Variations in kidney arginase activity in chicks given diets low in available zinc
by Ellen Terry Dixon Bray

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Home Economics
Montana State University

Abstract:
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At 10 days, before the appearance of zinc deficiency symptoms, there was little difference in kidney arginase in chicks given the basal sesame meal and casein diets; that of egg albumen, however, was significantly lower,. Supplements of zinc and/or histidine had no effect.

At 21 days, the addition of histidine significantly increased arginase activity of chicks given the casein diets but had no effect on leg deformities or growth. Additions of zinc and/or histidine to sesame meal diets prevented the leg deformities and increased the weight of the chick but did not increase arginase activity, It appears that destruction of arginine by arginase was not a factor in improving leg scores of chicks given sesame meal diets.

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Date January 27, 1971

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ABSTRACT

The purpose of this study was to determine the relationship of kidney arginase activity to zinc deficiency symptoms and the effect of zinc and/or histidine supplements. Three experiments were conducted with day-old chicks fed sesame meal, casein or egg albumen diets low in available zinc.

At 10 days, before the appearance of zinc deficiency symptoms, there was little difference in kidney arginase in chicks given the basal sesame meal and casein diets; that of egg albumen, however, was significantly lower. Supplements of zinc and/or histidine had no effect.

At 21 days, the addition of histidine significantly increased arginase activity of chicks given the casein diets but had no effect on leg deformities or growth. Additions of zinc and/or histidine to sesame meal diets prevented the leg deformities and increased the weight of the chick but did not increase arginase activity. It appears that destruction of arginine by arginase was not a factor in improving leg scores of chicks given sesame meal diets.

CHAPTER I

INTRODUCTION

Importance of Study

For normal growth, the chick, as a member of the animal kingdom, must make proteins containing about 22 amino acids. However, the body is unable to synthesize all of these amino acids. Amino acids which cannot be synthesized and must be supplied by the diet are called essential amino acids. Arginine is one of the essential amino acids for the chick.

The dietary requirement for arginine varies according to the type of protein used in the diet. Casein is low in arginine, therefore, the pure amino acid must be added. When this is done the chick's requirement is greater than the normal requirement. Relatively little research has been done, however, with the variations in dietary arginine requirement of chicks fed sesame or egg albumen diets. The level of dietary arginine has been considered to be adequate in egg albumen while the level of arginine in sesame meal was unknown.

Leg deformities have occurred on zinc-deficient diets when sesame and casein-gelatin were the sources of protein. They did not occur on egg albumen diets unless excess arginine was added. Arginine may play some role in the occurrence of leg deformities.

The enzyme arginase is involved in the metabolism of L-arginine to urea. The body levels of arginine, therefore, are partially controlled by arginase. Chicks are unable to synthesize arginine through the urea cycle as many mammals do.

Purpose of the Study

The purpose of this study was to determine the relationship of arginase to the leg deformities and poor growth which occur in diets low in available zinc. Adequate levels of zinc will prevent both of these abnormalities but histidine alleviates only the leg deformities on sesame diets. The effect of addition of zinc, or histidine or various combinations on arginase activity was to be determined. This would give some clue as to arginine metabolism in this deficiency. *In vitro* studies have shown that zinc decreases arginase activity. Histidine and arginine have been shown to increase arginase activity when fed with certain diets. The effect of any of these variations on arginase activity was to be determined in relation to the condition of the zinc-deficient chicks.

CHAPTER II

REVIEW OF LITERATURE

Arginine

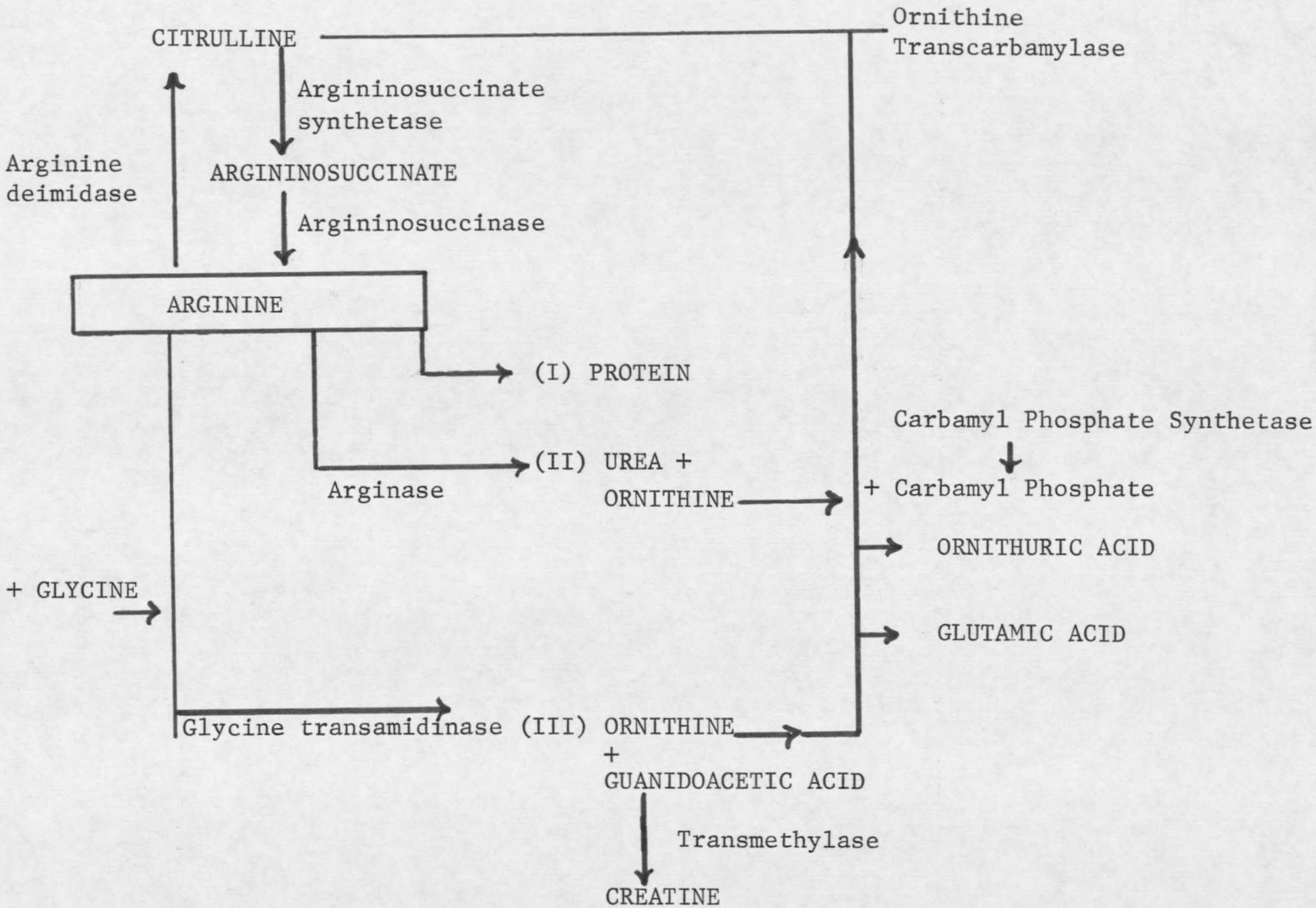
Arginine is an essential amino acid to the chick. This amino acid must come from the diet because the chick is unable to synthesize it.

Ingested arginine can follow three pathways but not all three pathways are present in all species. As shown in Figure 1 [1], these are: (I) protein synthesis, (II) formation of ornithine and urea through the action of the enzyme arginase and (III) production of guanidoacetic acid in the presence of glycine and the enzyme, glycine transamidinase. The chick can use pathway (I) for protein synthesis and pathway (III) for the formation of creatine. Synthesis of muscle creatine places a requirement on dietary arginine which is over and above the arginine needed for building tissue [2]. Creatine combines with phosphate to form creatine phosphate which is an important energy reservoir for the cell [3].

In pathway (II), other species can synthesize citrulline from ornithine which can lead further to the synthesis of arginine. The chick, however, is unable to complete this cycle because it lacks two necessary enzymes, carbamyl phosphate synthetase and ornithine transcarbamylase [4]. Sufficient arginine, therefore, must be supplied by the diet to provide for protein synthesis and meet the energy requirements.

FIGURE 1[1]

Schematic representation of arginine metabolism



[1]Adapted from G.H. Smith and D. Lewis [1].

Measurement of urea as an indication of arginine metabolism is difficult in the chick because birds excrete most nitrogenous waste as uric acid instead of urea as mammals do. The amount of urea present in chick excreta is very small and arises only from the breakdown of dietary arginine [5].

Arginine in Protein

Since the protein, casein, is low in arginine, the pure amino acid must be added to the diet for normal growth [6]. Normally the chick requirement for arginine is 1.2 percent [7]. When casein is the source of protein in the diet, the arginine requirement is approximately 1.7 percent [8]. O'Dell ascribes this to a more rapid absorption of the free amino acid leading to a more rapid breakdown to urea by kidney arginase [2]. Chicks fed some plant proteins, such as corn + soybean meal, had a normal arginine requirement [2]. Sesame meal used in this experiment is a plant protein source. Its amino acid composition and its effects on arginase activity were unknown. Egg albumen contains sufficient arginine to meet the chick's normal requirement [6]. Arginase activity in chicks fed this protein has not been investigated previously as far as this investigator knows.

Factors Affecting Arginase Activity

Besides the amino acid composition of a protein, other factors have been shown to influence arginase activity. The chick's minimum requirement for lysine is 1.1 percent [7]. Casein contributes 1.69 percent and egg albumen 1.32 percent [6]. The lysine content of sesame meal is low but lysine is added to meet the chick's normal requirement [9]. Two percent lysine or 1.6 percent histidine added to a casein diet caused growth depression and an increase in arginase activity [10,11]. Supplementation with 1 percent arginine prevented the growth depression [10].

Strains of chicks have been developed which differ in dietary arginine requirements. Chicks with a high requirement for arginine had levels of arginase activity three to four times greater than chicks with a low requirement [12]. The high-requirement chicks were particularly sensitive to excess dietary lysine; arginase activity was increased and growth was depressed [12]. The broiler chicks used in this laboratory probably were of the type which have a high requirement for arginine [13].

Glycine is necessary for optimal growth of the chick. It can depress arginase activity when supplied in the diet [10], possibly by pathway (III) as seen in Figure 1. The glycine level of casein is .52 percent and egg albumen is .77 percent [6]. The level of glycine in sesame meal was not known but previous studies have shown that only lysine and zinc additions were needed for optimal growth [9].

In vitro studies have shown that arginase was activated by Mn^{++}, Co^{++} and Ni^{++}; added Zn^{++} produced a marked depression in arginase activity [14].

Leg Deformities

Leg deformities occurred on zinc-deficient diets when protein sources were casein-gelatin or sesame meal [9]. Addition of 1 percent histidine prevented the leg deformities which occurred on sesame diets [15] but in this experiment did not affect those which occurred on casein-gelatin diets. The zinc content of the tibia was not increased [15]. Leg deformities did not appear on egg albumen diets [16]. Addition of 2 percent arginine, however, did cause leg deformities which were prevented by an addition of 1 percent histidine [17]. An increase in the zinc of the tibia was also found [18].

Zinc Requirement

Chicks fed a sesame meal diet need 60 mg/kg of zinc to obtain normal growth and prevent leg abnormalities [9]. Casein diets have a requirement of 10 mg/kg [9]. The addition of 10 mg/kg of zinc to egg albumen diets is sufficient to meet the zinc requirement of the chick [19].

Species

Zinc deficiency symptoms are produced in other species, e.g., the rat [20] but it is only the chick that shows leg deformities.

Histidine will affect leg deformities but will not affect any of the other obvious symptoms of zinc deficiency [15]. This apparently is one specific physiological effect.

CHAPTER III

METHODOLOGY

Sample

The chick is the species chosen for this investigation because the fowl is the only small animal which shows leg deformities as a zinc deficiency symptom. Three experiments were conducted using day-old Cornish breed chicks, "Cornish King".[1] Duplicate groups of 4 chicks for 13 rations, in experiments 1 and duplicate groups of 10 chicks for 6 rations, in experiments 2 and 3 were randomly distributed in the battery. In experiments 2 and 3, nine day-old chicks were divided into groups of three and sacrificed before receiving food and water.

Variation in Time

Day-old chicks were sacrificed to establish a base line for arginase, body weight and tibia zinc content. Ten days was chosen as one sampling point because this is a critical period for the chick on zinc-deficient diets. At this time those which are still surviving will probably survive the rest of the experiment. The effect of zinc on weight may or may not appear depending upon how rapidly the chicks grow. Overt leg deformities are not present. By 21 days zinc deficiency symptoms which include leg deformities and lack of growth appear.

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

In all three experiments, three chicks from each group were sacrificed at 10 days. The two kidneys and the right tibia were taken and stored at -70°C until time for analysis. The same procedure was followed in experiments 2 and 3 for 21 day-old chicks.

Chick Care

The chicks were housed in a stainless steel battery[2] to minimize contamination from environmental zinc. Food and deionized water were given *ad libitum*.

The chicks were weighed as a group weekly and individually at time of sacrifice.

Criteria for Measurement

The criteria for determining the effect of feeding the various diets for 21 days were:

(1) Body weight.

(2) Leg score (Appendix A).

(3) Kidney arginase (Appendix A).

(4) Tibia zinc as indicative of body zinc status (Appendix A).

The same criteria were used for the 10 day old chicks except for determination of leg scores.

The significance of these criteria was determined statistically by Duncan's multiple choice test [22].

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[2]Petersime Incubator Company, Gettysburg, Ohio.

Diets

The diets were composed so as to contain 20 percent protein and 10 percent fat. Sesame meal, casein or egg albumen were the basal protein sources (Table I). The basic amino acids of these were determined using a Beckman 120C amino acid analyzer;[3] adjustments were made to meet chick's requirement (Table II). When additions of histidine, zinc, glycine, manganese, or pyridoxine and folic acid were made, the sucrose was decreased accordingly.

[3] Courtesy of Dr. Kenneth Hapner, Chemistry Department, Montana State University, Bozeman.

TABLE I

Composition of Basal Diets per Kilogram

Ingredients			
	g	g	g
Casein[1]	230		
Sesame[2]		345	
Egg Albumen[3]			260
Vitamin mix[4]	5	5	5
MHA (Ca Salt)[5]	3.3		
Choline.Cl (70%)	3	3	3
Vitamin D oil[6]	1.5	1.5	1.5
Santoquin oil[7]	2.5	2.5	2.5
Corn oil[8]	96	66.7	96
Mineral mix[9]	60	60	60
$MgCO_3$	1.5		1.5
L-arginine.HCl[10]	10.9		
L-lysine.HCl[11]		9	
Biotin mix[12]			1
Glycine[13]			2.5
Sucrose	586.2	507.2	567

[1]Vitamin-free casein. This contained 87.70 percent protein to give 20.1 percent of protein in the ration.

[2]Texas 61 Sesame meal, extracted with cold hexane. This contained 58.4 percent protein to give 20.1 percent of protein and 7.9 percent of fat to give 29.3 g. of fat in the ration.

[3]Egg white solids (albumen), Armour Creameries, Chicago, Illinois. This contained 78.38 percent protein to give 20.3 percent of protein in the ration.

[4]The vitamin mix contained in g: vitamin B_{12} (as 0.1% mix) 4.0; menadione bisulfite soidum 0.9; biotin 0.04; pyridoxine.HCl 1.0; folic acid 1.0; riboflavin 2.0; d-Ca pantothenate 6.0; thiamine mononitrate 2.0; niacin 10.0; vitamin A (250,000 IU/g) 8.0; DL-a-tocopheryl acetate (250 IU/g) 40. Made to 1,000 g with sucrose.

[5]Calcium salt of methionine hydroxyanalogue. Sigma Chemical Company, St. Louis, Missouri.

[6]Twenty-five mg cholecalciferol in 1,000 ml Mazola which gave 1,500 ICU/kg of ration.

Footnotes (continued)

[7]Santoquin, Monsanto Company, St. Louis, Missouri. Ten g/200 ml of corn oil.

[8]Mazola.

[9]The mineral mix contained the following in g: $CaHPO_4.2H_2O$ 5,440; $CaCO_3$ 2,984; K_2HPO_4 2,222; NaCl 1,200; $MgCO_3$ 35; Fe Citrate 66.6; KI 0.52; $CuSO_4.5H_2O$ 6.68; $MnSO_4.H_2O$ 66.6.

[10]L-arginine.HCl.

[11]L-lysine.HCl.

[12]Biotin mix. One-hundred mg of biotin in 100 g. sucrose.

[13]Glycine.

The ingredients of footnotes 1, 4, 6, 10, 11, 12 and 13 came from Nutritional Biochemicals Corporation, Cleveland, Ohio.

TABLE II

Chick Requirement and Amino Acid Content of Sesame,
Casein and Egg Albumen Diets

Amino Acid	Chick Requirement[1]	Sesame	Vitamin-Free Casein	Egg Albumen
	%	%	%	%
Lysine	1.1	0.56[2]	1.84	1.89
Histidine	0.4	0.52	0.68	0.6
Arginine	1.2	2.7	0.89[3]	1.53
Glycine	1.0	1.1	0.52[4]	0.77[5]

[1]Reference [7].

[2]Brought to (0.56 + 0.72) 1.28 percent by addition of lysine (Table I).

[3]Brought to (0.89 + 0.9) 1.79 percent by addition of arginine (Table I).

[4]Reference [6].

[5]Brought to (0.25 + 0.77) 1.02 percent by addition of glycine (Table I).

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to determine the relationship of arginase activity to the occurrence of leg deformities and poor growth in chicks fed diets low in available zinc.

Amino Acid Analysis

Casein and egg albumen vary in amino acid content from lot to lot; the amino acid content of the sesame meal was not known. Amino acid analyses were necessary to see how well the proteins used met the chick's requirements; also the content of the basic amino acids in each of the proteins might have an influence on the chick performance and interpretation of the results.

By analysis, in g/100g of meal, Texas 61 sesame, as fed contained: 1.61 lysine, 1.52 histidine, 7.90 arginine and 3.20 glycine; the vitamin-free casein, 8.00 lysine, 2.97 histidine and 3.52 arginine; the egg albumen, 7.28 lysine, 2.30 histidine and 5.88 arginine. The basic amino acids contributed by a dietary level of 20 percent protein was compared to the chick's minimum requirement (Table II) and suitable adjustments were made as follows.

Lysine.--Lysine was added to the sesame meal diet because it contained only 0.56 percent. The levels in casein and egg albumen diets were 1.84 percent and 1.89 percent respectively. While this was somewhat higher than the minimum requirement, an increase in arginase activity was not found until an excess of 2 percent lysine was added to the diet [10]. Therefore, lysine-arginine interaction was not considered a factor in these experiments.

Histidine.--Histidine was adequate in all three diets. When 1 percent histidine was added, about four times the chick's minimum requirement was supplied.

Arginine.--The arginine level of sesame meal diets was 2.7 percent. The level of arginine in casein diets was 0.89 percent. The latter was supplemented with 0.9 percent arginine to bring the level to 1.8 percent which is close to 1.7 percent suggested by Snyder et al. [8] for optimum growth. Egg albumen diets had 1.53 percent which surpassed the minimum requirement.

Glycine.--Sesame meal diets met the minimum requirement. It was low in casein diets, 0.52 percent, but in Experiment 1 it was not changed because it was desirable to find out the effect on growth of arginine alone. In one group in Experiment 2, 1 percent glycine was added which gave 1.52 percent. This is one and one-half times the chick's minimum requirement. Without the addition of glycine, optimum growth was obtained with the addition of zinc to the diet (Table VII). In egg albumen the level was found to be 0.77 percent. It was deemed necessary to add 0.25 percent glycine because, in contrast to casein, the addition of zinc alone did not lead to optimum growth. The additional glycine, however, still did not prevent the poor growth

(Table V). Meeting the chick's minimum requirement for glycine, therefore, was not the limiting factor in this egg albumen diet.

It is seen by the above discussion that the basal diets as fed were quite adequate in the basic amino acids in meeting the chick's minimum requirements.

Chick starter was composed of corn, barley, soybean meal and meat meal as protein sources with the necessary vitamin and mineral supplements.[4] The chicks which were fed this diet, therefore were considered to be a "normal" control.

Arginase Activity

Variation With Age and Basal Diet

The day-old chicks in Experiments 2 and 3, which were used as a baseline, were found to be fairly uniform in total arginase activity and arginase per gram of kidney. The tibia zinc content and body weight were also comparable (Tables IV and VII).

By 10 days the arginase activity of the chicks fed the sesame meal and casein basal diets had increased about 20 to 30 times (Tables IV and VII). Chicks fed the basal egg albumen diet, however, had an increase of only about five times (Table V). This comparison among the three proteins may have several explanations. O'Dell found

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[4]Three hundred sixteen mg/kg of zinc by analysis.

chicks to have a lower requirement for arginine with a plant protein diet of corn-soybean meal in contrast to a casein diet supplemented with arginine [2]. This plant protein, however, had a level of 1.7 percent arginine while the sesame meal in this study had an inherent arginine content of 2.7 percent which is about two times the chick's minimum requirement (Table II). This may account for the high arginase activity found in our plant protein diet. Generalities as to arginase activity with different proteins cannot be made until the inherent arginine content of the protein being used in the experiment is known.

At 21 days a further increase in arginase activity was found in chicks given the basal sesame meal diet. Little change in arginase activity in chicks given the basal casein diet occurred (Table VII). Consequently, arginase activity at 21 days in chicks given the basal sesame meal diet was significantly higher than that of casein (Table VIII). This may signify an adaptation with time by the sesame meal chicks to the very high level of arginine. Egg albumen diets were not continued for chicks of this age period.

Variation with Supplements

Sesame Meal

Zinc and/or Histidine.--At 10 days, in Experiment 1, before the gross appearance of the zinc deficiency symptoms, supplements of zinc and/or histidine had little effect on arginase activity (Table III).

TABLE III

Kidney Arginase and Zinc Status of Chicks Fed Sesame Meal at 10 Days in Experiment 1

	10 Day[1]				
	Kidney Arginase[2]		Weight		Tibia
Variations	Total	Per Gram	Kidney	Chick	Zinc
	uM/hr[3]	uM/hr[3]	gm[3]	gm[3]	ppm
0 Zn	1857a	957a	1.95a	138a	56 ± 3
0 Zn + Hist	1685a	848a	2.07a	134b	58 ± 5
30 Zn + Hist	2120a	1010a	2.10a	141a	127 ± 17
60 Zn	1714a	805a	2.14a	153a	142 ± 4
Chick Starter	200b	179b	1.15b	97c	179 ± 13

[1] Mean of six chicks for each group except basal ration which contains five chicks.

[2] According to [23,24].

[3] $P (< 0.05)$ by Duncan's Multiple Range Test, values followed by the same letter are not significantly different.

TABLE IV

Kidney Arginase and Zinc Status of Chicks Fed Sesame Meal Diets at 10 Days and at 21 Days in Experiment 3

Variations	Kidney Arginase[2] Total uM/hr[3]	Kidney Arginase[2] Per Gram uM/hr[3]	Weight Kidney gm[3]	Weight Chick gm[3]	Tibia Zinc ppm	Leg Score[4]
10 Day[1]						
0 Zn	2356a	1573a	1.47a	105b	44±2	
0 Zn + Hist	1186a	853a	1.48a	101b	51±1	
30 Zn	1707a	1071a	1.58a	124a	85±3	
30 Zn + Hist	1826a	1007a	1,81a	129a	74±1	
60 Zn	2365a	1477a	1,56a	126a	146±43	
21 Day[1]						
0 Zn	4230a	1592a	2,65b	203b	107±15	3.0
0 Zn + Hist	4158a	1221a	3.51b	228b	96±29	0.9
30 Zn	4055a	1179a	3.46b	300a	209±36	1.7
30 Zn + Hist	5800a	1409a	4,25a	325a	236±26	0.6
60 Zn	4952a	1286a	3,95a	342a	377±9	0.4
Chick Starter	446b	133b	3,33b	313a	700±0	0.2
Day Old Chick	68	71	0,95	41	40	

[1]Mean of six chicks for each group. [2]According to [23,24].

[3]P(<0.05) by Duncan's Multiple Range Test, values followed by the same letter are not significantly different.

[4]Graded from 0 to 4 increasing in severity; a difference of 1 was considered significant.

In Experiment 2, growth was improved by these supplements although arginase activity was still not affected (Table IV). Utilization of dietary zinc is seen by the increase in tibia zinc content.

At 21 days the lack of growth and leg deformities characteristic of zinc-deficient diets were seen (Table IV). Addition of zinc increased growth and decreased leg scores. Supplements of histidine decreased leg scores only. The addition of both zinc and histidine improved growth and leg scores. There was no increase in arginase activity to indicate that the improvement in these physiological conditions was due to the destruction of arginine by pathway II (Figure 1). The improvement in leg scores apparently was due to the addition of zinc and/or histidine rather than to destruction of arginine by arginase since there was no increase in arginase activity.

Egg Albumen

This experiment was conducted only for 10 days consequently leg deformities as a result of zinc-deficiency were not investigated. The growth rate of these chicks was lower than those fed casein or sesame meal diets.

Zinc.--The low level of arginase activity of egg albumen was not affected by the addition of zinc to the diet although zinc did cause a significant increase in chick weight over that of the basal diet (Table V). The utilization of dietary zinc is also seen in the tibia zinc content.

TABLE V

Kidney Arginase and Zinc Status of Chicks Fed Egg Albumen Diets at 10 Days in Experiment 1

	10 Day[1]				
	Kidney Arginase[2]		Weight		Tibia
Variations	Total	Per Gram	Kidney	Chick	Zinc
	uM/hr[3]	uM/hr[3]	gm[3]	gm[3]	ppm
0 Zn	251a	319a	0.74a	67b	27±2
10 Zn	113a	136a	0.84a	79a	111±11

[1]Mean of six chicks for each group.

[2]According to [23,24].

[3]P(<0.05) by Duncan's Multiple Range Test, values followed by the same letter are not significantly different.

Casein-Arginine

Histidine.--At 10 days, in Experiment 1, zinc deficiency symptoms were not visibly apparent; histidine had no effect on arginase activity or growth. Histidine significantly depressed growth at 10 days in Experiment 2 but there was still no difference in arginase activity (Tables VI and VII). At 21 days arginase activity was significantly increased but the effect of histidine on growth had disappeared (Table VII). The increased destruction of arginine, however, was not accompanied by a decrease in leg deformities. The increased arginase activity which was found is in accordance with findings by Austic et al. [10] that the addition of 1.6 percent dietary histidine increased arginase activity.

At 10 days the glycine supplement counteracted the depression in growth caused by histidine and resulted in the lowest arginase of the experiment (Tables VII, $P < 0.05$). Its further effect was noted at 21 days in the increase in growth ($P<0.05$) and decrease of the high arginase due to histidine. Glycine appeared to counteract the effect of histidine both on growth and arginase with little effect on leg scores. This is in accord with previous studies which have shown glycine to suppress arginase activity in vivo [10].

Zinc.--At 10 days the utilization of dietary zinc was seen by the increase in growth and the tibia zinc content (Tables VI and VII). It had no significant effect, however, on arginase activity.

TABLE VI

Kidney Arginase and Zinc Status of Chicks Fed Casein-Arginine Diets at 10 Days in Experiment 1

	10 Day[1]				
	Kidney Arginase[2]		Weight		Tibia
Variation	Total	Per Gram	Kidney	Chick	Zinc
	uM/hr[3]	uM/hr[3]	gm[3]	gm[3]	ppm
0 Zn	1135a	922b	1.24a	97a	74 ± 5
0 Zn + Hist	1651a	1223a	1.38a	104a	92 ± 10
10 Zn	1091a	822b	1.31a	115a	168 ± 8
10 Zn + Hist	1707a	1194a	1.34a	97a	189 ± 45
0 Zn + High Mn	2299a	1775a	1.25a	93a	87 ± 4
0 Zn + Folic Acid + High Pyridoxine	971b	789b	1.22a	98a	113 ± 9

[1]Mean of six chicks for each group.

[2]According to [23,24].

[3]$P (< 0.05)$ by Duncan's Multiple Range Test, values followed by the same letter are not significantly different.