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THE EFFECTS OF SUPPLEMENTAL MICROBIAL PHYTASE ON NUTRIENT UTILIZATION IN BROILER CHICKENS

by

SYLVESTER SEBASTIAN

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SUPPLEMENTAL PHYTASE AND NUTRIENT UTILIZATION IN CHICKENS

Dedicated to my late father,

Christi Sebastian,

who dared to dream that one day I might be what I am today

ABSTRACT

Doctor of Philosophy Animal Science

Sylvester Sebastian

THE EFFECTS OF SUPPLEMENTAL MICROBIAL PHYTASE ON NUTRIENT UTILIZATION IN BROILER CHICKENS

The influence of microbial phytase on growth performance, availability of macro and trace minerals, apparent ileal digestibility (AID) and apparent "fecal" digestibility (AFD) of amino acids (AA) and CP were investigated. The optimum level of dietary Ca and P for the maximum efficacy of supplemental phytase in broiler chickens was also studied. Phytase supplementation (600 U / kg) to a low P diet increased ($P < 0.05$) the body weight gain and feed consumption but had no effect ($P > 0.05$) on feed efficiency in broiler chickens at 21 d. The efficacy of phytase, particularly in stimulating growth, was higher in male than female chickens. The relative retention of Ca, P, Cu, Zn and N increased by addition of phytase to a low P diet but phytase had no effect ($P > 0.05$) on the retention of Mg, Mn and Fe. Phytase supplementation increased ($P < 0.05$) plasma P and reduced ($P < 0.05$) plasma Ca level compared to control and had no effect ($P > 0.05$) on plasma Zn, Cu, and Mg. Phytase increased ($P < 0.05$) the ash content in both tibia head and shaft but had no effect ($P > 0.05$) on mineral proportions in the tibia ash; however, it increased ($P < 0.05$) the DM percentage of P and Ca in the tibia head. Dietary Ca levels had a significant effect ($P < 0.05$) on the response to supplemental phytase. The optimum growth performance and mineral utilization were achieved at the low (0.6%) level of dietary Ca with phytase compared with the recommended (1.0%) or high (1.25%) levels of dietary Ca. Microbial phytase supplementation increased ($P < 0.035$) the AID of most of the AA in female chickens at 28-d; it had no effect ($P > 0.05$) on AID of any of the AA in male chickens. Addition of phytase did not have any effect ($P > 0.05$) on AFD of any of the AA in male chickens but increased ($P < 0.05$) the AFD of Asp, Glu, Gly, Thr, and Ser in females. Phytase supplementation had no effect ($P > 0.05$) on either AID or AFD of CP and AA at 21-d. In summary, phytase

supplementation increased the growth performance, availability of P, Ca, Cu, Zn and N, plasma P, and tibia ash and reduced plasma Ca; it also increased the AID and AFD of most of the AA, particulary in female chickens at 28-d. The efficacy of microbial phytase was high when dietary P and Ca levels were low.

RÉSUMÉ

Docteur en Philosophie Zootechnie

Sylvester Sebastian

LES EFFETS DE LA SUPPLÉMENTATION DE LA PHYTASE MICROBIENNE SUR L'UTILISATION DES ÉLÉMENTS NUTRITIFS PAR LE POULET DE CHAIR.

Nous avons étudié l'influence de la phytase microbienne sur les performances de croissance, sur la disponibilité des minéraux mineurs et majeurs, sur la digestibilité apparente au niveau de l'iléum (DAI) et sur la digestibilité apparente au niveau des fèces (DAF) pour les acides aminés (AA) et la protéine brute (PB). Le niveau optimum du Ca et du P de la ration afin de maximiser l'efficacité de la phytase microbienne pour le poulet à chair a aussi été étudié. La supplémentation de phytase à raison de 600 U/ kg à une ration déficiente en phosphore augmente ($P < 0.005$) le gain de poids vif et la consommation alimentaire mais n'a aucun effet ($P > 0.05$) sur l'efficacité alimentaire des poulets à chair de 21 jours. L'efficacité de la phytase stimule particulièrement la croisssance des mâles comparée à celle des femelles. La rétention relative du Ca, P, Cu, Zn et N augmente par l'addition de phytase à une ration basse en P, mais la phytase n'a aucun effet ($P > 0.05$) sur la rétention du Mg, Mn et Fe. La supplémentation de phytase augmente ($P < 0.05$) le P dans la plasma sanguin et réduit ($P < 0.05$) le Ca du plasma sanguin lorsque comparée à la ration témoin et n'a pas d'effet ($P > 0.05$) sur le niveau de Zn, Cu et Mn du plasma sanguin. La phytase augmente ($P < 0.05$) la quantité de cendres de la tête et de la diaphyse du tibia mais n'a pas d'effets ($P > 0.05$) sur la quantité de minéraux présents dans les cendres. Par contre, le pourcentage de matière sèche augmente ($P < 0.05$) pour le P et le Ca dans la tête du tibia. La quantité de Ca de la ration a un effet signicatif ($P < 0.05$) sur l'effet de la phytase. La performance de croissance optimale et de l'utilisation maximum des minéraux a été atteinte à des niveaux restraints de Ca (0.6%) de la ration avec la phytase ajoutée plutôt qu'aux niveaux

recommandés de 1.0% et 1.25%. La supplémentation de phytase microbienne augmente ($P < 0.035$) la DIA de la plupart des AAs pour les femelles à 28 jours, mais n'a pas d'effet ($P > 0.05$) sur la DAI d'aucun AAs chez le mâle. L'addition de phytase n'a eu aucun effet ($P > 0.05$) sur la DAF des AAs chez le mâle mais augmente ($P < 0.05$) la DAF de l'Asp, Glu, Gly, Thr, et de la Ser chez la femelle. La supplémentation de la phytase n'a eu aucun effet ($P > 0.05$) sur la DAI, la DAF, la PB ou les AAs à 21 jours. En résumé la supplémentation de phytase augmente les performances de croissance, la disponibilité du P, Ca, Cu, Zn et de N, le P dans le plasma sanguin et les cendres du tibia, mais réduit la quantité de Ca dans le plasma. La phytase augmente aussi la DAI et la DAF de la pluspart des AAs particulièrement chez la femelle à 28 jours. L'efficacité de la phytase microbienne était plus elevée lorsque le P et le Ca de la ration étaient bas.

RESUMEN

Doctor en Filosofía — Ciencia Animal

Sylvester Sebastian

EFECTO DE LA SUPLEMENTACION DE FITASA MICROBIANA EN LA UTILIZACION DE NUTRIENTES DE POLLOS BROILERS.

El efecto de la suplementación de fitasa microbiana (FiM) en la tasa de crecimiento, la disponibilidad en la dieta de elementos minerales mayores y menores, la digestibilidad aparente de los aminos acidos (AA) y la proteína cruda (PC) al nivel del ileón (DAI) y en la fecas (DAF) fué investigada en pollos broilers. También se estableció el nivel óptimo de P y Ca que permite obtener la máxima eficacia de la enzima FiM suplementada en la dieta de pollos broilers. La suplementación de la enzima FiM (600 U/kg) a una dieta de pollos broilers con bajo contenido de P aumenta ($P < 0.05$) la ganancia de peso vivo y el consumo de alimento pero no tiene efecto ($P > 0.05$) en la eficiencia de conversión del alimento de los pollos hasta los 21 d de edad. La eficacia de la FiM, especialmente en estimular el crecimiento, fué mayor en los pollos machos que en las hembras. La retención relativa de Ca, P, Cu, Zn y N aumentó con la suplementación de la FiM en dietas con bajo contenido de P; sin embargo, la FiM no tuvo efecto ($P > 0.05$) en la retención de Mg, Mn y Fe. En el grupo suplementado con FiM aumentó ($P < 0.05$) el P y redujo ($P < 0.05$) el Ca en el plasma sanguíneo comparado con el grupo control. Esta suplementación no tuvo ningún efecto en el contenido de Zn, Cu y Mg en el plasma. La enzima FiM aumentó ($P < 0.05$) el contenido de ceniza tanto en la cabeza como en el eje de la tibia pero no tuvo ningún efecto ($P > 0.05$) en la concentración de minerales de la tibia; sin embargo, la FiM aumentó el contenido de P y Ca en la materia seca de la cabeza de la tibia. El nivel de Ca en la dieta tuvo un efecto significativo ($P < 0.05$) en la respuesta a la suplementación de FiM. La respuesta óptima de crecimiento y de utilización de los minerales se obtuvo cuando la FiM fué suplementada a una dieta con bajo (0.6%) contenido de Ca en

comparación a dietas con un nivel de Ca recomendado (1.0%) o alto (1.25%). La suplementación de FiM aumentó (P <0.035) la ADI en la mayoría de los AA en la pollos hembras a los 28 d de edad; no tuvo efecto (P >0.05) en la DAI en ningúno de los AA en los pollos machos. La suplementación de FiM no mostró ningún efecto (P >0.05) ya sea en la DAI o DAF de la PC o AA a los 21 d de edad. En resumen, la suplementación de FiM aumentó la tasa de crecimiento, la disponibilidad de P, Ca, Cu, Zn de la dieta y la ceniza de la tibia y además redujo el Ca en el plasma sanguíneo; también aumentó la DAI y DAF en la mayoría de los AA especialmen-te en pollas hembras a los 28 d de edad. La eficacia de la suplementación de FiM fué alta en la dieta que contenía bajos niveles de P y Ca.

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GUIDELINES CONCERNING THESIS PREPARATION

Faculty of Graduate Studies and Research, McGill University

This thesis is presented as a series of three papers corresponding to section B.2. of the "GUIDELINES CONCERNING THESIS PREPARATION" (Revised January 1993) from the Faculty of Graduate Studies and Research at McGill University. These guidelines require that the following statement be quoted in the thesis:

"Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provided that these copies are bound as an integral part of the thesis.

If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory.

The thesis must still conform to all other requirements of the 'Guidelines Concerning Thesis Presentation' and should be in a literary form that is more than a mere collection of manuscripts published or to be published. The thesis must include, as separate chapters or sections: (1) a Table of Contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and /or summary.

Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Ph. D.

Oral Defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers.

The complete text of the above must be cited in full in the introductory sections of any theses to which it applies (this will inform the external reader of Faculty Regulations)."

Section II of this thesis has already been published as indicated on the title page of the chapter. Section III has been accepted for publication and section IV is in preparation for submission. Sections II, III and IV are authored by S. Sebastian, S. P. Touchburn, E. R. Chavez and P. C. Laguë. Drs. S. P. Touchburn and E. R. Chavez were the student's co-supervisors. Dr. P. C. Laguë, a member of the thesis committee, provided valuable advice and help in taking blood samples from the chickens.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

In recent years, sophisticated knowledge of the nutrient requirements of poultry and the nutrient content of feed ingredients have contributed, in part, to high levels of production and feed conversion efficiency in the modern poultry industry. Thus, major improvements in poultry nutrition in the future are unlikely to come from the discovery of new nutrients or refining nutrient requirements; instead, improvements in the efficiency of production must rely on obtaining maximum nutrient utilization from feedstuffs (Classen and Bedford, 1991). It is recognized that a considerable proportion of the nutrient content of feeds is not digested and absorbed by poultry. Some feedstuffs are overlooked or under-utilized because of poor nutrient availability, high levels of non-starch polysaccharides and / or the presence of anti-nutritional factors.

The availability of nutrients in plant materials is affected by natural complexing agents. This is particularly true in the case of seeds such as legumes, cereals and oil seeds containing phytic acid, known as myo-inositol hexaphosphate. Phytate, being a strong acid, can form various salts with important minerals such as phosphorus, calcium, magnesium, copper, zinc, potassium and iron, thus reducing their solubility (Erdman, 1979). Phytate also complexes with proteins making them less soluble (Cheryan, 1980). Knuckles and Betschart (1978) have shown that phytate strongly inhibits alpha-amylase activity by binding calcium which is needed for the activity and stability of this enzyme. This inhibition may lead to low digestibility of starch, thereby reducing the availability of energy.

Poultry diets consist largely of seeds (cereal grains) and products derived from

seeds (oil seed meal, cereal grain by-products). Unfortunately, many grains, oil seed meals and plant derived products contain high levels of phytic acid which contains 60-80% of the total phosphorus present (Simons *et al.*, 1990). Phosphorus in the phytate form is not available to poultry because they lack the enzyme, phytase, which hydrolyzes phytate into inorganic phosphorus and inositol. The limited ability of simple-stomached animals to utilize phosphorus from feedstuffs of plant origin presents two kinds of problems to animal nutritionists. The first involves the formulation of diets that satisfy physiologic needs for phosphorus. The second involves the environmental impact of unutilized dietary phosphorus which is excreted in the feces. In order to meet the phosphorus requirements of the animal, nutritionists have traditionally supplemented the diet with inorganic phosphorus. This is not only an expensive dietary ingredient, but also fails to address problems such as over supplementation and mineral binding. Over supplementation can lead to phosphorus pollution. Recent legislation in many countries has forced the feed industry to look for alternate ways to make phytate phosphorus more available to the animal. Due to economic and environmental concerns, there has been renewed interest in utilizing the enzyme phytase to reduce the need for phosphorus supplementation and to improve phosphorus utilization of feedstuffs.

The research on dietary phytase supplementation is intensifying because of the recent development in genetic engineering of microorganisms that are capable of producing large amounts of enzymes. It is well documented that microbial phytase supplementation improves the availability of phytate-P in poultry. Theoretically, phytase supplementation should increase the availability of other minerals, protein and amino

acids bound to phytic acid. However, there is very little information about the effect of phytase supplementation on the availability of other minerals, energy, protein and amino acids and the optimum dietary conditions to achieve maximum phytate degradation with supplemental phytase within the gastrointestinal tract.

The objectives of the experiments described in this thesis were:

1) To investigate the role of microbial phytase supplementation on growth performance and the availability of minerals such as phosphorus, calcium, copper, zinc, manganese, magnesium and iron in young broiler chickens fed corn-soybean diets.

2) To study the optimum levels of dietary calcium, phosphorus and calcium-phosphorus ratio for the maximum efficacy of phytase in corn-soybean diets fed to young broilers.

3) To study the effect of phytase on apparent ileal and "fecal" digestibilities of protein and amino acids in 21 and 28-d-old broiler chickens fed a corn-soybean meal diet.

Chapter I

LITERATURE REVIEW

LITERATURE REVIEW

1.1 Phytate

1.1.1 Introduction

Phytate (myoinositol hexaphosphate) is an organic complex which occurs naturally in many feeds of plant origin. Phytates are found in all seeds where they are generally regarded as the primary storage form of both phosphate and inositol. (Cosgrove, 1966). Phytic acid rapidly accumulates in seeds during the ripening period accompanied by other storage substances such as starch and lipids (Nahapetian and Bassiri, 1976). The amount of phytic acid varies from 0.50 to 1.89% in cereals, from 0.40 to 2.06% in legumes, and from 2.0 to 5.2% in oil seeds (Reddy *et al.*, 1982).

1.1.2 Structure of phytic acid

Phytate can be considered as a unique compound since it bears six phosphate groups on one 6-carbon molecule of low molecular weight (660). The structure of phytic acid, a naturally occurring anti-nutrient in seeds, has been a subject of controversy. A large number of conflicting conclusions about the precise structure of phytic acid exist despite the use of apparently sound methodology (Cheryan, 1980). The structure (Figure 1) proposed by Anderson (1914) is now generally accepted since many of the physicochemical properties, interactions and nutritional effects can be best explained by this model. On the basis of the Anderson structure, the proper name for phytic acid is myo-inositol-1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate). At neutral pH, phosphate groups in phytic acid have either one or two negatively charged oxygen atoms; hence, various cations could strongly chelate between two phosphate groups or weakly with a

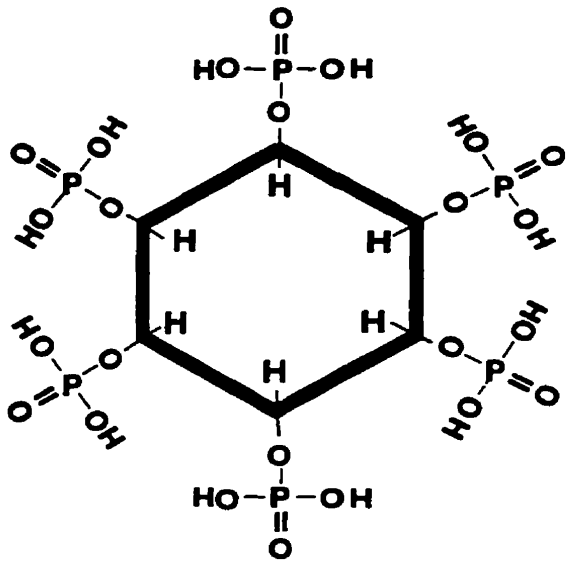

Fig. 1 Structure of phytic acid proposed by Anderson (1914)

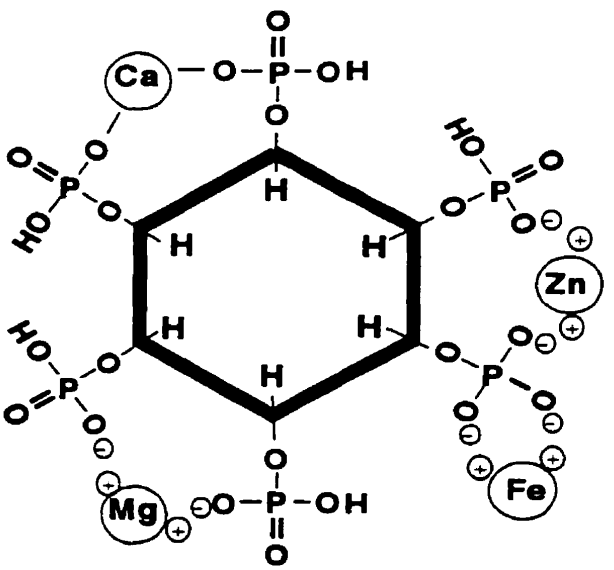

Fig. 2 Phytic acid chelate at neutral pH (Erdman, 1979)

single phosphate group (Figure 2). Thus, phytate has been recognized as a nutrient, because it contains phosphorus. In contrast, it is also considered to be toxic, because it binds various essential metals and reduces their availability for absorption from the diet. (Reddy, 1982).

1.1.3 Biological functions

Phytic acid serves several important physiological functions during dormancy and germination of seeds; these include initiation of dormancy (Gibson and Ullah, 1990), antioxidant protection during dormancy (Graf, 1986) and storage of P, high-energy phosphoryl groups and cations for use during germination (Williams, 1970). It has also been suggested that phytic acid acts as a carrier or storage site for trace minerals during plant growth on the basis of its strong chelating powers (Cosgrove, 1966). Gupta and Venkatasubramanian (1975) have suggested that phytic acid plays a mycological role in the field, preventing aflatoxin production in soybean seeds by making zinc unavailable to the mold.

1.1.4 Occurrence of phytic acid in feedstuffs

Phytate P constitutes the major portion of total P in cereal seeds, grain legumes and oil-bearing plants. In general, the proportion of phytate P varies from 60 to 80% of the total P in these materials (NRC, 1994). The concentration of phytate P in feedstuffs depends largely on the part of the plant from which they are derived. Oilseed meals and cereal by-products contain large amounts of phytate P compared to grain legumes (Ravindran *et al.*, 1995a). Roots and tubers contain small amounts of phytate P; in vegetative, non-storage organs of plants such as the leaves, phytate P is either absent or

present only in trace amounts. The phytic acid in cereals is not uniformly distributed within the kernel, but associated with specific morphological components in the seed (Oberleas, 1973). In wheat and rice, the endosperm is almost devoid of phytate, but the aleurone layer of the kernel and the bran contain substantial amounts. In rice, more than 80% of the phytate is present in the outer bran (O'Dell *et al.*, 1972). In dicotyledonous seeds including oilseeds and grain legumes, phytate is distributed throughout the kernel in subcellular inclusions known as globoids (Erdman, 1979). In contrast, phytates in soybean are unique in that, although associated with globoids, they appear to have no specific site of localization. Phytate concentration in plant materials may vary depending on the stage of maturity, degree of processing, cultivar, climatic factors, water availability, soil factors, location and the year during which they are grown (Reddy *et al.*, 1982).

1.2 Nutritional implications of phytate

1.2.1 Digestion of phytate and its bioavailability

In mature cereal grains, legumes, and oil seeds, the major portion of the total P is present in the form of phytic acid. The utilization of P when present in the form of phytate depends on the species, the age of the animal, the level of phytase which hydrolyses the phytic acid in the intestinal tract of the specific species, and nutritional factors. Phytate is generally regarded as being less biologically available than most inorganic P compounds. In mammals, extremely low levels of the phytase enzyme have been isolated from the brush border region of the small intestine (Cooper and Gowing, 1983). Phytase produced by the ruminal microflora is mainly responsible for the

increased rate of phytate P digestibility in ruminants. There is some indication that the ability of swine to utilize phytate P improves with age. Nelson (1976) measured the amount of natural phytate hydrolyzed by chickens and laying hens. He found that 4- and 9-wk old chicks and laying hens, respectively, hydrolyzed 0, 3 and 8 % of the natural phytate when the diet contained corn as the only grain source.

1.2.2 Bioavailability of phytate phosphorus for poultry

Nelson (1967) was the pioneer in demonstrating that natural phytate P was poorly utilized by poultry. However, it is now becoming increasingly clear that phytate P is digested and utilized to some extent by poultry. Reports indicate that phytate P utilization by poultry ranges from zero (Nelson, 1976) to over 50% (Edwards, 1983; Mohammed *et al.*, 1991). Nelson (1976) determined the hydrolysis of phytate P from different diets by 4- and 9-week-old broiler chickens and by laying hens. On corn-based diets, the amounts hydrolyzed were 0, 3 and 8%, respectively. The corresponding values for wheat-corn based diets were 8, 13 and 13%, respectively. In contrast, Temperton and Cassidy (1964 a,b) reported that chicks could absorb and retain 60% of ingested phytate P. Edwards (1983) also reported high phytate P retention values (37 - 56%) for chicks. In the studies of Ballam *et al.* (1984), phytate hydrolysis values ranging from 3 to 42% were determined, depending on the level of dietary Ca and the source of fibre. Thus, wide disagreement exists among researchers concerning the ability of poultry to utilize phytate P. The disagreement appears to be due, in part, to the complex nature of factors influencing phytate hydrolysis, including the source of phytase, age of birds, and dietary levels of Ca and vitamin D_3.

1.2.3 Phytate hydrolysis in poultry

In order for the phytate P to be utilized, phytate must be hydrolyzed into inorganic P within the digestive tract. Although non-enzymatic cleavage of phytates has been suggested by some workers (Hegsted *et al.*, 1954), the dephosphorylation of phytic acid is largely by the action of an enzyme called phytase (myo-inositol hexaphosphate phophohydrolase). Phytase comprises a family of enzymes that catalyze the stepwise removal of inorganic orthophosphate from the phytic acid, via inositol pentaphosphate to monophosphate as intermediatory products (Nayni and Markakis, 1986). This lysis can occur in the digestive tract or in the feedstuff prior to consumption by the bird. The degradation of phytate in the digestive tract of poultry may be attributed to the action of phytase from one or more of three possible sources. These sources include, (1) intestinal phytase in digestive secretions, (2) phytase activity originating from microbes resident in the intestinal tract or (3) endogenous phytase activity present in some feedstuffs. A controversy exits concerning the presence of intestinal phytase activity in poultry. Bitar and Reinhold (1972) claimed that activity is found in the mucosa of the small intestine in poultry. Davies and Motzak (1978) also reported that a phytase obtained from homogenate of the chick intestinal mucosa hydrolyzed sodium phytate. However, the presence of intestinal phytase in poultry has not been confirmed in other studies (Moore and Veum, 1983). Whether phytase or other phosphatases of the intestinal mucosa play a role in phytate hydrolysis has yet to be clearly established (Moore and Veum, 1983). Cooper and Gowing (1983) found that intestinal phytase activity in rats was somewhat similar to, but distinct from alkaline phosphatase. In a recent study with chicks, Maenz

et al. (1995) have shown that intestinal brush border membrane contains phytase activity that is independent of non-specific phosphatase enzyme. They also reported that at optimum pH, at least two distinct enzymes contribute to total phytase activity in the brush border. However, the available data suggest that endogenous phytase activity in intestinal secretions of poultry is inconclusive and that endogenous phytase activity in the intestinal mucosa of poultry is extremely low, at least in the younger birds.

Phytase is known to be produced by fungi, bacteria, yeast and by rumen and soil microorganisms. Filamentous fungi, particularly those from the *Aspergillus* genus (*Aspergillus ficuum* and *Aspergillus niger*) have been extensively studied as rich sources of microbial phytase. The microbes in the rumen of ruminant animals produce phytase which effectively hydrolyzes phytate (Taylor, 1965). Nelson *et al.*, (1976) reported that total hydrolysis of phytate occurred in the digestive tracts of calves and steers. There is some evidence indicating that bacterial phytase may be active in the digestive tract of poultry.

It has long been established that some feed ingredients have endogenous phytase activity. Phytases are present in most cereals, but their activity varies widely amongst cereals (Bartnik and Szafranska, 1987). Rye, wheat and barley contain high levels of phytase activity, whereas corn, oats, sorghum and oilseeds contain little or no enzyme. In a series of experiments, Temperton and his co-workers (1965 a,b) provided indirect evidence to show that endogenous plant phytase is effective in feeds. Using diets containing 32 to 36% wheat and 10% barley meal, without feedstuffs of animal origin and inorganic P supplements, they demonstrated that chicks were able to utilize phytate

P for bone calcification. The data of Scheuermann *et al.* (1988) show that wheat phytase can act on phytates from other ingredients as well. Thus it is possible to avoid the use of inorganic phosphate supplements by employing a combination of these ingredients. It is relevant to note that the optimum pH for plant phytase activity is in the range of 4.0 - 6.0 (Reddy *et al.*, 1982), though some activity may be retained at pH 3.0. It is possible that the amount of active phytase in feed ingredients can vary depending on cultivar, age and /or drying and storage conditions. High temperatures employed during ingredient processing or during pelleting of diets can also influence the native phytase activity of plant ingredients. Plant phytase activity is not altered by such treatments at temperatures between 47 and 62^{0}C (Pointillart, 1988), but higher temperatures (70-80^0 C) can cause partial or total inactivation. It should be noted that the activity of phytase in the gastrointestinal tract may be governed by a number of variables including source, pH and the presence of metal ions. Microbial phytase has a broader pH activity range than plant phytases (Eeckhout and de Paepe, 1991) and therefore are more effective within the gastrointestinal environment.

1.2.4 Factors influencing phytate phosphorus utilization

The hydrolysis and absorption of phytate P by monogastric animals are complex processes that are influenced by factors such as: dietary levels of Ca, inorganic P (or available P), vitamin D_3, age and type of birds, dietary ingredients and feed processing.

a) Dietary Calcium and Phosphorus levels.

Phytate P utilization by poultry has been shown to be influenced by both Ca and

P levels in the diet (Mohammed *et al.*, 1991), but the effects of dietary Ca are much greater. At very high levels of Ca, phytate hydrolysis may be completely prevented (Taylor, 1965). Under practical feeding conditions where Ca and P must be added to the diets for maximum performance and bone calcification, phytate P is utilized very little by poultry. This adverse influence has been confirmed in several studies where Ca was added at recommend levels (Nelson, 1976). Balance studies by Nott *et al.*, (1967), demonstrated that when Ca intake was adequate for optimum shell quality, hens were unable to utilize phytate P. Ballam *et al.* (1984) found that chicks fed diets containing 1.0% Ca hydrolyzed less phytate than those fed diets with 0.85% Ca. Similarly, Mohammed *et al.* (1991) reported that phytate P utilization was increased by 15% when dietary Ca levels were reduced form 1% to 0.5%. In their studies the adverse effects of a high-phytate, low-inorganic P diet on chicks were overcome by lowering the Ca level and/or increasing the vitamin D_3 concentration. The Ca:total P ratio also plays a role on phytate P utilization (Wise, 1983). A high Ca level or a Ca:total P ratio of 2:1 results in lowered digestion of phytate, owing to the formation of an insoluble Ca-phytate complex in the intestine (Nelson, 1967). Vandepopuliere *et al.* (1961) found that chicks fed a diet with a Ca:total P ratio of 1:1 performed better than those fed a diet with a 2:1 ratio. Harms *et al.* (1962) showed that narrowing the Ca:total P ratio from 2:1 to 1:1 improved the availability of P from phytic acid to a greater extent than supplementing feeds with inorgainc P.

b) Dietary vitamin D_3 level

Early research clearly demonstrated that phytate utilization was depressed by

feeding diets marginal or deficient in vitamin D_3 (Ewing, 1963). Several recent reports also indicated that the addition of vitamin D_3 markedly enhanced the amount of phytate P retained by chickens (Edwards, 1991; Mohammed *et al.*, 1991). In these studies, the phytate P utilization was increased from 31-50% to 68-87%. The improved utilization of phytate P in response to vitamin D_3 supplementation may be attributed to one or more of the following three mechanisms: (1) increased synthesis or activity of intestinal phytase (Shafy *et al.*, 1992) (2) increased phytate hydrolysis (Mohammed *et al.*, 1991) via stimulation of Ca absorption, thus rendering the phytate more soluble and available for utilization, and (3) enhanced transport of P (Wassermann and Taylor, 1973; Tanaka and De Luca, 1974).

c) Age of birds

It is generally conceded that older birds hydrolyze phytate P to a greater extent than do chicks (Peeler, 1972), due to the fact that there is more of the phytase activity present in the gastrointestinal tract of older birds. The available literature suggest that with plant-derived ingredients about one-third of the total P is available to young chicks and perhaps up to one-half available to hens. Edwards *et al.* (1989) reported that the ability of poultry to utilize phytate P increases with age. Based on chromic oxide balance studies, they found that 21-d-old broilers utilized phytate P better than 14-and 7-d-old broilers. In this study, a significant effect of sex was also observed, with males retaining more phytate P than females. Ashton *et al.* (1960) also indicated increased utilization of ^{32}P-labelled Ca-phytate P by 6-wk-old chicks compared to 4-wk-old chicks. However, Nelson (1976) investigating the hydrolysis of phytate P by 4- and 9-wk-old broilers in

balance experiments, observed only a slight increase in utilization by the older birds. Interestingly, the ability of laying hens to utilize phytate P appears to decline with advancing age. Scheideler and Sell (1987) reported that the retention of phytate P was quite high at 34 wks of age, averaging 46.7%, but decreased to 9.1 and 16.5% at 50 and 72 weeks of age, respectively. The reasons for this decline with age are unclear.

d) Type of dietary ingredients

As discussed earlier, phytate P utilization in poultry diets can be improved by incorporating plant-derived ingredients with known phytase activity. It has been demonstrated that phytate P in diets based on such ingredients, with no feedstuffs of animal origin, are well utilized by young growing chicks, pullets (Temperton *et al.*, 1965 b), laying hens (Salmon *et al.*, 1969; Couch and Creger, 1970), and turkey poults (Temperton and Cassidy 1964 c) for production purposes and bone growth. Differences in the solubility of phytate from different sources have been reported by de Boland *et al.* (1975). They found that the phytate in soybean meal was more soluble than that in sesame meal. This suggests differences in the extent of hydrolysis of phytates from different feedstuffs within the gastrointestinal tract, if one presumes that the soluble phytate is a better substrate for enzymatic degradation.

e) Effect of fibre

There is some evidence to indicate that certain sources of fibre have significant influence on phytate P utilization. In studies reported by Ballam *et al.* (1984) hydrolysis of phytate was reduced by cotton seed hulls but unaffected by wheat bran. The dietary levels of fibre may also be expected to influence phytate hydrolysis, but this aspect has

not been investigated to date. Further research is needed to clearly establish any influence of fibrous feedstuffs on phytate P utilization.

f) Genotype of birds

Limited evidence indicates that there may be breed and strain differences in the utilization of phytate P. Edwards (1983) reported the average retention of phytate P by Leghorn chickens to be greater than that by meat-type broilers. In this study, considerable differences were also noted between the three broiler strains tested in their ability to utilize the phytate P.

1.2.5 Effect of phytate on bioavailability of other nutrients

a) Effects on mineral bioavailability

The structure of the phytic acid is suggestive of tremendous chelating potential. It is a strong acid and forms a wide variety of insoluble salts with di- and trivalent cations at neutral pH (Oberleas, 1973), potentially rendering these minerals unavailable for intestinal absorption. Experiments with animals have suggested that phytic acid in plant foods complexes with dietary essential minerals such as Ca, Zn, Cu, Fe, and Mg, and makes them biologically unavailable for absorption (Oberleas, 1973). The mechanism by which phytate affects mineral nutrition in not clearly understood. Most investigations suggest that the formation of phytate-mineral complexes in the intestinal tract prevent the metal absorption (Oberleas, 1973). Vohra *et al.* (1965) using titration curves of phytate as free acid in the presence of single cations, reported that phytate formed complexes with minerals in the following descending order: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$. In a similar study, Maddaiah *et al.* (1964) found that at physiological pH, Zn

formed the most insoluble salt with phytic acid. The decreasing order of stability of salts was found to be $Zn^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Ca^{2+}$. Although Ca has the lowest binding affinity, the greatest impact of phytate on mineral nutrition (other than P) is on Ca bioavailability. It is now known that phytate lowers the bioavailability of several nutritionally important minerals; such interference may lead to increased mineral requirements of animals. Most of the studies leading to conclusions on the role of phytate in impairing the bioavailability of minerals have been conducted on humans and rats. Although some species differences with regard to mineral availability have been reported (O'Dell *et al.*, 1972), it is likely that the findings have general application to poultry. In poultry nutrition, some attention has been given to the effect of phytate in increasing the requirements of Ca. Nelson *et al.* (1968) found that the Ca requirement of White leghorn chicks fed a purified diet containing no phytate was 0.5%; the requirement was increased to 0.95% on a practical diet containing 12.5% phytic acid. Nelson (1984) suggested that the dietary Ca requirement of poultry must be expressed in terms of available rather than total Ca. Thus, if diets were to contain ingredients high in phytate, more Ca would be required to offset the portion that was unavailable as insoluble Ca-phytate. However, interest in phytate continues to be more in relation to P availability, rather than Ca availability, owing to the expense of supplying P in the diet relative to the provision of Ca. Zinc becomes a limiting mineral with high-phytate diets, since it forms a highly insoluble salt at pH 6.0 (Maddaiah *et al.*, 1964), the approximate pH of the upper intestine where most of the absorption of minerals occurs. Zinc is also significantly affected by the phytate-Ca synergism. The effect of phytate on Zn availability of chicks

has been demonstrated by several workers (Edwards, 1966; Lease, 1966). O'Dell (1962) reported that growth rate was lowered when phytic acid was added to chick diets; this depression was overcome by the addition of supplemental Zn. Kratzer *et al.*, (1959) showed that autoclaving of soybean protein increased the availability of zinc to turkey poults and suggested that this increase was due to the destruction of phytic acid. The effect of phytate on the availability of minerals other than Ca and Zn has received only little attention in poultry nutrition. McWard (1969) found that addition of 4% phytic acid-soyprotein complex to a balanced diet containing 75 ppm supplemental Mg depressed growth and increased mortality of chicks, which was attributed to a decreased Mg availability. Davis *et al.* (1961) showed that diets containing isolated phytate lowered the availability of Cu and Mn for chicks.

Several reports indicate that the lower phosphates of inositol (inositol mono-, bis-, tris- and tertra-phosphates), which are formed during the step-wise dephosphorylation of phytate, may have nutritionally less effects on mineral availability (Tao *et al.*, 1986; Sandberg *et al.*, 1987). It has been suggested that at least five of the six possible sites on inositol need to be phosphorylated in order to exert an inhibitory effect on the intestinal absorption of Zn and Ca. It would appear therefore, that the influence of phytate on mineral utilization may also vary depending on the degree of phytate degradation and the proportion of lower phosphates of inositol present in the gut.

b) Effect on protein availability

The nutritional significance of phytate is further complicated by protein-mineral-phytate interactions (de Rahm and Jost, 1979; Cosgrove, 1980) and their inhibitory

effects on proteolytic enzymes (Caldwell, 1992). The association between phytate and protein begins in seeds during ripening when phytate accumulates primarily in the protein-rich aleurone layers of monocotyledonous seeds and in the protein bodies of dicotyledonous seeds. (Prattley, 1982). Carnovale *et al.* (1988) reported that phytic acid-protein interaction affects the protein availability of legumes negatively and that the nature of the protein source plays a prominent role. It has been shown that phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Barre *et al.*, 1956). The interaction between phytic acid and proteins is thought to be of the ionic type and is dependent on pH (de Rham and Jost, 1979). Phytic acid is known to form complexes with protein at both acidic and alkaline pH. Careen (1980) offered the following explanation: at low pH (about pH 2) phytic acid is strongly negatively charged while proteins are strongly positively charged, thus phytate-protein complexes may be formed. At high (alkaline) pH, a strong protein-phytate interaction is suggested (O'Dell and de Boland, 1976) which is apparently different than at low pH in that the electrostatic effects are minor. Multivalent cations such as calcium are thought to mediate such phytate-protein complexes. This interaction between phytic acid and proteins leads to decreased solubility of proteins (Saio *et al.*, 1967). The reduced solubility of protein as a result of a phytate-protein complex can adversely affect certain functional properties of the protein which are dependent upon their hydration and solubility (Cheryan, 1980). O'Dell and de Boland (1976) suggested that the accessibility of binding groups is the key factor that determines phytate binding to the protein. Thus, it not surprising that conflicting results have been reported from in vivo experiments with

regard to phytate intake and protein utilization. While some have suggested that phytic acid does not affect protein digestibility (Thompson and Serraino, 1986), others have found improvements in protein utilization with decreasing levels of phytate (Atwal *et al.*, 1980; Knuckles *et al.*, 1985). Dietary phytate levels were correlated significantly with fecal nitrogen output, indicating that the lowered digestibility may have resulted from phytate-bound protein. However, it should be noted that changes in fecal nitrogen output may also be associated with other components of the diets, such as cell wall polysaccharides and lignins (Low, 1985).

Phytate is known to inhibit a number of digestive enzymes such as pepsin (Camus and LaPorte, 1976), α amylase (Deshpande and Cheryan, 1984) and trypsin (Singh and Krikorian, 1982; Caldwell, 1992). This may be due to the non-specific nature of the phytate-protein interactions. Inhibition may also result from the chelation of Ca ions which are essential for the activity of trypsin and α-amylase, or possibly to an interaction with the substrate for these enzymes (Liener, 1989). These negative influences may also be partly responsible for the effects of phytate on protein utilization. However, the extent to which the inhibition of enzyme activity by phytate contributes to its overall anti-nutritional effect remains uncertain. Anderson (1985) suggested that the main nutritional effect of phytate-protein complex formation is the reduction in mineral availability. But several recent studies with supplemental dietary phytase provide indirect evidence that phytate-protein interactions can interfere with protein and amino acid digestibility (Mroz *et al.*, 1994; Yi *et al.*, 1994a). Further research is warranted to understand the full implications of phytate on the protein quality of feedstuffs.

c) Effect on starch digestibility

Alpha-amylase occurs in nearly all plants, animals, and microorganisms. It is well known that calcium ions are required for the activity and maximum stabilization of alpha-amylase. Cawley and Mitchell (1968) reported that phytate suppressed the alpha-amylase activity in sprouted wheat meal by complexing the calcium ion necessary for enzyme activity. In a study with human saliva, it has been shown that at pH 4.15, myo-inositol hexaphosphate (phytate) and myo-inositol-2-monophosphate reduced starch digestion by salivary alpha-amylase to 8.5 and 78.3 % respectively, of the control. Thomson and Yoon (1984) reported that the addition of phytic acid to wheat reduced the starch digestibility by 60 % compared to that of the control treatment.

1.3 Phytase

1.3.1 Origin and Characteristics

Phytase (myo-inositol hexaphosphate phosphohydrolase) is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. Phytase is widely distributed in plants, animals and fungi. Phytase acts on myo-inositol hexaphosphate to yield inositol and orthophosphate, via inositol penta- to monophosphates as intermediary products. Phytase has been isolated from a number of plant sources such as kidney beans, wheat grain, canola seed and corn. In addition, phytase has been isolated from bacterial (Pseudomonas and *Bacillus subtilus*) and fungal (*Saccharomyces cerevisiae* and selected *Aspergillus* strains) sources. Extremely low levels of phytase have been isolated in monogastrics but the microflora present in the rumen are responsible for increased phytase activity observed in ruminants. Commercial production of phytase for use as an

exogenous enzyme supplement to feedstuffs is most easily achieved by microbial cultures. *Aspergillus niger* was the most active mold isolated with phytase activity. *Aspergillus ficuum* strain was shown to produce the highest concentration of phytase enzymes. Unlike many other enzymes, phytase from *Aspergillus ficuum* was found to be remarkably thermostable. In purified form, the enzyme retains 40 % of its activity after being subjected to heating at 68^0 C for 10 minutes. The phytase being an acid phosphatase retains its activity over a wide pH range with optimum enzymatic activity between pH 5 and 6.5.

1.3.2 Microbial Phytase as a potential means to increase the availability of nutrients

Nelson *et al.* (1971) were among the first to demonstrate the efficacy of a microbial phytase obtained from *Aspergillus ficuum* in hydrolyzing the phytate P in plant-derived ingredients. Until recently, very little research had been done in this area because of the difficulties in obtaining large quantities of enzyme and the high cost involved in the enzyme production. However, this may no longer be a limiting factor because of developments in genetic engineering of microorganisms that are capable of producing large amounts of phytase. In addition to other enzymes, renewed research in dietary phytase has been conducted with the realization that phytase provides a cost-effective alternative to inorganic P supplementation in regions with dense populations and intensive livestock production, where excessive P in animal waste is a national concern (Campbell and Bedford, 1992).

a) Effects of microbial phytase on phytate phosphorus availability

Nelson *et al.* (1976) incubated soybean meal with crude phytase extracts prior to

feeding and found that chicks utilized the hydrolyzed phytate P as efficiently as the P from inorganic sources. Phytate P in un-incubated soybean meal was not utilized by chicks. In a subsequent study (Nelson *et al.*, 1971) where fungal phytase was added directly to a corn-soybean diet that was low in P, an increased bone ash content indicating hydrolysis and utilization of phytate P was observed. Similarly Rojas and Scott (1969) reported almost complete hydrolysis of phytate in cottonseed meals following treatment with a phytase from *Aspergillus ficuum*. In a study with broiler chickens, Simons *et al.* (1990) have shown that microbial phytase supplementation to a low-P corn-soybean diet increased the availability of P by 60% and the amount of P in the droppings decreased by 50% and growth rate and feed conversion ratio on the low-P diet containing microbial phytase were comparable to or even better than those obtained on the control diet. There are several other studies indicating that microbial phytase supplementation increased the availability of phytate P in broiler chickens (Nelson *et al.*, 1968; Denbow *et al.*, 1995; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996) and turkeys (Yi *et al.*, 1995; Qian *et al.*, 1996a). Reported improvements in P availability by phytase supplementation are generally in the range of 20 to 45%. The amount of phytate P released by microbial phytase is dependent on the level (Kornegay *et al.*, 1996) and source (Simons *et al.*, 1990) of added phytase and dietary phytate, dietary level of non-phytate P (Ravindran *et al.*, 1995b; Yi *et al.*, 1995), dietary Ca level (Schoner *et al.*, 1993), vitamin D_3 level (Edwards, 1993; Qian *et al.*, 1995) and Ca:P ratio (Schoner *et al.*, 1993; Qian *et al.*, 1995). Other factors that are reported to influence phytate hydrolysis include temperature, moisture, pH and incubation time (Han and Wilfred, 1988; Simons *et al.*,

1990; Nair *et al.*, 1991). Unlike the plant phytase, microbial phytase is active over a wide range of pH, from 2.5 to 5.5 (Simons *et al.*, 1990) and thus it can be active within the proventriculus. Studies involving cannulated pigs have shown that over 85 % of the hydrolysis of phytate by microbial phytase takes place in the stomach (Jongbloed *et al.*, 1992). A similar scenario probably exists in poultry with phytate hydrolysis mainly occurring within the proventriculus. This may explain, at least in part, the effectiveness and consistency of microbial phytase compared to those of plant origin. Obviously more research is needed to resolve the factors that may influence the efficacy of phytase enzyme in poultry diets and these factors include dietary levels of Ca, P and vitamin D_3. Future studies should also define the influence of Ca:non-phytate P ratio rather than Ca: total P ratio. Edwards (1993) indicated that vitamin D_3 and dietary phytase activity may function synergistically to enhance phytate P utilization. Studies indicate that high Ca levels may be detrimental to phytase activity (McCuiag *et al.*, 1972; Lei *et al.*, 1994). Efficacy of microbial phytase is probably different for various classes of poultry, due to differences in P and Ca requirements. However, little is known about this aspect. Studies reported by Simons *et al.* (1992) show that supplemental phytase is effective in layer diets despite their high Ca contents.

b) Effect of microbial phytase on availability of cations

As previously discussed, phytic acid has tremendous chelating potential and forms a variety of complexes with cations and proteins, rendering these nutrients biologically unavailable. Theoretically, when phytic acid is hydrolysed by microbial phytase, all minerals bound to it will be released. There is ample evidence to indicate that microbial

phytase supplementation improves the availability of Ca in broiler chickens (Schoner *et al.*, 1991; Broz *et al.*, 1994; Yi *et al.*, 1994b; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996) and turkeys (Qian *et al.*, 1996a). In a broiler study designed to measure the effect of phytase on Ca availability, Schoner *et al.* (1994) reported that 500 U of microbial phytase were equivalent to 0.35 g Ca, as measured by body weight gain and 0.56 g Ca as measured by phalanx ash. Qian *et al.* (1996a) reported that both P and Ca retention were sensitive to the addition of phytase at varying nonphytate levels of Ca:total P ratios in turkeys. Calcium retention increased linearly as the amount of supplemented phytase increased, and decreased as the Ca:total P ratio became wider and as the level of nonphytate increased (Qian *et al.*, 1996a).

Studies on the influence of phytase on other phytate-bound minerals are limited. Pallauf *et al.*, (1992) found that phytase addition increased the apparent absorption of Mg, Zn, Cu and Fe by up to 13, 13, 7, and 9%, respectively, in pigs. The addition of 800 U/kg of phytase to a diet containing 27 mg /kg of Zn increased the retention of Zn and decreased Zn excretion of chicks (Thiel and Weigand,1992). Thiel *et al.*, (1993) reported that the femoral Zn content of chicks fed a diet containing 30 mg Zn /kg plus 700 U of phytase /kg diet was equal to that of chicks fed a diet containing 39 mg of Zn/ kg without phytase. Roberson and Edwards (1994) reported that phytase supplementation had no effect on Zn retention in broiler chicks, but improved its retention when given along with vitamin D_3. In a study conducted in our laboratory (Sebastian, *et al.*, 1996), it has been shown that microbial phytase supplementation increased the relative retention of Zn by 62.5 percentage units compared to control in broiler chickens fed a low P corn-

soybean meal diet. Yi and his coworkers (1996a) reported that Zn retention was linearly increased by adding microbial phytase in broiler chickens fed a low Zn basal diet and they indicated that 0.9 mg of Zn was released per 100 U of phytase over a range of 150 to 600 U phytase.

Aoyagi and Baker (1995) studied the effect of microbial phytase supplementation on dietary Cu utilization in chickens; they found that phytase supplementation to soybean meal had no effect on Cu availability and in fact it reduced the Cu bioavailability by 21%. Sebastian *et al.*, (1996) showed that microbial phytase supplementation increased the relative retention of Cu by 19.3 percentage units in broiler chickens fed a low P corn-soybean meal diet. Studies on the influence of microbial phytase on other minerals are very limited.

c) Effects of microbial phytase on availability of protein and amino acids

Theoretically, phytase supplementation must also be able to release the phytate-bound protein for utilization, but published data on this aspect are scanty. Officer and Batterham (1992) observed improvements of 7-12% in the ileal digestibility of protein and essential amino acids in pigs fed with a supplemental microbial phytase. Several studies have reported that phytase supplementation improved nitrogen digestibility in pigs (Mroz *et al.*, 1994; Yi *et al.*, 1994a) and nitrogen retention in broiler chickens (Farrell *et al.*, 1993) and in laying hens (Van der Klis and Versteegh, 1991). Yi *et al.* (1996b) determined the effect of phytase on N and amino acid digestibility in female turkeys; they found that at a dietary level of 0.45% nonphytate P, adding phytase to 22.5% CP diets tended to improve the apparent and true ileal digestibility of N and amino acids with the

exception of cystine or methionine. However, in a recent study with broiler chickens, Newkirk and Classen (1995) showed that supplementation of either semi-purified phytase or crude phytase had no significant effect on crude protein digestibility. The possible effect of phytase on protein and amino acid utilization is of immense practical interest and needs to be confirmed.

d) Effect of microbial phytase on growth performance in broiler chickens

There are several studies which indicate that microbial phytase supplementation increases body weight gain and feed intake in broiler chickens (Simons, *et al.*, 1990; Broz *et al.*, 1994; Denbow *et al.*, 1995; Michell and Edwards, 1996; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996), turkeys (Ravindran *et al.*, 1995b; Yi *et al.*, 1996b) and pigs (Beers and Jongbloed, 1992; Cromwell *et al.*, 1993; Lie *et al.* 1994; Young *et al.*, 1993; Qian *et al.*, 1996b). The improvements in growth performance observed in chickens fed a low P diet with phytase may be due to: a) the release of minerals from the phytate-mineral complex b) the utilization of inositol by animals as suggested by Simons *et al.*, (1990) c) increased starch digestibility as suggested by Knuckles and Betschart (1987) or d) increased availability of protein. However, Perney *et al.*, (1993) reported that dietary phytase supplementation to a corn-soybean diet did not improve the body weight gain and feed intake but increased the toe and tibia ash and plasma inorganic phosphorus in broiler chickens. Several reports have indicated that microbial phytase supplementation had no significant effect on feed-gain ratio in broiler chickens (Simons *et al.*, 1990; Broz *et al.*, 1994; Denbow *et al.*, 1995; Sebastian *et al.*, 1996) as a result of a simultaneous increase in body weight gain and feed intake.

e) Influence of dietary calcium and calcium:phosphorus ratio on the efficacy of microbial phytase

Excess dietary Ca will interfere with the availability of P as well as Mg, Mn, and Zn (NRC, 1994). The dietary Ca and the Ca:phytate ratio may be important factors that determine the extent of phytate hydrolysis (Wise, 1983; Ballam *et al.*, 1984). Excess dietary Ca can progressively precipitate all the phytate by forming an insoluble Ca-phytate complex in the intestine (Nelson and Kirby, 1987); consequently phytate P as well as Ca itself, is largely unavailable for absorption (Wise, 1983). High levels of dietary Ca and Mg are known to reduce intestinal phytase activity in chicks (McCuaig *et al.*, 1972). Only a few studies have investigated the influence of dietary Ca level (Ca:P ratio) on the efficacy of phytase in broiler chickens. Schoner *et al.*, (1993) reported that microbial phytase supplementation to a diet containing a high level of dietary Ca with a constant level of P (0.35%) reduced body weight gain and feed intake and P and Ca retention compared to a diet containing low dietary Ca plus microbial phytase. In a study with turkeys, Qian *et al.*, (1996a) showed that the largest response to dietary Ca:total P and supplemental phytase was achieved when the dietary Ca:total P ratio was low, with phytase supplementation of 600 U / kg of the diet. Lei *et al.* (1994) reported that supplemental microbial phytase improved the phytate P utilization more efficiently at moderately low levels of dietary Ca than at normally recommended levels in pigs.

1.3.3 Economics of using microbial phytase

In the long term, economics will determine the acceptability of phytase by the feed industry. This will depend not only on the magnitude of its efficacy, but also on the

cost, product stability and ease of application. Yi *et al.* (1994b) estimated that the cost of adding phytase enzyme to broiler diets is 1.3 times the cost of adding an equivalent amount of inorganic P. One can speculate that much attention will be centered in future studies on the effects of phytase on nutrients other than P and this, in turn, may increase the cost effectiveness of the enzyme.

1.4 Summary and conclusion of literature review

Phytate is a naturally occurring organic complex in plant materials. It is generally assumed that two-thirds of the total P in feedstuffs of plant origin is phytic acid and is biologically less available to poultry due to a lack of phytase that hydrolyzes this compound. Phytase, either of microbial origin or endogenous to certain ingredients, must be present in poultry diets in order to hydrolyze significant amounts of phytate. The structure of phytic acid is suggestive of tremendous chelating power. Phytic acid being a strong acid forms a wide variety of insoluble salts with di- and trivalent cations. Phytic acid is also known to complex with protein under both acidic and alkaline conditions and subequently to reduce the availability of protein. Recent studies indicate that phytic acid reduces the activity of pepsin, trypsin and α-amylase. Developments in genetic engineering have paved the way for the production of large quantities of microbial phytase. It is well documented that microbial phytase supplementation increases the availability of phytate P; however, data on the influence of phytase on trace minerals is scanty. In future, the effect of phytase supplementation on the availability of other minerals including trace minerals should be investigated. One area requiring immediate attention is the optimum dietary conditions to achieve maximum phytate degradation with

supplemental phytase within the gastrointestinal tract. Obviously more research is needed to resolve the factors that may influence the efficacy of phytase enzyme in poultry diets and these factors include dietary levels of Ca, P and vitamin D_3. Future studies should also define the influence of the ratio of Ca:non-phytate P ratio rather than that of Ca: total P. The role of inositol liberated from phytic acid by microbial phytase has not been yet investigated. It is imperative that the effects of phytase on the bioavailability of protein and amino acids be evaluated.

Preface to Chapter II

Chapter II is comprised of materials contained in a manuscript by S.Sebastian, S. P. Touchburn, E. R. Chavez and P. C. Laguë which has been published in Poultry Science. The format has been changed to be consistent within this thesis, according to the guidelines set by the Faculty of Graduate Studies and Research. All literature cited in this chapter is listed in the reference section at the end of the thesis. Each table for chapter II is presented at the end of this chapter.

In this experiment, the role of supplemental microbial phytase on growth performance, relative retention of Ca, P, Cu and Zn and bone mineralization in broiler chickens fed a low P corn-soybean meal diet was investigated.

Chapter II

The Effects of Supplemental Microbial Phytase on the Performance and Utilization of Dietary Calcium, Phosphorus, Copper and Zinc in Broiler Chickens Fed Corn-Soybean Based Diets

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The Effects of Supplemental Microbial Phytase on the Performance and Utilization of Dietary Calcium, Phosphorus, Copper and Zinc in Broiler Chickens Fed Corn-Soybean Based Diets

ABSTRACT

A 3-wk feeding trial with 180 sexed day-old broiler chickens was conducted to study the efficacy of microbial phytase (Natuphos 1000) on growth performance, relative retention of P, Ca, Cu, and Zn, and mineral contents of plasma and bone. Treatments involved a normal P level corn-soybean diet, a low P diet and a low P plus phytase (600 Phytase units / kg) diet. Phytase supplementation increased ($P \leq 0.05$) body weight in male and female chickens by 13.2 and 5.8 %, respectively at 21 d. The improvements yielded body weights comparable to those obtained on the normal P diet. Phytase supplementation overcame ($P \leq 0.05$) the depression of feed intake observed on the low P diet. Treatments had no effect ($P > 0.05$) on feed-gain ratio. Phytase supplementation of the low P diet increased ($P \leq 0.05$) the relative retention of total P, Ca, Cu and Zn by 12.5, 12.2, 19.3 and 62.3 percentage units, respectively, in male chickens. However, in female chickens, phytase supplementation had no effect ($P > 0.05$) on the relative retention of any minerals measured except Zn. Microbial phytase increased the plasma P by 15.7 % and reduced ($P \leq 0.05$) the Ca concentration by 34.1%, but had no effect ($P > 0.05$) on plasma concentrations of Cu or Zn. Phytase supplementation increased the percentage ash in both head and shaft portions of dry, fat-free tibia bone to a level comparable to that of the normal P diet. Phytase supplementation had no effect ($P > 0.05$) on the concentration of any of the minerals measured in whole tibia ash but did

increase ($P \leq 0.05$) the DM percentage of P and Ca in tibia-head of male chickens by .65 and 1.4 percentage units, respectively. These results show that microbial phytase supplementation of a low P diet increased growth and relative retention of total P, Ca, Cu, and Zn and improved bone mineralization in broiler chickens.

(*Key words*: phytase, broiler, phosphorus, calcium, zinc, copper)

INTRODUCTION

Plant materials are the major constituents of poultry diets. About two-thirds of the phosphorus of plant origin is present as phytic acid in the form of myo-inositol phosphates (Cromwell,1980). Phosphorus in the phytic acid form is poorly available to monogastric animals because they lack phytase, the enzyme that hydrolyzes phytic acid into inositol and orthophosphate (Peeler, 1972). It is well documented that microbial phytase supplementation improves the availability of phytate-bound phosphorus in broiler chickens (Nelson *et al.*, 1971; Simons *et al.*, 1990; Roberson and Edwards, 1994). However, there is little information about the availability of trace minerals when the broiler diet is supplemented with microbial phytase.

Phytate, being a strong acid can form various salts with the important minerals such as calcium, magnesium, copper, zinc, iron and potassium, thus reducing their solubility (Eardman, 1979). Nutritionally more important is the fact that maximum binding of zinc-calcium-copper-phytate as well as copper-calcium-phytate occurs at pH 6, which is the normal pH of the duodenum where maximum absorption of divalent cations takes place (Oberleas, 1973). When phytic acid is hydrolyzed by microbial phytase it may release all phytate-bound minerals. However, in a very recent study,

Aoyagi and Baker (1995) have shown that microbial phytase supplementation reduced the copper utilization by 50 % in chickens fed soybean meal. The authors speculated that phytase may have increased the zinc bioavailability in soybean meal and the released zinc might have had an antagonized effect on absorption of copper. In another study with broiler chickens, Roberson and Edwards (1994) have shown that phytase addition to a corn-soybean meal diet did not affect the zinc retention whereas phytase plus 1,25-$(OH)_2D_3$ increased zinc retention. The lack of information and contradictions concerning the efficacy of phytase on the availability of trace minerals indicate the need for more investigation.

Therefore, the objectives of this study were to determine the effects of microbial phytase supplementation on the performance of broiler chickens fed low phosphorus corn-soybean meal diets, and to study the efficacy of microbial phytase on the apparent availability of Ca, P, and trace minerals such as Cu and Zn and on the mineral contents of bone and plasma.

MATERIALS AND METHODS

Experimental Design: A total of 180 sexed day-old Ross x Indian River broiler chicks were purchased from a commercial hatchery[1], wing-banded and weighed individually prior to the experimentation. Fifteen birds were assigned to each of 12 pens (6 pens of each sex) and housed in thermostatically controlled Petersime[2] battery brooder

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[1] René Poirier Ltée, St. Félix-de-Valois, QC. Canada JOK 2MO

[2] Gettysburg, OH 45328

with raised wire floors. All birds had *ad libitum* access to water and experimental diet from day-old to 21 d and they received 24 h light / d. Each of three dietary treatments was replicated two times per sex. The experimental design was a completely randomized one with factorial arrangements of treatments. The experimental diets (Table 2.1) were as follows: 1) corn-soybean meal diet, no enzyme (control) ; 2) corn-soybean (low phosphate), no enzyme;3) corn-soybean (low phosphate) plus Phytase (a commercially available microbial phytase preparation, Natuphos 1000[3], was added at 600 Phytase Units / kg diet). According to the manufacturer's instructions, one unit of phytase is defined as the quantity of enzyme which sets free 1 μmol of inorganic phosphorus per minute from .0015 mol / L sodium phytate at pH 5.5 at 37 C. Individual body weights of chickens and group feed consumption data were recorded on days 7, 14 and 21.

Apparent Availability of Minerals

On the first 3 d of week 2 and 3 of the experiment, the daily feed consumption and total fecal output were recorded. A representative sample of excreta and feed from each pen was freeze-dried, ground, and analyzed for mineral content. Calcium, copper, and zinc were determined by flame atomic absorption spectrophotometer[4] after wet ashing with HNO_3. Phosphorus was determined by the alkalimeter ammonium molybdate method (AOAC, 1990) and the color intensity read in a UV/VIS spectrophotometer[5] at

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[3] BASF Canada, Inc., Georgetown, ON. Canada L7G 4R7.

[4] Perkin Elmer, Model 2380, Norwalk, CT. 60521

[5] Beckman, Model DU-20, Fullerton, CA. 92713

400 nm. All samples were assayed in duplicate. The difference in the mineral content of the feed consumed and of the feces excreted was used to calculate the apparent availability of minerals.

Plasma Analysis

On the last day of the experiment, heparinized blood was obtained by cardiac puncture from three randomly selected chickens in each replicate. Plasma was separated immediately by centrifugation of blood for 10 min. at 2,000 x g. Plasma calcium, copper, zinc, and total phosphorus were determined as indicated in the mineral analyses of the feed and excreta samples.

Bone Analysis

The birds used for blood sampling were killed immediately afterwards by cervical dislocation and the left tibia was removed. After removing any adhering tissue, both ends (heads) were separated from the shaft portion of the tibia for separate mineral analysis. Both head and shaft portions were freeze-dried, fat extracted, and then analyzed for ash minerals (AOAC, 1990) on a fat free dry basis. Bone calcium, copper, zinc, and phosphorus were determined as indicated in the mineral analyses of feed and excreta.

Statistical Analysis

The data were analyzed using the general linear models procedure for analysis of variance (SAS Institute, 1990). Significant differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955) with a 5% level of probability.

RESULTS

Feed Intake, Body Weight and Feed to Gain Ratio

The effects of phytase supplementation on growth performance are summarized in Table 2.2 Treatment effect on body weight was significant ($P < 0.05$) at 14 and 21 d. There was a significant effect of sex on body weight at d 7,14, and 21. However, interaction between treatment and sex was not significant ($P > 0.05$) for any of the growth variables measured. Compared to the normal P diet, the low P diet consistently reduced the feed intake as well as the live weight of both male and female chickens throughout the experiment although the difference reached significant levels ($P \leq 0.05$) within each sex only at 21 d. Phytase supplementation of the low P diet consistently increased the body weight of both male and female chickens compared to those fed the low P diet; however, the improvement in body weight was significant ($P \leq 0.05$) only at 21 d where phytase supplementation increased the body weight in male and female chickens by 13.2 and 5.8 %, respectively. Dietary phytase supplementation did not affect ($P > 0.05$) the efficiency of feed conversion for either male or female chickens throughout the experiment.

Apparent Availability of Minerals

The effects of phytase supplementation on relative retention of minerals are summarized in Table 2.3. The treatment effects were significant ($P \leq 0.05$) mostly at d 17, whereas effect of sex and the interaction between treatment and sex were not significant ($P > 0.05$) for any of the minerals measured. The low P diet did not change ($P > 0.05$) the relative retention of P. Phytase supplementation of the low P diet

increased ($P \leq 0.05$) the P retention by 12.4 percentage units in males and even though the improvement in P retention in female chickens was not significant ($P > 0.05$), it showed a 6.5 percentage units improvement. The low P diet reduced ($P \leq 0.05$) the Ca retention by 9.1 percentage units in male chickens at 17 d but the reduction in the females was not significant ($P > 0.05$). Phytase supplementation of the low P diet increased ($P \leq 0.05$) Ca retention by 12.2 percentage units in males but this increment was not observed in female chickens. The low P diet significantly reduced ($P \leq 0.05$) the retention of Cu in male chickens but the reduction observed in female chickens was not significant ($P > 0.05$). Phytase supplementation increased ($P \leq 0.05$) the retention of Cu by 19.3 percentage units in male chickens whereas there was no improvement ($P > 0.05$) in female chickens compared to the low P diet.

There was a significant reduction in the retention of Zn in both male and female chickens fed the low P diet. Phytase supplementation increased ($P \leq 0.05$) the Zn retention by 62.3 percentage units in male chickens and by 44.3 percentage units in female chickens.

Plasma Minerals

The effects of phytase supplementation on plasma mineral levels are summarized in Table 2.4. Treatment effect was significant for P and Ca but not for Cu or Zn. The effect of sex on plasma minerals was not significant ($P > 0.05$) except for P ($P < 0.04$). The interaction between treatment and sex was not significant for any of the plasma minerals measured.

The low P diet reduced ($P \leq 0.05$) plasma P in both male and female chickens

by 19.1 and 26.3 %, respectively. Phytase supplementation increased the plasma P in male and female chickens by 15.7 and 20.7 %, respectively and the improvement became significant ($P \leq 0.05$) when the values for both sexes were combined. The values for phytase supplemented birds were comparable with plasma P levels in chickens fed the normal P diet. The low P diet signficantly increased ($P \leq 0.05$) plasma Ca concentration in both male and female chickens by 34.1 and 22.6 %, respectively. Phytase enzyme supplementation reduced ($P \leq 0.05$) plasma Ca for both male and female chickens by 11.5 and 16.7 %, respectively. Plasma Cu and Zn were not significantly affected ($P > 0.05$) by either the low P diet or phytase supplementation.

Bone Minerals

The effect of phytase on mineral concentrations in the ash of tibia are summarized in Table 2.5. The treatment effect on tibia ash content of head and shaft portions was significant ($P \leq 0.05$). However the effect of sex and interaction of treatment and sex were not significant. The low P diet significantly reduced ($P \leq 0.05$) the ash content of both the head and the shaft portion of the tibia bone in both sexes. However, the reduction in ash content was more significant ($P < 0.0004$) in the head portion compared to the shaft portion ($P < 0.008$) in both sexes. It is interesting to note that the mineral concentrations in the ash of the tibia shaft were relatively constant and were not affected ($P > 0.05$) by either the low P diet or phytase supplementation. The effects of treatment, sex and their interaction on P and Ca concentrations in the tibia bone ash were not significant. Neither the low P diet nor phytase supplementation caused any change ($P > 0.05$) in the concentration of P and Ca in the ash of either portion of tibia for

either sex. One exception was that the low P diet increased ($P \leq 0.05$) the Ca concentration in the ash of tibia head of male chickens. The treatment effects on the concentrations of Cu and Zn were significant ($P \leq 0.05$) for the ash of the head portion but not for the shaft portion of the tibia. The low P diet significantly increased ($P \leq 0.05$) Cu concentration in the head by 2.7 percentage units in males and by 3.3 percentage units in females. Phytase supplementation to a low P diet significantly decreased ($P \leq 0.05$) the Cu concentration in the head portion by 2.5 percentage units in male and by 2.2 percentage units in female chickens. The low P diet significantly increased ($P \leq 0.05$) Zn concentration in the ash of the tibia head by 188 ppm in female chickens. Phytase supplementation, although not significant, decreased Zn concentration by 130 and 140 ppm in male and female chickens, respectively.

The effects of phytase on the minerals present in the DM of tibia are summarized in Table 2.6. The treatment effects on the concentrations in tibia DM were significant for P and Ca but not for Cu and Zn. The low P diet significantly reduced ($P \leq 0.05$) the P content in the tibia head by 1.2 and 1.6 percentage units in male and female chickens, respectively. Neither the low P diet nor phytase supplementation to a low P diet had effect ($P > 0.05$) on any mineral content in the DM of the tibia shaft, except low P diet significantly ($P \leq 0.05$) reduced the P content in tibia shaft of male chickens. Phytase supplementation to a low P diet significantly increased ($P \leq 0.05$) the P content in the tibia head by .65 percentage units in male chickens. The low P diet significantly reduced ($P \leq 0.05$) Ca content in the DM of tibia head by 1.9 and 4.2 percentage units in male and female chickens, respectively. Phytase supplementation to a low P diet

significantly increased ($P \leq 0.05$) Ca content in tibia head by 1.4 percentage units in male chickens but the improvement in female chickens was not significant. Neither a low P diet nor phytase supplementation to a low P diet had any effect ($P > 0.05$) on the Cu or Zn levels in whole tibia bone.

DISCUSSION

The results of this study show the effects of microbial phytase supplementation on growth performance, apparent availability of minerals as well as plasma and bone mineral contents as indications of nutrient utilization by broiler chickens fed a corn-soybean diet. Phytic acid has been regarded as the primary storage form of both phosphate and inositol in almost all seeds (Cosgrove, 1966). Approximately 66 % of the P in corn and 61 % of the P in soybean meal is in the form of phytic acid (Nelson *et al.*, 1968). The inability of young broiler chickens to utilize phytic acid has been clearly demonstrated in this study by a slower growth rate (Table 2.2), a low concentration of plasma P and reduced bone mineralization observed in chickens fed the low P diet (Table 2.5) in which most of the P was provided by corn and soybean meal. Phytase supplementation of the low P diet yielded a significant improvement in body weight for both sexes at 21 d, compared to chicks fed the low P diet without phytase supplementation. The body weights achieved by phytase supplementation were comparable to those obtained in the control diet (Table 2.2) which contained a source of inorganic P to satisfy the requirement (NRC, 1994). The efficacy of phytase on improving body weight was greater for male chickens (13.2 %) than for females (5.8 %). Similar observations of improved body weight with phytase supplementation have

been reported for broiler chickens (Simons *et al.*, 1990; Broz *et al.*, 1994). The improvements in growth performance observed in the chickens fed phytase may be due to: a) the release of minerals from the phytate-mineral complex and b) the utilization of inositol by animals as suggested by Simons *et al.*,(1990) or c) increased starch digestibility as suggested by Knuckles and Betschart (1987) or d) increased availability of protein. Phytate also complexes with proteins making them less soluble (Smith and Rackis, 1957). It has been shown that phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Hill and Tyler, 1954). It may be possible that phytase liberates proteins from the complex, making them more available to the animal. However, further investigations are needed to determine the effect of phytase on availabilities of starch, protein and inositol. Phytase supplementation overcame the depression of growth rate observed on the low P diet. As a result of the simultaneous increase in both body weight and feed intake, no significant differences in feed to gain ratio were observed. Our findings might be the result of a combination of improvement in nutrient utilization not only of minerals but also of energy and protein. These results agree with the findings of Simons *et al.* (1990) and Perney *et al.* (1993), who did not find any significant improvements in feed conversion of broiler chickens fed a corn-soybean diet supplemented with phytase.

As expected, phytase supplementation increased the relative retention of P by 12.5 percentage units in male chickens (Table 2.3), which agrees with results of previous studies dealing with chickens (Simons *et al.*, 1990; Broz *et al.*, 1994) and pigs (Young *et al.*, 1993; Lei *et al.*, 1994; Mroz *et al.*, 1994; Bruce and Sundstol, 1995). It is

uncertain why female chickens failed to show significant improvement in the relative retention of P (Table 2.3). Phytase supplementation also improved the relative retention of Ca in male chickens. This improvement was expected because phytase liberates Ca from the calcium-phytate complex and as the availability of P increases, the availability of Ca also increases because both are part of the same complex.

In this experiment, phytase supplementation to the low P diet significantly improved the retative retention of both Zn and Cu. This contradicts recent studies in which phytase supplementation to soybean meal did not improve the utilization of Cu (Aoyagi and Baker, 1995) or Zn (Roberson and Edwards, 1994). Phytase supplementation increased the Zn relative retention by almost 62.3 percentage units compared to the low P diet without phytase supplementation (Table 2.3). This highly significant improvement in Zn relative retention may be due to higher availability of Zn from the phytate-mineral complex. This observation emphasizes the need to reevaluate the Zn requirement when the broiler diet is supplemented with phytase. Even though the phytase supplementation significantly increased the relative retention of Cu compared to the low P diet without phytase, it failed to reach the level of Cu relative retention obtained in the normal P diet. The possible explanation for this observation is that the higher concentration of Zn as the result of phytase activity induces the intestinal synthesis of metallothionein (Blalock *et al.*, (1988), a cysteine-rich metalloprotein, which binds zinc, copper and other divalent cations; copper is much more tenaciously bound to metallothionein than zinc and metallothionein appears to serve primarily as a negative regulator of copper absorption (Cousins, 1985). L'Abbe and Fischer (1984) have also

shown that excess dietary Zn aggravates the signs of low Cu status in rats. We have no obvious explanation why the low P diet significantly reduced the apparent relative retention of Cu and Zn. It may be possible that the higher content of Ca relative to P in the low P diet increased the intestinal pH and reduced the soluble fraction of minerals, consequently reducing their availability for absorption (Shafey, 1993).

Plasma P was increased by phytase supplementation to a level comparable to that of the control diet (Table 2.4) and this concurs with other studies reported in chickens (Perney *et al.*, 1993; Broz *et al.*, 1994) and pigs (Young *et al.*, 1993). The low P diet significantly increased the plasma Ca for both male and female chickens. This was expected because a low P diet normally results in an elevated ionized Ca in the plasma. This depresses the release of parathyroid hormone (PTH) thus reducing the PTH inhibition on tubular reabsorption of phosphate and permitting the urinary excretion of additional Ca absorbed from the gut during low P diet feeding (Tayler and Dacke, 1984). In contradiction to our observation, some recent studies have shown that phytase had no significant effect on plasma Ca (Edwards,1993; Roberson and Edwards, 1994). Phytase supplementation did not show any significant effect on plasma Zn (Table 2.4) and this agrees with Roberson and Edwards (1994) who found no significant effect on plasma Zn when a corn-soybean diet supplemented with phytase was fed to broiler chicks; these authors suggested that an adequate level of Zn in the diet might be responsible for the failure to show the significant effect on plasma Zn. Phytase supplementation likewise did not affect the plasma Cu (Table 2.4).

The percentage of tibia ash was significantly improved by addition of dietary

phytase (Table 2.5), an observation which agrees with the previous studies dealing with chickens (Nelson, 1971; Perney *et al.*, 1993; Broz *et al.*, 1994) and pigs (Young *et al.*, 1993). The improvement in ash percentage in tibia bone is a good indication of increased bone mineralization due to the fact that there is an increased availability of P, Ca, Zn, and Cu from the phytate-mineral complex by the action of phytase. The shaft portion of the bone represents the more rigid state of the bone, while the head is a more active state of change and consequently more susceptible to variation due to availability of minerals. The concentrations of all minerals measured were high in the shaft portion compared to the head portion except for Cu which was more concentrated in the head portion (Table 2.5). Either low P or phytase supplementation did not affect the concentration of P, Ca, Cu and Zn in whole tibia ash (both head and shaft portions); however, phytase supplementation significantly improved the content of P and Ca in the DM of tibia head. In agreement with our observation, Broz *et al.* (1994) showed that phytase supplementation of a corn-soybean diet increased the tibia ash percentage in chickens. However, they could not find any significant difference in P and Ca concentration in tibia ash of broiler chickens. In a recent study with pigs, Young *et al.* (1993) found that phytase supplementation increased bone weight, ash percentage, weight of ash, weight of Ca and P in the dry fat-free third metatarsal bone but the concentration of P in the ash had not been affected by phytase supplementation. The ash, Cu and Zn of the head of the tibia bone was significantly affected by the dietary treatment while in the shaft portion only the ash content was affected. Dietary treatments had an effect on Ca and P levels in the tibia head DM but not in that of Cu and Zn.

Dietary microbial phytase supplementation to a low P corn-soybean diet improved the growth, the feed consumption, the apparent availability of Ca, P, Cu, and Zn, plasma P, percentage of ash, and content of Ca and P in the DM of tibia head of broiler chickens. The efficacy of phytase, particularly in stimulating growth, is higher in male than female chickens. The results obtained in this study clearly indicate the importance of reevaluating mineral requirements, particularly Zn, of broiler chickens when the diet is supplemented with phytase enzyme. This study shows that microbial phytase not only reduces the need for inorganic P but also serves to reduce the need for some other minerals in the diet.

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Table 2.1. Composition of experimental diets (%)

	Corn-soybean	Corn-soybean (low-P)
Ingredient		
Corn, ground	58.0	58.7
Soybean meal	32.1	32.1
Fish meal	3.0	3.0
AV- fat[1]	3.3	3.3
Calcium carbonate	1.8	1.9
Calcium phosphate	0.8	-
Salt	0.2	0.2
Vitamin -mineral premix[2]	0.5	0.5
DL-Methionine	0.2	0.2
L-Lysine. HCl	0.1	0.1
Analyzed composition		
Calcium	1.4	1.3
Total phosphorus	0.7	0.5
Copper (ppm)	9.0	7.8
Zinc (ppm)	70.9	68.9
Calculated composition		
Crude protein	22.3	22.4
ME (Kcal/kg)	3119	3143
Available phosphorus (%)	0.46	0.33
Lysine	1.3	1.3
Methionine + Cysteine	0.9	0.9

[1] Animal-vegetable fat blend

[2] Vitamin-mineral premix supplied the following per kilogram of diet: calcium, 1000 mg; phosphorus, 450 mg; magnesium, 25 mg; sodium chloride, 550 mg; selenium, 0.04 mg; iron, 25 mg; manganese, 22 mg; copper, 1.6 mg; zinc, 16 mg; iodine, 0.14 mg; retinyl acetate, 925 IU; cholecalciferol, 275 IU; dl-α-tocopherol acetate, 5 IU; choline, 40.8 mg; menadione sodium bisulfite, 0.19 mg; riboflavin, 1.9 mg; vitamin B_{12} , 2.2 mg; niacin, 8.1 mg; pantothenic acid, 2.2 mg; biotin, 0.013 mg; folic acid, 0.16 mg; thiamin, 0.5 mg; pyridoxine, 0.9 mg; salinomycin sodium, 7.5 mg

Table 2.2. The effect of phytase supplementation on feed intake (g), body weight (g) and feed to gain ratio (g:g) of broiler chickens fed corn-soybean diets for 21 days.

Diet	Sex	0 to 7 days			0 to 14 days			0 to 21 days		
		Feed intake	Body weight	Feed: gain	Feed intake	Body weight	Feed: gain	Feed intake	Body weight	Feed: gain
Corn-soy (Control)	Male	154	143	1.52	477	355	1.53	910[a]	639[a]	1.52
Corn-soy (low P)		133	130	1.48	394	303	1.49	764[b]	549[b]	1.50
Corn-soy (low P) + phytase		147	136	1.57	449	332	1.54	867[ab]	622[a]	1.49
SEM		9.4	5.7	0.02	28.4	15.2	0.01	30.1	20.5	0.02
Corn-soy (Control)	Female	135	128	1.60	450	320	1.62	842[a]	587[a]	1.54
Corn-soyn (low P)		124	120	1.57	379	286	1.57	733[b]	514[c]	1.55
Corn-soy (low P) + phytase		131	118	1.75	397	287	1.63	768[ab]	544[b]	1.53
SEM		7.6	6.8	0.04	19.8	8.6	0.03	20.9	4.3	0.02
Corn-soy (Control)	Male	145	136	1.56	463[a]	337[a]	1.57	876[a]	613[a]	1.53
Corn-soy (low P)	and	128	125	1.52	387[b]	294[b]	1.52	748[b]	532[b]	1.52
Corn-soy (low P) + phytase	Female	139	127	1.66	423[ab]	309[ab]	1.58	817[ab]	583[ab]	1.51
SEM		6.6	5.5	0.03	17.3	12.3	0.02	25.5	18.6	0.02
Source of variation		Probabilities								
Treatment		.231	.267	.063	.054	.034	.262	.007	.004	.909
Sex		.077	.032	.025	.175	.018	.037	.020	.004	.432
Treatment * Sex		.826	.824	.576	.756	.543	.783	.474	.403	.963

[a,b,c] Means within columns, within sex classification, with no common superscripts differ significantly ($P < .05$)

Table 2.3. The effect of phytase supplementation on relative retention (%) of total phosphorus, calcium, copper and zinc at different ages in broiler chickens fed corn-soybean diets.

		Phosphorus		Calcium		Copper		Zinc	
Diet	Sex	10 d	17 d	10 d	17 d	10 d	17 d	10 d	17 d
Corn-soy (Control)	Male	48.5^{b}	51.0^{b}	34.2	40.7^{a}	3.6	8.5^{a}	16.6^{ab}	8.2^{ab}
Corn-soy (low P)		51.3^{ab}	51.0^{b}	36.5	31.7^{b}	-0.8	-24.6^{c}	14.1^{b}	-27.6^{b}
Corn-soy (low P) + phytase		57.6^{a}	63.5^{a}	36.7	43.9^{a}	-4.9	-5.4^{b}	41.3^{a}	34.7^{a}
SEM		1.51	1.08	2.83	1.36	5.58	1.96	6.02	8.42
Corn-soy (Control)	Female	48.7	48.7	33.9	39.8	1.3^{ab}	4.2	9.4	-0.1^{ab}
Corn-soy (low P)		53.8	52.7	41.5	36.5	5.1^{a}	-10.0	7.3	-28.3^{b}
Corn-soy (low P) + phytase		54.7	59.2	32.2	36.7	-8.4^{b}	-10.7	-0.8	16.1^{a}
SEM		1.81	2.83	3.19	4.39	2.59	6.96	3.48	7.44
Corn-soy (Control)	Male	48.6^{b}	49.8^{b}	34.1	40.2	2.4	6.3^{a}	13.0	4.0^{b}
Corn-soy (low P)	and	52.6^{a}	51.9^{b}	39.0	34.1	2.2	-17.3^{b}	10.7	-27.9^{c}
Corn-soy (low P) + phytase	Female	56.2^{a}	61.3^{a}	34.4	40.3	-6.68	-8.1^{b}	20.3	25.4^{a}
SEM		1.12	1.50	2.07	2.37	2.78	3.99	7.75	5.70
Source of variation		Probabilities							
Treatment		.011	.003	.266	.170	.266	.010	.209	.001
Sex		.934	.378	.988	.691	.988	.702	.003	.206
Treatment * Sex		.329	.421	.356	.257	.356	.171	.017	.560

^{a,b,c} Means within columns, within sex classification, with no common superscripts differ significantly ($P < .05$)

Table 2.4. The effect of phytase supplementation on concentrations of plasma total phosphorus, calcium, copper and zinc in 21-d old broiler chickens fed corn-soybean diets.

Diet	Sex	Phosphorus	Calcium	Copper	Zinc
		(mg/dL)		(μg/dL)	
Corn-soy (Control)	Male	16.4[a]	10.8[b]	26	508
Corn-soy (low P)		13.3[b]	14.4[a]	24	520
Corn-soy (low P) + phytase		15.4[ab]	12.8[a]	16	436
SEM		0.62	0.42	6.0	40
Corn-soy (Control)	Female	15.6[a]	11.5[b]	21	406
Corn-soy (low P)		11.5[b]	14.1[a]	18	505
Corn-soy (low P) + phytase		13.8[ab]	11.8[b]	18	388
SEM		0.63	0.19	5.0	42
Corn-soy (Control)	Male	16.0[a]	11.2[c]	23	457
Corn-soy (low P)	and	12.4[b]	14.3[a]	21	512
Corn-soy (low P) + phytase	Female	14.6[a]	12.3[b]	17	412
SEM		0.55	0.29	3.0	30
Source of variation		Probabilities			
Treatment		.003	.0002	.529	.140
Sex		.036	.503	.512	.168
Treatment * Sex		.746	.081	.715	.612

[a,b,c] Means within columns, within sex classification, with no common superscripts differ significantly ($P < .05$)

Table 2.5. The effect of phytase supplementation of corn-soybean diets on the tibial bone content of ash ,total phosphorus, calcium, copper and zinc in 21-d old broiler chickens.

		Ash (%)		Phosphorus		Calcium		Copper		Zinc	
Diet	Sex	Head	Shaft	Head	Shaft	Head	Shaft	Head	Shaft	Head	Shaft
Corn-soy (Control)	Male	34.4[a]	57.3[a]	13.8	16.4	30.9[b]	36.9	12.0[b]	9.1	445	462
Corn-soy (low P)		25.5[b]	51.4[b]	13.9	15.2	34.2[a]	36.8	14.8[a]	9.0	618	487
Corn-soy (low P) + phytase		30.9[ab]	54.4[ab]	13.5	14.8	32.6[ab]	35.6	12.3[ab]	8.6	488	474
SEM		1.34	1.15	0.19	0.47	0.59	2.28	0.56	0.24	39.1	31.9
Corn-soy (Control)	Female	33.7[a]	57.4[a]	14.5	15.4	38.7	41.9	10.5[b]	7.0[b]	388[b]	392
orn-soy (low P)		23.5[b]	52.0[b]	14.2	14.3	38.8	38.4	13.8[a]	7.9[a]	576[a]	460
Corn-soy (low P) + phytase		30.3[a]	55.8[ab]	13.9	14.6	34.4	35.3	11.6[ab]	7.8[a]	436[ab]	430
SEM		0.83	1.18	0.88	0.66	1.70	2.41	0.67	0.17	36.1	15.1
Corn-soy (Control)	Male	34.1[a]	57.4[a]	14.2	15.9	34.8	39.4	11.3[b]	8.0	416	427
orn-soy (low P)	and	24.5[c]	51.7[b]	14.1	14.8	36.5	37.6	14.3[a]	8.5	597	474
Corn-soy (low P) + phytase	Female	30.6[b]	55.1[a]	13.7	14.7	33.5	35.4	11.9[b]	8.2	462	452
SEM		0.74	0.73	0.38	0.40	1.69	1.62	0.47	0.43	26.2	20.5
Source of variation		Probabilities									
Treatment		.0004	.008	.766	.147	.136	.303	.006	.181	.007	.256
Sex		.262	.469	.410	.203	.004	.315	.092	.002	.154	.063
Treatment * Sex		.805	.725	.954	.744	.141	.553	.789	.045	.980	.705

[a,b,] Means within columns, within sex classification, with no common superscripts differ significantly ($P < .05$)

Table 2.6. The effect of phytase enzyme supplementation on the DM percentage of total phosphorus, calcium, copper and zinc present in the head and shaft portion of tibia of 21-d old broiler chickens fed corn-soybean diets.

		Phosphorus		Calcium		Copper		Zinc	
Diet	Sex	Head	Shaft	Head	Shaft	Head	Shaft	Head	Shaft
Corn-soy (Control)	Male	5.4[a]	10.1[a]	10.6[a]	21.2	4.1	5.2	153	265
Corn-soy (low P)		4.2[c]	8.5[b]	8.7[b]	18.9	3.8	4.7	153	250
Corn-soy (low P) + phytase		4.9[b]	8.7[b]	10.1[a]	19.3	3.8	4.7	151	257
SEM		.09	.26	.20	1.30	.19	.14	9.0	17.3
Corn-soy (Control)	Female	5.7[a]	9.5	12.8[a]	23.8	3.6	4.0	131	225
Corn-soy (low P)		4.1[b]	8.2	8.6[b]	19.4	3.3	4.1	135	240
Corn-soy (low P) + phytase		4.9[ab]	8.9	9.9[ab]	19.3	3.5	4.4	132	240
SEM		.23	.34	.65	1.32	.19	.09	11.9	8.8
Corn-soy (Control)	Male	5.5[a]	9.8[a]	11.7[a]	22.5[a]	3.9	4.6	142	245
Corn-soy (low P)	and	4.1[c]	8.3[b]	8.6[b]	19.2[b]	3.5	4.4	144	245
Corn-soy (low P) + phytase	Female	4.9[b]	8.8[b]	10.0[b]	19.3[b]	3.7	4.5	142	249
SEM		.11	.21	.45	.88	.18	.24	8.3	10.8
Source of variation		Probabilities							
Treatment		.0007	.007	.002	.075	.273	.235	.960	.951
Sex		.825	.331	.196	.373	.024	.0004	.063	.091
Treatment * Sex		.583	.537	.083	.579	.711	.026	.973	.585

[a,b,c] Means within columns, within sex classification, with no common superscripts differ significantly ($P < .05$)

Preface to Chapter III

Chapter III is comprised of materials contained in a manuscript by S.Sebastian, S. P. Touchburn, E. R. Chavez and P. C. Laguë which has been submitted to Poultry Science. The format has been changed to be consistant within this thesis, according to guidelines set by the Faculty of Graduate Studies and Research. All literature cited in this chapter is listed in the reference section at the end of the thesis. Each table for chapter III is presented at the end of this chapter.

In the previous experiment, it was demonstrated that phytase supplementation increased the growth performance, relative retention of Ca, P, Cu and Zn and bone mineralization in broiler chickens fed a low P corn-soybean meal diet. In this experiment, the influence of dietary Ca on the efficacy of supplemental microbial phytase and the optimum dietary conditions namely dietary Ca and P levels in order to maximize the efficacy of supplemental microbial phytase were investigated in broiler chickens fed a corn-soybean meal diet.

Chapter III

Efficacy of Supplemental Microbial Phytase at Different Dietary Calcium Levels on Growth Performance and Mineral Utilization of Broiler Chickens

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Efficacy of Supplemental Microbial Phytase at Different Dietary Calcium Levels on Growth Performance and Mineral Utilization of Broiler Chickens

ABSTRACT

A 3-wk feeding trial with 240 sexed, day-old broiler chickens was conducted to determine the efficacy of microbial phytase at different levels of dietary Ca on performance and utilization of minerals in broiler chickens fed a low P corn-soybean diet. The experimental design was a 3 x 2 x 2 factorial arrangement of treatments; Ca at 0.6, 1.0,and 1.25%; phytase at 0 and 600 phytase U / kg diet; sexes. Since there were no significant differences between sexes response to dietary treatments, data of males and females were pooled. Phytase supplementation, regardless of Ca level, increased ($P \leq 0.005$) feed intake, ($P \leq 0.0001$) body weight, and ($P \leq 0.025$) feed efficiency at 21 d; the optimum levels of body weight, feed intake, and feed efficiency were obtained at the low (0.6%) dietary Ca plus phytase. The retentions of P, Ca and, N were increased ($P \leq 0.05$) by phytase supplementation. Even though the maximum retention of P and N were obtained at the 1.0 and 1.25% Ca levels, respectively, they were not significantly different from the values obtained at the 0.6% Ca. The increasing level of dietary Ca decreased plasma P ($P \leq 0.05$) and Cu ($P \leq 0.06$). Phytase supplementation had the opposite effect; i.e. increased plasma P ($P \leq 0.031$) and Cu ($P \leq 0.018$). The maximum level of plasma P was obtained with phytase at the 1.0% Ca level but this value was not significantly different from the value obtained with phytase at the 0.6% Ca level. Phytase supplementation increased ($P \leq 0.036$) the ash content of both tibia

head and shaft but had no effect on mineral contents in the ash. The optimum level of ash content was observed with the 0.6% Ca diet plus phytase. The results show that microbial phytase supplementation to a low P diet improved growth performance and mineral utilization in broiler chickens. Dietary Ca levels had a significant effect on the response to phytase; the optimum growth performance and mineral utilization were achieved at the low (0.6%) level of dietary Ca supplemented with phytase.

(*Key words*: phytase, broiler, dietary calcium, minerals, bioavailability)

INTRODUCTION

Phytate is a naturally occurring complex compound that is a main natural organic source of P in animal feedstuffs of plant origin. The phosphate present in the phytate is not available for absorption unless it is liberated from the inositol molecule by either intrinsic feed, intestinal, or microbial phytase (Sandberg *et al.*, 1993). It has been well documented that supplementation of microbial phytase increases the bioavailability of phytate P (Simons *et al.*, 1990; Denbow *et al.*, 1995) and of trace minerals such as Cu and Zn (Sebastian *et al.*, 1996) and improves body weight gain (Schoner *et al.*, 1993; Broz *et al.*, 1994) in broiler chickens.

The mineral level of the diet can also affect the degree of phytate degradation (Sandberg *et al.*, 1993). Calcium, the major dietary divalent cation for many species, can progressively precipitate the phytate by forming the extremely insoluble Ca-phytate complex in the intestine (Nelson and Kirby, 1987); consequently phytate P, as well as Ca itself, is largely unavailable for absorption. It has been reported that high levels of Ca in the diet of rats (Nahapetian and Young, 1980), poultry (Scheideler and Sell, 1987),

and pigs (Moore and Tyler 1955) decreased the availability of phytate P considerably. Mohammed *et al.* (1991) reported that diets containing both low levels of Ca and elevated levels of cholecalciferol permitted a greater utilization of phytate P and reduced the requirement of inorganic P for chickens.

High levels of dietary Mg and Ca are known to reduce intestinal phytase activities in chicks (McCuaig *et al.*, 1972). A recent study with pigs suggests that supplemental microbial phytase in a corn-soybean meal diet improves phytate P utilization more effectively at moderately low levels of dietary Ca than at normally recommended levels (Lei *et al.*, 1994). Qian *et al.* (1996a) found that the improvements caused by phytase supplementation were negatively affected when the Ca:P ratio was increased in turkey diets.

Published data are scanty on the efficacy of phytase at different levels of dietary Ca in broiler chickens. The objective of this experiment was to determine the efficacy of supplemental microbial phytase at three levels (low, recommended, and high) of dietary Ca, on the performance and utilization of macro- and trace minerals in broiler chickens fed a low P corn-soybean meal diet.

MATERIALS AND METHODS

Experimental Design

A total of 240 sexed, day-old Ross x Indian River broiler chicks was purchased from a commercial hatchery[1], wing-banded and weighed individually. Ten birds were

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[1] René Poirier Ltée, St. Félix-de-Valois, QC. Canada JOK 2MO

assigned to each of 24 pens (12 pens of each sex) and housed in thermostatically controlled Petersime[2] battery brooders with raised wire floors. All birds were exposed to continuous fluorescent light. Feed and water were provided *ad libitum* throughout the 21-d trial. Each of six treatments was replicated two times per sex. The experimental design was completely randomized with a 3 x 2 x 2 factorial arrangement of treatments. The variables included dietary levels of Ca, phytase[3] and sex: Ca at 0.66% (low), 1.0% (recommended), 1.25% (high); phytase at 0 and 600 phytase units (PU) / kg diet. According to the manufacturer's instructions, one unit of phytase is defined as the quantity of enzyme that frees 1 μmol of inorganic P / min from 0.0015 mol sodium phytate at pH 5.5 at 37°C. The calculated Ca:total P (P_t) ratios of the diets containing 0.66, 1.0 and 1.25% Ca were 1.25, 2.08, and 2.60, respectively. The low level of dietary available P (0.3%) was below the NRC (1994) requirement in all experimental diets to ensure maximum response from microbial phytase supplementation. The composition of the basal diets is given in Table 3.1. Individual body weights of chickens and group feed consumption data were recorded on Days 7, 14, and 21.

Relative Retention of Minerals

On the first 3 d of the 2nd and 3rd wk of the experiment, the daily feed consumption and total excreta were recorded. A representative sample of excreta from each 24-h collection was taken from each pen and freeze-dried, ground, and analyzed for

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[2] Gettysburg, OH 45328

[3] Natuphos 1000, BASF Canada, Inc., Ville St. Laurent, QC. Canada H4T 1Y4

mineral content. Feed samples were subjected to proximate analysis and mineral contents. Calcium, Cu, Zn, Mn, Mg and Fe were determined by flame atomic absorption spectrophotometer[4] after wet ashing with HNO_3. Phosphorus was determined by the alkalimeter ammonium molybdate method (AOAC, 1990) and the color intensity was read in a UV/VIS spectrophotometer[5] at 400 nm. Nitrogen content was determined by Leco® nitrogen analyzer[6]. All samples were assayed in duplicate. The difference in the mineral consumed and the amount of mineral excreted in the feces was used to calculate the relative retention of minerals.

Plasma Analysis

On the last day of the experiment, heparinized blood was obtained by cardiac puncture from three randomly selected chickens in each replicate of each treatment. Plasma was separated immediately by centrifugation for 10 min at 2,000 x *g*. Plasma Ca, Cu, Zn, Mg, and P were determined as indicated in the mineral analyses of the feed and excreta samples.

Bone Analysis

The birds used for blood sampling were killed immediately afterwards by cervical dislocation and the left tibia was removed. After removing any adhering tissue, both ends (heads) were separated from the shaft portion of the tibia for separate mineral analysis.

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[4] Model 2380, Perkin Elmer, Norwalk, CT 06856

[5] Model DU-20, Beckman, Fullerton, CA 92634

[6] Model FP-428, Leco® corporation, St. Joseph, MI 49085

Both head and shaft portions were freeze-dried, fat extracted, and then analyzed for ash minerals (AOAC, 1990) on a fat-free dry basis. Bone Ca, Cu, Zn, Mg, Mn and P were determined as indicated in the mineral analyses of feed and excreta.

Statistical Analysis

The data were analyzed using the General Linear Models procedure for analysis of variance (SAS Institute, 1990). Significant differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955) with a 5% level of probability. There were no significant differences between sexes of broiler response to dietary treatments; thus, data of males and females were pooled.

RESULTS

Feed Intake, Body Weight and Feed Efficiency

The effects of phytase supplementation at different levels of Ca on growth performance are summarized in Table 3.2. The interaction of Ca by phytase was not significant ($P > 0.05$) for any of the growth variables measured except for the feed intake and body weight at 14 d. Phytase supplementation to both the 1.0 and the 1.25% Ca diets increased ($P \leq 0.05$) the body weight at 14 d; however, the feed intake at 14 d was significantly reduced at the 1.25% Ca level but recovered to normal level with phytase supplementation. Regardless of phytase level, dietary Ca supplementation at the level of 1.25% reduced ($P \leq 0.05$) feed intake and body weight compared to the 0.6 and 1.0% dietary Ca at 21 d. Dietary phytase supplementation, regardless of Ca level, increased ($P \leq 0.005$) feed intake, ($P \leq 0.0001$) body weight, and ($P \leq 0.025$) feed efficiency, as measured by feed:gain ratio, at 21 d. Even though numerically the highest

feed intake and body weight and the lowest feed: gain ratio were obtained with 1.0% dietary Ca plus phytase at 21 d, these values were not significantly different from the values obtained with 0.6% Ca with phytase. Thus, it was concluded that phytase supplementation of the diet containing 0.6% Ca resulted in optimum broiler performance.

Relative Retention of Minerals

The effects of phytase supplementation at different dietary Ca levels on the relative retention of minerals and N are summarized in Table 3.3. The statistical interaction of Ca by phytase was significant ($P \leq 0.05$) only for the retention of Cu, Fe, Mn, and N. Regardless of dietary Ca level, phytase supplementation increased ($P \leq 0.008$) the relative retention of P and N ($P \leq 0.059$), decreased the relative retention of Cu, and had no effect ($P > 0.05$) on the relative retention of Ca, Mg, Fe, Mn, and Zn. Regardless of phytase level, dietary Ca at 1.0% level increased ($P \leq 0.05$) the retention of P compared to both 0.6 and 1.25% Ca levels. Although, the maximum retention of P was obtained at the 1.0% Ca with phytase, this value did not differ significantly from the value obtained at the 0.6% Ca with phytase. Calcium retention was reduced ($P \leq 0.001$) by increasing levels of dietary Ca; the maximum Ca retention was obtained at the 0.6% Ca level with supplemental phytase. At the low dietary Ca level phytase supplementation only significantly affected Cu retention, but at a recommended (1.0%) Ca level, phytase supplementation decreased ($P \leq 0.05$) retention of Cu and Mn. Phytase supplementation at a high level of dietary Ca increased ($P \leq 0.05$) the retention of N and Mn. Even though the maximum retention of N was observed at the 1.25% Ca with phytase, it did not differ significantly from the value obtained at the 0.6% Ca with

phytase. These results indicate that phytase supplementation to a diet containing 0.6% Ca showed the optimum level of relative retention of N and Ca.

Plasma Minerals

The effects of phytase supplementation at different dietary Ca levels on plasma minerals are summarized in Table 3.4. The interaction of Ca by phytase was significant ($P \leq 0.0001$) only for plasma Ca. At the low or recommended Ca levels, phytase supplementation did not have any significant effect but at a high Ca level, phytase supplementation maintained the plasma Ca at a normal level, which was otherwise elevated without phytase supplementation. The increasing level of dietary Ca decreased ($P \leq 0.05$) plasma P and ($P \leq 0.06$) Cu; the effect on Mg was unclear. Dietary phytase supplementation had the exactly opposite effect; i.e. increased ($P \leq 0.031$) plasma P and ($P \leq 0.018$) Cu and decreased ($P \leq 0.009$) plasma Ca. The maximum level of plasma P was obtained at the 1.0% Ca level with phytase, however, this value did not significantly differ from the value obtained at the 0.6% Ca with phytase. Plasma Zn and Mg were not affected ($P > 0.05$) by phytase supplementation.

Bone Minerals

The effects of phytase supplementation at different levels of Ca on ash content in tibia head and mineral concentrations in the ash of tibia head are summarized in Table 3.5. The interaction of Ca with phytase was not significant for ash content or its mineral composition except for Fe and Cu. Tibia head Cu and Fe were decreased and increased, respectively, with increasing levels of dietary Ca. Regardless of the Ca level, phytase supplementation increased ($P \leq 0.0003$) ash content in tibia head and reduced ($P \leq$

.047) Mn and Cu ($P \leq 0.003$) contents in tibia head ash; however, phytase supplementation had no effect ($P > 0.05$) on P, Ca, Mg, Zn and Fe content in the ash of the tibia head. Regardless of phytase level, dietary Ca level had no effect ($P > 0.05$) on ash content in tibia head. The maximum ash content was observed at the 1.0% dietary Ca level with phytase supplementation. The increasing level of dietary Ca decreased ($P \leq 0.05$) Mg content in tibia head ash; however, Ca content itself in tibia head ash was not significantly affected. The maximum content of Mg and Zn in the ash of the tibia head were obtained with low Ca diets without phytase supplementation. Phytase supplementation to the 1.25% Ca diet reduced ($P \leq 0.05$) Cu content and to the 0.6% Ca diet increased ($P \leq 0.05$) Fe content of the tibia head ash.

The effects of phytase supplementation at different levels of dietary Ca on ash content in tibia shaft and mineral concentrations in the ash of tibia shaft are summarized in Table 3.6. The interaction of Ca with phytase was significant ($P \leq 0.041$) only for Mg and Zn. Regardless of the Ca level, phytase supplementation to the diet increased ($P \leq 0.036$) total ash content in the tibia shaft but it did not affect ($P > 0.05$) the concentrations of any of the minerals measured. Phytase supplementation at the 0.6% Ca diet resulted in the ash content of tibia shaft that was equivalent to that obtained with the 1.0 and 1.25% Ca plus phytase. The increasing level of dietary Ca decreased ($P \leq 0.003$) P, Mg, ($P \leq 0.0001$) and Zn ($P \leq 0.010$) contents and had no effect ($P > 0.05$) on Ca, Cu, Fe, and Mn in the ash of the tibia shaft. Regardless of phytase supplementation, the highest content of P, Mg, and Zn were recorded with diets containing 0.6% Ca. Phytase supplementation to the 1% Ca diet increased ($P \leq 0.05$)

Mg content in tibia shaft ash.

DISCUSSION

The study presented herein shows the effects of microbial phytase supplementation on growth performance and on macro- and trace mineral utilization at different levels of Ca in broiler diets. Recent studies in broilers on the effect of microbial phytase supplementation were almost exclusively conducted with a single level of Ca (Simons *et al.*, 199; Borz *et al.*, 1994). The present study allowed us to evaluate the efficacy of microbial phytase at different levels of dietary Ca. The results clearly confirm that phytase supplementation to a corn-soybean meal diet improves the growth performance of broiler chickens as assessed by increased body weight, feed intake, and feed efficiency. These findings are in agreement with previous studies in broiler chickens (Simons *et al.*, 1990; Sebastian *et al.*,1996), and in turkeys (Qian *et al.*, 1996a). The improvements observed in the growth performance, retention of P, plasma P, and tibia ash of chickens fed a phytase-supplemented diet are indications of the effectiveness of phytase in this experiment.

Phytate P utilization by poultry has been shown to be influenced by both Ca and P levels in the diet (Mohammed *et al.*, 1991) but the effects of dietary Ca are much greater. Phytate hydrolysis may be completely prevented at very high levels of Ca (Taylor, 1965). Previous studies have shown that high levels of Ca in the diets of rats (Nahapetian and Young, 1980) and of poultry (Scheideler and Sell, 1987) decreased the availability of phytate P considerably. These observations are in agreement with the results of the present study which showed that high (1.25%) dietary Ca decreased the

availability of phytate P as assessed by the lowest retention of P, reduced feed intake and body weight compared to the low (0.6%) or recommended (1.0%) dietary Ca. These findings are in agreement with previous reports which showed that high dietary Ca level depresses the growth rate in broiler chickens (McDonald and Solvyns, 1964), likely caused by decreased availability of other minerals. Similarly, Mohammed *et al.* (1991), reported that phytate P utilization was increased by 15% when dietary Ca levels were reduced from 1.0 to 0.5%. The possible explanations for the decreased phytate P availability at high dietary Ca may be, as suggested by Kornegay (1996), due to: 1) the precipitation of phytate by Ca through the formation of extremely insoluble Ca-phytate complexes that are less accessible to phytase (Wise, 1983); 2) the direct depression of phytase activity resulting from extra Ca competing with phytate for the active sites of the enzyme (McCuaig *et al.*, 1972) or 3) increased intestinal pH resulting from increased dietary Ca, which reduces the soluble fraction of minerals, hence limits their availability for absorption (Shafey *et al.*, 1991).

The results of the present study revealed that phytase supplementation, at the low level of dietary Ca, showed the optimum response for the growth performance (increased body weight, feed intake and feed efficiency) compared to recommended or high dietary Ca levels. In a recent study with pigs, it has been shown that supplemental microbial phytase improved the phytate P utilization more efficiently at moderately low levels of dietary Ca than at normally recommended levels (Lei *et al.*, 1994). Schoner *et al.*, (1993) reported that increasing levels of dietary Ca progressively decreased broiler growth performance response to phytase. In a study with turkeys, Qian *et al.* (1996a)

showed that the largest response to dietary Ca:P_t and supplemental phytase was achieved when the dietary Ca:P_t ratio was low (1.1:1) with phytase supplementation of 600 U/ kg of diet.

Phytate, being a strong acid, can form various salts with essential minerals, thus reducing their solubility and ultimately their absorption (Sandberg and Svanberg, 1991). When phytate is hydrolyzed by microbial phytase it may release all constituent minerals, myo-inositol and inorganic phosphates. In the present study, phytase supplementation at the low (0.6%) level of dietary Ca showed the optimum level of retention of N, P, and Ca which agrees with the results of previous studies with chickens (Schoner *et al.*, 1993) and turkeys (Qian *et al.*, 1996a). The improvement in the retention of P by phytase supplementation at the low Ca level indicates that there was less Ca-phytate complexes formed; thus, more phytate molecules were exposed to phytase hydrolysis. In our study, the optimum improvement in the relative N retention may indicate that a phytate-protein complex was partially cleaved by microbial phytase; our observations are in agreement with similar studies in turkeys (Yi *et al.*, 1995) and in pigs (Mroz *et al.*, 1994). We do not have a reasonable explanation why phytase supplementation failed to improve the retention of Mg, Zn, Fe, and Mn; however, these findings are partially in agreement with previous published data in which phytase supplementation had no effect on Zn retention in broiler chickens (Roberson and Edwards, 1994). To the authors' knowledge, there are no data published on the effect of phytase on the retention of Mg, Mn and Fe in poultry; however, previous studies with pigs showed that microbial phytase had no effect on Mg and Mn (Adeola, 1996).

Results from blood analysis indicate that plasma P and Cu were increased by phytase supplementation. These results concur with other studies in chickens (Broz *et al.*, 1994; Mitchell and Edwards, 1996; Sebastian, *et al.*, 1996) and pigs (Young *et al.*, 1993). The increase in plasma P gives further evidence of phytate P utilization by microbial phytase as Mitchell and Edwards (1996) suggested that bone ash and plasma P are the most sensitive and reliable methods for determining the animal's P need. As we expected, the maximum levels of plasma P and Cu were observed in the low level (0.6%) Ca diet supplemented with phytase, which agrees with the results of a previous study with pigs (Lei *et al.*, 1994). It is unknown why plasma Cu was increased while Cu retention was reduced by phytase supplementation; in contradiction to our observation, Adeola (1996) reported a reduction in serum Cu while absorption of Cu was increased by phytase supplementation.

The percentage of tibia ash in both head and shaft portions was improved by the addition of phytase regardless of Ca levels. These results are in agreement with previous studies in chickens (Broz *et al.*, 1994) and in pigs (Young *et al.*, 1993). The improvement in ash percentage in the tibia indicates an increase in bone mineralization consequent to an increase in the availability of minerals liberated by phytase from the phytate-mineral complex. In the tibia shaft, phytase supplementation at the 0.6% Ca increased the ash content to a level comparable to that obtained with the 1.0 or 1.25% Ca plus phytase. Qian *et al.* (1996a) found higher toe ash content of turkeys fed a low Ca:P_t diet. Phytase supplementation did not affect the concentrations of any minerals measured in either the head or the shaft of the tibia; however, the maximum content of

Mg, Zn and Cu in tibia head ash were obtained with the low Ca diets.

In conclusion, the data obtained from this research show that dietary microbial phytase supplementation (600 U / kg diet) improved the body weight, feed intake, feed efficiency, and relative retention of P, Ca, and N. It also increased the levels of plasma P and Cu, and the ash content of tibia head and shaft of broiler chickens fed a corn-soybean meal diet. Dietary Ca levels had a significant effect on the response to supplemental phytase; the optimum growth performance, retention of P, Ca, and N, the ash content of the tibia shaft and plasma P, and Cu were achieved at the low (0.6%) level of dietary Ca supplemented with microbial phytase.

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Table 3.1. Composition of experimental diets[1]

	Calcium (%)		
	0.6	1.0	1.25
Ingredients	(%)		
Corn, ground	60	59.25	58.75
Soybean meal (48% CP)	32.1	32.1	32.1
Fish meal (65% CP)	3.0	3.0	3.0
AV-fat[2]	3.3	3.3	3.3
Calcium carbonate (32% Ca)	0.4	1.15	1.7
Calcium phosphate (25% Ca; 19% P)	0.2	0.2	0.15
Salt	0.2	0.2	0.2
Vitamin-mineral mixture[3]	0.5	0.5	0.5
DL- Methionine	0.2	0.2	0.2
L-Lysine. HCl	0.1	0.1	0.1
Analyzed composition			
Calcium	0.53	0.92	1.20
Total phosphorus	0.54	0.53	0.54
Magnesium	0.15	0.15	0.15
Copper (ppm)	8.9	8.9	9.6
Zinc (ppm)	56.5	49.3	55.18
Manganese (ppm)	34.5	30.4	35.1
Crude protein	23.2	23.2	23.4
Calculated composition			
ME (Kcal/kg)	3186	3160	3144
Available Phosphorus	0.31	0.31	0.30
Lysine	1.36	1.34	1.34
Methionine + Cysteine	0.93	0.92	0.92

[1]Calculated to meet or exceed the requirements of broiler starter diet (NRC, 1994)
[2]Animal-vegetable fat blend
[3]Vitamin-mineral premix supplied the following per kilogram of diet: calcium, 1000 mg; phosphorus, 450 mg; magnesium, 25 mg; sodium chloride, 550 mg; selenium, 0.04 mg; iron, 25 mg; manganese, 22 mg; copper, 1.6 mg; zinc, 16 mg; iodine, 0.14 mg; retinyl acetate, 925 IU; cholecalciferol, 275 IU; dl-α-tocopherol acetate, 5 IU; choline, 40.8 mg; menadione sodium bisulfite, 0.19 mg; riboflavin, 1.9 mg; vitamin B_{12} , 2.2 mg; niacin, 8.1 mg; pantothenic acid, 2.2 mg; biotin, 0.013 mg; folic acid, 0.16 mg; thiamin, 0.5 mg; pyridoxine, 0.9 mg; salinomycin sodium, 7.5 mg

Table 3.2. The effect of phytase supplementation at different levels of calcium on feed intake (g), body weight (g) and feed to gain ratio (g:g) of broiler chickens fed a low-P (0.3% available phosphorus) corn-soybean meal diet

Treatment		0 to 7 days			0 to 14 days			0 to 21 days		
Calcium (%)	Phytase (PU/kg)	Feed intake	Body weight	Feed: gain	Feed intake	Body weight	Feed: gain	Feed intake	Body weight	Feed: gain
0.60	0	145	140	1.55	430[a]	324[ab]	1.53	780	591	1.45
0.60	600	149	134	1.68	424[a]	317[abc]	1.55	811	616	1.40
1.0	0	142	133	1.58	411[a]	302[c]	1.58	783	555	1.55
1.0	600	145	141	1.50	429[a]	335[a]	1.48	830	636	1.40
1.25	0	131	125	1.55	359[b]	278[d]	1.53	701	530	1.45
1.25	600	141	137	1.48	412[a]	313[bc]	1.50	771	577	1.45
SEM		1.88	1.75	0.03	6.42	4.43	0.02	10.4	8.67	0.02
ANOVA	df	Probabilities								
Treatment	5	0.011	0.096	0.141	0.002	0.0002	0.502	0.0006	0.0001	0.054
Calcium	2	0.004	0.256	0.147	0.003	0.002	0.800	0.0005	0.001	0.346
Phytase	1	0.156	0.160	0.842	0.024	0.001	0.287	0.005	0.0001	0.025
Calcium x Phytase	2	0.273	0.074	0.099	0.039	0.007	0.266	0.217	0.104	0.099
Main effects										
Calcium										
0.6		150[a]	137	1.61	427[a]	321[a]	1.54	805[a]	604[a]	1.43
1.0		144[a]	137	1.54	420[a]	319[a]	1.53	807[a]	596[a]	1.48
1.25		136[b]	131	1.51	385[b]	296[b]	1.51	736[b]	554[b]	1.45
Phytase										
0		141	132	1.56	400	301	1.55	761	559	1.48
600		146	137	1.55	422	322	1.51	804	610	1.41

[a-d] Means within each column with no common superscripts differ significantly($P < 0.05$).

Table 3.3. The effect of phytase supplementation at different levels of calcium on relative retention of minerals in 17-d old broiler chickens fed a low-P (0.3% available phosphorus) corn-soybean meal diet

Treatment		Relative retention (%)							
Calcium	Phytase	P	Ca	Mg	Zn	Cu	Fe	Mn	N
(%)	(PU/kg)								
0.60	0	54.7	64.1	19.2	-11.3	-0.2^{cd}	20.0^{bc}	9.6^{b}	66.9^{b}
0.60	600	57.6	67.6	21.2	11.2	9.1^{bc}	27.1^{b}	9.5^{b}	69.2^{ab}
1.0	0	57.5	51.1	23.6	-1.0	25.8^{a}	22.6^{bc}	5.5^{b}	70.8^{a}
1.0	600	61.2	46.9	22.2	-15.8	6.9^{d}	15.7^{c}	-2.3^{c}	68.6^{ab}
1.25	0	53.2	43.2	24.5	-10.5	23.4^{a}	37.2^{a}	3.7^{bc}	67.4^{b}
1.25	600	59.0	43.6	28.1	-11.8	17.8^{ab}	29.1^{ab}	17.9^{a}	70.8^{a}
SEM		0.81	2.11	0.99	3.39	2.87	1.81	1.56	0.48
ANOVA	df	Probabilities							
Treatment	5	0.036	0.0001	0.139	0.199	0.0001	0.0017	0.0004	0.038
Calcium	2	0.010	0.001	0.040	0.352	0.002	0.0007	0.003	0.254
Phytase	1	0.008	0.951	0.451	0.742	0.007	0.302	0.314	0.059
Calcium x Phytase	2	0.662	0.135	0.515	0.077	0.001	0.041	0.0009	0.021
Main effects									
Calcium									
0.6		56.1^{b}	65.9^{a}	20.2^{b}	-0.03	4.5^{b}	23.6^{b}	9.6^{a}	68.0
1.0		59.5^{a}	49.0^{b}	22.9^{ab}	-8.38	9.4^{b}	19.1^{b}	1.6^{b}	69.7
1.25		56.1^{b}	43.5^{c}	26.3^{a}	-11.2	20.6^{a}	33.2^{a}	10.8^{a}	69.1
Phytase									
0		55.2	52.8	22.4	-7.6	16.3	26.6	6.3	68.3
600		59.2	52.7	23.8	-7.6	6.6	23.9	8.3	69.6

[a-d]Means within each column with no common superscripts differ significantly ($P < 0.05$).

Table 3.4. The effect of phytase supplementation at different levels of calcium on plasma minerals in 21-d old broiler chickens fed a low-P (0.3% available phosphorus) corn-soybean meal diet

Treatment		Plasma				
Calcium	Phytase	P	Ca	Mg	Zn	Cu
(%)	(PU/kg)		(mg/dL)		(μg/dL)	
0.60	0	14.2	11.3[b]	2.03	175	9
0.60	600	13.9	11.4[b]	2.02	170	20
1.0	0	12.8	11.4[b]	1.81	170	11
1.0	600	14.6	12.2[b]	1.92	168	15
1.25	0	10.6	14.6[a]	2.07	185	7
1.25	600	12.8	11.6[b]	1.95	162	9
SEM		0.37	0.27	0.03	4.0	1.0
ANOVA	df			Probabilities		
Treatment	5	0.005	0.0001	0.036	0.850	0.027
Calcium	2	0.005	0.0001	0.013	0.929	0.060
Phytase	1	0.031	0.009	0.852	0.367	0.018
Calcium x Phytase	2	0.146	0.0001	0.152	0.634	0.236
Main effects						
Calcium						
0.6		14.1[a]	11.3[b]	2.03[a]	172	15
1.0		13.7[a]	11.8[b]	1.87[b]	169	13
1.25		11.7[b]	13.1[a]	2.01[a]	174	8
Phytase						
0		12.5	12.4	1.9	176	9
600		13.8	11.7	1.9	167	14

[a,b]Means within each column with no common superscripts differ significantly ($P < 0.05$).

Table 3.5. The effect of phytase supplementation at different levels of calcium on the total ash and its mineral components in the head portion of the tibia in 21-d old broiler chickens fed a low-P (0.3% available phosphorus) corn-soybean meal diet

Treatment		Tibia head							
Calcium	Phytase	Ash	P	Ca	Mg	Zn	Cu	Fe	Mn
(%)	(PU/kg)	(% of DM)	(% of ash)			(ppm in ash)			
0.60	0	25.1	15.3	31.5	.92	600	16.9[a]	515[c]	15.7
0.60	600	26.5	14.5	30.3	.83	426	14.9[ab]	640[ab]	14.6
1.0	0	26.2	14.5	32.3	.72	360	14.0[bc]	555[bc]	15.6
1.0	600	29.5	14.7	32.9	.81	382	14.1[bc]	633[ab]	16.1
1.25	0	24.0	14.1	32.2	.75	468	16.1[a]	708[a]	18.2
1.25	600	28.8	13.4	30.2	.75	408	12.6[c]	636[ab]	15.3
SEM		0.51	0.22	0.55	0.02	23.5	0.79	16.9	0.34
ANOVA	df	Probabilities							
Treatment	5	0.002	0.220	0.890	0.010	0.023	0.002	0.003	0.031
Calcium	2	0.074	0.087	0.642	0.004	0.010	0.021	0.010	0.099
Phytase	1	0.0003	0.294	0.362	0.955	0.140	0.003	0.087	0.047
Calcium x Phytase	2	0.167	0.600	0.797	0.071	0.258	0.038	0.009	0.073
Main effects									
Calcium									
0.6		25.8	14.9	30.9	0.87[a]	530[a]	15.9[a]	577[b]	15.2
1.0		27.9	14.6	32.2	0.76[b]	371[b]	14.0[b]	594[b]	15.8
1.25		26.4	13.7	31.2	0.75[b]	437[ab]	14.3[b]	672[a]	16.7
Phytase									
0		25.1	14.6	32.0	0.79	476	15.7	593	16.5
600		28.2	14.1	30.9	0.80	417	13.9	636	15.3

[a-c]M eans within each column with no common superscripts differ significantly ($P < 0.05$).

Table 3.6. The effect of phytase supplementation at different levels of calcium on the total ash and its mineral components in the shaft portion of tibia in 21-d old broiler chickens fed a low-P (0.3% available phosphorus) corn-soybean meal diet

Treatment		Tibia shaft							
Calcium	Phytase	Ash	P	Ca	Mg	Zn	Cu	Fe	Mn
(%)	(PU/kg)	(% of DM)	(% of ash)			(ppm in ash)			
0.60	0	43.6	17.7	38.8	.90[a]	639[a]	11.6	415	12.3
0.60	600	48.7	15.7	34.6	.83[ab]	431[b]	10.5	433	10.9
1.0	0	46.3	15.4	34.4	.65[d]	376[b]	9.7	405	11.8
1.0	600	48.2	15.5	35.9	.78[bc]	414[b]	10.1	395	12.5
1.25	0	44.2	14.2	34.6	.61[d]	432[b]	10.8	417	12.6
1.25	600	49.4	14.4	33.9	.69[cd]	439[b]	9.2	381	11.3
SEM		0.93	0.32	0.63	0.03	23.2	0.28	11.4	0.24
ANOVA	df	Probabilities							
Treatment	5	0.346	0.009	0.227	0.0003	0.005	0.153	0.861	0.214
Calcium	2	0.885	0.003	0.266	0.0001	0.010	0.154	0.650	0.596
Phytase	1	0.036	0.249	0.337	0.209	0.128	0.144	0.694	0.134
Calcium x Phytase	2	0.710	0.132	0.173	0.041	0.017	0.273	0.669	0.138
Main effects									
Calcium									
0.6		46.1	16.7[a]	36.7	0.86[a]	535[a]	11.1	423	11.6
1.0		47.2	15.5[ab]	35.1	0.72[b]	395[b]	9.9	400	12.1
1.25		46.8	14.3[b]	34.2	0.65[b]	436[b]	10.0	399	11.9
Phytase									
0		44.7	15.7	35.9	.72	482	10.7	412	12.2
600		48.8	15.2	34.7	.76	428	9.9	402	11.5

[a-d]Means within each column with no common superscripts differ significantly ($P < 0.05$).

Preface to Chapter IV

Chapter IV is comprised of materials contained in a manuscript by S. Sebastian, S. P. Touchburn, E. R. Chavez and P. C. Laguë which has been submitted to Poultry Science. The format has been changed to be consistent within this thesis, according to the guidelines set by the Faculty of Graduate Studies and Research. All literature cited in this chapter is listed in the reference section at the end of the thesis. Each table for chapter IV is presented at the end of this chapter.

In the previous experiments, it was demonstrated that phytase supplementation increased the growth performance, relative retention of Ca, P, Cu and Zn and bone mineralization in broiler chickens, and the maximum efficacy of supplemental phytase was achieved at low levels of dietary Ca and P. In this experiment, the effect of phytase supplementation on apparent ileal and fecal digestibilities of crude protein and amino acids in broiler chickens fed a corn-soybean meal diet were studied.

Chapter IV

Apparent Digestibility of Protein and Amino Acids in Broiler Chickens Fed a Corn-Soybean diet Supplemented with Microbial Phytase

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Apparent Digestibility of Protein and Amino Acids in Broiler Chickens Fed a Corn-Soybean diet Supplemented with Microbial Phytase

ABSTRACT

The effect of microbial phytase supplementation on CP and amino acid (AA) digestibility was investigated in a 28-d trial using 360 sexed, day-old broiler chickens fed corn-soybean meal diets. A completely randomized experimental design with a 3 x 2 factorial arrangement of treatments was used. The variables included P-Ca levels and phytase: P-Ca levels were: normal P-normal Ca (0.45% available P [P_a], 1.0% Ca), low P-normal Ca (0.35% P_a, 1.0% Ca) and low P-low Ca (0.35 P_a and 0.6% Ca); and phytase at 0 and 600 units / kg diet. Phytase supplementation increased body weight gain ($P < 0.004$) and feed intake ($P < 0.006$) at 19-d in male chickens; in females, phytase increased ($P < 0.003$) only body weight gain at 19-d. The low P-normal Ca diet reduced ($P < 0.05$) feed intake and body weight gain in both sexes at 7, 14, and 19-d, compared to the normal P-normal Ca diet; the low P-low Ca diet overcame the above depression to a level comparable to that of the normal P-normal Ca diet. Microbial phytase increased ($P < 0.065$) the apparent ileal digestibility (AID) of CP, but had no influence on AID of any AA except Met and Phe in male broiler chickens at 28-d. However, adding phytase increased the AID of all AA except Lys, Met, Phe and Pro in female broiler chickens at 28-d. The low P-normal Ca diet reduced ($P < 0.05$) the AID of Arg, Phe, Asp and Ser in male chickens and of all the AA except Met and Pro in

females compared to the normal P-normal Ca diet. The low P-low Ca diet overcame the depression of the AID of the AA caused by the low P-normal Ca diet to a level comparable to that of the normal P-normal Ca diet in both sexes. Phytase supplementation did not have any effect ($P > 0.05$) on apparent "fecal" digestibility (AFD) of any of the AA in male chickens; however in female chickens it increased the AFD of Thr, Asp, Glu, Gly, and Ser. The low P-normal Ca diet reduced ($P < 0.05$) the AFD of all the AA in male chickens and of Arg, His, Phe, Thr, Val, Asp, Ala, Glu, Gly and Ser in females. The low P-low Ca diet increased ($P < 0.05$) the AFD of all the AA reduced by low P-normal Ca to a level comparable to that of the normal P-normal Ca diet in both sexes. In summary, phytase supplementation increased growth performance in both sexes, and AID and AFD of AA particularly in female chickens at 28-d; the optimum growth performance and AA digestibilities were obtained in the low P-low Ca diet supplemented with microbial phytase.

(*Key words*: broilers, Phytase, P-Ca levels, crude protein, amino acids, digestibility)

INTRODUCTION

Phytic acid, myo-inositol phosphorylated on all of its six hydroxyl groups, can ionically bind minerals and proteins in aqueous medium (de Rham and Jost, 1976). The interactions among phytic acid, minerals, and protein appear to be primarily responsible for the adverse nutritional effects of a high-phytate diet (Cheryan, 1980). Phytic acid has the ability to bind protein at acidic, alkaline and neutral pH (Anderson, 1985). The interaction between phytic acid and protein leads to decreased solubility of protein and eventually reduces its utilization (Cheryan, 1980).

It is well documented that microbial phytase supplementation enhances the phytic acid hydrolysis of and increases the availability of minerals bound to the phytic acid. Theoretically, phytase supplementation must also be able to release the phytate bound protein for utilization. It has been reported that phytase supplementation improved nitrogen digestibility in pigs (Yi *et al.*, 1994a), and nitrogen retention in broiler chickens (Farrell *et al.*, 1993) and laying hens (Ven der Klis and Versteegh, 1991). In contrast, Newkirk and Classen (1995) did not find any significant effect on crude protein digestibility by either semi-purified phytase or crude phytase supplementation of broiler diets. In a recent study with female turkeys, Yi *et al.* (1996b) showed that phytase supplementation enhanced the ileal digestibility of N and AA. Mroz *et al.* (1994) reported that phytase supplementation enhanced the apparent digestibility of CP and AA except for cystine and proline. There is no published data on the apparent ileal digestibility of protein and AA in broiler chickens. The objective of this experiment was to investigate the efficacy of microbial phytase supplementation on apparent digestibility of protein and AA in broiler chickens fed either normal P or low P or low P-low Ca corn-soybean meal diets.

MATERIALS AND METHODS

Experimental Design

A total of 360 sexed, day-old broiler chicks was purchased from a commercial hatchery[1], wing-banded and weighed individually. Ten birds were assigned to each of 36

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[1] René Poirier Ltée, St. Félix-de-Valois, QC. Canada J0K 2M0

pens (18 pens of each sex) and housed in thermostatically controlled Petersime[2] battery brooders with raised wire floors. All birds were exposed to continuous fluorescent light. Feed and water were provided *ad libitum* throughout the 28-d trial. Each of six treatments was replicated three times per sex. The experimental design was completely randomized with a 3 x 2 factorial arrangement of treatments. The variables included P-Ca levels and phytase[3]: P-Ca levels were: normal P-normal Ca (0.45% available P [P_a], 1.0% Ca), low P-normal Ca (0.35% P_a, 1.0% Ca) and low P-low Ca (0.35 and 0.6%, respectively); and phytase at 0 and 600 units / kg diet. The percentage composition of the basal diets is shown in Table 4.1 and the AA analysis of the diets is shown in Table 4.2. Individual body weights of chickens and group feed consumption data were recorded on days 7, 14, and 19. All birds were cared for according to the Canadian Council on Animal Care guidelines (CCAC, 1993).

Sample Collection and Chemical Analysis

The digestibilities of protein and AA were measured at 21 and 28 d. During this period, to attain uniformity in feed intake and digesta content, the feed troughs were removed for 30 min, then replaced for two hours before the birds were taken out for the measurements. Five and four birds were randomly selected from each replicate at 21 and 28 d, respectively, for the measurement of ileal apparent digestibility and apparent total tract digestibility of protein and AA. After euthanasia with an intra-venous injection of

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[2] Gettysburg, OH 45328

[3] Natuphos® 1000, BASF Canada, Inc., Ville St. Laurent QC. Canada H4T 1Y4

pentobarbitone, each bird was immediately dissected and the ileum located, defined as extending from Meckel's diverticulum to the ileo-cecal junction; the distal 40 mm of the ileum were tied off and excised. This segment was bisected transversely and its contents were gently squeezed out into a plastic cup. A digesta sample from the rectum was also obtained to determine the apparent "fecal" digestibility. The digesta from the gizzard were analyzed to determine the rate of disappearance of CP and AA from this organ. At 21-d, the digesta of five birds in a pen were pooled to accumulate enough samples for the analysis; at 28-d, the digesta of two birds out of four were pooled separately in order to have more replicates of analysis. The pooled samples were freeze-dried, ground through 1-mm mesh screen and immediately prepared for the analysis of crude protein and AA. Digesta and feed were hydrolyzed for 24 h with 6 N HCl at 110^0C for the determination of AA by HPLC[4] using the Pico-Tag system from Waters chromatography systems[5]. The cystine and tryptophan, being partly destroyed under acid hydrolysis, were not determined. Nitrogen analysis was carried out with a Leco nitrogen analyzer[6]. Chromic oxide was analyzed using a flame atomic absorption spectrophotometer[7] after wet digestion with concentrated nitric acid and perchloric acid (Emerson, 1975). All samples were assayed in duplicate.

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[4] Model Waters 700 WISP Satellite; model 510 HPLC pump, Waters, Houston, TX77054

[5] Waters chromatography division / millipore corporation, Milford, MA 01757

[6] Model FP-428, Leco® corporation, St. Joseph, MI 49085

[7] Model 2380, Perkin Elmer, Norwalk, CT 06856

Calculations and Statistical Analysis

Apparent ileal and fecal digestibility coefficients of crude protein and AA were estimated by using 0.2% chromic oxide (Cr_2O_3) as an indigestible marker. The following formula was used to calculate the digestibility coefficients:

$$AD_{AA} = 100 - \left(100 * \frac{[AA]_{digesta} * [Cr]_{diet}}{[AA]_{diet} * [Cr]_{digesta}}\right)$$

Where AD_{AA} = apparent digestibilities of individual AA (%)

$[AA]_{digesta}$ = AA concentration in the digesta

$[AA]_{diet}$ = AA concentration in the diet

$[Cr]_{digesta}$ = chromic oxide concentration in the digesta

$[Cr]_{diet}$ = chromic oxide concentration in the diet

The observed very low level of Cr_2O_3 in the digesta from the gizzard (0.04 to 0.05%, compared to 0.5 to 0.6 % Cr_2O_3 in the ileal digesta) was thought to be due to leaching off of Cr_2O_3 from the gizzard contents. We verified this supposition by soaking the feed containing Cr_2O_3 for 15 min. in water; only 0.006% of Cr_2O_3 was left in the feed while the rest entered the liquid phase. Since Cr_2O_3 was easily washed out while solid feed particles were held in the gizzard, we used acid detergent fibre (ADF) as insoluble marker to calculate the rate of disappearance of CP and AA from the gizzard. In order to validate the usage of ADF for this purpose in the gizzard, we compared the ileal and fecal digestibility values by using both ADF and Cr_2O_3 as insoluble markers (Table 4.25). The following formula was used to calculated the disappearance of CP and AA from the gizzard.

$$DA_{AA} = 100 - \left(100 * \frac{[AA]_{gizzard} * [ADF]_{diet}}{[AA]_{diet} * [ADF]_{gizzard}}\right)$$

Where DA_{AA} = disappearance of individual AA (%)

$[AA]_{gizzard}$ = AA concentration of digesta in the gizzard

$[AA]_{diet}$ = AA concentration in the diet

$[ADF]_{gizzard}$ = ADF content of digesta in the gizzard

$[ADF]_{diet}$ = ADF content in the diet

The data on performance, apparent ileal and "fecal" digestibilities and disappearance of AA from the gizzard were analyzed using the General Linear Models procedure for analysis of variance (SAS Institute, 1990). Significant differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955) with a 5% level of probability.

RESULTS

Feed Intake, Body Weight Gain and Feed Efficiency.

Main effects: The growth performance of male and female chickens fed the various dietary treatments are shown in Tables 4.3 and 4.4, respectively. The main effect of microbial phytase (600 U/ kg diet) indicates increased body weight gain ($P < 0.004$) and feed intake ($P < 0.006$) at 19-d in maless; however phytase supplementation did not show any significant ($P > 0.05$) effect on body weight gain or feed intake at both 7 and 14-d. In females, phytase supplementation increased ($P < 0.003$) only body weight gain at 19-d (Table 4.4). Phytase had no influence ($P > 0.05$) on feed to gain ratio in either

males or females at 7, 14 or 19-d. The P-Ca levels in the diet showed significant effects on feed intake ($P < 0.02$), body weight gain ($P < 0.003$), and feed to gain ratio ($P < 0.007$) at 14-d and on feed intake ($P < 0.012$) and body weight gain ($P < 0.004$) at 19-d in both males and females. The low P-normal Ca diet reduced ($P < 0.05$) feed intake, and body weight gain in both males and females at 7, 14, and 19-d compared to the normal P-normal Ca diet. It is interesting to note that the low P-low Ca diet overcame the depression of feed intake and body weight gain in both males and females at 7, 14 and 19-d to a level comparable to that of the normal P-normal Ca diet. The interactions of P-Ca level by phytase were nonsignificant ($P > 0.05$) for any growth performance variables.

Apparent Ileal Digestibility of Crude Protein and Essential Amino acids at 28-d

Main effect: The apparent ileal digestibility (AID) coefficients of CP and essential amino acids (EAA) of male and female chickens fed the various dietary treatments for 28-d are shown in Tables 4.5 and 4.6, respectively. The main effect of microbial phytase indicated that phytase increased the AID of CP ($P < 0.069$); but had no influence on AID of any EAA except Met and Phe which were reduced ($P < 0.05$) by phytase in males (Table 4.5). However, phytase supplementation significantly increased the AID of all of the EAA except Lys, Met, and Phe in females at 28-d (Table 4.6). The P-Ca level had a significant effect on the AID of Arg and Phe in males and of all of the EAA except Met in females. The low P-normal Ca reduced ($P < 0.05$) the AID of Arg and Phe in males and of all of the EAA except Met in females compared to the normal P-normal Ca diet. The Low P-Low Ca diet overcame the depression of the AID of AA caused by

low P-normal Ca to a level comparable to that of the normal P-normal Ca diet in both males and females. The interaction of P-Ca level by phytase was significant for the AID of CP, His, Lys, Met, and Thr in males; but in females, such interactions were nonsignificant ($P > 0.05$) for the AID of CP and all of the EAA. The AID values of CP and all of the EAA were consistently higher in females (86.3%) than in males (84.8%).

Diet effect: Dietary treatments affected ($P < 0.001$ to 0.054) the AID of CP and all of the EAA except Leu, Ile, Lys, and Val in males and of all of the EAA except Met in females. In males, the maximum values of the AID for Ile , Leu, and Lys were observed in the low P-low Ca diet without phytase and for Arg, His, Phe, Thr and Val were observed in the normal P-normal Ca diet supplemented with phytase. In the case of females, the maximum values of AID for all of the EAA and CP were found in normal P-normal Ca diet supplemented with phytase. The minimium values of the AID for all EAA were observed in the low P-normal Ca diet with or without phytases supplementation in both sexes. Among all of the EAA, Met and Arg had the highest AID values in both males and females; the lowest AID was observed for Ile in males and for Thr in females.

Apparent Ileal Digestibility of Nonessential Amino acids at 28-d

Main effect: The AID coefficients of nonessential amino acids (NEAA) of male and female chickens fed the various dietary treatments for 28-d are shown in Tables 4.7 and 4.8, respectively. Phytase supplementation had no influence on AID of any of the NEAA in males (Table 4.7); however, phytase supplementation increased ($P < 0.002$ to 0.03) all of the NEAA except Pro in females (Table 4.8). The P-Ca level had a

significant effect on Asp, and Ser ($P < 0.03$) in males and on all of the NEAA except Pro ($P > 0.002$ to 0.05) in females. The low P-normal Ca diet reduced ($P < 0.05$) the AID of Asp and Ser in males and of all the NEAA except Pro in females; however, the low P-low Ca diet overcame the reduction in the AID of NEAA to a level comparable to that of the normal P-normal Ca diet. The interaction of P-Ca level by phytase was significant ($P < 0.004$ to 0.028) for Ala, Gly, and Pro in males; however, such interaction was nonsignificant ($P > 0.05$) for all of the NEAA in females. The AID values for all of the NEAA were higher in females (86.1%) than in males (85.0%).

Diet effect: Dietary treatments affected ($P < 0.0007$ to 0.058) the AID of Ala, Gly, Pro, and Ser in males and of all of the NEAA except Pro in females. The maximum AID values for all of the NEAA except Pro, were achieved in the normal P-normal Ca diet supplemented with phytase in both males and females. The minimum AID values for all NEAA were observed in the low P-normal Ca diet with or without phytase in both sexes except Asp in males. Phytase supplementation to the low P-normal Ca diet reduced ($P < 0.05$) the AID of Ala, Gly, and Pro in males. The highest value of the AID was observed for Glu in both males (92.4%) and females (93.4%). The lowest value of the AID was observed for Ser in both sexes.

Apparent "Fecal" Digestibility of Crude Protein and Essential Amino acids at 28-d

Main effects: The apparent "fecal" digestibility (AFD) coefficients of CP and EAA of male and female chickens fed the various dietary treatments for 28-d are shown in Tables 4.9 and 4.10, respectively. Phytase supplementation did not have any effect ($P > 0.05$) on AFD of any of the EAA in both sexes except Thr in females. The P-Ca level

significantly affected ($P < 0.001$ to 0.04) the ADF of all of the EAA in males and of Arg, His, Phe, Thr and Val in females. The low P-normal Ca diet reduced ($P < 0.004$ to 0.03) the AFD of all of the EAA in males; in females it reduced the AFD only for Arg, His, Phe, Thr, and Val. The low P-low Ca diet increased ($P < 0.05$) the AFD of all the EAA affected by low P-normal Ca to a level comparable to that of the normal P-normal Ca diet in both sexes. The interaction of P-Ca level by phytase was nonsignificant ($P > 0.05$) for the AFD of CP and for all of the EAA in both sexes.

Diet effect: Dietary treatments had a significant effect ($P < 0.0001$ to 0.002) on the AFD of all of the EAA except Met in males; but in females, dietary treatment was significant only for Arg, His, Phe, and Thr. The maximum values for the AFD of all the EAA were obtained in the normal P-normal Ca diet supplemented with phytase in females; however, in males, only Arg, His, Leu, Phe, and Thr showed their maximum values in the normal P-normal Ca diet. Among the EAA, Arg had the highest AFD value in both males and females fed a normal P-normal Ca diet supplemented with phytase. The minimum values for the AFD of all the EAA were observed in the diet containing low P-normal Ca without phytase in both sexes. The lowest AFD was observed for Thr in both sexes. The AFD values of CP and EAA were consistently higher in female than in male.

Apparent "Fecal" Digestibility of Nonessential Amino acids at 28-d

Main effects: The apparent "fecal" digestibility coefficients for the NEAA of male and female chickens fed the various dietary treatments for 28-d are shown in Tables 4.11 and 4.12, respectively. Phytase supplementation did not affect ($P > 0.05$) AFD of any

of the NEAA in males (Table 4.11); however in females phytase supplementation increased (P <0.01 to 0.05) AFD of Asp, Glu, Gly, and Ser (Table 4.12). The P-Ca levels also had an influence (P < 0.0001 to 0.015) on AFD of all of the NEAA in both males and females, except Pro in females. The low P-normal Ca diet reduced (P < 0.05) AFD of all NEAA in both males and females except Pro in females. Lowering the Ca level in the low P-normal Ca diet increased (P <0.05) AFD of all the NEAA affected by a low P-normal Ca diet to a normal level. The interaction of P-Ca level by phytase was nonsignificant (P > 0.05) for the AFD of any of the NEAA in both sexes.

Diet effects: Dietary treatments had a significant (P < 0.0001 to 0.05) effect on AFD of all of the NEAA, except Pro, in both males and females. The maximum AFD values for all the NEAA except Pro and Ala, were obtained in both sexes fed a normal P-normal Ca diet supplemented with microbial phytase; the maximum value for Pro was obtained for both sexes in the low P-low Ca diet without phytase supplementation. Among the NEAA, Glu had the highest AFD value in both sexes. The minimum AFD values for all the NEAA were obtained in the low P-normal Ca diet without phytase supplementation. Among the NEAA, Ser had the lowest AFD value in both sexes. The AFD values of all of the NEAA were higher in female than in male.

Disappearance of Crude Protein and Essential Amino Acids from the Gizzard at 28-d

Main effects: The rates of disappearance of CP and EAA from the gizzard in male and female chickens fed the various dietary treatments for 28-d are shown in Tables 4.13 and 4.14, respectively. Adding phytase to the diets had no impact (P > 0.05) on the rate of disappearance of CP or any of the EAA from the gizzard in either males or females.

The P-Ca levels significantly ($P < 0.0001$ to 0.054) affected the rate of disappearance of CP and all of the EAA from the gizzard in both males (Table 4.13) and females (Table 4.14). In fact the low P-normal Ca reduced ($P < 0.05$) the rate of disappearance of CP and all of the EAA from gizzard in both males and females. The rate of disappearance of CP and all of the EAA was not affected ($P > 0.05$) by lowering the Ca level in the low P-normal Ca diet in either males or females. It was interesting to observe an interaction of P-Ca level by phytase that significantly ($P < 0.0001$ to 0.055) affected the rate of disappearance of all of the EAA except Arg in males and Met in both sexes. There was no difference ($P > 0.05$) in the rate of disappearance of CP and EAA between sexes.

Diet effects: The dietary treatment had a significant ($P < 0.0001$ to 0.05) effect on the disappearance of CP and all the EAA from the gizzard in both males and females. The maximum rate of disappearance of CP and all of the EAA occurred in both males and females fed a normal P-normal Ca diet with or without microbial phytase. Overall, the highest value for the disappearance of EAA was observed in Met for males and females.

Disappearance of Nonessential Amino Acids from the Gizzard at 28-d

Main effects: The rates of disappearance of NEAA from the gizzard in male and female chickens fed the various dietary treatments for 28-d are shown in Tables 4.15 and 4.16, respectively. Microbial phytase supplementation had no effect ($P > 0.05$) on the rates of disappearance of CP or any of the NEAA in either males (Table 4.15) or females (Table 4.16). The P-Ca level significantly ($P < 0.0001$ to 0.001) affected the rates of

disappearance of all of the NEAA except Pro from the gizzard in both males and females. The low P-normal Ca diet reduced ($P < 0.05$) the rates of disappearance of all the NEAA from the gizzard in both males and females compared to the normal P-normal Ca diet. The reduction of Ca level in the low P-normal Ca diet had no impact ($P > 0.05$) on the rates of disappearance of any of the NEAA from the gizzard in either males or females. The interaction of P-Ca level by phytase was not significant ($P > 0.05$) for the disappearance of any of the NEAA except Tyr in males; but in the females, such interaction was significant ($P < 0.05$) for Ala, Glu, Gly, Ser, and Tyr.

Diet effects: The dietary treatment had a significant effect ($P < 0.0001$ to 0.004) on the rates of disappearance of all NEAA except Pro from the gizzard of both males and females. The maximum rate occurred in the normal P-normal Ca diet supplemented with microbial phytase in both males and females. The normal P-normal Ca diet with or without phytase promoted a higher rate of disappearance of NEAA in males than in females. The highest of rate of disappearance was observed for Asp in both male and female chickens fed a normal P-normal Ca diet supplemented with microbial phytase.

The correlation coefficients for the disappearance of AA (EAA and NEAA) from the gizzard versus their AID for males and females were 0.68 and 0.70, respectively; the corresponding values for AFD were 0.63 and 0.66.

Apparent Ileal Digestibility of Crude Protein and Essential Amino acids at 21-d

Main effects: The apparent ileal digestibility coefficients of CP and EAA of male and female chickens fed the various dietary treatments for 21-d are shown in Tables 4.17 and 4.18, respectively. None of the experimental treatments, diet, P-Ca level, phytase

supplementation or interaction of P-Ca level by phytase had effect ($P > 0.05$) on CP or AID of any of the EAA in either males or females. Two exceptions were that reducing the Ca level of the low P diet enhanced the AID of His in males and of Thr in females. There was no difference between the average AID values of the EAA observed at 21 and 28-d in either males (85.1 vs 84.8%) or females (85.7 vs 86.1%).

Apparent Ileal Digestibility of Nonessential Amino acids at 21-d

Main effects: The apparent ileal digestibility coefficients of the NEAA of male and female chickens fed the various dietary treatments for 21-d are shown in Tables 4.19 and 4.20, respectively. Neither dietary treatment nor P-Ca level nor phytase supplementation nor interaction of P-Ca level by phytase had any influence ($P > 0.05$) on AID of any of the NEAA in either males or females. There was no difference in the average AID values observed between males and females (84.3 vs 84.7%) at 21-d. There was a slight increase in the average AID values of the NEAA observed at 28-d, compared to that of 21-d for both males (85.6 vs 84.3%) and females (86.6 vs 84.8%).

Apparent "Fecal" Digestibility of Crude protein and Essential Amino acids at 21-d

Main effects: The apparent "fecal" digestibility coefficients of CP and EAA of male and female chickens fed the various dietary treatments for 21-d are shown in Tables 4.21 and 4.22, respectively. Phytase supplementation had no influence ($P > 0.05$) on the AFD of either CP or any of the EAA in either sex with the exception of His and Thr in females. The P-Ca level significantly ($P < 0.0009$ to 0.050) affected the AFD of CP and all of the the EAA in females (Table 4.22); in males, such significant ($P < 0.0006$ to 0.056) effect was observed in all EAA except Lys, but not for CP. The low P-low Ca

diet increased ($P < 0.05$) the AFD of all of the EAA except Lys in males and of CP and all of the EAA in females compared to the low P-low Ca diet. The interaction of P-Ca level by phytase was nonsignificant ($P > 0.05$) for AFD of CP or any of the EAA in both sexes except His and Thr in females.

Diet effects: Dietary treatments had a significant ($P < 0.001$ to 0.059) effect on the AFD of Arg, His, Ile, and Thr in both sexes and of Lys, Val and Phe in females. The maximum values for AFD of all of the EAA were observed in both sexes in the low P-low Ca diet with or without phytase. The lowest AFD among EAA was observed for Thr in both males and females in the low P-normal Ca diet without phytase supplementation. It is interesting to note that the highest value for AFD was observed for Arg among EAA in both male and female chickens fed the low P-low Ca diet supplemented with phytase. The average AFD values at 21-d was higher (86.3 %) in males than in female (85.1%). There was an increase in the average AFD values of the EAA observed at 21-d, compared to that at 28-d for both males (86.3 vs 82.9%) and females (85.1 vs 84.2%).

Apparent "Fecal" Digestibility of Nonessential Amino acids at 21-d

Main effects: The apparent "fecal" digestibility coefficients of NEAA of male and female chickens fed the various dietary treatments for 21-d are shown in Tables 4.23 and 4.24, respectively. Phytase supplementation had no effect ($P > 0.05$) on AFD of any of the NEAA in either sex. The P-Ca level had a significant ($P < 0.010$ to 0.05) effect on AFD of Gly, Ser, and Tyr in both sexes; the low P-low Ca diet increased ($P < 0.05$) AFD of Gly, Ser, and Tyr, compared to that of the low P-normal Ca diet.

Diet effects: The dietary treatment had no effect ($P > 0.05$) on AFD on any of the NEAA in males; however, dietary treatment had a significant ($P < 0.025$) effect on Gly, Ser, and Tyr in females. The maximum values of AFD of all of the NEAA were observed in the low P-low Ca diet supplemented with phytase in both sexes except for Asp in females. The highest value for AFD was observed for Glu in the low P-low Ca diet plus phytase in both sexes; the lowest AFD value was observed for Ser in male and for Gly in females. The average AFD values observed in males and females (84.3 and 83.8%, respectively) were similar at 21-d. In males, there was a slight increase in the average AFD values of the NEAA observed at 21-d compared to that at 28-d (84.3 vs 83.2%); in females, the average AFD value obtained at 28-d was higher than that of 21-d (85.3 vs 83.8%).

DISCUSSION

The study presented herein shows the effects of microbial phytase supplementation at different P-Ca levels on AID and AFD of CP, EAA and NEAA in both male and female chickens fed a corn-soybean meal diet for 28-d. In agreement with previous studies (Denbow *et al.*, 1995; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996), phytase supplementation increased body weight gain and feed intake in both sexes at 19-d. However, phytase supplementation did not affect the feed to gain ratio because of the simultaneous increase in body weight gain and feed intake, an observation which agrees with those of Simons *et al.*, (1990), Perney *et al.*, (1993), Denbow *et al.* (1995) and Sebastian *et al.*, (1996). The absence of a significant interaction of P-Ca level by phytase in this study indicates that the influence of phytase on growth performance is independent

of P-Ca levels in the diet. The observations of reduced feed intake and weight gain in chickens fed a low P-normal Ca diet may be due to: 1) inadequate supply of P in the diet to meet the P requirement, 2) reduction of appetite as a result of the low level of P or 3) reduction in the solubility of mineral complexes as a consequence of increased ileal pH by the relatively high dietary Ca level (Shafey *et al.*, 1991). These observations clearly indicate the importance of maintaining the ileal Ca:P ratio in broiler diets in order to obtained optimum growth performance. The low P-low Ca diet overcame the depression of feed intake and body weight gain observed in the low P-normal Ca diet; in fact the low P-low Ca diet increased the body weight gain and feed intake to a level comparable to that of the normal P-normal Ca diet. This study clearly revealed that phytase supplementation showed the optimum response for growth performance when added to a low P-low Ca diet.

There is no published data on the AID coefficients of CP or AA as influenced by supplementary microbial phytase in diets of broiler chickens. The results of this experiment indicate that the addition of 600 U of phytase / kg diet increases AID of EAA as well as NEAA in female chickens; however, the magnitude of response to phytase in males was very low. The reason for the lower response of phytase observed in males is not clear. It is interesting to note that AID values of CP and all AA were higher in females than in males. The observation of improved availability of CP and AA by microbial phytase supports the notion that phytic acid binds the protein and AA and makes them unavailable (Cosgrove, 1980).

Phytic acid does form complexes with proteins, the nature of which is governed

by pH. Phytic acid is strongly negatively charged in the low pH range while proteins are strongly positively charged, as a result a phytate-protein complex can be formed (Saio, 1967). Although both proteins and phytic acid have a net negative charge above pH 5, multivalent cations such as Ca, Mg or Zn are thought to mediate the formation of phytate-protein complexes (Okubo *et al.*, 1976). The complexing of phytate with proteins can change the protein structure, which in turn decreases solubility, digestibility, and functionality of the protein (Yi *et al.*, 1996b). Saio *et al.*, (1967) showed that excess Ca ions interact with protein and phytate to further decrease the solubility of proteins. It has been demonstrated that phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Barre, 1956). When microbial phytase cleaves the ester bond to release P from phytate, it also frees proteins bound to phytate and increases the availability of protein and AA for absorption.

The increase in the ileal digestibility of AA may also indicate that phytate-protein complexes were cleaved by microbial phytase to some extent. Phytate is also known to inhibit a number of digestive enzymes such as pepsin (Camus and LaPorte, 1976), and trypsin (Singh and Krikorian, 1982; Caldwell, 1992). These negative influences may also be partly responsible for the effects of phytate on protein utilization. Another possible reason for the improvement in AA digestibility is that phytase supplementation, by reducing phytic acid level in the diet, reduces the inhibitory effect of phytic acid on pepsin and trypsin, thereby increasing the digestibility of proteins and AA. In a recent study with female turkeys, Yi *et al.* (1996b) showed that microbial phytase supplementation to a diet containing 0.45% nonphytate P improved ileal and true

digestibilities of N and most of the EAA and NEAA at both 22.5 and 28.0% CP levels; at 0.60% nonphytate P level, phytase supplementation increased the ileal and true digestibilities of N and all of the AA at 22.5% CP level but not at the level of 28.0% CP. In contrast, a study with pigs showed that microbial phytase supplementation increased the AID of only Met and Arg, but did not influence the AID of other AA (Mroz *et al.*, 1994). In this study, the improvement on AID of AA by phytase supplementation was in a range of 1.8 (Arg) to 4.3 (Thr) percentage units, which is higher than that observed in a study dealing with female turkeys (Yi *et al.*, 1996b). We have no obvious explanation for the decreased and increased digestibilities in the low P-normal Ca and the low P-low Ca diets, respectively. It may be possible that the former increases the ileal pH due to the presence of the relatively high Ca level compared to P in the diet (Shafey *et al.*, 1991); at the consequently high pH, the excess Ca in the low P-normal Ca diet plays an important role in the formation of a phytate-calcium-protein complex which is extremely insoluble (Saio *et al.*, 1967) and thus reduces the availability of protein (and AA). The low P-low Ca diet prevents the situations of increased ileal pH and excess Ca, thereby reducing the formation of the insoluble complex. The range (76.8 to 91.8% and 76.2 to 90.6%) of average AID and AFD values of all AA reported in this study, are within the range reported previously in chickens (Payne *et al.*, 1968; Green *et al.*, 1987) and pigs (Mroz *et al.*, 1994); however, the values reported by Yi *et al.*, (1996b) for AID in the turkey were consistently higher for all AA. The highest AID and AFD values were observed for Met and Pro among the EAA and NEAA, respectively, which agree with the results of Yi *et al.* (1996b) who obtained the maximum AID value

for Met in turkeys.

The efficacy of phytase on the digestibility of AA in females was further confirmed by the observation of increased AFD of Asp, Glu, Gly, Thr, and Ser in female but not in males at 28-d, emphasizing the need of further research to better understand these phenomena. It is not clear why phytase failed to show prominent effects on the AFD of the other AA in females. It may be possible that the contribution of microbial protein and bacterial synthesis of some AA in the hind gut and caeca of chickens (Parsons *et al.*, 1981) overshadows the effect of phytase. In contrast, Mroz *et al.* (1994) reported that microbial phytase supplementation in pigs enhanced the AFD of all AA except for cystine and proline. As expected, AID of CP and all the AA was higher than AFD of the same in both males and females at 28-d, further indicating the potential contribution of microbes in the hind gut. These observations suggest that recovery of AA from the ileum represents a sensitive index of digestibility coefficient that is not confounded by cecal and intestinal bacteria. Contradictory to our observation, the results of Mroz *et al.* (1994) in pigs show that AID values of all AA are lower than the AFD values. As observed in the case of AID, the AFD values of CP and all AA were numerically higher in females than males. It is not known why phytase supplementation did not show any prominent effect on either AID or AFD in both sexes at 21-d.

Curiosity prodded us to study the possibility of there being any relation between the disappearance of AA in the gizzard and their apparent digestibility. As mentioned earlier, ADF instead of Cr_2O_3 was used to calculate the disappearances of CP and AA

from the gizzard. Comparison of their digestibilities in the ileum and rectum (Table 4.25) calculated by using ADF and Cr_2O_3 indicated no differences ($P > 0.05$) between these two markers. Phytase supplementation had no influence on the disappearance of any of the AA. The average maximum disappearance of AA from the gizzard was observed for Met (81.1 and 81.1%) among the EAA and for Pro (85.4 and 84.3%) among the NEAA in both males and females; surprisingly, the AID and AFD values were also maximum for the very same AA in both sexes. The correlation analysis indicates that there is a postive correlation (r = 0.70) between the disappearances of AA from gizzard and their digestibility; however, the biological advantages of this observation are yet to be investigated.

In conclusion, the data obtained from this research showed that dietary microbial phytase supplementation (600 U / kg diet) improved growth performance in both sexes and increased the AID of all AA except Lys, Met, Phe and Pro in females at 28-d. However, phytase had no influence on the AID of any of the AA except Phe in males; thus, the response to phytase supplementation is sex dependent. Phytase supplementation increased the AFD of Asp, Glu, Gly and Ser in females but not in males at 28-d. The growth performance and digestibility of AA observed in the low P-low Ca diet were comparable to those observed in the normal P-normal Ca diet at 28-d. In this study, phytase supplementation in the low P-low Ca diet showed optimum response for growth performance in both sexes, and for AID and AFD of AA particularly in female chickens at 28-d.

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Table 4.1. Composition of experimental diets[1]

	Normal P-normal Ca	Low P-normal Ca	Low P-low Ca
Ingredients		(%)	
Corn, ground	57.5	57.4	59
Soybean meal (48% CP)	32	32	32
Fish meal (65% CP)	4.1	4.1	4
AV-fat[2]	3.5	3.8	3.5
Calcium carbonate (32% Ca)	1.0	1.7	0.5
Dicalcium phosphate (25% Ca; 19% P)	0.9	0	0
Salt	0.2	0.2	0.2
Vitamin-mineral mixture[3]	0.5	0.5	0.5
DL- Methionine	0.2	0.2	0.2
L-Lysine. Hcl	0.1	0.1	0.1
Analyzed composition			
Calcium	0.95	1.05	0.58
Total phosphorus	0.63	0.48	0.44
Crude protein	22.8	22.7	22.4
Calculated composition			
ME (Kcal/kg)	3150	3174	3197
Available Phosphorus	0.49	0.32	0.31
Lysine	1.10	1.0	1.10
Methionine + Cysteine	0.90	0.96	0.90

[1]Calculated to meet or exceed the requirements of broiler starter diet (NRC, 1994)
[2]Animal-vegetable fat blend
[3]Vitamin-mineral premix supplied the following per kilogram of diet: calcium, 1000 mg; phosphorus, 450 mg; magnesium, 25 mg; sodium chloride, 550 mg; selenium, 0.04 mg; iron, 25 mg; manganese, 22 mg; copper, 1.6 mg; zinc, 16 mg; iodine, 0.14 mg; retinyl acetate, 925 IU; cholecalciferol, 275 IU; dl-α-tocopherol acetate, 5 IU; choline, 40.8 mg; menadione sodium bisulfite, 0.19 mg; riboflavin, 1.9 mg; vitamin B_{12} , 2.2 mg; niacin, 8.1 mg; pantothenic acid, 2.2 mg; biotin, 0.013 mg; folic acid, 0.16 mg; thiamin, 0.5 mg; pyridoxine, 0.9 mg; salinomycin sodium, 7.5 mg

Table 4.2. Amino acid analysis of experimental diets[1]

	Normal P-normal Ca	Low P-normal Ca	Low P-low Ca
Amino acids		(%)	
Essential			
Arg	1.67	1.64	1.72
His	0.54	0.54	0.53
Ile	0.57	0.56	0.58
Leu	1.85	1.84	1.89
Lys	1.17	1.05	1.10
Met	0.48	0.43	0.46
Phe	0.89	0.85	0.88
Thr	0.92	0.92	0.96
Val	1.00	0.98	1.03
Non-essential			
Ala	1.23	1.25	1.29
Asp	2.37	2.26	2.45
Glu	4.22	4.17	4.32
Gly	0.99	0.99	1.02
Pro	1.01	0.95	0.96
Ser	1.21	1.23	1.27
Tyr	0.92	0.92	0.96
Total amino acid	21.03	20.58	21.43

[1] The diets were hydrolyzed with 6 N HCl at 110°C and analyzed for amino acid contents by HPLC using the Peco-Tag system from Waters chromatography systems.

Table 4.3. The effect of phytase supplementation on feed intake (g), body weight (g) and feed to gain ratio (g:g) of male broiler chickens fed corn-soybean diets for 19 d

Diet		0 to 7 d			0 to 14 d			0 to 19 d		
P-Ca level	Phytase (U/kg)	Feed intake	Weight gain	Feed: gain	Feed intake	Weight gain	Feed: gain	Feed intake	Weight gain	Feed: gain
Normal P-normal Ca	0	132	104	1.26	451^{a}	335^{a}	1.34^{bc}	702^{ab}	558^{a}	1.26
Normal P-normal Ca	600	127	101	1.26	445^{a}	334^{a}	1.33^{c}	742^{a}	557^{a}	1.33
Low P-normal Ca	0	121	91	1.32	396^{b}	264^{c}	1.50^{a}	581^{c}	467^{b}	1.24
Low P-normal Ca	600	127	99	1.28	429^{ab}	298^{b}	1.44^{ab}	668^{ab}	519^{a}	1.29
Low P-low Ca	0	131	99	1.32	428^{ab}	291^{bc}	1.47^{a}	641^{bc}	513^{ab}	1.25
Low P-low Ca	600	134	104	1.30	441^{a}	303^{b}	1.46^{a}	728^{a}	571^{a}	1.27
SEM		1.49	1.51	0.011	5.72	6.86	0.018	15.7	10.1	0.013
ANOVA						Probabilities				
Diet		0.090	0.101	0.512	0.037	0.002	0.014	0.005	0.004	0.537
P-Ca level		0.051	0.068	0.256	0.018	0.0003	0.002	0.006	0.004	0.604
Phytase		0.464	0.216	0.324	0.510	0.079	0.313	0.004	0.014	0.126
P-Ca level * Phytase		0.171	0.222	0.781	0.231	0.233	0.695	0.568	0.153	0.774
Main effects										
P-Ca level										
Normal P-normal Ca		130^{ab}	103^{a}	1.26	448^{a}	335^{a}	1.34	722^{a}	557^{a}	1.30
Low P-normal Ca		124^{b}	95^{b}	1.30	412^{b}	281^{b}	1.47	624^{b}	494^{b}	1.27
Low P-low Ca		132^{a}	101^{ab}	1.30	435^{ab}	297^{b}	1.47	684^{a}	542^{a}	1.26
Phytase										
0		127	98	1.30	425	298	1.43	641	513	1.25
600		129	101	1.28	439	311	1.41	712	549	1.29

^{a,b,c} Means within columns with no common superscripts differ significantly ($P < .05$)

Table 4.4. The effect of phytase supplementation on feed intake (g), body weight (g) and feed to gain ratio (g:g) of female broiler chickens fed corn-soybean diets for 19 d

Diet		0 to 7 d			0 to 14 d			0 to 19 d		
P-Ca level	Phytase (U/kg)	Feed intake	Weight gain	Feed: gain	Feed intake	Weight gain	Feed: gain	Feed intake	Weight gain	Feed: gain
Normal P-normal Ca	0	127^{ab}	94	1.35	429^{a}	295^{ab}	1.45^{abc}	675^{a}	495^{ab}	1.37
Normal P-normal Ca	600	126^{ab}	97	1.31	433^{a}	313^{a}	1.39^{c}	670^{ab}	523^{a}	1.29
Low P-normal Ca	0	124^{b}	88	1.41	393^{b}	255^{c}	1.54^{a}	589^{b}	438^{b}	1.34
Low P-normal Ca	600	116^{c}	89	1.32	397^{b}	263^{bc}	1.51^{ab}	590^{b}	461^{b}	1.28
Low P-low Ca	0	128^{ab}	97	1.31	417^{ab}	289^{ab}	1.45^{abc}	620^{ab}	487^{ab}	1.27
Low P-low Ca	600	131^{a}	99	1.32	427^{a}	300^{a}	1.42^{bc}	699^{ab}	561^{a}	1.25
SEM		1.25	1.45	0.05	4.80	6.03	0.05	13.5	11.40	0.016
ANOVA						Probabilities				
Diet		0.003	0.112	0.256	0.024	0.011	0.025	0.031	0.004	0.237
P-Ca level		0.001	0.022	0.485	0.004	0.002	0.007	0.012	0.003	0.198
Phytase		0.297	0.409	0.117	0.430	0.165	0.085	0.233	0.012	0.084
P-Ca level * Phytase		0.054	0.986	0.242	0.915	0.902	0.767	0.207	0.319	0.719
Main effects										
P-Ca level										
Normal P-normal Ca		126^{a}	96^{a}	1.33	431^{a}	304^{a}	1.42^{b}	673^{a}	509^{a}	1.33
Low P-normal Ca		120^{b}	89^{b}	1.36	395^{b}	259^{b}	1.52^{a}	589^{b}	449^{b}	1.31
Low P-low Ca		129^{a}	98^{a}	1.32	422^{a}	294^{a}	1.44^{b}	659^{a}	524^{a}	1.26
Phytase										
0		126	93	1.36	413	279	1.48	628	473	1.33
600		124	95	1.31	419	292	1.44	653	515	1.27

[a,b,c] Means within columns with no common superscript differ significantly ($P < .05$)

Table 4.5. Effect of phytase on apparent ileal digestibility of protein and essential amino acids in 28-d-old male chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Normal P-normal Ca	0	80.7^{ab}	90.6^{ab}	84.0^{ab}	77.9	87.1	86.3^{ab}	89.7^{bc}	87.1^{ab}	75.1^{b}	82.7
Normal P-normal Ca	600	83.5^{a}	92.2^{a}	88.0^{a}	81.1	88.6	88.5^{ab}	91.0^{abc}	87.9^{a}	82.0^{a}	85.8
Low P-normal Ca	0	83.9^{a}	90.5^{ab}	85.6^{ab}	78.7	88.1	89.6^{a}	93.6^{a}	84.5^{abc}	76.1^{b}	83.3
Low P-normal Ca	600	76.7^{b}	87.9^{b}	81.4^{b}	73.0	84.3	84.5^{b}	87.8^{c}	80.5^{c}	73.6^{b}	79.2
Low P-low Ca	0	83.2^{a}	91.6^{a}	85.2^{ab}	82.5	89.5	90.2^{a}	91.5^{ab}	86.9^{ab}	79.0^{ab}	85.6
Low P-low Ca	600	80.4^{ab}	90.1^{ab}	82.7^{b}	76.4	86.4	86.0^{ab}	89.5^{bc}	82.5^{bc}	75.2^{b}	81.6
SEM		0.722	0.402	0.657	0.986	0.598	0.649	0.526	0.717	0.864	0.749
ANOVA						Probabilities					
Diet		0.024	0.035	0.054	0.063	0.164	0.065	0.023	0.007	0.042	0.089
P-Ca level		0.462	0.051	0.207	0.186	0.391	0.747	0.955	0.007	0.164	0.196
Phytase		0.069	0.268	0.469	0.118	0.128	0.061	0.027	0.046	0.902	0.245
P-Ca level * Phytase		0.012	0.066	0.023	0.074	0.144	0.037	0.013	0.161	0.017	0.071
Main effects											
P-Ca level											
Normal P-normal Ca		82.1	91.4^{a}	86.0	79.5	87.9	87.4	90.3	87.5^{a}	78.5	84.3
Low P-normal Ca		80.3	89.2^{b}	83.5	75.9	86.2	87.0	90.7	82.5^{b}	74.8	81.3
Low P-low Ca		81.8	90.8^{ab}	84.0	79.5	87.9	88.1	90.5	84.7^{ab}	77.1	83.6
Phytase											
0		82.6	90.9	84.9	79.7	88.2	88.7	91.6	86.2	76.7	83.9
600		80.2	90.0	84.0	76.8	86.5	86.4	89.4	83.6	76.9	82.2

$^{a-c}$Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.6. Effect of phytase on apparent ileal digestibility of protein and essential amino acids in 28-d-old female chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Normal P-normal Ca	0	83.8	91.7[a]	86.1[a]	81.0[a]	89.1[a]	90.1[a]	91.6	88.7[ab]	77.2[a]	85.1[a]
Normal P-normal Ca	600	83.5	92.9[a]	88.2[a]	83.0[a]	90.7[a]	90.1[a]	92.0	89.7[a]	81.4[a]	87.3[a]
Low P-normal Ca	0	79.5	87.7[b]	81.9[b]	75.2[b]	86.0[b]	86.5[b]	90.6	82.0[c]	70.7[b]	80.6[b]
Low P-normal Ca	600	83.0	90.7[a]	85.7[ab]	81.0[a]	89.2[a]	88.8[ab]	90.8	86.4[ab]	78.2[a]	84.7[a]
Low P -lowCa	0	82.4	90.9[a]	84.6[ab]	80.9[a]	88.8[a]	89.7[a]	91.4	85.9[abc]	77.8[a]	84.6[a]
Low P-low Ca	600	83.2	92.1[a]	85.7[ab]	81.7[a]	89.7[a]	89.4[a]	91.7	84.9[bc]	79.2[a]	85.6[a]
SEM		0.549	0.403	0.564	0.730	0.416	0.376	0.289	0.653	0.912	0.577
ANOVA						Probabilities					
Diet		0.222	0.001	0.036	0.017	0.024	0.032	0.720	0.004	0.011	0.018
P-Ca level		0.190	0.001	0.035	0.028	0.046	0.012	0.292	0.001	0.034	0.021
Phytase		0.214	0.008	0.035	0.020	0.015	0.336	0.596	0.185	0.008	0.021
P-Ca level * Phytase		0.324	0.390	0.540	0.289	0.411	0.227	0.994	0.130	0.298	0.460
Main effects											
P-Ca level											
Normal P-normal Ca		83.6	92.3[a]	87.1[a]	82.4[a]	89.9[a]	90.1[a]	91.8	89.2[a]	79.3[a]	86.2[a]
Low P-normal Ca		81.2	89.2[b]	83.8[b]	78.1[b]	87.6[b]	87.6[b]	90.7	84.2[b]	74.4[b]	82.6[b]
Low P-low Ca		82.8	91.5[a]	85.1[ab]	81.3[a]	89.3[ab]	89.6[a]	91.5	85.4[b]	78.5[b]	85.1[ab]
Phytase											
0		81.9	90.1	84.2	79.0	88.0	88.8	91.2	85.9	75.3	83.4
600		83.2	91.9	86.5	82.1	89.9	89.4	91.5	87.0	79.6	85.9

[a,b,c] Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.7. Effect of phytase apparent ileal digestibilities of nonessential amino acids in 28-d-old male chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Normal P-normal Ca	0	84.6	88.3	90.1	80.3^{abc}	88.7^{bc}	79.2^{ab}	84.9
Normal P-normal Ca	600	87.9	91.9	92.4	85.1^{a}	91.5^{ab}	84.5^{a}	88.2
Low P-normal Ca	0	86.9	86.6	90.6	81.8^{ab}	93.9^{a}	77.2^{b}	86.2
Low P-normal Ca	600	81.0	85.9	87.8	75.9^{c}	87.0^{c}	75.8^{b}	80.9
Low P-low Ca	0	87.5	85.7	91.0	81.8^{ab}	92.4^{ab}	80.9^{ab}	87.5
Low P-low Ca	600	84.0	84.8	89.9	79.6^{bc}	91.3^{ab}	78.1^{b}	83.5
SEM		0.701	0.787	0.498	0.785	0.642	0.846	0.826
ANOVA					Probabilities			
Diet		0.058	0.094	0.156	0.014	0.015	0.036	0.089
P-Ca level		0.387	0.026	0.210	0.081	0.416	0.025	0.280
Phytase		0.152	0.648	0.547	0.411	0.136	0.812	0.190
P-Ca level * Phytase		0.028	0.367	0.097	0.009	0.004	0.082	0.060
Main effects								
P-Ca level								
Normal P-normal Ca		86.3	90.1^{a}	91.3	82.7	90.1	81.8^{a}	86.6
Low P-normal Ca		84.0	86.3^{b}	89.2	78.8	90.5	76.5^{b}	83.5
Low P-low Ca		85.7	85.2^{b}	90.5	80.7	91.9	79.5^{ab}	85.5
Phytase								
0		86.3	86.8	90.6	81.3	91.7	79.1	86.2
600		84.3	87.5	90.0	80.2	90.0	79.4	84.2

[a-c]Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.8. Effect of phytase on apparent ileal digestibility of nonessential amino acids in 28-d-old female chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Normal P-normal Ca	0	87.9[a]	89.2[ab]	91.7[ab]	82.8[a]	90.9	80.7[a]	86.9[a]
Normal P-normal Ca	600	89.7[a]	92.6[a]	93.4[a]	85.0[a]	92.1	84.5[a]	89.5[a]
Low P-normal Ca	0	83.9[b]	83.2[c]	88.1[c]	76.2[b]	91.3	72.8[b]	82.4[b]
Low P-normal Ca	600	86.9[a]	89.5[ab]	91.5[ab]	81.5[a]	90.7	80.0[a]	87.4[a]
Low P -low Ca	0	87.4[a]	85.2[bc]	90.9[b]	81.1[a]	93.0	79.9[a]	86.2[a]
Low P-low Ca	600	88.2[a]	86.8[bc]	91.9[ab]	82.7[a]	92.8	81.2[a]	87.0[a]
SEM		0.481	0.742	0.398	0.696	0.358	0.902	0.601
ANOVA					Probabilities			
Diet		0.010	0.0007	0.001	0.003	0.287	0.003	0.016
P-Ca level		0.006	0.002	0.004	0.003	0.084	0.005	0.045
Phytase		0.029	0.002	0.002	0.012	0.824	0.008	0.011
P-Ca level * Phytase		0.566	0.248	0.286	0.363	0.580	0.278	0.281
Main effects								
P-Ca level								
Normal P-normal Ca		88.8[a]	90.9[a]	92.6[a]	83.9[a]	91.5	82.6[a]	88.2[a]
Low P-normal Ca		85.4[b]	86.3[b]	89.8[b]	78.8[b]	91.0	76.4[b]	84.9[b]
Low P-low Ca		87.8[a]	86.0[b]	91.4[a]	81.9[a]	92.9	80.6[a]	86.6[ab]
Phytase								
0		86.4	85.9	90.2	80.3	91.7	77.8	85.2
600		88.3	89.6	92.3	83.1	91.9	81.9	88.0

[a-c]Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.9. Effect of phytase on apparent "fecal" digestibility of protein and essential amino acids in 28-d-old male chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Normal P-normal Ca	0	80.0[a]	91.3[a]	84.5[ab]	78.9[a]	87.9[a]	86.8[ab]	90.0	87.9[a]	76.1[a]	83.5[a]
Normal P-normal Ca	600	79.1[a]	92.0[a]	86.2[a]	80.5[a]	89.1[a]	86.9[ab]	89.7	88.0[a]	79.2[a]	84.5[a]
Low P-normal Ca	0	71.1[c]	86.0[c]	76.7[c]	67.9[c]	82.3[c]	78.7[c]	87.2	78.8[b]	65.5[c]	73.1[c]
Low P-normal Ca	600	73.1[bc]	87.2[bc]	77.7[c]	71.1[bc]	83.6[bc]	82.2[bc]	86.1	79.4[b]	69.9[bc]	77.0[bc]
Low P-low Ca	0	80.3[a]	91.4[a]	85.0[a]	82.1[a]	89.4[a]	89.3[a]	91.5	86.4[a]	77.5[a]	85.4[a]
Low P-low Ca	600	77.6[ab]	89.8[ab]	82.0[b]	76.5[ab]	86.6[ab]	84.4[ab]	88.9	82.7[ab]	75.1a[b]	81.5[ab]
SEM		0.889	0.531	0.793	1.25	0.688	0.922	0.629	0.941	1.08	1.03
ANOVA						Probabilities					
Diet		0.002	0.0003	0.0001	0.002	0.003	0.005	0.143	0.001	0.0002	0.0004
P-Ca level		0.0003	0.0001	0.0001	0.002	0.0004	0.001	0.043	0.0001	0.0001	0.0001
Phytase		0.707	0.873	0.903	0.888	0.909	0.767	0.267	0.530	0.293	0.849
P-Ca level * Phytase		0.413	0.324	0.168	0.169	0.245	0.090	0.706	0.441	0.196	0.134
Main effects											
P-Ca level											
Normal P-normal Ca		79.6[a]	91.7[a]	85.4[a]	79.7[a]	88.5[a]	86.9[a]	89.8[a]	83.0[a]	76.6[a]	84.0[a]
Low P-normal Ca		72.1[b]	87.6[b]	77.2[b]	69.5[b]	82.9[b]	80.4[b]	86.7[b]	79.1[b]	67.7[b]	75.0[b]
Low P-low Ca		78.9[a]	90.6[a]	83.4[a]	79.3[a]	88.0[a]	86.9[a]	90.2[a]	84.5[a]	76.3[a]	83.4[a]
Phytase											
0		77.1	89.6	82.1	76.3	86.5	84.9	89.6	84.3	73.0	80.7
600		76.6	89.7	81.9	76.0	86.4	84.5	88.2	83.4	74.9	81.0

[a-c]Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.10. Effect of phytase on apparent "fecal" digestibility of protein and essential amino acids in 28-d-old female chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Normal P-normal Ca	0	81.1	91.2[a]	86.8[a]	82.5	89.9	89.0	92.5	89.0[a]	79.0[a]	86.2
Normal P-normal Ca	600	80.4	92.4[a]	87.1[a]	82.7	90.0	89.3	90.9	89.0[a]	81.1[a]	86.5
Low P-normal Ca	0	74.3	87.7[b]	77.8[b]	75.2	86.2	84.0	89.8	81.7[b]	66.5[b]	79.5
Low P-normal Ca	600	80.2	90.0[ab]	83.8[a]	79.8	88.7	87.1	89.8	86.0[a]	76.8[a]	83.6
Low P-low Ca	0	79.1	90.7[a]	82.2[a]	79.7	88.7	87.8	90.1	86.0[a]	76.1[a]	83.3
Low P-low Ca	600	81.0	91.5[a]	84.5[a]	80.4	89.3	87.0	90.2	84.9[ab]	79.1[a]	84.4
SEM		0.906	0.424	0.772	0.910	0.459	0.658	0.425	0.669	0.218	0.784
ANOVA						Probabilities					
Diet		0.252	0.019	0.003	0.188	0.175	0.333	0.428	0.006	0.003	0.107
P-Ca level		0.254	0.006	0.001	0.073	0.074	0.123	0.158	0.002	0.005	0.040
Phytase		0.187	0.066	0.095	0.305	0.225	0.689	0.567	0.338	0.015	0.222
P-Ca level * Phytase		0.328	0.717	0.116	0.503	0.547	0.518	0.671	0.125	0.199	0.532
Main effects											
P-Ca level											
Normal P-normal Ca		80.7	91.8[a]	86.9[a]	82.6	89.9	89.2	91.7	89.0[a]	80.1[a]	86.4[a]
Low P-normal Ca		79.3	88.8[b]	80.8[b]	77.5	87.4	85.8	89.8	83.9[b]	71.7[b]	81.6[b]
Low P-low Ca		80.0	91.1[a]	84.4[a]	80.1	89.0	87.4	90.1	85.4[b]	77.8[a]	83.9[ab]
Phytase											
0		78.2	89.9	82.9	79.1	88.2	87.2	90.8	85.6	73.9	83.0
600		80.5	91.3	85.1	81.0	89.3	87.7	90.3	86.6	79.0	84.8

[a,b]Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.11. Effect of phytase on apparent "fecal" digestibility of nonessential amino acids in 28-d-old male chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Normal P-normal Ca	0	84.9[a]	88.1[ab]	90.3[a]	80.4[a]	89.2[a]	79.9[a]	85.7[a]
Normal P-normal Ca	600	86.5[a]	90.6[a]	91.8[a]	82.2[a]	89.8[a]	82.6[a]	87.3[a]
Low P-normal Ca	0	76.8[b]	78.3[c]	84.9[c]	69.6[b]	87.1[ab]	70.1[b]	78.3[c]
Low P-normal Ca	600	78.3[b]	83.5[b]	86.1[bc]	71.9[b]	82.8[b]	73.3[b]	80.2[bc]
Low P -lowCa	0	87.1[a]	84.4[b]	90.8[a]	79.4[a]	91.2[a]	79.7[a]	87.5[a]
Low P-low Ca	600	83.5[a]	83.7[b]	89.3[ab]	77.0[a]	88.9[a]	78.4[a]	83.9[ab]
SEM		0.952	0.936	0.614	0.996	0.816	0.933	0.853
ANOVA					Probabilities			
Diet		0.0007	0.0008	0.0007	0.0001	0.039	0.0001	0.001
P-Ca level		0.0001	0.0002	0.0001	0.0001	0.015	0.0001	0.0002
Phytase		0.922	0.113	0.690	0.670	0.180	0.245	0.975
P-Ca level * Phytase		0.272	0.272	0.356	0.309	0.288	0.311	0.182
Main effects								
P-Ca level								
Normal P-normal Ca		85.7[a]	89.3[a]	91.1[a]	81.3[a]	89.5[a]	81.2[a]	86.5[a]
Low P-normal Ca		77.5[b]	80.9[b]	85.5[b]	70.8[b]	85.0[b]	71.7[b]	79.2[b]
Low P-low Ca		85.3[a]	84.0[b]	90.1[a]	78.2[a]	90.0[a]	79.0[a]	85.7[a]
Phytase								
0		82.9	83.6	88.7	76.5	89.2	76.6	83.8
600		82.8	85.9	89.1	77.1	87.2	78.1	83.8

[a-c]Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.12. Effect of phytase on apparent "fecal" digestibility of nonessential amino acids in 28-d-old female chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Normal P-normal Ca	0	88.2[a]	90.7[a]	91.7[a]	81.3[a]	91.0	82.9[a]	88.1[a]
Normal P-normal Ca	600	88.8[a]	90.7[a]	92.4[a]	82.8[a]	91.2	83.8[a]	88.9[a]
Low P-normal Ca	0	81.5[b]	79.8[c]	86.4[b]	70.1[b]	87.5	69.3[b]	82.1[b]
Low P-normal Ca	600	85.1[ab]	88.8[ab]	90.7[a]	78.9[a]	90.3	79.4[a]	85.9[ab]
Low P-low Ca	0	85.8[ab]	83.1[bc]	90.2[a]	79.1[a]	92.2	79.2[a]	85.2[ab]
Low P-low Ca	600	86.3[ab]	84.8[abc]	90.7[a]	80.4[a]	91.1	81.8[a]	86.5[ab]
SEM		0.743	0.005	0.530	0.142	0.686	0.132	0.675
ANOVA					Probabilities			
Diet		0.051	0.001	0.014	0.013	0.491	0.0003	0.044
P-Ca level		0.013	0.002	0.015	0.011	0.242	0.0005	0.017
Phytase		0.256	0.030	0.049	0.058	0.638	0.011	0.116
P-Ca level * Phytase		0.568	0.062	0.188	0.224	0.523	0.075	0.546
Main effects								
P-Ca level								
Normal P-normal Ca		88.5[a]	90.6[a]	92.0[a]	82.1[a]	91.1	83.4[a]	88.5[a]
Low P-normal Ca		83.3[b]	84.3[b]	88.6[b]	74.3[b]	88.9	70.3[b]	84.0[b]
Low P-low Ca		86.1[ab]	84.0[b]	90.4[ab]	79.8[a]	91.7	80.5[a]	85.8[ab]
Phytase								
0		85.2	84.5	89.4	76.9	90.2	77.1	85.1
600		86.7	88.1	91.3	80.7	90.8	81.7	87.1

[a-c]Means within column with no common superscript differ significantly ($P < 0.05$)

Table 4.13. Effect of phytase on disappearance of protein and essential amino acids from gizzard in 28-d-old male chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Normal P-normal Ca	0	78.2[a]	85.8[a]	82.0[a]	75.9[a]	80.6[a]	86.2[a]	86.9[a]	82.3[a]	79.8[a]	81.1[a]
Normal P-normal Ca	600	79.1[a]	86.4[a]	83.9[a]	76.2[a]	81.7[a]	85.0[ab]	85.8[ab]	81.5[a]	82.7[a]	81.9[a]
Low P-normal Ca	0	67.1[b]	73.4[b]	68.9[c]	56.8[c]	65.1[b]	75.9[c]	77.6[c]	63.1[c]	68.5[c]	66.3[c]
Low P-normal Ca	600	68.5[b]	76.8[b]	72.9[bc]	62.7[bc]	70.2[b]	79.4[c]	77.6[c]	68.5[bc]	73.1[bc]	70.7[bc]
Low P-low Ca	0	68.4[b]	78.5[b]	75.6[b]	65.9[b]	71.2[b]	80.6[bc]	81.3[bc]	71.4[b]	74.6[b]	72.7[b]
Low P-low Ca	600	65.1[b]	75.2[b]	70.5[bc]	56.5[c]	65.2[b]	75.7[c]	77.5[c]	62.4[c]	69.4[bc]	66.7[c]
SEM		1.20	1.06	1.18	1.69	1.40	0.924	0.912	1.64	1.10	1.28
ANOVA						Probabilities					
Diet		0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0003	0.0001	0.0001	0.0001
P-Ca level		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Phytase		0.862	0.844	0.855	0.629	0.973	0.513	0.239	0.474	0.583	0.876
P-Ca level * Phytase		0.431	0.159	0.055	0.026	0.056	0.052	0.496	0.025	0.026	0.032
Main effects											
P-Ca level											
Normal P-normal Ca		78.7[a]	86.1[a]	83.0[a]	76.1[a]	81.2[a]	85.6[a]	86.4[a]	81.9[a]	81.2[a]	81.6[a]
Low P-normal Ca		67.8[b]	75.1[b]	70.9[b]	59.7[b]	67.7[b]	77.7[b]	77.7[b]	65.8[b]	70.8[b]	68.6[b]
Low P-low Ca		66.8[b]	76.8[b]	73.1[b]	61.2[b]	68.2[b]	78.1[b]	79.4[b]	66.9[b]	72.0[b]	69.7[b]
Phytase											
0		71.2	79.6	75.5	66.2	72.3	80.9	81.9	72.3	74.2	73.4
600		70.9	72.9	75.8	65.1	72.4	80.0	80.3	70.7	75.1	73.2

[a,b,c] Means within each column with no common superscript differ significantly ($P < 0.05$)

Table 4.14. Effect of phytase on disappearance of protein and essential amino acids from gizzard in 28-d-old female chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Normal P-normal Ca	0	73.6^{b}	80.1^{b}	75.8^{b}	67.1^{b}	74.5^{b}	80.0^{b}	81.6	75.6^{ab}	73.6^{b}	74.9^{b}
Normal P-normal Ca	600	78.7^{a}	85.8^{a}	83.5^{a}	76.4^{a}	81.0^{a}	84.7^{a}	84.3	81.1^{a}	83.1^{a}	82.3^{a}
Low P-normal Ca	0	70.6^{b}	76.8^{bc}	73.5^{bc}	63.8^{b}	72.4^{b}	78.7^{b}	81.6	69.2^{c}	69.8^{bc}	71.7^{b}
Low P-normal Ca	600	69.2^{bc}	77.2^{bc}	74.9^{b}	63.1^{b}	71.9^{b}	77.1^{bc}	79.1	69.9^{bc}	72.7^{bc}	71.2^{bc}
Low P-low Ca	0	70.9^{b}	78.6^{bc}	75.6^{b}	64.0^{b}	72.2b	80.5^{ab}	81.3	70.9^{bc}	74.2^{b}	72.7^{b}
Low P-low Ca	600	65.1^{c}	75.2^{c}	70.3^{c}	56.7^{c}	66.3^{c}	73.9^{c}	78.6	60.1^{d}	68.5^{c}	67.1^{c}
SEM		0.938	0.763	0.853	1.25	0.968	0.800	0.587	1.31	0.960	0.957
ANOVA						Probabilities					
Diet		0.0001	0.0001	0.0001	0.0001	0.0001	0.0009	0.052	0.0001	0.0001	0.0001
P-Ca level		0.0001	0.0001	0.0001	0.0001	0.0001	0.003	0.054	0.0001	0.0001	0.0001
Phytase		0.596	0.395	0.277	0.796	0.987	0.392	0.426	0.336	0.069	0.728
P-Ca level * Phytase		0.009	0.005	0.0002	0.001	0.003	0.003	0.082	0.0007	0.0001	0.0005
Main effects											
P-Ca level											
Normal P-normal Ca		76.2^{a}	82.9^{a}	79.6^{a}	71.7^{a}	77.8^{a}	82.4^{a}	82.9^{a}	78.3^{a}	78.4^{a}	78.6^{a}
Low P-normal Ca		69.9^{b}	77.0^{b}	74.1^{b}	63.5^{b}	72.1^{b}	77.9^{b}	80.3^{ab}	69.6^{b}	71.3^{b}	71.4^{b}
Low P-low Ca		68.1^{b}	76.9^{b}	72.9^{b}	60.4^{b}	69.2^{b}	77.2^{b}	79.9^{b}	65.5^{c}	71.4^{b}	69.9^{b}
Phytase											
0		71.7	78.5	74.9	64.9	73.0	79.7	81.5	71.9	72.5	73.1
600		71.0	79.4	76.2	65.4	73.0	78.6	80.6	70.3	74.7	73.5

^{a,b,c,d} Means within each column with no common superscript differ significantly ($P < 0.05$)

Table 4.15. Effect of phytase on disappearance of nonessential amino acids from gizzard in 28-d-old male chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Normal P-normal Ca	0	80.4[a]	86.1[a]	85.8[a]	81.3[a]	87.8	81.5[a]	81.3[a]
Normal P-normal Ca	600	82.6[a]	86.7[a]	86.6[a]	82.5[a]	88.3	83.1[a]	83.0[a]
Low P-normal Ca	0	65.6[b]	75.2[b]	73.4[b]	69.4[b]	83.9	68.9[b]	64.8[c]
Low P-normal Ca	600	69.6[b]	77.4[b]	76.4[b]	71.2[b]	81.4	71.9[b]	72.2[b]
Low P-low Ca	0	71.7[b]	79.1[b]	77.6[b]	73.8[b]	88.1	73.7[b]	74.2[b]
Low P-low Ca	600	66.6[b]	76.4[b]	74.5[b]	69.8[b]	82.8	69.8[b]	68.9[bc]
SEM		1.33	0.991	1.09	1.12	0.99	1.16	1.39
ANOVA					Probabilities			
Diet		0.0001	0.0001	0.0001	0.0001	0.163	0.0001	0.0001
P-Ca level		0.0001	0.0001	0.0001	0.0001	0.086	0.0001	0.0001
Phytase		0.816	0.988	0.836	0.825	0.206	0.903	0.496
P-Ca level * Phytase		0.072	0.324	0.234	0.214	0.471	0.152	0.032
Main effects								
P-Ca level								
Normal P-normal Ca		81.5[a]	86.4	86.1[a]	81.9[a]	88.1	82.4[a]	82.1[a]
Low P-normal Ca		67.6[b]	76.3	74.8[b]	70.3[b]	82.7	70.4[b]	68.5[b]
Low P-low Ca		69.1[b]	77.8	76.1[b]	71.8[b]	85.5	71.8[b]	71.6[b]
Phytase								
0		72.6	80.1	78.9	74.8	86.7	74.8	73.4
600		72.9	80.1	79.1	74.5	84.2	74.9	74.7

[a,b,c] Means within each column with no common superscript differ significantly ($P < 0.05$)

Table 4.16. Effect of phytase on disappearance of nonessential amino acids from gizzard in 28-d-old female chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Normal P-normal Ca	0	74.8[a]	80.6[b]	80.5[b]	75.2[b]	83.5	75.7[b]	75.2[b]
Normal P-normal Ca	600	81.7[a]	86.2[a]	85.7[a]	82.4[a]	86.9	83.4[a]	82.8[a]
Low P-normal Ca	0	71.4[bc]	77.7[b]	77.7[b]	71.7[bc]	84.8	72.3[bc]	73.7[bc]
Low P-normal Ca	600	70.8[bc]	77.6[b]	77.3[b]	70.8[bc]	79.6	72.5[bc]	73.1[bc]
Low P-low Ca	0	73.4[b]	79.0[b]	78.9[b]	73.9[bc]	87.9	74.6[bc]	73.2[bc]
Low P-low Ca	600	67.2[c]	78.0[b]	76.1[b]	69.6[c]	83.1	70.2[c]	69.2[c]
SEM		0.983	0.781	0.771	0.896	0.993	0.894	0.887
ANOVA					Probabilities			
Diet		0.0001	0.004	0.001	0.0001	0.181	0.0001	0.0001
P-Ca level		0.0001	0.001	0.0006	0.0001	0.304	0.0001	0.0001
Phytase		0.965	0.273	0.579	0.567	0.262	0.391	0.474
P-Ca level * Phytase		0.001	0.091	0.030	0.001	0.129	0.001	0.001
Main effects								
P-Ca level								
Normal P-normal Ca		78.2[a]	83.4[a]	83.1[a]	71.7[a]	85.2	79.3[a]	79.1[a]
Low P-normal Ca		71.1[b]	77.6[b]	77.4[b]	71.3[b]	82.2	72.3[b]	73.5[b]
Low P-low Ca		70.3[b]	78.5[b]	77.5[b]	71.7[b]	85.5	72.4[b]	71.2[b]
Phytase								
0		73.1	79.1	79.1	73.6	85.3	74.1	74.1
600		73.2	80.5	79.7	74.3	83.2	75.2	75.1

[a,b,c] Means within each column with no common superscript differ significantly ($P < 0.05$)

Table 4.17. Effect of phytase on apparent ileal digestibility of protein and essential amino acids in 21-d-old male chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Low P-normal Ca	0	79.9	88.7	83.5	77.3	86.8	87.5	89.6	87.2	72.2	81.9
Low P-normal Ca	600	76.9	89.8	83.4	75.8	84.7	85.2	86.1	85.1	73.5	79.2
Low P-low Ca	0	83.0	92.5	89.7	84.1	89.2	89.3	91.6	89.8	79.3	85.7
Low P-low Ca	600	77.7	91.1	89.0	82.0	88.0	88.0	88.4	88.1	78.3	83.4
SEM		1.59	0.756	1.21	1.64	1.14	1.29	1.15	1.10	1.62	1.38
ANOVA						Probabilities					
Diet		0.534	0.340	0.080	0.252	0.603	0.785	0.449	0.591	0.320	0.544
P-Ca level		0.542	0.113	0.014	0.064	0.266	0.449	0.387	0.270	0.080	0.230
Phytase		0.213	0.945	0.821	0.576	0.502	0.538	0.185	0.451	0.900	0.456
P-Ca level * Phytase		0.731	0.418	0.870	0.921	0.851	0.873	0.970	0.963	0.772	0.967
Main effects											
P-Ca level											
Low P-normal Ca		78.4	89.2	83.4	76.6	85.7	86.4	87.9	86.2	72.8	80.9
Low P-low Ca		80.4	91.8	89.4	83.1	88.6	88.6	90.0	88.9	79.0	84.6
Phytase											
0		81.5	90.6	86.6	80.7	88.0	88.4	90.6	88.5	75.7	83.8
600		77.3	90.5	86.2	78.9	86.3	86.6	87.2	86.6	76.1	81.6

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$)

Table 4.18. Effect of phytase on apparent ileal digestibility of protein and essential amino acids in 21-d-old female chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Low P-normal Ca	0	80.5	89.7	83.7	798.1	88.1	88.4	90.1	88.1	74.2	83.1
Low P-normal Ca	600	79.2	90.6	87.4	80.9	87.3	86.0	87.5	86.9	75.5	81.9
Low P-low Ca	0	77.2	90.9	86.5	80.8	86.7	86.9	90.0	87.9	77.0	82.6
Low P-low Ca	600	81.7	92.1	88.5	83.7	89.3	89.2	91.2	89.0	80.7	85.0
SEM		0.797	0.409	0.803	0.850	0.552	0.642	0.571	0.513	1.00	0.680
ANOVA						Probabilities					
Diet		0.231	0.481	0.173	0.316	0.421	0.317	0.103	0.605	0.094	0.470
P-Ca level		0.803	0.232	0.191	0.203	0.781	0.508	0.090	0.414	0.038	0.360
Phytase		0.313	0.341	0.280	0.184	0.447	0.981	0.471	0.933	0.163	0.679
P-Ca level * Phytase		0.078	0.917	0.562	0.735	0.157	0.092	0.070	0.305	0.470	0.234
Main effects											
P-Ca level											
Low P-normal Ca		79.8	90.2	85.5	80.0	87.7	87.2	88.8	87.5	74.9[b]	82.5
Low P-low Ca		79.5	91.5	87.5	82.3	88.0	88.0	90.6	88.4	78.8[a]	83.8
Phytase											
0		78.9	90.3	85.1	80.0	87.4	87.6	90.0	88.0	75.6	82.9
600		80.4	91.5	87.9	82.3	88.3	87.6	89.4	87.9	78.1	83.5

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$)

Table 4.19. Effect of phytase on apparent ileal digestibility of nonessential amino acids in 21-d-old male chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Low P-normal Ca	0	83.9	84.9	89.0	77.5	88.4	76.5	84.2
Low P-normal Ca	600	81.3	85.7	88.7	77.5	84.4	73.5	83.5
Low P-low Ca	0	87.2	85.3	90.7	82.0	89.5	82.1	89.2
Low P-low Ca	600	85.6	85.6	90.1	80.1	86.7	80.9	87.3
SEM		1.56	1.26	0.892	0.141	1.24	0.179	1.28
ANOVA					Probabilities			
Diet		0.651	0.997	0.847	0.671	0.559	0.331	0.392
P-Ca level		0.290	0.944	0.420	0.271	0.527	0.096	0.117
Phytase		0.543	0.858	0.785	0.757	0.226	0.565	0.627
P-Ca level * Phytase		0.886	0.935	0.992	0.754	0.821	0.800	0.819
Main effects								
P-Ca level								
Low P-normal Ca		82.6	85.3	88.7	77.5	86.4	75.0	83.8
Low P-low Ca		86.4	85.5	90.4	81.1	88.1	81.5	88.3
Phytase								
0		85.6	85.1	89.8	79.8	88.9	79.3	86.7
600		83.4	85.7	89.3	78.8	85.5	77.2	85.4

Table 4.20. Effect of phytase supplementation on apparent ileal digestibility of nonessential amino acids in 21-d-old female broiler chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Low P-normal Ca	0	85.1	86.1	89.8	80.0	89.3	78.3	86.1
Low P-normal Ca	600	83.1	85.4	89.1	77.7	87.2	77.4	86.5
Low P-low Ca	0	84.0	83.0	89.3	78.6	85.8	78.1	86.3
Low P-low Ca	600	86.7	86.3	91.3	82.1	90.0	81.1	88.1
SEM		0.079	0.716	0.446	0.802	0.866	0.855	0.618
ANOVA					Probabilities			
Diet		0.341	0.378	0.311	0.235	0.330	0.293	0.693
P-Ca level		0.390	0.464	0.344	0.326	0.839	0.277	0.514
Phytase		0.826	0.387	0.479	0.697	0.531	0.431	0.421
P-Ca level * Phytase		0.121	0.185	0.142	0.081	0.097	0.192	0.591
Main effects								
P-Ca level								
Low P-normal Ca		84.1	85.7	89.5	78.8	80.3	77.9	86.3
Low P-low Ca		85.3	84.6	90.3	80.4	87.9	80.0	87.2
Phytase								
0		84.6	84.5	89.6	79.3	87.5	78.2	86.2
600		84.9	85.8	90.2	79.9	88.6	79.6	87.3

Table 4.21. Effect of phytase on apparent "fecal" digestibility of protein and essential amino acids in 21-d-old male chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Low P-normal Ca	0	75.2	88.8^{c}	80.0^{b}	78.1^{bc}	87.2	86.4	89.4	86.9	70.5^{b}	81.9
Low P-normal Ca	600	65.3	90.1^{bc}	83.3^{b}	76.5^{c}	85.6	84.3	86.8	85.4	72.0^{b}	79.9
Low P-low Ca	0	78.7	92.0^{ab}	89.5^{a}	84.7^{ab}	89.7	88.1	90.8	89.8	77.3^{ab}	85.6
Low P-low Ca	600	80.0	93.4^{a}	91.3^{a}	86.5^{a}	91.0	90.3	91.8	91.3	83.7^{a}	87.5
SEM		2.96	0.614	1.54	1.62	0.975	1.14	0.832	1.00	2.03	1.31
ANOVA						Probabilities					
Diet		0.311	0.010	0.003	0.042	0.210	0.313	0.148	0.136	0.055	0.147
P-Ca level		0.144	0.002	0.0006	0.007	0.056	0.113	0.053	0.032	0.015	0.036
Phytase		0.465	0.106	0.148	0.968	0.936	0.970	0.581	0.993	0.228	0.980
P-Ca level * Phytase		0.344	0.951	0.670	0.476	0.435	0.339	0.240	0.407	0.447	0.403
Main effects											
P-Ca level											
Low P-normal Ca		70.3	89.5^{b}	81.6^{b}	77.3b	86.4	85.4	88.1	86.1^{b}	71.3^{b}	80.9^{b}
Low P-low Ca		79.4	92.7^{a}	90.4^{a}	85.6a	90.3	89.2	91.3	90.5^{a}	80.5^{a}	86.6^{a}
Phytase											
0		77.0	90.4	84.7	91.4	88.5	87.2	90.1	88.3	73.9	83.8
600		72.6	91.8	87.3	81.5	88.3	87.3	89.3	88.3	77.9	83.7

$^{a-c}$ Means within columns with no common superscript differ significantly ($P < 0.05$)

Table 4.22. Effect of phytase on apparent "fecal" digestibility of protein and essential amino acids in 21-d-old female chickens

Diet											
P-Ca level	Phytase	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
	(U/kg)					(%)					
Low P-normal Ca	0	68.6	87.2^{b}	77.5^{b}	72.8^{b}	84.9	82.8^{b}	86.6	85.1^{b}	66.7^{b}	77.1^{b}
Low P-normal Ca	600	74.4	91.7^{a}	87.7^{a}	80.9^{a}	87.6	84.5^{ab}	88.0	88.0^{ab}	75.9^{a}	81.6^{ab}
Low P-low Ca	0	75.9	92.2^{a}	89.6^{a}	84.3^{a}	89.4	88.5^{a}	90.4	90.3^{a}	79.8^{a}	85.6^{a}
Low P-low Ca	600	78.7	92.2^{a}	90.2^{a}	83.6^{a}	89.5	89.0^{a}	90.0	89.5^{a}	80.5^{a}	84.7^{a}
SEM		1.55	0.793	1.63	1.65	0.767	1.02	0.737	0.790	1.82	1.27
ANOVA						Probabilities					
Diet		0.103	0.036	0.001	0.016	0.092	0.053	0.228	0.059	0.002	0.045
P-Ca level		0.050	0.042	0.001	0.009	0.032	0.010	0.060	0.022	0.0009	0.014
Phytase		0.123	0.077	0.008	0.117	0.275	0.0513	0.3715	0.406	0.022	0.359
P-Ca level * Phytase		0.574	0.078	0.014	0.066	0.321	0.725	0.531	0.148	0.039	0.183
Main effects											
P-Ca level											
Low P-normal Ca		71.5	89.5	82.6	76.8	86.3	83.6	87.3	86.5	71.3	79.4
Low P-low Ca		77.3	92.2	89.9	83.9	89.4	88.8	90.2	89.9	80.2	85.1
Phytase											
0		72.2	89.7	83.6^{b}	78.5	87.1	85.7	88.5	87.7	73.3^{b}	81.3
600		76.6	92.0	89.0^{a}	82.2	88.6	86.7	89.0	88.8	78.2^{a}	83.1

^{a,b} Means within columns with no common superscript differ significantly ($P < 0.05$)

Table 4.23. Effect of phytase on apparent "fecal" digestibility of nonessential amino acids in 21-d-old male chickens

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Low P-normal Ca	0	83.2	85.0	88.2	73.4	86.9	79.4	84.5
Low P-normal Ca	600	81.2	86.1	88.2	74.2	84.9	73.0	83.8
Low P-low Ca	0	86.1	83.6	89.9	78.5	88.1	80.1	89.4
Low P-low Ca	600	88.6	88.8	92.6	83.2	89.9	84.9	90.8
SEM		1.14	1.05	0.842	1.76	1.06	1.89	1.29
ANOVA					Probabilities			
Diet		0.289	0.385	0.198	0.175	0.468	0.095	0.122
P-Ca level		0.089	0.743	0.073	0.052	0.192	0.025	0.024
Phytase		0.914	0.164	0.377	0.397	0.970	0.714	0.865
P-Ca level * Phytase		0.421	0.345	0.402	0.541	0.460	0.271	0.622
Main effects								
P-Ca level								
Low P-normal Ca		82.2	85.5	88.2	73.8[b]	85.9	74.2[b]	84.2[b]
Low P-low Ca		87.3	86.2	91.3	80.8[a]	89.0	82.5[a]	90.1[a]
Phytase								
0		84.6	84.3	89.0	75.9	87.5	77.8	86.9
600		84.9	87.5	90.4	78.7	87.4	78.9	87.3

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$)

Table 4.24. Effect of phytase on apparent "fecal" digestibility of nonessential amino acids in 21-d-old female chickens.

Diet								
P-Ca level	Phytase	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
	(U/kg)				(%)			
Low P-normal Ca	0	78.8	83.9	87.0	69.8[b]	86.2	73.7[b]	81.4[b]
Low P-normal Ca	600	82.4	86.7	89.0	76.4[ab]	86.6	78.1[ab]	86.2[ab]
Low P-low Ca	0	86.2	84.7	90.7	79.2[a]	88.8	80.8[a]	88.9[a]
Low P-low Ca	600	86.4	85.6	91.1	80.6[a]	89.2	81.4[a]	88.6[a]
SEM		1.23	0.937	0.733	1.55	0.755	1.14	1.11
ANOVA					Probabilities			
Diet		0.060	0.807	0.182	0.029	0.432	0.029	0.025
P-Ca level		0.015	0.939	0.056	0.012	0.121	0.010	0.010
Phytase		0.338	0.402	0.379	0.100	0.807	0.142	0.178
P-Ca level * Phytase		0.390	0.674	0.549	0.253	0.967	0.258	0.128
Main effects								
P-Ca level								
Low P-normal Ca		80.6	85.3	87.9	73.1	86.4	75.9	83.8
Low P-low Ca		86.3	85.1	90.8	79.9	89.0	81.1	88.8
Phytase								
0		82.5	84.3	88.1	74.5	87.5	77.2	85.2
600		84.4	86.1	90.0	78.5	87.9	79.8	87.4

[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$)

Table 4.25. Comparison of apparent ileal (AID) and fecal (AFD) digestibilities (%) calculated by using either Cr_2O_3 or ADF as a marker[1]

Digestibility:	AID		AFD	
Marker:	Cr_2O_3	ADF	Cr_2O_3	ADF
Amino acids				
Asp	83.5	83.5	83.0	81.2
Glu	89.9	90.1	90.3	89.3
Ser	77.0	77.4	77.9	75.7
Gly	79.2	79.7	78.2	76.0
His	82.9	83.5	83.8	82.1
Arg	90.3	90.6	90.8	89.9
Thr	75.1	75.5	75.5	73.1
Ala	85.4	85.8	85.9	84.5
Pro	93.6	93.4	92.9	91.9
Tyr	85.5	85.8	86.4	85.0
Val	83.3	83.6	84.0	82.4
Met	90.1	90.5	90.8	89.9
Ile	80.1	80.5	80.9	78.9
Leu	87.7	88.0	88.8	87.6
Phe	87.7	87.9	87.5	86.1
Lys	90.0	90.1	89.2	88.1
CP	80.4	80.9	78.5	76.3

[1] There is no statistical difference ($P > 0.05$) between digestibilities calculated by using Cr_2O_3 and ADF

Chapter V

GENERAL DISCUSSION

GENERAL DISCUSSION

Introduction

Poultry diets consist largely of seeds and seed by-products which contain high levels of phytic acid. Phytic acid being a strong acid, can form various salts with important minerals and also complex with proteins and make them less available (Cheryan, 1980). Chickens cannot completely hydrolyze the phytic acid due to lack of endogenous phytase. The main objectives of this thesis were to study the effect of microbial phytase supplementation on growth performance, availability of macro and trace minerals, and the AID and AFD of crude protein and amino acids and to investigate the influence of dietary P and Ca levels on the efficacy of supplemental microbial phytase in broiler chickens fed corn-soybean meal diets.

Microbial phytase on growth performance

The inability of young broiler chickens to utilize phytic acid has been clearly demonstrated in our studies (Chapters II, III and IV), by a slower growth rate, a low concentration of plasma P and reduced bone mineralization observed in chickens fed the low P diet in which most of the P was provided by corn and soybean meal. The observations of reduced feed intake and weight gain in chickens fed a low P diet may be due to: 1) inadequate supply of inorganic P in the diet to meet the P requirement, 2) reduction of appetite as a result of low level of P or 3) reduction in the solubility of mineral complexes as a consequence of increased ileal pH by the relatively high dietary Ca level (Shafey *et al.*, 1991). Phytase supplementation of the low P diet yielded a significant improvement in body weight for both sexes at 21 d, compared to chicks fed

the low P diet without phytase supplementation. The body weights achieved by phytase supplementation were comparable to those obtained in the control diet which contained a source of inorganic P supplemented to satisfy the requirement (NRC, 1994). The efficacy of phytase in improving body weight was greater for male chickens (13.2 %) than for females (5.8 %) (Chapter II). The improvements in growth performance observed in the chickens fed phytase may be due to: a) the release of minerals from the phytate-mineral complex or / and b) the utilization of inositol by animals as suggested by Simons *et al.*,(1990) or / and c) increased starch digestibility as suggested by Knuckles and Betschart (1987) or / and d) increased availability of protein. Phytase supplementation overcame the depression of growth rate observed in chickens fed a low P diet. As a result of the simultaneous increase in both body weight and feed intake, no significant differences in feed to gain ratio were observed (Chapters II and IV).

Microbial phytase on macro and trace mineral utilization

As expected, phytase supplementation increased the relative retention of P by 12.5 percentage units in male chickens, which agrees with results of previous studies dealing with chickens (Simons *et al.*, 1990) and pigs (Lei *et al.*, 1994). It is uncertain why female chickens failed to show significant improvement in the relative retention of P (Chapter II). Phytase supplementation also improved the relative retention of Ca in male chickens (Chapter II). This improvement was expected because phytase liberates Ca from the calcium-phytate complex and as the availability of P increases, the availability of Ca also increases because both are part of the same complex. In this study (Chapter II) phytase supplementation in the low P diet significantly improved the relative retention

of both Zn and Cu by almost 62.3 and 19.3 percentage units, respectively. This highly significant improvement in Zn relative retention may be due to higher availability of Zn from the phytate-mineral complex. This observation emphasizes the need to re-evaluate the Zn requirement when the broiler diet is supplemented with phytase. The reduction in the apparent relative retention of Cu and Zn in the low P diet may be due to the fact that the higher content of Ca relative to P in the low P diet increased the intestinal pH and reduced the soluble fraction of minerals, consequently reducing their availability for absorption (Shafey, 1993). Microbial phytase supplementation had no influence on the relative retention of Mg, Fe, or Mn (Chapter III). To the author's knowledge, there are no data published on the effect of phytase on the retention of Mg, Mn and Fe in poultry; however, previous studies with pigs showed that microbial phytase had no effect on Mg and Mn (Adeola, 1996).

Plasma P was increased by phytase supplementation to a level comparable to that of the control diet and this concurs with other studies reported in chickens (Broz *et al.*, 1994). The low P diet significantly increased the plasma Ca for both male and female chickens. This was expected because a low P diet normally results in an elevated ionized Ca in the plasma. This depresses the release of parathyroid hormone (PTH) thus reducing the PTH inhibition on tubular reabsorption of phosphate and permitting the urinary excretion of additional Ca absorbed from the gut during low P diet feeding (Tayler and Dacke, 1984). Phytase supplementation did not show any significant effect on plasma Zn, Cu (Chapter II), and Mg (Chapter III).

The percentage of tibia ash was significantly improved by the addition of dietary

phytase (Chapter II). The improvement in ash percentage in tibia bone is a good indication of increased bone mineralization due to the fact that there is an increased availability of P, Ca, Zn, and Cu from the phytate-mineral complex by the action of phytase. The shaft portion of the bone represents the more rigid state of the bone, while the head is a more active state of change and consequently more susceptible to variation due to availability of minerals. The concentrations of all minerals measured were high in the shaft portion compared to the head portion except for Cu which was more concentrated in the head portion (Chapter II). Phytase supplementation did not affect the concentration of P, Ca, Cu and Zn in whole tibia ash (both head and shaft portions); however, phytase supplementation significantly improved the content of P and Ca in the DM of tibia head.

Efficacy of microbial phytase at different levels of dietary Ca

We studied the effects of microbial phytase supplementation on growth performance and on macro- and trace mineral utilization at different levels of Ca in broiler diets (Chapter III). The optimum levels of body weight, feed intake and feed efficiency were obtained at the low (0.6%) dietary Ca level plus phytase. The observation of decreased availability of phytate P as assessed by the lowest retention of P, reduced feed intake and body weight at the high (1.25%) dietary Ca level compared to the low (0.6%) or recommended (1.0%) level support the notion that phytate hydrolysis is reduced at very high levels of Ca due to the formation of insoluble Ca-phytate complex. Even though the maximum retentions of P and N were obtained at the 1.0 and 1.25% Ca levels, respectively, they were not significantly different from the values at 0.6% Ca

level (Chapter III). The improvement in the retention of P by phytase supplementation at the low Ca level indicates that there was less Ca-phytate complex formed; thus, more phytate molecules were exposed to phytase hydrolysis. The improvement in the relative N retention may indicate that a phytate-protein complex was partially cleaved by microbial phytase.

As expected, the maximum levels of plasma P and Cu were observed in the low level (0.6%) Ca diet supplemented with phytase, which agrees with the results of a previous study with pigs (Lei *et al.*, 1994). In the tibia shaft, phytase supplementation of the 0.6% Ca diet increased the ash content to a level comparable to that obtained with the 1.0 or 1.25% Ca plus phytase. Phytase supplementation did not affect the concentrations of any minerals measured in either the head or the shaft of the tibia; however, the maximum content of Mg, Zn and Cu in tibia head ash were obtained with the 0.6 % Ca diets.

Effect of microbial phytase on apparent ileal and "fecal" digestibility

We also studied the effects of microbial phytase supplementation at different P-Ca levels on AID and AFD of CP, EAA and NEAA in both male and female chickens fed a corn-soybean meal diet for 21 and 28-d (Chapter IV). Addition of 600 U of phytase / kg diet increased the AID of EAA as well as NEAA in female chickens; however, the magnitude of response to phytase in male chickens was very low. It is interesting to note that AID values of CP and all the AA were numerically higher in female than in male chickens. The increase in the ileal digestibility of amino acids may indicate that the phytate-protein complexes were partially cleaved by microbial phytase. Another possible

reason for the improvement in amino acid digestibility is that phytase supplementation, by reducing phytic acid level in the diet, decreases the inhibitory effect of phytic acid on pepsin and trypsin, thereby increasing the digestibility of proteins and amino acids. In this study, the improvement on AID of amino acids by phytase supplementation was in a range of 1.8 (Arg) to 4.3 (Thr) percentage units, which is higher than that observed in a study dealing with female turkeys (Yi *et al.*, 1996). We have no obvious explanation for the decreased and increased digestibilities in the low P-normal Ca and the low P-low Ca diets, respectively. The range (76.8 to 91.8% and 76.2 to 90.6%) of average AID and AFD values of all amino acids reported in this study, are within the range reported previously in chickens (Green *et al.*, 1987) and pigs (Mroz *et al.*, 1994); however, the values reported by Yi *et al.*, (1996) for AID in the turkey were consistently higher for all amino acids. The efficacy of phytase on the digestibility of amino acids in female chickens was further confirmed by the observation of increased AFD of Asp, Glu, Gly, Thr, and Ser in female but not in male chickens at 28-d, emphasizing the need for further research to better understand these phenomena. It is not clear why phytase failed to show prominent effects on the AFD of the other amino acids in female chickens. It may be possible that the contributions of microbial protein and bacterial synthesis of some amino acids in the hind gut and caeca of chickens (Parsons *et al.*, 1981) overshadows the effect of phytase. As expected, AID of CP and all the amino acids were higher than AFD of the same in both male and female chickens at 28-d. These observations suggest that recovery of amino acids from the ileum may represent a sensitive index of digestibility coefficient that is not confounded by protein synthesis as a result of cecal and intestinal

bacterial activity. Phytase supplementation did not show any prominent effect on either AID or AFD in either sex at 21-d. Dietary P-Ca levels had a significant effect on both AID and AFD of CP and all the AA. The low P-normal Ca diet consistently reduced the AID and AFD of CP and all AA, compared to the normal P-normal Ca diet; the low P-low Ca diet increased the digestibility of CP and all AA to a level comparable to the normal P-normal Ca diet. It clear that optimum level of CP and AA digestibility can be achieved in a diet containing low levels of P and Ca supplemented with microbial phytase. In summary, phytase supplementation increased the growth performance, availability of P, Ca, Cu, Zn and N, plasma P, and tibia ash and reduced plasma Ca; it also increased the AID and AFD of most of the AA particulary in female ch

CONCLUSIONS

CONCLUSIONS

From the results of these experiments, the following conclusions can be drawn.

Dietary microbial phytase supplementation (600 U / kg diet) to a low P corn-soybean diet improved the body weight gain and feed consumption in broiler chickens at 21-d. The efficacy of phytase, particularly in stimulating growth, is higher in male than in female chickens.

Addition of phytase to a low P corn-soybean diet increased the apparent availability of Ca, P, Cu, Zn, and N but not Mg, Mn and Fe. Phytase supplementation increased plasma P and reduced plasma Ca and had no effect on plasma Cu, Zn and Mg.

Phytase increased the ash content in both tibia head and shaft but had no effect on mineral content in the ash; however, it increased the DM percentage of P and Ca in tibia head.

Dietary Ca levels had a significant effect on the response to supplemental phytase. The optimum growth performance and mineral utilization were achieved at a low (0.6%) level of dietary Ca with phytase compared to the recommended (1.0%) or a high (1.25%) level of dietary Ca.

Microbial phytase supplementation increased the AID of most of the AA in female chickens at 28-d; it had no effect on AID of any of the AA in male chickens. Addition of phytase did not have any effect on AFD of any of the AA in male chickens but increased the AFD of Asp, Glu, Gly and Ser in female chickens. There was a positive correlation between disappearance of AA from the gizzard and their digestibility.

Finally, phytase supplementation not only reduces environmental pollution by

reducing P and N levels in feces but also serves to reduce the need for certain minerals and amino acids in the broiler diet.

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CLAIMS OF ORIGINALITY AND CONTRIBUTION TO KNOWLEDGE

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To the author's knowledge, the following aspects of this study constitute original contributions to knowledge:

The apparent ileal and "fecal" digestibility of amino acids measured here as influenced by microbial phytase in broiler chickens has not been reported to date. The observed increased AA digestibility indicates that supplemental microbial phytase provided more efficient utilization of protein and AA and resulted in less N in the feces; In future, phytase supplementation will reduce the amount of protein and AA required in feeds and consequently reduce the contribution of poultry to nitrogenous waste pollution of the environment.

This was the first comparison of the effects of supplemental microbial phytase on apparent ileal digestibility (AID) and apparent "fecal" digestibility (AFD) in both male and female broiler chickens. The observation of higher AID compared to AFD suggests that recovery of AA from the ileum represents a superior index of digestibility that is not confounded by intestinal microbes.

There has been no previous report comparing the effect of microbial phytase on the digestibility of amino acids in male and female broiler chickens. The increased AID of AA in female but not in male chickens indicates that the response to phytase in AID of AA is sex dependent.

ADF was shown to be as good as Cr_2O_3 as a marker for the estimation of ileal and "fecal" digestibilities in broilers. This observation suggests that in future we can avoid adding Cr_2O_3 and the concern about the uniformity of its distribution in the feed.

This study was the first one to demonstrate the efficacy of supplemental microbial phytase on the relative retention of a wide range of minerals: Ca P, Cu, Zn, Mg, Mn, and Fe in broiler chickens. The observation of increased retention of P, Ca, Cu and Zn but not Mg, Mn and Fe suggests that phytase is selective in liberating the minerals from the phytic acid.

This was the first report of the effects of microbial phytase on the ash and mineral

contents of Ca P, Cu, Zn, Mg, Mn, and Fe in tibia head and shaft separately and showed them to differ in the head and shaft in broiler chickens.

This thesis provides the first information on the disappearance of amino acids from the gizzard as influenced by microbial phytase.

A positive correlation ($r = 0.7$) was shown between the disappearance of amino acids from the gizzard and their digestibility.

This thesis provides the first report on the effect of microbial phytase on Mg and Zn levels in plasma.

APPENDIX

APPENDIX I

PROTOCOL FOR HYDROLYSATE AMINO ANALYSIS

Protocol used for hydrolysate amino analysis in the Crampton Nutrition Laboratory using the Pico-Tag system from Waters Chromatography Division, Millipore Corporation (Milford, MA, USA).

1. Start oven, mark tubes with a diamond pen.
2. Weigh approximately 5 mg of feed sample into a marked 6x50 mm culture tube.
3. Add 200 μl 6N HCl.
4. Add a drop each of phenol, 2-mercaptoethanol and octanoic acid. Vortex.
5. Put culture tube into a Waters reaction vial and close the cap.
6. Submerge the vial in liquid nitrogen to freeze the contents.
7. Mount the vial on the vacuum station, apply vacuum and allow the contents to thaw under vacuum, thus releasing the dissolved oxygen. Purge the vial with nitrogen, then reevacuate. Purge with nitrogen for the second time and immediately close the vial with an airtight cap.
8. Place the vial in the oven at 110^0 C and hydrolyze for exactly 24 hours.
9. Cool to room temperature. Filter the hydrolysate through a microcentrifuge tube filter (PGC cat #352-121 0.45 μm) by centrifuging at 3000 x G for 5 minutes in a Beckman microfuge 12.
10. Transfer 10 μl norleucine internal standard and 10 μl of the hydrolysate (in that order) into a 6x50 mm culture tube.
11. Prepare 3 standards using 5, 10 and 20 μl of amino acid standard and add 10 μl of norleucine internal standard.
12. Put tubes into a reaction vial. Mount on vacuum station and evaporate to dryness (below 50 millitor on the vacuum gauge = approximately 45 minutes).
13. Add 50 μl of Waters redrying solution (methanol :water : triethylamine (TEA),

2:2:1), mix and vacuum to dryness. Repeat once.

14. Add 50 μl of PITC derivatization reagent (methanol:water:triethylamine: phenylisothiocyanate (PITC), 7:1:1:1). Vortex and let stand for 20 minutes.
15. Evaporate to dryness under vacuum.
16. Reconstitute sample with 200 μl WATERS sample diluent for HPLC analysis.
17. Centrifuge at 3000 rpm for 5 minutes to settle the precipitates.
18. Transfer supernatant with a pasteur pipette to a plastic insert for HPLC analysis.

Table A.1 Gradient parameters for HPLC amino acid analysis

Time (min.)	Flow rate (ml / min.)	% A	% B	Curve
0	1.0	100	0	-
1.0	1.0	88	12	5
3.0	1.0	80	20	6
11.0	1.0	60	40	6
11.5	1.0	25	75	6
13.0	1.0	0	100	6
13.5	1.5	0	100	6
14.0	1.5	100	0	6
20.0	1.5	100	0	6
20.5	1.0	100	0	0

Figure A.1 Chromatogram of Amino acid standards (15 cm Pico-Tag Column)

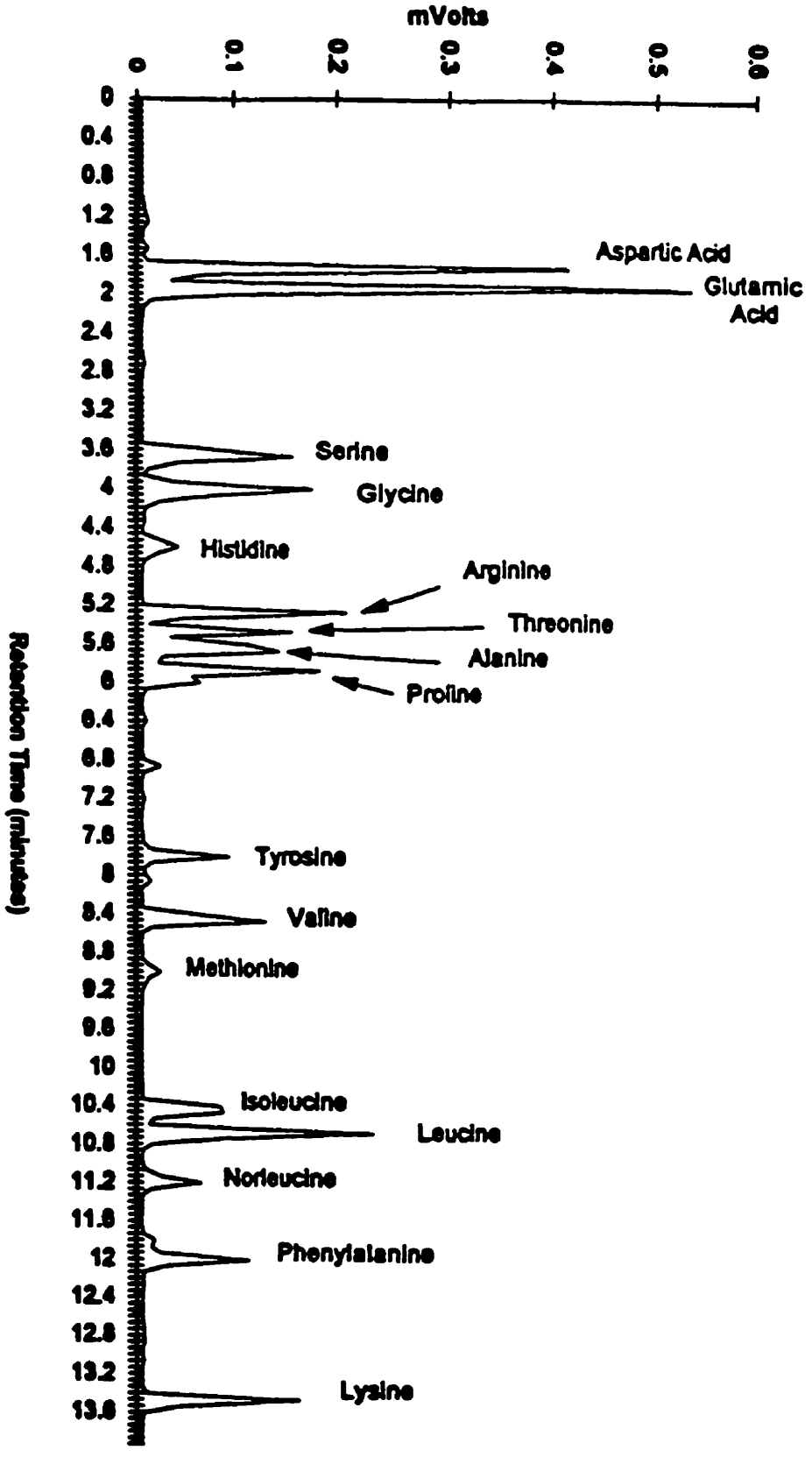

mVolts
0
0.1
0.2
0.3
0.4
0.5
0.6
Retention Time (minutes)
0
0.4
0.8
1.2
1.6
2
2.4
2.8
3.2
3.6
4
4.4
4.8
5.2
5.6
6
6.4
6.8
7.2
7.6
8
8.4
8.8
9.2
9.6
10
10.4
10.8
11.2
11.6
12
12.4
12.8
13.2
13.6
Aspartic Acid
Glutamic Acid
Serine
Glycine
Histidine
Arginine
Threonine
Alanine
Proline
Tyrosine
Valine
Methionine
Isoleucine
Leucine
Norleucine
Phenylalanine
Lysine

Table A.2 The average content of Cr_2O_3 and ADF present in the diets and digesta obtained from the gizzard, ileum and rectum .

Treatment		Digesta		
	Feed	Gizzard	Ileum	Rectum
		Cr_2O_3 (%)		
Normal P-normal Ca	0.17	0.06	0.56	0.59
Noramal P-normal Ca + phytase	0.17	0.05	0.53	0.64
Low P-normal Ca	0.17	0.05	0.64	0.50
Low P-normal Ca + phytase	0.18	0.06	0.58	0.50
Low P-low Ca	0.17	0.06	0.76	0.63
Low P-low Ca +phytase	0.19	0.06	0.55	0.56
		ADF (%)		
Low P-low Ca	3.37	6.68	11.1	9.68

Table A.3 The data base of apparent ileal and fecal digestibilities (%) of AA and disappearance (%) of AA from the gizzard at 28-d

Diet	Trt	phy	Sex	organ	Rep	Dup	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Normal P-normal Ca	2	0	M	G	1	1	86.8	86.4	83.2	82.5	82.6	85.9	82.5	82.1	89.0	83.5	83.1	87.4	78.5	82.8	84.0	86.6
Normal P-normal Ca	2	0	M	G	1	2	83.4	83.1	77.7	77.6	78.6	83.7	74.7	76.1	85.6	76.1	76.7	83.4	70.6	76.4	78.6	83.4
Normal P-normal Ca	2	0	M	G	2	1	82.5	82.2	77.4	75.1	78.1	82.1	76.2	75.7	85.9	77.0	75.8	82.0	69.9	76.2	78.5	83.0
Normal P-normal Ca	2	0	M	G	2	2	81.7	80.4	76.4	77.9	75.8	81.7	73.6	75.2	82.9	76.4	76.9	83.4	68.5	74.4	76.8	82.1
Normal P-normal Ca	2	0	M	G	3	1	92.2	92.1	88.9	88.5	91.0	91.6	87.6	88.5	94.0	88.6	88.4	92.4	85.7	88.8	89.3	92.1
Normal P-normal Ca	2	0	M	G	3	2	90.1	89.1	86.1	86.4	86.1	90.0	84.0	84.9	90.0	85.6	85.9	92.8	82.7	85.4	86.7	90.2
Normal P-normal Ca	2	0	M	I	1	1	88.3	90.4	79.8	79.7	85.0	91.2	76.4	87.0	88.8	87.0	84.7	91.6	80.5	89.0	89.2	88.6
Normal P-normal Ca	2	0	M	I	1	2	90.9	89.0	78.7	78.0	82.0	88.1	70.9	83.3	86.4	83.1	81.3	89.6	75.0	86.1	85.4	84.3
Normal P-normal Ca	2	0	M	I	2	1	90.0	91.9	80.1	80.7	85.2	92.8	77.5	87.1	90.3	86.9	85.0	90.3	81.5	89.5	89.6	88.7
Normal P-normal Ca	2	0	M	I	2	2	90.0	92.1	82.5	84.6	87.6	92.6	79.9	87.8	92.3	88.5	86.6	91.5	82.2	89.4	88.9	89.6
Normal P-normal Ca	2	0	M	I	3	1	92.1	92.6	82.1	83.9	86.9	91.8	77.4	86.8	91.5	87.1	85.2	91.5	81.0	89.5	89.3	88.4
Normal P-normal Ca	2	0	M	I	3	2	78.4	84.8	71.8	74.7	77.3	86.8	68.6	75.5	83.1	77.0	73.7	83.8	67.3	79.3	80.0	78.1
Normal P-normal Ca	2	0	M	R	1	1	83.4	87.3	73.4	73.7	79.7	87.9	67.0	79.4	84.8	80.8	77.0	86.4	72.7	84.4	84.9	81.6
Normal P-normal Ca	2	0	M	R	1	2	91.0	90.7	83.6	82.0	85.6	91.0	78.5	86.8	88.6	87.0	85.5	91.6	80.9	89.1	88.6	87.9
Normal P-normal Ca	2	0	M	R	2	1	92.4	93.8	83.4	83.2	87.2	93.4	80.1	87.9	93.0	88.3	87.0	92.1	83.4	90.7	91.0	90.1
Normal P-normal Ca	2	0	M	R	2	2	89.6	91.8	81.7	84.1	86.7	93.4	79.5	86.7	91.4	88.5	86.2	90.8	82.3	89.5	89.5	89.7
Normal P-normal Ca	2	0	M	R	3	1	88.9	90.7	81.1	81.7	85.8	92.0	78.3	86.7	91.1	86.1	84.9	90.1	79.9	88.8	88.5	87.7
Normal P-normal Ca	2	0	M	R	3	2	83.2	87.8	76.2	77.8	82.0	90.1	73.3	82.1	86.3	83.5	80.3	88.8	74.2	84.7	84.7	84.0
Normal P-normal Ca	2	0	F	G	1	1	82.2	81.3	77.2	76.4	75.9	80.6	75.3	76.1	85.8	77.0	77.0	84.4	70.2	76.4	78.0	81.5
Normal P-normal Ca	2	0	F	G	1	2	78.6	79.1	74.2	74.2	73.8	79.0	71.4	73.7	82.1	74.3	73.6	79.9	64.7	72.7	73.8	77.5
Normal P-normal Ca	2	0	F	G	2	1	81.7	81.8	76.7	76.5	78.8	81.2	75.4	75.9	84.2	76.6	75.5	82.2	68.0	76.0	76.6	81.2
Normal P-normal Ca	2	0	F	G	2	2	79.3	78.3	73.9	74.3	73.1	78.0	72.1	72.4	82.2	74.0	74.2	79.1	64.9	71.9	73.4	78.4
Normal P-normal Ca	2	0	F	G	3	1	83.8	83.1	78.6	77.2	79.1	82.9	76.7	77.1	86.6	77.5	77.4	85.2	70.2	76.9	78.0	83.0
Normal P-normal Ca	2	0	F	G	3	2	78.4	79.5	73.6	72.9	74.0	79.0	70.9	73.3	79.9	73.6	72.0	79.3	64.5	73.3	74.0	78.6
Normal P-normal Ca	2	0	F	I	1	1	83.5	89.3	71.5	78.0	80.9	89.4	66.1	84.0	87.5	80.9	79.3	88.5	74.3	85.6	85.2	87.5
Normal P-normal Ca	2	0	F	I	1	2	90.1	91.4	82.7	83.2	87.5	91.8	79.8	88.7	90.2	88.1	85.9	92.1	81.1	89.1	88.1	90.1
Normal P-normal Ca	2	0	F	I	2	1	95.9	95.0	88.7	89.6	91.5	93.8	86.0	91.1	95.5	90.9	89.2	94.9	86.0	91.5	91.8	93.2
Normal P-normal Ca	2	0	F	I	2	2	82.7	88.0	73.5	75.4	79.6	88.6	69.1	83.8	87.1	82.1	80.3	88.1	75.6	86.0	85.2	86.8
Normal P-normal Ca	2	0	F	I	3	1	93.2	94.0	84.1	85.8	88.4	93.8	80.5	90.3	93.3	90.1	88.2	93.7	85.3	91.7	91.5	91.2
Normal P-normal Ca	2	0	F	I	3	2	89.9	92.6	83.9	84.9	88.8	92.8	81.5	89.6	92.0	89.3	87.8	92.3	83.9	90.7	90.2	91.9
Normal P-normal Ca	2	0	F	R	1	1	89.1	89.9	83.3	78.7	84.8	87.8	78.2	87.1	91.3	86.9	85.5	92.2	81.9	89.3	87.9	88.3
Normal P-normal Ca	2	0	F	R	1	2	90.9	92.8	84.8	85.3	88.6	92.4	81.4	90.0	92.0	89.8	88.1	93.0	85.7	91.5	90.5	91.7
Normal P-normal Ca	2	0	F	R	2	1	93.8	93.6	83.0	77.7	85.7	93.6	78.4	87.5	92.5	88.2	86.2	92.3	83.1	90.5	90.7	89.4
Normal P-normal Ca	2	0	F	R	2	2	83.5	88.4	78.3	79.3	87.3	89.5	75.1	86.2	86.9	86.4	83.8	91.2	79.3	87.8	86.7	87.4
Normal P-normal Ca	2	0	F	R	3	1	92.1	92.1	82.7	82.0	86.3	91.8	78.4	88.1	90.6	87.8	85.7	92.9	81.6	89.6	88.8	88.6
Normal P-normal Ca	2	0	F	R	3	2	94.7	93.5	85.5	85.0	88.0	92.5	82.7	90.4	92.7	89.8	88.0	93.5	83.2	90.4	89.5	90.5

Diet	Trt	phy	Sex	organ	Rep	Dup	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Normal P-normal Ca	3	1	M	G	1	1	88.0	87.2	85.0	85.0	86.2	87.9	85.2	83.7	89.1	84.8	84.1	88.1	79.2	82.6	83.4	86.8
Normal P-normal Ca	3	1	M	G	1	2	85.3	85.5	82.8	81.8	82.5	85.5	82.0	82.3	87.4	82.8	81.8	84.9	75.0	81.4	81.4	83.7
Normal P-normal Ca	3	1	M	G	2	1	83.2	82.8	79.9	78.9	81.3	82.8	80.2	78.6	83.7	79.6	78.9	82.5	71.2	78.0	77.8	80.8
Normal P-normal Ca	3	1	M	G	2	2	86.5	85.2	81.9	82.3	81.9	86.6	81.6	80.1	86.9	82.2	80.4	85.9	74.8	78.5	79.9	85.3
Normal P-normal Ca	3	1	M	G	3	1	87.2	88.5	83.4	82.0	83.7	85.9	82.2	84.4	91.0	83.1	82.0	85.2	76.6	83.7	81.6	84.8
Normal P-normal Ca	3	1	M	G	3	2	90.0	90.8	86.1	85.2	87.8	89.8	85.3	86.5	91.9	85.8	84.8	88.4	80.9	86.1	85.2	88.7
Normal P-normal Ca	3	1	M	I	1	1	90.2	91.1	80.0	81.1	84.9	91.8	77.8	85.8	90.6	86.0	83.6	89.6	77.7	87.3	87.1	86.7
Normal P-normal Ca	3	1	M	I	1	2	86.9	90.2	79.5	80.6	83.9	91.2	75.8	83.1	90.8	84.6	81.2	86.9	75.8	85.1	84.9	84.4
Normal P-normal Ca	3	1	M	I	2	1	89.3	90.0	80.6	80.6	84.5	90.6	77.5	84.8	87.9	85.6	82.9	89.1	78.1	86.7	86.4	85.8
Normal P-normal Ca	3	1	M	I	2	2	91.7	92.6	84.6	85.3	89.1	92.3	81.6	88.7	92.8	89.1	86.1	90.7	82.1	89.5	88.7	88.7
Normal P-normal Ca	3	1	M	I	3	1	96.7	95.3	91.1	91.6	92.8	93.6	89.5	92.6	93.6	92.0	90.6	94.9	86.5	91.5	90.2	92.7
Normal P-normal Ca	3	1	M	I	3	2	96.7	95.3	91.1	91.6	92.8	93.6	89.5	92.6	93.6	92.0	90.6	94.9	86.5	91.5	90.2	92.7
Normal P-normal Ca	3	1	M	R	1	1	91.6	92.5	82.9	83.8	87.4	93.2	81.1	87.3	91.3	88.2	86.3	91.0	82.2	89.5	89.4	88.8
Normal P-normal Ca	3	1	M	R	1	2	89.5	91.5	82.8	82.1	85.5	92.4	78.5	84.8	90.7	86.2	83.1	89.0	78.6	88.4	87.1	85.1
Normal P-normal Ca	3	1	M	R	2	1	94.5	93.1	84.7	83.8	86.9	91.6	81.1	86.9	89.1	88.3	85.2	91.0	80.7	88.5	87.7	88.1
Normal P-normal Ca	3	1	M	R	2	2	87.7	90.2	83.3	80.1	85.2	90.9	78.2	82.6	89.3	84.4	79.5	84.7	75.5	86.9	85.7	83.2
Normal P-normal Ca	3	1	M	R	3	1	91.0	91.5	81.6	83.3	86.3	90.9	78.9	88.1	89.3	88.0	85.7	91.3	81.9	89.5	87.8	86.8
Normal P-normal Ca	3	1	M	R	3	2	89.4	92.4	80.1	80.4	86.0	93.1	77.1	89.5	89.1	88.6	86.8	91.1	84.4	91.6	90.5	89.4
Normal P-normal Ca	3	1	F	G	1	1	82.8	82.0	79.1	78.1	79.5	82.2	80.0	76.7	83.2	79.0	77.7	80.2	70.9	76.2	76.7	80.8
Normal P-normal Ca	3	1	F	G	1	2	87.0	86.2	83.4	83.3	84.0	87.0	83.4	81.7	87.6	83.6	83.0	85.4	76.8	81.3	81.9	86.1
Normal P-normal Ca	3	1	F	G	2	1	85.7	85.2	82.4	81.5	82.7	85.8	82.7	81.2	86.0	82.9	81.9	83.9	75.8	80.1	80.7	84.5
Normal P-normal Ca	3	1	F	G	2	2	87.1	86.6	84.1	84.2	84.0	86.5	83.5	83.2	88.9	83.0	83.2	84.7	77.0	81.6	81.6	86.5
Normal P-normal Ca	3	1	F	G	3	1	88.6	88.1	85.7	84.9	86.3	88.9	85.7	84.0	87.9	86.1	85.1	88.4	81.1	84.0	84.7	87.7
Normal P-normal Ca	3	1	F	G	3	2	85.9	86.5	83.6	82.8	84.8	84.9	83.3	83.6	88.1	82.2	82.8	83.3	76.8	82.9	81.0	83.0
Normal P-normal Ca	3	1	F	I	1	1	92.3	92.3	85.8	83.3	87.3	92.7	83.4	90.1	90.5	89.5	88.4	92.6	84.7	91.3	89.7	89.9
Normal P-normal Ca	3	1	F	I	1	2	94.2	94.9	87.9	88.0	91.8	94.8	85.1	91.8	94.3	91.6	89.6	93.2	86.7	92.4	91.9	92.4
Normal P-normal Ca	3	1	F	I	2	1	95.8	95.1	87.6	87.6	90.0	93.6	85.1	91.7	92.6	92.2	89.9	93.9	86.4	92.1	91.5	91.8
Normal P-normal Ca	3	1	F	I	2	2	97.1	95.8	87.4	87.6	89.8	94.6	84.3	91.6	92.5	91.8	89.2	93.8	86.2	92.0	91.7	91.9
Normal P-normal Ca	3	1	F	I	3	1	86.4	90.6	77.7	80.0	83.7	89.4	74.4	85.1	92.0	84.7	82.0	88.5	77.8	87.2	85.7	86.8
Normal P-normal Ca	3	1	F	I	3	2	89.9	91.7	80.6	83.8	86.3	92.1	76.5	88.1	90.8	87.6	84.8	89.8	80.3	89.0	87.6	87.9
Normal P-normal Ca	3	1	F	R	1	1	92.2	93.4	85.1	85.9	89.4	93.1	83.2	90.3	91.8	89.5	87.7	91.9	84.4	90.9	89.9	91.2
Normal P-normal Ca	3	1	F	R	1	2	93.1	94.1	89.5	87.7	89.0	92.9	85.2	87.9	93.9	89.7	85.8	91.6	83.0	90.0	89.1	87.9
Normal P-normal Ca	3	1	F	R	2	1	92.6	93.5	85.8	85.0	88.9	94.3	83.6	91.0	92.3	91.3	89.9	92.0	86.7	92.3	91.8	91.3
Normal P-normal Ca	3	1	F	R	2	2	89.4	90.2	81.5	79.9	85.0	90.2	78.7	87.5	88.9	86.9	84.7	89.6	79.1	87.9	86.4	88.0
Normal P-normal Ca	3	1	F	R	3	1	92.7	93.3	84.7	84.4	87.7	93.2	82.8	89.8	93.5	89.5	87.9	92.1	84.0	90.7	90.0	90.1
Normal P-normal Ca	3	1	F	R	3	2	84.0	89.6	76.6	74.1	82.3	90.7	73.0	86.2	87.1	86.4	83.1	88.3	79.1	88.3	87.0	85.7

Diet	Trt	phy	Sex	organ	Rep	Dup	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Low P-normal Ca	4	0	M	G	1	1	76.0	73.2	70.1	70.8	70.0	74.3	68.8	65.8	79.6	74.5	68.9	80.1	65.4	69.8	75.4	78.5
Low P-normal Ca	4	0	M	G	1	2	80.0	77.4	73.9	73.5	73.7	78.4	73.8	70.0	90.0	70.3	70.3	82.8	61.1	67.8	63.5	81.3
Low P-normal Ca	4	0	M	G	2	1	76.4	75.4	70.1	70.9	69.8	74.1	69.7	67.2	88.4	64.8	67.8	79.8	61.0	68.3	66.6	78.3
Low P-normal Ca	4	0	M	G	2	2	80.8	79.8	75.1	74.7	75.5	80.0	74.4	72.5	86.4	72.9	72.5	80.8	63.2	70.8	64.9	80.4
Low P-normal Ca	4	0	M	G	3	1	70.0	69.0	64.0	65.8	63.5	66.9	64.0	62.0	85.0	48.4	62.8	70.9	49.8	60.9	62.2	69.2
Low P-normal Ca	4	0	M	G	3	2	67.9	65.7	60.7	60.4	60.7	66.4	60.3	56.3	74.7	58.0	55.8	71.6	40.6	53.5	46.0	68.1
Low P-normal Ca	4	0	M	I	1	1	88.8	91.0	78.4	82.1	86.7	90.8	78.5	88.2	94.3	87.9	85.1	93.4	82.3	89.8	89.2	91.4
Low P-normal Ca	4	0	M	I	1	2	85.9	91.5	79.6	82.4	84.9	91.2	76.1	87.7	93.9	87.2	83.5	92.8	78.9	88.4	81.8	89.5
Low P-normal Ca	4	0	M	I	2	1	85.8	89.4	77.5	81.1	85.1	89.1	77.4	85.9	92.0	87.1	82.8	96.5	78.3	87.4	86.3	88.4
Low P-normal Ca	4	0	M	I	2	2	83.5	89.2	79.0	79.8	85.3	89.9	75.6	84.2	95.8	84.2	81.0	92.4	73.8	85.4	79.1	86.8
Low P-normal Ca	4	0	M	I	3	1	88.2	90.0	72.0	80.6	85.6	89.8	74.8	87.0	92.8	85.3	82.3	92.8	77.3	87.7	85.6	90.2
Low P-normal Ca	4	0	M	I	3	2	87.6	92.4	76.6	84.7	85.6	92.1	74.3	88.6	94.5	85.8	85.2	93.6	82.0	90.0	85.1	91.2
Low P-normal Ca	4	0	M	R	1	1	75.1	81.2	65.2	64.9	74.5	83.2	60.7	70.1	82.6	74.1	65.7	80.7	60.7	79.7	79.9	71.8
Low P-normal Ca	4	0	M	R	1	2	78.3	84.7	73.7	70.5	75.8	86.1	67.4	76.6	90.0	77.5	71.6	83.3	66.8	82.0	74.8	77.6
Low P-normal Ca	4	0	M	R	2	1	80.4	87.8	68.2	74.4	81.8	88.0	69.2	83.4	88.3	84.5	80.3	80.0	77.5	87.4	86.2	87.1
Low P-normal Ca	4	0	M	R	2	2	79.4	84.8	72.8	71.7	76.5	85.3	66.2	75.3	93.1	75.5	71.8	87.4	62.0	78.9	70.1	77.3
Low P-normal Ca	4	0	M	R	3	1	84.9	89.3	75.1	74.3	82.0	89.7	75.1	83.9	89.5	86.6	82.0	89.4	79.5	87.7	87.1	87.5
Low P-normal Ca	4	0	M	R	3	2	71.8	81.6	65.6	61.8	69.9	83.5	54.4	71.2	79.4	71.6	67.2	82.6	61.1	78.1	74.6	70.7
Low P-normal Ca	4	0	F	G	1	1	78.6	79.5	74.5	73.6	75.1	78.3	72.0	73.4	87.5	79.1	73.5	82.8	67.2	75.5	75.0	81.1
Low P-normal Ca	4	0	F	G	1	2	77.9	78.0	71.6	71.3	71.4	76.3	69.8	70.4	83.3	69.0	71.3	78.5	63.2	70.6	64.2	77.7
Low P-normal Ca	4	0	F	G	2	1	80.9	79.8	74.8	74.3	75.3	80.0	71.2	73.5	85.6	77.9	74.5	86.6	68.7	76.2	78.0	82.6
Low P-normal Ca	4	0	F	G	2	2	78.5	78.5	73.5	72.5	73.9	78.0	71.0	71.7	84.6	75.1	71.5	80.9	63.0	71.4	65.4	77.6
Low P-normal Ca	4	0	F	G	3	1	74.1	72.7	67.4	67.5	71.2	73.5	65.7	66.8	83.7	70.6	68.5	80.2	59.9	68.4	69.9	78.2
Low P-normal Ca	4	0	F	G	3	2	76.5	77.7	72.0	71.4	74.2	75.0	69.2	72.4	83.9	70.9	71.0	80.3	61.0	72.1	62.9	74.9
Low P-normal Ca	4	0	F	I	1	1	82.0	88.1	73.2	76.2	82.4	87.9	72.2	84.7	92.5	84.3	81.9	91.8	77.4	87.7	86.5	87.7
Low P-normal Ca	4	0	F	I	1	2	81.0	88.7	72.2	75.1	79.2	88.5	65.7	83.0	91.4	80.6	79.1	90.2	74.3	85.2	77.2	85.6
Low P-normal Ca	4	0	F	I	2	1	82.7	86.9	74.0	76.7	83.3	87.2	72.9	84.7	91.7	83.5	81.3	92.0	76.2	86.8	85.0	87.6
Low P-normal Ca	4	0	F	I	2	2	88.5	91.3	80.3	80.8	85.2	91.4	77.3	88.1	89.4	87.3	85.5	91.7	80.8	89.4	85.4	88.6
Low P-normal Ca	4	0	F	I	3	1	82.2	86.4	63.7	72.9	80.7	83.9	66.2	81.3	91.6	80.3	77.8	88.7	72.6	84.0	82.0	85.7
Low P-normal Ca	4	0	F	I	3	2	82.7	87.1	73.1	75.4	80.5	87.2	69.9	81.8	91.0	78.6	77.9	88.9	70.2	82.9	75.9	83.5
Low P-normal Ca	4	0	F	R	1	1	83.3	89.6	71.6	77.9	82.3	88.6	72.5	85.5	94.2	83.6	82.5	91.4	78.8	88.3	86.8	89.4
Low P-normal Ca	4	0	F	R	1	2	73.1	84.0	63.9	62.0	70.1	86.2	54.2	76.0	84.5	76.6	73.5	89.0	68.7	82.9	75.3	78.1
Low P-normal Ca	4	0	F	R	2	1	67.6	79.4	53.3	54.1	68.8	83.2	51.5	73.8	76.7	78.2	73.3	86.7	70.8	83.7	79.5	80.4
Low P-normal Ca	4	0	F	R	2	2	87.5	91.5	82.0	82.1	85.8	92.1	79.1	88.8	91.8	88.1	86.9	93.9	83.0	90.6	85.8	89.5
Low P-normal Ca	4	0	F	R	3	1	85.4	87.6	73.4	74.9	83.4	88.9	72.9	83.7	94.0	84.5	82.7	91.0	79.3	87.9	85.8	87.3
Low P-normal Ca	4	0	F	R	3	2	81.9	86.4	71.3	69.6	76.6	87.4	69.0	81.1	84.0	81.5	78.1	86.9	70.6	83.9	76.8	82.2

Diet	Trt	phy	Sex	organ	Rep	Dup	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Low P-normal Ca	5	1	M	G	1	1	76.3	75.0	69.5	69.6	72.4	74.9	70.8	67.8	89.5	73.5	69.1	79.1	63.2	70.5	72.2	79.4
Low P-normal Ca	5	1	M	G	1	2	74.5	73.0	69.6	68.7	69.7	72.8	72.0	67.1	75.5	67.5	68.2	72.3	57.7	66.9	61.3	75.5
Low P-normal Ca	5	1	M	G	2	1	80.2	79.8	75.3	73.7	76.5	79.6	75.0	72.5	82.8	74.9	73.5	80.0	69.1	75.4	78.1	83.1
Low P-normal Ca	5	1	M	G	2	2	82.3	82.7	78.2	76.2	78.8	82.9	78.6	77.1	83.0	78.1	76.6	83.8	70.2	76.9	72.3	83.2
Low P-normal Ca	5	1	M	G	3	1	79.8	78.0	73.9	74.5	73.3	78.7	74.2	71.9	88.2	74.1	72.9	81.1	65.6	71.7	70.2	81.1
Low P-normal Ca	5	1	M	G	3	2	71.2	69.0	65.1	64.7	66.9	72.0	67.7	61.8	69.8	65.3	64.4	70.0	50.5	60.1	56.7	74.4
Low P-normal Ca	5	1	M	I	1	1	85.4	86.7	76.3	74.6	82.4	88.9	73.6	80.0	86.1	81.7	79.0	88.2	73.2	84.5	81.8	82.9
Low P-normal Ca	5	1	M	I	1	2	85.0	84.6	76.4	73.7	78.6	85.5	74.4	81.7	79.0	81.9	79.1	87.6	70.4	83.4	78.1	83.3
Low P-normal Ca	5	1	M	I	2	1	93.1	92.5	79.7	81.5	86.0	91.7	78.4	87.6	89.5	88.7	85.6	92.2	82.6	90.4	87.2	89.6
Low P-normal Ca	5	1	M	I	2	2	96.1	96.0	84.5	84.0	88.5	92.5	81.1	89.4	90.6	89.7	87.6	92.6	84.2	91.5	85.7	92.1
Low P-normal Ca	5	1	M	I	3	1	74.3	82.7	65.4	67.8	75.5	83.3	63.3	71.5	95.4	65.2	69.3	81.7	61.1	76.4	76.1	78.1
Low P-normal Ca	5	1	M	I	3	2	81.5	84.3	72.3	73.6	77.4	85.7	70.7	76.0	81.6	77.9	74.9	84.6	66.3	79.8	74.1	81.1
Low P-normal Ca	5	1	M	R	1	1	89.5	89.9	78.5	76.2	81.9	90.3	73.8	81.3	84.4	83.7	81.1	89.3	78.0	87.9	88.2	84.6
Low P-normal Ca	5	1	M	R	1	2	76.5	79.5	66.5	64.4	71.3	81.3	63.6	73.4	73.4	73.9	70.5	83.7	60.9	77.9	70.7	75.9
Low P-normal Ca	5	1	M	R	2	1	87.6	88.1	77.1	73.6	80.4	89.7	73.1	84.4	86.4	85.6	82.4	88.3	77.8	87.9	82.4	86.7
Low P-normal Ca	5	1	M	R	2	2	93.2	93.2	81.3	81.6	84.4	92.6	79.0	88.0	90.4	88.2	86.1	89.6	82.5	90.4	83.6	90.6
Low P-normal Ca	5	1	M	R	3	1	74.0	82.8	67.6	68.7	74.7	84.8	65.0	71.8	84.1	75.4	70.8	83.5	64.4	79.2	79.3	77.5
Low P-normal Ca	5	1	M	R	3	2	80.0	83.0	68.7	67.0	73.6	84.7	65.0	70.7	78.0	74.3	70.8	82.5	62.8	78.1	72.2	77.7
Low P-normal Ca	5	1	F	G	1	1	79.8	79.0	74.7	74.6	75.3	78.0	74.9	73.6	87.5	74.7	73.9	82.2	66.4	74.2	71.7	79.9
Low P-normal Ca	5	1	F	G	1	2	75.3	74.5	69.5	68.0	71.7	75.2	70.5	66.5	76.8	70.0	68.0	74.9	58.1	66.6	64.4	74.0
Low P-normal Ca	5	1	F	G	2	1	84.7	84.8	80.7	78.7	82.9	83.5	80.1	80.7	91.0	81.0	79.2	84.4	73.2	81.2	78.8	84.0
Low P-normal Ca	5	1	F	G	2	2	77.4	77.8	72.2	70.4	74.2	77.5	72.3	70.5	79.0	71.8	70.3	79.4	61.2	70.8	65.9	76.2
Low P-normal Ca	5	1	F	G	3	1	70.7	70.2	66.1	64.2	71.3	71.8	66.7	62.7	67.6	68.4	66.0	74.4	59.4	68.0	72.4	73.2
Low P-normal Ca	5	1	F	G	3	2	77.5	77.4	71.8	69.4	73.8	77.6	71.8	70.7	75.5	72.9	69.7	79.4	60.8	70.5	66.5	75.5
Low P-normal Ca	5	1	F	I	1	1	86.7	90.0	77.0	77.6	82.5	90.5	74.9	84.2	92.4	83.9	82.1	89.1	77.6	87.5	84.5	86.7
Low P-normal Ca	5	1	F	I	1	2	94.1	93.7	83.5	85.4	88.2	92.7	81.8	88.7	92.4	89.0	86.7	92.7	83.0	90.2	86.0	89.5
Low P-normal Ca	5	1	F	I	2	1	88.6	91.7	78.8	80.7	86.7	89.8	76.8	87.1	90.8	88.6	85.4	91.0	83.2	90.5	90.2	90.8
Low P-normal Ca	5	1	F	I	2	2	90.1	91.2	78.3	81.5	84.6	89.9	76.4	86.7	88.8	86.2	82.9	90.4	78.1	87.4	82.2	87.7
Low P-normal Ca	5	1	F	I	3	1	87.1	91.0	80.8	81.2	85.6	90.0	79.3	88.0	90.1	89.1	86.5	91.4	85.5	91.5	91.1	90.9
Low P-normal Ca	5	1	F	I	3	2	90.5	91.4	81.4	82.7	86.4	91.2	79.7	86.8	89.8	87.3	84.5	90.0	78.9	88.2	84.2	87.0
Low P-normal Ca	5	1	F	R	1	1	87.1	89.7	76.4	76.2	82.2	89.6	73.3	82.0	95.8	82.0	81.0	88.2	76.9	86.7	85.7	86.5
Low P-normal Ca	5	1	F	R	1	2	90.0	90.1	78.3	75.8	82.6	89.3	74.0	81.3	87.9	82.5	79.5	87.6	74.9	86.1	80.4	83.4
Low P-normal Ca	5	1	F	R	2	1	86.0	89.0	76.5	78.6	82.9	87.4	74.5	84.1	85.2	85.5	82.8	90.6	79.3	88.6	88.2	86.5
Low P-normal Ca	5	1	F	R	2	2	90.4	90.9	80.5	77.6	82.3	90.6	77.4	85.9	88.1	86.9	83.6	88.6	78.9	88.1	82.8	86.3
Low P-normal Ca	5	1	F	R	3	1	86.6	91.4	80.5	80.8	85.1	90.5	79.1	88.6	93.4	88.9	87.5	91.2	85.3	91.6	91.6	91.4
Low P-normal Ca	5	1	F	R	3	2	92.9	93.4	84.2	84.2	87.6	92.5	82.5	88.5	91.3	89.7	87.4	92.3	83.4	90.8	87.5	88.7

Diet	Trt	phy	Sex	organ	Rep	Dup	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Low P-low Ca	6	0	M	G	1	1	80.4	79.4	75.0	75.2	79.8	78.6	76.9	74.7	94.3	78.0	75.6	84.8	70.7	76.3	77.7	82.0
Low P-low Ca	6	0	M	G	1	2	80.9	78.7	75.3	74.9	75.8	81.0	75.8	72.0	78.2	74.2	73.2	82.0	66.0	70.5	70.0	80.0
Low P-low Ca	6	0	M	G	2	1	79.1	77.3	72.4	73.0	74.5	77.3	72.8	70.4	93.3	73.0	73.0	81.3	66.7	72.1	74.4	82.5
Low P-low Ca	6	0	M	G	2	2	84.1	83.1	80.3	80.3	80.1	84.2	80.7	78.5	88.2	79.7	78.8	83.5	71.4	76.6	72.8	83.6
Low P-low Ca	6	0	M	G	3	1	69.2	68.1	63.6	63.4	65.3	68.4	64.5	60.9	91.5	65.0	61.4	75.4	53.6	59.7	64.5	74.4
Low P-low Ca	6	0	M	G	3	2	81.1	79.0	76.2	76.2	78.2	81.3	76.8	73.9	83.5	75.5	74.6	81.0	67.1	72.0	68.8	81.2
Low P-low Ca	6	0	M	I	1	1	85.6	91.3	80.6	82.0	85.0	91.3	79.3	87.5	95.1	87.8	86.3	92.1	85.0	90.9	90.7	91.4
Low P-low Ca	6	0	M	I	1	2	88.4	93.0	85.4	84.8	88.3	93.6	83.0	89.9	92.2	89.7	88.4	92.7	85.3	91.3	86.3	91.0
Low P-low Ca	6	0	M	I	2	1	81.5	88.0	72.8	75.1	78.8	88.4	70.8	82.2	93.6	82.8	80.3	87.7	76.7	85.4	85.6	88.4
Low P-low Ca	6	0	M	I	2	2	87.9	92.3	85.8	85.4	88.2	92.5	84.6	90.3	91.4	90.0	88.2	93.5	84.2	90.8	86.8	90.6
Low P-low Ca	6	0	M	I	3	1	85.5	91.8	81.1	83.3	86.9	92.3	79.4	88.5	93.6	88.1	86.3	92.5	83.6	90.0	89.8	91.5
Low P-low Ca	6	0	M	I	3	2	85.1	90.6	79.6	80.4	83.9	91.5	76.9	86.7	88.5	86.8	84.1	90.4	80.4	88.2	82.0	88.2
Low P-low Ca	6	0	M	R	1	1	87.4	92.2	82.6	82.5	86.6	90.8	81.4	88.6	97.2	88.8	88.3	92.4	86.2	91.4	91.3	92.3
Low P-low Ca	6	0	M	R	1	2	85.8	91.7	82.0	80.6	85.8	93.6	79.9	88.6	90.5	89.3	87.1	90.9	84.2	90.8	85.0	89.9
Low P-low Ca	6	0	M	R	2	1	84.0	90.4	79.6	78.1	83.9	91.0	77.2	86.5	89.9	86.9	84.4	91.9	81.4	89.2	86.6	89.0
Low P-low Ca	6	0	M	R	2	2	83.5	89.8	80.1	77.9	84.0	90.0	77.7	86.4	87.1	86.8	84.2	92.1	79.9	88.2	82.4	86.7
Low P-low Ca	6	0	M	R	3	1	82.0	90.2	76.2	78.3	83.7	90.6	73.9	85.3	95.8	85.9	83.7	89.7	80.4	88.3	88.3	89.4
Low P-low Ca	6	0	M	R	3	2	83.6	90.7	77.6	79.3	85.9	92.3	74.8	86.9	86.6	87.3	84.7	92.2	80.5	88.8	84.5	88.8
Low P-low Ca	6	0	F	G	1	1	72.8	71.6	67.8	68.4	69.7	72.5	67.6	65.4	92.2	68.1	65.4	79.6	55.2	64.1	68.8	77.7
Low P-low Ca	6	0	F	G	1	2	78.4	76.9	72.7	72.4	74.4	78.2	72.8	69.9	77.8	69.0	71.2	77.0	60.9	67.7	64.5	79.0
Low P-low Ca	6	0	F	G	2	1	81.3	82.0	78.0	77.7	78.7	81.4	78.1	77.3	93.3	77.9	76.3	83.8	69.7	76.3	78.2	84.6
Low P-low Ca	6	0	F	G	2	2	75.9	78.2	73.2	71.7	75.4	76.7	71.7	74.9	79.2	73.4	72.0	80.0	61.1	73.7	68.2	72.5
Low P-low Ca	6	0	F	G	3	1	82.6	81.3	77.4	76.3	76.7	80.6	77.2	74.7	96.8	74.1	75.2	84.4	67.7	74.4	76.2	86.5
Low P-low Ca	6	0	F	G	3	2	83.1	83.8	78.6	77.0	79.0	82.5	78.1	78.2	88.1	77.4	76.3	83.3	69.6	77.0	70.0	82.6
Low P-low Ca	6	0	F	I	1	1	86.4	90.3	81.9	82.1	84.7	90.8	79.3	86.6	96.2	85.8	84.5	91.3	80.5	87.9	87.7	90.7
Low P-low Ca	6	0	F	I	1	2	82.8	89.9	78.2	78.1	83.3	90.1	75.9	86.2	89.5	85.7	83.1	89.4	79.0	88.2	82.7	88.1
Low P-low Ca	6	0	F	I	2	1	81.7	90.7	76.8	78.6	83.4	91.0	75.6	86.4	93.4	85.5	84.1	90.7	81.0	88.9	88.6	90.3
Low P-low Ca	6	0	F	I	2	2	86.3	91.2	78.4	79.4	83.2	90.5	75.8	86.8	90.6	86.4	83.6	90.9	80.5	88.5	83.2	87.8
Low P-low Ca	6	0	F	I	3	1	84.8	89.6	78.6	81.1	83.0	88.9	76.2	86.3	95.4	82.8	82.8	92.4	77.6	87.1	85.7	89.2
Low P-low Ca	6	0	F	I	3	2	89.6	93.8	85.4	87.0	90.1	94.3	84.2	91.8	92.9	91.1	89.5	93.5	86.6	92.4	87.8	92.3
Low P-low Ca	6	0	F	R	1	1	83.0	89.5	81.1	80.4	84.3	91.1	78.5	86.5	93.3	86.9	85.1	90.4	81.9	88.8	88.8	90.3
Low P-low Ca	6	0	F	R	1	2	86.6	91.6	82.8	81.5	85.7	92.1	80.4	87.3	91.7	88.0	85.6	90.5	82.5	90.3	84.3	87.9
Low P-low Ca	6	0	F	R	2	1	83.6	91.2	75.6	79.4	82.9	90.8	72.7	87.3	96.5	83.7	84.4	91.6	81.5	89.7	89.2	91.1
Low P-low Ca	6	0	F	R	2	2	85.2	91.0	81.4	80.9	86.2	92.1	79.8	88.1	89.6	88.6	86.3	91.1	83.1	90.3	86.7	88.5
Low P-low Ca	6	0	F	R	3	1	73.5	84.3	73.1	68.3	79.1	85.7	66.4	75.6	90.7	74.5	70.9	84.1	64.1	81.3	80.4	78.4
Low P-low Ca	6	0	F	R	3	2	86.6	92.8	81.2	84.2	87.2	92.5	79.1	90.1	91.4	89.4	87.8	92.7	85.3	91.5	86.6	90.7

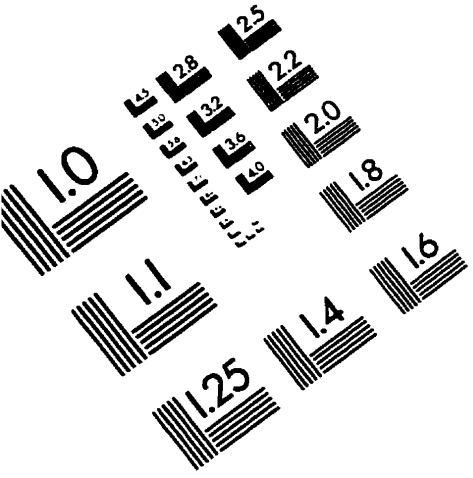
Diet	Trt	phy	Sex	organ	Rep	Dup	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Low P-low Ca	7	1	M	G	1	1	76.3	74.1	68.7	69.6	68.6	74.8	69.4	64.7	88.1	66.8	64.6	78.7	53.7	63.8	66.0	79.8
Low P-low Ca	7	1	M	G	1	2	77.7	75.3	70.8	69.9	72.2	77.5	69.8	66.1	76.0	70.1	66.5	76.2	57.3	64.2	60.0	75.4
Low P-low Ca	7	1	M	G	2	1	77.4	75.8	70.6	70.8	69.1	75.2	72.2	66.7	92.1	69.4	66.6	79.3	57.2	66.6	67.4	80.9
Low P-low Ca	7	1	M	G	2	2	74.2	71.2	66.8	68.0	67.5	73.7	66.2	62.4	76.1	65.8	63.8	73.1	53.3	59.2	56.5	71.8
Low P-low Ca	7	1	M	G	3	1	75.7	74.9	70.0	68.5	73.0	74.2	69.9	69.6	82.5	70.1	69.0	78.8	57.5	69.1	63.4	72.0
Low P-low Ca	7	1	M	G	3	2	77.1	75.9	71.9	72.0	72.9	75.9	69.5	70.4	82.3	71.2	70.1	78.8	60.2	68.6	61.0	74.3
Low P-low Ca	7	1	M	I	1	1	84.0	88.3	75.6	77.4	78.6	88.3	71.0	79.9	93.7	78.4	77.0	88.3	69.6	83.1	80.9	83.7
Low P-low Ca	7	1	M	I	1	2	84.6	88.5	76.6	78.0	81.8	89.1	73.4	81.3	87.9	84.0	80.3	87.6	75.6	85.1	79.5	80.6
Low P-low Ca	7	1	M	I	2	1	83.9	90.1	78.3	78.2	82.8	91.1	76.4	84.7	92.5	84.1	82.9	90.6	78.1	87.5	86.9	89.5
Low P-low Ca	7	1	M	I	2	2	86.3	91.0	82.1	81.4	84.7	91.1	78.8	86.1	90.0	85.4	83.6	89.7	79.1	87.9	82.3	87.0
Low P-low Ca	7	1	M	I	3	1	83.0	89.5	76.1	79.1	82.6	89.1	73.9	84.4	94.0	81.6	80.9	90.3	74.9	86.0	80.2	87.3
Low P-low Ca	7	1	M	I	3	2	87.0	92.0	80.1	83.2	85.9	91.8	77.9	87.3	90.0	87.4	85.2	90.7	81.2	89.0	85.4	88.4
Low P-low Ca	7	1	M	R	1	1	84.8	89.3	79.5	78.2	82.5	90.6	75.5	81.2	93.3	82.0	79.7	89.3	73.8	85.4	84.2	85.2
Low P-low Ca	7	1	M	R	1	2	86.2	89.9	80.0	79.1	83.1	91.1	77.0	83.6	88.1	85.2	82.2	89.4	76.9	87.1	82.0	82.9
Low P-low Ca	7	1	M	R	2	1	85.2	91.3	79.8	80.2	84.2	90.7	77.9	86.7	90.8	87.0	84.6	88.0	80.4	89.0	86.8	88.9
Low P-low Ca	7	1	M	R	2	2	78.2	86.1	75.5	72.0	78.9	87.2	70.0	79.0	82.4	79.8	76.3	85.2	70.3	83.4	79.0	77.7
Low P-low Ca	7	1	M	R	3	1	84.3	90.4	77.6	78.4	82.7	89.9	74.8	86.6	93.9	83.7	83.7	91.7	79.8	88.9	83.4	88.6
Low P-low Ca	7	1	M	R	3	2	83.7	88.7	78.0	74.3	79.9	89.4	75.5	83.8	85.0	85.5	82.2	89.5	77.6	85.8	80.8	83.1
Low P-low Ca	7	1	F	G	1	1	73.1	73.2	66.5	66.4	64.8	71.2	65.3	65.6	92.1	67.3	65.3	78.4	53.3	66.0	58.2	71.9
Low P-low Ca	7	1	F	G	1	2	75.3	74.1	68.4	68.5	69.7	75.4	66.3	66.2	78.3	66.9	66.0	76.3	56.6	64.9	59.2	74.0
Low P-low Ca	7	1	F	G	2	1	78.5	75.2	69.4	70.2	68.1	75.1	67.5	66.8	89.7	71.2	66.9	81.9	55.8	65.0	60.2	74.4
Low P-low Ca	7	1	F	G	2	2	84.1	83.4	78.5	77.9	78.7	83.4	76.9	76.3	85.8	76.1	76.4	84.1	70.8	75.8	71.2	82.1
Low P-low Ca	7	1	F	G	3	1	86.0	79.9	72.0	69.5	71.9	74.9	69.9	63.4	75.9	67.0	63.8	77.3	52.2	61.2	56.4	74.0
Low P-low Ca	7	1	F	G	3	2	71.1	71.0	66.5	65.1	68.5	71.4	64.9	65.0	76.8	67.0	64.4	73.4	51.5	64.7	55.3	67.6
Low P-low Ca	7	1	F	I	1	1	88.0	92.6	82.7	83.6	86.2	92.0	80.5	89.1	94.3	87.6	86.4	92.7	82.8	90.3	85.7	90.1
Low P-low Ca	7	1	F	I	1	2	86.3	91.5	78.5	81.0	83.9	92.2	75.9	87.8	89.7	86.1	84.9	90.9	80.8	89.6	85.4	88.4
Low P-low Ca	7	1	F	I	2	1	83.6	89.3	77.6	78.8	81.2	89.6	75.0	84.0	94.6	81.7	81.1	90.9	75.2	85.5	79.4	86.7
Low P-low Ca	7	1	F	I	2	2	88.4	92.6	82.1	84.4	87.5	92.5	81.2	89.5	91.1	88.7	87.2	91.9	84.0	91.2	87.0	90.0
Low P-low Ca	7	1	F	I	3	1	85.3	91.8	81.4	81.9	86.3	92.3	79.2	88.1	93.4	87.1	85.1	91.0	81.8	89.8	85.6	89.9
Low P-low Ca	7	1	F	I	3	2	89.3	93.8	85.2	86.2	88.9	93.9	83.6	90.7	93.9	91.0	89.0	92.9	85.9	91.7	86.2	91.2
Low P-low Ca	7	1	F	R	1	1	85.8	91.2	81.7	80.4	83.7	91.4	79.8	88.2	94.1	85.8	86.1	92.3	82.2	90.2	85.1	89.4
Low P-low Ca	7	1	F	R	1	2	87.2	92.2	82.6	82.0	86.1	93.4	81.1	89.0	90.7	89.0	87.6	91.4	84.3	91.1	87.0	89.5
Low P-low Ca	7	1	F	R	2	1	86.3	91.4	82.2	82.4	85.4	91.9	80.3	87.8	95.4	87.5	86.3	93.0	82.2	89.8	84.8	89.9
Low P-low Ca	7	1	F	R	2	2	78.3	86.0	76.7	72.4	79.5	88.0	71.5	77.6	83.1	79.9	74.9	83.3	69.2	84.1	80.1	77.2
Low P-low Ca	7	1	F	R	3	1	89.0	93.6	86.4	85.9	88.6	93.2	84.4	91.0	94.4	91.1	89.8	93.3	87.0	92.6	88.6	92.1
Low P-low Ca	7	1	F	R	3	2	82.4	89.9	81.0	79.2	83.5	91.2	77.9	84.3	89.1	85.8	81.8	88.0	77.3	88.1	83.8	84.1

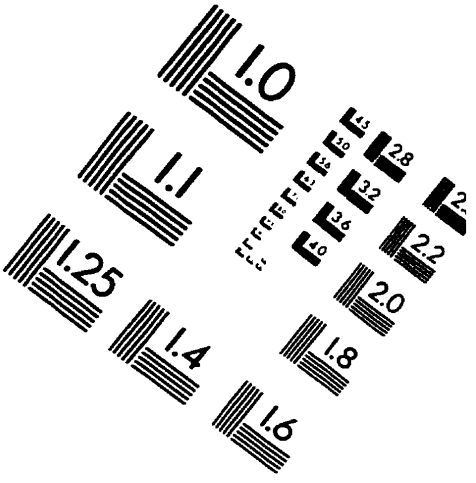
Table A.4 The data base of apparent ileal and fecal digestibilities (%) of AA at 21-d

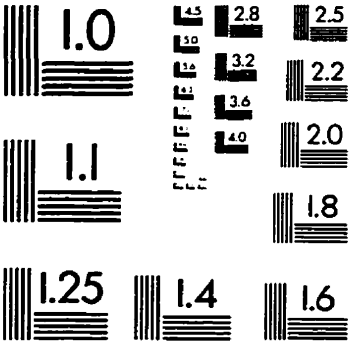
Diet	Trt	Phy	Sex	Organ	Rep	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Low P-normal Ca	4	0	M	I	1	88.6	89.5	79.0	79.3	86.6	88.8	74.2	84.9	89.0	85.3	82.8	90.1	77.4	87.0	86.8	86.5
Low P-normal Ca	4	0	M	I	2	82.6	87.7	72.9	74.5	81.0	87.9	67.5	81.6	86.7	81.5	79.6	88.4	75.3	85.5	86.1	86.7
Low P-normal Ca	4	0	M	I	3	83.5	89.7	77.5	78.7	82.9	89.3	74.9	85.3	89.4	85.7	83.3	90.3	79.2	88.0	88.5	89.4
Low P-normal Ca	4	0	M	R	1	87.5	89.3	77.3	76.0	81.5	89.6	71.8	84.9	89.3	86.5	83.2	89.9	79.5	88.5	88.0	87.4
Low P-normal Ca	4	0	M	R	2	83.6	87.8	75.4	74.5	80.8	89.0	70.5	82.0	86.8	83.4	80.8	88.8	76.0	85.5	85.7	86.0
Low P-normal Ca	4	0	M	R	3	83.9	87.4	73.5	69.8	77.6	87.9	69.2	82.6	84.6	83.7	81.8	89.5	78.8	87.6	86.9	85.9
Low P-normal Ca	4	0	F	I	1	84.4	89.4	76.2	78.6	81.2	88.7	70.9	84.3	88.9	84.9	81.8	89.8	78.6	87.7	87.7	88.4
Low P-normal Ca	4	0	F	I	2	85.4	88.8	77.2	79.4	84.1	88.9	73.6	83.1	87.7	84.8	81.7	88.7	76.6	86.9	86.7	85.5
Low P-normal Ca	4	0	F	I	3	88.5	91.3	81.6	82.0	85.8	91.5	78.2	88.0	91.3	88.5	85.8	92.0	82.2	89.7	90.0	91.2
Low P-normal Ca	4	0	F	R	1	76.5	82.8	70.3	64.7	72.8	83.7	61.2	73.0	82.5	76.9	71.3	82.1	65.9	80.9	81.3	78.0
Low P-normal Ca	4	0	F	R	2	89.1	90.9	77.3	76.7	82.8	90.4	72.6	84.3	89.6	86.1	82.9	90.6	79.5	88.5	88.8	87.1
Low P-normal Ca	4	0	F	R	3	86.2	87.2	73.4	67.9	77.1	87.5	66.3	79.2	86.6	81.3	77.2	87.0	72.9	85.3	85.1	83.4
Low P-normal Ca	5	1	M	I	1	90.8	91.1	74.2	79.5	82.4	90.2	71.5	84.5	85.6	84.7	81.0	88.9	76.1	86.8	86.8	88.0
Low P-normal Ca	5	1	M	I	2	90.9	92.9	83.4	85.0	88.3	93.0	82.3	89.5	91.4	89.8	87.6	92.0	84.4	90.9	91.2	92.2
Low P-normal Ca	5	1	M	I	3	75.4	81.4	62.9	68.1	79.4	86.2	66.7	69.9	76.2	76.0	70.8	77.6	67.0	76.4	77.7	75.5
Low P-normal Ca	5	1	M	R	1	87.3	88.5	71.0	69.2	80.0	89.0	66.2	81.6	84.3	82.3	78.4	86.9	74.8	86.0	84.9	84.9
Low P-normal Ca	5	1	M	R	2	90.8	92.3	82.4	83.7	87.1	92.2	81.0	88.6	89.8	89.6	86.6	91.3	83.1	90.2	90.2	89.9
Low P-normal Ca	5	1	M	R	3	80.2	83.9	65.4	69.8	82.7	89.2	68.9	73.4	80.7	79.5	74.7	82.2	71.5	80.7	81.0	78.2
Low P-normal Ca	5	1	F	I	1	85.3	89.6	77.8	78.4	89.0	91.1	75.5	83.8	88.5	87.6	82.5	87.8	82.4	88.1	85.1	86.9
Low P-normal Ca	5	1	F	I	2	87.5	89.1	78.3	78.0	87.5	90.4	77.1	82.9	86.6	87.3	82.3	88.8	80.8	87.2	87.8	85.1
Low P-normal Ca	5	1	F	I	3	83.3	88.6	76.0	76.6	85.6	90.4	73.8	82.6	86.6	84.6	81.0	85.9	79.6	86.5	87.7	86.0
Low P-normal Ca	5	1	F	R	1	87.6	89.9	79.9	78.4	88.4	93.1	75.3	84.0	87.8	86.9	82.9	88.6	82.9	88.4	88.9	86.5
Low P-normal Ca	5	1	F	R	2	86.4	88.3	75.3	75.1	87.5	91.2	75.7	81.1	85.8	85.8	80.7	87.8	79.6	87.0	87.4	83.2
Low P-normal Ca	5	1	F	R	3	86.0	88.7	79.2	75.6	87.3	90.9	76.7	82.1	86.2	85.9	81.2	87.5	80.2	87.5	87.8	83.7
Low P-low Ca	6	0	M	I	1	88.0	92.5	85.7	86.1	92.9	94.0	84.2	90.6	91.8	92.3	89.1	92.4	87.9	91.6	92.1	92.3
Low P-low Ca	6	0	M	I	2	81.6	88.0	77.3	77.2	86.5	89.9	75.3	83.0	85.1	85.8	81.9	90.1	79.5	86.3	86.4	85.4
Low P-low Ca	6	0	M	I	3	86.4	91.7	83.2	82.8	89.8	93.6	78.4	88.0	91.4	89.6	86.3	92.4	85.0	89.9	90.9	90.3
Low P-low Ca	6	0	M	R	1	83.7	90.1	80.1	78.1	89.1	92.1	77.8	86.9	89.2	90.6	86.2	90.5	86.0	90.5	90.4	89.4
Low P-low Ca	6	0	M	R	2	79.6	87.1	74.8	73.3	86.6	90.8	70.7	81.2	82.7	84.4	81.2	89.4	79.6	86.4	86.8	83.5
Low P-low Ca	6	0	M	R	3	87.6	92.6	85.5	83.9	92.6	93.1	83.5	90.1	92.3	93.1	89.4	92.7	88.3	92.2	92.0	91.4
Low P-low Ca	6	0	F	I	1	86.0	91.4	82.7	82.6	89.7	93.7	79.4	86.9	90.2	89.4	86.0	91.7	84.5	89.6	90.3	89.1
Low P-low Ca	6	0	F	I	2	78.8	86.4	73.6	73.6	82.4	87.6	72.6	79.5	79.9	82.2	78.1	87.6	75.6	83.0	84.6	83.7
Low P-low Ca	6	0	F	I	3	84.2	90.1	78.1	79.6	87.5	91.5	79.0	85.6	87.4	87.2	83.8	90.7	82.2	87.6	88.8	87.8

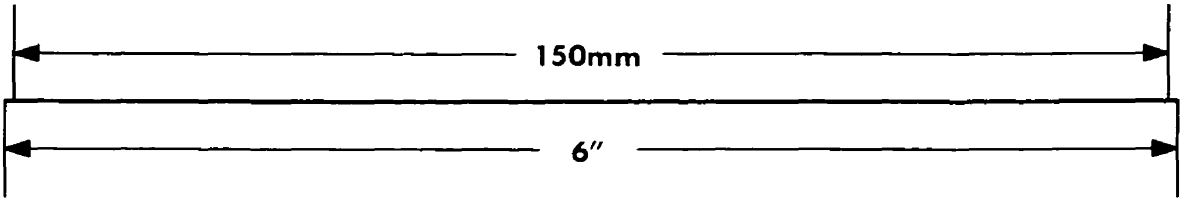
Diet	Trt	Phy	Sex	Organ	Rep	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Ile	Leu	Phe	Lys
Low P-low Ca	6	0	F	R	1	87.2	92.2	84.6	82.7	90.9	93.8	81.5	87.9	92.6	90.8	87.2	91.3	85.6	90.5	91.2	89.9
Low P-low Ca	6	0	F	R	2	82.8	88.9	78.8	76.0	87.9	90.7	78.3	83.7	85.3	86.7	83.0	88.5	82.2	87.5	88.8	86.7
Low P-low Ca	6	0	F	R	3	84.1	90.8	78.9	79.0	90.0	92.1	79.8	87.0	88.4	89.4	86.5	91.4	85.2	90.1	91.0	89.1
Low P-low Ca	7	1	M	I	1	86.2	89.7	79.5	79.5	89.3	89.1	75.8	84.5	86.4	85.5	81.8	87.4	80.4	87.1	86.4	86.6
Low P-low Ca	7	1	M	I	2	83.3	88.8	78.9	78.0	86.7	89.9	77.5	83.2	84.8	85.2	81.5	86.9	79.5	86.4	86.7	86.4
Low P-low Ca	7	1	M	I	3	87.4	91.9	84.3	82.8	90.9	94.5	83.0	89.0	88.9	91.3	86.9	90.8	86.2	90.5	91.1	90.9
Low P-low Ca	7	1	M	R	1	87.6	92.2	85.6	85.0	92.6	93.5	83.9	88.8	91.3	91.0	87.5	92.5	86.5	91.3	91.6	90.4
Low P-low Ca	7	1	M	R	2	89.5	92.3	82.3	80.0	88.7	92.2	81.2	86.4	87.2	88.5	85.3	91.0	84.0	89.2	89.0	88.3
Low P-low Ca	7	1	M	R	3	89.4	93.4	86.8	84.5	92.6	94.5	86.1	90.7	91.2	93.0	89.7	92.0	89.0	92.5	93.2	92.3
Low P-low Ca	7	1	F	I	1	86.0	91.6	81.4	81.9	87.7	92.2	80.5	86.4	90.6	88.2	84.8	90.4	83.9	89.3	89.6	88.3
Low P-low Ca	7	1	F	I	2	87.3	91.2	82.8	83.2	89.7	92.8	80.7	86.8	89.5	88.9	84.9	92.0	83.3	88.9	88.5	89.2
Low P-low Ca	7	1	F	I	3	85.6	91.1	81.2	81.3	88.1	91.3	80.8	86.9	89.9	87.3	85.3	91.4	83.9	89.7	88.9	90.1
Low P-low Ca	7	1	F	R	1	84.7	90.9	81.6	79.6	90.6	92.3	80.9	85.5	89.2	88.5	83.9	89.6	83.2	89.2	88.8	87.9
Low P-low Ca	7	1	F	R	2	85.1	90.7	81.0	80.2	90.9	91.9	79.7	85.8	88.9	89.5	84.3	90.0	83.3	89.1	90.2	90.0
Low P-low Ca	7	1	F	R	3	87.1	91.5	81.7	81.9	89.4	92.4	80.9	87.9	89.6	87.8	85.8	90.5	84.2	90.2	89.5	89.2

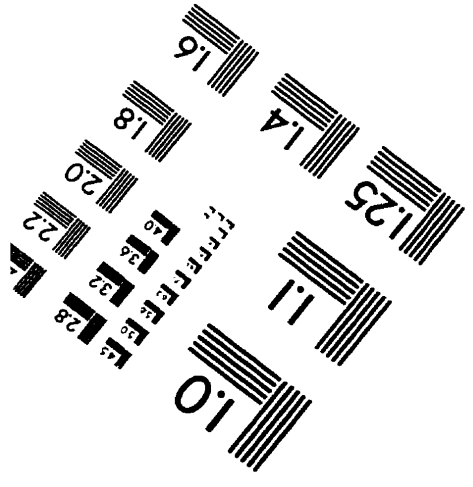
IMAGE EVALUATION
TEST TARGET (QA-3)

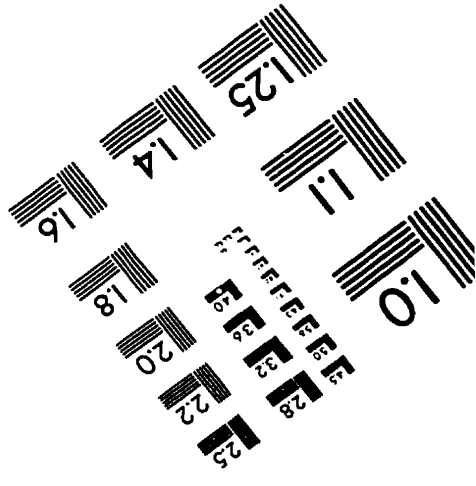












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