The effects of various forms of egg albumen on nutrition of the chick
by Nancy Ann Dowding Hettich

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:
The original purpose of the study was to compare the effect of raw and of cooked egg albumen as a source of protein in low-Zn diets when fed to chicks. Chicks fed the cooked egg white diets had high mortality in contrast to those fed raw egg white. Investigation of this difference included: variation of amino acids and yitamin content of the diet; source of chick; source of egg white; age of chick; and use of a Salmonella bactericide. All of these were ineffective in reducing mortality. Mortality could be greatly reduced by feeding chick starter 5 days before feeding the cooked egg albumen. High mortality was not observed when chicks were fed whole cooked eggs.

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Date June 8, 1973

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by

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

of

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ABSTRACT

The original purpose of the study was to compare the effect of raw and of cooked egg albumen as a source of protein in low-Zn diets when fed to chicks. Chicks fed the cooked egg white diets had high mortality in contrast to those fed raw egg white. Investigation of this difference included: variation of amino acids and vitamin content of the diet; source of chick; source of egg white; age of chick; and use of a Salmonella bactericide. All of these were ineffective in reducing mortality. Mortality could be greatly reduced by feeding chick starter 5 days before feeding the cooked egg albumen. High mortality was not observed when chicks were fed whole cooked eggs.

CHAPTER I

INTRODUCTION

The source of protein used in zinc deficient diets has been found to affect the response of the chick to a zinc deficiency. When sesame meal (1), isolated soy protein (2, 3, 4) or casein (3, 5) were used as the source of protein, leg deformities appeared. The addition of histidine to sesame or isolated soy protein (ISP) diets (1, 3) resulted in prevention of leg deformities without a change in the zinc status of the chick. Histidine however, did not have this effect when added to casein diets (3). This difference in the response of the two vegetable proteins and the animal protein raised the question of possible differences between sources of protein.

Egg albumen is a animal protein, low in zinc, commonly used in animal experiments and also forms a part of diets of humans. In early studies, chicks fed egg albumen diets did not develop leg deformities (6, 7, 8). Recently, however, when chicks were fed low zinc egg albumen diets moderate leg deformities have been reported (5).

Many of the recent studies using egg albumen use the raw dried form. This protein is generally cooked before it is consumed by man. Casein, on the other hand, as the protein of milk, is usually consumed with a minimum of processing. The form in which the protein is consumed could have an effect on zinc utilization. Before a valid

comparison of animal proteins could be made, it seemed that the effect of cooking egg albumen should be explored.

The original objective of this study was to compare the effects of Zn deficient raw or cooked egg albumen diets, and supplements to each of zinc and several amino acids. The criteria for measuring Zn deficiency to be used were the occurrence and prevention of leg deformities; zinc content of the tibia as a measure of zinc intake; and weight gain. Tibia alkaline phosphatase (AP) activity may be reduced by zinc deficiency (9, 10, 11, 12) and for this reason was also used as a criterion. It was found in the initial experiment that cooking the egg albumen led to very high mortality. This problem had no solution available from a literature search. Therefore, a wide range of possibilities had to be explored. These were supplements of vitamins, amino acids, and zinc; use of an antibiotic; removal of dietary NaCl; variation in chick age, in the source of egg white, and the source of chicks.

CHAPTER II

REVIEW OF LITERATURE

Some Factors Which Influence Zn Deficiency in the Chick

Amino Acid Supplements

Either histidine or arginine has an effect on the response of the chick, depending on the protein source. These effects may be contradictory and therefore each is discussed separately and in combination.

Histidine. Histidine added to ISP (2, 3) or sesame (1) diets low in zinc prevented the occurrence of leg deformities. Additions of histidine have also decreased leg scores of chicks fed low-zinc egg albumen diets (5). However, histidine supplements to casein diets low in Zn did not have this effect (3, 5).

Two aspects of bone metabolism have been shown to be affected by histidine supplements. Histidine has been shown to be effective in increasing sulfate incorporation in the epiphyseal plate and primary spongiosia of chick bone, regardless of Zn status (13). Sulfur in bone is found in the acid mucopolysaccaride, chondroitin sulfate, which is a constituent of the ground substance of bone (14). A reduced incorporation of sulfur into the epiphyseal plate cartilage has been found in chicks fed Zn deficiency diets (13). Histidine has also caused an

increase in tibia AP activity concomitant with the prevention of leg deformities when chicks were fed ISP (3) or sesame diets (1).

Dietary histidine did not affect histamine levels in tissues (15). This indicates that histidine was not being converted to histamine and was not alleviating deformities by correcting defective histamine storage.

The effect of histidine appears to be on bone metabolism rather than on growth, as histidine in general has not improved growth. Body weight of chicks fed casein (5) or egg albumen diets low in zinc was not improved by histidine supplements (5, 8).

Arginine. Effects of added arginine vary with the level of the arginine supplement. The addition of 1% arginine to ISP or casein diets low in zinc did not result in increased severity of leg deformities (3). Additions of 2% arginine to ISP or egg albumen diets, or 3.4% to casein diets did result in somewhat more severe deformities (5).

Histidine plus Arginine. The interaction of histidine with added arginine also varies. Histidine supplements did not decrease leg scores of chicks fed casein diets when arginine was supplemented at 0.6%, 1.4% (5) or 2% (3). Histidine reduced leg deformities when 3.4% arginine (5) was added. Histidine had no effect on leg scores when added to egg white diets with a 2% arginine supplement (5).

Severity of leg deformities has been reduced by the inclusion of histidine with supplements of agrinine to ISP diets (3).

Alkaline Phosphatase

Alkaline phosphatase (AP), a zinc-containing metalloenzyme (16), is found in many tissues of the body. Although the exact functions of AP remain unknown, one of them is thought to be related to bone formation. Several possibilities for the physiological role of AP in this process exist. It has been suggested that AP promotes the calcification of bone by a breakdown of organic phosphate compounds to form a high phosphate ion concentration (16). It also appears to be necessary for normal cell mauration and degeneration in non-calcifying epiphyseal plate tissues (17). AP may also be involved in the formation of collagen based on the observation that vesicles rich in AP and hydroxyproline are located near fibrils of chick bone cells that presumably will contribute to collagen fibers when extruded (18).

Types of AP

It has been found that the AP of chick intestine, bone and liver can be differentiated by their reactions to denaturing agents. Bide found that intestinal AP was not affected by treatment with 4 M urea, but bone and liver AP were (19). These differences were used to determine the origin of serum AP and some evidence was found for

intestinal AP as the source of serum AP. It has also been suggested that serum AP originates mainly from bone, with lesser amounts contributed by the liver (20).

In the human, there are three classifications, intestinal, non-intestinal (bone and liver) and placental (21). Intestinal AP of the human is more resistant to treatment with urea than non-intestinal or placental (22). It can be inhibited by L-phenylalanine (23) and resists inhibition by bile acids (24). Its mobility is not affected by incubation with neuraminadase (25). L-homoarginine has been found to inhibit AP of the liver and bone (26). Isoenzymes found in the serum of normal individuals have been found to be heterogeneous.

AP Activity

A reduced AP activity has been reported in tibia, intestine and serum of Zn deficient birds (9, 10, 11, 12). The role of zinc in the function of this enzyme has two suggested explanations. One is that Zn is used to synthesize AP, and the other is that Zn may act as a transport mechanism for other substances needed to produce AP in cells (17).

AP activity can be affected by factors other than zinc. Supplementary histidine has also been found to increase tibia AP activity significantly in Zn deficient chicks fed sesame meal (1) or ISP diets (3), concomitant with alleviation of leg deformities. AP

was not increased on casein low-zinc diets and leg scores were not improved (3).

CHAPTER III

METHODOLOGY

The techniques for chick care given below were used throughout the study. The methods for cooking egg albumen were solved by trial and error during the course of the study (Appendix A). The basal diets given in Table I was used throughout the study. Details of supplements and omissions are given with each section of results.

Sample

One day old Cornish Kings (Cornish X White Rock) chicks[1] were used as the experimental animal. In the initial experiment, chicks on each diet were randomly distributed in duplicate groups of 10 each without regard to sex. Varying numbers of chicks were used in additional experiments.

The chicks were housed in a stainless steel battery[2] in order to decrease Zn contamination from the environment. Feed and deionized water were given ad libitum.

Chicks were weighed on day 1, 7, and 14 as a group. They were weighed individually on day 21 at the time of sacrifice.

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[1] Quality Hatchery, Billings, MT.

[2] Petersime Incubator Company, Gettysburg, OH.

TABLE I

Other ingredients of egg albumen diets per kilogram

Ingredients	g
Vitamin mix[1]	5.0
Choline Cl (70%)	3.0
Vitamin D oil[2]	1.5
Santoquin oil[3]	2.5
Corn oil[4]	96.0
Glycine[5]	3.0
Mineral mix[6]	60.1
$MgCO_3$	2.5
Biotin mix[7]	1.5
Zinc mix[8]	0.04-0.05
Sucrose[9]	656.6

[1]Vitamin mix provided the following per kg of ration: vit. B_{12} (as 0.1% mix) 20 ug; menadione bisulfite sodium, 5 mg; biotin, 0.6 mg; pyridoxine HCl, 6.0 mg; folic acid, 5.0 mg; riboflavin, 15 mg; d-Ca pantothenate, 30 mg; thiamine mononitrate, 100 mg; niacin, 100 mg; vitamin A palmitate, 10,000 IU and DL-a-tocopheryl acetate, 50 IU.

Variation 1: Pyridoxine.HCl, folic acid, d-Ca pantothenate and riboflavin were increased to provide per kg: Pryridoxine.HCl, 30 mg; folic acid, 12 mg; d-Ca pantothenate, 100 mg; riboflavin, 36 mg.

Variation 2: All vitamins were increased with the exception of vitamin A and DL-a-tocopheryl acetate to provide per kg: vitamin B_{12} (as 0.1% mix), 40 ug; menadione bisulfite sodium, 5.0 mg; biotin, 0.6 mg; pyridoxine.HCl, 15 mg; folic acid, 5 mg; riboflavin, 15 mg; d-Ca pantothenate, 90 mg; thiamine mononitrate, 50 mg; niacin, 100 mg.

[2]Twenty-five mg cholecalciferol in 1000 ml Mazola to give 1500 ICU/kg of ration.

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

[4]Mazola.

[5]Glycine, Nutritional Biochemicals, Cleveland, OH.

[6]Mineral mix: see J. of Nutr. 96: 126 (1968) for composition.

[7]Biotin mix. 100 mg of biotin in 100 g of sucrose.

[8]Zinc mix. 116 g of $ZnCO_3$ made to 600 g with sucrose. 0.1 g contributes 10 ppm of zinc in 1 kg.

[9]Supplements of amino acids or zinc were added at the expense of sucrose. L-arginine.HCl, L-histidine.HCl.H_2O, threonine, and lysine. HCl were obtained from Sigma Chemical Company, St. Louis, MO. Methionine as methionine hydroxy analog was obtained from Nutritional Biochemical Corporation, Cleveland, OH.

Diets

Several forms of egg albumen were studied. Two lots of dried egg white solids, RDEW[3] and RDEW72[4] were investigated in the raw and in the cooked form, CDEW and CDEW72. Pasteurized frozen egg whites[5] were cooked and dried. Fresh egg whites[6] separated from whole eggs were cooked. Yolks from the fresh egg whites were cooked and added to the CDEW. Whole eggs[6] were cooked and used also. Methods for preparation of the egg white are in Appendix A. Each protein source was added to provide 20% protein to the ration (260 g/kg). The remainder of the basal diets is given in Table I. Chick starter[7] or isolated soy protein[8] + 20 ppm Zn was fed to the control groups.

The criteria used, which have been shown to be useful in measuring zinc deficiency in this and other laboratories, were the following:

1) body weight

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[3]Armour and Co., Chicago, IL., November, 1971.

[4]Armour and Co., Chicago, IL., December, 1972.

[5]Pacific Egg Products, Northwest, Inc., Marysville, WA.

[6]M.S.U. Poultry Department, Bozeman, MT.

[7]Ceretana, Montana Flour Mills, Great Falls, MT.

[8]Assay Protein, C-1, Skidmore Enterprises, Cincinnati, OH.

2) leg scores as indicative of deformities

3) analysis of Zn content of the tibia

4) analysis of AP activity of tibia

At the end of the initial experiment using two preparations of egg white, an additional criterion, mortality rate, was also used.

The procedures for zinc or AP analysis can be found in Appendix B.

Statistical analysis for significance at the 5% probability level was done by Duncan's Multiple Rang- Test (27) for the initial experiment.

CHAPTER IV

RESULTS AND DISCUSSION

The objective of the study was to compare the effects of Zn deficient raw or cooked egg albumen diets on the development of leg deformities in the chick. Dietary variations were to be correlated with tibia AP activity, zinc content of the tibia and growth.

Initial Experiment Using Two Forms of Egg Albumen

Day-old chicks were fed basal rations containing either dried (RDEW), or reconstituted dried egg albumen that had been cooked (CDEW) (Appendix A, pg. 38). Supplements to each were 1% histidine; 2% arginine; 2% arginine plus 1% histidine; or 30 ppm Zn. Commercial chick starter[9] was fed as a control group.

Amino acid supplements did not significantly affect growth of chicks fed either CDEW or RDEW (Table II). Body weights of chicks fed either CDEW or RDEW + 30 ppm Zn were significantly increased from those fed either basal ration. Growth, however, was significantly less for these groups compared to those fed chick starter. Chicks fed egg white diets containing 80 ppm Zn have been found to have low body weights (5).

Chicks developed few leg deformities when fed either basal ration (Table II). In another laboratory, leg deformities have been

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[9]Ceretana, Montana Flour Mill, Great Falls, MT.

TABLE II

Effect of zinc, histidine and arginine on chicks fed two preparations of egg albumen for 3 weeks.[1]

Product	Zn	Amino Acids	Mortality	BWT[4]	Left Tibia			Right Tibia[2]			Leg Score[7]
					Wt[5]	Total Zn[6]	Per g[5]	Wt	AP Activity[3] Total	AP Activity[3] Per g	
	ppm		%	g	g	ppm	ppm	g			
Chick Starter			0	307a	7.80	525	67	2.52a	385a	153a	0.0
CDEW	5		70	127cd	3.10	106	34	1.06c	117c	109c	1.3
	5	Arg	90	108d				0.92c	78c	85c	2.0
	5	Hist	70	144c	3.65	150	41	1.17c	117c	98c	0.9
	5	Arg + Hist	85	133c				1.25c	94c	69c	3.0
	30		35	232b	5.72	583	102	1.88b	366b	191a	0.3
RDEW	5		15	114d	3.03	129	39	1.16c	119dc	102cd	0.4
	5	Arg	0	107d	2.84	108	38	0.96d	76c	79c	1.2
	5	Hist	30	108d	2.74	149	54	0.91d	113dc	124d	0.0
	5	Arg + Hist	15	137c	3.71	118	32	1.91cd	135c	113dc	0.6
	30		0	154b	4.13	398	96	1.38b	235b	169a	0.1

[1]Values in each column for each product by the same letter are not significantly different ($P < 0.05$) from each other. Each supplement within a product is compared to chick starter.

[2]Mean of six individual tibiae; wet weight.

[3]Milligrams of p-nitrophenol liberated in 30 minutes at 37°.

[4]Mean of six individual chicks.

[5]Mean of two composite samples, three tibiae each; wet weight.

[6]Mean of two composite samples, three tibiae each.

[7]In increasing severity from 0 to 4.

reported in chicks fed low-zinc egg white diets (5). Leg deformities have also been reported to occur when casein (3, 5), sesame meal (7) or ISP (3, 5) were the source of protein in low-zinc diets.

Additions of 2% arginine to the basal diet in this experiment did not cause a significant increase in leg deformities. In contrast, addition of 2% arginine to low-zinc egg white diets has been reported to increase the severity of deformities greatly (5). This may have been due to a different source of egg albumen. Additions of 1% arginine, however, to ISP or casein diets low in zinc did not significantly increase the severity of deformities (3).

Since few leg deformities developed in this experiment, the possible effects of the 1% histidine supplements on the alleviation of deformities was not apparent. Coleman, et. al. (5) have reported decreased leg scores by addition of histidine when chicks were fed low-zinc egg albumen diets with or without arginine. Histidine supplements have also been reported to alleviate leg deformities of chicks fed ISP (3, 5) or sesame diets (1) that were low in zinc. This effect was not seen in other studies when chicks were fed casein diets (3, 5).

Histidine did not cause an increase in AP activity for either basal ration, nor did any leg deformities develop. It appears that for egg albumen histidine in itself has little effect on tibia AP. While leg deformities occur on low-zinc casein diets, supplements of histidine did not improve leg scores nor did tibia AP increase (1, 3).

Addition of histidine increased AP activity when chicks were fed ISP or sesame protein diets, concomitant with the alleviation of leg deformities.

The arginine supplements to the basal CDEW or RDEW did not decrease tibia AP. Supplements of arginine + histidine caused no apparent change in AP activity (Table II).

When the requirement of the chicks for zinc was met, for both preparations of egg albumen AP increased significantly. The AP per gram of tibia for chicks fed either preparation + 30 ppm Zn or those fed chick starter (78 ppm Zn) were not significantly different. This suggests that 30 ppm Zn provided enough Zn for the full activity of AP.

The total Zn content of the tibia for either basal ration was comparable. The amino acid supplements did not change this significantly. Tibia zinc was higher for chicks fed 30 ppm Zn supplements indicating that the chick was utilizing Zn. Total Zn of the tibia was similar for chicks fed chick starter (78 ppm Zn) and those fed cooked egg albumen + 30 ppm Zn. That of chicks fed dried raw egg albumen + 30 ppm Zn was somewhat lower. These results indicated that the 30 ppm Zn was enough to meet the chick's requirement for Zn.

Mortality for chicks fed the basal cooked egg albumen was high, 70% (Table II). The amino acid supplements did not reduce this. In contrast, chicks fed raw egg albumen had a low mortality, 15%

(Table II). A similar difference was seen between the Zn supplemented groups: CDEW 35% and RDEW 0%. This suggests that the mortality may be related to the cooking process.

Most mortality occurred within the first 10 days of the experiment. Chicks were unable to maintain their balance and had a tendency to walk backward rather than forward. The typical head retraction characteristic of Vitamin E deficiency was not seen. Such a condition has not been found to be described in the literature. This condition was not familiar to two poultry scientists.[10, 11] After appearance of these symptoms, death occurred in 3-6 hours.

In a later experiment, a live chick, exhibiting these symptoms, was examined for pathological lesions of the brain and liver.[12] None were found and the diagnosis was left open.

As a result of the high mortality of chicks fed basal CDEW diets, it did not appear that Zn deficiency and factors which might influence AP activity could be dealt with until the cause of the mortality was determined. The subsequent experiment, described

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[10]Oral Communication. Dr. C. H. Hill, Dept. Poultry Science, North Carolina State University, Raleigh, N.C.

[11]Oral Communication. Dr. Don Turk, Poultry Science Depart., Clemson University, Clemson, S.C.

[12]Courtesy of William J. Quinn, D.V.M., Livestock Sanitary Board, Diagnostic Laboratory, Bozeman, MT.

below, were conducted in an attempt to ascertain the cause of high mortality.

Whole egg protein is ranked second among proteins for biological value and is often used as a reference protein (28). Egg albumen is ranked fourth. Most recent dietary studies using egg albumen have been with the raw dried product (7, 8, 5, 29). During the commercial processing of egg albumen the necessity to kill Salmonella organisms found in egg and egg products (30) has led to many manipulations of the albumen. For this reason, several sources of egg albumen were investigated.

Although mortality appeared to be related to the cooking process, other factors were also investigated. Cornish King chicks were obtained from two hatcheries,[13] and Leghorn-Bantams[14] were also used in order to eliminate the possibility that one breed or source of chick would be more susceptible than the other. Supplements of amino acids (Table V), vitamins (Table IV) and 60 ppm Zn (Table VI) were also made to the ration. Since mortality occurred very early in the chick's life, variations in the age the chicks were fed the cooked egg white were made (Table IX).

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[13] Quality Hatchery, Billings MT. and a Washington Hatchery.

[14] M.S.U. Poultry Department, Bozeman, MT.

Supplements

Vitamins

Biotin. In early work, raw egg white fed to rats resulted in high mortality (31). This was later found to be due to the binding of the essential vitamin, biotin, by avidin (32). If biotin deficiency were the cause of mortality, then it would be expected that the higher mortality would occur with raw egg white diets, but this was not the case in the first experiment conducted.

Avidin has been found to be destroyed in fresh egg by heating to 80°C for 5 minutes (31). In reconstituted Chinese dried egg white heating to 85°C for twenty minutes destroyed the avidin. The cooked egg white in this experiment was heated to 80°C and held for 10 minutes. Most of the avidin should have been destroyed. In subsequent experiments biotin was routinely added to the cooked egg white diets. Mortality was not reduced and did not appear to be due to a biotin deficiency.

Other Vitamins. In vitamin Variation 1, pryridoxine, folic acid, Ca-pantothenate and riboflavin were increased (Table I) to provide 10 times the minimum requirement (33) for each. In vitamin Variation 2, all vitamins with the exception of A, D, and E (Table I, III) were increased. The vitamins increased in Variation 1 were

TABLE III

Amount of vitamins provided by variation 2

Vitamin	Amount times the minimum requirement
Vitamin B_{12}	4.4
Menadione bisulite sodium	9.4
Biotin[1]	6.6
Pyridoxine.HCl	5.0
Folic Acid	4.0
Riboflavin	4.0
d-Ca Pantothenate	9.0
Thiamine	25.0
Niacin	4.0

[1]Biotin was added separately

selected because the type of symptoms observed could be related to the functioning of the nervous system. Previously, day-old chicks showed high mortality which could be prevented by pantothenic acid injections (34).

Mortality and growth of chicks fed low or high Zn CDEW diets were little different between the two vitamin variations (Table IV, II). Although mortality was somewhat reduced from that in Experiment 1, vitamin variation 2 was used in other experiments and this was not found (Table VI, VII, VIII, IX, X). As in the initial experiment, a difference in growth and mortality was seen between the low and high Zn groups.

TABLE IV

Effect of Zn and vitamin variation on body weight and mortality of chicks fed cooked or raw egg albumen diets for 3 weeks

Product	Zn	Vitamins	No.[1]	Mean Body Wt.	S.D.[2]	Mortality
	ppm			g		%
CDEW	5	Variation 1	3	142	11.5	54[3]
	4	Variation 2	3	153	21.3	55[3]
	30	Variation 1	3	258	14.6	23[3]
	30	Variation 2	6	291	27.4	30[4]
RDEW	4	Variation 1	6	142	24.1	0[4]
	4	Variation 2	6	118	5.5	0[4]
	30	Variation 2	6	192	20.0	0[4]
Chick Starter[5]			6	282	36.5	12[3]

[1]Number of chicks used to determine mean body weight.

[2]Standard deviation.

[3]Based on one group of 10 chicks.

[4]Based on duplicate groups of 10 chicks each.

[5]As a standard.

Vitamins A, E, and D were not increased because of the amount present in the original vitamin mix. It provided five times the minimum requirement for vitamin A and seven and one half times the requirement for vitamin D. The requirement of the chick for vitamin E

is not known, however, including three times the amount ordinarily added had little effect on mortality.

Amino Acids

Supplements of arginine, histidine, or arginine + histidine in the initial experiment had no effect on reducing mortality. Other amino acid supplements were used in an attempt to reduce mortality.

Lysine. The egg albumen was found by analysis to contain 1.89% lysine (7). This is above the minimum requirement of the chick of 1.1% for lysine. Lysine poses two possibilities. An excess of lysine has been shown to cause an increase in the chick's requirement for arginine (35). Chicks of the HA strain had severely depressed growth rates when fed excess lysine. The present basal ration contained 1.53% arginine, slightly above the chick's minimum requirement of 1.2% for arginine.

Scott (36) found that by autoclaving egg albumen at 121°C for 10 minutes the availability of lysine was reduced from 74% to 37%. If cooking at 80°C had affected its availability, the lysine could be less than that of the chick's requirement.

Supplements of 0.4% lysine were made to the basal CDEW ration and to CDEW + 30 ppm Zn. Mortality for chicks fed the basal diet was reduced from that in Experiment 1 (Table V). That of chicks fed 30 ppm, however, was not reduced.

TABLE V

Effect of some amino acid supplements on mortality of chicks fed egg albumen diets for 3 weeks

Product	Zn	Amino Acid	No.[1]	Mean Body Wt.	S.D.[2]	Mortality
	ppm			g		%
CDEW	5	0.4 Lys	3	141	8.6	46[3]
	5	0.4 Met	3	170	33.8	69[3]
	4	0.2 Met	6	143	18.4	60[4]
	30	0.4 Lys	3	283	11.2	38[3]
	30	0.4 Met	3	334	16.0	23[3]
	30	0.2 Met	6	296	25.7	35[4]
RDEW	4	0.2 Met	6	119	6.5	5[4]
	30	0.2 Met	6	196	16.7	5[4]
ISP[5]	20		6	259	40.9	3[4]

[1]Number of chicks used to determine mean body weight.

[2]Standard deviation.

[3]Based on one group of 10 chicks.

[4]Based on duplicate groups of 10 chicks each.

[5]ISP + 20 ppm Zn was used as a standard.

Methionine. Zn deficiency has been found to increase greatly the conversion of methionine to CO_2 (37). An increased requirement for methionine could be the result. It was calculated that the egg albumen contained 3.7% methionine, supplying 0.96% to the ration. The minimum requirement of the chick for methionine is 0.4% (33). Supplements of 0.2 and 0.4% were made to the basal CDEW and CDEW + 30 ppm Zn.

Supplements of 0.2% methionine were also made to RDEW and RDEW + 30 ppm Zn. Mortality was little reduced by either level of methionine supplement (Table V).

Methionine and Threonine. Despite the incidence of high mortality of chicks fed CDEW, those that survived tended to grow better than chicks fed RDEW diets. It was thought that if the poor growth was due to poor utilization of the protein, an increased growth might be obtained if nitrogen retention could be improved. Supplements of methionine plus threonine have been found to reduce urinary nitrogen excretion in rats fed protein free diets (38). Methionine alone or in combination with any other essential amino acid did not reduce the excretion of nitrogen.

Additions of 0.5% methionine + 0.5% threonine were made to the RDEW plus 30 ppm Zn ration. The average body weight for this group, 89 g, was not improved over body weight for other chicks fed RDEW plus 30 ppm Zn, 109 g.

Zinc

Some manipulations of egg white to destroy Salmonella organisms call for the addition of alkali polyphosphates, and are selected from a group which includes sodium tripolyphosphate, tetrasodium pyrophosphate, tetrapolyphosphate, sodium hexametaphosphate, and sodium acid pyrophosphate (39). These commonly act as sequestering agents (40).

If any of these were added to the egg albumen used, zinc might not be available to the chick. Considering the concentration of zinc in the tibia (Table II) it did not appear that zinc was being bound. However, 60 ppm Zn was added to the CDEW ration as a check to levels of dietary Zn. Mortality was not reduced from that of groups fed 30 ppm Zn (Table VI). Growth of those fed either 30 ppm or 60 ppm was not different.

TABLE VI

Effect of Zn supplements to egg albumen diets on mortality and body weight of chicks at 3 weeks

Product	Zn	No.[1]	Mean Body Wt.	S.D.[2]	Mortality
	ppm		g		%
CDEW	30	6	258	28.2	35[3]
CDEW	60	6	274	22.8	40[3]
Chick Starter	(78)	6	282	33.5	8[4]

[1]Number of chicks used to determine mean body weight.

[2]Standard deviation.

[3]Based on duplicate groups of 10 chicks each.

[4]Based on one group of 12 chicks.

Antibiotics

Salmonella infections may be caused by a large number of species of the genus (41). Most species are named after the place they were first isolated. Contamination can come from humans and other animals. Important sources are poultry and eggs. Salmonella infections are known to cause digestive upsets in humans.

The RDEW was stated to be Salmonella negative.[15] However, during the cooking and subsequent drying process, contamination could have occurred. Samples of all RDEW and CDEW, frozen and fresh cooked egg white were tested for Salmonella[16] and found to be negative. A live chick exhibiting the typical symptoms was also tested, and Salmonella montevidea was isolated.

Furacin[17] (5-nitro-2 furaldehyde semicarbazone) which is effective against Salmonella was used. The CDEW contained either 4 or 30 ppm Zn. RDEW was supplemented with 2% arginine plus 1% histidine at both levels of Zn.

Chicks fed either CDEW diet experienced the same mortality as had been found in previous experiments (Table VII). Infections with Salmonella was apparently not the cause of high mortality.

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[15] As determined by the manufacturer (U.S.D.A. Methods of Analysis).

[16] Courtesy of B. Hubbell, D.V.M., Livestock Sanitary Board, Veternary Diagnostic Laboratory, Bozeman, MT.

[17] Furacin was added to deionized drinking water at the rate of 10.36 g per 2.5 gal.

TABLE VII

Effect of an antibiotic on body weight and mortality of chicks[1] fed raw and cooked egg albumen

Product	Zn	Amino Acid	No.[2]	Mean Body Wt.	S.D.[3]	Mortality[4]
	ppm			g		%
CDEW	4		3	70	2.5	85
CDEW	30		6	110	13.2	40
RDEW	4	Arg + Hist	6	62	27.8	5
RDEW	30	Arg + Hist	6	109	8.7	0

[1]Leghorn-Bantams, M.S.U. Poultry Department, Bozeman, MT.

[2]Number of chicks used to determine mean body weight.

[3]Standard deviation.

[4]Based on duplicate groups of 10 each.

Removal of dietary NaCl

The pH of raw egg white is 7.0 - 9.3 depending on the age of the egg (42). HCl may be used to lower the pH to a range of 6.5 - 8.0 during the pasteurization process (39). Before drying the egg white is returned to the normal pH.

Turkey poults fed diets of spray-dried egg albumen developed cardiovascular symptoms resulting in high mortality rates (29). This was found to be due to the large amount of sodium contributed by the egg albumen.

Optimal growth has been found to be produced in chicks using 0.12% chlorine (43). Growth of chicks fed diets containing 0.06% chlorine depends upon the amount of sodium in the diets (44). High mortality resulted when low chlorine was fed at levels of 0.89 and 1.05% added sodium (43).

The pH of RDEW and CDEW was 6.9, suggesting that the pH had been lowered during pasteurization. For this reason NaCl was omitted from the mineral mix of the CDEW + 4 ppm Zn diet.

The pH of raw frozen egg whites was found to be 9.2. It was found necessary to lower the pH to 7 with HCl in this laboratory in order to coagulate the albumen during the cooking process. NaCl was omitted from the mineral mix of a cooked frozen egg white + 4 ppm Zn diet.

Mortality for all groups was high. Of the chicks fed CDEW with or without NaCl, 91% died. One hundred per cent of the chicks fed the frozen egg white diets with or without dietary NaCl died by day 10 of the experiment.

Sodium and chlorine levels of CDEW and RDEW as well as fresh raw egg white, and fresh or frozen cooked dried egg white were determined[18, 19] (Table VIII). Frozen cooked egg white contained the

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[18]Courtesy Chemistry Station Analytical Laboratory, M.S.U. Agriculture Experiment Station, Bozeman, MT.

[19]Courtesy Mr. Vincent Haby, Plant and Soils Testing Laboratory, M.S.U., Bozeman, MT.

greatest amount of chlorine, but this was probably due to the addition of HCL. Fresh cooked egg white contained 2.11% chlorine, while chlorine levels were lowest and nearly equal for both CDEW and RDEW.

In the present experiments mortality was much lower for chicks fed RDEW, although chlorine and sodium levels were similar for both the RDEW and CDEW. It does not appear that excess levels of sodium or chlorine were the cause of high mortality on the CDEW rations.

TABLE VIII

Sodium and chlorine levels of various sources and preparations of egg albumen

Product	Chlorine[1] %	Sodium[2] mg/100g
Frozen egg white, cooked	3.12	____
CDEW	1.87	175
RDEW	1.84	188
Fresh raw egg white	____	211
Fresh cooked egg white	2.11	____

[1]Courtesy Chemistry Station Analytical Laboratory, M.S.U. Agriculture Experiment Station, Bozeman, MT.

[2]Courtesy Mr. Vincent Haby, Plant and Soils Testing Laboratory, M.S.U., Bozeman, MT.

Variation in Age of Chick

In previous experiments most mortality occurred by day 10. Such chicks as survived usually grew at least as well as those fed RDEW. To determine if mortality would be reduced if the CDEW was fed at a later age of the chick, RDEW basal rations were fed for one, two or three weeks. At the end of each period, chicks were changed to basal CDEW diets.

Mortality was reduced in groups changed at two and at three weeks. As the length of time chicks were fed RDEW before the CDEW increased, mortality decreased (Table IX).

The protein of the chick starter diet is vegetable in origin. Day-old chicks fed this for five days, and CDEW + 5 ppm Zn for the subsequent 16 days did not experience any mortality (Table IX). Another vegetable protein diet, ISP + 2 ppm Zn, was fed for five days before feeding CDEW. The mortality for this group was also considerably lower than for those chicks fed CDEW (Table IX).

TABLE IX

Effect of chick age on mortality produced by CDEW

	Mean Body Wt. at Change	Mean Final Body Wt.[1]	S.D.[2]	Mortality
	g	g		%
7 d RDEW[3] - 14 d CDEW[3]	58	113	15.3	40[4]
14 d RDEW[3] - 7 d CDEW[3]	65	109	23.6	25[4]
21 d RDEW[3] - 21 d CDEW[3]	69	129	30.0	16[5]
5 d Chick Starter - 16 d CDEW[3]	66	184	5.3	0[5]
5 d ISP[6] - 16 d CDEW[3]	57	170	26.4	25[7]

[1]Mean body weight of six chicks, except for 21d RDEW - 21d CDEW mean body weight of 5.

[2]Standard deviation.

[3]Supplement of 4 ppm Zn except 5 d ISP - 16d CDEW supplement of 5 ppm Zn.

[4]Based on duplicate groups of 10 each.

[5]Based on one group of 6.

[6]Supplement of 2 ppm Zn.

[7]Based on one group of 8.

Variation in Source of Egg Albumen

Results of previous experiments led to interest in the effects of different sources of egg white. Fresh or frozen egg white was cooked, and a new lot of dried egg white solids, (RDEW72) was cooked,

CDEW72 (Appendix A, pg.38). Each was fed with a supplement of 4 ppm Zn. A supplement of 10% fresh cooked egg yolk (Appendix A, pg. 39). was also made to a diet containing CDEW.

Mortality for groups fed either the frozen cooked egg white or the fresh cooked egg white was 100% (Table X). This was greater than that for CDEW of previous experiments. The new lot of commercial dried egg white showed a similar mortality with and without cooking.

The addition of one part egg yolk to ten parts of egg white reduced mortality to 50% from 72%. Whole cooked eggs, three parts egg yolk to one part of egg white greatly reduced mortality.

TABLE X

The effect of variation of source of egg albumen on the body weight and mortality of chicks fed these diets for 3 weeks

	Zn	No.[1]	Mean Body Wt.	S.D.[2]	Mortality[3]
	ppm		g		%
Fresh Cooked Egg White	4	—	—		100
Frozen Cooked Egg White	4	—	—		100
CDEW + 10% Yolk	4	5	157	44.4	50
CDEW72	5	6	127	35.5	40
RDEW72	5	6	98	23.3	30

[1]Number of chicks used to determine mean body weight.

[2]Standard deviation.

[3]Based on one group of 10 each for each treatment.

Variation In Mortality Due to Zn Supplementation

Mortality was found to vary somewhat from experiment to experiment for each level of zinc supplement. This could be due to variation in chicks as experimental animals. Chicks fed 4-5 ppm Zn regardless of other supplements were found to have an average mortality of 72%. Those fed 30 ppm Zn regardless of other supplements had an average mortality of 32%. These were statistically compared and mortality for those fed supplements of 30 ppm Zn was significantly lower (P 0.05) than those fed 4 - 5 ppm Zn. Therefore, it appears that variations within each level of Zn supplement was due to chick variation rather than effect of other treatments.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The original objective of this study was to compare the effect on chicks of low-zinc diets based on cooked or raw egg albumen. The effect of arginine or histidine supplements on the development of leg deformities was to be included. Tibia AP activity, zinc content of the tibia, and growth were to be correlated with dietary variations.

In the first experiment, few deformities developed on either basal ration. Chicks fed cooked egg white diets experienced very high mortality rates. Those fed the uncooked egg white had little mortality. In order to accomplish the first objective mortality had to be reduced. A search of the literature offered no solution; therefore a wide range of possibilities had to be explored.

The source of egg white was investigated. Fresh or frozen egg white was cooked. The mortality was higher, as 100% of the chicks on either source died by day 10.

Supplements of 0.4% lysine, 0.2 and 0.4% methionine, increased dietary vitamins, or 60 ppm Zn were not effective in reducing mortality. The mechanics of processing some egg albumen involves changes in pH, for this reason the NaCl was omitted. This had little effect in reducing mortality. Addition of "Furacin," an antibiotic effective against Salmonella infection, was also ineffective.

The effect of supplements of cooked egg yolk to egg white in reference to mortality was investigated. One part of egg yolk to ten parts of egg white reduced mortality slightly. Whole cooked eggs, three parts of egg yolk to one part of egg white, greatly reduced mortality.

The influence of age of chicks at which cooked egg white was fed appeared to be involved since most mortality occurred by the 10th day of the experiments. Chicks were fed raw egg albumen for one, two or three weeks, and at the end of each period were fed cooked egg white. Mortality was reduced for those groups changed at two and three weeks. Day-old chicks were also fed chick starter or ISP diets 5 days prior to feeding the cooked egg white. This was found to be effective in greatly reducing mortality.

Conclusions

1. Few leg deformities developed on either low Zn raw (RDEW) or cooked (CDEW) egg white diets. Histidine or arginine had little effect on the development of deformities or AP activity.
2. The egg albumen was apparently affected by cooking, as those fed CDEW + 5 ppm Zn had high mortality, average of 72% in contrast to those fed RDEW + 5 ppm Zn, average of 11%. For CDEW + 30 ppm Zn, mortality averaged was 32%

and for RDEW mortality 1%. Additions of 60 ppm Zn did not further reduce mortality.

Variations in diet; source of chicks; source of egg white; use of a Salmonella bactericide; and age at which the chicks were fed the cooked egg white were investigated with the following conclusions:

1. Mortality was not related to the source of egg white. Fresh or frozen egg white which had been cooked caused high mortality.
2. High mortality was apparently not due to a deficiency of any of the known vitamins, lysine, methionine or sodium or chlorine toxicity.
3. High mortality was prevented when chicks were fed whole cooked eggs. Mortality was decreased slightly by supplements of 10% egg yolk.
4. High mortality was apparently not due to the source of breed of chick as all chicks fed CDEW experienced high mortality.
5. Salmonella infection was apparently not the cause of mortality.
6. The high mortality rate could be prevented when chicks were fed either ISP or chick starter 5 days before feeding CDEW.

Recommendations

Before cooked egg albumen can be used to study the effects of Zn deficiency, further study must be done to determine what will prevent the high mortality of chicks fed these diets and yet allow for the appearance of Zn deficiency symptoms. Determination of the per cent of egg yolk supplements which will reduce mortality without feeding whole egg should be done, as results in this laboratory indicate that whole egg contains enough Zn to meet the chick's requirements. Fractionation of the egg yolk should also be done. If the unknown factor is contained in either fraction, then only that fraction would need to be added to the diet. This might also lead to the isolation of the factor. The period of time chicks are fed chick starter or ISP should be reduced to the point where mortality is prevented and Zn deficiency symptoms will appear. It would also be of interest to know if high mortality is characteristic only of baby chicks, and experiments using young of other species and egg white diets might provide the answer.

APPENDICES

APPENDIX A

METHODS OF EGG ALBUMEN PREPARATION

Cooked Egg Albumen

For Dried Egg White Solids (CDEW, CDEW72)

500 g of raw egg white solids were mixed with 2000 ml of glass distilled water. This mixture was divided in half and placed in two aluminum-foil-lined baking pans 9" X 13" X 2½". Better mixing was obtained by using the fingers. These pans were placed in a 350°F (176°C) oven. After approximately 10 minutes the egg white was stirred and the temperature checked. When the egg white mix reached 70°C the oven was turned to 250°F (121°C). This oven temperature maintained the temperature of the egg white at 80°C. This temperature was maintained for 10 minutes.

The egg white was removed and spread on paper in front of fans at room temperature. It was then broken into pieces as small as possible. After drying for approximately one hour, it was put through a Waring blender, filling the blender until the blades were just covered. It was then put back on the drying papers in front of the fans until dry. During this time it was stirred occasionally. After drying it was put through a Waring blender again until all of the egg white was a fine powder. It was then milled in a micro-Wiley mill and stored in the refrigerator until used.

For Frozen and Fresh Egg White

One liter of frozen egg white brought to pH 7 with approximately 3 ml of 6 N HCl, or one liter of fresh egg white separated by hand from whole eggs was put into baking pans previously described. This was then put into an oven preheated to 235°F(113°C). This temperature was maintained for 15 minutes, and stirred at the end of this time. The internal temperature of the egg white was 80°C. The oven temperature was lowered to 175°F (89°C) and maintained for 10 minutes. The egg white was dried the same as the dried egg white solids.

For Egg Yolk

Egg yolks were separated from whole eggs, and frozen at a -25°C. Before cooking they were thawed at room temperature. The oven was preheated to 250°F (121°C). The egg yolks were put into an aluminum-foil lined pan 9" X 13" X 2½", and placed in the oven. At the end of 25 minutes the egg yolks had reached a temperature of 75°C and were removed from the oven. Drying was done as previously described.

For Whole Egg

Whole eggs were mixed so that yolks were broken, and placed in an aluminum-foil lined pan. This was placed in a preheated 250°F (121°C) oven. At the end of 15 minutes the temperature of the egg was 70°C. The oven temperature was lowered to 175°F (89°C) and

maintained for 10 minutes. Drying was done as previously described.

APPENDIX B

METHODS OF ANALYSIS

Leg Scores

Chick tibiae were scored for leg deformities with the following designation:

0 - normal development of legs

1 - slight deformity - one leg

2 - slight deformity - both legs

3 - severe deformity - one leg

4 - severe deformity - both legs

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

Collection of Tibiae

Tibiae were removed from 3 chicks per group of ten in each cage. All flesh was removed, and the tibiae were immediately frozen and stored at $-25^{o}C$ until time of analysis. The right tibia were weighed individually and used for AP analysis. The left were weighed as a group of three from each cage, and used in Zn analysis.

Tibia Zinc Analysis

The three weighed tibia were dried at 55°C for 4 hours and reweighed.

The dried tibiae were wrapped in a paper towel, crushed and tied into a small packet. Fat was removed by extraction in a Soxhlet apparatus: 20 hours with 95% ethanol, plus 20 hours with ethyl ether. Packets were left in the hood at room temperature overnight; bones were removed, weighed. They were ashed at 600°C for 24 hours, cooled in desiccators and weighed. The ash was dissolved in 6 N HCl. After evaporation of the 6 N HCl, the ash was dissolved in 2 N HCl to be equivalent to 0.1 N HCl in the final dilution. Zn was analyzed for by an atomic absorption method[20] using a Perkin-Elmer 290-B Spectrophotometer. The wave length was set at 2138 A; acetylene was the fuel, the oxidant air. The standard was metallic Zn dissolved in 0.1 N HCl.

Alkaline Phosphatase Method

This method is based on that of Starcher and Kratzer (12). The p-nitrophenol liberated from the p-nitrophenyl phosphate by AP is measured at 410 mu.[21]

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[20] Courtesy Mr. Vincent Haby, Plant and Soils Testing Laboratory, M.S.U., Bozeman, MT.

[21] Spectron 20, Bausch and Lomb.

Solutions[22]

1. 2 N HCl: 17.7 ml of concentrated acid in 100 ml H_2O.

2. 1 M $MgCl_2$: 0.94 g $MgCl_2$ in 100 ml H_2O.

3. 0.17 N NaOH: 6.9 g per liter.

4. Substrate: a) 4.45 g 2-amino-2 methyl-propanol[23] plus 9 ml 2 N HCl; dilute to 50 ml with water. Adjust to pH 10 with concentrated NaOH or 6 N HCl.

   b) The above solution is added to 210 mg p-nitrophenyl phosphate[24] dissolved in 50 ml of H_2O. Add finally, 0.2 ml of 1 M $MgCl_2$. Pipette 1.5 ml into each large test tube, and store at -25°C.

Tissue Preparation

1. Snip up with scissors into Virtis homogenizer cup one weighed tibia; add enough deionized water to cover. Homogenize in an ice bath. The time of homogenization and speed is varied with the size.

2. Place in a chilled 35 ml centrifuge tube and dilute with water to the nearest ml. Keep in an ice bath. Centrifuge at 2500 rpm for 1 minute. Do six samples plus a blank in one run.

3. An aliquot of the homogenate is diluted. This dilution may vary from 0.8 ml to 10 ml to 0.1 ml to 20 ml depending on tissue, age of chick and the ration fed. Use a Cyclomixer to insure a uniform mixture. Add 0.2 ml of the aliquot and 0.3 ml of deionized water to the warmed buffer substrate tubes.

4. Place in a water bath at 37°C for exactly 30 minutes.

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[22]Deionized water is used throughout the procedure.

[23]Sigma Chemical Company, St. Louis, MO. [24]Ibid.

5. Remove and add 4.5 ml of 0.17 N NaOH to each tube to stop the reaction.

6. Pour tube contents into a spectrophotometer[25] tube. Use the blank to standardize the machine. Read per cent transmission at 410 mu. Convert to O.D. and obtain ug of p-nitrophenol liberated by comparison with the standard curve.

7. For the 0.1 to 10 ml dilution multiply the ug of p-nitrophenol by a factor of 0.5. This times the mls of homogenate equals the total mg of p-nitrophenol in the tibia. Divide by the weight of the tibia to obtain mg/g of bone.

Preparation of Standard Curve

1. Working standard: (Stable one day) Accurately pipette into a 100 ml volumetric flask 0.5 ml p-nitrophenol. standard solution. Add 0.17 m NaOH to make 100 ml, mix thoroughly.

2. Pipette the solutions indicated in the columns below into seven labeled test tubes.

Tube No.	working standard ml	0.17 M NaOH ml	T %	O.D.	Equiv. p-nitrophenol ug
1	.50	6.0			3.48
2	1.00	5.5			6.95
3	1.50	5.0			10.43
4	2.00	4.5			13.90
5	3.00	3.5			20.85
6	4.00	2.5			27.80
7	5.00	1.5			34.75

3. Read and record the per cent transmittance of each tube at 410 mu using 6.5 ml of 0.17 N NaOH as a blank. This gives a range of approximately 14-80% transmittance. A curve is prepared each day the analyses are done. Optical density, found on a chart which comes with the Spectrophotometer, is plotted as the ordinate. Equivalents of p-nitrophenol are plotted on the abcissa.

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[25]Spectron 20, Bausch and Lomb.

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