

The effect of pantothenic acid on chicks fed cooked and raw egg white diets and on zinc utilization
by Mona Salama Ibrahim

A thesis submitted in partial fulfillment of the requirement for the degree of MASTER OF SCIENCE
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Abstract:
The purpose of this study was to determine the optimum level of pantothenic acid (PA) necessary to prevent mortality and to promote growth of chicks fed diet Based on either cooked dried egg white (CDEW) or raw dried egg white (RDEW) diet. Also studied was whether zinc (Zn) utilization would be affected by the optimum level of PA on both diets. There was a difference in requirement of PA for the chick in either CDEW and RDEW diets. Chicks fed CDEW diet required 9 times the suggested requirements of PA (9PA) for best growth in contrast to 3PA in RDEW diet. High PA level in the early life of the chick fed CDEW proved to be necessary for growth and survival. Optimum dietary PA in either diets apparently did not cause an added 3 ppm of dietary Zn to be used as efficiently as 6 ppm as was indicated by body weight, alkaline phosphatase (AP) concentration and tibia Zn content.

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Date Jan. 24, 1978

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TABLE OF CONTENTS

		Page
LIST OF TABLES		v
LIST OF FIGURES		vi
ABSTRACT		vii
CHAPTER		
I.	INTRODUCTION	1
	Purpose of the study	3
II.	REVIEW OF LITERATURE	4
	Pantothenic Acid	4
	Egg Albumen	7
	Alkaline Phosphatase	10
III.	METHODOLOGY	12
	Sample	12
	Chick Care	12
	Diets	13
	Experimental Design	13
	Analysis of Data	18
IV.	RESULTS AND DISCUSSION	19
V.	CONCLUSION, SUMMARY AND RECOMMENDATIONS	30
	Conclusion	30
	Summary	32
	Recommendations	34
LITERATURE CITED		36
APPENDICES		
	Egg Albumen Preparation	43
	Methods of Analysis	44

LIST OF TABLES

Table		Page
I.	Composition of basal diets	14
II.	Composition of vitamin mix in chick diets and estimated vitamin requirement for the chick	16
III.	Effect of variation in PA on the growth and zinc status of leghorn chicks fed CDEW and RDEW diets for four weeks	20
IV.	Effect of variation of dietary zinc on growth, mortality, zinc status and tibia AP activity in broiler chicks fed CDEW and RDEW diets for three weeks	25

LIST OF FIGURES

Figure		Page
I.	Biosynthesis of coenzyme A from pantothenic acid ---	5

ABSTRACT

The purpose of this study was to determine the optimum level of pantothenic acid (PA) necessary to prevent mortality and to promote growth of chicks fed diet based on either cooked dried egg white (CDEW) or raw dried egg white (RDEW) diet. Also studied was whether zinc (Zn) utilization would be affected by the optimum level of PA on both diets. There was a difference in requirement of PA for the chick in either CDEW and RDEW diets. Chicks fed CDEW diet required 9 times the suggested requirements of PA (9PA) for best growth in contrast to 3PA in RDEW diet. High PA level in the early life of the chick fed CDEW proved to be necessary for growth and survival. Optimum dietary PA in either diets apparently did not cause an added 3 ppm of dietary Zn to be used as efficiently as 6 ppm as was indicated by body weight, alkaline phosphatase (AP) concentration and tibia Zn content.

CHAPTER 1

INTRODUCTION

Interest in zinc as an essential trace element has been renewed since the 1960's when evidence for its need by man was shown. The primary features of zinc deficiency syndrome in man are growth failure and hypogonadism (1). In earlier experiments chicks fed a low zinc diet showed a lack of growth and exhibited leg defects that were characterized by shortening and thickening of leg bones and enlargement of the hock joint (2). These leg defects were designated as "arthritis-like" (3). Zinc deficiency can be produced in other species of animals, e.g. the rat (4,5). However, the avian species readily exhibit leg deformities as a zinc deficiency symptom (2,6,7). In a recent study by Simkin (8), human patients with chronic, refractory rheumatoid arthritis taking oral zinc supplements showed an improvement with regard to arthritis symptoms. These encouraging results suggest further studies with zinc deficiency.

The source of protein used in a zinc deficient diet has a marked effect on zinc deficiency syndrome in chicks. Leg deformities appeared when sesame meal (9), isolated soy protein (ISP), (3,10,11,12) or casein (11,13) were used as the source of protein. The addition of histidine to diets containing ISP (3,11) or sesame meal (9), prevented the leg deformities characteristic of zinc deficiency. It was also noted that supplementary histidine has been found to increase tibia alkaline phosphatase (AP), a zinc-containing enzyme, activity significantly in

zinc deficient chicks fed sesame meal (9,11) or ISP diets (11),attendant with alleviation of leg deformities. Histidine did not have the same effect on leg deformities or on AP when casein was used as a protein source (11). In order to determine whether the above characteristics were a property of casein or a property shared by other animal proteins, egg white (egg albumen) was used as a protein source to produce zinc deficiency in this laboratory (14).

Egg albumen, an animal protein, low in zinc has been used in animal experiments in raw dried form by several workers (4,9,15,16) to produce zinc deficiency symptoms. Egg albumen in raw dried form has been found to produce moderate leg deformities on chicks fed low-zinc egg albumen diets (10). Egg albumen also forms a part of diets of humans. This protein is generally cooked before it is consumed by man. Diets containing raw dried egg white (RDEW), which had been reconstituted with water and heated to 80°C (CDEW), were fed to the chicks (15,14). Chicks that were fed these CDEW diets experienced very high mortality rates. A fairly consistent mortality of 50-75% occurred when low zinc (5-6 ppm) diets were fed to the chicks. Approximately a 30% mortality rate occurred when sufficient zinc (30 ppm) was fed. RDEW diets caused little mortality regardless of zinc level (14).

In successive experiments, variations in diets, sources of chicks, source of egg white, and use of a bactericide for the chicks fed the CDEW were investigated and found to have little effect on mortality

(15). It was found that (14) a drop in mortality rate was obtained by increasing pantothenic acid (PA), by 14-18 times the suggested minimum (17) in CDEW and RDEW diets. It was noted that mortality occurred in the early life of the chick usually at the 10th to 12th day, suggesting that there is a demand for extra PA early in life of the chicks fed CDEW (14). Before CDEW can be used to study the effect of zinc deficiency, further work should be done to determine the relationship and the effect of PA level of chicks fed CDEW and RDEW diets.

The objectives in this study were to determine (1) the optimum level of PA necessary to prevent mortality and to promote growth of chicks fed either CDEW or RDEW diets, (2) whether zinc utilization would be affected by the optimum level of PA in both diets.

CHAPTER II

REVIEW OF LITERATURE

Pantothenic Acid (PA)

PA is one of the B vitamins and thus cannot be synthesized by animals (18). Most micro-organisms are able to make this vitamin from its precursors, pantoic acid and β-alanine. Both animals and bacteria can convert PA via pantetheine to coenzyme A which is an important catalyst of biological acetylation reactions (18,19), Figure 1. PA has a molecular weight of 219 and consists of α, γ-dihydroxy β, β' dimethyl butyric acid and β- alanine joined by a peptide linkage (20,21).

Identification of PA as part of coenzyme A molecule has implicated PA in a large number of important biochemical reactions (21,22). Coenzyme A influences the metabolism of carbohydrates, proteins and lipids; it is necessary for fatty acid oxidation and synthesis; synthesis of cholesterol and phospholipids; and essential for the formation of porphyrin, the pigment portion of the hemoglobin molecule.

In growing laboratory animals, the earliest evidence of PA deficiency is a decline in the rate of growth (23,24). Fertility of rats is impaired and sudden death has been noted in several species, often precipitated by hemorrhagic destruction of the adrenal glands (25).

Jukes (26) noted that PA deficiency in the chicks results in the classical dermatitis in the corners of the mouth, toes and upper surface of the foot. Other signs of the deficiency include poor

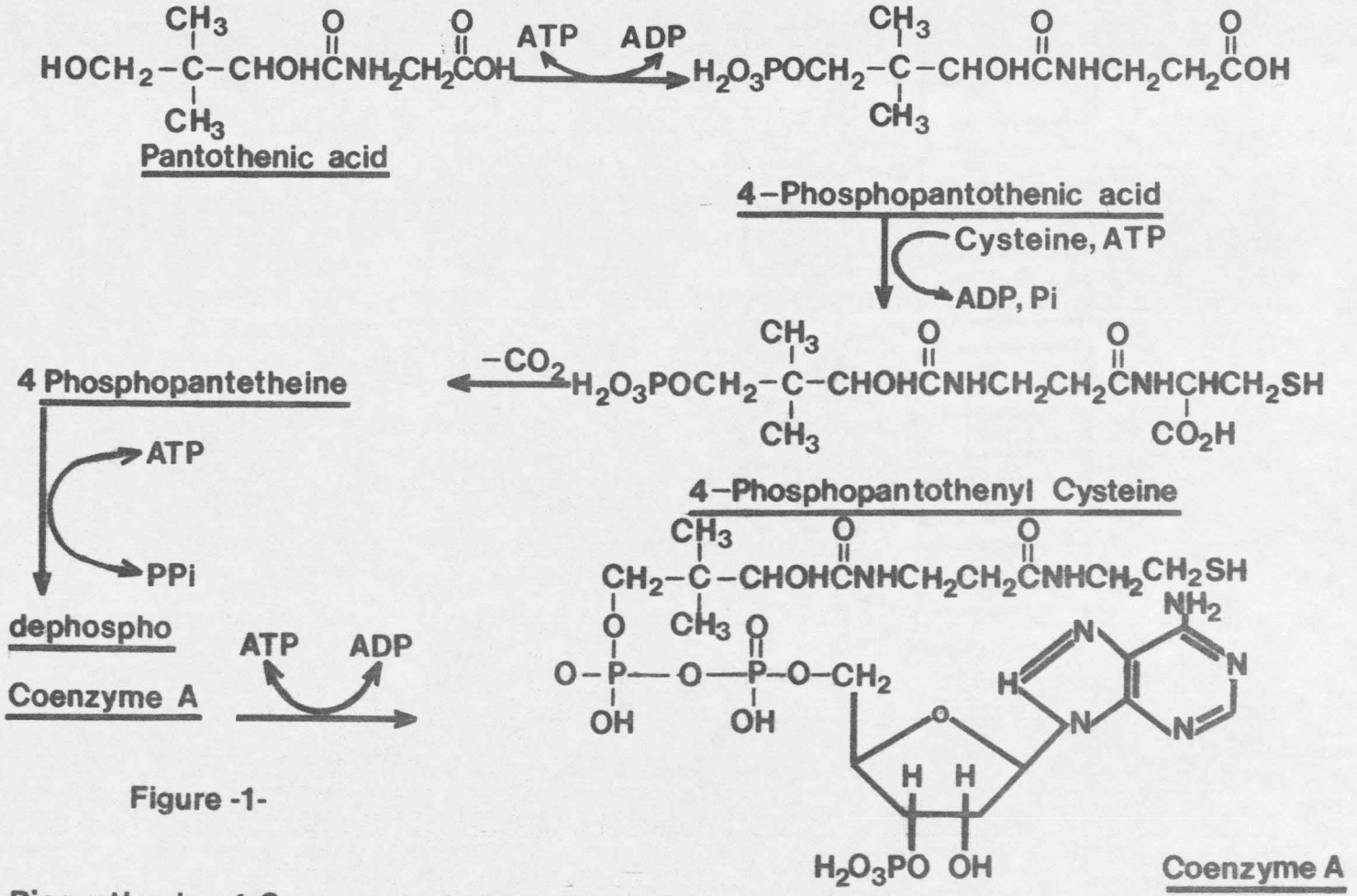


Figure -1-

Biosynthesis of Coenzyme A from Pantothenic acid

Adapted from Brown, G. M. (18)

feathering, spinal cord degradation, incoordination, paralysis, involution of the thymus, fatty degradation of the liver and death. Hence, PA has been variously known as the antidermatitis, liver filtration, yeast or the chick antipellagra factor (22).

It is suggested that (17) 10 mg/kg of feed of PA are required to produce satisfactory growth and feathering in the chick. In work done by Lease (14) chicks that were fed low zinc CDEW diets with 3 and/or 9 times the suggested minimum amount of PA, experienced a high mortality rate. A drop in mortality rate was obtained by increasing PA by 14 to 18 times the suggested minimum. Egg white in these experiments (14) was heated to 80°C for 10 minutes. In early work (26), PA was destroyed in corn meal, wheat middlings, and casein ingredients when heated at 120°C for 36 hours. Thus the destruction of PA in the cooking process was not the prime factor in mortality (14).

Studies showed that (27) low PA in the diet of the hen does not permit adequate carry over for maximum growth by the offspring or for prevention of mortality. It was also suggested that (28) the PA content of the eggs laid was directly proportional to that of the content of the diet of the hen. In a study of the effect of diet on the PA of eggs, Snell and his associates (29) noted that the increase of PA in the diet of the hen would result in greater stores in eggs which in turn will affect PA storage level in the chick. Chicks that were hatched from high PA eggs were fed CDEW (14); mortality was very low, feathering was

good and there were no signs of crusting at the corners of the mouth regardless of level of zinc supplements. In earlier experiments (27) oral or intraperitoneal PA administered to chicks early enough, during the first few hours after hatching, largely prevented mortality.

Hodges et al. (30) assumed that coenzyme A, the active derivative of PA, has functions which are thought to be disturbed and thus caused the metabolic changes observed in the animals.

Egg Albumen

Egg contents consist of approximately 65% white and 35% yolk. The egg white consists of about 87.6% water, 10.9% protein, 1.1% carbohydrate and trace of fat (31,32). Protein makes up 82.8% of the 12% solids in the white. Egg has been designated the reference protein against which other proteins are measured as good source of amino acids (33). Egg white is one of the best examples of a natural colloidal solution of a protein (33,34).

Egg white has been used in animal experiments in raw dried form by several workers to produce zinc deficiency symptoms (35,4,36,37). Leg deformities have been reported when the chicks were fed low-zinc egg white diets (13). Chicks fed egg white diets containing sufficient zinc (80 ppm) have still been found to have low body weights with

reduction in tibia zinc concentration (13). Coleman et al. (13) reported that the additions of 2% arginine to low-zinc RDEW diets increased the leg deformities greatly and resulted in significant growth depression. Also, a moderate decrease in leg scores was obtained by the addition of a histidine supplement to low-zinc RDEW diets.

When RDEW (14,15) was cooked and used for basal diets a fairly consistent mortality of 50-75% occurred when low zinc (4-6 ppm) was fed. A trial with rats (14) showed no difference in mortality or growth between CDEW and RDEW diets. It was also noted that (14) some of the chicks fed CDEW showed crusting at the corner of the mouth, gummy eyelids, and a "backing-up" syndrome where by the chicks approached the feed and then spontaneously backed away from it. Death usually occurred in the chicks at 10 to 12 days of life. The RDEW diet chicks did not show this syndrome and little mortality regardless of zinc level. No mortality occurred with a control group fed a commercial chick starter (14,15).

The presence of avidin in the egg white, which binds the essential vitamin, biotin, may be destroyed by heating the egg white to 80°C for five minutes (38). However, it was also found that avidin is more resistant to destruction by heat in some kinds of egg white than others (38). Biotin was increased to 14 times the suggested minimum (17) and the cooked egg white that has been used in this laboratory station (14) was heated to 80°C and held for 10 minutes. Most of the avidin

should have destroyed.

When the whole egg was cooked and used as a source of dietary protein (14,15) mortality rate decreased from 72% to 10% with a diet containing 22 ppm zinc. When approximately equal parts of yolk (240 g) and CDEW (260 g) (dietary zinc 13 ppm), were mixed and fed to the chicks, a marked drop in mortality resulted. Lesser amounts had a decreased or no effect (14). Extraction of cooked yolk with hexane or water did not reduce the protective effect. Since hexane removes fat soluble substances the protective factor was not a fat. PA is a water soluble vitamin, the majority of PA in foods and biological materials exsists as coenzyme A and other bound forms. Enzymatic hydrolysis is required for microbiological assays in order to measure the free forms (20). Therefore some of the protective effect of the yolk could be due to PA (14). Addition of yolk to RDEW diets had no apparent effect on the chick status.

Dewar, et al. (35) found that turkey poults fed diets of spray-dried egg albumen developed cardiovascular symptoms which led to high mortality rates, due to the large amount of sodium in the egg albumen. In this laboratory (15) sodium and chlorine levels of CDEW and RDEW diets were determined and found to be nearly equal for both diets. However, the mortality rate was higher for chicks fed CDEW. It was assumed that excess levels of sodium or chlorine do not appear to cause high mortality in CDEW rations.

Aklaline Phosphatase

Alkaline phosphatase is a zinc-containing metalloenxyme (39). AP is thought to be related to bone formation (40). It has been suggested (41) that AP is involved in the formation of collagen, the organic matrix of bone. AP may also be involved in promoting the calcification of bone by producing high phosphate compounds (39). It has been observed that in all types of calcification there is an increase in AP concentration with the deposition of bone salts in developing bones (42).

It has been found (43) that the AP of the chick's bone, liver and intestine can be differentiated by their reaction to denaturation by urea digestion.

Chicks on zinc deficient diets exhibit reduced AP activity in bone, intestinal tissues and serum (44,7,45,6). However, later work by Lease (9) chicks on low zinc deficient diets did not show reduced AP activity in intestinal tissues. In the zinc deficient chick the epiphyseal cartilage, the area of growth or elongation of the long bone was found to be abnormal (41,46,12). The cartilage tunnels in the epiphyseal-diaphyseal joint changed to a narrow zone where penetration of the cartilage by tunnels was significantly reduced (46,47).

It was suggested that (41,9) zinc may play two roles in AP enzyme function. Zinc is necessary for activity of AP since histidine

is essential for AP activities, it is also thought that zinc has a role in binding histidine to the AP molecule.

Tibia AP activity of chicks fed a low zinc diet varied with the source of amino acid supplements. Supplementary histidine in low zinc casein diets, did not have an effect on tibia AP activity or improvement in leg scores (9,11). On the other hand, supplementary histidine was found to increase tibia AP activity significantly in zinc deficient chicks fed sesame meal (9) or ISP diets (11), concomitant with alleviation of leg deformities.

To determine whether the above finds were characteristics of casein, or a property shared by other animal protein, egg white was used as a protein source in this laboratory (14).

CHAPTER III

METHODOLOGY

Sample

Two factorial experiments were conducted using one-day old chicks. Leghorn[1] chicks were used in the first experiment that was continued for 28 days. Broiler[1] chicks were used in the second experiment and were terminated at 21 days, the difference in time was to allow approximately equal growth between the two chick groups. The chicks on each diet were randomly distributed into duplicate group of 10 chicks in each cage without regard to sex.

Chick Care

The chicks were housed in a stainless steel battery[2] in order to decrease zinc contamination from the environment. Diets and deionized water were given ad libitum. Chicks were weighed as a group initially and weekly. Individual weights were recorded at the time of sacrifice. Before sacrifice chick tibiae were scored for leg deformities (Appendix, page 44). Tibiae were removed after sacrifice and used for AP analysis and zinc analysis (Appendix, page 44).

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[1] H & N Inc., Richmond, WA

[2] Petersime Incubator Company, Gettysburg, OH

Diets

Dried egg white solids[1] were investigated in the raw and cooked form. The method of preparation of egg white at 80°C is given (Appendix, page 43). Each diet of CDEW and RDEW provided 20% protein in the ration (260 g/kg). The basal diets given in Table I were used throughout the study. Composition of the vitamin mix in the basal diet and estimated vitamin requirement for the chicks is in Table II. Variation of PA supplement on the basal diet is given in Table II.

Experimental Design

The first experiment was designed to determine the optimum level of PA needed for chicks fed either CDEW or RDEW diets and if there was a difference in the requirement between these two diets. The PA was added and adjusted in the vitamin mix so that it was at 3 or 9 times the suggested minimum requirement (17), in both CDEW and RDEW diets. The zinc supplement was constant at 6 ppm throughout the study. Selected groups of chicks were given 500 ml of PA drinking water[2] in addition to the PA level that was offered in the CDEW and RDEW diets.

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[1] Spray processed dried egg white solids. Armour Food Company, Albert Lea, MN.

[2] 0.2 gm of Pa/500 ml water for 10 chicks

TABLE I

Composition of basal diets

Ingredient	g/kg
RDEW[1] or CDEW[2]	260.0
Vitamin mixture[3]	5.0
Choline.Cl (70%)	3.0
Oil[4]	46.0
Vitamin D oil[5]	1.5
Santoquin oil[6]	2.5
Glycine[7]	1.5
Mineral mixture[8]	60.3
$MgCO_3$	2.5
Biotin mixture[9]	1.5
Zinc mixture[10]	0.06
Sucrose	616.34

[1]Armour Cloverbloom, spray processed dried egg white solids. Armour Food Company, Albert Lea, MN.

[2]See Appendix (page 43).

[3]See Table II

[4]A 1:1 mixture of corn oil (Mazola) and soybean oil (Crisco).

[5]Supplied 1,500 IU of cholecalciferol/kg.

[6]Santoquin, Monsanto Company, St. Louis, MO. A 1:1 mixture of corn oil (Mazola) and soybean oil (Crisco) added to 20 gm Santoquin (ethoxquin) to make 400 gm. 25 ml. is used to give 0.0125% in the diet.

[7]Sigma Chemical Company, St. Louis, MO 63178.

[8]Supplied per kg of diet: (in gm) $CaHPO_4.2H_2O$, 27.2; $CaCO_3$, 14.9; K_2HPO_4, 11.1; NaCl, 6.0; (in mg) $MgCO_3$, 175; Fe citrate, 533; $MnSo_4.H_2O$, 333; KI, 2.6 and $CuSO_4 5H_2O$); 334.

[9]100 mg of biotin in 100 g of cusrose, in addition to that supplied by the vitamin mix.

[10]$ZnCO_3$ in sucrose, 0.1 g supplied 10 ppm zinc/kg of diet.

TABLE II

Composition of vitamin mix in chick diets and estimated vitamin requirement for the chick

Ingredient	Per kg of diet	Estimated requirement as the vit. per se.(17)
Vitamin B_{12} (mg)	0.04	0.009
Menadione bisulfite sodium (mg)	5.00	0.53
Biotin (mg)	0.6	0.09
Pyridoxine.HCl (mg)	15.0	3.00
Folic acid (mg)	5.0	1.2
Riboflavin (mg)	15.0	3.6
D-Ca pantothenate (mg)[1,2]		10.0
ThiamineHCl (mg)	50.0	1.8
Niacin (mg)	100.0	27.0
Retinyl palmitate (IU)	10,000	1500
DL-a-tocopheryl acetate (IU)	50	10

[1,2]Supplied 90 or 30 mg per kg of diet.

The PA water was offered from day one until consumed (max. 3 days). Thereafter deionized water was available. The PA water supplement was offered to investigate the effect of concentrated oral PA administration in the early life of the chick (27). A control group was fed commercial chick starter[1]. The experiment was conducted for 28 days.

In experiment 2, the zinc supplement was 3 or 6 ppm + the optimum PA level in CDEW and RDEW diets that was found in experiment 1. A control group was fed CDEW + 9 times the suggested minimum requirement (17), of PA and 30 ppm zinc. The experiment was conducted for 21 days.

The criteria used in measuring the effect of various treatments for these experiments were the following:

(1) mortality rate

(2) body weight

(3) leg scores as indicative of deformity (Appendix, page 44).

(4) analysis of zinc content of the tibia (Appendix, page 44).

(5) in experiment 2, analysis of AP activity of tibia (Appendix, page 45).

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[1] Formax, Con Agra, Inc., Bozeman, MT

The data were analyzed by standard analysis of variance method and the means were separated for the controlled variables by Duncans Multiple Range test (48). Results from personal observations will be discussed but not subjected to statistical analysis.

CHAPTER IV

RESULTS AND DISCUSSION

In experiment one, variation in PA supplements significantly ($P< 0.05$) affected growth of chicks fed CDEW or RDEW diets (Table IV). Body weight of the chicks (4 weeks) fed CDEW + 9 times the suggested minimum requirement of PA (9PA) (17), was higher than chicks fed CDEW + 3PA. In previous work done in this laboratory (14) it was found out that when PA was increased to 13.5 and 18 times the requirement in CDEW diets it reduced mortality and the chicks grew well. This suggested that there is a need for a higher level of PA in these chicks for normal growth and feathering.

PA added to the water (PA water) apparently had no effect on body weight when it was supplied to chicks that were fed CDEW + 9PA only. The addition of PA water to CDEW + 3PA increased the growth of the chicks and made it comparable to the growth of both CDEW + 9PA + PA water and CDEW + 9PA only. Beer et al. (27) have suggested that oral PA offered to the chicks early in life, during the first few hours, will promote growth as well as prevent mortality. Thus, the early administration of PA water to the chick in the first three days of life had its impact on the growth in this laboratory. It is assumed that the PA water supplied the chicks with enough stores of PA that they could use it to promote continuing good growth. Chicks that were fed CDEW + 3PA + PA water diet showed a significant ($P< 0.05$) increase in body weight

TABLE III

Effect of variation in PA on the growth and zinc status of leghorn chicks fed CDEW and RDEW diets for 4 weeks.[1]

Product	Zn	PA	Mortality	BWT[2]	Left Tibia			
					Leg Score[3]	Wt[4]	Zn[4]	F-E[5]
	ppm	g/kg	%	g		g	ug/g	
CDEW	6	9	0	167A	1.8	1.68A	26.09C	2.94
CDEW	6	g + PA water	0	173A	.8	1.63A	21.09C	2.83
CDEW	6	3	10	140B	1.4	1.33AB	17.69C	3.45
CDEW	6	3 + PA water	0	172A	1.0	1.60A	24.26C	2.98
RDEW	6	9	15	90C	.4	0.74C	42.36B	6.29
RDEW	6	3	0	132B	1.6	1.27AB	16.95C	3.54
RDEW	6	3 + PA water	15	128B	1.9	1.28AB	18.23C	4.05
Chick Starter	-	-	24	114B	0	1.14AB	92.63A	5.41

[1]Values in each column for each product with the same letter are not significantly different ($P < 0.05$).

[2]Body weight - Mean of six individual chicks.

[3]In increasing severity from 0 to 4.

[4]Mean of two composite samples, three tibia each; wet weight.

[5]Feed Efficiency - see footnote in page (21).

over chicks fed CDEW + 3PA only. This also tends to support their experiments (27,14), which suggested that high level of PA is needed early in the life of the chicks.

There was no significant difference ($P < 0.05$) in body weight with the addition of PA water, when the chicks were fed RDEW + 3PA. On the other hand when the chicks were fed RDEW + 9PA they showed a significantly ($P < 0.05$) lower body weight. Apparently 9PA added to RDEW diet was an excess that resulted in growth depression of the chick. This also demonstrated that the chicks on RDEW diets do not need as high PA supplements or stores as the chicks on CDEW diets.

The chicks fed CDEW + 9PA with or without PA water and 3PA + PA water have a higher body weight than chicks fed RDEW + 9PA and 3PA with or without PA water. However, chicks that were fed CDEW + 3PA were comparable in weight to the chicks fed RDEW + 3PA with or without PA water. This showed that with an ample supply of PA, chicks on CDEW diet have a greater potential for growth than chicks fed RDEW diet. When feed efficiency was calculated[1] (Table III), chicks fed CDEW diets apparently showed better growth per gm of food than chicks fed RDEW diets. This suggested that chicks fed CDEW diets used the feed efficiently for growth. In recent work, (14,15) chicks that were fed RDEW

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[1] $\text{Feed Efficiency} = \frac{\text{Total Feed Consumed}}{\text{Total Body Weight Gain}}$

had a lower body weight than chicks fed CDEW diets.

In previous trials to investigate the above findings various supplements were added to RDEW diets (14). The effects of several amino acids supplements, arginine, histidine or a combination of the two or of methionine were investigated with chicks fed low zinc RDEW and CDEW diets (14,15), because of their known reaction on zinc deficiency (13,9,11). There was no apparent change in body weight of chicks fed RDEW diets. In another trial (14), the addition of the whole egg to the RDEW or CDEW diets so the proportion of white to yolk was the same as whole egg did not help in bringing up the growth of the RDEW chicks to those given CDEW diets. Since there is no apparent answer to the above reaction, one can only speculate that there are some other factors beside PA, zinc, constituents of egg yolk and certain amino acids involved. This point need to be explored further. Nielsen et al. (13) also found that RDEW diets fed to the chicks resulted in lower body weights than chicks fed isolated soybean protein or casein diets.

Chicks fed CDEW and RDEW diets showed only moderate leg scores. However, chicks that were given 9PA had a slightly lower leg scores than those given 3PA in both CDEW and RDEW diets. The higher PA level in the diet may affect the degree of leg score of chicks fed either CDEW or RDEW diets.

Mortality (10%) was low when the chicks were fed the CDEW diets in comparison to previous experiments (14), where mortality ranged

from 50-75%. Lower mortality rates could be due to the high level of PA that was supplied early in the diet of the chick. Also, it could be due to the diet of the breeding hen. The chicks were obtained from a commercial hatchery[1] and the composition of their diet is unknown. A study by Snell et al. (29) suggested that the greater the PA in the diet of the hen, the greater the storage level in the chicks. Also, chicks that were hatched from high PA eggs and were fed CDEW diets had a low mortality rate (14). The breed of the chicks seemed to have little effect on the reaction to the CDEW diet (14).

Chicks that were fed commercial chick starter[2], which was supposed to be a control group, showed a low body weight and a high mortality rate compared to previous chicks fed chick starter in this laboratory (14). This may have been due to a bad lot of feed and thus, it cannot be considered as a control group in this experiment.

Tibia zinc was significantly ($P < 0.05$) higher in chicks fed the chick starter diet. When this diet was analyzed for zinc[3], a content of 250 ppm was found. The amount of zinc required for normal growth of the chicks is 50 mg/kg of feed (17).

---

[1] H & N Inc., Richmond, WA

[2] Formax, Con Agra Inc., Bozeman, MT

[3] Varian Techtron AA-6, Varian Techtron, TPY LT., Melbourne, Australia, Courtesy Chemistry Station Analytical Laboratory, Montana State University

With the exception of chicks fed RDEW + 9PA, PA variations in both CDEW and RDEW diets had no apparent effect on tibia zinc content (ug/g). Chicks that were fed RDEW + 9PA showed significantly higher tibia zinc content. Since the growth was poor for this group, it resulted in a low tibia weight and this could lead to a higher concentration of zinc (ug/g). However if the tibia weight of this group were comparable to the tibia weight of the other RDEW diet chicks, the ug/g of zinc content would be 33.35 (Table III). This showed that RDEW + 9PA chicks absorbed and stored as much zinc as the other RDEW groups. Apparently, high PA did not interfere with these processes.

It was found in the first experiment that 9PA and 3PA in CDEW and RDEW diets respectively were the optimum levels to promote growth and prevent mortality. These were therefore adopted for experiment 2.

In the second experiment (Table IV) when zinc levels were varied in the diets, body weight at 3 weeks varied also. Chicks that were fed CDEW + high zinc diet (30 ppm) were used as a positive control group. In the broiler chick under normal conditions the body weight is expected to double every week.[1] The body weight in this group surpassed this criterion; leg score and mortality were zero. Thus this group was evidently suitable for a positive control.

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[1]Personal observation by Dr. J. Lease

TABLE IV

Effect of variation of dietary zinc on growth, mortality, zinc status and tibia AP activity in broiler chicks fed CDEW and RDEW diets for 3 weeks.[1]

Product	Zn	PA	Mortality	BWT[2]	Left Tibia			Right Tibia[5] AP activity[6]		F-E[7]
					Leg Score[3]	Wt[4]	Zinc[4]			
	ppm	g/kg	%	g		g	ug/g	Total	Per g	
CDEW	30	9	0	408A	0	4.14A	82.36A	643.83A	154.77A	1.51
CDEW	6	9	10	241B	2.6	2.75B	14.56B	236.18B	85.03B	1.39
CDEW	3	9	55	94D	2.1	0.96D	10.47B	40.94C	44.04C	----[8]
RDEW	6	3	5	131C	1.15	1.39C	13.63B	45.4C	32.28C	2.99
RDEW	3	3	25	93D	1.3	0.80D	18.92B	26.96C	34.13C	----[8]

[1]Values in each column for each product by the same letter are not significantly different ($P < 0.05$).

[2,3,4]See footnote 2,3,4 in Table II.

[5]Mean of six individual tibia; wet weight.

[6]Milligrams of P-nitrophenol liberated in 30 minutes at 37°.

[7]See footnote 5 in Table II.

[8]Not calculated due to high mortality rate.

Chicks fed CDEW + a lower zinc level diet (6 ppm) had a significantly ($P < 0.05$) lower body weight than the control group. When the zinc level was still lower (3 ppm), body weight was significantly ($P < 0.05$) lower than chicks fed CDEW + 6 ppm zinc.

Body weight was also lower in RDEW diets when chicks were fed low zinc (3 ppm) in comparison with chicks fed the higher zinc level (6 ppm). Since zinc deficient chicks exhibit retarded growth (2), the above findings were typical reactions to low zinc levels in the diet. Chicks fed CDEW + 6 ppm zinc had a higher body weight than chicks fed RDEW + 6 ppm zinc. Also chicks fed CDEW + 6 ppm zinc showed better growth per gm of food than chicks fed RDEW diets (Table IV). Chicks fed CDEW or RDEW + 3 ppm zinc experienced high mortality, thus the feed efficiency could not be calculated. There was no significant difference ($P < 0.05$) between body weight of the surviving chicks fed CDEW or RDEW + 3 ppm zinc.

In chicks fed CDEW diets mortality was high (55%) when the chicks were supplied with 3 ppm of zinc in contrast to 10% with 6 ppm of zinc and 0% with 30 ppm of zinc. This high mortality rate may be caused by the low zinc level in the diet; also it could be the result of the reaction of the chicks to CDEW diets that was seen earlier (14). It was suggested (14) that CDEW could interfere with the utilization of dietary PA by binding it so it would not be used, and/or inhibit the conversion of PA to coenzyme A.

Chicks fed RDEW + 3 ppm of zinc experienced about 25% mortality in contrast to 5% mortality when the chicks were fed 6 ppm zinc. This could be due to low zinc level in the diet.

Leg scores were somewhat higher in chicks fed low zinc + CDEW diets than the chicks fed low zinc RDEW diets.

Except for the control group, there was no significant difference ($P < 0.05$) in ug/g of tibia zinc content between chicks given 3 and 6 ppm of zinc in CDEW and RDEW diets. Zinc is involved in at least 20 metalloenzymes and zinc-activated enzymes in the metabolic processes (49). Processes which are profoundly affected by experimental zinc deficiency include DNA metabolism (50,51,52,54) RNA metabolism (50,53, 54,55) protein metabolism (54,56,57,58) and mucopolysaccharide metabolism (59,47,60). These effects of zinc deficiency are reflected in the intact animal by poor utilization of dietary nitrogen and sulfur (61) and prompt growth arrest (50). When zinc was offered at 3 and 6 ppm it is possible that the chicks have used the zinc that was available in the diets to facilitate the other necessary metabolic process that required zinc rather than deposit it in the tibia which acts as a storage area.

Even though zinc was at 6 ppm in both RDEW and CDEW diets the chicks on CDEW diets used it more efficiently for growth as indicated by significant ($P < 0.05$) increase in body weight and AP concentration in tibia (Table IV). This could be interpreted that the unknown factors

that depress the growth of the chicks on RDEW diet might be destroyed in the cooking process of the egg white. This should be explored further.

For CDEW diets, the total AP content of the tibia was significantly higher ($P < 0.05$) in the control group (30 ppm zinc) than the other groups which indicated that the level of zinc in the diet was sufficient for high activity (40,9) for this zinc-containing enzyme (38). AP concentrations in the tibia were significantly lower ($P < 0.05$) when the chicks were fed 3 ppm zinc in contrast to 6 ppm zinc. Zinc level in the diet affected the total AP concentration. Chicks on zinc deficient diets were found to exhibit reduced AP activity in bone (44,7,6,9).

For RDEW diets, there was no significant difference ($P < 0.05$) in AP content (total or ug/gm) when the chicks were fed 3 or 6 ppm of zinc. Apparently zinc at 6 ppm was used for the other necessary metabolic processes (49) and it was not enough to affect AP concentration in the tibia. A decrease in serum AP has been used clinically as a clue to the diagnosis of zinc deficiency in humans (62).

AP was significantly higher ($P < 0.05$) in the chicks fed CDEW + 6 ppm than the chicks fed RDEW + 6 ppm zinc. This tends to support the previous findings that chicks fed CDEW diets used the zinc more efficiently.

With the optimum level of PA in each diet there was no significant difference ($P < 0.05$) in AP concentration between chicks fed CDEW + 3 ppm zinc and RDEW + 3 ppm zinc. Since both groups experience a high

mortality rate and a low body weight, the zinc supplement at 3 ppm is low and is not sufficient for the chicks to continue growth.

When body weight, AP concentration or tibia content of zinc were used as indication of zinc utilization, optimum dietary PA apparently did not cause an added 3 ppm of dietary zinc to be used as efficiently as 6 ppm for either CDEW or RDEW diets.

CHAPTER V

CONCLUSIONS, SUMMARY AND RECOMMENDATIONS

Conclusions

From these two experiments the following conclusions may be drawn:

1. For CDEW diets, availability of PA water early in the life of the chicks increased the growth when chicks were fed 3PA and made them comparable to the group fed CDEW + 9PA with or without PA water. A high level of PA is apparently needed early in the life of the chicks.

2. PA at 9 mg/kg in RDEW diets caused growth depression and was apparently in excess for the chicks. PA water did not have a significant effect ($P < 0.05$) on body weight when chicks were fed 3PA.

3. In experiment 1, body weights of chicks fed RDEW diets were significantly lower ($P < 0.05$) than body weights of CDEW chicks. There appear to be some other factors beside PA, zinc, certain amino acids or constituents of egg yolk involved.

4. Chicks fed CDEW and RDEW diets showed only moderate leg scores. Chicks fed 9PA had slightly lower leg scores than those given 3PA in both CDEW and RDEW diets. Higher PA level in the diet may affect the leg scores of the chicks in both diets.

5. PA variation had no apparent affect on tibia zinc content (ug/g) in both diets except when the chicks were fed RDEW + 9PA. Chicks fed this latter diet had a significantly ($P < 0.05$) higher tibia zinc content. This was attributed to general poor growth and low bone weight.

6. In experiment 2, chicks fed CDEW or RDEW diets + 3 ppm zinc showed a significantly ($P < 0.05$) lower body weight and a higher mortality than chicks fed 6 ppm zinc. Mortality was 55% for chicks on CDEW diets in contrast to 25% for chicks on RDEW diets. CDEW could interfere with the utilization of dietary PA by binding it so it would not be used, and/or inhibit the conversion of PA to coenzyme A.

7. There was no significant difference ($P < 0.05$) in ug/g or total tibia zinc between chicks given 3 and 6 ppm zinc in CDEW and RDEW diets. It is possible that the chicks used such zinc as was supplied for metabolic processes rather than depositing it in the tibia.

8. Chicks fed CDEW + 6 ppm zinc used it more efficiently for growth and AP synthesis than those fed RDEW.

9. Zinc level in the CDEW diets affected the total AP concentration. The higher the zinc in the diet the higher AP concentration in the tibia.

10. Since at 3 ppm in CDEW or RDEW diets appears to be insufficient

for the chicks to grow well.

11. The optimum level of PA in CDEW or RDEW diets apparently did not increase the utilization of zinc in the chicks fed low zinc diets as shown by the criteria mentioned.

Summary

Two factorial experiments with chicks were conducted to determine (1) the optimum level of PA necessary to prevent mortality and to promote growth of chicks fed either CDEW or RDEW diets and (2) whether zinc utilization would be affected by the optimum level of PA in both diets. Growth, mortality, zinc content of tibia and tibia AP concentration (2nd experiment) were the criteria used to evaluate dietary variation.

In experiment 1, variation of PA supplements significantly ($P < 0.05$) affected the growth and mortality of the chicks fed CDEW and RDEW diets. Chicks fed CDEW + 9PA showed good growth and no mortality in contrast to lower body weight and 10% mortality when chicks were fed CDEW + 3PA.

Chicks that were fed RDEW + 9PA showed a significantly ($P < 0.05$) lower body weight than chicks fed RDEW + 3PA; also 15% mortality in contrast to no mortality respectively.

The addition of PA water significantly affected ($P < 0.05$) the growth and the mortality when supplied to the chicks fed CDEW + 3PA.

Body weight and mortality were comparable to that of chicks fed CDEW + 9PA with or without PA water. Chicks fed 3PA in RDEW diet did not improve by the addition of PA water. Thus 9PA for CDEW diets and 3PA for RDEW diets were determined to be the optimum level of PA.

In the second experiment using the optimum level of PA in either CDEW or RDEW diets, zinc was supplied in both diets at 3 or 6 ppm.

The chicks fed either CDEW or RDEW with 6 ppm zinc had a lower mortality and significantly ($P < 0.05$) higher body weight than when fed 3 ppm zinc.

There was no significant difference ($P < 0.05$) in ug/g zinc in tibia when the chicks were fed low zinc CDEW or RDEW diets. However, chicks fed CDEW + 6 ppm zinc showed significantly ($P < 0.05$) higher tibia AP concentrations than when fed CDEW + 3 ppm or RDEW + 3 or 6 ppm zinc.

On the basis of growth and tibia AP concentration, chicks on CDEW + 6 ppm zinc diets tend to utilize the zinc more efficiently than chicks fed RDEW diets.

Apparently an optimum level of PA did not increase the zinc utilization for the chicks fed either low zinc diet. The state of egg white seems to be more important than the level of PA in the diet.

Recommendations

Further research should be done to investigate the following:

1. Factors other than PA, zinc, constituents of egg yolk and certain amino acids (arginine, histidine and methionine) should be investigated to find the reason for the growth depression of chicks fed RDEW diets.
2. Since chicks fed either RDEW or CDEW cooked at 80°C showed different adverse reactions; different cooking temperatures or other procedures which might change the characteristics of RDEW should be explored further.
3. AP activity and concentration in the tibia should be investigated with the variation of PA level in the diets. Also, with chicks fed RDEW diets + 30 ppm zinc.

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APPENDICES

EGG ALBUMEN PREPARATION

Cooked Egg Albumen

A 250 gm portion of raw egg white solids[1] was mixed with 1000 ml of distilled water in a baking pan 9" x 13" x 2.5". Mixing was done by a hand-held electric mixer at low speed for about two minutes. The mixture was placed in an oven preheated to 400°F (176°C), after 10 minutes the egg white reached a temperature of 70°C. It was stirred and oven temperature reduced to 300°F (120°C) so that the mixture was maintained at 80°C for the remaining 10 minutes of cooking time. The egg white had a firm texture similar to that of hard-cooked eggs. The egg white was removed and cut with scissors into pieces about 1.5 x 1.5 cm, spread in a thin layer on brown paper and partially dried at a room temperature in front of fans. The pieces were stirred occasionally. The resulting dried product was ground in a Waring blendor, filling the blendor until the blades were just covered. It was then replaced on the drying papers in front of the fans until thoroughly dried. During that time it was stirred occasionally. The dried cooked egg white was ground in Waring blendor to a fine powder. It was then milled in a micro-Wiley mill to pass through a No. 20 sieve and stored air tight in the refrigerator until used.

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[1]Spray processed dried egg white solids. Armour Food Co., Albert Lea, MN.

METHODS OF ANALYSIS

Leg Scores

Before sacrifice, chick tibiae were scores 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 three chicks per group of ten in each cage. All flesh was removed, and the tibiae were immediately frozen and stored at $-30^{\circ}C$ until time of analysis. The right tibia was weighed individually and used for AP analysis. The left were weighed as a group of three from each cage, and used in zinc analysis.

Tibia Zinc Analysis

The three weighed tibiae were dried at 50°C for four hours and reweighed. The dried tibiae were ashed at 600°C for 24 hours. The resulting ash was cooled in a desiccator 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 and diluted with distilled water so that the final dilution was 0.1 N HCl. Zinc was analyzed by atomic absorption.[1]

Alkaline Phosphatase Method

This method is based on that of Starcher and Katzer (7). The p-nitrophenol liberated from the p-nitrophenyl phosphate by AP is measured at 410 mu.[2]

Solutions[3]

1. 2 N HCl: 17.7 ml of concentrated acid diluted to 100 ml H_2O).
2. 1 M $MgCl_2$: 0.94 g $MgCl_2$ dissolved and diluted to 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 plus 9 ml 2 N HCl, dilute to 50 ml with water. Adjust to pH 10 with concentrated NaOH or 6 N HCl.[4]

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[1] Varian Techtron AA-6, Varian Techtron, Tpy Lt, Melbourne, Australia. Courtesy Chemistry Station Analytical Laboratory, Montana State University.

[2] Spectron 20, Bausch and Lomb.

[3] Deionized water is used throughout the procedure.

[4] Sigma Chemical Company, St. Louis, MO.

b) The above solution is added to 210 mg p-nitrophenyl phosphate[1] dissolved in 60 ml of H_2O. Add finally, 0.4 ml of 1 M $MgCl_2$. Pipette[2] 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 of the bone.

2. Place in a chilled 35 ml centrifuge tube and dilute with water to the nearest ml. Keep in an ice bath.

3. An aliquot of the homogenate is diluted. This dilution may be from 0.4 ml to 10 ml or 0.1 ml to 20 ml depending on tissue, age of chick and the ration fed. Mix well. Add 0.2 ml of the diluted 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.

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 tube.[2] 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. For 0.2 to 10 ml dilution multiply the ug of p-nitrophenol by a factor of 0.25. For 0.4 to 10 ml dilution multiply the ug of p-nitrophenol by a factor of 0.125. For 0.1 to 20 ml dilution multiply ug of p-nitrophenol by homogenate. This times the homogenate equals the total mg of

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[1] Sigma Chemical Company, St. Louis, MO.
[2] Spectron 20, Bausch and Lomb.

p-nitrophenol in the tibia. Divide by the weight of the tibia to obtain mg AP per gm 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.[1] Add 0.17 m NaCl to make 100 ml, mix thoroughly.

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

Tube No.	Working p-nitrophenol 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 percent 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 abscissa.

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[1] Sigma Chemical Company, St. Louis, MO.

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The effect of pantothenic acid ...

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