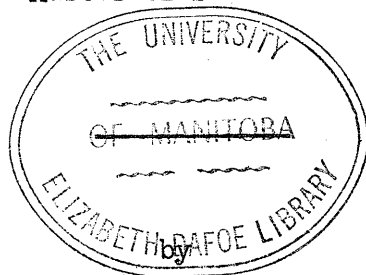


A STUDY OF INTER-RELATIONSHIPS BETWEEN
DIETARY LEVELS OF IRON PHOSPHORUS
AND CALCIUM FOR THE CHICK

A Thesis
Presented to
the Faculty of Graduate Studies and Research
The University of Manitoba

In Partial Fulfillment
of the Requirements for the Degree
Master of Science



Donald Waddell

September 1963

ACKNOWLEDGEMENTS

For making this work possible as well as helping in its execution, the author is deeply indebted to Dr. J. L. Sell, Assistant Professor of Poultry Science, University of Manitoba. The helpful criticism and guidance of Professor G. C. Hodgson, head of the Poultry Division, Department of Animal Science, University of Manitoba is also gratefully acknowledged.

The author is indebted to Mr. J. A. McKirdy and his staff for their assistance in chemical analysis. Technical assistance given by Mr. William Guenter is also appreciated.

This project was assisted by a grant from the National Research Council of Canada and a bursary from the Manitoba Hatchery Association for which the author is grateful.

ABSTRACT

A STUDY OF INTER-RELATIONSHIPS BETWEEN DIETARY LEVELS OF IRON, PHOSPHORUS AND CALCIUM FOR THE CHICK

Two experiments were conducted utilizing a semi-purified ration in which the major ingredients were corn starch, dextrose and dried egg white. Broiler type chicks were used in both experiments. In the first experiment (1262) a complete factorial arrangement of 6 ration treatments was used. The levels of iron (Fe) and phosphorus (P) studied were: 13, 26, and 39 p.p.m.; and 0.30 and 1.0% respectively. In the second experiment (1562), the following levels of iron, phosphorus and calcium (Ca) were tested: 16, 36, 56, 76, 96 p.p.m.; 0.10, 0.35, 0.60, 0.85 and 1.10%; and 0.20, 0.60, 1.0, 1.4, 1.8% respectively. A three dimensional, central composite design was used to assign the ration treatments.

In both experiments, weight gain proved to be a relatively poor criterion for iron status as compared to hemoglobin concentration (Hb). Both Fe and P exerted significant ($P < .01$) main effects on Hb in experiment 1262. At each level of dietary Fe, increasing the phosphorus level from 0.30 to 1.0% reduced Hb. Regardless of the P level, increasing the Fe level from 13 to 26 or to 39 p.p.m. increased Hb. Cytochrome c content of heart tissue failed to indicate any consistent trend related to Fe or P treatments. Changes in dietary Fe level failed to cause any significant effect on bone ash values. In experiment 1562, an increase

in dietary P, when Ca and Fe were held constant, resulted in a significant ($P \leq .05$) decrease in Hb. Simultaneously, added P, caused a reduction in the percent of dietary Fe retained. An increase in ration Ca, at a given P level, also reduced Hb when the rations contained 56 p.p.m. or less of Fe but exerted little influence on Fe retention. Varying the levels of Fe did not cause any consistent change in bone ash.

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	3
Iron, Calcium and Phosphorus Inter-relationships	4
Iron as it Affects Blood Components and Liver Stores	8
EXPERIMENTAL PROCEDURE	11
Part 1 - Iron and Phosphorus Studies Maintaining a Constant Calcium Phosphorus Ratio	11
RESULTS	17
Weight Gain and Feed Consumption	17
Blood Components	22
Cytochrome c Content of Heart Tissue	22
Bone Ash and Ether Extract Content of Tibia	25
DISCUSSION	31
EXPERIMENTAL PROCEDURE	33
Part 11 - Studies with Iron, Calcium and Phosphorus using a Three Dimensional Central Composite Design	33
RESULTS	42
Weight Gains	42
Blood Components	46
Hemoglobin	46
Hematocrit	54
Red Blood Cell Count	59
Mean Corpuscular Hemoglobin	63
Mean Corpuscular Volume	69
Mean Corpuscular Hemoglobin Concentration ...	74
Heart Weights as a Percentage of Body Weight ...	77
Iron Content of Livers	82
Percent Dietary Iron Retained	89
Percent Bone Ash in Right Tibia	92
DISCUSSION	100
SUMMARY AND CONCLUSIONS	113

LIST OF TABLES

TABLE		PAGE
1	Composition of Basal Rations - Experiment 1262 c-4	12
11a	Analysis of Variance for Weight Gains of Chicks at 2, 3 and 4 weeks of age	18
11b	Weight Gains of Chicks at 2, 3 and 4 weeks of age	19
111a	Analysis of Variance for Feed Efficiency of Chicks at 2, 3 and 4 weeks of age	20
111b	Feed-Efficiency of Chicks at 2, 3 and 4 weeks of age	21
1Va	Analysis of Variance for Hemoglobin Concentration and Hematocrit Values of Chicks at Four Weeks of Age	23
1Vb	Hemoglobin Concentrations and Hematocrit Values of Chicks at Four Weeks of Age ...	24
Va	Analysis of Variance for Cytochrome c Content of Heart Tissue of Chicks at Four Weeks of Age	26
Vb	Cytochrome c Content of Heart Tissue of Chicks at Four Weeks of Age	27
V1a	Analysis of Variance for the Percent Bone Ash and Percent Ether Extract in the Tibias of Chicks at Four Weeks of Age ...	28
V1b	Percent Bone Ash and Percent Ether Extract in the Right Tibias of Chicks at Four Weeks of Age	29
V11	Dietary Variables and Code Values	34
V111	Composition of Ration 14 - Experiment 1562 c-4	35

TABLE		PAGE
LX	Weight Gains of Chicks from 1 - 3 weeks of age	45
X	Analysis of Variance for Weight Gain Data and Weight Gains of Chicks at Three Weeks of Age (2 ³ Portion)	47
XI	Circulating Hemoglobin Concentrations of Chicks at Three Weeks of Age	50
XII	Analysis of Variance for Hemoglobin Data and Circulating Hemoglobin Concentrations of Chicks at Three Weeks of Age (2 ³ portion)	51
XIII	Hematocrit Values of Chicks at Three Weeks of Age	56
XIV	Analysis of Variance for Hematocrit Data and Hematocrit Values of Chicks at Three Weeks of Age (2 ³ Portion)	58
XV	Red Blood Cell Counts of Chicks at Three Weeks of Age	61
XVI	Analysis of Variance for Red Blood Cell Count Data and Red Blood Cell Counts of Chicks at Three Weeks of Age (2 ³ Portion)	62
XVII	Mean Corpuscular Hemoglobin of Chicks at Three Weeks of Age	66
XVIII	Analysis of Variance for Mean Corpuscular Hemoglobin Data and Mean Corpuscular Hemoglobin of Chicks at Three Weeks of Age (2 ³ Portion)	67
XIX	Mean Corpuscular Volumes of Chicks at Three Weeks of Age	71
XX	Analysis of Variance for Mean Corpuscular Volume Data and Mean Corpuscular Volumes of Chicks at Three Weeks of Age (2 ³ Portion)	73
XXI	Mean Corpuscular Hemoglobin Concentrations of Chicks at Three Weeks of Age	76

TABLE		PAGE
XXII	Analysis of Variance for Mean Corpuscular Hemoglobin Concentration Data and Mean Corpuscular Hemoglobin Concentrations of Chicks at Three Weeks of Age (2^3 Portion)	78
XXIII	Heart Weights of Chicks at Three Weeks of Age	81
XXIV	Analysis of Variance for Heart Weight Data and Heart Weights of Chicks at Three Weeks of Age (2^3 Portion)	83
XXV	Liver Iron of Chicks at Three Weeks of Age	86
XXVI	Analysis of Variance for Liver Iron Data and Liver Iron Contents of Chicks at Three Weeks of Age (2^3 Portion)	88
XXVII	Iron Retention by Chicks from 11 to 21 Days of Age	91
XXVIII	Analysis of Variance for Iron Retention Data and Iron Retained by Chicks from 11 to 21 days of Age (2^3 Portion)	93
XXIX	Percentage Bone Ash in the Right Tibia of Chicks at Three Weeks of Age	96
XXX	Analysis of Variance for Bone Ash Data and Bone Ash Percentage of Chicks at Three Weeks of Age (2^3 Portion)	98

LIST OF FIGURES

FIGURE		PAGE
1	Spatial arrangement of mean weight gains of chicks from 1 - 3 weeks of age	44
2	Spatial arrangement of mean circulating hemoglobin concentrations of chicks at 3 weeks of age	49
3	Spatial arrangement of mean hematocrit values of chicks at 3 weeks of age	55
4	Spatial arrangement of red blood cell count of chicks at 3 weeks of age	60
5	Spatial arrangement of mean corpuscular hemoglobin of chicks at 3 weeks of age	65
6	Spatial arrangement of mean corpuscular volume of chicks at 3 weeks of age	70
7	Spatial arrangement of mean corpuscular hemoglobin concentration of chicks at 3 weeks of age	75
8	Spatial arrangement of mean heart weights of chicks at 3 weeks of age	80
9	Spatial arrangement of mean liver iron content of chicks at 3 weeks of age	85
10	Spatial arrangement of mean percentage iron retained by chicks from 11 to 21 days of age	90
11	Spatial arrangement of mean percent bone ash in the right tibia of chicks at 3 weeks of age	95

INTRODUCTION

Research in nutrition, particularly in the last decade, has devoted considerable attention to ways and means of increasing productivity of domestic animals through the use of new feed additives and adjustments in the quantities of essential nutrients included in the ration. Recently, much research has been conducted in an effort to re-evaluate the dietary requirement of domestic animals for various minerals. In this regard, Davis (1960) said;

"No longer is it possible to discuss (or study) any element in terms of its requirement without considering the other nutrients in the diet whether these be protein, carbohydrate, or other mineral elements ...

In the overall nutrition of human or animal a deficiency or toxic excess of any given element must be considered against the background of the levels of other nutrients which are present in the diet."

There has been much interest in the dietary inter-relationships between calcium and phosphorus, and trace elements such as magnesium, zinc, aluminium and iron. Most research on this subject has been done with rats, and much attention has been focused on the inter-relationships between either calcium or phosphorus and specific trace minerals. Consequently, little information is available concerning possible nutritionally important inter-relationships which may exist simultaneously between calcium, phosphorus and a specific trace mineral. It was the objective of this study, to examine the dietary inter-relationships between calcium,

phosphorus and iron in the chicken. The study was conducted in two parts. Part 1 dealt with the effect of various phosphorus and iron levels (maintaining a constant calcium to phosphorus ratio) on weight gain, feed efficiency and blood components. Part 11 dealt with the effects of various combinations of calcium, phosphorus and iron levels, on weight gains, blood components and utilization of the three elements.

REVIEW OF LITERATURE

Mineral inter-relationships have been the subject of numerous investigations. Pensack et al (1958) tested the effects of calcium and phosphorus on zinc requirements of chickens. Zinc levels of six, twenty and forty parts per million (p.p.m.) from varied sources were tested in combination with calcium: phosphorus ratios of 1.0:0.6, 2.0:0.6 and 1.0:1.2. The results showed that zinc was equally available from all sources tested and that neither excessive calcium nor excessive phosphorus deleteriously affected zinc availability. Roberson and Schaible (1960) also working with chicks found that 2.23 percent calcium in the ration caused significant growth depression and symptoms of zinc deficiency when zinc was present at 36 p.p.m. At the same level of calcium and 96 p.p.m. of zinc, growth was normal and zinc deficiency symptoms did not occur. A phosphorus level of 1.10 percent in the presence of either 36 or 96 p.p.m. of zinc did not significantly affect growth.

Forbes (1961) reported that calcium and phosphorus can have an adverse effect on magnesium utilization. Rats were used as experimental subjects in a balance trial employing a 3 x 3 factorial design. Weight gains were reported to be markedly depressed by excess calcium. On the other hand, phosphorus increased weight gains especially in the presence of high calcium levels while magnesium slightly improved weight gains. Visible symptoms of magnesium deficiency were most readily produced on high calcium - high phosphorus diets and were alleviated by magnesium supplementation. Magnesium balance was improved by added magnesium and by added phosphorus, but was adversely affected by added calcium when a

low magnesium diet was fed. The calcium effect was slight on high magnesium diets. The data indicate that calcium was more detrimental than phosphorus to magnesium utilization. O'Dell (1960) also reported evidence of calcium, phosphorus and magnesium inter effects. Excess dietary magnesium was reported to have caused a loss of calcium from the body when the diet was low in phosphorus but not when phosphorus was adequate. Conversely, the consumption of excess calcium accentuated the symptoms of magnesium deficiency in an animal that consumed a deficient diet. O'Dell stated that there is evidence to show that excess phosphorus will substantially increase the magnesium requirement. Phosphorus was said to exert its effect chiefly by decreasing absorption of magnesium but there was limited evidence to suggest that it also had a direct metabolic effect.

Iron, Calcium and Phosphorus Inter-relationships

Cox et al (1931) reported that when guinea pigs were fed rations containing iron and/or aluminum salts, inorganic phosphorus levels in the blood were substantially lowered. The ash, calcium and phosphorus contents of the bones were reduced to seventy percent of normal after the diet containing aluminum had been fed for 12 weeks. Similiar but less marked effects when excess iron was fed were observed. The addition of mono-sodium phosphate equivalent (on a molar basis) to the level of aluminum or iron in the diet entirely prevented the occurrence of these symptoms. From these findings, it was postulated that aluminum and iron induced a phosphorus deficiency by precipitation of alimentary phosphorus as ferric and aluminum phosphates.

Deobald and Elvehjem (1935) performed similar work with chicks. Day-old chicks fed normal rations to which large amounts of soluble iron and aluminum salts (0.9 percent iron, 0.44 percent aluminum) were added developed severe rickets in one to two weeks and were all dead within three weeks. Levels of iron and aluminum salts equivalent to one half and three quarters of the amount necessary to unite with all the ration phosphorus to form insoluble ferrous phosphate and aluminum phosphate reduced bone ash to twenty-five percent and reduced blood phosphorus to 2-4 mg/100 cc. of serum at three weeks of age. The addition of sodium acid phosphate in amounts sufficient to unite with the added metals allowed rapid growth and normal bone formation.

Studies on the effect of ferric chloride on both calcium and phosphorus were performed by Rehm and Winters (1960). Using rats, these workers made comparisons of the amounts of total ash, calcium and phosphorus in the bodies of animals fed unsupplemented diets with the amounts in the bodies of animals fed a diet supplemented with enough ferric chloride to combine with one half the phosphorus of the diet. It was shown that the addition of ferric chloride considerably reduced the amounts of total ash, calcium, and phosphorus at the end of thirty days. These results were obtained even though food intake was equalized. It was concluded by the authors that large amounts of ferric chloride had a detrimental effect on calcium and phosphorus metabolism.

The detrimental effect of iron on calcium and phosphorus utilization appears to be one of mutual antagonism. Evidence of this in regard to calcium, was reported by Kletien (1940). The addition of one percent calcium carbonate