Effect of certain amino acid, aspirin and zinc supplements on the zinc-deficiency syndrome of chicks given four protein sources
by Susan Lynne Smith Davis

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Home Economics
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Abstract:
Four basal protein rations, either low in available zinc (sesame meal) or low in zinc casein-gelatin, egg albumen and soybean meal) were fed to day-old chicks for three weeks. Supplements of zinc, histidine, glutamic acid or aspirin, or a combination of zinc and histidine or aspirin were fed. Chick weights, leg deformities (indicated by leg score and length to width ratio), and tibia zinc and nitrogen content were determined. Tibia of chicks given sesame, 0 and 60 mg/kg zinc and histidine, or histidine, 30 mg/kg zinc; casein-gelatin, 0 and 10 mg/kg zinc and histidine, or histidine, 5 mg/kg zinc rations were separated into four fractions on the basis of probable time of deposition of collagen. The nitrogen of each fraction was measured. The hydroxyproline content of the tibia of chicks given sesame rations with and without histidine was determined as a measure of collagen.

Growth of chicks fed the basal plant protein rations was better than chicks fed basal animal protein rations. Additions of 60 mg/kg zinc to sesame, 10 mg/kg zinc to casein-gelatin and 10 mg/kg zinc to egg albumen rations was sufficient to prevent zinc deficiency symptoms.

Addition of 1 percent histidine to the basal sesame rations decreased leg deformities but had little effect on growth. Addition of 1 percent glutamic acid did not prevent zinc deficiency symptoms.

One percent histidine added to casein-gelatin rations had little effect on either criteria.

Short tibia deformity of chicks fed sesame rations was alleviated with 1 percent aspirin, but growth of chicks was depressed.

Addition of 1 percent histidine to sesame rations resulted in a significant increase in total tibia nitrogen content, concomitant with decreases in leg deformities, but growth was only moderate. Zinc or zinc and histidine additions significantly improved growth and leg deformities but not tibia nitrogen content. Tibia nitrogen of casein-gelatin fractions were increased only with. 10 mg/kg zinc.

The addition of histidine to the basal sesame rations also resulted in increases in hydroxyproline. However, both the increases in tibia nitrogen of fractions and hydroxyproline disappeared when values were calculated per gram of bone or nitrogen.

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Date June 8, 1970

EFFECT OF CERTAIN AMINO ACID, ASPIRIN AND ZINC SUPPLEMENTS ON THE ZINC-DEFICIENCY SYNDROME OF CHICKS GIVEN FOUR PROTEIN SOURCES

by

SUSAN LYNNE SMITH DAVIS

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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Approved:

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Head, Major Department

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Chairman, Examining Committee

____
Graduate Dean

MONTANA STATE UNIVERSITY
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ABSTRACT

Four basal protein rations, either low in available zinc (sesame meal) or low in zinc (casein-gelatin, egg albumen and soybean meal) were fed to day-old chicks for three weeks. Supplements of zinc, histidine, glutamic acid or aspirin, or a combination of zinc and histidine or aspirin were fed. Chick weights, leg deformities (indicated by leg score and length to width ratio), and tibia zinc and nitrogen content were determined. Tibia of chicks given sesame, 0 and 60 mg/kg zinc and histidine, or histidine, 30 mg/kg zinc; casein-gelatin, 0 and 10 mg/kg zinc and histidine, or histidine, 5 mg/kg zinc rations were separated into four fractions on the basis of probable time of deposition of collagen. The nitrogen of each fraction was measured. The hydroxyproline content of the tibia of chicks given sesame rations with and without histidine was determined as a measure of collagen.

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The addition of histidine to the basal sesame rations also resulted in increases in hydroxyproline. However, both the increases in tibia nitrogen of fractions and hydroxyproline disappeared when values were calculated per gram of bone or nitrogen.

I. INTRODUCTION

"Arthritis" properly defined, means an inflammation of a joint and causes the loss of many man hours in industry alone. Rheumatoid arthritis, the most damaging of all forms of arthritis has an estimated 3,000,000 to 5,000,000 victims. The cause and cure remain unknown; the symptoms include acute and painful inflammation of several or all major joints (1,2). Spontaneous remission of arthritic symptoms often occur; this makes it difficult to assay treatment.

Experimental animals could prove useful in determining which drugs actually alleviate the arthritis symptoms. To date, arthritis symptoms have not been produced in experimental animals by dietary means. Recently, however, it has been shown that chicks fed certain low-zinc diets have leg deformities which resemble forms of rheumatoid arthritis. Inclusion of high dietary levels of the amino acid histidine, decreased the occurrence and severity of leg deformities in chicks given low-zinc sesame and isolated protein diets but growth of the zinc content of the tibia was not affected (3,4,5).

Preliminary experiments suggested that inclusion of histidine also led to an increased nitrogen content of the tibia (6). Collagen is the main nitrogen-containing constituent of bone and cartilage. Variations in collagen content or composition have been found when severe crippling deformities of bones and joints occurred in humans (7).

The purpose of this study is:

1. To determine the effect of four protein sources, low in available zinc, on chick body weight, leg deformities and tibia zinc content.

2. To determine the effect on body weight, leg deformities and tibia zinc content when various amounts of zinc or histidine or a combination of zinc and histidine are added.

3. To determine the effect on body weight, leg deformities and tibia zinc content when an antiarthritic agent alone and with added zinc is added to sesame rations.

4. Depending upon the results of objectives one and two, to determine the effect of various zinc-amino acid treatments on the total nitrogen content of chick tibiae and four chick tibia fractions.

5. To analyze fractionated chick tibia for hydroxyproline as a measure of collagen if a difference in per cent nitrogen is found.

II. REVIEW OF LITERATURE

Zinc deficiency symptoms can be produced in rats, poultry, pigs and humans. Of these, only young fowl develop deformities of the leg bone. Japanese quail exhibited poor growth, abnormal tibiae and poor feathering (8). Poults, which were fed a ration deficient in zinc, developed an enlarged hock condition. Deformities in chicks are characterized by shortened, thickened leg bones with enlarged hocks and resemble the inflammed joints of human arthritis (9).

Composition of Bones

Bone is composed of three major components: two organic and one inorganic.

Organic

Mucopolysaccharides.--Mucopolysaccharides, an organic bone component, composes the ground substance of bone called mucoid (11). It is a nitrogenous substance which is acid in nature.

Collagen.--Collagen, also a nitrogenous bone component, is the chief organic portion of bone and cartilage (12). It is part of the bone matrix within which growth in length occurs. Calcification of bone may depend upon an interaction between collagen fibrils and mucopolysaccharides (12). Any change in early collagen formation could show up in more mature bone since calcification is complete only in mature bone.

The structural properties of the bone depend on the way in which the basic elements (calcified collagen fibrils) are distributed in the tissue (14).

A recent study suggested that one of the functions of the zinc-containing enzyme, alkaline phosphatase, is important for collagen synthesis (13). The enzyme functions in the maturation of the chick epiphyseal plate cartilage and is markedly decreased in a zinc deficiency (13).

The measurement of collagen is a two-step process: (1) extraction; and (2) the determination of hydroxyproline.

Collagen varies in extractability with age. Collagen extracted with NaCl will contain the most recently synthesized molecules and the narrowest age range (15). When heated above a certain temperature, as in autoclaving, three stranded collagen molecules can be reduced to a mixture of random coil components called gelatin (16). Acid solutions will extract the older fibrils in addition to the newer ones (17).

Determination of hydroxyproline as a measure of collagen content in comparison to the total nitrogen of the fraction, is based on research using both colorimetric methods (18,37) and an amino acid analyzer.

Lathryism, a bone disorder in chicks, is caused by ingestion of the pea, Lathyrus Odoratus; it is thought to be due to failure to form normal insoluble collagen (19). Collagen formation is not interfered with in lathyrism, but the collagen, already present, is more extractable (20). The failure could be due to a loss of intramolecular cross linking, previously formed intermolecular bonds being disrupted, or possible alteration in collagen.

Calcium, copper and zinc ions partially relieve lathyrism symptoms (21). Collagen from copper-deficient chicks was more soluble than collagen from control chicks, resulting in bone abnormalities (22).

Approximately 65 percent of the bone is inorganic and is termed the bone salt portion. The bone salts consist primarily of calcium and phosphate as hydroxyapatite. Magnesium, sodium, citrate and carbonate ions are present in minor amounts (12). The inorganic materials are deposited in the collagen matrix as growth progresses. The zinc-containing enzyme, alkaline phosphatase, is thought to be active in promoting deposition of the inorganic constituents (12). Although alkaline phosphatase levels are reduced in zinc-deficient rats (23), the level of dietary zinc does not affect the percentage of bone ash in the chick.

Causes of Zinc Deficiencies Resulting in Bone Deformities

Zinc Requirement

The zinc requirement of the normal chick is given as approximately 35 mg/kg of food (24). Because of various factors, as discussed below, this requirement can vary greatly.

Effect of Phytic Acid

Phytic acid is a phosphate ester of inositol found in plants and occurs as a calcium, magnesium complex. By binding available zinc in plant proteins, chicks fed these proteins may become zinc deficient (25).

Plant protein rations.--Some sesame meals have zinc present as a component of or associated with an insoluble calcium • magnesium • phytate complex (26). When a sesame meal, Texas 61 containing 150 ppm of zinc, is used as a source of protein, leg deformities, feather abnormalities, low tibia zinc content, and poor growth occurred in chicks (39). It required 60 mg/kg of added zinc to prevent the deficiency symptoms. This meal, therefore, not only makes zinc unavailable (because of the phytic acid content) but also has a high capacity to bind added zinc. Although autoclaving the meal does not significantly reduce the phytin content, chicks fed this meal showed little signs of a zinc deficiency. The zinc was thought to be released through other means than the decrease of phytic acid (27).

Soybean meal is another protein ingredient of chick rations and contains about 40 percent as much zinc or phytate phosphorus as the sesame meal (28). Soybean proteins make zinc available by means other than through releasing it from the phytate complex (26). When fed to chicks, no abnormalities and slightly poor growth resulted.

Isolated soy protein, however, has a high affinity for zinc and increases the amount of zinc necessary to prevent deficiency symptoms (29,30). In the production of the isolated soy protein, the components of the soybean meals that make zinc available are thought to be lost (26). From 15-40 mg/kg of added zinc are necessary for normal growth of chicks fed isolated soy protein.

Animal protein rations.--The presence of phytic acid is not necessary for production of a zinc deficiency. Animal protein diets, which are low in zinc produce severe zinc deficiency symptoms.

Chicks given egg white rations grew poorly, developed open sores and bleeding, but did not have leg deformities (31).

Casein-gelatin rations, which have a low zinc content, do not bind zinc in the ration. Leg deformities, poor weight gain, and poor feathering have been found when chicks are given a casein-gelatin diet. Some researchers have found that 0.4%-2.0% phytic acid, when added to casein diets reduced the zinc available to the chick (30,32,33). Others found that the addition of 0.5% phytic acid to a casein hydrolysate diet did not reduce available zinc (31). Casein hydrolysate diets cause poor growth and abnormal feathering in chicks but not leg deformities (23). From 9 to 20 mg/kg of added zinc were found to be sufficient for normal growth (30,31).

Effect of Minerals

Calcium.--High dietary calcium decreases the availability of zinc to the chick when a phytate-containing ration is fed. *In vitro* studies showed that zinc • calcium • phytate complexes are more insoluble than zinc phytate complexes at the intestinal pH of the chick (28).

Magnesium.--Since the chemical behavior of magnesium is similar to that of calcium, the effect of added dietary magnesium was also studied (31). Increased magnesium in some sesame diets decreased the zinc available to the chick, but available zinc was not decreased on a soybean meal ration until 60 mg/kg of zinc were added with 3,000 mg/kg of magnesium. Up to 3,000 mg/kg of added magnesium did not decrease the zinc available to chicks fed casein (non-phytate containing) rations.

Copper, Manganese and Vitamin D_3.--This can partially be explained by the fact that copper and manganese deficiencies, in themselves cause bone deformities (22,31). Supplements of copper, manganese or varying amounts of Vitamin D_3 did not prevent the leg deformities of the zinc-deficient chick.

Compounds Which Alleviate Zinc Deficiency Symptoms

Ethylenediaminetetraacetic

Ethylenediaminetetraacetic (EDTA), a chelating agent, when added to either sesame or isolated soy protein diets, increased growth and reduced leg deformities. Since EDTA, itself, does not stimulate growth, it was theorized that EDTA makes phytate or protein-bound zinc more available (38,39).

Anti-Arthritic Agents

Several anti-arthritic agents (i.e., cortisone, aspirin, indomethacin, phenylbutazone) have been added to isolated soy protein diets (34).

Although they prevented some leg deformities, they also had harmful side effects such as high mortality rates or perforated chick gizzards.

Histidine

It is possible that histidine plays a role in correcting zinc deficiency symptoms through the variation in the amount of soluble collagen, since collagen content is thought to be related to crippling deformities (10).

One percent histidine, added to isolated soy protein diets, decreased the severity of bone disorders in chicks given an isolated soy protein diet. It did not counteract the low weight gain or low tibia-zinc content (31). When added to a sesame meal diet, it significantly decreased leg deformities. Weight gain or tibia zinc content, however, were not affected (35).

Zinc deficiency symptoms caused by casein-gelatin diets were not counteracted by histidine (29).

A recent study suggested that the tibiae of chicks given a sesame meal diet low in available zinc, supplemented with histidine had a higher nitrogen content than tibia of chicks not given supplementary histidine (29). Since a difference in growth due to histidine supplementation did not occur, the increase in nitrogen content of the tibiae may be due specifically to changes in bone structure rather than a general increase in body nitrogen.

In an effort to determine how histidine counteracted leg deformities, histidine was added to a Ca • Mg • Zn phytate complex, isolated from sesame meal and labeled with $zinc^{65}$ (3). The low absorption of Zn^{65} from the phytate complex by the chick was not increased by inclusion of histidine. It was hypothesized that histidine prevents leg deformities by other means than by releasing zinc from a phytate complex (29).

Recent work showed that 2 percent of dietary arginine added to egg albumen rations has been found to have a synergistic effect on the zinc deficiency symptoms (36). Low zinc, 2 percent (added) arginine diets, produced not only more severe leg deformities, but also poorer growth and lower tibia-zinc content (36). The chick's dietary requirement of arginine is 1.2 percent. These deformities were corrected by the addition of 1 percent histidine.

III. METHODOLOGY

Sample

Two factorial experiments were conducted using day old White Rock (f) X Cornish (M) chicks[1] without differentiating according to sex. The chicks were randomly distributed into duplicate groups of 10 for the first experiment and 10 or 13 for the second.

Chick Care

The chicks were housed in a stainless steel battery[2] to minimize contamination from environmental zinc. Feed and deionized water were given *ad libitum*. Each cage of chicks was weighed as a group for the first two weeks and individually the third week. Leg scores (Appendix A, p. 42) were determined at the same time. Chicks were sacrificed at 3 weeks of age.

Rations were comprised of four basal protein sources, sesame, casein-gelatin, egg albumen and soybean, plus the necessary vitamins and minerals (Table I). Additions, histidine, zinc, aspirin and glutamic acid, are shown in Table II.

Criteria for Measurement

The criteria used to determine the effects of feeding the various rations for three weeks were:

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[1]Obtained from Quality Hatchery, Billings, Montana.

[2]Petersime Incubator Company, Gettysburg, Ohio.

TABLE I. Composition of Basal Diets

	Texas 61 Sesame	Casein-Gelatin	Soybean Meal	Egg White
	g	g	g	g
Texas 61 sesame meal[1]	300	--	--	--
Casein, vitamin free[2]	--	230	--	--
Gelatin[3]	--	100	--	--
Egg white[4]	--	--	--	200
Soybean meal[5]	--	--	400	--
Vitamin mix[6]	5.0	5.0	5.0	5.0
MHA (Ca salt)[7]	--	3.3	3.3	--
Choline chloride (70%)	3.0	3.0	3.0	3.0
Vitamin D[8]	1.5	1.5	1.5	1.5
Santoquin[9]	2.5	2.5	2.5	2.5
Corn oil[10]	94.0	96.0	96.0	96.0
Salts[11]	60.1	60.1	60.1	60.1
$MgCO_3$	--	1.5	--	1.5
Biotin[12]	--	--	--	10.0
Lysine[13]	9.0	--	--	--
Sucrose	524.9	497.1	428.6	620.4
	1000.0	1000.0	1000.0	1000.0

[1]Texas 61 sesame meal extracted with petroleum ether, twice.

[2]Nutritional Biochemicals Corporation, Cleveland, Ohio.

[3]Pharmaceutical grade gelatin, P. Leiner & Sons, America, Inc., St. Clair, Michigan.

[4]Egg white solids (albumen), Armour, Chicago, Illinois.

[5]50 percent protein, commercial soybean meal, Farmer's Elevator, Bozeman, Montana.

[6]The vitamin mix contained: (in gm.) vitamin B_{12} (as 0.1% mix), 4; menadione bisulfite sodium 0.9; biotin, 0.04; pyridoxine · HCl, 1.0; folic acid, 1.0; niacin, 10.0; riboflavin, 2.0; D-Ca pantothenate, 6.0; thiamin mononitrate, 2.0; vitamin A (250,000 IU/gm) 8; vitamin E (250 IU/gm), 40. The basic mix was made to 1000 gm with sucrose.

[7]Calcium salt of methionine hydroxyanalogue.

[8]Vitamin D_3, 1000 IU/ml.

[9]Santoquin, Monsanto Company, St. Louis, in corn oil, 0.05 g/ml.

[10]Mazola.

(continued)

Footnotes Table I (continued)

[11]The salt mix contained: (in gm) $CaHPO_4 \cdot 2H_2O$, 5440; $CaCO_3$, 2984; K_2HPO_4, 2222; NaCL, 1200; $MgCO_3$, 25; Fe citrate, 66.6; $MnSO_4 \cdot H_2O$, 66.6; KI, 0.52; $CuSO_4 \cdot 5H_2O$, 6.68.

[12]Biotein, 40 mg per 100 gm sucrose.

[13]L-lysine · HCl, Nutritional Biochemicals, Cleveland, Ohio.

TABLE II. Additions to the Basal Diets (at the expense of sucrose).

Protein Source	Histidine[1]	Zinc[2]	Aspirin[3]	Glutamic Acid[4]
	1%	mg/kg	1%	1%
SESAME EXPERIMENT 1		60		
	+	0		
	+	30		
		0	+	
		30	+	
		0		
EXPERIMENT 2	+	0		
CASEIN-GELATIN EXPERIMENT 1		10		
	+	0		
	+	5		
EXPERIMENT 2	+	0		
EGG ALBUMEN		10		
		20		
	+	0		
	+	10		
	+	20		
SOYBEAN MEAL	+	0		

[1]Histidine HCl, monohydrate, Nutritional Biochemicals, Cleveland, Ohio.

[2]$ZnCO_3$ mix, $ZnCO_3$ in sucrose; 1 gm = 10 mg/kg of Zn for 10 kg ration.

[3]Aspirin, St. Josephs.

[4]L-glutamic acid, Nutritional Biochemicals, Cleveland, Ohio.

1) Body weight;

2) Leg scores as indicative of deformity (Appendix A, p. 42);

3) Ratio of length to width of the tibia (Appendix A, p. 44);

4) Analysis of tibia for zinc (Appendix A, p. 45);

5) Analysis for total nitrogen (Appendix A, p. 46); and

6) Analysis of four fractions of each set of tibiae for nitrogen (Appendix A, p. 47).

The significance of these criteria were determined statistically with Duncan's multiple choice test.[3,4]

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[3]Steel, Robert G.D., James H. Torrie, 1960. Principles and Procedures of Statistics, (New York: McGraw-Hill Book Co.), pp 107-109.

[4]The author would like to acknowledge Dr. Ervin P. Smith for his help with the statistical testing.

IV. RESULTS AND DISCUSSION

Results

The purpose of this study was to determine the results of feeding sesame, casein-gelatin, egg albumen and soybean rations, with zinc or amino acid supplements, to chicks, and to determine, by various analyses, the degree of deformity, if any, that resulted.

The following results are based upon the number of chicks which were alive at three weeks (Table III). The only mortality of chicks occurring on basal sesame rations was with sesame and aspirin. The mortality of chicks on egg albumen and casein-gelatin without zinc was high. The addition of zinc tended to correct this.

Protein Sources

Sesame.--Chicks given the basal diet (experiments 1 and 2) exhibited low body weight, and severe leg deformities (Table III, Figure 1). Leg deformities were also evident by low length to width ratios. Tibia zinc content, although higher than that of the day-old chick was still low.

The addition of 60 mg/kg of zinc significantly increased the weight of the chicks and decreased the leg scores (experiment 1). Supplemental zinc was deposited in the tibiae as evidenced by the relatively high tibia zinc content. Length to width ratio was significantly greater.

Casein-Gelation.--Chicks fed the basal rations grew poorly, had severe leg deformities and very low tibia zinc content (experiment 1). The length to width ratio was high (Table IV).

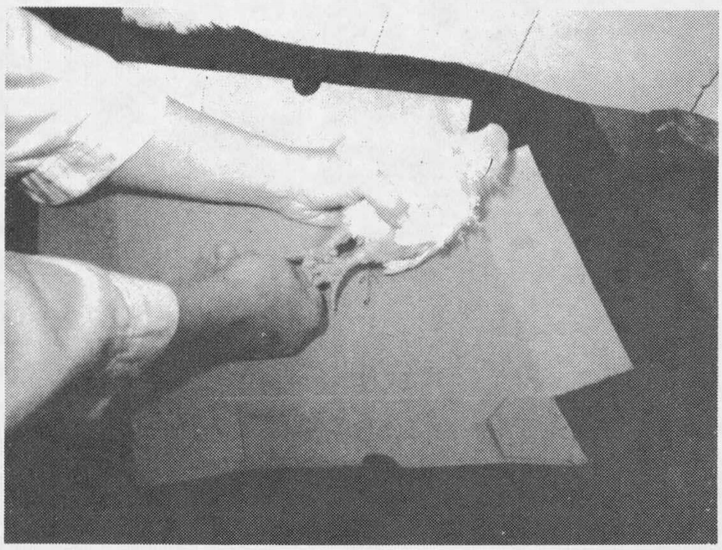

Figure 1. Effect of Texas 61 Sesame Meal Fed to Chicks. Severe Deformity at Arrow.

TABLE III. Mortality of 20 Chicks Given Several Protein Sources for Three Weeks.

Protein Source	Added Zinc	Experiment 1	Experiment 2
	mg/kg	%	%
SESAME	0	0	0
	60	0	
Sesame +	0	0	0
1% Histidine	30	0	
Ses+Glut Acid	0	0	
Sesame +	0	5	
1% Aspirin	30	20	
CASEIN-	0	45	3 1/
GELATIN	10	0	
Casein-Gel +	0	35	11 1/
1% Histidine	5	20	
EGG ALBUMEN	0		43 1/
	10		5
	20		0
Egg Albumen	0		29 1/
+	10		0
1% Histidine	20		0
SOYBEAN	0		5
Soy + Histidine	0		0

1/ Percentages from these rations were based on 26, 26, 23 and 24 chicks respectively.

TABLE IV. Criteria Mean to Judge the Extent of Zinc Deficiencies at Three Weeks in Chicks Fed Several Protein Sources.

Protein Source	EXPERIMENT 1			Tibia		
	Added Zinc	Weight	Leg Score	l/w. Ratio	Zinc	Nitrogen
	mg/kg	g			Total	% 1/
SESAME	0	198e 2/	3.6	9.12a	103	5.41bc
	60	291b	1.0	11.88c	375.5	5.38bc
Sesame + 1% Histidine	0	237c	1.2	11.89c	139.0	5.69a
	30	306a	1.5	11.73c	295.0	5.26cd
Sesame + 1% Glut. Acid	0	209d	3.1	9.26a	70.0	4.94f
Sesame + 1% Aspirin	0	107g	1.4	14.55g	102.5	4.39g
	30	115f	1.0	13.63e	73.5	5.09ef
CASEIN-GELATIN	0	85h	2.7	14.37f	38.0	5.19de
	10	287b	1.1	10.49b	307.5	5.31bcd
Cas-Gel + 1% Histidine	0	106g	3.0	12.94d	53.0	5.33bcd
	5	194e	1.6	12.81d	117.5	5.46b
	EXPERIMENT 2					
SESAME	0	173e	3.2	11.05a	64.0	5.60a
Sesame + 1% Histidine	0	190b	2.4	12.49b	80.5	5.56ab
CAS-GEL	0	197a	2.0	12.24b	242.5	5.18d
Cas-Gel + 1% Histidine	0	161d	1.85	12.89c	213.0	5.20d
EGG ALBUMEN	0	58g	1.4	15.84fg	35	4.57g
	10	116f	.33	15.50de	110.0	4.70fg
	20	122f	.30	15.73ef	230.0	4.70fg
Egg Albumen + 1% Histidine	0	68g	.90	16.12gh	47.0	4.74efg
	10	121f	.60	16.34h	125.0	4.85ef
	20	131e	.26	15.36d	231.5	4.90e
SOYBEAN	0	177c	.7	13.77c	89.0	5.14bc
Soybean + 1% Histidine	0	184b	.16	13.35c	86.0	5.28cd

1/ Percentage was based on dried, fat-free bones.
2/ P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

Chick body weight, leg score and tibia length to width ratio were significantly improved with the addition of 10 mg/kg of zinc, (experiment 1). Supplemental zinc was deposited in the tibia as shown by the significantly higher tibia zinc content (Table IV).

In experiment 2, a different lot of casein was used. On subsequent analysis, the casein had a larger amount of zinc. As a result, chicks given the basal ration did not exhibit deformities or poor growth. Therefore, a discussion of these results is not included in the following sections.

Egg Albumen.--Very poor growth and only slight leg deformities were obtained with the basal ration. Leg deformities may not have developed because of the poor growth; the high length to width ratio might also be attributed to poor growth. Tibia zinc content was very low.

Another condition, baldness, occurred (Figure 2); the chicks lost all the feathers on top of their head. The addition of zinc made no difference in the degree of baldness; the condition may have been related to other ration ingredients.

With the additions of 10 mg/kg of zinc, chick body weight was significantly increased (Table IV). The intake of added zinc was evident by increased tibia zinc content. The addition of 20 mg/kg of zinc did not improve growth over that with 10 mg/kg zinc nor did it increase the tibia content. Even with added zinc, chicks did not grow well and length to width ratios were high.