



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
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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.

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January 27, 1971

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GIVEN DIETS LOW IN AVAILABLE ZINC

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ELLEN TERRY DIXON BRAY

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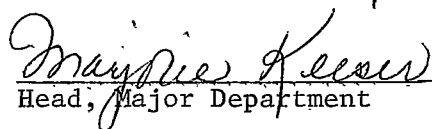
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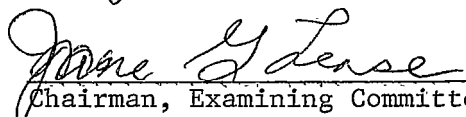
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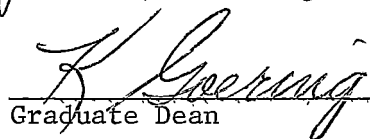
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ABSTRACT

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

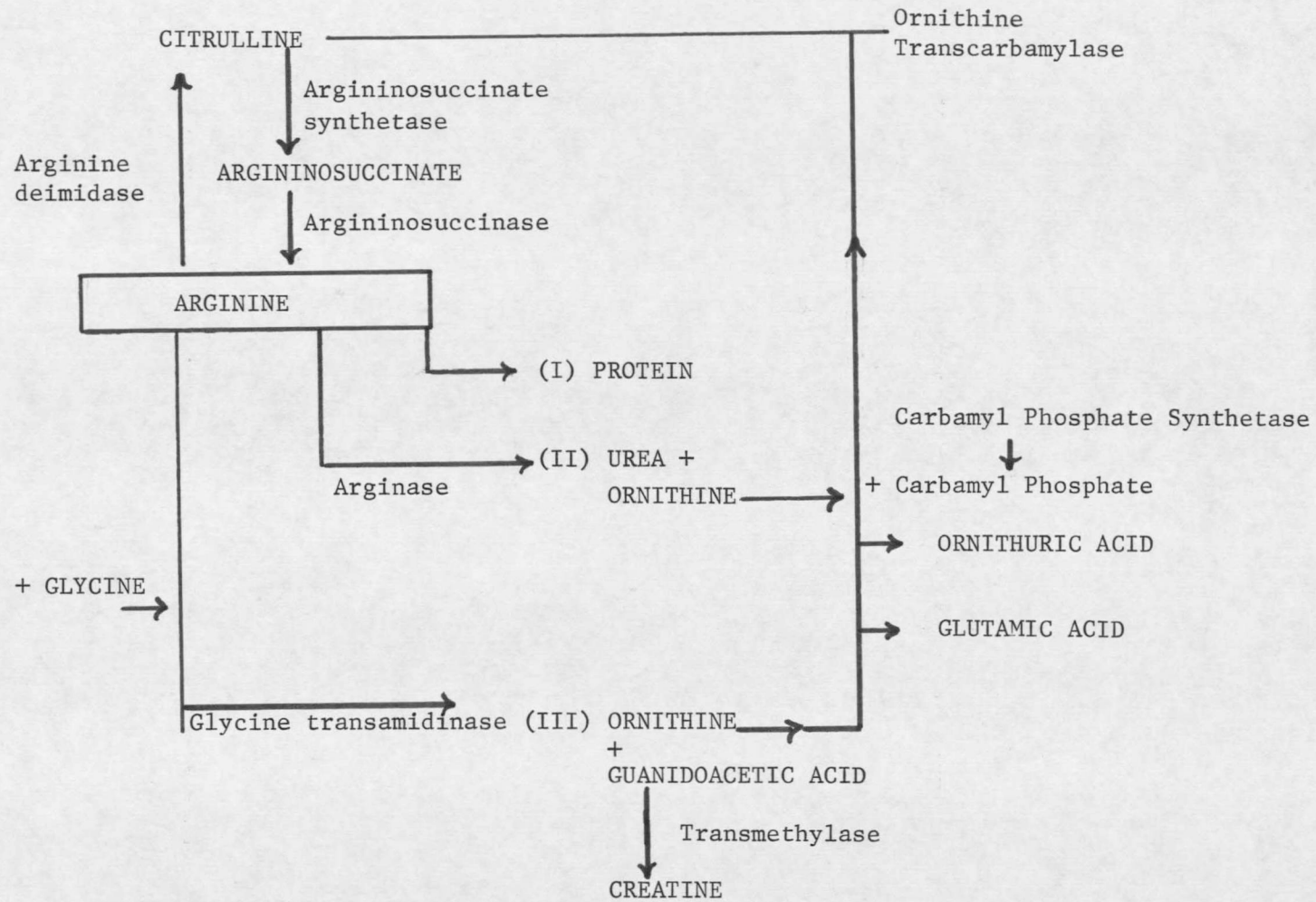
Arginine is an essential amino acid to the chick. This amino acid must come from the diet because the chick is unable to synthesize it.

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 1¹

Schematic representation of arginine metabolism



¹Adapted 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.

¹Obtained 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 battery² 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].

²Petersime 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.

³Courtesy 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

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

²Texas 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.

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

⁴The vitamin mix contained in g: vitamin B₁₂ (as 0.1% mix) 4.0; menadione bisulfite sodium 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- α -tocopheryl acetate (250 IU/g) 40. Made to 1,000 g with sucrose.

⁵Calcium salt of methionine hydroxylanalogue. Sigma Chemical Company, St. Louis, Missouri.

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

Footnotes (continued)

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

⁸Mazola.

⁹The mineral mix contained the following in g: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ 5,440; CaCO_3 2,984; K_2HPO_4 2,222; NaCl 1,200; MgCO_3 35; Fe Citrate 66.6; KI 0.52; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 6.68; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 66.6.

¹⁰L-arginine.HCl.

¹¹L-lysine.HCl.

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

¹³Glycine.

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

¹Reference [7].

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

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

⁴Reference [6].

⁵Brought 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

⁴Three 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

Variations	10 Day ¹		Weight		Tibia
	Kidney Arginase ²				Zinc
	Total	Per Gram	Kidney	Chick	
	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

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

²According to [23,24].

³P(<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	10 Day ¹					
	Kidney Arginase ²		Weight		Tibia	
	Total uM/hr ³	Per Gram uM/hr ³	Kidney gm ³	Chick gm ³	Zinc ppm	
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						
					Leg Score ⁴	
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	

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

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

⁴Graded 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

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

¹Mean of six chicks for each group.

²According to [23,24].

³P(<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

Variation	10 Day ¹		Weight		Tibia
	Kidney Arginase ²				Zinc
	Total uM/hr ³	Per Gram uM/hr ³	Kidney gm ³	Chick 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

¹Mean of six chicks for each group.

²According to [23,24].

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

TABLE VII

Kidney Arginase and Zinc Status of Chicks Fed Casein-Arginine Diets at 10 Days and at 21 Days in Experiment 2

Variations	10 Day ¹					Leg Score ⁴
	Kidney Arginase ²		Weight		Tibia	
	Total uM/hr ³	Per Gram uM/hr ³	Kidney gm ³	Chick gm ³	Zinc ppm	
0 Zn	1926a	1781a	1.12a	88a	69 [±] 4	
0 Zn + Hist	1223a	1656a	0.83b	60c	58 [±] 7	
10 Zn	1388a	1073a	1.31a	98a	90 [±] 1	
10 Zn + Hist	3216a	2487a	1.33a	99a	125 [±] 0	
0 Zn + Hist + 1% Glycine	576b	509b	1.14a	85b	58 [±] 7	
	21 Day ¹					Leg Score ⁴
	Kidney Arginase ²		Weight		Tibia	
	Total uM/hr ³	Per Gram uM/hr ³	Kidney gm ³	Chick gm ³	Zinc ppm	
0 Zn	1072b	589b	1.96b	136c	188 [±] 62	1.2
0 Zn + Hist	4923a	2431a	1.99b	162c	229 [±] 26	1.1
10 Zn	2661b	1308b	2.10b	203b	325 [±] 15	0.4
10 Zn + Hist	2778b	1197b	2.41b	226b	393 [±] 7	0.4
0 Zn + Hist + 1% Glycine	1506b	658b	2.31b	208b	255 [±] 20	0.7
Chick Starter	735c	223c	3.32a	306a	688 [±] 37	0.2
Day Old Chick	41	59	0.70	40	34	

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

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

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

By 21 days zinc-deficiency symptoms were apparent. Leg scores on the basal diet were not as high as previous studies have indicated they should have been [9]. The casein diet used in this experiment was found to contain a higher level of zinc than previously suspected. Leg scores were decreased, however, by the dietary zinc and there was a significant increase in chick weight (Table VII). Zinc also appeared to suppress arginase activity. The addition of zinc to the histidine supplemented diet appeared to counteract the effect of histidine because arginase activity was significantly lowered. The suppression caused by zinc was in accord with in vitro studies by Greenberg [14].

Manganese.--The effect of added dietary manganese, about 20 times the chick's requirement, was determined on arginase activity in Experiment 1 (Table VI) at 10 days since in vitro studies have shown manganese activates arginase [14]. Manganese has no relation to the zinc status of the chicks. Since zinc was already a factor in these experiments it was decided to try one of the other ions known to affect arginase activity and see if the in vitro studies held true when tested in vivo. Only a slight increase in arginase activity over the basal diet or the basal diet plus zinc was found. It had no effect on growth.

Pyridoxine and Folic Acid.--Pyridoxine plays an important role in amino acid metabolism. Jones [21] suggested an interrelationship

among arginine, aspartic acid and pyridoxine as an indication of another route of arginine catabolism. Lysine significantly increased arginase activity even when pyridoxine was increased to about 100 times the chick's requirement [21]. In Experiment 1 (Table VI) when a similar level of pyridoxine was used, arginase activity was significantly lowered from that of any of the other variations in the diet. No difference appeared in growth, therefore, a decrease in arginase activity was not beneficial at this time.

Chick Starter

Chick starter served as a base diet in all three experiments (Tables III, IV and VII). Arginase activity was significantly lower than that of sesame meal or casein diets. Tibia zinc content and good growth showed utilization of dietary zinc and leg scores were insignificant.

Comparison of Proteins

Histidine.--Leg deformities of chicks given sesame meal diets were prevented by the addition of histidine. There was, however, no significant increase in arginase activity. This prevention, therefore, probably was not due to an increase in destruction of arginine, mediated through an increase in arginase by dietary histidine. The preventative effect of histidine apparently was exerted in another way,

TABLE VIII

Comparison of Kidney Arginase of Chicks Fed Casein-Arginine,
Sesame Meal, Egg Albumen and Chick Starter Diets

Protein	Supplement	10 Day		21 Day	
		Kidney Arginase ¹		Kidney Arginase ¹	
		Total	Per Gram	Total	Per Gram
		uM/hr ³	uM/hr ³	uM/hr ³	uM/hr ³
Casein	0 Zn	1531a	1352a	1072b	589b
Sesame Meal	0 Zn	2129a	1293a	4230a ³	1593a ⁵
Egg Albumen	0 Zn	251b	319a		
Casein	0 Zn + Hist	1437a	1440a	4924a	2431a
Sesame Meal	0 Zn + Hist	1435a	851a	4158a	1221b ⁶
Casein	10 Zn + Hist	2462a	1840a	2778b	1197a
Sesame Meal	30 Zn + Hist	1972a	1008a	5800a ⁴	1409a
Casein	10 Zn	1239a	947a	2661b	1308a
Sesame	60 Zn	2040a	1141a	4952a ⁴	1286a
Egg Albumen	10 Zn	113b	136b		
Chick Starter				591c	178b ⁴

¹According to [23,24].

²By Duncan's Multiple Range Test, values followed by the same letter are not significantly different.

³P(<0.06).

⁴P(<0.01).

⁵P(<0.10).

⁶P(<0.08).

With casein, histidine addition did not prevent the occurrence of leg deformities but arginase activity, thus destruction of arginine, was increased.

Since the effect of added dietary histidine is opposite in the two proteins, both in reference to leg deformities and to arginase activity, it would appear that arginine metabolism is not involved in the production of zinc deficiency with these proteins.

Zinc and Histidine.--The combination of a zinc plus histidine supplement to the sesame meal diet had the same lack of effect on arginase activity as did zinc or histidine alone. Growth was significantly increased over that of histidine alone and leg scores were reduced. Again, reduction of zinc deficiency symptoms was not accompanied by an increased destruction of arginine. Arginase activity of chicks given the casein diets with this combination was significantly lower than that of histidine alone. Weight was significantly increased and leg scores decreased.

With casein, as with sesame meal, correction of zinc deficiency symptoms was not accompanied by an increase in arginine destruction. The role of zinc in lowering of the higher arginase activity in the presence of histidine, seen above, may be an interplay of the effect of zinc and of histidine on arginase [14,10] unrelated to correction of zinc deficiency symptoms.

Chicks fed chick starter showed a high tibia zinc content (Tables III, IV and VII), and no zinc deficiency symptoms. Arginase activity

was significantly lower than casein or sesame meal chicks at 10 and at 21 days. Two factors may have contributed to this low arginase activity. First, the plant protein composition [2] and second, the high zinc content [14].

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was conducted to determine the relationship of arginase activity to zinc deficiency symptoms in chicks fed zinc-deficient diets. Diets contained sesame meal, casein or egg albumen as the basal protein sources. Supplements were zinc, histidine, glycine, manganese or folic acid and pyridoxine. Chick weight, leg deformities, kidney arginase and tibia-zinc content were determined at 1, 10 and 21 days.

At 10 days, before the appearance of zinc deficiency symptoms, arginase activity of chicks fed sesame meal or casein basal diets had increased 20 to 30 times that of day-old chicks. That of chicks fed the basal egg albumen diet, however, had increased only about five times.

By 21 days, addition of 1 percent histidine to the basal sesame diet decreased leg deformities but had little effect on growth or kidney arginase. Addition of zinc or zinc and histidine increased growth and prevented leg deformities but arginase activity was not affected. Arginase activity of the sesame meal diets at this time was significantly higher than that of the casein diets.

At 21 days the addition of zinc or zinc and histidine to the casein diets increased growth and prevented leg deformities but did not change arginase activity. The addition of 1 percent histidine

alone, however, while significantly increasing arginase activity, did not increase growth or decrease leg deformities. While the addition of 1 percent glycine appeared to counteract the effect of histidine by lowering arginase activity and growth was increased, no large change in leg deformities appeared.

Conclusions

The addition of zinc and/or histidine had little effect on arginase activity of chicks given sesame meal diets. The decrease in leg scores obtained with the additions of zinc and/or histidine to the sesame diet was probably the result of these two factors rather than arginase playing some role in decreasing the leg scores through the destruction of arginine since arginase activity did not increase.

The low leg scores on the casein diets were probably due to the fact that casein had a higher amount of zinc than suspected at the time of the experiment. While the addition of histidine alone caused a significant increase in arginase activity the relation of histidine to arginase and leg deformities could be clarified if a greater degree of leg deformities were produced.

Recommendations

Further research involving egg albumen diets in relation to the effect of added arginine, histidine and zinc should be done.

Since the casein used in this experiment was found to be high in zinc, further research with a casein lower in zinc in relation to arginase activity would also be valuable.

APPENDICES

APPENDIX A
METHODS OF ANALYSES

Leg Scores

Chick tibiae were scored with the following designations:

- 0 - normal leg development;
- 1 - slight deformity in one leg;
- 2 - slight deformity in both legs;
- 3 - severe deformity in one leg; and
- 4 - severe deformity in both legs.

The degree of leg deformity was determined by the shortness of the tibia and the knobs on the tibia joint. A difference of 1 in leg scores was considered significant.

Tibia Zinc Analysis

1. The right tibia was removed from the same chicks used for arginase analysis and put in plastic bags and stored at -30°C until time of analysis.
2. The extraneous flesh was removed and after dipping in boiling deionized water for 1 minute, all excess tissue, including the epiphyseal plate cartilage, removed and dried at 50°C for 3 hours.
3. The three bones from each cage were wrapped together in paper toweling, crushed and securely tied; they were extracted in a Soxhlet extractor for 20 hours with 95 percent alcohol, 20 hours with ethyl ether, dried at 50°C in a vacuum over at 5 lb/in^2 pressure and weighed.

4. The extracted bones were ashed at 550°C for 24 hours, cooled in a desiccator and weighed.
5. The ash, dissolved in 0.1 N HCl was analyzed for zinc by an atomic absorption method. A Perkin-Elmer 290-B Spectrophotometer was used; the wave length was 2138Å; acetylene was the fuel, the oxidant was air. Metallic zinc was dissolved in 0.1 HCl for the standard. The concentration was varied from 0 to 5 ppm over 0 to 100 percent transmission.¹

Kidney Care

The kidneys were put in individual glass vials and placed immediately on dry ice until frozen. The vials were stored in plastic bags at -70°C until immediately before analysis. This procedure was desirable because of lability of the enzyme arginase.

Arginase Method

Kidneys were taken rather than livers because they have higher arginase activity [23]. The general method used for analysis of kidney arginase which is based on the breakdown of arginine by arginase to urea, was that of Tamir and Ratner [23] and Van Slyke and Archibald [24].

¹Courtesy of Mr. Vincent Haby, Plant and Soil Science, Montana State University, Bozeman.

Solutions²

1. 0.02 M. potassium phosphate: 6.96 g. of K_2HPO_4 to 2 liters with H_2O . Adjust pH to 7.5. (Takes 13-14 drops of 18N NaOH.)
2. 0.1 M $MnCl_2 \cdot 4H_2O$: 19.79 g. of $MnCl_2 \cdot 4H_2O$ to 1 liter with H_2O .
3. 0.1 M glycine buffer: 7.51 g. glycine to 1 liter with H_2O . Adjust pH to 9.5. (About 6 drops 18 N NaOH.)
4. 0.85 M arginine-HCl: Dissolve 9.00 g. arginine.HCl in small amount of deionized H_2O . Add 1.6 ml 18 N NaOH. Make up to 50 ml with H_2O . Adjust pH to 9.5. (About 14 drops 18 N NaOH.)
5. Dilute arginine solutions for standard: 5 ml of 0.85 M. arginine. HCl diluted to 25 with $SO_4 \cdot PO_4$ mix. (0.285 M arginine.)
6. 15% trichloroacetic acid: 15 g. was made to 100 ml with H_2O .
7. 0.85% saline solution: 8.5 g. NaCl in 1000 ml of H_2O .
8. Stock urea: 4uM = 24 mg. (0.024 gm.) urea in 100 ml H_2O . Store in refrigerator. Prepare monthly.
9. Working standard: 0.4uM urea/ml = 1 ml stock urea solution to 10 ml with $SO_4 \cdot PO_4$ mixture.
10. $H_2SO_4 \cdot H_3PO_4$ mixture: 90 ml conc. H_2SO_4 in 2 liter beaker in ice bath. Slowly add 270 ml syrupy phosphoric acid (H_3PO_4). Stir gently with glass rod. Slowly add 600 ml H_2O . Make to 1 liter.
11. Color reagent: 3% isonitrosopropiophenone = 3 gm. a-iso-nitrosopropiophenone to 100 ml with 95% ethanol. Keep at room temperature.³

Method

1. Six samples were run at one time. Fourteen 15 ml centrifuge tubes were set up in a rack. Two aliquots are taken from each original sample so tubes are labeled accordingly including 2 to

²Deionized water was used throughout.

³Sigma Chemical Company, St. Louis, Missouri.

be used as blanks. 0.5 ml of 0.1 M, glycine buffer and 0.05 ml of 0.1 M MnCl_2 are put in each tube. (Use lambda self-regulating pipette to measure the MnCl_2 .)

2. Weigh kidney on analytical balance.
3. Homogenize kidney in Virtus homogenizer in about 10 ml of cold 0.02 M K_2HPO_4 . (Keep K_2HPO_4 on ice.) Hold container under homogenizer for about 1 minute. Make homogenate up to 20 or 30 ml (depending on size of kidney) in graduated 35 ml centrifuge tubes with cold phosphate solution. Shake well.
4. Immediately take 2 aliquots and dilute to 10 ml with cold saline solution. Mix well. Immediately add 0.8 ml of each dilution to the centrifuge tube containing the glycine buffer and MnCl_2 . Incubate for exactly 30 minutes at 38°C in a water bath.
5. While incubation is proceeding, make up the standards. Use etched numbered large test tubes, consecutive in number. Add 1, 2, 3, 4, 5, 6, 7 ml of working urea standard. Add 8, 7, 6, 5, 4, 3, 2 of $\text{H}_2\text{SO}_4\cdot\text{H}_3\text{PO}_4$ mixture. Each tube should contain 9 ml. Set aside.
6. Fill large kettle half full of water and put to boil.
7. At the end of the 30 minute incubation period add 1 ml of the TCA solution to the blanks. To homogenates and blanks add immediately in succession 0.65 ml. (Use graduate 1 ml pipette) of 0.85 M arginine solution. Mix. Allow all to stand for 10 minutes at 38°C .
8. Add 1.0 ml of 15% TCA solution to homogenates only, to halt reaction. Let stand in water bath for 10 minutes. Centrifuge for 10 minutes at 2500 rpm. Put one set of the aliquots in the freezer for possible future use.
9. Pour, carefully, supernatant into small (8-9 ml) test tubes. Anywhere from .1 to 2.5 ml of each aliquot is added to 15 ml $\text{H}_2\text{SO}_4\cdot\text{H}_3\text{PO}_4$ solution. (These large tubes are prepared in lots of 50 beforehand, corked and left at room temperature.) Mix well.
10. 10 ml of each homogenate- $\text{H}_2\text{SO}_4\cdot\text{H}_3\text{PO}_4$ solution is put in a large, etched numbered test tube.
11. The standards are arranged. 1 ml of dilute arginine solution is added to serve as a control for unreacted arginine.

12. To all tubes add 0.5 ml of 3% isonitrosopropiophenone, consecutively.
13. Immediately mix and insert stopper fitted with a capillary tube and place tubes in foil-covered wire basket. Place basket in the large kettle of boiling water. Cover with foil. Keep boiling exactly 1 hour. At the end of exactly 60 minutes place wire basket in ice bath for 1 minute. Read immediately in Spectron 20 at 540 mu.

Calculations

A graph is prepared for each day that an experiment is run with the optical density readings from the standards as the ordinate and micromoles of urea present in 10 ml of standard solution as the abscissa. The optical density readings from the samples are plotted on the curve to get the micromoles of urea present in 10 ml of solution. Micromoles of urea/ml of working standard are as follows:

<u>ml of working standard</u>	<u>uM of urea/10 ml $\text{SO}_4\text{.PO}_4$</u>
1	0.4
2	0.8
3	1.2
4	1.6
5	2.0
6	2.4
7	2.8

Calculations were carried out with this equation: $\frac{A}{D} \times \frac{B}{E} \times C$.

A is the total acidified digest volume (constant) 3 ml = 0.8 of dilute homogenate, 0.5 of 0.1 M glycine buffer, 0.05 of 0.1 M MnCl_2 ; 0.65 of 9.85 M arginine.HCl solution, 1.00 of TCA.

B is 15 ml of $\text{H}_2\text{SO}_4\text{.H}_3\text{PO}_4$ (constant) plus sample aliquot.

C is used to convert the experimental value to an hour basis.
Six (constant) is the ratio of 60 minutes to the time used for digestion (10 minutes).

D is the volume of aliquot used with H_2SO_4, H_3PO_4 mixture. Varies from .1 to 2.5 ml.

E is the aliquot used for urea measurement.

The factor is used to figure E dilute.

E dilute: Factor X U. U is the number of uM urea in the analyzed 10 ml aliquot as read from curve on graph.

E original: Factor X U X V. V is the volume to which 1 volume of the original arginase preparation was diluted. Homogenate was always 20 or 30 ml and 1 or 2 ml were always taken from this. Adjust accordingly. This value is the amount of uM urea/ml for total kidney.

Example: Homogenate = 30 ml and 2 ml was taken. Multiply E dilute by 15.

To figure the urea per gram of kidney, E original is divided by the weight of the kidney.

LITERATURE CITED

- 1 Smith, G.H. and D. Lewis. 1963. Arginine in poultry nutrition. 2. Chick arginase. Brit. J. Nutr. 17:433.
- 2 O'Dell, B.J., O.A. Laerdal, A.M. Jeffay and J.E. Savage. 1958. Arginine metabolism in the growing chick. Poultry Sci. 37:817.
- 3 Guyton, A.C. 1968. Textbook of Medical Physiology. W.B. Saunders Co. p. 971.
- 4 Cohen, P.P. and G.W. Brown, Jr. 1960. Comparative Biochemistry, Vol. 2. Academic Press, Inc. p. 161.
- 5 Scott, M.L., C. Nesheim and R.J. Young. 1969. Nutrition of the Chicken. M.L. Scott and Associates. p. 101.
- 6 Block, R.J. 1956. Amino Acid Handbook. Charles C. Thomas, Publisher. pp. 258, 266.
- 7 Nutrition requirements of Poultry, 1966. National Academy of Sciences. National Research Council, Publication 1345. p. 6.
- 8 Snyder, J.M., W.D. Morrison and H.M. Scott. 1956. The arginine requirement of chicks fed purified and corn-soya diets. Poultry Sci. 35:852.
- 9 Lease, J.G. and W.P. Williams, Jr. 1967. The effect of added magnesium on availability of zinc with some high protein feedstuffs. Poultry Sci. 45:242.
- 10 Austic, R.E. and M.C. Nesheim. 1970. Role of kidney arginase in variations of the arginine requirement of the chick.
- 11 Jones, J.D. 1964. Lysine-arginine antagonism in the chick. J. Nutr. 84:313.
- 12 Nesheim, M.C. 1968. Kidney arginase activity and lysine tolerance in strains of chickens selected for a high or low requirement of arginine. J. Nutr. 95:79.
- 13 _____, Personal communication. Fed. Proc. Meeting. April 1970.
- 14 Greenberg, D.M., A.E. Bagot and O.A. Roholt, Jr. 1956. Liver arginase. III. Properties of highly purified arginase. Arch. Biochem. Biophys. 62:446.

- 15 Lease, J.G. 1968. Effect of histidine or soybean meal additions of Zn deficiency symptoms. Fed. Proc. 27:1498.
- 16 Nielsen, F.H., M.L. Sunde and W.G. Hoekstra. 1966. Effect of dietary amino acid source on the zinc-deficiency in chicks. J. Nutr. 89:24.
- 17 Coleman, B.W., E.M. Reimann, R.H. Grummer and W.G. Hoekstra. 1969. Antagonistic effect of dietary arginine on zinc deficiency in chicks. Fed. Proc. 28:2806.
- 18 Coleman, B.W. Personal communication. Fed. Proc. Meeting. April 1969.
- 19 Davis, Susan S. Effect of certain amino acid, aspirin and zinc supplements on the zinc-deficiency syndrome of chicks given four protein sources. M.S. Thesis. Montana State University, Bozeman, 1970.
- 20 Wilson, E.D., K.H. Fisher and M.E. Fuqua. 1961. Principles of Nutrition. John Wiley and Sons, Inc. p. 163.
- 21 Jones, J.D., S.J. Petersburg and P.C. Burnett. 1967. The mechanism of the lysine-arginine antagonism in the chick: Effect of lysine on digestion, kidney arginase, and liver transamidinase. J. Nutr. 93:103.
- 22 Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. 11:1.
- 23 Tamir, H. and S. Ratner. 1963. Enzymes of arginine metabolism in chicks. Arch. Biochem. Biophys. 102:249.
- 24 Van Slyke, D.D. and R.M. Archibald. 1946. Gasometric and photometric measurement of arginase activity. J. Biol. Chem. 165:293.

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