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Effects of biotin, vitamin-B6, and zinc on growth and trace mineral status of young male rats fed adequate and excess protein

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EFFECTS OF BIOTIN, VITAMIN B-6, AND ZINC ON GROWTH AND TRACE MINERAL STATUS OF YOUNG MALE RATS FED ADEQUATE AND EXCESS PROTEIN

by

Wilda Dixon Wade

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APPROVAL PAGE

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This study investigated the effects of biotin, vitamin B-6, and zinc supplements on growth of and trace mineral deposition in young male rats. The animals were kept on experiment for 4 weeks and had free access to food and water while on experiment. Two levels of protein (15% and 30%) were used to provide diets with adequate and excess protein. Supplements to these basic diets included 2 levels of biotin (0 ppm and 4 ppm), 2 levels of zinc (0 ppm and 50 ppm), and 3 levels of vitamin B-6 (0 ppm, 50 ppm, and 100 ppm). Criteria used to evaluate the responses of animals to the dietary treatments included weight gain, and liver copper, iron, and zinc deposition.

Analyses of variance indicated that growth was significantly increased when the diets were supplemented with zinc, vitamin B-6, and biotin. Maximum growth was observed in rats fed diets containing 4 ppm of biotin, 50 ppm of vitamin B-6, and 50 ppm of zinc.

Animals fed excess protein had higher liver copper levels than animals fed adequate protein. Zinc supplementation was associated with decreases in liver copper deposition. Although biotin alone tended to enhance liver copper deposition, this effect was counteracted by the presence of zinc. Increases in vitamin B-6 supplementation were associated with decreases in liver copper deposition. Liver iron levels were

highest in animals fed the 30% protein diets, and the presence of additional zinc in the diet reduced the amount of iron being incorporated into the liver. Biotin and vitamin B-6 supplements had no apparent effect on liver iron deposition. None of the dietary supplements had any effect on liver zinc deposition in this study.

This study indicates that the effects of zinc on animal performance may be dependent upon the amount of biotin and vitamin B-6 present in the diet. The results of this study also support findings from previous studies that zinc offers some protection against excess incorporation of iron into the liver which could possibly result in a toxic condition.

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The author wishes to dedicate this document to the memory of her grandmother, who would have been a century old this year, for her strength, courage and advice to "never give up."

Last but not least, the author wishes to express a deep sense of a "greater spirit" that provided the strength and led the way.

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CHAPTER I

INTRODUCTION

The interrelationships between various nutrients and the effect of one nutrient on a biological parameter as influenced by the presence or absence of other nutrients are currently focal points for many investigations. One such interrelationship under investigation involves vitamin B-6 and zinc, two nutrients which are closely associated with protein and amino acid metabolism. Although vitamin B-6 and zinc have separate biological functions regarding protein metabolism and growth, there is evidence that the presence or absence of either nutrient will affect action of the other nutrient in a biological system.

Biotin deficiency had been considered to be rare since the vitamin is produced by the microflora in the large intestines. Recent investigations have recognized biotin-dependent and biotin-responsive genetic disorders (inherited carboxylase deficiencies) in children and adults (Bonjour, 1981; Cowan, 1984; Hommes, 1986; Mock, 1986). These inherited disorders affect carbohydrate, amino acid, and fatty acid metabolism. Dakshinamurti and Bhagavan (1985) and Bonjour (1984) described the deficiency symptoms of biotin in animals. Alaouri, Essatra, and McClain (1985) have reported that biotin and zinc deficiency symptoms are similar and that an interrelationship exists between the vitamin and mineral.

The purpose of this study was to investigate the effects of biotin, vitamin B-6, and zinc on growth in and trace mineral status of young male rats fed adequate and excess protein. It was to be determined if increases in dietary biotin had any influence on growth and trace mineral parameters effected by a vitamin B-6-zinc dietary interrelationship.

CHAPTER II

REVIEW OF THE LITERATURE

Growth of and protein utilization in several animal species are increased when the diet is supplemented with zinc (Hardie-Muncy & Rasmussen, 1979; Magee & Grainger, 1979; Motsinger & Magee, 1980; Oberleas & Prasad, 1969; O'Dell, Newberne, & Savage, 1958). Magee and Grainger (1979) also reported that the apparent utilization of a suboptimal protein (7.5%) level of dried egg white solids by rats could be improved with a zinc supplement of 50 ppm. Vitamin B-6, as a component of pyridoxal phosphate, is involved with amino acid metabolism, synthesis, and utilization which has a definite influence on growth of an animal (Everett, Mitchell, & Benevenga, 1979; Hawkins, Leonard, & Coles, 1959). Daghir and Shah (1973) reported that growth and feed efficiency in chicks fed high protein diets were significantly increased when the dietary level of vitamin B-6 was increased. Berger, Gebhardt, and Hoffman (1973) also reported that nitrogen retention was higher in chicks supplemented with vitamin B-6 than in chicks fed vitamin B-6 deficient diets. Vitamin B-6 and zinc requirements appear to be dependent upon the level and the source of dietary protein (Cerecedo & Foy, 1946; Cerecedo, Foy, & DeRenze, 1948; Dirige & Beaton, 1969; Forbes

& Yohe, 1960; Hardie-Muncy & Rasmussen, 1979; Magee & Grainger, 1979; Oberleas, Muhrer, & O'Dell, 1965; O'Dell, Burpo, & Savage, 1972; Schweigert, Sauberlich, Elvehjem, & Bauman, 1946; Stuart, Keyelsen, Weaver, & Erdman, 1986).

A number of studies (Evans & Johnson, 1979; Hsu, 1965; Ikeda, Hosotani, Ureda, Kotake, & Sakakbara, 1979; Prasad, Lyall, & Nath, 1982) have shown that zinc status is influenced by dietary vitamin B-6. Son and Magee (1987) reported that increases in growth of rats associated with adequate protein (15%) occurred only when the diet contained at least 50 ppm of zinc, while increases in growth associated with vitamin B-6 supplements occurred only when the protein was marginal (7.5%). The results of Son and Magee (1987) indicated that a zinc-vitamin B-6 interaction had a direct influence on the utilization of dietary protein and on copper and zinc status in young rats.

Dried egg white solids has been used as the protein source in most of the zinc-deficient studies involving rats conducted at the University of North Carolina-Greensboro because it has a low zinc content, and a zinc deficiency state can be produced without subjecting the protein to an extraction procedure to remove the mineral. According to Teklad Test Diets, the supplier of the dried egg white solids, the heat treatment used for the preparation of this protein does not destroy all of the avidin, and approximately 99.5%

of the original amount (0.5%) remains (personal communication). Avidin is a compound known to reduce the bioavailability of biotin, a vitamin which can be synthesized by intestinal microorganisms. Most vitamin mixtures commercially available provide approximately 0.2 ppm of biotin, and this was the level used for previous studies conducted by Magee and Grainger (1979), Motsinger and Magee (1980), and Son and Magee (1987). This level, however, may be inadequate if a high level (20-30%) of dried egg white solids is the protein source. Personnel at Teklad Test Diets (personal communications) indicated that in commercial test diets containing the high protein level, the biotin level is increased to 4 ppm. Son (1984) found that young rats fed a 30% level of protein provided by dried egg white solids which contained 0.2 ppm biotin did not grow as well as rats fed a 15% level of protein. Thus, there is the possibility the level of biotin was not adequate when protein was high (30%) in the diet. Klevay (1976) reported 2 ppm of biotin was necessary to maximum growth in young rats fed 20% egg white, while Kramer, Briske-Anderson, Johnson, and Holoman (1984) found a level of 2.4 ppm of biotin was necessary for growth of young rats fed this level of egg white. The purpose of this study will be to investigate effects of biotin, vitamin B-6, and zinc in young male rats fed adequate (15%) and excess (30%) protein levels (as dried egg white solids) and to determine if increases in dietary biotin have any influence on growth and trace mineral parameters effected by a vitamin B-6-zinc dietary interrelationship.

CHAPTER III

EXPERIMENTAL METHODS

Methods and Procedures

The purpose of this study was to investigate the effect of different levels of biotin, vitamin B-6, and zinc on the growth and liver deposition of copper, iron, and zinc in young male rats fed adequate and excess protein. Parameters used for evaluation included food consumption, weight gain, and liver copper, iron, and zinc concentrations.

This study was a 2 x 2 x 2 x 3 factorial design. Twenty-four dietary combinations included two levels of protein, two levels of biotin, three levels of vitamin B-6, and two levels of zinc. The diets were randomly assigned to a replication, and a total of six replications were used.

Design

A randomized block design, involving a 2 x 2 x 2 x 3 factorial treatment arrangement, was used for the study. Factors included two levels of protein (15% and 30%), two levels of zinc supplement (0 ppm and 50 ppm), two levels of biotin supplements (0 ppm and 4 ppm), and three levels of vitamin B-6 supplements (0 ppm, 50 ppm, and 100 ppm). Animals were maintained on experiment for 4 weeks, and parameters used for evaluation included food consumption, weight gain, and liver copper, iron, and zinc concentrations. The study involved 24 dietary combinations, and a total of six replications were utilized.

Diets

Dried egg white solids[1] was used as the source of protein. Sources of the respective dietary supplements included zinc sulfate,[2] pyridoxine HCl[3], and biotin.[3] In addition to dried egg white solids, the basal diets (without supplements) contained 44.4% or 63.0% cornstarch[4] (depending upon protein level), 10% hydrogenated vegetable oil,[5] 4% mineral mix,[6] 2% vitamin mix (minus biotin and vitamin B-6),[7] 2% vitamin A and vitamin D mix,[8] and 2% cellulose.[9] The compositions of the diets, mineral mix, and vitamin

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[1]Teklad Test Diets, Madison, WI.

[2]Mallinkcrodt Chemical Works, St. Louis, MO.

[3]ICN Nutritional Biochemicals, Cleveland, OH.

[4]Teklad Test Diets, Madison, WI.

[5]Harris Teeter Food Stores, Charlotte, NC.

[6]Wesson Modified Osborne-Mendel Mineral Mix, Teklad Tests Diets, Madison, WI.

[7]All vitamins were purchased from ICN Biochemicals, Cleveland, OH.

[8]The mix contained 3,000 units of Vitamin A palmitate/gram and 400 units of Vitamin D/gram. The vitamins were purchased from Teklad Test Diets, Madison, WI.

[9]Alphacel, ICN Nutritional Biochemicals, Cleveland, OH.

mixtures are shown in Appendix A (Appendix Tables A-1-4). Representative samples of all diets were analyzed for copper, iron, and zinc by atomic absorption spectrophotometry.[10] The nonsupplemented zinc diets contained approximately 7.0 ppm of zinc. Copper and iron contents of all diets averaged approximately 2.7 ppm and 78.2 ppm, respectively. Appendix Table A-5 shows the copper, iron, and zinc analyses of the diets.

Animals

One hundred and forty-four male weaning Sprague-Dawley rats[11] were used for the study. These animals were approximately 21 days of age when received, and the average initial weight of the animals was 54 grams (range 50 to 59 grams). The animals were randomly assigned to individual stainless steel, wire bottom cages within a replication according to initial body weights. The 24 experimental diets (treatments) were also randomly assigned to cages within a replication. Food and distilled water were provided ad libitum. The animals were maintained on experiment for 4 weeks in an environmental controlled environment. Food consumption records were kept on individual animals, and the animals were weighed at weekly intervals.

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[10] Thermo Jarrell Ash Corporation, Model Video 12E, Franklin, MA.

[11] Holtzman Company, Madison, WI.

At the end of the experimental period, animals from five randomly selected replications were decapitated. The livers were removed and weighed. A small amount of each liver was removed, weighed, and oven dried at 60°C to constant weight for moisture determination. The remainder of the liver was wet ashed with hot nitric and perchloric acids on a hot plat. The ash of each liver was dissolved in 0.3 ml of 0.6N HCl and diluted with redistilled water to 25 ml. All glassware used was acid washed. Representative samples of diets were also prepared for mineral analyses as were the livers. Copper, iron, and zinc concentrations of livers and diets were determined by atomic absorption spectrophotometer.

Mineral Analyses

The copper, iron, and zinc contents of livers and diets were determined by atomic absorption spectrophotometer. Commercial standards, analytical AA grade, were purchased for each mineral. Each standard was diluted to appropriate dilutions with redistilled water and standard calibration curve was determined. Aliquots of liver and diet samples were rediluted with redistilled water and read against copper, iron, and zinc standards. All glassware used was acid washed. The copper, iron, and zinc concentrations were read at 324.7 nm, 24.3 nm, and 213.9 nm, respectively. An air-acetylene mixture was used for the flame.

Statistical Analyses

All statistical analyses were determined by SAS computerized program.[12] The growth and minerals data were analyzed by analysis of variance with a significance difference based on the .05 level of probability. A covariance analysis was used to determine if the differences in weight gains were due to diet or food consumption. Least significant differences (LSD) values were determined to provide the differences between individual means which were needed to show significance at the .05 and .01 levels of probability.

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[12] SAS Institute Inc., SAS for linear models: A guide to Anova and GLM procedures. Cary, NC: SAS Institute Inc., 1985, p. 231.

CHAPTER IV

RESULTS

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

Weight Gain

The effects of protein levels and dietary supplements on the growth of young rats used in this study are given in Table 1. The animals receiving the non-zinc supplemented diets exhibited poorer growth than those on zinc supplemented diets. Animals receiving diets deficient in zinc, biotin, and vitamin B-6 combined grew better than animals receiving biotin supplements alone with adequate protein level. Maximum growth was obtained with diets containing 50 ppm of zinc, 4 ppm of biotin, and 50 ppm of vitamin B-6. Zinc supplementation stimulated growth better with adequate protein than with excess protein, and increases in zinc supplementation were associated with highly significant increases ($p < .01$) in weight gain (Appendix C, Table C-1). A difference of 98 grams contributed to a significant increase in percent weight gain (Appendix D,

Table 1

Effects of Protein Levels and Dietary Supplements on Growth of Young Rats

Protein Level %	Zinc Supplement (ppm)	Biotin Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
			0	50	100
			4 Week Weight Gain (gram)[1]		
15	0	0	57 ± 5	51 ± 7	58 ± 8
	0	4	40 ± 6	49 ± 7	82 ±20
	50	0	91 ± 5	157 ±11	157 ± 8
	50	4	129 ± 9	199 ± 8	191 ±13
30	0	0	28 ± 5	69 ±16	50 ± 6
	0	4	33 ± 4	56 ± 4	61 ±11
	50	0	74 ± 4	126 ± 5	150 ± 4
	50	4	128 ±14	211 ±12	197 ± 7

LSD[2] .05: 30

.01: 34

[1]Each value is a mean of 6 animals ±SEM.

[2]Least significant differences at specified probability levels.

Table D-1). Increases in supplements of biotin or vitamin B-6 were associated with highly significant increases ($p < 0.01$) in weight gain (Appendix C, Table C-2).

Interpretations of a significant zinc x biotin interaction ($p < .01$) suggest that zinc stimulates growth and that biotin further enhances the effect of zinc on growth, regardless of protein and vitamin B-6 levels (Appendix D, Table D-2). A zinc x vitamin B-6 interaction was highly significant ($p < .01$) on the effects of growth, regardless of level of protein or biotin (Appendix C, Table C-1). The addition of zinc and vitamin B-6 supplements in diets were synergistic in increasing weight gain, regardless of the protein or biotin levels (Appendix D, Table D-3). Vitamin B-6 significantly increased the growth of rats receiving biotin supplements, regardless of protein and zinc levels (Appendix D, Table D-4).

The effects of the various dietary regimen on percent weight gain are shown in Table 2. All of the interactions previously discussed were significant in percent weight gain. In addition, a significant protein x vitamin B-6 interaction was revealed in the statistical analysis of covariance of percent weight change (Appendix C, Table C-2). Vitamin B-6 apparently improved growth of animals fed adequate dietary protein (15%) more effectively than it did the growth of animals fed excess dietary protein (30%). The significant increase ($p < .01$) in maximum percent weight change was 217 (Appendix D, Table D-5).

Table 2

Effects of Protein Levels and Dietary Supplements on Percent Weight Change of Young Rats

Protein Level %	Zinc Supplement (ppm)	Biotin Supplement (ppm)	Vitamin B-6 Supplement (ppm) 0	50	100
			Percent Weight Change[1]		
15	0	0	107	51	107
15	0	4	75	92	149
15	50	0	171	293	290
15	50	4	128	375	354
30	0	0	53	123	93
30	0	4	61	104	113
30	50	0	138	235	279
30	50	4	240	391	368

[1]Each value is a mean of 6 animals.

When weight gains were adjusted for food consumption by covariance analysis (Appendix C, Table C-3), the effects of the diets on growth were still significant. Thus, the differences in weight gains observed in animals fed the various diets were not due to variations in food consumption.

Interpretation of a significant ($p < .05$) zinc x biotin x vitamin B-6 interaction suggest that increases in biotin supplementation decreases growth, but the addition of zinc supplementation significantly stimulated growth. The effects of zinc and biotin combined were greater on growth than either alone. Maximum growth was observed with 50 ppm of zinc, 4 ppm of biotin, and 50 ppm of vitamin B-6 (Appendix D, Table D-6).

Liver Copper

The effects of dietary supplements on liver copper deposition in animals fed adequate and excess protein are shown in Table 3. Maximum copper values were found in animals receiving no zinc supplements (Appendix D, Table D-7), and increases in zinc were associated with highly significant decreases ($p < .01$) in liver copper levels (Appendix C, Table C-4). Although the main effect of biotin on liver copper deposition was not statistically significant, the presence of biotin tended to enhance liver copper deposition. Biotin, however, could not alleviate the adverse effect of

Table 3

Effects of Protein Levels and Dietary Supplements on Liver Copper Deposition in Young Rats

Protein Level %	Zinc Supplement ppm	Biotin Supplement ppm	Vitamin B-6 Supplement (ppm) 0	50	100
			Liver Copper (mcg/g Dry Weight)[1]		
15	0	0	12.5 ±0.7	13.3 ±1.0	13.7 ±0.9
			15.1 ±1.8	13.4 ± .7	14.1 ±1.8[2]
	50	0	7.4 ±1.4	9.9 ±1.2[2]	8.6 ±0.8[2]
	50	4	9.8 ±1.6	10.5 ±0.8	11.2 ±0.7[2]
30	0	0	14.1 ±1.5	14.5 ±1.5	12.7 ±0.7
	0	4	22.0 ±5.2	14.8 ±0.4	12.3 ±1.3
	50	0	13.0 ±3.5	7.2 ±0.6	8.1 ±1.3
	50	4	8.6 ±1.4	10.2 ±0.9	10.4 ±1.0
LSD[3] .05:	1.4				
.01:	1.9				

[1]Each value is the mean of 5 animals ±SEM, unless otherwise indicated.

[2]Mean of 4 animals.

[3]Least significant differences at specified probability levels.

zinc on liver copper deposition, and the presence of both of these supplements in the diet was associated with significant reduction ($p < .01$) in liver copper deposition (Appendix D, Table D-8). Significant increases ($p < .05$) in liver copper were associated with increases in dietary protein, but the presence of vitamin B-6 in the diet appeared to have an adverse effect on liver copper, regardless of zinc or biotin levels (Appendix D, Table D-9). Supplements of vitamin B-6 were associated with decreases in liver copper deposition when zinc was deficient in the diet, but appeared to have little effect when adequate zinc was present in the diet (Appendix D, Table D-10). Vitamin B-6 supplements tended to decrease liver copper levels in the presence and absence of biotin regardless of level of protein (Appendix D, Table D-11). There was also evidence that vitamin B-6 supplementation was able to counteract the adverse effects of both biotin and zinc on liver copper deposition, regardless of protein levels (Appendix D, Table D-12).

Liver Iron

Table 4 shows the effects of dietary supplements on liver iron deposition in animals fed adequate and excess protein. Highly significant increases ($p < .01$) in liver iron deposition were observed as the protein level of the diet was increased from 15% to 30%. Supplements of zinc were associated with highly significant ($p < .01$) decreases in liver iron deposition

Table 4

Effects of Protein Levels and Dietary Supplements on Liver Iron Deposition in Young Rats

Protein Level %	Zinc Supplement ppm	Biotin Supplement ppm	Vitamin B-6 Supplements (ppm) 0	50	100
			Liver Iron (mcg/gm Dry Weight)[1]		
15	0	0	598 ±109	720 ±196	681 ± 33
	0	4	723 ±176	762 ± 60	609 ±144
	50	0	470 ±106	287 ± 49	421 ± 83
	50	4	349 ± 69	360 ± 34	439 ±141[2]
30	0	0	830 ± 83	1112 ±272	721 ± 88
	0	4	1508 ±515	1242 ±204	896 ±267
	50	0	725 ±349	535 ± 86	546 ±115
	50	4	472 ± 57	509 ± 64	369 ± 37
LSD[3] .05:	146				
.01:	193				

[1]Each value is the mean of 5 animals ±SEM, unless otherwise indicated.

[2]Mean of 4 animals.

[3]Least significant differences at specified probability levels.

(Appendix C, Table C-5). The main effects of biotin and vitamin B-6 did not appear to be statistically significant, but supplements of biotin were associated with some increase in liver iron deposition. The presence of vitamin B-6 showed a tendency to adversely affect liver iron deposition. (Appendix C, Table D-13).

Liver Zinc

The effects of dietary supplements on liver zinc deposition of young rats fed adequate and excess protein are given in Table 5. None of the main effects of biotin, vitamin B-6, protein, or zinc and none of the interactions between these factors were found to be statistically significant in this study (Appendix C, Table C-5). Higher zinc deposition in the livers was found in animals fed the 30% protein level than in animals fed 15% protein, and the presence of vitamin B-6 was associated with slight increases in liver zinc deposition (Appendix D, Table D-14). Biotin and zinc supplements were found to have only a small nonsignificant influence on liver zinc, and the effects of these two factors appeared to be dependent upon the amount of protein and/or vitamin B-6 that was present in the diet.

Table 5

Effects of Protein Levels and Dietary Supplements on Liver Zinc Deposition of Young Rats

Protein Level %	Zinc Supplement ppm	Biotin Supplement ppm	Vitamin B-6 Supplement (ppm) 0	50	100
			Liver Zinc (ug/gram dry weight)[1]		
15	0	0	81 ±18	62 ±13	73 ±13
	0	4	71 ±18	89 ± 7	85 ±37[2]
	50	0	65 ±12	68 ±17	89 ±22[3]
	50	4	59 ± 8	56 ±15	76 ±12[3]
30	0	0	81 ± 8	104 ±36	83 ±15
	0	4	85 ±22	73 ± 7	51 ± 7
	50	0	77 ±22	66 ±10	103 ±41
	50	4	79 ±22	100 ±42	110 ±42
LSD[4] .05:	61				
.01:	63				

[1]Each value is the mean of 5 animals ±SEM, unless otherwise indicated.

[2]Mean of 3 animals.

[3]Mean of 4 animals.

[4]Least significant differences at specified probability levels.

CHAPTER V

DISCUSSION

Results of this study support the findings of Son and Magee (1987) that rats fed adequate protein (15%) in the form of dried egg white solids require at least 50 ppm of zinc for adequate growth. Son (1984) also reported that zinc supplements increased growth of animals fed excess protein (30%), but the results of this study did not support this observation. Even when a biotin supplement was added to a diet containing 30% protein to replace the biotin lost by the effect of avidin present in dried egg white solids, animals fed this diet did not show increased growth over animals fed diets containing 15% protein. This observation was not totally unexpected because the utilization of many nutrients, including protein, apparently decreases as the levels of such nutrients are increased in the diet. The results of this study did indicate that growth of young rats appears to be dependent on the presence of biotin, vitamin B-6, and zinc in the diet.

Zinc is an essential nutrient involved in protein and amino acid metabolism. Zinc-dependent enzymes, such as RNA and DNA polymerase and thymidine kinase, associated with nucleoprotein metabolism essential for growth, are depressed in a zinc deficiency. Amino acid metabolism and/or utilization are also reduced in animals fed zinc-deficient diets Hsu, Anthony, & Buchanan, 1969; Hsu & Woosley, 1972; Theuer & Hoekstra, 1966). Several of the enzymes associated with protein and amino acid metabolism apparently are very sensitive to zinc depletion (Prasad, 1988). Neary and Divan (1970) also reported that pyridoxal kinase, the enzyme which catalyzes the conversion of vitamin B-6 analogs to pyridoxal phosphate, is reduced in zinc deficiency. Pyridoxal phosphate, the active form of vitamin B-6, is involved with amino acid absorption and in amino acid metabolic reactions such as transamination, deamination, and transsulfuration which provide necessary amino acids for protein biosynthesis. Although zinc and vitamin B-6 are associated with different mechnaisms involving protein biosynthesis and growth, this study supports previous findings that adequate amounts of both are needed in the diet for maximum protein utilization by growing animals.

Biotin supplements were associated wtih increases in weight gains of young rats in this study, and the results of this study suggest that the effects of vitamin B-6 and/or zinc on certain parameters may be dependent upon the amount of biotin in the diet. Alaouri, Essatra, and McClain (1985) reported that a biotin supplement of 0.2 ppm added to a 20% dried egg white solid diet fed to weanling rats could partially alleviate growth depression associated with zinc deficiency (0.2 ppm and 2.0 ppm). These researchers also reported that biotin (0.2 ppm) could improve the growth of animals receiving adequate zinc (12 ppm and 150 ppm), but that a supplement of 2 ppm of biotin resulted in a decrease in weight gain. A similar trend was observed by this researcher (Wade, unpublished data, 1989) in a preliminary experiment in which weanling rats were fed zinc-deficient diets supplemented with biotin. The source of protein in this experiment was dried egg white solids, and increases in growth were observed when a 4 ppm supplement of biotin was added to diets with inadequate and excess protein. Although the growth of the animals in this preliminary study was still subnormal because the diets were deficient in zinc, the results did indicate biotin did have an effect on growth.

Physiological symptoms of deficiencies of vitamin B-6 or zinc appear to be similar in rats; and based on the results of previous studies, the symptoms of a biotin or a zinc deficiency are also similar. Growth retardation, alopecia, dermal lesions, immunodeficiency, and infertility are common to each of these nutrients (Cunnane, 1988). Animals in this study receiving diets deficient in biotin, vitamin B-6, and zinc exhibited loss of hair around the eyes, enlarged pupils, dermal lesions on the feet, and loss of hair on the abdominal area. Zinc, however, appeared to be the nutrient that was primarily responsible for alleviating these symptoms observed in rats in this study.

The decrease of liver copper deposition associated with physiological increases of dietary zinc and the increase in liver copper deposition in zinc deficient rats observed in this study support the supposition of Gawthrone and Howell (1987) that the level of copper or zinc in a diet will affect the utilization of the other mineral. Although dietary copper was not a factor involved with this study, Magee and Matrone (1960) have reported that copper supplements could alleviate the adverse effect of zinc on copper utilization in young rats.

The increases in liver copper levels associated with increases in dietary protein observed in this study are in agreement with the findings of previous researchers (Motsinger & Magee, 1980; Son & Magee, 1986). Vitamin B-6 supplements, however, were associated with decreases in liver copper deposition, regardless of protein level and when zinc was deficient in the diet. There is the possibility that the effect of vitamin B-6 on liver copper may be due to the size of the liver observed in animals with and without zinc supplementation. Son and Magee (1986) reported the effects of zinc and vitamin B-6 were not necessarily the same when total copper content of the liver was used as a parameter instead of reporting copper on a per gram dry weight of liver tissue.

Vitamin B-6 may influence liver copper deposition in the absence of zinc and may spare the effect of a marginal zinc deficiency on weight gain. If zinc is deficient in the system, it would be expected that copper utilization would be increased since the competitive effect of zinc is absent. The possible role of biotin in copper metabolism remains unclear at this time, although the results of this study suggest that the presence of biotin influences liver copper deposition.

Significant accumulations of liver iron observed in animals fed increases in dietary protein were significantly reduced when zinc was supplemented in the diet. Although there was no evidence that the excess liver iron had any adverse effects on the animals in this study, excessive iron accumulation in a system can result in toxic situations uncer certain conditions. Son and Magee (1986) previously suggested that zinc may offer some protection against a possibility of an iron toxicity developing in young animals, and the results of this study support this suggestion.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

Young male rats of the Sprague-Dawley variety were fed adequate and excess protein supplemented with 2 levels of biotin, 2 levels of zinc, and 3 levels of vitamin B-6. Parameters used to evaluate the effects of the various dietary regimens fed to the animals were weight gain, and liver copper, liver iron, and liver zinc deposition.

The addition of 50 ppm of zinc to the diet was associated with highly significant increases ($\underline{p} < .01$) in weight gain. Biotin and vitamin B-6 supplements were associated with highly significant ($\underline{p} < .01$) increases in weight gain. The effect of zinc, vitamin B-6, and biotin on weight gains were 84, 34, and 21 grams, respectively. Significant interactions between zinc x vitamin B-6 and zinc x biotin suggest vitamin B-6 and biotin were synergistic on the effects of zinc on growth. There was also evidence that the growth of young rats was better when both biotin and vitamin V-6 were in the diet. Maximum growth occurred with adequate protein, 50 ppm of zinc, 4 ppm of biotin, and 50 ppm of vitamin B-6.

Zinc supplementation was associated with highly significant decreases in liver copper deposition; whereas increases

in dietary protein were associated with significant increases in liver copper, vitamin B-6 diminished the effect of protein. Biotin appeared to promote a further decrease in liver copper associated with zinc supplementation. A vitamin B-6 effect on reducing liver copper occurred only when the diet was not supplemented with zinc. Vitamin B-6 supplements decrease liver copper in the presence or absence of biotin and also reverses the effect of biotin and zinc combination on liver copper deposition.

Highly significant increases ($p < .01$) in liver iron were observed with increases in protein and highly significant ($p < .01$) decreases in liver iron are associated with increases in dietary zinc. Biotin slightly increases liver iron, whereas vitamin B-6 slightly decreases liver iron.

Liver zinc deposition was not significantly influenced by protein, biotin, vitamin B-6, or zinc, and no interactions were of significance. The level of protein in the diet seems to increase zinc deposition.

There were similarities of significant effects of dietary components on parameters evaluated. Increases in dietary protein levels were generally associated with substantial increases in growth, liver copper, and liver iron. Biotin supplementation increased weight gain, but had no apparent effect on liver copper and liver iron. Supplements of zinc were associated with increases in weight gain, decreases in liver copper and liver iron, but no effect on liver zinc.

Vitamin B-6 supplements increase weight gain, decrease liver copper in the absence of zinc, and in the presence or absence of biotin. Several interactions between the same nutrients occurred in growth and liver copper. The two-way interactions were zinc x vitamin B-6, zinc x biotin, vitamin B-6 x biotin, and protein x vitamin B-6. A three-way interaction occurred between zinc x vitamin B-6 x biotin.

Recommendations

Since a supplemental level of 4 ppm of biotin was associated with decreases in growth in the absence of zinc and vitamin B-6, another study could be designed to study the effects of levels of biotin in the diet between the minimum recommended for rats (0.2 ppm) and 4 ppm. Lower levels of supplemental biotin may also interact differently with zinc and vitamin B-6 than was observed in this study.

The effects of biotin, vitamin B-6, and zinc on the exact mechanisms associated with growth cannot be determined from this study. Thus, studies which are designed to study specific mechanisms involved with amino acid absorption and metabolism as influenced by these three nutrients may provide additional information at the cellular level which might be helpful in elucidating the specific roles of biotin, vitamin B-6, and zinc. Nitrogen balance studies conducted on rats fed diets similar to the ones used in this study may also yield additional information.

Although zinc apparently influences the effect of vitamin B-6 in the diet, the exact mechanism of how zinc is associated with vitamin B-6 metabolism remains an enigma. Studies which are designed to study the effect of zinc on vitamin B-6 utilization in young rats or the effect of zinc on certain vitamin B-6 dependent enzyme systems associated with amino acid utilization and/or metabolism may provide useful information for the clarification of an interaction between zinc and vitamin B-6 suggested in this study.

Biotin has an influence on growth in the absence or presence of zinc, but its role in zinc deficiency needs further study since it partially alleviates the physical symptoms. Investigation of the biotin and zinc enzyme systems would help to elucidate some additional effects of this interaction.

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APPENDIX A

COMPOSITION OF THE EXPERIMENTAL DIETS

Table A-1

Composition of Basal Diets

Ingredients	Protein Level 15%	30%
	gram/2 kilogram	
Dried egg white solids 80% purity	375	750
Cornstarch	1265	890
Hydrogenated vegetable oil	200	200
Mineral mix	80	80
Vitamin mix (minus biotin and vitamin B-6)	40	40
Cellulose	40	40
Vitamin A and D mix	20	20

Table A-2

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

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

[a]Teklad Test Diets, Madison, Wisconsin.

Table A-3

Composition of Vitamin Mixture (Minus Biotin and Vitamin B-6)

Constituents[a]	gm/2 kg Mix
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[b]	1732.00

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

[b]Teklad Test diets, Madison, Wisconsin.

Table A-4

Diet Combinations

Diet	Protein Level (%)	Vitamin B-6 Supplement (ppm)	Zinc Supplement (ppm)	Biotin Supplement (ppm)
1	15	0	0	0
2	15	0	0	4
3	15	0	50	0
4	15	0	50	4
5	15	50	0	0
6	15	50	0	4
7	15	50	50	0
8	15	50	50	4
9	15	100	0	0
10	15	100	0	4
11	15	100	50	0
12	15	100	50	4
13	30	0	0	0
14	30	0	0	4
15	30	0	50	0
16	30	0	50	4
17	30	50	0	0
18	30	50	0	4
19	30	50	50	0
20	30	50	50	4
21	30	100	0	0
22	30	100	0	4
23	30	100	50	0
24	30	100	50	4

Table A-5

Trace Mineral Content of Dried Egg White Solids[a] Diets

Mineral	mcg/gm dry wt[b]
Copper	2.7
Iron	78.0
Zinc	7.0[c]

[a]Teklad Test Diets, Madison, WI.

[b]Atomic absorption spectrophotometry, Thermo Jarrell Ash Corporation, Model Video 12E, Franklin, MA.

[c]Nonsupplemented zinc diet.

APPENDIX B

RAW DATA

Table B-1

Total Wet Weight of Livers

Diet	Replicates 1	2	3	4	5	Mean
			grams			
1	4.12	4.65	5.69	4.33	4.17	4.59
2	3.85	2.61	2.50	2.15	5.83	3.39
3	6.88	7.21	3.05	5.61	7.18	5.99
4	10.20	7.59	6.94	6.13	8.38	7.84
5	4.05	3.50	4.51	3.90	3.72	3.94
6	4.94	4.40	2.91	2.39	4.58	3.84
7	11.02	8.28	8.09	8.38	8.62	8.88
8	14.40	12.61	10.84	13.22	8.69	11.95
9	3.59	6.20	3.73	3.56	5.02	3.68
10	4.81	3.41	4.21	2.93	8.79	4.83
11	7.01	7.72	10.23	8.14	9.62	8.54
12	7.23	13.31	12.69	11.45	10.31	11.00
13	2.70	3.88	2.81	1.99	4.09	3.09
14	2.93	3.37	3.29	2.60	3.31	3.10
15	5.92	4.63	5.03	6.39	4.21	5.24
16	8.01	4.23	13.18	8.48	6.79	8.26
17	3.55	3.57	4.19	3.05	6.91	4.25
18	2.89	4.26	4.12	5.21	4.61	4.22
19	6.88	8.89	9.23	8.60	7.75	8.27
20	10.39	11.81	11.31	11.62	10.55	11.14
21	3.53	3.24	3.05	3.90	5.02	3.75
22	4.75	3.06	4.59	8.31	4.50	5.04
23	9.25	8.29	8.82	7.87	10.27	8.90
24	10.45	13.38	11.82	12.19	11.03	11.86

Table B-2

Wet Weight of Livers

Diet	Replicates 1	2	3	4	5	Mean
			grams			
1	3.79	4.42	5.38	3.96	3.95	4.30
2	3.68	2.40	2.07	1/97	5.57	3.14
3	6.61	7.02	6.54	5.37	6.83	6.47
4	9.82	7.34	6.74	5.78	8.15	7.60
5	3.76	3.31	4.26	3.64	3.52	3.04
6	4.65	4.11	2.63	2.11	4.32	3.56
7	10.61	7.85	7.83	8.15	8.37	8.56
8	13.96	12.21	10.52	13.01	8.41	11.62
9	3.30	6.03	3.46	3.22	4.75	4.15
10	4.51	3.22	3.82	2.77	8.42	4.55
11	6.64	7.44	9.87	7.81	9.17	8.19
12	6.80	12.97	12.34	11.12	9.95	10.64
13	2.29	3.23	2.69	1.62	3.89	2.74
14	2.68	2.86	3.00	2.18	2.97	2.74
15	5.52	4.64	4.59	6.05	4.00	4.96
16	7.70	3.97	12.96	8.17	6.51	7.86
17	3.76	3.41	3.78	2.71	6.83	4.02
18	2.66	3.93	3.93	4.99	4.61	4.02
19	6.65	8.62	9.05	8.29	7.59	8.04
20	9.97	11.51	10.97	11.38	10.29	10.82
21	3.27	2.94	2.54	3.70	4.84	3.46
22	4.36	2.86	4.42	8.00	4.18	4.76
23	9.02	7.98	8.48	7.59	9.90	8.59
24	9.98	13.01	11.53	11.94	10.67	11.42

Table B-3

Dry Weight of Livers

	Replicates					
Diet	1	2	3	4	5	Mean
			grams			
1	1.21	1.41	1.75	1.12	1.27	1.35
2	1.23	0.75	0.62	0.60	1.92	1.02
3	2.11	2.31	2.04	1.70	2.25	2.08
4	2.62	1.89	2.10	1.83	2.55	2.20
5	1.11	1.04	1.39	1.14	1.14	1.17
6	1.48	1.33	0.81	0.64	1.38	1.13
7	2.98	2.51	2.39	2.67	2.57	2.62
8	4.28	3.55	3.21	3.97	2.61	3.52
9	1.01	1.88	1.09	1.06	1.51	1.31
10	1.36	0.98	1.19	0.79	2.62	1.39
11	2.21	2.34	3.14	2.48	2.74	2.58
12	2.28	4.03	3.77	3.34	3.21	3.33
13	0.73	1.00	0.88	0.52	1.31	0.89
14	0.90	0.86	0.99	0.70	0.89	0.87
15	1.78	1.51	1.37	1.93	1.24	1.57
16	2.55	1.30	3.92	2.67	2.08	2.50
17	1.05	1.11	1.13	0.87	1.94	1.22
18	0.87	1.28	1.26	1.63	1.141	1.29
19	2.18	2.78	2.76	2.69	2.53	2.59
20	3.09	3.52	3.31	3.84	3.15	4.70
21	1.08	0.93	0.79	1.17	1.60	1.11
22	1.38	0.92	1.38	2.50	1.36	1.51
23	2.92	2.55	2.72	2.41	3.09	2.74
24	3.14	4.37	3.67	3.66	3.44	3.66

Table B-4

Percent Dry Weight of Livers

Diet	Replicates 1	2	3	4	5	Mean
	grams					
1	31.91	31.88	32.48	28.26	32.29	31.36
2	33.53	31.43	29.67	30.43	34.47	31.91
3	31.81	31.90	32.30	31.93	32.96	32.18
4	26.70	25.70	31.12	31.73	31.30	29.31
5	29.37	31.55	32.66	31.42	32.50	31.50
6	31.84	32.40	30.99	30.32	32.04	31.51
7	28.12	32.02	30.57	32.75	30.05	30.78
8	30.70	29.70	30.50	30.52	30.99	30.48
9	30.48	31.21	31.64	32.85	31.72	31.58
10	30.10	30.40	31.10	28.57	31.15	30.46
11	33.24	31.41	31.77	31.72	29.82	31.59
12	33.56	31.07	30.57	30.03	32.22	31.49
13	31.94	31.08	32.80	31.79	33.66	32.25
14	33.47	30.21	32.99	31.89	29.85	31.68
15	32.17	33.73	29.82	31.98	30.92	31.72
16	31.94	32.70	30.22	32.72	33.30	32.18
17	31.84	32.48	29.80	31.98	29.39	31.10
18	32.61	32.42	32.09	32.72	32.08	32.38
19	32.76	32.21	30.51	32.48	33.30	32.25
20	30.97	30.61	30.20	33.75	30.62	31.23
21	33.07	30.60	31.26	31.71	32.95	31.92
22	31.61	32.09	31.18	31.21	32.61	31.74
23	32.34	32.00	32.06	31.79	31.92	32.02
24	31.48	33.606	31.84	30.65	32.22	31.96

Table B-5

Total Weight Gain of Rats

Diet	Replicates 1	2	3	4	5	6	Mean
	grams						
1	54	71	54	73	41	47	57
2	54	30	32	36	62	26	40
3	98	85	84	90	112	77	91
4	157	129	94	114	132	147	129
5	55	40	31	58	79	43	51
6	67	65	34	32	60	37	49
7	153	149	138	208	151	140	157
8	237	195	185	198	191	189	199
9	37	84	41	51	55	78	58
10	65	53	44	42	148	137	82
11	128	148	166	148	163	186	157
12	126	213	197	198	207	204	191
13	24	42	21	11	33	38	28
14	25	34	22	29	46	40	33
15	79	59	76	70	72	89	74
16	116	82	191	129	125	127	128
17	43	51	42	38	109	131	69
18	40	64	58	68	51	55	56
19	106	124	143	121	136	127	126
20	175	207	178	233	227	246	211
21	46	43	32	72	59	45	50
22	72	32	56	111	50	44	61
23	151	142	148	162	159	138	150
24	188	215	218	197	179	186	197

Table B-6

Total Food Consumption of Rats

	Replicates 1	2	3	4	5	6	Mean
	grams						
1	104	199	232	221	208	177	190
2	229	174	200	200	195	296	175
3	148	210	183	201	212	332	214
4	237	198	177	218	247	180	210
5	155	177	175	196	196	154	176
6	117	186	168	160	183	280	182
7	330	252	300	240	319	262	284
8	287	325	310	357	350	160	315
9	179	262	191	185	200	307	221
10	306	181	185	200	244	224	223
11	300	202	383	313	250	320	295
12	176	423	251	338	372	310	312
13	145	166	124	752	161	204	159
14	243	143	87	161	123	215	162
15	198	177	161	195	292	233	209
16	228	210	289	182	246	224	230
17	232	206	213	163	268	261	223
18	246	218	251	238	232	307	249
19	156	270	225	222	235	230	223
20	328	275	320	370	370	390	319
21	247	146	207	184	210	225	203
22	149	150	164	300	172	153	181
23	272	220	204	340	210	254	250
24	340	360	303	311	394	362	345

Table B-7

Liver Copper Concentrations

Diet	Replicates 1	2	3	4	5	Mean
			mcg/gram dry weight			
1	13.0	12.6	10.3	14.7	12.0	12.5
2	14.0	21.2	17.1	15.5	10.2	15.6
3	13.0	6.06	5.8	6.0	6.0	7.4
4	5.2	14.1	8.6	12.6	8.5	9.8
5	12.2	13.9	10.8	13.1	16.6	13.3
6	11.3	14.1	14.4	15.0	12.3	13.4
7	10.0	-	6.7	12.1	10.8	9.9
8	9.5	10.6	10.8	8.6	13.2	10.5
9	13.4	11.2	13.3	16.8	13.9	13.7
10	-	16.2	12.0	18.0	10.3	14.1
11	8.3	10.8	-	7.3	8.0	8.6
12	13.3	-	10.1	10.7	10.8	11.2
13	13.4	13.6	13.6	19.4	10.3	14.1
14	14.2	42.9	13.4	23.7	15.8	22.0
15	7.0	11.8	26.8	9.4	9.7	13.0
16	6.0	11.6	5.8	7.7	12.2	8.6
17	20.0	16.2	12.4	11.8	12.6	14.5
18	13.8	11.6	13.3	12.6	13.0	12.8
19	6.5	7.8	8.2	5.2	8.2	7.2
20	10.8	10.4	9.4	7.7	12.9	10.2
21	11.8	15.3	12.0	13.7	11.1	12.7
22	12.0	16.6	12.3	8.5	11.9	12.3
23	5.1	11.4	5.1	9.2	9.8	8.1
24	10.6	10.5	10.5	10.0	10.3	10.4

Table B-8

Liver Iron Concentrations

Diet	Replicates 1	2	3	4	5	Mean
	mcg/gram dry weight					
1	852	857	401	536	343	598
2	1058	696	1169	460	233	723
3	769	298	460	635	189	470
4	318	600	301	352	176	349
5	1049	1077	346	978	147	720
6	928	750	806	771	555	762
7	320	320	434	148	211	287
8	250	436	376	321	417	360
9	740	645	778	627	614	681
10	66	860	678	837	600	609
11	666	500	229	472	240	421
12	819	-	489	240	209	439
13	973	767	1072	642	698	830
14	2203	3152	1138	664	381	1508
15	767	270	2062	197	328	725
16	663	520	362	445	369	472
17	2062	1088	1186	802	421	1112
18	1879	1348	1387	800	795	1242
19	706	772	466	346	383	535
20	575	401	660	317	591	509
21	928	631	833	784	427	721
22	1672	1323	694	619	174	896
23	489	489	660	896	198	546
24	440	329	247	445	385	369

Table B-9

Liver Zinc Concentrations

	Replicates					
Diet	1	2	3	4	5	Mean
			mcg/gram dry weight			
1	114	129	43	78	39	82
2	132	41	91	42	49	71
3	104	35	61	48	75	65
4	34	73	54	58	78	59
5	107	53	45	71	33	62
6	76	103	69	99	99	89
7	57	12	99	105	68	68
8	105	53	51	61	10	56
9	124	47	63	65	66	73
10	-	45	33	158	-	85
11	124	46	-	58	128	89
12	41	-	80	90	94	76
13	82	93	50	84	95	81
14	63	275	51	90	169	85
15	56	54	-	55	141	77
16	58	51	59	59	168	79
17	239	62	67	36	116	104
18	50	59	69	96	89	73
19	95	29	68	67	69	66
20	130	37	47	34	254	100
21	104	54	71	133	55	83
22	36	75	50	53	41	51
23	73	59	53	62	267	103
24	50	50	82	92	276	110

APPENDIX C

STATISTICAL ANALYSIS

Table C-1

Analysis of Variance of Weight Gain Data

Source of Variation	Degrees of Freedom	Sum of Squares		Mean Squares
Total	143	548031		
Diet	23	485989		21130
Protein	1		1438	1438
Biotin	1		23896	23896**
Zinc	1		346627	346627**
B-6	2		61913	30957**
Protein x B-6	2		1343	672
Protein x Biotin	1		1185	1185
Protein x Zinc	1		4	4
B-6 x Zinc	2		18529	9265**
Biotin x Zinc	1		21438	21438**
B-6 x Biotin	2		613	357
Protein x B-6 x Biotin	2		591	296
Protein x B-6 x Zinc	2		2278	1139
Protein x Biotin x Zinc	1		1363	1368
B-6 x Biotin x Zinc	2		3504	1752*
Protein x B-6 x Biotin x Zinc	2		1269	635
Error	120	62043		517

*Significant ($p < .05$)
**Highly Significant ($p < .01$)

Table C-2

Analysis of Covariance of Percent Weight Gain

Source of Variation	Degrees of Freedom	Sum of Squares		Mean Squares
Total	143	189.754		
Diet	24	170.651		7.110
Eaten	1		106.8	106.800**
Protein	1		0.002	0.002
Biotin	1		1.04	1.040
Zinc	1		49.65	49.650**
B-6	2		0.515	0.258
Protein x B-6	2		1.030	0.518*
Protein x Biotin	1		.005	0.005
Protein x Zinc	1		.000	0.00
B-6 x Zinc	2		3.277	1.639**
Biotin x Zinc	1		5.408	5.408**
B-6 x Biotin	2		0.852	0.476
Protein x B-6 x Biotin	2		0.248	0.124
Protein x B-6 x Zinc	2		0.274	0.137
Protein x Biotin x Zinc	1		0.213	0.213
B-6 x Biotin x Zinc	2		1.472	0.736*
Protein x B-6 x Biotin x Zinc	2		0.320	0.160
Error	119	19.103		0.161

*Significant ($p < .05$)
**Highly significant ($p < .01$)

Table C-3

Analysis of Covariance of Weight Gain Data

Source of Variation	Degrees of Freedom	Sum of Squares		Mean Squares
Total	143	548031		
Diet	24	491107		20463
Eaten	1		312166	312166**
Protein	1		24	24
Biotin	1		1596	1596
Zinc	1		139339	139339**
B-6	2		1539	1539
Protein x B-6	2		2440	1220
Protein x Biotin	1		26	26
Protein x Zinc	1		2	2
B-6 x Zinc	2		8887	4444**
Biotin x Zinc	1		14927	14927**
B-6 x Biotin	2		2619	1310
Protein x B-6 x Biotin	2		661	331
Protein x B-6 x Zinc	2		666	333
Protein x Biotin x Zinc	1		640	640
B-6 x Biotin x Zinc	2		4517	2254*
Protein x B-6 x Biotin x Zinc	2		1058	514
Error		56924		478

*Significant ($p < .05$)
**Highly significant ($p < .01$)

Table C-4

Analysis of Variance of Liver Copper Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
Total	115	2548.33	
Diet	23	1168.75	50.82
Protein	1	5.59	5.59
Biotin	1	46.62	46.62
Zinc	1	646.15	646.15**
B-6	2	52.79	52.79
Protein x B-6	2	106.73	53.37*
Protein x Biotin	1	0.55	0.55
Protein x Zinc	1	5.82	5.82
B-6 x Zinc	2	42.68	21.34
Biotin x Zinc	1	1.90	1.90
B-6 x Biotin	2	16.77	8.39
Protein x B-6 x Biotin	2	2.51	1.26
Protein x B=6 x Zinc	2	11.07	5.54
Protein x Biotin x Zinc	1	8.79	8.79
B-6 x Biotin x Zinc	2	136.72	68.36*
Protein x B-6 x Biotin x Zinc	2	84.07	42.04
Error	92		15.00

*Significant ($p < .05$)
**Highly significant ($p < .01$)

Table C-5

Analysis of Variance of Liver Iron Data

Source of Variation	Degrees of Freedom	Sum of Squares		Mean Squares
Total	118	25294886		
Diet	23	10036680		436377
Protein	1		1891342	1891342**
Biotin	1		389257	289157
Zinc	1		5045951	5045951**
B-6	2		349538	174769
Protein x B-6	2		289257	194629
Protein x Biotin	1		39741	39741
Protein x Zinc	1		389291	389291
B-6 x Zinc	2		317173	158587
Biotin x Zinc	1		518672	518672
B-6 x Biotin	2		67933	33967
Protein x B-6 x Biotin	2		70232	35116
Protein x B-6 x Zinc	2		42842	21421
Protein x Biotin x Zinc	1		351031	351031
B-6 x Biotin x Zinc	2		407183	203592
Protein x B-6 x Biotin x Zinc	2		77442	38721
Error	95	15258206		160613

*Significant ($p < .05$)
**Highly significant ($p < .01$)

Table C-6

Analysis of Variance of Liver Zinc Data

Source of Variation	Degrees of Freedom	Sum of Squares Squares		Mean Squares
Total	114	245908		
Diet	23	27540		1197
Protein	1		4352	4352
Biotin	1		60	60
Zinc	1		31	31
B-6	2		1371	686
Protein x B-6	2		478	239
Protein x Biotin	1		57	57
Protein x Zinc	1		2379	2379
B-6 x Zinc	2		6476	3238
Biotinx Zinc	1		533	533
B-6 x Biotin	2		653	327
Protein x B-6 x Biotin	2		622	311
Protein x B-6 x Zinc	2		1005	503
Protein x Biotin x Zinc	1		5483	5483
B-6 x Biotin x Zinc	2		137	69
Protein x B-6 x Biotin x Zinc	2		3904	1952
Error	91	218367		2400

*Significant ($p < .05$)
**Highly significant ($p < .01$)

APPENDIX D

GROUP MEAN TABLES

Table D-1

Main Effects of Vitamin B-6, Biotin, and Zinc Supplements on Growth of Young Rats Fed Different Protein Levels

		4 Week Weight Gain[1]		
Factor	Level	Gram	Percent	
Protein	15%	105[2a]	196	(60)
	30%	99[a]	183	(60)
Vitamin B-6	0 ppm	72[a]	135	(48)
	50 ppm	115[b]	214	(48)
	100 ppm	118[bc]	219	(48)
Biotin	0 ppm	88[a]	165	(72)
	4 ppm	114[b]	213	(72)
Zinc	0 ppm	53[a]	98	(72)
	50 ppm	151[b]	281	(72)

[1]Number in parenthesis is the number animals used for each mean.

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

Table D-2

Weight Gain of Young Rats Fed Zinc and Biotin Supplements Regardless of Protein and Vitamin B-6 Levels

Zinc Supplement (ppm)	Biotin Supplements (ppm)[1]	
	0	4
	4 Week Weight Gain (gram)	
0	52[2a]	53[a]
50	125[b]	176[c]

[1]Each number is a mean of 24 animals.

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

Table D-3

Weight Gain of Young Rats Fed Zinc and Vitamin B-6 Supplements Regardless of Protein and Biotin Levels

Zinc supplement ppm	Vitamin B-6 Supplements (ppm)		
	0	50	100
	4 Week Weight Gain (gram)[1]		
0	39[2a]	56[ac]	62[ac]
50	106[b]	172[c]	174[c]

[1]Each number is a mean of 24 animals.

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

Table D-4

Weight Gain of Young Rats Fed Biotin and Vitamin B-6 Supplements, Regardless of Protein and Zinc Levels

Biotin Supplement (ppm)	Vitamin B-6 Supplements (ppm)		
	0	50	100
	4 Week Weight Gain (gram)[a]		
0	70[2a]	100[bc]	103[bc]
4	82[abc]	129[b]	133[b]

[1]Each value is a mean of 24 animals.

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

Table D-5

Weight Gain of Young Rats Fed Two Levels of Protein and Vitamin B-6 Supplement, Regardless of Zinc or Biotin Levels

Protein Level %	Vitamin B-6 Supplement (ppm)		
	0	50	100
	4 Week Weight Gain		
15	83 (155)[2a]	113 (212)[b]	117 (217)[b]
30	73 (135)[a]	112 (208)[ab]	111 (211)[ab]

[1]Each number is mean of 24 animals.

[2]Numbers in parenthesis are % weight gain.

[3]Values not showing common superscript are significantly different ($p < .05$).

Table D-6

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

Zinc Supplement (ppm)	Biotin Supplement (ppm)	Vitamin B-6 Supplements (ppm)		
		0	50	100
		4 Week Weight Gain (gram)		
0	0	42^{abc}	60^{abc}	54^{abc}
0	4	36^{2a}	53^{abc}	71^{b}
50	0	82^{bc}	141^{d}	153^{d}
50	4	129^{d}	187^{e}	194^{e}

[1]Each number is a mean of 12 animals.

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

Table D-7

Main Effects of Vitamin B-6, Biotin, and Zinc Supplements on Liver Copper Deposition in Young Rats Fed Different Protein Levels

Factor	Level	Liver Copper (mcg/gram Dry Weight)[1]
Protein	15%	11.7[1a] (60)[2]
	30%	12.2[a] (56)
Vitamin B-6	0 ppm	12.9[a] (40)
	50 ppm	11.5[a] (39)
	100 ppm	11.4[a] (37)
Biotin	0 ppm	11.3[a] (58)
	4 ppm	12.6[a] (58)
Zinc	0 ppm	14.3[b] (59)
	50 ppm	9.6[a] (57)

[1]Means not sharing common superscript are significantly different ($p < .01$).

[2]Number in parenthesis is the number of animals.

Table D-8

Liver Copper of Young Rats Fed Zinc and Biotin Supplements, Regardless of Protein or Vitamin B-6

Zinc Supplement (ppm)	Biotin Supplement (ppm)	
	0	4
	Liver Copper mcg/gram dry weight	
0	13.5[b] (30)	15.0[c] (29)
50	9.0[2a] (28)	10.1[a] (29)

[1]Number in parenthesis indicates the number animals used for mean values.

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

Table D-9

Liver Copper Deposition in Young Rats Fed Protein and Vitamin B-6 Supplements, Regardless of Zinc and Biotin Levels

Protein Level %	Vitamin B-6 Supplements (ppm)		
	0	50	100
	Liver Copper (mcg/gm Dry Weight)		
15	11.3[a] (20)	11.9[a] (19)	12.0[a] (17)
30	14.4[c] (20)	11.2[a] (20)	10.9[2ab] (20)

[1]Number in parenthesis indicates the number of aniamls used for mean values.

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

Table D-10

Liver Copper of Young Rats Fed Zinc and Vitamin B-6 Supplements, Regardless of Protein or Biotin

Zinc Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
	9	50	100
	Liver Copper (mcg/gram dry weight)		
0	16.0[c] (20)	13.5[b] (20)	13.2[b] (19)
50	9.7[a] (20)	9.4[2a] (19)	9.5[a] (18)

[1]Number in parenthesis indicates the number animals used for mean values.

[2]Means not sharing a common superscript are significant ($p < .05$).

Table D-11

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

Biotin Supplement	Vitamin B-6 Supplement (ppm) 0	50	100
	Liver Copper (mcg/gram dry weight)		
0	11.7[2a]	11.3[3a]	10.8[3]
4	14.0[b]	11.8[a]	11.9[4a]

[1]Each value is a mean of 20 animals, unless otherwise indicated.

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

[3]Mean of 19 animals.

[4]Mean of 18 animals.

Table D-12

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

Zinc Supplement (ppm)	Biotin Supplement (ppm)	Vitamin B-6 Supplement (ppm)		
		0	50	100
		Liver Copper (mcg/gram dry weight)		
0	0	13.3[c]	13.9[c]	13.2[c]
0	4	18.8[e]	13.1[c]	13.1[c]
50	0	10.2[b]	8.4[a]	8.3[a]
50	4	9.2[abc]	10.4[b]	10.8[b]

[a]Each value is a mean of 10 animals, unless otherwsie indicated.

[b]Mean of 9 animals.

[c]Values not sharing common superscript are significantly different ($p < .05$).

Table D-13

Main Effects of Vitamin B-6, Biotin, and Zinc Supplements on Liver Iron Deposition in Young Rats Fed Different Protein Levels

Factor	Level	Liver Iron (mcg/gram Dry Weight)[1]
Protein	15%	535[1a] (60)[2]
	30%	787 (59)
Vitamin B-6	0 ppm	710[bc] (40)
	50 ppm	691[b] (40)
	100 ppm	586[a] (39)
Biotin	0 ppm	637[a] (60)
	4 ppm	687[a] (59)
Zinc	0 ppm	867[b] (60)
	50 ppm	457a (59)

LSD .05: 146

.01: 193

[1]Means not sharing common superscript letters are significantly different ($p < .01$).

[2]Number in parenthesis is the number of aniamls.

Table D-14

Main Effects of Vitamin B-6, Biotin, and Zinc Supplements on Liver Zinc Deposition in Young Rats Fed Different Protein Levels

Factor	Level	Liver Zinc (mcg/gram Dry Weight)[1]
Protein	15%	72[2a] (56)
	30%	84[a] (59)
Vitamin B-6	0 ppm	75[a] (40)
	50 ppm	77[a] (40)
	100 ppm	84[a] (35)
Biotin	0 ppm	79[a] (58)
	4 ppm	78[a] (57)
Zinc	0 ppm	78[a] (58)
	50 ppm	79[a] (57)

[1]Number in parenthesis is the number of animals.

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