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The effects of 5, 10, and 20% levels of soybean meal and several fractions of soybean meal on the growth, hemoglobin levels, and liver copper and iron levels of rats fed 0.8% of zinc were determined in an attempt to characterize the factor(s) in soybean meal which alleviates symptoms of zinc toxicity. Fractions of soybean meal were obtained from ashing, methanol or water extractions, and chloroform fractionation of a methanol extract. Each fraction and level of soybean meal was added to a basal diet at the expense of equal amounts of cornstarch and fed to young male rats for four weeks.

All levels of soybean meal markedly increased the weight gains of the zinc-fed rats; however, the soybean meal did not prevent the anemia or depressed liver copper and iron levels associated with zinc toxicity. The 20% level of soybean meal offered much greater protection against subnormal growth than the 5 and 10% levels of the meal, but did not completely reverse the toxic effect of zinc on growth.

The factor(s) in soybean meal which alleviates the subnormal growth of zinc-fed rats appears to be organic in nature and extractable with methanol or water. An attempt to further characterize the factor(s) in the methanol extract as lipid or non-lipid in nature was unsuccessful.

A STUDY OF THE ZINC TOXICITY ALLEVIATING

FACTOR(S) IN SOYBEAN MEAL

by

Mary Virginia Durkin

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APPROVAL SHEET

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CHAPTER I

INTRODUCTION

The effects of toxic levels of the essential trace elements on experimental animals are often studied to gain information concerning the role of these minerals in animal nutrition. The dietary level required to produce symptoms of toxicosis varies considerably among the different minerals. Animals can consume much greater concentrations of zinc without adverse effects than of many other metallic elements. However, when toxic levels of zinc are fed in the diet, a wide range of metabolic processes in the cells and tissues is affected.

The first report of zinc toxicity by Sutton and Nelson in 1937 indicated that dietary levels of 0.5 and 1.0% of zinc cause growth depression, anemia, and reproductive failure in young rats. Subsequent studies have revealed many more symptoms of zinc toxicity. These include decreased liver copper and iron levels, decreased catalase and cytochrome oxidase activities, and interference with the normal deposition of calcium and phosphorus in the bones.

The adverse effect of zinc on the growth and mineral metabolism of young rats has often been studied through the use of dietary supplements. Growth depression can be alleviated with supplements of calcium and phosphorus, liver extract, distiller's dried solubles, and soybean meal. The fact that these latter three materials reverse the subnormal growth of zinc-fed rats suggests that some necessary growth factor may be involved in the interference of zinc with growth. Characterization of the factor(s) in liver extract, distiller's dried solubles, and soybean meal might lead to an explanation of the effect of toxic levels of zinc on the growth of young rats.

CHAPTER II

REVIEW OF LITERATURE

Although Sutton and Nelson (1) reported that 0.5% and 1.0% levels of dietary zinc caused growth depression in young rats more than three decades ago, the exact nature of the interference of zinc with growth has not been determined. Grant-Frost and Underwood (2) reported that a level of 0.5% of zinc caused a marked decrease in food consumption as well as growth and maintained that the effect of zinc on the growth of rats was largely due to reduced food consumption. Sadasivan (3), however, found no decrease in food intake at a 0.5% level of zinc but observed decreased food consumption in rats fed 1.0% level of zinc.

Several investigators (4, 5, 6, 7) have shown that supplements of either liver extract, distiller's dried solubles, soybean meal, or calcium and phosphorus alleviate the growth depressing effect of high levels of zinc. Magee and Spahr (7) reported that 5% supplemental levels of distiller's dried solubles and liver extract offered greater protection against subnormal growth in zinc-fed rats than the same level of soybean meal. Although the addition of 20% of distiller's dried solubles completely alleviated the adverse effect of zinc toxicity on weight gain, the effects of increasing the levels of liver extract or soybean meal supplements to 20% have not been reported. The results of McCall, Mason, and

Davis (8) suggest that increasing the level of soybean meal in the diet would cause marked improvement in the growth of zinc-fed rats. Their data indicated that the growth response of rats fed zinc and a level of 40% of soybean meal as the sole source of dietary protein was essentially the same as that of rats fed the control diet.

Magee and Matrone (5) reported that the factor(s) in liver extract which alleviates the subnormal growth of zinc-fed rats is organic in nature and can be extracted with methanol. Recently, Magee[1] has obtained data which indicates that the alleviating factor(s) in distiller's dried solubles is extractable with water or methanol. No detailed study of the factor(s) detected in soybean meal has been undertaken to date.

The objectives of this study were to determine the effects of 5, 10, and 20% levels of soybean meal on the growth of rats fed a toxic level of zinc and to investigate the nature of the factor(s) in soybean meal which alleviates the subnormal growth of zinc-fed rats.

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[1] Aden C. Magee, 1968. Unpublished data.

CHAPTER III

EXPERIMENTAL PROCEDURES

This study compares the effects of a.) the three levels of soybean meal supplements, b.) various soybean meal fractions, and c.) fractions from a methanol extract of soybean meal on the weight gains of rats fed a high level of zinc. The influence of these supplements on the hemoglobin levels, liver copper levels, and liver iron levels of the zinc-fed rats was also considered. Since a number of different methods were used to prepare the supplements for this study, procedures related to the individual experiments will be discussed separately. The general procedures common to all of the experiments will be described in the following paragraphs.

Young male rats[1] of the Sprague-Dawley strain (3 weeks of age) were used for all experiments conducted during the study. The rats were housed in individual wire bottom cages and given free access to food and water. The animals in each experiment were randomized into replications according to initial body weights. The test treatments within each replication were randomly assigned to individual cages. The length of each experiment was four weeks. The animals were weighed weekly, and their food consumption was recorded.

The composition of the basal diet used in this study is shown in

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

Table I. The supplements tested were added to the basal diet at the expense of equal amounts of cornstarch. High zinc diets contained 0.8% of zinc as zinc carbonate.

At the end of each experiment, oxyhemoglobin determinations were made on blood samples obtained from the tails of all animals according to the method of Shenk et al.(9).

At the termination of experiments I and 2, four rats from each test diet were sacrificed. In experiment 3, all animals were sacrificed. The liver of each sacrificed animal was removed and prepared for subsequent mineral analyses.

Approximately one-tenth of each liver was dried to constant weight at 35°C to obtain dry weight data. The remainder of each liver was ashed with nitric and perchloric acids. The liver ash was dissolved in I ml. of 0.6N HCl, and the solution was brought up to a volume of 25 ml. with redistilled water.

The copper and the iron contents of the livers were determined by the methods of Parks et al.(10) and Kitzes et al.(11), respectively, as modified by Matrone et al.(12). A randomized block design was used for each experiment, and all data were subjected to an analysis of variance. The effect of food intake on weight gain was determined by covariance analysis. Statements of significance are based on odds of at least 19 to 1($p \leq 0.05$). Least significant difference (L.S.D.) values were calculated to give an indication of the difference required between two treatment means to show significance.

Experiment I

The purpose of this experiment was to compare the effects of

TABLE I

COMPOSITION OF THE BASAL DIET

Constituents	Per cent
Casein[a]	22.1
Cornstarch[b]	59.9
Vegetable fat[c]	10.0
Mineral mix[d]	4.0
Vitamin mix[e]	2.0
Cellulose[f]	2.0
Oleum percomorphum[g]	

[a]Vitamin Test Casein, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[b]Globe Easy-flow Cornstarch 3366, Corn Products Sales Company, Greensboro, North Carolina.

[c]Crisco, Proctor and Gamble Company, Cincinnati, Ohio.

[d]Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio. The composition of this salt mixture is listed as: (in per cent) $CaCO_3$ 21.000; $CuSO_4 \cdot 5H_2O$, 0.039; $FePO_4 \cdot 2H_2O$, 1.470; $MnSO_4$, 0.020; $Mg\ SO_4$, 9.000; $KAl(SO_4)_2 \cdot 12H_2O$, 0.009; KCl, 12.000; KH_2PO_4, 31.000; KI, 0.005; NaCl, 10.5000; NaF, 0.057; and $Ca_3\ (PO_4)_2$, 14.900.

[e]Each 100 gm of vitamin mix contained: (in milligrams) 0.1% vitamin B_{12} (with mannitol), 0.1; biotin, 1; folic acid, 5; Thiamine·HCl, 25; pyridoxine · HCl, 25; 2-methyl-naphthoquinone, 50; riboflavin, 50; nicotinic acid, 50; Ca pantothenate, 150; p-aminobenzoic acid, 500; (in grams) inositol, 5: choline chloride, 7.5; DL-methionine, 30; and cornstarch, 56.6. All vitamins and methionine were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[f]Alphacel, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[g]Each kilogram of diet contained 24 drops of oleum percomorphum, Mead Johnson and Company, Evansville, Indiana.

5, 10, and 20% levels of soybean meal on the growth, hemoglobin levels, and liver copper and iron levels of rats fed 0.8% of zinc.

Experiment 2

The purpose of this experiment was to compare the effects of soybean meal ash and materials obtained from water and methanol extractions of soybean meal on the growth, hemoglobin levels, and liver copper and iron levels of rats fed 0.8% of zinc. The ash, methanol extract, water extract, and solvent extracted meal were obtained from amounts of soybean meal equivalent to a level of 20% of meal in the diet. All solid fractions were dried at 60°C prior to incorporation into the test diets. The liquid fractions were added to appropriate amounts of cornstarch, and the mixtures were dried at 60°C before addition to the proper test diets.

The inorganic portion of soybean meal was prepared by ashing 25 gm. amounts of the meal with nitric and perchloric acids on a hotplate. Most of the ashed material was removed from the beakers with a spatula and incorporated into the test diet. The remaining ash which adhered to the beakers was dissolved in 0.6N HCl, and the resulting solutions were added to cornstarch, dried, and incorporated into the ash test diet.

The solvent extracts were prepared by fractionating 50 gm. and 100 gm. amounts of soybean meal with 700 ml. of water and 500 ml. of methanol, respectively. All extractions were run 18 hours in Soxhlet extractors. When methanol was used as the solvent, solids precipitated from each extract. These precipitates were dispersed in water and added to cornstarch.

Experiment 3

The purpose of this experiment was to compare the effects of chloroform-soluble and chloroform-insoluble fractions of a methanol extract on the growth, hemoglobin levels, and liver copper and iron levels of rats fed 0.8% of zinc. The amount of methanol extract used in the fractionation was equivalent to a level of 20% of soybean meal in the diet.

One thousand grams of soybean meal was soaked in methanol 24 hours and then extracted 18 hours with approximately 1500 ml. of methanol in a large modified Soxhlet extractor. The methanol extract was filtered, and 40% of the extract was dried onto cornstarch. Another 40% of this methanol extract was concentrated to a thick slurry in a flash evaporator at 56-58°C under reduced pressure. The slurry was extracted with chloroform until no color remained in the chloroform layer. The chloroform extracts were dried onto cornstarch at 40°C under vacuum. The chloroform-insoluble fraction of the extract was dispersed in water, and this dispersion was added to cornstarch.

CHAPTER IV

RESULTS AND DISCUSSION

The data obtained from this study are presented in Appendix A, Tables 1-12, and Appendix C, Tables 1-3.

Experiment I

Supplements of 5, 10, and 20% levels of soybean meal resulted in highly significant increases ($p \leq 0.01$) in the weight gains of the rats fed 0.8% of zinc (Table 2). Although the 20% level of soybean meal offered much greater protection against growth depression than the 5 and 10% levels of meal, it did not completely alleviate the toxic effect of zinc on weight gain.

Supplementing the zinc diet with soybean meal did not prevent the marked decreases in hemoglobin, liver copper, and liver iron levels associated with zinc toxicity. The mean liver copper level of rats fed 10% of soybean meal plus zinc was significantly higher ($p \leq 0.05$) than that of rats fed zinc without supplement, but the 5 and 20% levels of soybean meal did not significantly lessen the effect of the high zinc diet on liver copper levels. The addition of soybean meal to the zinc diet was associated with further but nonsignificant reductions in hemoglobin and liver iron levels.

When soybean meal was added to the basal diet, the weight gains of the rats increased as the level of meal was increased in the diet, and the rats fed 10 and 20% levels of soybean meal were

TABLE 2

RESPONSE OF RATS TO VARIOUS LEVELS OF ZINC AND SOYBEAN MEAL

Level of Soybean meal Supplement	Level of Added Zinc	Weight Gain at 4 weeks[a]	Hemoglobin Level[a]	Liver Constituents[b] Cu	Fe
%	%	gm	gm/100 ml blood	mcg/gm dry weight	
0	0	176	12.81	12.39	248.79
0	0.8	96	6.58	3.98	139.30
5	0	190[c]	13.81[c]	9.12	244.00
5	0.8	124	5.85	5.17	126.37
10	0	193	13.99	9.39	248.91
10	0.8	126	6.48	6.61	115.65
20	0	196[c]	13.17[c]	10.10	288.81
20	0.8	160	6.70	3.02	121.19
$L.S.D._{0.05}^{d}$		17	3.50	2.70	63.84
$L.S.D._{0.01}^{d}$		23	4.68	3.67	86.88

[a]Each figure is the mean of seven animals unless otherwise indicated.
[b]Each figure is the mean of four animals.
[c]Mean of six animals.
[d]Least significant difference at specified probability levels.

significantly heavier ($p \leq 0.05$) than the rats fed the basal diet. The addition of soybean meal to the basal diet caused nonsignificant increases in hemoglobin and liver iron levels. At all levels of supplementation, the liver copper levels of the rats fed soybean meal were lower than those of rats fed the basal diet; the difference was significant for the 5 and 10% levels but not the 20% level of supplementation.

Experiment 2

The methanol and water extracts were the only fractions of soybean meal tested that alleviated the growth depression of rats fed 0.8% of zinc (Table 3). The mean weight gain of the rats consuming the zinc diet with the methanol extract and the rats consuming the zinc diet with the water extract were essentially the same. The weight gains of the rats fed either of these extracts were significantly higher ($p \leq 0.01$) than those of the rats fed only zinc; however, the effects of these extracts on the growth of the zinc-fed rats were less than the effect obtained with a level of 20% of soybean meal in experiment 1. The decreased growth responses of the rats fed the extracts suggest that part of the factor(s) was deactivated or made unavailable by the extraction procedures.

Addition of the other soybean meal fractions or soybean meal itself to zinc diets resulted in no significant differences in the weight gains of the rats fed these supplements and the weight gains of rats fed zinc without supplement. The insignificant weight increase of the rats receiving 20% of soybean meal plus zinc was unexpected in view of the highly significant ($p \leq 0.01$) growth

TABLE 3

RESPONSE OF ZINC-FED RATS TO VARIOUS FRACTIONS OF SOYBEAN MEAL

Supplement	Level of Added Zinc	Weight Gain at 4 weeks[a]	Hemoglobin Level[a]	Liver Constituents[b] Cu	Fe
	%	gm	gm/100 ml blood	mcg/gm dry weight	
None	0	171	13.04	13.84	254.86
None	0.8	98[c]	5.69[c]	3.88	121.35
20% Soybean meal	0	198	12.96	10.82	227.70
20% Soybean meal	0.8	102[c]	6.34[c]	2.81	106.48
Soybean meal ashe[e]	0.8	83[c]	8.31[c]	4.13	152.85
Methanol extract[e]	0.8	136[c]	5.80[c]	4.40	108.32
Methanol extracted soybean meal	0.8	84	5.86	3.15	168.41
Precipitate from methanol extract	0.8	98	5.52	4.62	104.16
Water extract[e]	0.8	135	5.14	4.59	95.17
Water extracted soybean meal	0.8	112[c]	5.42[d]	3.86	141.45
$L.S.D.^{f}_{0.05}$		23	1.10	2.36	46.89
$L.S.D.^{f}_{0.01}$		30	1.47	3.19	63.32

[a]Each figure is the mean of seven animals unless otherwise indicated.
[b]Each figure is the mean of four animals.
[c]Mean of six animals.
[d]Mean of five animals.
[e]Equivalent to 20% soybean meal.
[f]Least significant difference at specific probability levels.

responses obtained with all three levels of soybean meal in experiment 1. The results of covariance analysis indicated that the growth responses in this experiment were not a reflection of food intake.

Supplementing the zinc diet with the various soybean meal fractions did not prevent marked reductions in hemoglobin, liver copper, and liver iron levels. Only the zinc-fed rats supplemented with the ash of soybean meal showed a significant increase ($p \leq 0.05$) in hemoglobin levels. Rats fed methanol-extracted soybean meal had significantly higher ($p \leq 0.05$) liver iron levels than the rats fed zinc alone, but no other supplement had a significant effect on liver iron. None of the supplements significantly increased the liver copper levels of the zinc-fed rats.

Experiment 3

The chloroform-insoluble fraction of the methanol extract was the only supplement that caused a significant increase ($p \leq 0.05$) in the weight gains of zinc-fed rats, and none of the supplements had a significant effect on the hemoglobin, liver copper, or liver iron levels of these rats (Table 4). After adjustments for food intake were made by covariance analysis, the weight gains of the rats on the various diets were not significantly different. Since zinc diets supplemented with 20% of soybean meal and methanol extract were included in this experiment, the reason for the lack of significant growth responses is not apparent. One of the factors involved may be depressed food consumption caused by infection because many of the rats exhibited symptoms commonly associated with respiratory disease.

TABLE 4

RESPONSE OF ZINC-FED RATS TO FRACTIONS OF A METHANOL EXTRACT OF SOYBEAN MEAL

Supplement	Level of Added Zinc	Weight Gain at 4 weeks[a]	Hemoglobin Level[a]	Liver Constituents[b] Cu	Fe
	%	gm	gm/100 ml blood	mcg/gm dry weight	
None	0	178	13.51	11.07	268.92
None	0.8	90	6.82	3.01	117.00
20% Soybean meal	0.8	97[b]	7.52[b]	2.64[b]	121.94[b]
Methanol extract[d]	0.8	106	7.00	3.32	129.20
Methanol extracted soybean meal[d]	0.8	120[c]	7.32[c]	1.98[c]	127.79[c]
Chloroform-soluble fraction of methanol extract	0.8	96	7.62	3.35	124.66
Chloroform-insoluble fraction of methanol extract	0.8	129	6.40	2.85	119.95
L.S.D.[e] 0.05		35	1.76	1.18	29.22
L.S.D. 0.01		47	2.38	1.59	39.46

[a]Each figure is the mean of six animals unless otherwise indicated.
[b]Mean of four animals.
[c]Mean of five animals.
[d]Equivalent to 20% soybean meal.
[e]Least significant difference at specific probability levels.

CHAPTER V

GENERAL DISCUSSION

The results of this study indicate that soybean meal contains an organic factor(s) which alleviates the subnormal growth of rats fed a toxic level of zinc and that this factor(s) can be extracted from the meal with water or methanol. An attempt was made to further characterize the factor(s) in the methanol extract; however, the varied growth responses of the rats do not permit definitive conclusions.

The solubility characteristics of the factor(s) in soybean meal give little indication of the type of compound which may be responsible for the alleviating effect of soybean meal. Identification of the factor(s) is further complicated by the fact that very little research on the compounds extracted from soybean meal with water or methanol has been reported. It has been established that water extraction of soybean meal removes most of the protein and that phytic acid is complexed with the protein (13). Alcoholic extracts of soybean protein precipitated from the water extract have been shown to contain phospholipids, triglycerides, saponins, genistein, and sitosterols (14). With the exception of the compounds mentioned, organic materials extracted from soybean meal with water or methanol have not been generally characterized.

Although the factor(s) which reverses the effect of zinc on growth has not been identified, the results of several investigators suggest that protein fractions which bind zinc may be involved. McCall _et al._(8) compared the growth of zinc-fed rats receiving equal amounts of protein from soybean meal or casein and found that the soybean meal provided greater protection than casein. These researchers proposed that specific protein compounds or structures in soybean meal may be responsible for this greater protection. Since zinc forms stable complexes with the imidazole group of histidine and the sulfhydryl group of cysteine (15), it is possible that these amino acids in soybean meal chelate with zinc to prevent its interference with growth. This possibility is further supported by the results of Rackis _et al._(13) which suggest that these amino acids may be concentrated in the water extract of soybean meal.

The alleviating effect of the factor(s) in soybean meal may also be due to the binding of zinc by phytic acid extracted with the soybean meal protein. The results of several studies (16, 17) suggest that the phytic acid extracted with the soybean protein reduces the availability of zinc in the diets of chickens by interfering with intestinal absorption. The possibility that phytic acid may be involved is further supported by the report of Allred _et al._(18) that the in vitro binding of zinc by soybean protein is reduced when part of the phytic acid is removed.

The soybean meal supplements used in this experiment did not reverse the effects of zinc on the hemoglobin levels of the rats. Since McCall _et al._(8) reported that 40 and 60% levels of soybean meal alleviate the anemia of zinc toxicity, it is possible that the levels of soybean meal used in this experiment did not permit utilization of sufficient quantities of iron and copper for normal hematopoiesis. Another possibility is that the higher levels of soybean meal used by McCall et al.(8) provided enough iron and copper to alleviate the adverse effect of zinc. However, the fact that the soybean meal ash significantly increased the hemoglobin levels of the zinc-fed rats suggests that most of the copper and iron in soybean meal may be present in forms which cannot be absorbed or utilized.

Settlemire and Matrone (19, 20) have proposed that zinc affects iron metabolism by a.) interfering with the incorporation of iron into or the release from ferritin and b.) shortening the life span of erythrocytes. Interference with ferritin impaired iron absorption and limited storage of iron as ferritin, whereas the shortened erythrocyte life span resulted in a greater excretion of red blood cell iron.

Only the 10% level of soybean meal alleviated the effect of zinc on liver copper levels. Since McCall et al.(8) reported that 40 and 60% levels of soybean meal slightly decreased the liver copper levels of zinc-fed rats, it seems possible that copper metabolism is affected by the level of some factor in the diet. The nature of the interference of zinc on copper metabolism has not been established. Studies (5, 21) have indicated that zinc decreases absorption and utilization of copper.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

The present study was conducted to determine the effects of 5, 10, and 20% levels of soybean meal supplements on the growth of rats fed 0.8% of zinc, and to investigate the nature of the factor(s) in soybean meal which alleviates the subnormal growth of the zinc-fed rats.

The basal diet was supplemented with soybean meal and fractions of soybean meal which were obtained from ashing, methanol or water extractions, and chloroform fractionation of a methanol extract. Each test diet was fed to young rats for a four week period. Weight gains, hemoglobin levels, and liver copper and iron levels were used to measure the response of the rats to the various supplements. All data were analyzed by analysis of variance and analysis of covariance.

Results from the first experiment showed that all three levels of soybean meal markedly increased the weight gains of the zinc-fed rats. The 20% level of soybean meal offered much greater protection against subnormal growth than the 5 and 10% levels of meal; however, the toxic effect of zinc was not reversed completely. The soybean meal did not prevent the anemia, or depressed liver copper and iron levels associated with zinc toxicity.

Data obtained from the second experiment indicated that the factor(s) in soybean meal which alleviates growth depression is organic in nature and that this factor(s) can be extracted from the meal with methanol and water. None of the soybean meal fractions improved the liver copper levels of the zinc-fed rats. The methanol-extracted soybean meal increased liver iron levels, but no other supplement had a significant effect on liver iron. Only the ash of soybean meal increased hemoglobin levels.

The results from the third experiment did not clearly indicate whether the factor(s) in the methanol extract is lipid or non-lipid in nature due to illness in many of the animals.

Recommendations for Further Investigations

The active factor(s) in soybean meal has been isolated in water or methanol extracts; however, the results of this study do not identify any specific component that is responsible for the alleviating effect of soybean meal on the growth of zinc-fed rats. Further isolation of the factor(s) from the water and/or methanol extracts would facilitate the identification of the active principle in soybean meal. Metabolic studies might elucidate the mechanism by which soybean meal reverses the adverse effect of zinc on growth.

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APPENDIX A

GROWTH, HEMOGLOBIN, TISSUE MINERAL DATA

TABLE I

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT I
ON GROWTH OF RATS

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 5% soybean meal
4. 5% soybean meal + 0.8% zinc
5. 10% soybean meal
6. 10% soybean meal + 0.8% zinc
7. 20% soybean meal
8. 20% soybean meal + 0.8% zinc

	Treatments							
Replications	1	2	3	4	5	6	7	8
				4 weeks weight gain (gm)				
1	175	73	(190)[a]	140	192	102	196	137
2	179	125	197	129	205	116	204	144
3	160	92	191	117	218	146	204	164
4	166	88	190	127	184	152	181	145
5	181	88	180	149	185	134	192	195
6	171	84	186	106	185	130	199	165
7	198	125	196	104	184	102	(196)	167
Total	1230	675	1330	872	1353	882	1357	1117
Mean	176	96	190	124	193	126	196	160

[a]( ) indicates calculated missing plot value

TABLE 2

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT I
ON HEMOGLOBIN LEVELS

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 5% soybean meal
4. 5% soybean meal + 0.8% zinc
5. 10% soybean meal
6. 10% soybean meal + 0.8% zinc
7. 20% soybean meal
8. 20% soybean meal + 0.8% zinc

	Treatments							
Replications	1	2	3	4	5	6	7	8
	gm/100 ml blood							
1	13.20	9.29	(13.81)[a]	5.60	14.23	5.31	11.60	4.51
2	13.12	5.40	13.49	7.83	14.54	6.86	13.86	6.29
3	13.66	5.69	13.49	4.91	13.29	5.20	12.94	6.72
4	12.43	7.72	12.94	5.31	14.86	6.72	13.12	5.89
5	11.20	5.89	15.14	5.43	12.94	6.97	13.66	6.17
6	12.94	6.97	12.94	6.97	14.03	7.14	13.86	4.57
7	13.12	5.11	14.86	4.91	14.03	7.14	(13.17)	5.77
Total	89.67	46.07	96.67	40.96	97.92	45.34	92.21	39.92
Mean	12.81	6.58	13.81	5.85	13.99	6.48	13.17	5.70

[a]( ) indicates calculated missing plot value.

TABLE 3
EFFECTS OF TREATMENTS TESTED IN EXPERIMENT I
ON LIVER COPPER

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 5% soybean meal
4. 5% soybean meal + 0.8% zinc
5. 10% soybean meal
6. 10% soybean meal + 0.8% zinc
7. 20% soybean meal
8. 20% soybean meal + 0.8% zinc

	Treatments							
Replications	1	2	3	4	5	6	7	8
	mcg/gm dry weight							
1	15.60	2.94	10.03	7.01	10.35	4.48	9.82	3.56
2	13.62	3.70	8.77	8.47	9.11	8.38	10.96	2.89
3	10.65	5.66	7.47	2.62	9.64	8.53	7.82	2.58
4	9.68	3.61	10.22	2.57	8.45	5.06	11.79	3.04
Total	49.55	15.91	36.49	20.67	37.55	26.45	40.39	12.07
Mean	12.39	3.98	9.12	5.17	9.39	6.61	10.10	3.02

TABLE 4

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT I
ON LIVER IRON

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 5% soybean meal
4. 5% soybean meal + 0.8% zinc
5. 10% soybean meal
6. 10% soybean meal + 0.8% zinc
7. 20% soybean meal
8. 20% soybean meal + 0.8% zinc

Replications	Treatments 1	2	3	4	5	6	7	8
	mcg/gm dry weight							
1	245.76	159.40	269.31	133.84	205.40	119.63	254.63	121.27
2	239.36	133.37	223.46	126.66	246.51	113.16	250.81	133.29
3	233.51	121.03	255.55	124.62	296.67	114.90	451.10	111.66
4	276.53	143.42	227.67	120.35	247.05	114.92	198.70	118.55
Total	995.16	557.22	975.99	505.47	995.63	462.61	1155.24	484.71
Mean	248.79	139.30	244.00	126.37	248.91	115.65	288.81	121.19

TABLE 5

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON GROWTH OF RATS

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% soybean meal
4. 20% soybean meal + 0.8% zinc
5. Soybean meal ash + 0.8% zinc
6. Methanol extract + 0.8% zinc
7. Methanol extracted soybean meal + 0.8% zinc
8. Precipitate from methanol extract + 0.8% zinc
9. Water extract + 0.8% zinc
10. Water extracted soybean meal + 0.8% zinc

	Treatments									
Replications	1	2	3	4	5	6	7	8	9	10
	4 weeks weight gain (gm)									
1	201	99	215	128	83	(136)	68	126	127	(112)
2	185	114	187	(102)	88	144	100	92	142	133
3	175	106	182	113	92	143	91	115	143	110
4	190	126	203	87	(83)	129	93	69	159	65
5	170	(98)[a]	189	85	80	130	126	102	142	134
6	179	59	234	104	79	110	75	111	136	128
7	100	87	178	94	74	163	35	75	96	101
Total	1200	689	1388	713	579	955	588	690	945	783
Mean	171	98	198	102	83	136	84	98	135	112

[a]( ) indicates calculated missing plot value.

TABLE 6

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON HEMOGLOBIN LEVELS

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% soybean meal
4. 20% soybean meal + 0.8% zinc
5. Soybean meal ash + 0.8% zinc
6. Methanol extract + 0.8% zinc
7. Methanol extracted soybean meal + 0.8% zinc
8. Precipitate from methanol extract + 0.8% zinc
9. Water extract + 0.8% zinc
10. Water extracted soybean meal + 0.8% zinc

	Treatments									
Replications	1	2	3	4	5	6	7	8	9	10
	gm/100 ml blood									
1	12.52	5.31	12.09	7.26	6.09	(5.80)	6.60	4.69	4.36	(5.42)
2	10.92	4.20	11.46	(6.34)	9.17	4.69	5.40	5.40	4.57	3.86
3	13.57	6.72	13.86	5.60	8.00	5.97	4.57	6.03	4.66	6.80
4	13.20	4.83	13.29	6.40	(8.31)	6.23	4.51	6.72	5.60	7.26
5	14.74	(5.69)[a]	14.03	7.03	9.72	5.40	6.29	4.91	5.43	4.51
6	12.77	7.26	13.49	5.89	8.66	7.09	5.43	4.51	4.74	4.69
7	13.57	5.83	12.52	5.89	8.23	5.40	8.23	6.40	6.66	(5.42)
Total	91.29	39.84	90.74	44.41	58.18	40.58	41.03	38.66	36.02	37.96
Mean	13.04	5.69	12.96	6.34	8.31	5.80	5.86	5.52	5.14	5.42

[a]( ) indicates calculated missing plot value.

TABLE 7

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON LIVER COPPER

Treatments	
1. Basal	6. Methanol extract + 0.8% zinc
2. Basal + 0.8% zinc	7. Methanol extracted soybean meal + 0.8% zinc
3. 20% soybean meal	8. Precipitate from methanol extract + 0.8% zinc
4. 20% soybean meal + 0.8% zinc	9. Water extract + 0.8% zinc
5. Soybean meal ash + 0.8% zinc	10. Water extracted soybean meal + 0.8% zinc

	Treatments									
Replications	1	2	3	4	5	6	7	8	9	10
	mcg/gm dry weight									
1	17.79	7.03	12.83	3.39	6.18	3.60	4.55	4.00	3.74	5.22
2	13.88	2.32	9.10	3.11	2.17	4.57	1.56	4.32	7.66	6.20
3	10.81	2.72	10.63	1.50	2.48	5.28	2.42	4.88	2.84	1.75
4	12.88	3.44	10.74	3.23	5.70	4.18	4.08	5.66	4.12	2.28
Total	55.36	15.51	43.30	11.23	16.53	17.63	12.61	18.86	18.36	15.45
Mean	13.84	3.88	10.82	2.81	4.13	4.40	3.15	4.72	4.59	3.86

TABLE 8

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 2
ON LIVER IRON

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% soybean meal
4. 20% soybean meal + 0.8% zinc
5. Soybean meal ash + 0.8% zinc
6. Methanol extract + 0.8% zinc
7. Methanol extracted soybean meal + 0.8% zinc
8. Precipitate from methanol extract + 0.8% zinc
9. Water extract + 0.8% zinc
10. Water extracted soybean meal + 0.8% zinc

	Treatments									
Replications	1	2	3	4	5	6	7	8	9	10
	mcg/gm dry weight									
1	240.34	130.96	225.68	86.23	119.99	117.15	155.24	109.10	90.84	106.44
2	193.88	146.14	213.50	92.13	143.84	100.93	157.43	97.55	102.98	235.06
3	279.96	100.10	208.09	136.95	154.72	101.93	215.11	100.39	96.17	113.80
4	305.26	108.21	263.51	110.63	192.84	113.28	145.87	109.58	90.69	110.49
Total	1019.44	485.41	910.78	425.94	611.39	433.29	673.65	416.62	380.68	565.79
Mean	254.86	121.35	227.70	106.48	152.85	108.32	168.41	104.16	95.17	141.45

TABLE 9

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON GROWTH OF RATS

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% soybean meal + 0.8% zinc
4. Methanol extract + 0.8% zinc
5. Methanol extracted soybean meal + 0.8% zinc
6. Chloroform-soluble fraction of methanol extract + 0.8% zinc
7. Chloroform-insoluble fraction of methanol extract + 0.8% zinc

Replications	Treatments 1	2	3	4	5	6	7
	4 weeks weight gain (gm)						
1	196	124	148	120	116	89	109
2	186	75	123	27	(120)	46	155
3	126	69	41	142	111	110	113
4	218	113	(97)[a]	123	137	111	142
5	180	59	(97)	100	121	108	117
6	163	100	76	125	115	114	138
Total	1069	540	582	637	720	578	774
Mean	178	90	97	106	120	96	129

[a]( ) indicates calculated missing plot value.

TABLE 10

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON HEMOGLOBIN LEVELS

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% Soybean meal + 0.8% zinc
4. Methanol extract + 0.8% zinc
5. Methanol extracted soybean meal + 0.8% zinc
6. Chloroform-soluble fraction of methanol extract + 0.8% zinc
7. Chloroform-insoluble fraction of methanol extract + 0.8% zinc

Replications	Treatments 1	2	3	4	5	6	7
	gm/100 ml blood						
1	13.12	6.77	5.40	7.94	6.09	5.63	6.97
2	14.86	7.09	6.09	8.97	(7.32)	9.37	5.31
3	13.66	10.54	4.52	6.66	7.49	6.03	7.37
4	12.34	6.40	(7.52)[a]	5.69	8.00	7.49	7.09
5	13.40	5.20	(7.52)	6.97	7.03	8.54	5.49
6	13.66	4.91	7.09	5.77	8.00	8.66	6.17
Total	81.04	40.91	45.14	42.00	43.93	45.72	38.40
Mean	13.51	6.82	7.52	7.00	7.32	7.62	6.40

[a]( ) indicates calculated missing plot value.

TABLE II

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3 ON LIVER COPPER

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% Soybean meal + 0.8% zinc
4. Methanol extract + 0.8% zinc
5. Methanol extracted soybean meal + 0.8% zinc
6. Chloroform-soluble fraction of methanol extract + 0.8% zinc
7. Chloroform-insoluble fraction of methanol extract + 0.8% zinc

Replications	Treatments 1	2	3	4	5	6	7
	mcg/gm dry weight						
1	9.52	3.68	2.44	4.28	1.57	3.23	2.65
2	10.20	3.49	1.07	3.28	(1.98)	4.90	2.53
3	11.37	4.27	3.40	2.79	1.38	1.61	2.73
4	11.70	2.30	(2.64)[a]	3.13	3.12	3.48	3.72
5	11.09	2.09	(2.64)	4.37	2.39	2.74	2.42
6	12.53	2.23	3.63	2.05	1.46	4.14	3.03
Total	66.41	18.06	15.82	19.90	11.90	20.10	17.08
Mean	11.07	3.01	2.64	3.32	1.98	3.35	2.85

[a]( ) indicates calculated missing plot value.

TABLE 12

EFFECTS OF TREATMENTS TESTED IN EXPERIMENT 3
ON LIVER IRON

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% Soybean meal + 0.8% zinc
4. Methanol extract + 0.8% zinc
5. Methanol extracted soybean meal + 0.8% zinc
6. Chloroform-soluble fraction of methanol extract + 0.8% zinc
7. Chloroform-insoluble fraction of methanol extract + 0.8% zinc

Replications	Treatments 1	2	3	4	5	6	7
	mcg/gm dry weight						
1	247.41	104.19	106.94	108.07	101.84	129.48	123.96
2	283.92	110.80	119.69	162.49	(127.79)	149.87	98.31
3	287.16	103.58	124.97	126.76	163.19	128.36	105.80
4	254.14	125.22	(121.94)[a]	114.49	144.68	136.92	144.20
5	356.82	156.91	(121.94)	109.36	117.57	105.58	136.05
6	284.07	101.34	136.14	154.00	111.69	97.81	111.36
Total	1713.52	702.04	731.62	775.17	766.76	747.97	719.68
Mean	268.92	117.00	121.94	129.20	127.79	124.66	119.95

[a]( ) indicates calculated missing plot value.

APPENDIX B

FOOD CONSUMPTION DATA

TABLE I

FOOD CONSUMED IN EXPERIMENT I

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 5% soybean meal
4. 5% soybean meal + 0.8% zinc
5. 10% soybean meal
6. 10% soybean meal + 0.8% zinc
7. 20% soybean meal
8. 20% soybean meal + 0.8% zinc

	Treatments							
Replications	1	2	3	4	5	6	7	8
	4 weeks food consumption (gm)							
1	399	237	(402)[a]	360	397	325	425	328
2	397	327	398	343	442	292	421	313
3	353	428	430	316	437	355	424	353
4	357	282	395	349	390	355	380	271
5	399	302	390	340	395	320	398	401
6	379	294	384	261	411	298	389	386
7	419	297	413	298	409	344	(406)	378
Total	2703	2167	2812	2267	2881	2289	2843	2430
Mean	386	310	402	324	412	327	406	347

[a]( ) indicates calculated missing plot value.

TABLE 2

FOOD CONSUMED IN EXPERIMENT 2

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% soybean meal
4. 20% soybean meal + 0.8% zinc
5. Soybean meal ash + 0.8% zinc
6. Methanol extract + 0.8% zinc
7. Methanol extracted soybean meal + 0.8% zinc
8. Precipitate from methanol extract + 0.8% zinc
9. Water extract + 0.8% zinc
10. Water extracted soybean meal + 0.8% zinc

	Treatments									
Replications	1	2	3	4	5	6	7	8	9	10
	4 weeks food consumption (gm)									
1	485	270	465	404	246	(333)	231	340	315	(307)
2	447	316	429	(308)	248	333	296	291	356	360
3	396	286	418	321	303	358	307	293	337	277
4	423	222	459	281	(258)	315	272	224	359	266
5	433	(265)[a]	418	280	239	314	313	318	340	343
6	389	213	482	307	270	300	253	318	341	382
7	300	267	414	254	242	377	187	262	253	215
Total	2873	1839	3085	2155	1806	2330	1859	2046	2301	2150
Mean	410	265	441	308	258	333	266	292	329	307

[a]( ) indicates calculated missing plot value.

TABLE 3

FOOD CONSUMED IN EXPERIMENT 3

Treatments

1. Basal
2. Basal + 0.8% zinc
3. 20% soybean meal + 0.8% zinc
4. Methanol extract + 0.8% zinc
5. Methanol extracted soybean meal + 0.8% zinc
6. Chloroform-soluble fraction of methanol extract + 0.8% zinc
7. Chloroform-insoluble fraction of methanol extract + 0.8% zinc

	Treatments						
Replications	1	2	3	4	5	6	7
	4 weeks food consumption (gm)						
1	414	289	330	308	284	252	271
2	403	251	274	125	(308)	187	334
3	286	221	179	330	280	263	271
4	456	324	(261)[a]	332	330	338	332
5	368	180	(261)	224	334	218	324
6	382	294	262	299	313	282	307
Total	2309	1559	1567	1618	1849	1540	1839
Mean	385	260	261	270	308	257	306

[a]( ) indicates calculated missing plot value.

APPENDIX C

ANALYSES OF VARIANCE

TABLE I

ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT I

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		weight gain	
Total	53	81,182	
Replications	6	1,190	
Treatments	7	69,728	9,961 **
Error	40	10,264	257
		hemoglobin level	
Total	53	799.46	
Replications	6	2.29	
Treatments	7	754.87	107.84 **
Error	40	42.30	1.06
		liver copper	
Total	31	386.02	
Replications	3	13.18	
Treatments	7	302.21	43.17 **
Error	21	70.63	3.36
		liver iron	
Total	31	190,831.24	
Replications	3	5,415.53	
Treatments	7	145,861.62	20,837.37 **
Error	21	39,554.09	1,883.52

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

TABLE 2

ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT 2

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		weight gain	
Total	64	121,053	
Replications	6	6,127	
Treatments	9	92,681	10,298 **
Error	49	22,245	454
		hemoglobin level	
Total	63	657.53	
Replications	6	11.90	
Treatments	9	595.60	66.17 **
Error	48	50.03	1.04
		liver copper	
Total	39	580.26	
Replications	3	26.75	
Treatments	9	481.39	53.48 **
Error	27	72.12	2.67
		liver iron	
Total	39	137,632.36	
Replications	3	1,531.10	
Treatments	9	107,898.73	11,988.74 **
Error	27	28,202.53	1,044.53

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

TABLE 3

ANALYSES OF VARIANCE OF DATA COLLECTED IN EXPERIMENT 3

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		weight gain	
Total	38	62,583	
Replications	5	6,038	
Treatments	6	33,402	5,567 **
Error	27	23,143	857
		hemoglobin level	
Total	38	289.09	
Replications	5	12.42	
Treatments	6	216.64	36.11 **
Error	27	60.03	2.22
		liver copper	
Total	38	382.37	
Replications	5	0.89	
Treatments	6	354.48	59.08 **
Error	27	27.00	1.00
		liver iron	
Total	38	155,031.65	
Replications	5	2,711.79	
Treatments	6	135,897.92	22,649.65 **
Error	27	16,421.94	608.22

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

APPENDIX D

ANALYSES OF COVARIANCE

TABLE I

ANALYSIS OF COVARIANCE OF WEIGHT GAIN AND FOOD CONSUMPTION DATA COLLECTED IN EXPERIMENT I

Source	d.f.	Sums of Squares and Products			Errors of Estimate		
		Sx^2	Sxy	Sy^2	Sum of Squares	d.f.	Mean Squares
Total	53	139,449	89,252	81,182			
Replications	6	8,674	2,280	1,190			
Diets	7	85,251	75,110	69,728			
Error	40	45,524	11,862	10,264	7,173	39	184
Diet + Error		130,775	86,972	79,992	22,151	46	
Difference for testing adjusted treatment means					14,978	7	2140 **

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

TABLE 2

ANALYSIS OF COVARIANCE OF WEIGHT GAIN AND FOOD CONSUMPTION DATA COLLECTED IN EXPERIMENT 2

		Sums of Squares and Products			Errors of Estimate		
Source	d.f.	Sx^2	Sxy	Sy^2	Sum of Squares	d.f.	Mean Squares
Total	64	343,140	190,568	121,053			
Replications	6	28,688	12,891	6,127			
Diets	9	239,088	146,081	92,681			
Error	49	75,364	31,659	22,245	8,946	48	186
Diet + Error		314,452	177,677	114,926	14,532	57	
Difference for testing adjusted treatment means					5,586	9	621 **

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

TABLE 3

ANALYSIS OF COVARIANCE OF WEIGHT GAIN AND FOOD CONSUMPTION DATA COLLECTED IN EXPERIMENT 3

		Sums of Squares and Products			Errors of Estimate		
Source	d.f.	Sx^2	Sxy	$S\ Sy^2$	Sum of Squares	d.f.	Mean Squares
Total	38	172,296	97,167	62,583			
Replications	5	31,185	13,134	6,038			
Diets	6	76,927	50,155	33,402			
Error	27	64,184	33,878	23,143	5,261	26	202
Diet + Error					6,503	32	
Difference for testing adjusted treatment means					1,242	6	207