INFORMATION TO USERS

This dissertation was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

A Xerox Education Company

EFFECT OF CHELATION ON TRACE MINERAL UTILIZATION BY POULTRY AND SWINE

by

Arthur A. Owen B.

A THESIS

Presented to the Faculty of

The Graduate College in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Department of Animal Science

Under the Supervision of Dr. E. R. Peo, Jr.

Lincoln, Nebraska

October, 1972

TITLE

EFFECT OF CHELATION ON TRACE MINERAL UTILIZATION

BY POULTRY AND SWINE

BY

Arthur A. Owen B.

APPROVED	DATE
Professor Ernest R. Peo, Jr.	October 10, 1972
Assoc. Prof. D. M. Danielson	" " "
Professor Foster G. Owen	" " "
Professor Robert M. Koch	" " "
Professor Thomas W. Sullivan	" " "

SUPERVISORY COMMITTEE

GRADUATE COLLEGE **UNIVERSITY OF NEBRASKA**

GRAD 52002 300 8-68(7)

"A mi patria Colombia, a la cual prestare con dedicacion mis conocimientos Y servicios por intermedio del Instituto Colombiano Agropecuario (ICA), agradezco y dedico."

A.A.O.B.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to his advisor, Dr. E. R. Peo, Jr., for his efforts, assistance and encouragement in the conduct of these experiments, the preparation of this dissertation and for his guidance and friendship during the course of graduate studies.

Appreciation is also expressed to Dr. T. W. Sullivan of the Poultry Science Department for assistance in collecting the chick data and the use of their facilities; to Dr. P. S. Cunningham for his help with the analysis of the statistical data.

Thanks are likewise extended to John Welch for assistance in developing laboratory procedures, to Robert W. Harms for his assistance in preparation of samples and their analysis and to other members of the Department of Animal Science and its graduate students for suggestions, advice and aid in collecting data.

To my wife, Ivonne, I express sentiments of gratitude for her patience, understanding and encouragement offered during the course of graduate studies, to my children, Albert, Arthur and Vivian, for their warmth and strength and to my family in Colombia.

A.A.O.B.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (continued)

INTRODUCTION

It is well established that man and all domestic animals require trace minerals in their diet for overall well being and optimal performance. The addition of trace mineral sources to the diets of domestic animals is a familiar practice to the nutritionist, animal scientist and feeder. Nevertheless, care must be taken when choosing the sources for a trace mineral mixture. Certain mineral salts are chemically incompatible, some are poorly available to the animal and some are unstable in a feed mixture. In the latter case the mineral element released from the unstable compound is destructive to vitamins, antibiotics and other feed additives present in the diet. Another important consideration in the choice of mineral sources is its solubility in an alkaline environment. It is necessary that the metal ion dissociate in the gastrointestinal tract prior to absorption. Once the mineral elements are released from their original carrier, they are in a highly reactive state and are subject to losses before absorption. These losses are due in part to the binding of the elements to electronegative sites on certain organic compounds forming a relatively large ring structure or "chelate." An example is the binding of zinc atoms with phytic acid, a naturally occurring chelating agent, which forms a zinc-phytic acid chelate. The element in this chelated form cannot be absorbed by the intestinal wall and is lost to metabolism. To counteract this effect, the nutritionist adds an excess of minerals to the diet to insure that some will escape complexing and thus be absorbed. However, all metal chelates do not jeopardize the element's absorption.

Recently nutritionists have found that certain synthetic chelating agents such as ethylenediamine tetraacetic acid (EDTA) added to the diet competes with the natural occurring chelating agents, such as phytic acid, for the metal ion. The metal-EDTA chelate releases the element at the intestinal wall or in some cases the whole complex is absorbed. In either instance the mineral is made available to the animal.

It was the purpose of the research reported herein to determine the effect of chelation on the utilization of certain trace elements by chicks and particularly growing-finishing swine since little research has been reported in this area.

REVIEW OF LITERATURE

Chelates have been used with success in agriculture for many years to enhance the absorption of trace minerals by plants from the soil (Wallace, 1962). As of late, animal nutritionists have become interested in the use of chelating agents to enhance the absorption of trace minerals, particularly zinc (Zn), by the chick and other domestic animals (Scott, 1965).

Chemistry

Before surveying the literature on the effects and use of chelating agents in animal nutrition, it is of interest, first to review some basic concepts about chelation.

Many compounds in nature are "looking" for a cation (an element with a deficiency of electrons) with which it can share its electrons, thereby forming a stable compound. One of the properties of a variety of organic molecules is the ability to form internal complexes with metals. Thus, chelates are cyclic compounds in which a metal is joined to a molecule by two or more of its donor groups (Martell and Calvin, 1952). Not restricted in its formation to the high energy covalent bonding which normally binds atoms together to form conventional molecules, a metal chelate is formed as a ring structure produced by attraction between the positively charged cations and two or more sites of high electronegativity in a chemical compound. The bonds are known as "coordinate" bonds and occur because of pecularities in the electron shells of the transition metals. The chemical compounds that complex with the metal ions are known as ligands. The most common electron

donating or electron sharing atoms in ligand molecules are nitrogen, oxygen and sulfur (Scott et al., 1969). In the coordinate bond, electrons are furnished by the complex forming ligand and if the ligand molcule contains only two electron donating groups, it is called a bidentate and only a single ring can be formed; if it contains three groups, it is a tridentate and two interlocking rings can be formed if proper spacial orientation is obtained. In some "polydentate" structures, such as porphyrins, many interlocking ring systems may be formed and the resulting structure has amazing stability (Bailar, 1971).

The two most important determinants of the formation of a complex are the pH of the solution which determines the amount of ionization of the ligand and the stability constant which expresses the tenacity of the particular bonds in the complex. The effect of pH on the comparative efficiency of ligands is considerable. If at a given pH value, two ligands are equally well ionized, their success in competing for traces of metal ions at that pH would be proportional to their stability constant alone. However, it is often the case that the degree of ionization of two molecular species is quite different at a given pH. Thus, at pH 7.3 the amount of anion (the chelating form) varies from 0.04% for proline to 2.45% for asparagine, or 60 fold. Asparagine will compete with proline five times as successfully for Fe^{+++} and seven times as successfully for Mn^{++} at that pH.

The affinity of a chelate for a metal may also be expressed quantitatively as a stability constant. Considering a general interaction between a chelating agent (A) and a cation (M), it may be expressed:

$$M + XA = MAx$$

The equilibrium constant K for this reaction is expressed mathematically as follows where the figures in parentheses are for activities of the respective ions:

$$K = \frac{(MAx)}{(M)(A)x}$$

However, the activities of the chelating ions are very difficult to measure and molar concentrations are substituted for activities under these conditions; an apparent formation constant K_f is used instead of equilibrium constants, i.e.,

$$K_f = \frac{(MAx)}{(M)\ (A)^x}$$

The log of K_f is equal to the stability constant.

The stability constant represents the number of moles of chelated metal ion in relation to the product of the number of moles metal ion and ligand remaining in the free state in the system (Chenoweth, 1956).

The metal ion having a higher stability constant theoretically can replace a metal ion of lower stability constant in a chelate, or at least chelates with the higher stability constant will be formed before that of a lower stability constant. The synthetic chelating agent EDTA has a very high stability constant with most of the essential trace minerals. EDTA when present in a system in adequate concentration, will pick up all of the polyvalent transition cations even in competition with most other chelating agents (Scott, 1965).

The number of electron pairs that the same metal ion can accept in the formation of a coordination complex is called the coordination

number of the ion; it determines the number of molecules or ions of complexing agents that will be bound to the metal ion. Coordinate number of metals are usually six, sometimes four and occasionally two or even eight (Chenoweth, 1956).

One must keep in mind that stability constants cited in the literature are maximum values and many chelating agents are determined under well defined in vitro conditions at definite optimum pH values and ionic concentrations. These conditions are rarely encountered in an intact animal. Sevem et al. (1960) pointed out that the in vivo stability of a metal chelate may be influenced by its inherent stability (indicated by its equilibrium constant, K), the competition of body cations for the chelating agent, pH and the tendency of the metal to form insoluble hydroxides, the distribution and metabolism of the chelate and the competition of physiological complexing radicals for the metal ion. Further the in vivo distribution of a chelate may have an influence on its stability, since compounds widely distributed in many body compartments may be exposed to greater physiological competition.

Scott (1965) suggested that chelates which influence biological systems can be classified into three groups in accordance with the function served by the chelate. Scott's classification is as follows: 1) Chelates that serve to transport and store metal ions. In these chelates the metal has no function of its own, it does not modify the properties of the chelating agent but does require an agent of such chemical and physical properties that the chelate is capable of being absorbed, transported in the bloodstream and passing across cell membranes to

deposit the metal ion where it is needed; 2) Chelates essential in metabolism. In this type the metal ion must be part of the chelate to perform its metabolic function. Heme and the cytochrome enzymes are chelates of this type; the hemoglobin molecule without its ion moiety would be incapable of transporting oxygen; and 3) The final group consists of those chelates which interfere with utilization of essential cations. Many such chelates are thought to be formed by "accident" and have no apparent useful biological purpose. Such is the case of the chelate formed by phytic acid and Zn which renders Zn non-available to the animal system.

Protein Source

The first hints of the importance of chelation in animal nutrition came about in an indirect manner. Several researchers conducting mineral studies with different species of animals, particularly poultry, found an association between the bird's Zn requirements and the source of dietary protein fed.

O'Dell and Savage (1957) in their original report on the need of Zn in poultry nutrition fed 50 white rock male chicks a basal diet containing 25% Drackett protein (isolated soybean prot(.n) and 50 ppm Zn by analysis. The basal diet was supplemented with 6.6 ppm and 56 ppm Zn and 5% distillers dried solubles. The birds receiving the supplemental Zn out performed the basal group and showed no shortening or thickening of the hocks. Since the basal diet was considered to contain adequate Zn, the authors concluded that the Zn in this diet was unavailable because the birds responded to additional Zn supplementation. Morrison

and Sarett (1958) found that a soybean protein basal diet containing 30 ppm Zn fed to chicks produced poor growth as compared to a casein-gelatin base diet containing equal amounts of Zn. Further when calcium was added in excess to the soybean protein diet, chick growth was depressed, but the addition of excess calcium to the casein-gelatin diet was without effect. Moeller and Scott (1958) conducted growth experiments with chicks receiving a glucose-Drackett protein diet and a glucose-egg white diet. Marked deficiency symptoms were observed on both basal diets. With the Drackett basal (13 ppm Zn) maximum growth was obtained with 20 ppm of supplemental Zn. In contrast, maximum growth on the laboratory prepared egg white basal was noted with only 10 ppm supplemental Zn.

Zeigler et al. (1961) found that the Zn requirement of chicks fed a purified diet containing casein was 12-14 mg. of total Zn per kg. of diet. Chicks fed a similar diet in which the casein protein was replaced by isolated soybean protein required 27-29 mg. of total dietary Zn per kg. of diet. Increasing the level of soybean protein in the diet increased the Zn requirement. The authors concluded that soybean protein appeared to contain a specific factor or property which increases the chicks requirement for Zn. Other researchers have found similar results with turkey poults (Kratzer et al., 1959), chickens (Kienholz et al., 1962) and with chicks (Davis et al., 1962; Edwards, H. M., 1966, and Nielsen and Hoekstra, 1966).

Smith et al. (1959) used five groups of eight three-week old pigs and fed them semi-purified diets containing 20.5% protein. All pigs

receiving isolated protein and soybean meal developed symptoms of parakeratosis, but there were no symptoms in the pigs fed milk protein. The pigs were reallotted, and significant increases in growth and the alleviation of parakeratosis were observed when Zn was added to soybean protein diet. The addition of Zn to milk protein diets did not improve growth. The same authors (Smith et al., 1961) conducted three experiments with 114 three-week old pigs to determine the Zn requirement of the growing pig when isolated soybean protein was fed in the diet and found the requirement to be 46 ppm. The dietary Zn requirement of the pig was lower when casein was substituted kilogram-for-kilogram for isolated soybean meal. Smith et al. (1962) in a study to determine the effect of various protein sources on dietary Zn requirements found that pigs receiving milk protein (containing 6-18 ppm Zn) with and without added Zn had superior growth to those on soybean protein and showed no signs of parakeratosis. All pigs receiving the soybean diets (containing 16-22 ppm Zn) showed typical Zn deficiency symptoms. The addition of 50 ppm Zn to the soybean protein diet alleviated the Zn deficiency symptoms; however, the addition of Zn to the milk protein diets did not improve performance.

From these results it appears that Zn in isolated soybean protein is bound so that it is absorbed less efficiently than the Zn in animal protein such as casein and egg-white.

Phytic Acid and Calcium Levels

One of the outstanding differences between animal and plant sources of protein is in their content of phytate, the hexaphosphate

ester of inositol. Most plant seeds, notably oilseed meals and cereal grains, store phosphorous as the organic ester. Smith and Rackis (1957) found when extracting proteins from soybean meal, phytin reacts with the protein to form complex products of varying composition.

In a study to test whether or not a casein-phytic acid complex would decrease availability of Zn to the growing chick, O'Dell and Savage (1960) added phytic acid to a casein base diet in an amount equivalent to that normally found in an isolated-soybean protein based diet. They found that Zn in the casein treated diet was less available than the untreated casein diet. Addition of calcium phytate to the casein base diet had little or no effect on Zn availability. They concluded that isolated soy protein has a high affinity for Zn. Oberleas et al. (1960) fed weanling pigs basal diets of casein (15% protein and 14 mg./kg. Zn) and soybean meal (15% protein and 25 mg./kg. Zn) to which phytic acid and/or Zn were added. Visual and histological analysis indicated that pigs receiving the soybean meal basal developed parakeratosis, whereas those receiving casein protein did not except when phytic acid was added. Zn supplementation at 100 mg./kg. prevented the negative effects of added phytic acid. Their data indicate that parakeratosis can be produced on a diet composed of animal protein if phytic acid is added at either low or high levels of dietary calcium.

Following their observation that phytic acid decreased Zn availability and produced parakeratosis in swine, Oberleas et al. (1961) used the rat to study phytic acid-Zn metabolic interrelationships. Weanling albino rats were fed diets based on casein and purified soy protein