated with highly significant increases in liver copper deposition regardless of the level of protein. Although the antagonistic interrelationship between zinc and copper in zinc toxicity has been reported by several workers, these data suggest an antagonistic interrelationship between zinc and copper at the level of 50 ppm of zinc, which was considered optimum for diets in this study. Further investigation of this antagonistic interrelationship should be considered at lower levels of zinc supplementation.

Results of this study also indicate an antagonistic interrelationship between zinc and iron. Liver iron deposition showed a marked decrease as the level of zinc increased. If zinc is deficient in the diet, however, it appears that high levels of liver iron are deposited, and these levels increase with increased dietary protein. These high levels of liver iron in zinc deficiency could have an adverse affect on the animal system. The data seem to indicate that zinc actually protects against iron toxicity. Further investigation should be considered at levels of zinc below 50 ppm.

The level of liver zinc deposition increased markedly with an increase in the protein level regardless of the level of dietary zinc. Zinc supplementation did not appear to affect liver zinc deposition except when the diet contained 30.0% protein. At this level there was an increase in liver zinc deposition with zinc supplementation at both levels of zinc. Since 30.0% protein is well above the optimum level of protein, further investigation does not seem warranted.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

Young male rats were fed diets containing various levels of zinc at various levels of protein. The effects of zinc and protein on growth, hemoglobin, and liver copper, iron, and zinc deposition were observed.

Results indicated that all animals receiving the zinc deficient diets exhibited poor growth rates regardless of the level of dietary protein. In the presence of adequate zinc, increasing levels of dietary protein were associated with highly significant increases in weight gain. Even when zinc was added to the diet containing a sub-optimal level of protein there was significant improvement in weight gain. There was also a highly significant zinc-protein interaction with respect to growth. Increasing the level of zinc above 50 ppm, however, appears to have an adverse effect on protein utilization.

Zinc supplementation resulted in a significant decrease in the high hemoglobin levels associated with zinc deficiency. The level of dietary protein had no apparent effect on hemoglobin formation.

Zinc supplementation was associated with highly significant decreases in liver copper. An antagonistic interrelationship therefore appears to exist between zinc and copper at the optimum level of zinc for diets in this study.

Liver iron deposition showed a marked decrease associated with zinc supplementation indicating an antagonistic interrelationship between zinc and iron. In zinc deficiency, however, high levels of liver iron are accentuated when dietary protein is increased. The data seem to indicate, therefore, that zinc actually protects against iron toxicity.

The level of zinc in the diet did not appear to have as much effect on liver zinc as did the level of dietary protein. A highly significant increase in liver zinc deposition was associated with an increase in the protein level.

Recommendations for Additional Investigation

The present study dealt with a high quality animal protein and demonstrated that normal growth could be obtained in young rats when 50 ppm of zinc was added to zinc-deficient diets. Since results also showed substantial weight gain associated with zinc supplementation at the sub-optimal protein level of 7.5%, additional studies could be done to determine growth at levels of protein between 7.5% and 15.0%, the point of maximal growth in this experiment. Zinc supplements from 0 to 50 ppm could be used since maximal growth was found at the level of 50 ppm of zinc.

Though zinc-deficient rats exhibit anorexia, which is partly responsible for depressed growth, highly significant differences in weight gain were still demonstratable in this study as a result of zinc supplementation. Studies conducted using paired feeding would be of value in distinguishing between the primary effects of reduced food intake.

This study investigated the effect of zinc supplements on the utilization of an animal protein, egg white solids (spray dried). The effect of zinc supplements on the utilization of other types of protein from both animal and plant sources could be investigated. The level of zinc required for utilization of certain plant proteins, particularly those containing phytic acid, may be higher than that found in this study using an animal protein.

Studies could be undertaken to see if zinc supplements could improve the utilization of a protein which is considered to be of poor quality because of an improper amino acid pattern or a limiting amino acid(s). Studies also could be undertaken to determine if zinc could improve the absorption of amino acids, enhance the synthesis of protein, or prevent amino acid catabolism, a phenomenon which occurs when the amino acids of the diet cannot be used for protein synthesis. Nitrogen balance trials to determine the effect of zinc on nitrogen retention may also be warranted.

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APPENDIX

TABLE 1

COMPOSITION OF INDIVIDUAL DIETS

Treatments

1 - 7.5% Protein
2 - 7.5% Protein + 50 ppm Zn
3 - 7.5% Protein + 100 ppm Zn
4 - 15.0% Protein
5 - 15.0% Protein + 50 ppm Zn
6 - 15.0% Protein + 100 ppm Zn
7 - 30.0% Protein
8 - 30.0% Protein + 50 ppm Zn
9 - 30.0% Protein + 100 ppm Zn

	Diets								
Constituents	1	2	3	4	5	6	7	8	9
	gm/2 kg								
Egg white solids	188	188	188	375	375	375	750	750	750
Dextrose	1452	1452	1452	1265	1265	1265	890	890	890
Vegetable Shortening	200	200	200	200	200	200	200	200	200
Mineral mix	80	80	80	80	80	80	80	80	80
Vitamin mix	40	40	40	40	40	40	40	40	40
Cellulose	40	40	40	40	40	40	40	40	40
Zinc carbonate	0	0.192	0.384	0	0.192	0.384	0	0.192	0.384
Oleum percomorphum	48 drops/2 kg in all diets								

TABLE 2

COMPOSITION OF SALT MIXTURE - W[a]

Constituent	Per cent
Calcium carbonate	21.000
Copper sulfate (5 H_2O)	0.039
Ferric phosphate	1.470
Manganous sulfate (anhyd.)	0.020
Magnesium sulfate (anhyd.)	9.000
Potassium aluminum sulfate	0.009
Potassium chloride	12.000
Potassium dihydrogen phosphate	31.000
Potassium iodide	0.005
Sodium chloride	10.500
Sodium fluoride	0.057
Tricalcium phosphate	14.900

[a]Product of ICN Pharmaceuticals, Cleveland, Ohio.

TABLE 3

COMPOSITION OF VITAMIN MIXTURE

Constituents	Amount per 100 gm mix
	mg
Biotin	1.0
Folic acid	5.0
Thiamine HCl	25.0
Pyridoxine HCl	25.0
Menadione (2-methyl-naphthoquinone)	50.0
Riboflavin	50.0
Nicotinic acid	50.0
Ca pantothenate	150.0
p-aminobenzoic acid	500.0
	gm
0.1% Vitamin B_{12} (mannitol trituration)	0.1
Inositol	5.0
Choline chloride	7.5
DL-methionine	30.0
Corn starch	56.6

TABLE 4

GROWTH DATA

Treatments

1 - 7.5% Protein
2 - 7.5% Protein + 50 ppm Zn
3 - 7.5% Protein + 100 ppm Zn
4 - 15.0% Protein
5 - 15.0% Protein + 50 ppm Zn
6 - 15.0% Protein + 100 ppm Zn
7 - 30.0% Protein
8 - 30.0% Protein + 50 ppm Zn
9 - 30.0% Protein + 100 ppm Zn

Treatments	Replications 1	2	3	4	5	6	7	Mean
	Growth Weight gain at 4 weeks (gm)							
1	33	44	43	49	44	32	28	39
2	77	59	102	88	84	96	92	85
3	81	104	77	63	86	87	101	85
4	32	31	46	50	38	41	56	42
5	171	136	139	159	167	131	149	150
6	136	155	110	117	156	142	152	138
7	46	45	47	35	40	42	30	41
8	142	131	122	139	115	103	123	125
9	103	139	145	130	144	141	141	135

TABLE 5

HEMOGLOBIN DATA

Treatments

1 - 7.5% Protein
2 - 7.5% Protein + 50 ppm Zn
3 - 7.5% Protein + 100 ppm Zn
4 - 15.0% Protein
5 - 15.0% Protein + 50 ppm Zn
6 - 15.0% Protein + 100 ppm Zn
7 - 30.0% Protein
8 - 30.0% Protein + 50 ppm Zn
9 - 30.0% Protein + 100 ppm Zn

Treatments	Replications 1	2	3	4	5	6	7	Mean
	Hemoglobin gm/100 ml blood							
1	14.94	14.23	15.92	19.09	18.23	14.86	16.83	16.30
2	(14.32)[a]	12.26	15.26	13.94	13.77	13.86	16.97	14.34
3	12.00	12.52	(13.00)[a]	12.77	13.49	12.43	16.37	13.26
4	16.83	16.60	13.57	10.12	14.94	15.49	19.37	15.27
5	13.49	13.86	13.66	14.14	13.49	15.14	15.14	14.13
6	16.26	13.03	13.86	14.86	15.37	14.34	17.83	15.08
7	15.80	14.23	18.23	15.26	17.09	17.57	16.60	16.40
8	15.49	12.26	14.23	14.03	14.86	14.14	15.92	14.42
9	14.86	14.94	14.43	12.26	13.77	14.86	19.83	14.99

[a]( ) indicates calculated missing plot.

TABLE 6

LIVER COPPER DATA

Treatments[a]

1 - 7.5% Protein
2 - 7.5% Protein + 50 ppm Zn
3 - 7.5% Protein + 100 ppm Zn
4 - 15.0% Protein
5 - 15.0% Protein + 50 ppm Zn
6 - 15.0% Protein + 100 ppm Zn
7 - 30.0% Protein
8 - 30.0% Protein + 50 ppm Zn
9 - 30.0% Protein + 100 ppm Zn

Treatments	Replications 1	2	3	4	5	Mean
	Copper mcg/gm dry weight					
1	5.18	11.47	7.19	4.88	4.68	6.68
2	6.01	5.36	5.36	3.32	2.20	4.45
3	1.58	8.77	3.33	4.89	4.34	4.58
4	6.01	(10.43)[a]	7.44	8.93	6.87	7.31
5	5.17	6.01	4.86	4.06	1.65	4.35
6	3.10	5.73	4.95	6.98	8.29	5.81
7	12.37	18.16	8.52	11.56	7.65	11.65
8	4.90	10.84	3.32	12.08	8.31	7.89
9	9.24	6.23	10.05	1.71	8.94	7.23

[a]( ) indicates calculated missing plot.

TABLE 7

LIVER IRON DATA

Treatments[a]

1 - 7.5% Protein
2 - 7.5% Protein + 50 ppm Zn
3 - 7.5% Protein + 100 ppm Zn
4 - 15.0% Protein
5 - 15.0% Protein + 50 ppm Zn
6 - 15.0% Protein + 100 ppm Zn
7 - 30.0% Protein
8 - 30.0% Protein + 50 ppm Zn
9 - 30.0% Protein + 100 ppm Zn

Treatments	Replications 1	2	3	4	5	Mean
	Iron mcg/gm dry weight					
1	452.95	441.91	520.61	599.16	403.16	483.56
2	258.24	210.74	239.21	174.20	237.40	223.96
3	240.67	260.51	280.74	219.04	239.62	248.12
4	458.91	(730.28)[a]	933.91	770.82	858.32	755.49
5	264.02	308.28	462.56	499.23	334.30	373.68
6	387.06	225.56	313.84	413.13	209.13	309.74
7	752.00	945.31	841.90	785.56	980.10	860.97
8	359.36	280.31	349.47	365.85	343.55	339.71
9	325.55	350.18	304.84	402.63	338.74	344.39

[a]( ) indicates calculated missing plot.

TABLE 8

LIVER ZINC DATA

Treatments[a]

1 - 7.5% Protein
2 - 7.5% Protein + 50 ppm Zn
3 - 7.5% Protein +100 ppm Zn
4 - 15.0% Protein
5 - 15.0% Protein + 50 ppm Zn
6 - 15.0% Protein +100 ppm Zn
7 - 30.0% Protein
8 - 30.0% Protein + 50 ppm Zn
9 - 30.0% Protein +100 ppm Zn

Treatments	Replications 1	2	3	4	5	Mean
	Zinc mcg/gm dry weight					
1	38.85	44.82	50.05	55.88	37.08	45.34
2	21.26	37.86	31.51	27.84	29.80	29.65
3	30.34	25.98	46.81	44.27	45.90	38.66
4	44.06	(60.47)[a]	65.54	55.74	93.50	64.71
5	45.84	27.41	52.79	42.96	52.32	44.26
6	35.37	45.36	59.75	49.76	76.64	53.38
7	62.75	58.67	47.30	48.62	80.85	59.64
8	58.84	54.21	55.93	63.84	70.54	60.67
9	73.00	85.89	59.18	47.53	112.95	75.71

[a]( ) indicates calculated missing plot.

TABLE 9

ANALYSIS OF VARIANCE OF WEIGHT GAIN, HEMOGLOBIN, AND LIVER COPPER, IRON, AND ZINC DATA

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Weight Gain			
Total	62	124698	
Replications	6	373	62
Treatments	8	115207	14401**
Zn	2	88045	44023**
Protein	2	18374	9187**
Zn x Protein	4	8788	2197**
Error	48	9118	190
Hemoglobin			
Total	60	227	
Replications	6	67	11
Treatments	8	59	7**
Zn	2	37	19**
Protein	2	5	2
Zn x Protein	4	17	4
Error	46	101	2
Liver Copper			
Total	43	500	
Replications	4	72	18
Treatments	8	218	27**
Zn	2	93	46**
Protein	2	113	57**
Zn x Protein	4	12	3
Error	31	210	7

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

TABLE 9--Continued

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Liver Iron			
Total	43	2283544	
Replications	4	45019	11255
Treatments	8	1997133	249642**
Zn	2	1535520	767760**
Protein	2	326937	163469**
Zn x Protein	4	134676	33669**
Error	31	241392	7787
Liver Zinc			
Total	43	15097	
Replications	4	2482	620
Treatments	8	8094	1012**
Zn	2	1263	631*
Protein	2	5703	2852**
Zn x Protein	4	1127	282
Error	31	4521	

*Significant ($p \leq 0.05$).

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