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EFFECTS OF ZINC AND VITAMIN B-6 SUPPLEMENTATION ON GROWTH AND MINERAL DEPOSITION OF YOUNG RATS FED VARIOUS LEVELS OF PROTEIN

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EFFECTS OF ZINC AND VITAMIN B-6 SUPPLEMENTATION ON GROWTH AND MINERAL DEPOSITION OF YOUNG RATS FED VARIOUS LEVELS OF PROTEIN

by

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Approved by

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APPROVAL PAGE

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TABLE OF CONTENTS

LIST OF TABLES

TABLE | Page

TABLE | Page

TABLE | Page

SON, SOOK MEE, Ph.D. Effects of Zinc and Vitamin B-6 Supplementation on Growth and Mineral Deposition of Young Rats Fed Various Levels of Protein. (1984)
Directed by Dr. Aden C. Magee. 102 pp.

The purpose of this study was to investigate the effects of zinc and vitamin B-6 supplementation on growth and mineral deposition of young rats fed various levels of protein. Dietary factors included three levels of protein (7.5, 15, and 30%), three levels of vitamin B-6 (1, 5, and 100 ppm), and three levels of zinc supplements (0, 50, and 100 ppm). Criteria used for evaluating animal responses to various test diets included weight gain, hemoglobin level, and copper, iron, and zinc deposition in the liver.

Results indicated that the addition of 50 ppm of zinc to the diet resulted in significant increase ($p < .01$) in weight gain, regardless of dietary protein level or vitamin B-6 supplementation. Increase in weight gains associated with increases in protein level occurred only when the diet contained adequate zinc, while increases in weight gains associated with increases in vitamin B-6 occurred only when the level of protein was marginal to inadequate (7.5%). Increasing levels of dietary protein were associated with highly significant increases ($p < .01$) in hemoglobin concentrations, while increases in zinc supplements resulted in highly significant decreases ($p < .01$) in hemoglobin levels.

As the level of dietary protein increased, the amount of copper deposited in the liver increased ($p < .01$). A 50 ppm zinc supplement

resulted in marked decreases in liver copper deposition, while increases in vitamin B-6 supplementation were associated wtih increases in liver copper deposition ($p < .05$). Maximum copper concentrations were observed in rats fed diets supplemented with 5 ppm of vitamin B-6.

Increasing the protein level of the diet was associated with increases in liver iron deposition ($p < .01$), while increases in zinc supplementation resulted in decreases in liver iron deposition ($p < .01$). Additional vitamin B-6 had no apparent effect on liver iron deposition in this study.

Liver zinc deposition was increased when the protein level was increased from suboptimal (7.5%) to adequate (15%). Vitamin B-6 and zinc supplementation also stimulated increases in liver zinc deposition ($p < .01$).

CHAPTER I

INTRODUCTION

Two nutrients which have essential roles related to protein and amino acid metabolism are vitamin B-6 and zinc, and deficiencies of either nutrient result in abnormalities in protein metabolism. The requirements for either vitamin B-6 or zinc are also dependent upon the quality and the quantity of dietary protein.

Pyridoxal phosphate, the phosphorylated form of vitamin B-6 is an important coenzyme for enzyme systems associated with amino acid absorption and metabolic reactions involving amino acids. Although an impairment of amino acid absorption results from a vitamin B-6 deficiency, the major effect of a deficiency of this vitamin would be the improper utilization of amino acids for biosynthetic and regulatory purposes. Increases in amino acid metabolism associated with growth processes or the utilization of dietary and body protein for energy would increase the need for vitamin B-6 in the diet.

Zinc has been identified as a mineral closely related to many aspects of growth, and the absence of zinc in the diet results in an impairment in growth and in the utilization of amino acids for protein biosynthesis. The need for zinc is particularly critical during early stages of growth in many animal species, including humans.

Since both of these nutrients are closely identified with the metabolism of protein, there is the possibility that certain interrelationships could exist between vitamin B-6 and zinc. There is also the possibility that the presence or absence of one nutrient in the system may have an effect on the action or function of the other nutrient. Support for such a possibility has been suggested from studies which have shown changes in the activities of some zinc metalloenzymes and insulin in vitamin B-6 deficient rats (Beaton, 1955; Beaton & Goodwin, 1954; Tulpule & Patwardhan, 1952). Some studies (Evans & Johnson, 1981; Hsu, 1965) indicated that the absorption and organ uptake of zinc was decreased when the diet was deficient in vitamin B-6. With the exception of these few studies, information pertaining to an interrelationship between vitamin B-6 and zinc is limited.

The purpose of this study was to investigate the effects of vitamin B-6 and zinc on growth and development of young rats fed varying levels of protein.

CHAPTER II

REVIEW OF LITERATURE

The relationship between dietary zinc and protein utilization has been reported by several researchers, and zinc supplementation significantly improved growth and apparent protein utilization in chickens, pigs, and rats (Hardie-Muncy & Rassmussen, 1979; Magee & Grainger, 1979; Motsinger & Magee, 1980; Oberleas & Prasad, 1969; O'Dell, 1969; O'Dell, Newberne, & Savage, 1958; O'Dell & Savage, 1957). Magee and Grainger (1979) also reported that a 50 ppm level of zinc supplement improved the apparent utilization of suboptimal levels of egg white protein by young rats.

Several studies have shown that amino acid utilization and/or incorporation are impaired in zinc-deficient animals. Increased oxidation of cystine, leucine, lysine, and methionine have been reported in zinc-deficient rats, and these oxidations are lessened when zinc supplements are added (Hsu, Anthony, & Buchanan, 1969; Hsu & Woosley, 1972; Theuer & Hoekstra, 1966). Hsu, Anthony, and Buchanan (1969) also reported that methionine incorporation into tissue proteins was decreased in zinc-deficient rats, while Swenerton, Shrader and Hurley (1969) found that zinc supplements stimualted the incorporation of ^{3}H-thymidine into regenerating livers of rats. Thompson, Griminger, and Evans (1976) observed decreased nitrogen losses in traumatized mature rats when the level of zinc in the diet was increased. Greger, Abernathy, and Bennett (1978), however, reported that nitrogen retention in adolescent females was not significantly affected by zinc intake.

Murthy, Klevay, and Petering (1974) reported that the zinc levels of aorta, liver, and heart did not change with either dietary zinc or copper, but the copper contents of liver, heart, and aorta responded to variations of dietary zinc or copper. Magee and Grainger (1979) reported a possible antagonistic interrelationship between zinc and copper and between zinc and iron when levels of dietary zinc, not generally considered toxic, were fed to young rats.

Vitamin B-6 also has a significant influence on protein and amino acid metabolism since pyridoxal phosphate is an important coenzyme involved with amino acid absorption, metabolism, synthesis, and utilization (Everett, Mitchell, & Benevenga, 1979; Hawkins, Leonard, & Coles, 1959). Vitamin B-6 requirements also appear to be dependent upon the quantity and quality of dietary protein as reported by several studies (Cerecedo & Foy, 1944; Cerecedo, Foy, & DeRenze, 1948; Dirige & Beaton, 1969; Schweigert, Sauberlich, Elvehjem, & Bauman, 1946). Daghir and Shah (1973) reported increase in dietary vitamin B-6 resulted in significant increases in weight gains, feed efficiency, and serum asparate transferase activity in chicks fed high levels (20 and 25%) of protein. Increased dietary levels of vitamin B-6 were also associated wtih increases in nitrogen retention in older chickens, while marked increases in nitrogen excretion were observed in chickens fed diets deficient in vitamin B-6 (Berger, Gebhardt, & Hoffman, 1973). Mice fed vitamin B-6 deficient, high protein diets also had lower tissue vitamin B-6 levels and higher mortality rates than did mice fed vitamin B-6 deficient, low protein diets (Miller & Bauman, 1945; Schweigert et al. 1946).

There is also some evidence that the absorption and/or retention of zinc is influenced by the presence of vitamin B-6. Hsu (1965) reported that zinc levels of plasma, liver, pancreas, and heart were lower in rats fed vitamin B-6 deficient diets than in animals fed adequate vitamin B-6 levels. Hsu (1965) also observed that the uptake of radioactive zinc by the plasma and liver of pyridoxine-deficient rats was significantly higher than that of their respective controls. These results are similar to the findings of Zeigler, Leach, Scott, Huegin, McEvoy and Strain (1964) that zinc-deficient rats retained more injected radioactive zinc in their tissues than those receiving a zinc-supplemented diet.

Prasad, Lyall, and Nath (1982) reported that a chronic vitamin B-6 deficiency resulted in a nonspecific increase in the in vitro intestinal uptake of zinc by rats. Evans and Johnson (1981) reported that daily zinc excretion decreased and zinc balance increased as the concentration of vitamin B-6 in the diet was increased. Daily absorption of zinc was greater in rats fed 10 or 40 ppm vitamin B-6 than in rats fed 2 and 4 ppm vitamin B-6.

Ikeda, Hosotani, Ureda, Kotake, and Sakakibara (1979), however, reported that conventional and germ-free rats fed vitamin B-6 deficient diets had essentially the same levels of zinc in pancreas, lungs, and testes as animals fed adequate vitamin B-6. The zinc contents of the kidneys of conventional rats and of the livers and spleens of the germ-free rats were increased when the diet was deficient in vitamin B-6. Gershoff (1968) reported no significant differences in tissue zinc, magnesium, or potassium in fasted control rats when compared to fasted vitamin B-6 deficient animals. Gubler, Cartwright, and Wintrobe (1947) reported that the absorption of copper and iron was increased in pyridoxine-deficient rats.

The purpose of this study was to investigate the effects of zinc and vitamin B-6 supplements on young rats fed low, adequate, and high levels of protein.

CHAPTER III

EXPERIMENTAL PROCEDURES

The purpose of this study was to determine the effects of zinc and vitamin B-6 supplementation on growth and copper, iron, and zinc status of young rats. Criteria used for the evaluation of the animal responses to various test diets included weight gain, hemoglobin concentration, and the levels of copper, iron, and zinc in the liver.

A 3^3 factorial design was utilized for the study, and the factors included were three levels of protein (7.5, 15, and 30%), three levels of vitamin B-6 (1, 5, and 100 ppm), and three levels of zinc supplements (0, 50, and 100 ppm). The source of protein was dried egg white solids.[1] Other dietary ingredients included 39 to 71% starch[2] (depending upon protein level), 10% vegetable oil,[3] 4% mineral mix,[4] 2% vitamin mix (minus vitamin B-6), 2% cellulose,[5] and 24 drops of oleum percomorphum[6] per kilogram of diet. Vitamin B-6 was provided

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[1]Teklad Test Diets, Madison, Wisconsin.

[2]Teklad Test Diets, Madison, Wisconsin.

[3]Hydrogenated Vegetable Oil, Teklad Test Diets, Madison, Wisconsin.

[4]Wesson Modified Osborne-Mendel Mineral Mix, Teklad Test Diets, Madison, Wisconsin.

[5]Alphacel, ICN Nutritional Biochemicals, Cleveland, Ohio.

[6]Mead Johnson and Company, Evansville, Indiana. The composition of this product is listed as 1250 USP units of vitamin A and 180 units of vitamin D per drop.

as pyridoxine hydrochloride, and zinc carbonate was the source of zinc. Analysis of the diets revealed that each contained approximately 4 ppm of copper and 77 ppm of iron. The non-zinc-supplemented diets contained approximately 2.4 ppm of zinc. The compositions of the mineral mix and the vitamin mix used in the study are given in Appendix A.

Small aliquots of each diet were collected periodically during the experimental period and combined into a composite sample. The diet samples were kept in a refrigerator until they could be analyzed for mineral content.

Two hundred and forty-three male weanling rats,[7] averaging 58 grams in weight initially, were used for the study. The animals were randomly assigned to nine replications according to initial body weight, and each animal was housed in a stainless steel wire bottom cage.

The 27 test diets were also randomly assigned to individual cages within each replication. Food and distilled water were given _ad libitum_, and individual food consumption records were kept on each animal. The animals were weighed at weekly intervals, and the weight gains were calculated.

A blood sample was taken from the tail of each animal after the four-week experimental period, and hemoglobin determinations were made on these samples by the method of Shenk, Hall, and King (1934). Animals from five randomly selected replications within treatment

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

were sacrificed, and the liver from each animal was removed. A small sample (0.5-1.0 gram) of each liver was removed and dried in an oven at 60° C to determine dry weight of the liver. The remainder of each liver was ashed with nitric and perchloric acids on a hot plate, and the ashed liver sample was dissolved in 3 ml of 0.6N HCL and diluted to 25 ml with redistilled water. Representative samples (approximately 1 gram) of the diets were also ashed and diluted in a similar manner.

Copper and iron determinations were made on the liver and diet samples by the methods of Kitzes, Elvehjem, and Schuette (1944) and Parks, Hood, Hurwitz, and Ellis (1943), as modified by Matrone, Peterson, Baxley, and Grinnels (1947), respectively. An atomic absorption spectrophotometer[8] was used for the determination of zinc in the livers and diets.

Some animals died before the end of the experimental period, and for the purpose of analysis an estimated parameter value was used for weight gain and hemoglobin concentrations of these missing animals. The estimator used was the mean of the animals receiving the diet common to the missing animals.

All data were analyzed by standard analysis of variance methods (Snedecor & Cochran, 1980). In order to determine if the differences in weight gains of animals were due to treatments or to food consumption, the weight gain data were subjected to a covariance analysis with total food consumed by an animal as the covariable. Significant differences between diet means were determined with the Least

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[8]Model 551, Instrumentation Laboratories, Wilmington, Massachusetts.

Significant Difference (LSD) and the Duncan's Multiple Range Test (Agricultural Research Service, USDA, 1957). In view of the physical limitations of the animal facilities, the study had to be conducted over three different four-week time intervals. The decision was made to confound the three time periods with protein level. Combining the data into one analysis of variance provided for tests of the protein x zinc and the protein x vitamin B-6 interaction and three factor interaction. Combination of the data for one analysis of variance also increased the sensitivity of the individual analysis and improved the precision of the tests.

CHAPTER IV
RESULTS

Detailed data obtained from this study are presented in Appendix B. Results of the statistical analyses are given in Appendix C, and group mean tables designed to assist with the interpretation of the statistical results are presented in Appendix D.

Weight Gain

All animals receiving non-zinc-supplemented diets exhibited poor growth, regardless of the level of dietary protein (Table 1). Increasing the level of dietary zinc was associated with highly significant increases ($p < .01$) in weight gains (Appendix C, Table C-1). The maximum growth in rats observed in this study was obtained wtih 50 ppm of zinc supplement, while increasing the zinc supplement to 100 ppm did not stimulate additional growth above that observed with the 50 ppm supplement (Appendix D, Table D-1). Zinc apparently stimulated growth in rats better when the level of dietary protein was adequate (15%) than when dietary protein was marginal (7.5%) or in excess (30%), because the average weight gain of rats fed the 15% protein level supplemented with zinc was approximately 20 grams higher than the average weight gains of rats fed either the zinc-supplemented low protein or zinc-supplemented high protein diets (Appendix D, Table D-2).

Increasing the vitamin B-6 level of the diets from 1 to 5 ppm resulted in a significant increase in weight gain (Appendix D,

Table 1

Effects of Zinc and Vitamin B-6 Supplements on Growth of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm) 0	50	100
		4 Week Weight Gain (gm)[1]		
7.5	1	41 ± 17	115 ± 15	115 ± 19[2]
	5	39 ± 10	132 ± 26	135 ± 14
	100	40 ± 12	137 ± 16	133 ± 25
15.0	1	46 ± 15[2]	140 ± 26	143 ± 25
	5	44 ± 5	147 ± 19	159 ± 12
	100	54 ± 15[2]	153 ± 11	146 ± 17
30.0	1	28 ± 13	113 ± 16	108 ± 17
	5	38 ± 12	123 ± 20[3]	106 ± 25[2]
	100	38 ± 13	122 ± 23	122 ± 24[2]
	LSD[4] 0.05: 17			
	LSD 0.01: 22			

[1]Each value is the mean of 9 animals ± SEM, unless otherwise indicated.

[2]Mean of 8 animals ± SEM.

[3]Mean of 7 animals ± SEM.

[4]Least significant differences at specified probability levels.

Table D-1), but the effects of vitamin B-6 supplementation was only noted when rats were fed a suboptimal level (7.5%) of protein or when the rats were fed at least 50 ppm of zinc (Appendix D, Tables D-3 and D-4). Vitamin B-6 supplementation had no apparent effect on weight gains of animals fed either 15 or 30% protein, regardless of the level of dietary zinc (Appendix D, Table D-4). The results of this study also suggest that as the protein level of the diet was increased or as the dietary level decreased, the influence of vitamin B-6 on growth was diminished. Interpretation of a significant protein x zinc interaction ($p < .05$) suggests when dietary protein was in excess (30%), the stimulatory effect of zinc on weight gain was not as great as it was when the protein level was either adequate (15%) or suboptimal (7.5%) (Appendix D, Table D-2).

Animals receiving 15% protein diets had significantly higher weight gains than animals fed diets containing 7.5% protein. Increasing the dietary protein level to 30% did not result in additional weight gains, and animals fed this protein level actually exhibited lower weight gains than animals fed 15% protein. These results were similar to the findings of Magee and Grainger (1979). Although the reason for the poorer growth observed in animals fed 30% protein is not clear, many of these animals did develop diarrhea during the latter stages of the experimental period. There is the possibility that this condition affected their ability to gain weight.

Although the animals fed the zinc-supplemented diets generally consumed more food during the experiment than did rats consuming the non-zinc-supplemented diets, adjusting the weight gains for food consumption by covariance analysis (Appendix C, Table C-2) revealed the effects of the diets on growth were still significant. Thus, it would appear that the differences in weight gains observed in animals fed the various dietary regimens were not due to differences in food consumption alone.

Hemoglobin Levels

The effects of zinc and vitamin B-6 supplements on hemoglobin levels of young rats fed varying levels of dietary protein are shown in Table 2. Although most of the hemoglobin values observed in animals fed the various dietary regimens were within normal ranges (13-17 gm/100 ml) for rats, analysis of the data revealed that increasing levels of dietary protein were associated with highly significant increases ($p < .01$) in hemoglobin concentrations (Appendix C, Table C-3). The highest overall hemoglobin levels were observed in animals which were fed the 30% protein diets (Appendix D, Table D-5). Increasing levels of dietary zinc were associated with highly significant decreases ($p < .01$) in hemoglobin concentrations in this study (Appendix C, Table C-3), although the significant adverse effect of zinc on hemoglobin level was not as great when the dietary level of protein was 30% (Appendix D, Table D-6). The effect of zinc on hemoglobin concentration is similar to the results of others (Magee & Grainger, 1979; Motsinger & Magee, 1980) and is probably being mediated through an effect on copper and/or iron metabolism. Vitamin B-6 supplements had no apparent effect on hemoglobin levels (Appendix D, Table D-5).

Table 2

Effects of Zinc and Vitamin B-6 Supplements on Hemoglobin Level of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm) 0	50	100
		gm/100 ml of Blood[1]		
7.5	1	13.5 ± 1.0	12.6 ± 1.7	12.3 ± 1.8[2]
	5	14.4 ± 0.8	12.2 ± 1.0	11.8 ± 0.4
	100	13.3 ± 1.5	12.0 ± 1.0	12.0 ± 1.5
15.0	1	14.8 ± 1.3[2]	11.9 ± 0.8	12.3 ± 0.8
	5	15.0 ± 1.0	12.2 ± 0.4	12.8 ± 1.2
	100	14.7 ± 1.3[2]	12.0 ± 0.8	12.1 ± 1.1
30.0	1	15.2 ± 2.1[2]	14.3 ± 1.0	14.6 ± 0.8
	5	16.0 ± 1.1	13.1 ± 0.6[3]	14.3 ± 0.3[2]
	100	14.8 ± 1.3	14.2 ± 0.8	14.1 ± 0.8[2]

LSD[4] 0.05: 1.0

LSD 0.01: 1.4

[1]Each value in the mean of 9 animales ± SEM, unless otherwise indicated.

[2]Mean of 8 animals ± SEM.

[3]Mean of 7 animals ± SEM.

[4]Least significant differences at specified probability levels.

Liver Copper

Table 3 shows the effects of zinc and vitamin B-6 supplements on liver copper deposition (in terms of mcg per gm of liver) in young rats fed varying levels of dietary protein. Analysis of the data indicated that highly significant increases ($p < .01$) in liver copper deposition were associated with increasing levels of dietary protein (Appendix C, Table C-4). Such increases would be expected since more copper and iron would be available for liver deposition when the diets contained 15 or 30% protein. Maximum copper deposition was observed in young rats fed 15% protein (Appendix D, Table D-9). Levels of liver copper observed in rats fed 30% protein, regardless of zinc or vitamin B-6 levels, were essentially the same as levels observed in animals fed 15% protein (Appendix D, Tables D-10 and D-11). The addition of 50 ppm zinc supplements to the diets resulted in highly significant decreases ($p < .01$) in liver copper deposition (on a per gm basis). Increasing the level of zinc supplementation to 100 ppm did not cause a further reduction in liver copper deposition (Appendix D, Table D-9). The effect of zinc on liver copper deposition is in agreement with previous findings (Magee & Grainger, 1979; Motsinger & Magee, 1980). Increasing the level of vitamin B-6 in the diet was associated with an increase in liver copper level which was significant at the .05 level of probability (Appendix C, Table C-4). The maximum increase in liver copper deposition, however, was observed when the dietary supplemental level of vitamin B-6 was 5 ppm. A supplement of 100 ppm of vitamin B-6 did not result in a significant increase in

Table 3

Effects of Zinc and Vitamin B-6 Supplements on Liver Copper of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm) 0	50	100
		mcg/g Dry Weight[1]		
7.5	1	8.4 ± 1.7	7.0 ± 0.6	5.9 ± 1.0
	5	11.3 ± 3.0	7.8 ± 2.7	7.9 ± 1.1
	100	9.5 ± 1.0	6.7 ± 1.1	7.3 ± 1.3
15.0	1	13.1 ± 5.8	10.0 ± 1.6	9.8 ± 2.5
	5	12.3 ± 2.6	11.5 ± 1.8	10.5 ± 2.1
	100	10.4 ± 2.4	10.1 ± 1.6	10.6 ± 3.0
30.0	1	8.1 ± 3.4	10.6 ± 0.3	7.7 ± 2.0
	5	12.2 ± 2.2	9.3 ± 1.3	11.5 ± 2.1
	100	11.1 ± 2.5	11.3 ± 2.2	10.5 ± 1.1

LSD[2] 0.05: 2.8

LSD 0.01: 3.8

[1]Each value is the mean of 5 animals ± SEM.

[2]Least significant differences at specified probability levels.

liver copper deposition over that which was observed with 1 ppm of vitamin B-6 supplementation (Appendix D, Table D-9). Significant increases in liver copper associated with vitamin B-6 supplementation were observed in the non-zinc and 100 ppm of zinc supplemented animals (Appendix D, Table D-12). The interrelationship between dietary vitamin B-6 and liver copper deposition noted in this study appears to be different from the findings of Gubler, Cartwright, and Wintrobe (1947) that copper absorption was increased in pyridoxine deficient rats.

Total liver copper levels expressed in terms of total copper per total liver are shown in Table 4. The addition of 50 ppm of zinc to the diet resulted in significant increases in total copper incorporation (Appendix D, Table D-13), and this observation is different than that observed when copper was expressed on a per gm basis. Total liver copper deposition significantly increased as the level of vitamin B-6 in the diet was increased from 1 ppm to 5 ppm. Levels of total liver copper observed in rats fed 5 ppm of vitamin B-6 supplements, however, were essentially the same as levels observed in animals fed 100 ppm of vitamin B-6 (Appendix C, Table C-5 and Appendix D, Table D-13).

Liver Iron

Liver iron deposition (on a per gm liver weight basis) in young rats fed the various dietary regimens used in the study is given in Table 5. Increases in dietary protein resulted in significant increases ($p < .01$) in liver iron deposition, while increases in zinc

Table 4

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Copper of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm)		
		0	50	100
		mcg/Total Dry Weight[1]		
7.5	1	12.4 ± 2.6	20.5 ± 3.9	19.5 ± 2.8
	5	13.6 ± 3.1	24.8 ± 4.2	27.0 ± 1.9
	100	13.8 ± 1.7	23.5 ± 26.2	26.2 ± 6.1
15.0	1	18.5 ± 9.7	25.9 ± 2.1	24.5 ± 6.1
	5	15.2 ± 0.8	30.7 ± 8.2	28.4 ± 5.9
	100	14.8 ± 0.9	28.3 ± 2.9	26.8 ± 5.2
30.0	1	9.4 ± 3.0	25.4 ± 4.1	16.5 ± 10.7
	5	13.8 ± 3.1	24.7 ± 4.4	22.2 ± 5.1
	100	14.4 ± 0.8	25.4 ± 2.6	26.1 ± 3.4

LSD[2] 0.05: 5.79

LSD 0.01: 7.62

[1]Each value is the mean of 5 animals ± SEM.

[2]Least significant differences at specified probability levels.

Table 5

Effects of Zinc and Vitamin B-6 Supplements on Liver Iron of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm)		
		0	50	100
		mcg/g Dry Weight[1]		
7.5	1	557 ± 90	279 ± 38	270 ± 44
	5	667 ± 213	239 ± 40	236 ± 39
	100	479 ± 127	237 ± 51	239 ± 38
15.0	1	461 ± 153	328 ± 39	309 ± 115
	5	608 ± 148	288 ± 45	307 ± 45
	100	462 ± 68	297 ± 72	310 ± 37
30.0	1	745 ± 242	389 ± 45	380 ± 63
	5	880 ± 322	341 ± 88	351 ± 87
	100	720 ± 238	372 ± 70	314 ± 37

LSD[2] 0.05: 154

LSD 0.01: 205

[1]Each value is the mean of 5 animals ± SEM.

[2]Least significant differences at specified probability levels.

supplementation were associated with marked decreases ($p < .01$) in liver iron deposition (Appendix C, Table C-6). The analysis also indicated protein x zinc and zinc x vitamin B-6 interactions which were significant at .05 level of probability. Interpretation of the significant protein x zinc interaction suggests that when supplements of 50 ppm or 100 ppm of zinc were present in the diet, the influence of protein level on liver iron deposition was not as great as it was when no zinc supplement was included in the diet (Appendix D, Table D-15). Interpretation of the significant zinc x vitamin B-6 interaction suggests the possibility that vitamin B-6 (at least at a 5 ppm dietary level) increased liver iron deposition in animals fed a zinc-deficient diet (Appendix D, Table D-16). When zinc was adequate in the diet, vitamin B-6 had no significant effect on liver iron deposition.

Total liver iron levels expressed in terms of total iron per total liver are shown in Table 6. If liver was expressed in terms of total amount, only an increase in protein level from 15% to 30% resulted in a significant increase in liver iron level (Appendix D, Table D-17). Zinc supplementation or vitamin B-6 supplementation had no effect on the total amount of iron expressed on a total liver weight basis (Appendix C, Table C-7).

Liver Zinc

Liver zinc concentrations (expressed on a per gm liver weight basis) of young rats fed the various dietary regimens used in the study are given in Table 7. Increases in either protein level, zinc

Table 6

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Iron Depositions of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm)		
		0	50	100
		mcg/Total Dry Weight[1]		
7.5	1	826 ± 143	810 ± 115	893 ± 150
	5	838 ± 353	792 ± 132	818 ± 70
	100	702 ± 210	826 ± 80	867 ± 236
15.0	1	622 ± 182	864 ± 352	731 ± 114
	5	748 ± 103	755 ± 150	833 ± 135
	100	677 ± 135	846 ± 255	801 ± 117
30.0	1	888 ± 281	944 ± 240	858 ± 186
	5	960 ± 245	890 ± 201	698 ± 252
	100	943 ± 250	1000 ± 319	794 ± 207

LSD[2] 0.05: 250

LSD 0.01: 328

[1]Each value is the mean of 5 animals ± SEM.

[2]Least significant differences at specified probability levels.

Table 7

Effects of Zinc and Vitamin B-6 Supplements on Liver Zinc of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm)		
		0	50	100
		mcg/g Dry Weight[1]		
7.5	1	57.8 ± 3.5	59.9 ± 8.4	55.1 ± 5.7
	5	75.0 ± 19.6	54.7 ± 11.8	59.6 ± 4.4
	100	59.3 ± 5.6	52.8 ± 12.3	66.4 ± 24.2
15.0	1	64.5 ± 7.5	75.1 ± 8.8	84.7 ± 19.6
	5	76.0 ± 16.5	90.0 ± 12.1	92.6 ± 8.0
	100	71.0 ± 6.7	83.2 ± 9.9	89.2 ± 22.1
30.0	1	63.4 ± 10.7	80.6 ± 5.0	78.1 ± 8.5
	5	76.3 ± 11.0	82.7 ± 17.6	92.2 ± 18.5
	100	68.1 ± 12.0	81.9 ± 15.9	80.8 ± 9.4
	LSD[2] 0.05: 16.4			
	LSD 0.05: 21.8			

[1]Each value is the mean of 5 animals ± SEM.

[2]Least Significant differences at specified probability levels.

supplementation, or vitamin B-6 supplementation resulted in highly significant increases ($p < .01$) in liver zinc deposition (Appendix C, Table C-8). These results were similar to previous findings which will be mentioned later in the text. Maximum liver zinc deposition (mcg per gm dry weight) was observed in animals fed 15% protein, a 100 ppm of zinc supplement, and a 5 ppm supplemental level of vitamin B-6 (Appendix D, Table D-18). Interpretations of a significant protein x zinc interaction ($p < .05$) indicated that increases in zinc supplementation were associated with slight, but not statistically significant, changes in liver zinc deposition in rats fed either 7.5 or 30% protein while, increases in zinc supplements were associated with significant increases in liver zinc deposition in rats fed 15% protein (Appendix D, Table D-19). Significant increases in liver zinc levels associated with 5 ppm of vitamin B-6 supplement were observed only in rats fed a 15% level of protein or in rats fed the non-zinc supplemented diets (Appendix D, Tables D-20 and D-21).

Total liver zinc levels expressed in terms of zinc per total liver are shown in Table 8. When liver zinc values were expressed on amount per total liver, a significant increase in liver zinc level was observed only in animals fed 15% protein while the total liver zinc amounts of animals fed either 7.5% or 30% protein were essentially the same (Appendix D, Table D-22). Increasing the levels of zinc or vitamin B-6 in the diets resulted in significant increases in total amounts of liver zinc incorporation (Appendix C, Table C-9).

Table 8

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Zinc Depositions of Young Rats Fed Various Levels of Protein

Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm)		
		0	50	100
		mcg/Total Dry Weight[1]		
7.5	1	86.0 ± 9.5	175.0 ± 32.8	183.1 ± 30.0
	5	89.6 ± 20.0	177.9 ± 15.2	209.0 ± 30.4
	100	86.8 ± 12.5	183.8 ± 13.9	225.8 ± 22.5
15.0	1	88.2 ± 6.5	195.4 ± 9.0	206.5 ± 25.5
	5	93.4 ± 12.0	237.3 ± 52.3	251.3 ± 32.6
	100	104.6 ± 19.7	234.1 ± 32.9	225.5 ± 32.4
30.0	1	76.3 ± 13.2	193.2 ± 23.4	180.1 ± 47.0
	5	85.8 ± 10.7	215.7 ± 27.6	176.3 ± 35.4
	100	89.0 ± 9.7	184.2 ± 15.1	202.3 ± 41.8
	LSD[2] 0.05: 32			
	LSD 0.01: 42			

[1]Each value is the mean of 5 animals ± SEM.

[2]Least significant differences at specified probability levels.

CHAPTER V

DISCUSSION

Results of this study support previous findings of Magee and Grainger (1979) that the addition of zinc to suboptimal protein (7.5%) diets of young rats was associated with highly significant increases ($p < 0.01$) in weight gains. A level of 50 ppm of zinc supplement also stimulated increased weight gains in young rats fed either 15% or 30% protein levels which were significant at the 0.01 level of probability. Increasing the level of dietary protein from a suboptimal level (7.5%) to an adequate level (15%) was associated with marked increases ($p < 0.01$) in weight gains only when zinc was adequate in the diet. Supplements of 5 ppm of vitamin B-6 resulted in highly significant increases ($p < 0.01$) in weight gains only when the level of dietary protein was suboptimal (7.5%), while extra vitamin B-6 had no apparent effect on weight gains when the dietary protein level was adequate. These findings would suggest the possibility that the utilization of suboptimal levels of protein for growth may be improved with increases in vitamin B-6 supplementation. The stimulatory effect of vitamin B-6 on weight gain was also noted only when the diets contain adequate zinc.

Zinc appears to have a major function related to the incorporation of amino acids into proteins or protein synthesis per se, while vitamin B-6 is generally associated with mechanisms, such as transamination and absorption, which make amino acids available for protein biosynthesis. There is the possibility that amino acids made available by the presence of adequate vitamin B-6 cannot be incorporated into body proteins without the presence of adequate zinc.

The overall effect of increasing protein level in the diet was an increase in hemoglobin concentration in the young rat, but this effect appeared to be lessened as the level of dietary zinc increased. The decreases in hemoglobin concentrations associated with increased dietary zinc observed in the study were similar to previous findings (Magee & Grainger, 1979; Motsinger & Magee, 1980). Since no effect of vitamin B-6 on hemoglobin level was observed in this study, it can be assumed that all diets contained sufficient vitamin B-6 for adequate hemoglobin formation.

The decreases in hemoglobin concentrations in young rats fed 50 ppm of zinc could be related to rapid increases in blood volumes associated with the increases in growth associated with zinc supplementation. Any increase in growth would be accompanied by an increase in blood volume which would require sufficient copper and iron to maintain normal hemoglobin concentrations.

Since this study, as well as other studies previously cited, has shown that the deposition of copper and iron into body storage sites, such as the liver was decreased with increases in dietary zinc levels, there is the possibility that less total copper and iron would be available for hemoglobin formation. Thus, the adverse effect of zinc on hemoglobin formation could be the result of the effect of this mineral on copper and iron status of the system. Settlemire and

Matrone (1967) also reported that zinc interferes with the incorporation of iron into ferritin and the release of iron from ferritin for hemoglobin formation.

Increases in dietary protein levels were generally associated with increases in liver iron, liver zinc, and liver copper deposition (on a per gram of dry weight). Zinc supplementation increased liver zinc deposition but was associated with decreases in the deposition of either copper or iron in the liver. Supplements of vitamin B-6 were associated with increases in liver copper and zinc deposition but had no apparent effect on liver iron concentration.

The increase in liver zinc deposition associated with an increase in vitamin B-6 supplementation observed in this study supports the findings of some researchers (Evans & Johnson, 1981; Hsu, 1965) but contradicts the findings of others (Gershoff, 1968; Ikeda et al., 1979). Ikeda et al. (1979) speculated that several factors could have been responsible for the differences of the effects of vitamin B-6 on zinc metabolism observed in various studies. Some of these factors, cited by Ikeda et al. (1979), included differences in diet compositions of various studies, varying food utilization, and the severity of vitamin B-6 deficiency imposed on the animals in the various studies. Evans and Johnson (1981) reported that the dominant zinc-binding ligand in rat intestine is a compound known as picolinic acid, and they found that a decrease in dietary vitamin B-6 level results in a decrease in kyureninase activity which is involved with the conversion of tryptophan to picolinic acid. Thus, if vitamin B-6 is deficient in the diet, less picolinic acid could be available for the absorption of zinc. Increasing the vitamin B-6 level of the diet, however, could stimulate an increase in picolinic acid and a subsequent increase in zinc absorption.

Although the antagonistic effect of a physiological level of zinc on liver copper and iron deposition on a per gram basis observed in this study supports previous results (Magee & Grainger, 1979; Motsinger & Magee, 1980), there is the possibility that the differences in liver copper and iron levels of non-zinc-supplemented animals and zinc-supplemented animals may be more closely related to liver size than to an interference with copper or iron incorporation into the liver. Increasing the level of zinc supplement to 50 ppm resulted in marked increases in total liver iron deposition in rats fed either 7.5% or 15% protein, while this level of zinc supplement was associated with decreases in total iron levels in rats fed 30% protein. This supplemented level of zinc, however, resulted in marked increases in total liver copper levels in all animals, regardless of protein level. The total liver weights (on a dry weight basis) of the zinc-supplemented rats, however, were approximately two times greater than the total liver weights of the non-zinc-supplemented rats, and when liver, copper, and iron values were calculated on a per gram of dry weight basis, the resulting values reflected a decrease in liver deposition associated with zinc supplementation.

The effect of vitamin B-6 on liver copper and zinc deposition (on a per gram of dry weight basis) appeared to be influenced by the level of zinc in the diet. Increases in liver copper associated with vitamin B-6 supplementation were observed in the non-zinc and 100 ppm of zinc-supplemented animals and increases in liver zinc deposition associated with vitamin B-6 supplementation was observed only in non-zinc-supplemented animals. There was also evidence that the effect of vitamin B-6 supplementation on liver copper and zinc deposition depended upon the level of protein in the diet. Increases in liver copper deposition associated with 5 ppm of vitamin B-6 supplementation was noted only when the level of protein in the diet was 7.5% or 30%. Increases in liver zinc deposition associated wtih vitamin B-6 supplementation only occurred when the level of dietary protein was 15%.

The increase in liver zinc deposition associated with an increase of dietary vitamin B-6 observed in this study supports the findings of previous reports (Evans & Johnson, 1981; Hsu, 1965) that zinc absorption and/or incorporation into body tissues is increased with higher dietary vitamin B-6 levels.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

Young male rats were fed diets consisting of three levels each of protein, vitamin B-6 supplements, and zinc supplements. Criteria used to evaluate the responses of the animals to the various diet combinations included weight gain, hemoglobin concentration, and copper, iron, and zinc deposition in the liver.

The addition of 50 ppm of zinc to the diet was associated with highly significant increases ($p < .01$) in weight gain, regardless of dietary protein level or vitamin B-6 supplement. Rats fed the zinc-supplemented high protein (30%) diet, however, showed total weight gains that were less than the weight gains of animals fed the zinc-supplemented low protein (7.5%) diet. This finding would suggest that above a certain level of protein intake, zinc supplementation does not improve protein utilization. Increases in weight gain associated with increases in protein level occurred only when the diet contained adequate zinc, while increases in weight gain associated with increases in vitamin B-6 occurred only when the level of protein was marginal to inadequate (7.5%).

Increasing levels of dietary protein were associated with highly significant increases ($p < .01$) in hemoglobin concentrations, while increases in zinc supplements resulted in highly significant decreases ($p < .01$) in hemoglobin levels. The hemoglobin levels of the zinc-fed rats, however, still appeared to be adequate because the animals did not exhibit low hemoglobin concentrations (5-7 mg hemoglobin/dl blood) which are characteristic of anemia in young rats.

As the level of dietary protein increased, the amount of copper deposited in the liver increased, and maximum copper deposition was observed in rats fed the 15% protein level. A 50 ppm of zinc resulted in marked decreases in liver copper deposition, while increases in vitamin B-6 supplementation were associated with increases in liver copper deposition. Maximum copper concentrations were observed in rats fed diets supplemented with 5 ppm of vitamin B-6.

The main effects of protein level, zinc supplementation, and vitamin B-6 supplementation on liver iron levels were to increase, to decrease, and to have no effect on iron deposition, respectively. The effect of zinc on liver iron deposition, however, appeared to be influenced by the amount of protein in the diet. Although the main effect of vitamin B-6 on liver iron was not statistically significant, interpretation of a significant vitamin B-6 x zinc interaction suggests that vitamin B-6 stimulates liver iron incorporation in a zinc-deficient animal but has no effect when zinc is adequate in the diet.

Liver zinc deposition was increased when the protein level was increased from suboptimal (7.5%) to adequate (15%). Vitamin B-6 and zinc supplementation also stimulated increases in liver zinc deposition.

Recommendations

Because of limited animal facilities, it was not feasible to conduct the 3^3 factorial design with nine replications as one experiment. The total number of animals required for the study as designed was 243, but only 180 cages were available. Thus, the decision was made to divide the study into three small experiments with the design being a 3^2 factorial (factors were zinc and vitamin B-6), and the nine resulting diets were fed in combination with the three protein levels over time. Although each separate experiment could be analyzed independently of the other two, statistical implications of some of the two-factor interactions and the three-factor interaction would not be possible. This type of problem possibly could be overcome with future studies of this magnitude by decreasing the number of replications or by utilizing confounding techniques associated with split-plot or incomplete block designs.

Since none of the diets used in this study were vitamin B-6 deficient, there is the possibility the influence of protein and/or zinc in the absence of vitamin B-6 could have yielded additional information regarding the interrelationship between the three nutrients in an animal system.

Whether to report the effect of dietary factors on tissue mineral concentrations in terms of mcg of mineral per gram of dry tissue weight or in terms of total mineral concentration in total tissue is an issue which has been raised as the result of this study. Although most researchers conducting similar types of research express tissue mineral levels on a relative basis (mcg per gram dry weight), there is a possibility that expressing tissue mineral levels on a total organ basis may give a better indication of the overall mineral status. The mineral contents on a per weight basis can vary considerably because of differences in organ weights. Analyses of additional organs, such as the pancreas, spleen, and kidney may also give additional information regarding mineral ststus. Another parameter which would possibly be helpful in interpreting results from a study of this type would be blood mineral concentrations.

If there is a true zinc vitamin B-6 interrelationship, as suggested by this study, the effects of zinc on vitamin B-6 body parameters should be observed in subsequent experiments.

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APPENDIX A

COMPOSITION OF THE EXPERIMENTAL DIETS

Table A-1

Composition of Vitamin Mixture

Constituents[1]	gm/2 kg Mix
Biotin	0.02
Folic acid	0.10
Thiamin HCL	0.50
Menadione (2-methyl-naphthoquinone)	1.00
Riboflavin	1.00
Nicotinic acid	1.00
Ca Pantothenate	3.00
P-aminobenzoic acid	10.00
0.1% vitamin B_{12} (Mannitol Trituration)	2.00
Inositol	100.00
Choline chloride	150.00
Corn starch[2]	1732.00

[1]All vitamins were purchased from ICN Biochemicals, Cleveland, Ohio.

[2]Teklad Test Diets, Madison, Wisconsin.

Table A-2

Composition of Wesson Modification of Osborne-Mendel Mineral Mix[1]

Constituents	Percent
Calcium carbonate	21.000
Cupric sulfate ($5H_2O$)	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

[1]Teklad Test Diets, Madison, Wisconsin.

APPENDIX B

RAW DATA

Table B-1

Effects of Zinc and Vitamin B-6 Supplements on Growth of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		4 Weeks Weight Gain (gm)									
0	1	44	36	51	46	39	69	43	5	38	41
50	1	102	138	105	128	101	114	122	129	96	115
100	1	79	130	(115)[1]	123	124	137	119	98	112	115
0	5	22	39	41	35	43	38	54	29	48	39
50	5	85	109	117	127	150	156	155	129	160	132
100	5	140	133	145	165	119	135	133	127	118	135
0	100	53	44	30	57	37	51	33	27	27	40
50	100	152	126	158	138	116	141	149	113	138	137
100	100	169	163	149	118	129	136	97	105	132	133

[1]( ) Estimated missing plot value.

Table B-2

Effects of Zinc and Vitamin B-6 Supplements on Growth of Young Rats Fed 15% Dietary Protein

Level of Supplementation Zinc (ppm)	Vitamin B-6 (ppm)	Replications 1	2	3	4	5	6	7	8	9	Mean
		4 Weeks Weight Gain (gm)									
0	1	43	37	82	45	31	41	49	(60)[1]	41	46
50	1	123	115	146	133	122	140	123	200	154	140
100	1	98	140	150	173	133	110	164	159	157	143
0	5	48	49	41	46	38	52	42	46	36	44
50	5	141	177	153	129	144	151	129	171	125	147
100	5	161	174	156	141	170	169	142	153	167	159
0	100	78	46	(57)[1]	46	37	65	52	36	68	54
50	100	139	148	150	162	141	145	171	158	160	153
100	100	169	146	124	136	145	125	143	160	169	146

[1]( ) Estimated missing plot value.

Table B-3

Effects of Zinc and Vitamin B-6 Supplements on Growth of Young Rats Fed 30% Dietary Protein

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		4 Weeks Weight Gain (gm)									
0	1	29	20	31	49	40	12	9	39	27	28
50	1	114	119	106	134	113	107	83	135	102	113
100	1	137	97	124	82	103	119	114	93	100	108
0	5	38	38	30	55	13	37	39	40	50	38
50	5	93	133	(123)[1]	96	139	(123)[1]	140	136	124	123
100	5	123	143	107	106	89	(106)[1]	92	124	64	106
0	100	34	13	45	28	44	36	41	44	59	38
50	100	98	156	124	120	90	147	132	97	131	122
100	100	159	99	142	144	119	95	112	(122)[1]	102	122

[1]( ) Estimated missing plot value.

Table B-4

Food Consumption of Young Rats Fed 7.5% Protein Diets Supplemented with Zinc and Vitamin B-6

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		4 Weeks Food Consumption (gm)									
0	1	198	191	213	185	221	265	204	144	252	208
50	1	324	379	286	363	264	361	349	336	292	328
100	1	230	359	(326)[1]	352	328	390	356	280	312	326
0	5	168	211	201	201	220	217	229	178	238	207
50	5	325	326	355	328	403	389	409	352	449	371
100	5	346	383	380	410	352	367	366	334	325	362
0	100	221	211	174	243	192	203	185	195	172	200
50	100	375	350	400	374	338	382	384	325	368	366
100	100	406	408	402	360	373	386	296	305	376	368

[1]( ) Estimated missing plot value.

Table B-5

Food Consumption of Young Rats Fed 15% Protein Diets Supplemented with Zinc and Vitamin B-6

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		4 Weeks Food Consumption (gm)									
0	1	147	164	197	169	126	154	165	(162)[1]	170	162
50	1	194	205	285	268	230	252	244	314	320	257
100	1	195	216	308	304	282	230	304	290	320	272
0	5	165	169	165	172	153	163	137	170	172	163
50	5	287	320	320	254	290	302	274	337	309	299
100	5	320	380	323	303	289	346	290	298	320	319
0	100	191	168	(167)[1]	128	169	159	164	152	203	167
50	100	238	309	270	316	282	319	305	335	326	300
100	100	341	245	281	288	307	269	310	320	337	300

[1]( ) Estimated missing value.

Table B-6

Food Consumption of Young Rats Fed 30% Protein Diets Supplemented with Zinc and Vitamin B-6

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		4 Weeks Food Consumption (gm)									
0	1	141	139	161	157	147	136	136	154	131	147
50	1	210	259	262	284	262	250	238	248	233	250
100	1	290	235	258	225	238	248	240	231	240	245
0	5	147	173	165	174	140	148	123	167	182	158
50	5	254	295	(272)[1]	239	309	(272)[1]	290	249	268	272
100	5	273	319	265	263	223	(255)[1]	222	277	200	255
0	100	149	133	192	156	161	168	169	154	184	163
50	100	240	318	281	282	263	293	263	230	267	271
100	100	325	250	284	307	254	256	224	(265)[1]	221	265

[1]( ) Estimated missing value.

Table B-7

Effects of Zinc and Vitamin B-6 Supplements on Hemoglobin Level of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		gm/100 ml Blood									
0	1	11.6	13.8	13.5	13.6	14.2	12.8	13.9	15.1	13.1	13.5
50	1	14.9	10.6	15.1	12.5	12.4	12.4	13.1	12.0	10.8	12.6
100	1	12.3	8.5	(12.3)[1]	12.3	13.4	11.4	13.0	14.9	12.5	12.3
0	5	13.4	13.5	15.5	14.2	14.2	15.7	14.2	14.6	14.3	14.4
50	5	11.9	13.9	10.7	12.8	11.4	13.3	12.2	11.4	12.2	12.2
100	5	12.4	12.1	11.3	11.9	11.7	11.8	12.0	11.8	11.1	11.8
0	100	10.0	12.8	13.7	13.6	13.2	13.8	12.8	15.4	14.6	13.3
50	100	12.9	11.3	13.0	11.3	10.9	10.5	12.3	12.3	13.3	12.0
100	100	12.5	10.3	11.5	12.4	12.0	12.6	15.1	10.5	10.8	12.0

[1]( ) Estimated missing value.

Table B-8

Effects of Zinc and Vitamin B-6 Supplements on Hemoglobin Level of Young Rats Fed 15% Dietary Protein

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		gm/100 ml Blood									
0	1	16.3	13.5	12.7	14.4	14.9	16.3	15.6	(14.8)[1]	14.7	14.8
50	1	11.1	11.8	12.9	11.1	13.3	12.1	12.1	12.0	11.0	11.9
100	1	12.4	13.7	11.1	12.1	12.9	11.8	12.3	13.1	11.6	12.3
0	5	15.9	15.5	13.7	15.1	14.3	15.9	15.8	13.7	15.6	14.5
50	5	11.6	12.4	12.0	12.3	12.3	12.8	11.7	11.7	12.6	12.2
100	5	14.0	13.5	11.7	14.4	11.8	11.5	12.3	13.9	12.0	12.8
0	100	12.9	13.5	(14.7)[1]	15.8	14.2	15.6	13.9	14.9	16.7	14.7
50	100	12.3	12.9	11.7	12.2	11.9	13.3	10.4	11.7	11.9	12.0
100	100	13.9	13.0	11.7	12.7	11.7	11.5	11.0	10.3	12.7	12.1

[1]( ) Estimated missing value.

Table B-9

Effects of Zinc and Vitamin B-6 Supplements on Hemoglobin Level of Young Rats Fed 30% Dietary Protein

Level of Supplementation		Replications									
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	6	7	8	9	Mean
		gm/100 ml Blood									
0	1	18.2	14.6	11.3	(15.2)[1]	15.3	17.3	14.2	14.9	15.9	15.2
50	1	15.5	12.7	13.9	14.5	14.4	14.0	14.4	13.0	15.8	14.3
100	1	14.3	15.5	14.5	14.9	13.5	14.2	15.5	13.6	15.4	14.6
0	5	15.4	16.1	15.4	15.3	18.7	15.9	15.7	14.9	16.5	16.0
50	5	14.1	13.0	(13.0)[1]	13.0	12.1	(13.0)[1]	13.1	13.0	12.9	13.0
100	5	14.9	15.4	16.4	13.3	13.1	(14.3)	13.9	14.2	13.1	14.3
0	100	15.6	14.2	14.3	17.3	15.3	15.3	13.3	13.0	14.9	14.8
50	100	14.5	13.9	15.3	14.3	14.9	15.1	13.5	12.9	13.4	14.2
100	100	12.9	14.5	14.1	15.1	13.7	13.1	15.3	(14.1)[1]	14.0	14.1

[1]( ) Estimated missing plot value.

Table B-10

Effects of Zinc and Vitamin B-6 Supplements on Liver Copper Level of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation: Zinc (ppm)	Level of Supplementation: Vitamin B-6 (ppm)	Replications: 1	2	3	4	5	Mean
		mcg/gm Dry Weight of Liver					
0	1	9.1	8.4	10.7	6.8	6.8	8.4
50	1	6.2	8.0	7.0	7.0	7.1	7.0
100	1	6.4	5.4	7.5	4.4	6.6	6.1
0	5	11.0	13.5	15.1	8.8	8.2	11.3
50	5	7.2	12.4	7.4	5.1	6.7	7.8
100	5	7.6	7.7	6.4	8.9	8.9	7.9
0	100	8.2	9.4	10.5	8.8	10.5	9.5
50	100	5.0	6.8	7.0	8.0	6.5	6.7
100	100	7.2	5.6	9.1	8.0	6.5	7.3

Table B-11

Effects of Zinc and Vitamin B-6 Supplements on Liver Copper Level of Young Rats Fed 15% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/g Dry Weight of Liver					
0	1	12.1	10.9	10.9	8.5	23.2	13.1
50	1	10.3	8.6	9.8	8.6	12.5	10.0
100	1	8.4	10.6	7.3	13.8	9.1	9.8
0	5	11.6	14.5	11.3	8.9	15.3	12.3
50	5	12.8	13.9	10.3	10.8	9.8	11.5
100	5	13.3	11.0	10.9	9.6	7.7	10.5
0	100	9.7	8.6	13.5	7.8	12.3	10.4
50	100	8.9	10.9	10.1	12.3	8.4	10.1
100	100	11.9	6.1	14.3	10.4	10.2	10.6

Table B-12

Effects of Zinc and Vitamin B-6 Supplements on Liver Copper Level of Young Rats Fed 30% Dietary Protein

Level of Supplementation: Zinc (ppm)	Vitamin B-6 (ppm)	Replications: 1	2	3	4	5	Mean
		mcg/g Dry Weight of Liver					
0	1	3.3	8.8	8.7	12.6	7.1	8.1
50	1	10.9	10.3	10.8	10.4	10.4	10.6
100	1	9.3	5.1	7.1	6.8	10.2	7.7
0	5	12.7	12.4	14.9	12.1	8.8	12.2
50	5	7.5	9.2	9.1	9.3	11.5	9.3
100	5	11.3	13.8	8.3	13.0	11.1	11.5
0	100	12.4	14.5	11.5	8.8	8.7	11.2
50	100	12.4	10.4	14.3	8.3	11.0	11.3
100	100	10.4	9.2	12.2	10.6	10.3	10.5

Table B-13

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Copper Level of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	14.1	12.5	14.9	8.2	12.4	12.4
50	1	20.2	27.2	19.7	17.6	17.7	20.5
100	1	18.5	20.5	22.2	15.0	21.0	19.5
0	5	10.3	16.3	17.4	12.6	11.2	13.6
50	5	19.9	31.6	24.6	23.4	24.7	24.8
100	5	29.3	26.0	28.3	24.3	26.8	27.0
0	100	15.3	13.8	15.8	12.2	12.0	13.8
50	100	23.9	21.0	21.9	24.2	26.5	23.5
100	100	32.2	32.2	22.9	18.0	25.8	26.2

Table B-14

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Copper Level of Young Rats Fed 15% Dietary Protein

Level of Supplementation Zinc (ppm)	Vitamin B-6 (ppm)	Replications 1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	17.7	14.1	12.0	13.2	35.4	18.5
50	1	26.6	23.5	27.6	23.8	27.9	25.9
100	1	13.7	27.6	27.8	25.4	28.1	24.5
0	5	14.5	16.3	14.6	14.5	15.7	15.2
50	5	29.7	44.1	28.0	30.0	21.6	30.7
100	5	34.4	32.9	28.8	26.5	19.5	28.4
0	100	15.3	14.1	13.9	14.5	16.1	14.8
50	100	23.2	30.1	30.1	29.9	28.3	28.3
100	100	33.7	21.7	28.2	28.8	21.5	26.8

Table B-15

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Copper Level of Young Rats Fed 30% Dietary Protein

Level of Supplementation: Zinc (ppm)	Level of Supplementation: Vitamin B-6 (ppm)	Replications: 1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	4.8	12.2	12.0	8.9	9.2	9.4
50	1	26.0	32.2	23.0	21.6	24.5	25.4
100	1	30.5	6.5	5.2	17.8	22.2	16.5
0	5	17.6	15.7	12.6	13.9	9.4	13.8
50	5	22.6	18.2	28.9	25.3	28.4	24.7
100	5	27.3	27.4	19.0	21.4	16.0	22.2
0	100	14.0	14.0	13.9	14.4	15.9	14.4
50	100	22.1	28.8	24.1	25.0	26.7	25.4
100	100	28.4	30.0	26.0	25.2	21.0	26.1

Table B-16

Effects of Zinc and Vitamin B-6 Supplements on Liver Iron Level of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/gm Dry Weight of Liver					
0	1	491	702	509	587	495	557
50	1	296	232	274	259	332	279
100	1	266	246	213	300	325	270
0	5	767	484	425	926	782	677
50	5	309	233	225	208	221	239
100	5	206	222	210	301	241	236
0	100	520	413	294	622	546	479
50	100	163	239	298	264	217	236
100	100	197	216	274	282	224	239

Table B-17

Effects of Zinc and Vitamin B-6 Supplements on Liver Iron Level of Young Rats Fed 15% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/g Dry Weight of Liver					
0	1	393	504	614	230	563	461
50	1	328	372	285	363	295	328
100	1	448	206	216	417	256	308
0	5	584	826	518	443	671	608
50	5	271	317	258	241	351	288
100	5	274	275	304	383	300	307
0	100	441	359	542	480	490	462
50	100	217	258	408	313	289	297
100	100	308	272	335	276	357	310

Table B-18

Effects of Zinc and Vitamin B-6 Supplements on Liver Iron Level of Young Rats Fed 30% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/g Dry Weight of Liver					
0	1	492	640	996	1010	588	745
50	1	348	438	417	406	366	389
100	1	309	466	418	370	337	380
0	5	403	922	1306	925	844	880
50	5	208	398	300	428	371	341
100	5	466	289	285	290	425	351
0	100	1043	551	906	576	525	720
50	100	448	340	443	342	290	372
100	100	330	325	345	319	249	314

Table B-19

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Iron Level of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation: Zinc (ppm)	Vitamin B-6 (ppm)	Replications: 1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	757	1036	712	714	912	826
50	1	968	792	812	646	831	810
100	1	771	937	701	1013	1044	893
0	5	720	584	486	1333	1066	838
50	5	850	596	750	950	814	792
100	5	790	746	934	824	796	818
0	100	963	608	444	867	624	702
50	100	773	738	929	802	888	826
100	100	886	1237	693	631	888	867

Table B-20

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Iron Level of Young Rats Fed 15% Dietary Protein

Level of Supplementation: Zinc (ppm)	Level of Supplementation: Vitamin B-6 (ppm)	Replications: 1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	575	645	677	355	857	622
50	1	845	1016	802	997	661	864
100	1	734	537	823	770	793	731
0	5	733	927	669	723	688	748
50	5	628	1005	702	668	774	755
100	5	712	822	805	1062	763	833
0	100	695	592	558	899	640	677
50	100	565	712	1219	760	974	846
100	100	873	960	659	763	750	801

Table B-21

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Iron Level of Young Rats Fed 30% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	714	885	1374	710	756	888
50	1	828	1370	885	845	791	944
100	1	1013	589	984	968	736	858
0	5	559	1172	1102	1063	905	960
50	5	626	789	952	1167	916	890
100	5	1124	574	654	478	611	688
0	100	1184	534	1099	945	954	943
50	100	800	943	1526	1025	705	1000
100	100	905	1064	736	756	509	794

Table B-22

Effects of Zinc and Vitamin B-6 Supplements on Liver Zinc of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/gm Dry Weight of Liver					
0	1	54.1	59.6	61.4	59.9	54.0	57.8
50	1	50.0	62.3	68.1	52.1	67.0	60.0
100	1	55.0	60.4	51.9	47.4	60.9	55.1
0	5	76.9	97.3	90.5	55.4	54.7	75.0
50	5	55.0	72.2	56.6	39.5	50.2	54.7
100	5	53.1	59.3	58.3	64.3	62.7	59.6
0	100	54.0	56.6	66.1	55.2	64.5	59.3
50	100	39.0	63.0	55.1	66.0	41.1	52.8
100	100	54.2	44.1	85.3	99.0	49.6	66.4

Table B-23

Effects of Zinc and Vitamin B-6 Supplements on Liver Zinc of Young Rats Fed 15% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/g Dry Weight of Liver					
0	1	56.1	71.5	73.1	59.2	62.6	64.5
50	1	79.2	66.0	70.4	71.2	88.4	75.1
100	1	104.6	79.6	62.5	105.7	71.0	84.7
0	5	88.9	85.4	72.5	48.6	84.4	76.0
50	5	92.6	104.5	76.4	78.9	97.5	90.0
100	5	99.1	96.2	83.0	99.7	85.0	92.6
0	100	76.1	60.8	74.8	67.7	75.8	71.0
50	100	72.4	89.6	92.2	89.4	72.4	83.2
100	100	99.0	58.9	106.2	72.9	109.0	89.2

Table B-24

Effects of Zinc and Vitamin B-6 Supplements on Liver Zinc of Young Rats Fed 30% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/g Dry Weight of Liver					
0	1	53.3	64.6	63.0	80.6	55.4	63.4
50	1	82.7	73.7	87.4	80.0	79.2	80.6
100	1	64.4	77.3	85.4	78.2	85.1	78.1
0	5	57.6	79.5	86.0	76.5	81.6	76.2
50	5	60.8	109.7	79.4	84.4	79.2	82.7
100	5	90.9	103.6	60.5	104.6	101.3	92.2
0	100	81.8	76.2	70.5	59.3	52.6	68.1
50	100	101.9	71.9	95.0	64.3	76.5	81.9
100	100	92.2	72.1	89.4	76.9	73.3	80.8

Table B-25

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Zinc Level of Young Rats Fed 7.5% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	83.4	88.0	85.9	72.9	99.5	86.0
50	1	163.4	212.5	201.7	130.2	167.6	175.1
100	1	159.6	230.1	170.8	159.6	195.4	183.1
0	5	72.2	117.5	103.8	79.8	74.5	89.6
50	5	151.3	184.3	188.8	180.4	184.8	177.9
100	5	203.5	199.5	258.9	176.1	207.0	209.0
0	100	100.2	83.3	99.7	76.9	73.8	86.8
50	100	184.9	194.3	171.4	200.1	168.1	183.8
100	100	243.5	252.4	212.4	221.2	196.4	225.8

Table B-26

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Zinc Level of Young Rats Fed 15% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	82.1	91.6	80.5	91.5	95.3	88.2
50	1	204.4	180.4	198.5	195.7	197.9	195.4
100	1	171.4	207.7	283.7	194.9	219.7	206.5
0	5	111.6	95.8	93.6	79.4	86.5	93.4
50	5	214.4	330.6	207.7	218.9	215.0	237.3
100	5	257.2	287.7	219.6	276.2	215.7	251.3
0	100	120.0	100.2	77.0	126.9	99.0	104.6
50	100	188.5	246.8	275.3	216.7	243.4	234.1
100	100	280.7	207.8	209.0	201.7	228.4	225.5

Table B-27

Effects of Zinc and Vitamin B-6 Supplements on Total Liver Zinc Level of Young Rats Fed 30% Dietary Protein

Level of Supplementation		Replications					
Zinc (ppm)	Vitamin B-6 (ppm)	1	2	3	4	5	Mean
		mcg/Total Dry Weight of Liver					
0	1	77.4	89.4	86.9	56.6	71.3	76.3
50	1	196.8	230.2	185.6	166.7	186.5	193.2
100	1	211.3	97.8	201.2	204.7	185.6	180.1
0	5	79.9	101.2	72.5	88.0	87.4	85.8
50	5	182.9	217.6	252.4	230.2	195.5	215.7
100	5	219.2	205.4	139.1	171.9	145.8	176.3
0	100	92.9	73.8	85.4	97.4	95.7	89.0
50	100	182.0	199.6	160.0	193.1	186.1	184.2
100	100	252.8	235.9	190.8	182.2	150.0	202.3

APPENDIX C

STATISTICAL ANALYSIS

Table C-1

Analysis of Variance of Weight Gain Data

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square
Total	235	538395	
Replication	8	1450	181
Treatment	26	472061	18156**
Protein	2	28367	14183**
Zinc	2	430197	215098**
B-6	2	4866	2433**
Protein x Zinc	4	3957	989*
Protein x B-6	4	362	90
Zinc x B-6	4	1165	291
Protein x Zinc x B-6	8	3149	394
Error	201	64884	323

*Significant ($p < .05$)

**Highly significant ($p < .01$)

Table C-2

Covariance Analysis of Weight Gain Data with Food Consumption

					Deviations from Regression		
Source of Variation	df	Sx^2	Sxy	Sy^2	df	Sd^2_{yx}	Mean Square
Total	234	1404809	740077	538395			
Replication	8	11379	2732	1451			
Treatments	26	1204513	648614	472061			
Error	200	188918	88732	64884	199	23208	117
Treatments plus Error	226	1393431	737345	536945	225	146772	
For testing adjusted means					26	123564	4753**

**Highly significant ($p < .01$)

Table C-3

Analysis of Variance of Hemoglobin Data

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square
Total	234		650		
Replication	8		6		.75
Treatment	26		382		14.69**
Protein		2		149	74.40**
Zinc		2		182	90.96**
B-6		2		4	2.10
Protein x Zinc		4		22	5.52**
Protein x B-6		4		3	.63
Zinc x B-6		4		12	2.97
Protein x Zinc x B-6		8		10	1.27
Error	200		262		1.31

**Highly significant ($p < .01$)

Table C-4

Analysis of Variance of Liver Copper Data

Source of Variation	Degrees of Freedom		Sum of Squares		Mean Square
Total	134		1022.95		
Replications	4		14.93		3.73
Treatment	26		470.55		18.10**
Protein		2		215.65	107.83**
Zinc		2		68.89	34.45**
B-6		2		52.35	26.18*
Protein x Zinc		4		27.20	6.80
Protein x B-6		4		32.75	8.19
Zinc x B-6		4		22.80	5.70
Protein x Zinc x B-6		8		50.91	6.36
Error	104		537.46		5.17

*Significant ($p < .05$)

**Highly significant ($p < .01$)

Table C-5

Analysis of Variance of Total Liver Copper Data

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square
Total	134		7115.66		
Replication	4		111.96		27.99
Treatment	26		4732.34		182.01**
Protein		2		414.95	207.48**
Zinc		2		3545.22	1772.61**
B-6		2		277.59	138.80**
Protein x Zinc		4		95.39	23.85
Protein x B-6		4		98.66	24.67
Zinc x B-6		4		148.96	37.24
Protein x Zinc x B-6		8		151.57	18.95
Error	104		2271.36		21.84

**Highly significant ($p < .01$)

Table C-6

Analysis of Variance of Liver Iron Data

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square
Total	134		5673833.28		
Replication	4		35703.00		8925.75
Treatment	26		4057421.70		156054.68**
Protein		2		539302.49	269651.25**
Zinc		2		2994864.64	1497432.30**
B-6		2		69518.81	34759.41
Protein x Zinc		4		247288.65	61822.16*
Protein x B-6		4		5834.55	1458.64
Zinc x B-6		4		190788.10	47697.03*
Protein x Zinc x B-6		8		9824.45	1228.06
Error	104		1580708.50		15199.12

*Significant ($p < .05$)

**Highly significant ($p < .01$)

Table C-7

Analysis of Variance of Total Liver Iron Data

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square
Total	134	5342664	
Replication	4	46101	11525
Treatment	26	1072302	41242*
Protein	2	329681	164840*
Zinc	2	88480	44240
B-6	2	5788	2894
Protein x Zinc	4	360372	90093
Protein x B-6	4	59551	14888
Zinc x B-6	4	117394	29348
Protein x Zinc x B-6	8	111036	13879
Error	104	4224260	40618

*Significant ($p < .05$)

Table C-8

Analysis of Variance of Liver Zinc Data

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square
Total	134		37930.07		
Replication	4		279.45		34.93
Treatment	26		19795.93		761.38**
Protein		2		11420.67	5710.34**
Zinc		2		2134.70	1067.35**
B-6		2		1783.68	891.84**
Protein x Zinc		4		2831.26	707.82**
Protein x B-6		4		174.98	43.75
Zinc x B-6		4		470.18	117.55
Protein x Zinc x B-6		8		980.46	122.56
Error	104		17854.70		171.68

**Highly significant ($\underline{p} < .01$)

Table C-9

Analysis of Variance of Total Liver Zinc Data

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square
Total	134	517671	
Replication	4	5775	
Treatment	26	442302	17012**
Protein	2	19044	9522**
Zinc	2	392976	196488**
B-6	2	8601	4300**
Protein x Zinc	4	9165	2291*
Protein x B-6	4	2454	614
Zinc x B-6	4	2524	631
Protein x Zinc x B-6	8	7538	942
Error	104	69595	669

*Significant ($p < .05$)

**Highly significant ($p < .01$)

APPENDIX D

GROUP MEAN TABLES

Table D-1

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements of Weight Gain

Factor	Level	4 Weeks Weight Gain (gram)
Protein	7.5%	98[1a] (80)[2]
	15.0%	116[b] (79)
	30.0%	87[c] (77)
Zinc	0 ppm	41[a] (79)
	50 ppm	131[b] (79)
	100 ppm	130[b] (78)
Vitamin B-6	1 ppm	95[a] (79)
	5 ppm	102[b] (78)
	100 ppm	105[b] (79)

[1]Means not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parentheses indicate the number of animals used for mean values.

Table D-2

Weight Gains of Young Rats Fed Three Levels of Protein and Zinc, Regardless of Vitamin B-6 Level

Protein Level (%)	Zinc Supplement (ppm)		
	0	50	100
	4 Week Weight Gain (gram)		
7.5	40.0[1]^{ab} (27)[2]	127.9^{d} (27)	128.3^{d} (27)
15.0	47.8^{b} (25)	146.3^{e} (27)	149.4^{e} (27)
30.0	34.3^{a} (27)	118.8^{d} (25)	111.6^{c} (25)

[1]Means not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parentheses indicate the number of animals used for mean values.

Table D-3

Weight Gains of Young Rats Fed Three Levels of Zinc and Vitamin B-6, Regardless of Protein Level

Zinc Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	4 Week Weight Gain (gm)		
0	37.9[1a] (26)[2]	40.3[a] (27)	43.5[a] (26)
50	122.4[b] (27)	134.8[c] (25)	137.0[c] (27)
100	122.1[b] (26)	134.5[c] (26)	134.1[c] (26)

[1]Means not sharing common superscript letters are significantly different ($\underline{p} < .05$)

[2]Numbers in parentehses indicate the number of animals used for mean values.

Table D-4

Weight Gains of Young Rats Fed Three Levels of Protein and Vitamin B-6, Regardless of Zinc Level

Protein Level (%)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	4 Week Weight Gain (gm)		
7.5	89.5[1a] (26)[2]	101.9[bc] (27)	103.3[cd] (27)
15.0	111.9[cde] (26)	116.7[de] (27)	120.0[e] (26)
30.0	84.2[a] (27)	85.4[a] (24)	92.7[ab] (26)

[1]Means not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parentheses indicate the number of animals used for mean variable.

Table D-5

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements on Hemoglobin Level

Factor	Level	Hemoglobin (gm/100 ml)
Protein	7.5%	12.7[1a] (80)[2]
	15.0%	13.1[b] (79)
	30.0%	14.5[c] (76)
Zinc	0 ppm	14.6[a] (78)
	50 ppm	12.7[b] (79)
	100 ppm	12.9[b] (78)
Vitamin B-6	1 ppm	13.5[a] (78)
	5 ppm	13.5[a] (78)
	100 ppm	13.2[a] (79)

[1]Means not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parenthesis indicate the number of animals used for mean values.

Table D-6

Hemoglobin Levels of Young Rats Fed Three Levels of Protein and Zinc, Regardless of Vitamin B-6 Level

Protein Level (%)	Zinc Supplement (ppm)		
	0	50	100
	Hemoglobin (gm/100 ml)		
7.5	13.7[1b] (27)[2]	12.2[a] (27)	12.0[a] (26)
15.0	14.9[c] (25)	12.0[a] (27)	12.4[a] (27)
30.0	15.3[d] (26)	13.9[b] (25)	14.3[b] (25)

[1]Means not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parentheses indicate the number of animals used for mean values.

Table D-7

Hemoglobin Levels of Young Rats Fed Three Levels of Protein and Vitamin B-6, Regardless of Zinc Level

Protein Level (%)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	Hemoglobin (gm/100 ml)		
7.5	12.8[1ab] (26)[2]	12.8[ab] (27)	12.4[a] (27)
15.0	13.0[ab] (26)	13.4[b] (27)	12.9[ab] (26)
30.0	14.7[c] (26)	14.6[c] (24)	14.4[c] (26)

[1]Means not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parentheses indicate the number of animals used for mean value.

Table D-8

Hemoglobin Levels of Young Rats Fed Three Levels of Zinc and Vitamin B-6, Regardless of Protein Level

Zinc Supplement (ppm)	Vitamin B-6 Supplement (ppm) 1	5	100
	Hemoglobin (gm/100 ml)		
0	14.5[1c] (25)[2]	15.5[d] (27)	14.2[c] (26)
50	12.9[ab] (27)	12.4[a] (25)	12.7[ab] (27)
100	13.1[b] (26)	12.9[ab] (26)	12.7[ab] (26)

[1]Mean not sharing common superscript letters are significantly different ($p < .05$)

[2]Numbers in parentheses indicate the number of animals used for mean values.

Table D-9

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements on Liver Copper

Factor	Level	Liver Copper (mcg/g Dry Weight)[1]
Protein	7.5%	8.0[2a]
	15.0%	10.9[b]
	30.0%	10.3[b]
Zinc	0 ppm	10.7[a]
	50 ppm	9.4[b]
	100 ppm	9.1[b]
Vitamin B-6	1 ppm	9.0[a]
	5 ppm	10.5[b]
	100 ppm	9.7[ab]

[1]Each value is the mean of 45 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-10

Liver Copper of Young Rats Fed Three Levels of Protein and Zinc, Regardless of Vitamin B-6 Level

Protein Level (%)	Zinc Supplement (ppm)		
	0	50	100
	Liver Copper (mcg/g Dry Weight)[1]		
7.5	9.7[2b]	7.2[a]	7.0[a]
15.0	11.9[c]	10.5[bc]	10.3[bc]
30.0	10.5[bc]	10.4[bc]	9.9[b]

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-11

Liver Copper of Young Rats Fed Three Levels of Protein and Vitamin B-6, Regardless of Zinc Level

Protein Level (%)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	Liver Copper (mcg/g Dry Weight)[1]		
7.5	7.1[2a]	9.0[bc]	7.8[ab]
15.0	11.0[d]	11.4[d]	10.4[cd]
30.0	8.8[abc]	11.0[d]	11.0[d]

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-12

Liver Copper of Young Rats Fed Three Levels of Zinc and Vitamin B-6, Regardless of Protein Level

Zinc Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	Liver Copper (mcg/g Dry Weight)[1]		
0	9.9^{2b}	11.9^{c}	10.3^{bc}
50	9.2^{ab}	9.5^{b}	9.4^{ab}
100	7.8^{a}	10.0^{b}	9.5^{ab}

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-13

Main Effects of Protein Level, Zinc Supplements or Vitamin B-6 Supplements on Total Liver Copper

Factor	Level	Total Liver Copper[1] (mcg/Total Dry Weight)
Protein	7.5%	20.1[2a]
	15.0%	23.7[b]
	30.0%	19.8[a]
Zinc	0 ppm	14.0[a]
	50 ppm	25.5[b]
	100 ppm	24.1[b]
Vitamin B-6	1 ppm	19.2[a]
	5 ppm	22.3[b]
	100 ppm	22.2[b]

[1]Each value is the mean of 45 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-14

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements on Liver Iron

Factor	Level	Liver Iron (mcg/g Dry Weight)[1]
Protein	7.5%	357[2a]
	15.0%	375[a]
	30.0%	499[b]
Zinc	0 ppm	621[a]
	50 ppm	308[b]
	100 ppm	302[b]
Vitamin B-6	1 ppm	414[a]
	5 ppm	436[a]
	100 ppm	381[a]

[1]Each value is the mean of 45 animals.

[2]Means not sharing common superscript letter are significantly different ($p < .05$).

Table D-15

Liver Iron of Young Rats Fed Three Levels of Protein and Zinc, Regardless of Vitamin B-6 Level

Protein Level (%)	Zinc Supplement (ppm)		
	0	50	100
	Liver Iron (mcg/g Dry Weight)[1]		
7.5	571[2c]	252[a]	248[a]
15.0	510[c]	305[ab]	311[ab]
30.0	782[d]	367[b]	348[ab]

[1] Each value is the mean of 15 animals.

[2] Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-16

Liver Iron of Young Rats Fed Three Levels of Zinc and Vitamin B-6, Regardless of Protein Level

Zinc Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	Liver Iron (mcg/g Dry Weight)[1]		
0	588[2b]	722[c]	554[b]
50	332[a]	289[a]	302[a]
100	322[a]	298[a]	287[a]

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-17

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements on Total Liver Iron

Factor	Level	Total Liver Iron[1] (mcg/Total Dry Weight)
Protein	7.5%	819[2ab]
	15.0%	764[a]
	30.0%	885[b]
Zinc	0 ppm	800[a]
	50 ppm	858[a]
	100 ppm	809[a]
Vitamin B-6	1 ppm	826[a]
	5 ppm	814[a]
	100 ppm	828[a]

[1]Each value is the mean of 45 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-18

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements on Liver Zinc

Factor	Level	Liver Zinc (mcg/g Dry Weight)[1]
Protein	7.5%	60.1[2a]
	15.0%	80.7[b]
	30.0%	78.2[b]
Zinc	0 ppm	67.9[a]
	50 ppm	73.4[b]
	100 ppm	77.6[b]
Vitamin B-6	1 ppm	68.8[a]
	5 ppm	77.7[b]
	100 ppm	72.5[b]

[1]Each value is the mean of 45 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-19

Liver Zinc of Young Rats Fed Three Levels of Protein and Zinc, Regardless of Vitamin B-6 Level

Protein Level (%)	Zinc Supplement (ppm) 0	50	100
	Liver Zinc (mcg/g Dry Weight)[1]		
7.5	64.0[2ab]	55.8[a]	60.4[ab]
15.0	70.5[b]	82.7[c]	88.8[c]
30.0	69.2[bc]	81.7[c]	83.7[c]

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-20

Liver Zinc of Young Rats Fed Three Levels of Protein and Vitamin B-6, Regardless of Zinc Level

Protein Level (%)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	Liver Zinc (mcg/g Dry Weight)[1]		
7.5	57.6[2a]	63.1[a]	59.5[a]
15.0	74.8[b]	86.2[c]	81.1[bc]
30.0	74.0[b]	83.7[bc]	76.9[bc]

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-21

Liver Zinc of Young Rats Fed Three Levels of Zinc and Vitamin B-6, Regardless of Protein Level

Zinc Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
	1	5	100
	Liver Zinc (mcg/g Dry Weight)[1]		
0	61.9[2a]	75.7[bc]	66.1[ab]
50	71.8[abc]	75.8[bc]	72.6[bc]
100	72.6[bc]	81.4[c]	78.8[c]

[1]Each value is the mean of 15 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).

Table D-22

Main Effects of Protein Level, Zinc Supplements, or Vitamin B-6 Supplements on Total Liver Zinc

Factor	Level	Total Liver Zinc[1] (mcg/Dry Weight)
Protein	7.5%	157.4[2a]
	15.0%	181.8[b]
	30.0%	155.9[a]
Zinc	0 ppm	88.8[a]
	50 ppm	199.6[b]
	100 ppm	206.6[b]
Vitamin B-6	1 ppm	153.8[a]
	5 ppm	170.7[b]
	100 ppm	170.7[b]

[1]Each value is the mean of 45 animals.

[2]Means not sharing common superscript letters are significantly different ($p < .05$).