# PHOSPHORUS DIGESTIBILITY OF CORN AND SOYBEAN MEAL (SBM) IN BROILERS: IN SEARCH OF A CONSISTENT AND RELIABLE MODEL TO QUANTIFY INDIVIDUAL INGREDIENT CONTRIBUTION

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by

COLWAYNE D. MORRIS

Dr. Jeffre Firman and Dr. David R. Ledoux, Thesis Supervisors

MAY 2017

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

PHOSPHORUS DIGESTIBILITY OF CORN AND SBM IN BROILERS: IN SEARCH OF A CONSISTENT AND RELIABLE MODEL TO QUANTIFY INDIVIDUAL INGREDIENT CONTRIBUTION.

presented by Colwayne D. Morris,

a candidate for the degree of Master of Science

and hereby certify that, in their opinion is worthy of acceptance.

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Dr. Jeffre Firman

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Dr. David R. Ledoux

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Dr. Daniel Shaw

_____

Dr. Roger Davin

*THIS THESIS IS*

*DEDICATED TO MY,*

*MOM SHARMIN ALEXIS MORRIS,*

*GRANDMOTHER ELIEEZE ALEXIS MORRIS*

*AND*

*MY LATE GRANDMOTHER KATHLEEN AGATHA DUNCAN*

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## Abstract

Two experiments were conducted to determine true digestibility of Phosphorus (P) in feed ingredients commonly in commercial broiler diets. A second objective was to determine if the true digestible P values of corn and soybean meal (SBM) would be additive when the two ingredients are fed in a typical commercial diet and finally how phytase supplementation affects true digestible P values.

In Experiment 1, P digestibility of individual ingredients with increasing doses of phytase supplementation in broilers was used to estimate true P digestibility (TPD) of corn, SBM and a formulated diet without or with phytase using the regression method approach in broilers, and verify the additivity of TPD of corn and SBM. Three diets with different inclusion levels each of corn, SBM, and a combination of corn- soybean meal (C-SBM) were prepared with and without phytase supplementation. Broiler chicks were raised on commercial starter diets in accordance to the World Poultry Science Association (WPSA) recommendations and with specific management and routine of the University of Missouri ACUC protocol. Then they were fed the experimental diets for a minimum of 7 d before content of the posterior half of the ileum was collected. Birds were randomly assigned to one of twenty treatments with eight replicate pens per treatment. All diets and digesta samples were analyzed in the same laboratory. Endogenous loss though not significant yielded high negative values for corn diets with and without phytase supplementation (being numerically less) for true ileal P digestibility from -281 to -196 mg/kg DMI and excreta P from -212 to -129 mg/kg DMI

respectively. True digestible P content vs P intake of corn, SBM and C-SBM diets supplemented with phytase were determined to be 30.4, 59.9, and 19.5% respectively.

In Experiment 2, Phosphorus ileal digestibility of corn, SBM and dicalcium phosphate is affected by dietary calcium level in broilers whereas endogenous phosphorus losses are not. The main objective of this study was to determine if digestibility values of ingredients would be additive when using the direct method approach. Also, to study the effect of calcium (Ca) (using two fixed Ca levels 0.35 and 0.85%) on apparent ileal P digestibility (AIPD) values of corn and dicalcium phosphate, on AIPD and TIPD values of SBM and on the estimations of endogenous P losses. The results in this study showed that the TIPD of SBM with 0.85% Ca was estimated to be 59.3%, that is 23 points lower than the TIPD estimation of SBM with 0.35% Ca (82%). The TIPD estimate values are greater than the AIPD values obtained from the SBM diets supplemented at different inclusion level. Increasing SBM level from low to medium improved AIPD in both Ca concentrations however, increasing SBM from 40 to 60% didn't further increase AIPD. Overall apparent P digestibility in diets with 0.35% Ca was higher than a similar diet with 0.85% Ca.

These studies indicate that estimates for true P digestibility should be higher than apparent P digestibility data. The negative Endogenous P Loss (EPL) values in Experiment 1 are a possible explanation for the lack of significant differences between AIPD and TIPD data for diets formulated with the fixed Ca:P as negative EPL. A possible consequence of regression equations that predict negative EPL also is the underestimation of true P utilization (TPU). Based on these findings, it is suggested that

the Ca concentration in broiler diets should be maintained low as realistically possible to maximize the utilization of other nutrients. Given that the addition of microbial phytase is currently routine in poultry diets and since the effect of phytase is diet-dependent, measurement of true P digestibility with a background of microbial phytase is of practical relevance. Using the regression approach, dietary phytase supplementation improved the true P digestibility in corn and SBM and a dietary P increases digestibility tend to decrease.

# CHAPTER I

# GENERAL INTRODUCTION

Phosphorus (P) is an expensive and most of all a critical macronutrient in poultry nutrition. An adequate dietary supply of P is essential for an animal's normal growth and production. This important mineral cannot be synthesized but, must be obtained from dietary sources and is a critical component during energy and lipid metabolism and during the synthesis of cell membranes (Hill et al., 2008). The primary constituents of diets for poultry and pigs are plant-based ingredients which come primarily from the seeds of plants; cereals, legumes, and oilseed products. Corn and soybean meal (SBM) are major feed ingredients used in poultry diets in the U.S., and it has been estimated that more than 60% of P in corn and SBM is in the form of phytate (Reddy et al., 1982). Soybean meal dominates the market with respect to protein supplements for poultry because of its consistency in nutrient content, it is readily available year-round, and has a high CP content making it an almost ideal protein supplement for poultry. Birds do not produce sufficient phytase, which is the enzyme required to break down phytate and release the bound P. Phytate P (myoinositol P) is not readily available to monogastric species because they lack the enzymes needed to hydrolyze phytate into (inorganic) phosphorus and inositol.

Feeds are, therefore, normally supplemented with P in the form of inorganic feed phosphates having a high and predictable available P content. To optimize the supply of dietary P for animals requires a sound knowledge regarding the availability of P from

feed raw materials, which is currently lacking in the poultry industry. As such, high levels of inorganic P (sometimes in excess of requirement) are usually supplemented in commercial poultry diets to meet the nutritional requirements of birds, thereby increasing the cost of poultry production. This approach has resulted in a large amount of P being present in poultry litter (Honeyman, 1993). Excessive P excretion leads to accumulation in the soil in areas with high animal stocking density, leading to P contamination of surface water or even eutrophication (Jongbloed and Kemme, 1990). The use of a well-defined criterion for P availability is therefore of paramount importance to ensure greater efficiency of utilization of dietary P and to reduce the excretion of P into the environment.

In recent years, there has been an increasing interest in improving the utilization dietary P for animals because of concerns over environmental pollution through excess P excretion, depletion of nonrenewable global inorganic phosphate deposits, and unpredictable prices of inorganic phosphate supplements (Cordell et al., 2009; Donohue and Cunningham, 2009). Therefore, highly available inorganic P sources are routinely incorporated into the diets for optimal growth performance of birds. Economically, P is the third most expensive component in the poultry diet after energy and protein, so studies on P availability in inorganic sources are of biological and economic importance. All animals need to absorb P, mainly as phosphate, from the intestine to meet their requirement. The amount needed varies widely, and depends on animal factors such as species, age, physiological state, targeted level of performance, and a commonly overlooked factor is the intestinal integrity of the animals (Shafey, 1993; al Masri, 1995;

Kerr et al., 2000). The desired concentration in the diet is fulfilled by combinations of different raw materials that vary in their content and forms of P as well as in the level of intrinsic phytase. In the commercial feed industry, the aim is avoiding deficiencies and maintaining animal health by optimizing dietary P concentration.

Broiler diets are typically formulated to contain between 8.0 and 10.0 g/kg total Ca. Tamim et al. (2004) reported a 44% reduction in phytate-P disappearance when birds were fed corn-soy diets with 5.0 g/kg added Ca compared with birds fed diets with no additional Ca. Conversely, it has been suggested that bone pathology in broilers is greatest when diets contain between 10 and 13 g/kg of Ca. Therefore, decreasing the amount of Ca in broiler diets may improve performance and nutrient availability; however, this should not be at the expense of increased leg problems. The P requirement of modern broilers is described as a requirement for NPP (Ross 708 Broiler Nutrition Specification, 2014). Non-phytate P in feedstuffs originates from phospholipids, nucleic acids, adenosine triphosphate, and phosphate molecules (Veum, 2010). Formulating diets to meet NPP requirements is based upon the assumption that NPP concentrations are unchanging between individual feedstuffs and are therefore 100% available for digestion and absorption (Rodehutscord, 2009). Additionally, the National Research Council (NRC) changed from listing available P values to NPP values of feedstuffs in consecutive editions of the Nutrient Requirements of Poultry without substantial differences in the published data itself (NRC, 1984). These terms are now considered to have different meanings, further complicating the understanding of true P availability of feedstuffs (Angel et al., 2002).

Different definitions to define available P (e.g. nonphytate P, and retainable P) have been proposed and several approaches are in use to determine P availability in feed ingredients for broilers (Shastak and Rodehutscord, 2013). The confusion in definitions resulted in an overestimation of true P requirements for poultry and resulting in excess P being added to diets thus, excreted (Angel et al., 2002; WPSA, 2013). Research publications used for amino acid and phosphorus recommendations in the last NRC are now, at best, from 1991 and at worst from 1947. Applegate and Angel (2014), recently concluded that the poultry science community has published substantial amounts of data in those areas to warrant an update to the ninth revised edition of the NRC Nutrient Requirements of Poultry. Limited information exists on true P digestibility in corn and SBM and how it is affected by microbial phytase. To the author's knowledge a comparison of the two methods, with and without phytase supplementation, using a regression approach, has never been done. Published data on endogenous losses of P in poultry are limited and available estimated values are shown to be affected by assay methodology, animal factors, and dietary factors such as Ca and nPP levels (al-Masri, 1995; Rodehutscord, 2009). Dilger and Adeola (2006) were the first authors to use the regression approach to estimate the true P digestibility and the endogenous P loss (EPL). Recently, Mutucumarana et al. (2014a, b) measured the true ileal digestibility of P in some feed ingredients for broiler chickens. Iyayi et al. (2013) also used that approach to evaluate the effect of phytase supplementation on P availability from two different ingredients (black-eyed pea and peanut flour).

The variation in P availability of different feed raw materials is high (Shastak and Rodehutscord, 2015), and it is generally accepted that the use of P, as a globally finite resource, can be optimized by considering the differences that exist in P availability of feed raw materials. Different response criteria and descriptive terms for available P have been used in the literature over the past seven decades (Shastak and Rodehutscord, 2013). These differences make it difficult to compare results obtained by using different techniques in different laboratories, and to compile comprehensive feedstuff tables needed by the industry. To improve this situation, the Working Group No 2: Nutrition of the European Federation of Branches of the World's Poultry Science Association proposed a standard protocol for the determination of P availability (WPSA, 2013). This protocol is based on using P digestibility measured at the terminal ileum of broiler chickens (prececal digestibility of P[pcdP], otherwise also referred to as ileal digestibility). The protocol defines assay details relevant for the outcome of the measurement, such as age of birds, minimum number of experimental replicates, diet composition, and P and calcium (Ca) levels in the diet. To provide meaningful and useful data to estimate P digestibility in the different diet compositions found around the world it is necessary to determine the available P in the main ingredients available for poultry that contain significant amounts of PP. Of the various approaches, measurement of preceacal digestible P is deemed to be the preferable method to assess P availability for poultry (WPSA, 2013; Rodehutscord, 2009). However, several research groups have recently encountered issues using the WPSA (2013) protocol to determine TPU in feed ingredients in poultry. These research groups include the Poultry Science Department of

Auburn University (K. Perryman and Professor Dozier), Poultry Science group from Massey University in New Zealand (R. K. Mutucumarana, V. and G. Ravindran, and A.J. Cowieson) and University of Missouri (D. Ledoux, R. Davin and C. Morris). There are several factors that are possibly making TPU data variable, inconsistent and not useful.

The apparent or TPD values in common feed ingredients for pigs have been determined by three approaches, namely regression analysis, the direct method, and the substitution method (Fan et al., 2001; Bohlke et al., 2005; Fang et al., 2007). Only limited attempts have been made to determine the digestible P content in feed ingredients for poultry and two approaches, namely the direct method (Leytem et al., 2008) and regression method (Dilger and Adeola, 2006), have been used. Corresponding data for other common feed ingredients are lacking.

This thesis here in are two studies, Chapter 3 and 4 following the guidelines of WPSA (2013) with some modifications to explore different approaches for determining TPD of individual feed ingredients (e.g. corn and soybean meal) used in broilers diets. To determine the additivity of true or apparent P digestibility values of feed ingredients in broilers. Where P digestibility of individual ingredients with increasing doses of phytase supplementation in broilers using the regression method approach in broilers, and verify the additivity of TPD of corn and SBM.

# CHAPTER II

# LITERATURE REVIEW

## History of Phosphorous

Phosphorous (P) is the twelfth most abundant element in the lithosphere and was first discovered and named in 1669 by a German chemist Henning Brand. The name P in Ancient Greece was the name for the planet Venus and is derived from Greek words, which roughly translates as light-bringer or light carrier. It is a non-metallic element and is widely distributed in the form of phosphates in soils, rocks, in the ocean, in living cells and in most foods (Corbridge., 2013). In the periodic table, P belongs to group 15, with an atomic number and atomic weight of 15 and 30.934, respectively.

Phosphate mines contain fossils, especially marine fossils, because phosphate is formed from the deposits of animal remains and excreta. The clear majority of P compounds produced are utilized as fertilizers. Phosphate is needed to replace the P that plants remove from the soil, and its annual demand is rising nearly twice as fast as the growth of the human population. Moreover, global P reserves are rapidly being depleted and it is estimated that current P reserves will be halved (relative to the reserves at the turn of the twentieth century) by 2040 or, more likely, by 2060.

The rapid depletion of non-renewable inorganic feed phosphate resources, together with their rising cost, indicate that there is an urgent need to develop a suitable method to measure for determining P digestibility in feed ingredients for poultry. Phytate P is

either unavailable to, or poorly utilized by the chick. In addition, phytic acid chelates mineral elements, including calcium, and thereby reduces their availability in whole or part. This requires transitioning from formulating diets on a non -phytate P (NPP) basis to a true P digestibility basis. The TPU, including TIPD and true P retention (TPR), of feedstuffs has been proposed by the World's Poultry Science Association (WPSA) as the best method to determine the true P availability of feedstuffs (WPSA, 2013).

## Importance of P for Broiler Nutrition

Research on the P requirement of broilers has been the subject of numerous investigations for many decades; however, the minimum requirement for this nutrient has still not been established with any certainty. Phosphorus is the second most abundant mineral in the animal body, and about 80% is found in the bones (Table 2.1). Phosphorus is essential for life since it is a key element in many biological roles. Phosphates (compounds containing the phosphate ion, $PO_4^{3-}$) are a component of DNA, RNA, ATP, and the phospholipids, which form all cell membranes. Phosphorus is required for the formation of the organic bone matrix as well as the mineralization of that matrix. The remaining 20% of body P is widely distributed in the fluids and soft tissues of the body, where it serves a range of essential functions (Suttle, 2010). Phosphorus is a vital macro-mineral which contributes to approximately 10 g/kg of body weight in birds. The skeleton and muscle tissues comprise of 850 and 60 g/kg, respectively, of the total P in the body. Phosphorus supports the skeletal system by providing a strong mechanical support together with calcium (Ca) as phosphates [$Ca_3(PO_4)^2$] and hydroxyapatites [$Ca10(PO_4)6(OH)_2$]). Bones are therefore able to serve

as a P source during periods of P deficiency. Non-skeletal P is a component of extracellular and intracellular fluids and is found in concentrations of 10 and 140 g/kg, respectively.

Through its involvement in these metabolic and structural processes, P is essential for animals to attain their optimum genetic potential in growth and feed efficiency as well as skeletal development. Because of the key role of P in growth and bone development and mineralization, the requirements of the animals for P are the highest during the time the animal is growing. Therefore, growing broilers usually require P fortification of vegetable diets with di-calcium phosphate (DCP) or mono-calcium phosphate (MCP).

Not surprisingly bone status is commonly used as an indicator of P and Ca adequacy in broiler diets. Well over 90% of Ca is found in the bones, where it combines with P to form calcium phosphate crystals or hydroxyapatite with the molecular formula $Ca10(PO_4)6(OH)_2$. The modern broiler chicken has been selected for rapid growth and increase in muscle mass, but this rapid growth may also be associated with poor leg health and lameness due to reduced bone mineralization. Reducing Ca and P in the diet can also cause broken bones and bloody meat during processing of the carcass. Bone breakage during catching and transportation creates problems during processing. Broken bones, especially fractured clavicle bones, may find their way into the meat, and must be removed at great expense. Hemorrhages in the meat are another major quality defect, which can lead to downgrading of the broiler carcass. This is very significant due to the increased current importance of selling cut-up chicken parts, in which the

emphasis is no longer only on yield but also on characteristics such as bloody breast meat and broken bones (Gregory and Wilkins, 1990).

## Phosphorus Sources and Their Availability in Poultry

The nutritive value of feed ingredients hence, level of P varies not only with source but also varies depending on the species, variety or cultivar, the season of the year, location, processing, and storage conditions (Ravindran et al., 1995; Rebollar and Mateos, 1999). Phosphorus concentrations in feed ingredients are similarly affected by the aforementioned factors. However, P concentration is known to differ more widely than those of other macro-minerals which are naturally found in the same feed ingredient. It is well known that trace minerals (Co, Cu, Fe, I, Mn, Mo, Se, and Zn, among others) are required for the normal functioning of basically all biochemical processes in the body. They are part of numerous enzymes and coordinate a great number of biological processes, and consequently they are essential to maintain animal health and productivity (Suttle, 2010; Lopez-Alonso, 2012).

Phosphorus in poultry feed formulations are primarily being supplied from plant-based feed ingredients commonly corn and soybean meal, combined with the use of phytase, and or inorganic mineral sources.

### Plant Based Ingredients

In general, seeds (cereal grains, legumes and oilseeds) have a greater P content than do forages some of the feedstuffs used in broiler production are summarized in Table 2.2.1. Phosphorus in plant-based sources is primarily found in the form of phytate or Ca-magnesium salts of phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate;

IP6). Phytate is commonly found in all plant seeds and their by-products, and serves as the chief storage form of P (Ravindran et al., 1995) and a source of P and other cations for germination (Williams, 1970). Phytate P comprised 60 to 80% of total P in the seeds of legumes and cereals (O'Dell et al., 1972). Phytic acid location in the grain components; three major components, bran, germ and endosperm varies depending on the type of grain (Figure 2.2).

It is therefore generally assumed that two-thirds of the total P in plant ingredients exists in this form and is poorly utilized by poultry and pigs (Ravindran et al., 1995; Viveros et al., 2000). Total and phytate P concentrations in commonly used cereals vary widely between and within the same feed ingredient. Oilseeds are comparatively rich sources of P compared to cereals, but also possess high phytate P concentrations.

**Figure 2.2.** The grain components; three major components—bran, germ and endosperm (CCF, 2013)

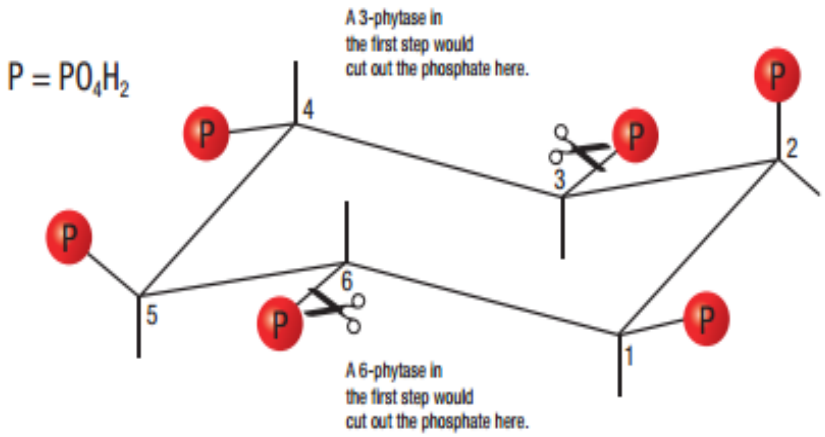


**Figure 3**. Structure of Phytate / Phytic acid-Source: Marounek et al., 2010

The availability of P among plant-based ingredients differs widely. Evidence from studies by Temperton and co-workers suggested that the availability of P in wheat is due partly to the endogenous phytase activity in these ingredients (Temperton and Cassidy, 1964a, b). Phytase activity in wheat (1,200 U/kg) and wheat bran (2,957 U/kg) are markedly higher than that in maize (12 U/kg) and sorghum (24 U/kg) (Eeckhout and De Paepe, 1994; Godoy et al., 2005). Oilseeds such as soybean meal (8 U/kg), peanut meal (3 U/kg) and rapeseed meal (16 U/kg) contain little or no phytase activity (Eeckhout and De Paepe, 1994). Inclusion of cereals such as wheat, rye and barley rich in intrinsic phytase activity has been shown to have a positive effect in phytate P hydrolysis and P utilization by animals (Selle and Ravindran, 2007). Therefore, plant breeding for low phytate, soluble phytate and high intrinsic phytase activity is an area of great interest to increase P utilization by animals (Diarra et al., 2010).

## The Use of Phytase

Phytate (inositol hexaphosphate), which is present in many plant-based feedstuffs, is the main P store in plants (Cosgrove, 1980; Pandey et al., 2001). Phytate passes undigested through the digestive tract of monogastric animals as they produce little or no intestinal phytase activity (Cooper and Gowing, 1983; Williams and Taylor, 1985; Wodzinski and Ullah, 1996; Maddaiah et al., 1964; Van Der Klis and Versteegh, 1996). Thus, monogastric feed must usually be supplemented with inorganic phosphate to meet the animal's P requirement. Moreover, the bioavailability of P present in phytate is generally poor (Camden et al., 2001; Rutherfurd et al., 2002). In addition, phytate can complex divalent cations as well as amino acids and proteins, and when present in diets for monogastric animals, can reduce the digestibility and absorption of the latter nutrients. Phytate-bound P is excreted in animal manure which is subsequently spread on farmland. This often contributes to eutrophication of surface waters, particularly in areas of intensive pig and poultry production (Common, 1989; Walsh et al., 1994).

Since the first release of a commercial phytase in 1990, an enzyme that dephosphorylates phytate, the poultry industry is now extensively using the different forms of phytase that have appeared on the market. It has been postulated that dietary microbial phytases can also improve the availability of minerals other than P, particularly those that can complex with phytate in the diet. Both fungal- and bacterial-derived phytases are commonly added to broiler diets and have been shown in many studies to improve the bioavailability of P (Rimbach et al., 1994; Camden et al., 2001; Rutherfurd et al., 2004a; Cowieson and Adeola, 2005), other minerals (such as calcium,

magnesium, potassium, and zinc; Ravindran et al., 2008; Santos et al., 2008; Saima et al., 2009), and amino acids, but particularly threonine (Sebastian et al., 1997; Camden et al., 2001; Rutherfurd et al., 2004a).

There are two classes of phytases (EC 3.1.3.8 and EC 3.1.3.26) that have been differentiated based on the first phosphate group in the phytate molecule to undergo phytase attack. The 3-phytases initially attack the carbon in the third position, whereas 6-phytases initially attack the carbon atom in the sixth position. The utilization of phytate P or phytate P hydrolysis is dependent upon the availability of extrinsic or intrinsic phytase enzyme to the monogastric animals. Phytate-bound P should be hydrolyzed into inorganic phosphates and inositol to make P available for poultry (Figure 2.1).

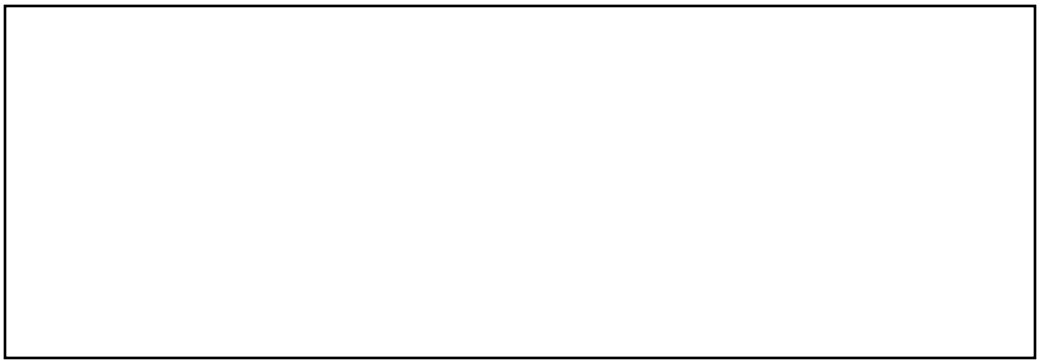

**Figure 2.** How do Enzymes Work (the induced fit model)

The induced-fit model, both enzyme and substrate undergo dynamic conformational changes upon binding. The enzyme contorts the substrate into its transition state, thereby increasing the rate of the reaction. *(Open Stax College, Biology)*

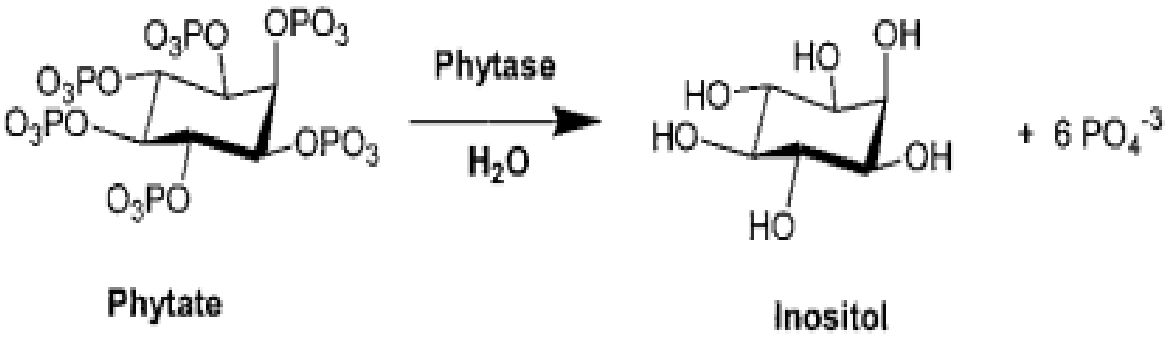


**Figure 2.1.** Mechanism of Phytate hydrolysis by Phytase (Garrett et al., 2004).

The addition of exogenous phytases in the diets of poultry has been shown to improve weight gain, mineral retention, energy utilization, and amino acid digestibility (Ravindran et al., 1999; Rutherfurd et al., 2002; Augspurger et al., 2003; Cowieson and Adeola, 2005). Phosphorus retention by broilers was improved from 50% to 60% by supplementing diets with a fungal phytase (Simons et al., 1990; Kornegay et al., 1996; Cowieson et al., 2009 and Selle et al., 2012). Phytases also reduced the phytate-P excretion when they were supplemented to diets with little available P (Selle et al., 2000). The common recommended dose of phytase is 500 FTU/kg in broiler diets for the destruction of 50 to 70% of phytate (Ravindran et al., 2006; Cowieson and Ravindran., 2007). The inorganic P equivalent of phytase reported in the literature is between 0.3 to 1.7 g/kg (Cowieson et al., 2011; Cowieson et al., 2013; Zyla et al., 2013; Cowieson et al., 2014). The activity measurements may differ significantly due to the methods of analysis (Dersjant-Li et al., 2015). However, in recent years, higher doses of phytase (three to four times the standard dose) are being used in poultry diets, showing some positive results in terms of nutrient availability and performance (Cowieson, 2010). It has been suggested that high phytase doses produce the complete de-phosphorylation of phytate

and release inositol (Ravindran, 2005), which is considered a growth promotor. Inositol is also known to have important metabolic roles, such as in fat metabolism and cell function, as well as being re-combing with P at a cellular level to form phytate, which is a potent anti-oxidant. Some studies like the one conducted by Zyla et al. (2004) addressed the positive effect of inositol supplementation in broilers. These authors observed that the supplementation of 0.10% inositol improved about FCR by 6.4% in broilers from 1 to 21 d of age.

## Mineral Sources of Phosphorus

Inorganic phosphates are usually used as mineral supplements in poultry diets, because of their high content and availability of P (Peeler, 1972; Waldroup, 1999). Table 2.3.2.2. lists the Inorganic feed phosphates and their respective total P (TP) (g/kg) and retainable P (RP) (% of TP). They occur naturally as rock phosphates which are widely distributed in Northern Europe, Africa, Asia, USA, China and Middle East (Mehmood et al., 2009; Van Kauwenbergh, 2010). Crude phosphates, which are of igneous and sedimentary origins, are obtained by surface (open cast or strip) mining or underground methods and are converted into orthophosphate after removing undesirable impurities such as cadmium, arsenic, chromium, zinc, copper, nickel, fluorine, and uranium (Mehmood et al., 2009; Van Kauwenbergh, 2010). Type of inorganic phosphate produced is mainly dependent on the manufacturing process. The major inorganic feed phosphates used in animal diets are different forms of calcium phosphates such as mono and dicalcium phosphates and defluorinated phosphates. Selection of an inorganic phosphate source for poultry diet formulations depends on a number of

factors, including biological availability, chemical composition, availability, cost, physical handling qualities, and free from harmful impurities.

Dicalcium, monocalcium, mono-di-calcium phosphates and de-fluorinated rock phosphate are the most commonly used forms of inorganic feed phosphates. The terms mono-and di-calcium phosphate (see Table 2.2.2) are commonly used in product descriptions; most commercial inorganic feed phosphates in the above categories are not pure products, but rather mixtures of MCP ($CaH_4P_2O_8$) and DCP ($CaHPO_4$) (Viljoen, 2001). The prececal P digestibility of the tested common inorganic P sources in broilers diets was 78.3% for MCP, 59.0% for DCP, 70.7% for MDCP and 31.5% for DFP (P. Bikker et al, 2016).

The ileal digestibility of the anhydrous DCP of 59.0% observed by Bikker et al. (2016) is in between the ATTR values of 76 and 53% for dehydrated DCP and anhydrous DCP, respectively, as measured in the study from Van der Klis and Versteegh (1992b) but closer to the value for the anhydrous form. However, the ileal P digestibility value of anhydrous DCP of 59.0% established in that study is substantially higher than the ileal P digestibility of anhydrous DCP of 25.0 to 30.0% observed in the study of Shastak et al. (2012). In the same study of Shastak et al. (2012), a ileal P digestibility of 54 to 67% for monosodium phosphate was found which is low compared to reported monosodium phosphate digestibility 66 to 78% (Van der Klis and Versteegh, 1996), and indicates that the differences in ileal P digestibility between DCP observed in the study of Shastak et al. (2012) may be attributed to systematic differences in experimental conditions and not to differences in DCP properties per se. The ileal P digestibility of MDCP of 70.7%

observed in Bikker et al. (2016) study was substantially lower than the ATTR value of 80.3% observed in a study from Van der Klis and Versteegh (1998a) in which MDCP was regarded as the only P source and tested at a retainable P concentration of 3.13 g/kg with a Ca:P ratio of 1.79. In another study of Van der Klis and Versteegh (1998b), two MDCP sources were tested resulting in ileal P digestibility values of 72.2 and 81.8%, indicating that substantial differences in MDCP qualities exist

## Phosphorus nutrition in poultry

Phosphorus, in addition to its function in bone formation, is also required in the utilization of energy and in structured components of cells (NRC, 1994). Only about 10 percent of the phytate P in corn and wheat is digested by poultry (Nelson, 1976). Phosphorous plays a critical role in many metabolic and structural processes, P is essential for animals to attain their optimum genetic potential in growth and feed efficiency as well as skeletal development. Because of the key role of P in growth and bone development and mineralization, the requirements of the animals for P are the highest during the time the animal is rapidly growing (Applegate and Angel, 2004). Phosphorus has also been known to control the appetite of animals and promote feed utilization (Suttle, 2010). Deficiency of P has been known to cause poor bone mineralization and rickets in poultry (Soares, 1995; Coon et al., 2002). The main clinical symptoms of rickets include rubbery beaks, swollen joints and enlargement of epiphysis. In older birds, dietary deficiency of P leads to osteomalacia resulting in weak bones (Coon et al., 2002).

Phosphorus is also an active component of 2,3-diphosphoglycerate, which controls the release of oxygen from hemoglobin. In fat metabolism, P actively participates in fatty acid transportation via formation of phospholipids (McDowell, 2003). Phosphorus plays a key role in the sodium (Na)-potassium ion pump and regulates transportation of metabolites through phospholipid bilayers.

There is also a global interest in improving the utilization of P by poultry due to concerns of environmental pollution through excess P excretion, depletion of non-renewable inorganic phosphate deposits, and increasing prices for these inorganic phosphate supplements thus increasing feed cost and cost of production. As indicated by Applegate and Angel (2008), substantial research has been conducted in broilers and laying hens to further define P needs since the 1980s-based publications that were the foundation for the recommendations in the current NRC (1994) publication (please refer to table 2.3). However, overfeeding of dietary P is common commercially, with excesses of 20 to 100% over published requirements commonly observed. In the United States, part of this overfeeding is due to a lack of a centralized, up-to-date source of information on poultry P requirements, especially broilers, (e.g., a current NRC publication), lack of information on digestible P in ingredients, and no information on ingredient concentrations with variability and number of analysis represented associated with it.

Additionally, when the NRC requirements (1994) were published, phytase was just entering the commercial market. Further, P nomenclature between the eighth (1984) and ninth (1994) NRC publications induced confusion in many, in that the 1984

publication used an available P (aP) nomenclature, but the 1994 publication used a nonphytate P (nPP) nomenclature without substantial change in the values resulting in feed ingredients having similar aP and nPP concentrations between these revisions. These terminologies are not synonymous, as aP refers to the P that is absorbed from the diet into the animal (i.e., feed P minus P within the distal ileum; a biologically available term) versus nPP, which is chemically determined term (total P minus phytate P). Since that time, substantial discussion has taken place among the poultry and swine scientific communities on development of consensus protocols for ingredient aP. The Nutrition Working Group of the European branch of the World's Poultry Science Association published (2013) consensus protocols for aP determinations, including procedures for the determination of nutrient hydrolysis per unit of enzyme inclusion.

## Digestion and Absorption of P in Poultry

Poultry diets composed of plant and animal based feedstuffs are theoretically sufficient to meet the P requirements of broilers. However, differences related to feedstuff P composition determine the efficacy of P utilization, and most of the P present in the diet is not readily digested or absorbed (Hill et al., 2008). Ravindran et al. (1998) and Sebatian et al. (1998) reported that poultry cannot produce enough amounts of endogenous phytase to hydrolyze and release P from phytate.

## Digestion of P in Poultry

Digestion may be defined simply as the preparation of food for absorption. In the broad sense, it may include mechanical forces (chewing or mastication; muscular contractions of the GI tract), chemical action (hydrochloric acid in the stomach; bile in

the small intestine), or hydrolysis of ingesta by enzymes produced in the GI tract or from microorganisms in various sites of the tract. The overall function of the various digestive processes is to reduce food to a molecular size or solubility that allows absorption and cellular utilization of the individual nutrients released in the process (Pond et al., 2005). The digestion of feed P is primarily determined by the form of P in which it is naturally present in feed ingredients (Hill et al., 2008). However, P needs to be available in the inorganic form (Pi) to be absorbed in the gastrointestinal tract (Gropper et al., 2008; Hill et al., 2008). Dietary P in the organic form must be first hydrolyzed into Pi by phytase enzyme such as phospholipase C and alkaline phosphatase to release P from bound forms (Ravindran et al., 1995; Gropper et al., 2008). Dietary factors such as vitamin $D_3$, zinc, manganese and molybdenum are known to increase the activity of alkaline phosphatase while magnesium and Ca have a negative effect (McCuaig and Motzok, 1972). Increased alkaline phosphatase activity has been observed when chickens were fed low P diets (Davies et al., 1970; McCuaig and Motzok, 1972). The metabolism of Ca and P is closely related, and a deficiency or an excess of either one will interfere with the utilization and metabolism of the other (Kebreab and Vitti, 2005). Calcium and P homeostasis is maintained through a complex feedback system described by many authors (Proszkowiec-Weglarz and Angel, 2013).

Phosphate transport in the intestine, (see Figure 1), the sodium-dependent phosphate transporters of the NaPi-IIb type are present at the luminal surface of the enterocyte (brush border membrane). The NaPi-IIb transporters are electrogenic and have high affinity for inorganic phosphate (Pi). Energy for this transport process is

provided by an inward down- hill sodium gradient, maintained by transport of Na+ from the cell via a Na+/K+ ATPase cotransporter at the basolateral membrane. The phosphate incorporated into the enterocytes by this mechanism is transferred to the circulation by poorly understood mechanisms. Phosphate absorption also occurs via a sodium-independent process (es), such as diffusional movement across the intercellular spaces in the intestine (Carpentar, 2010).

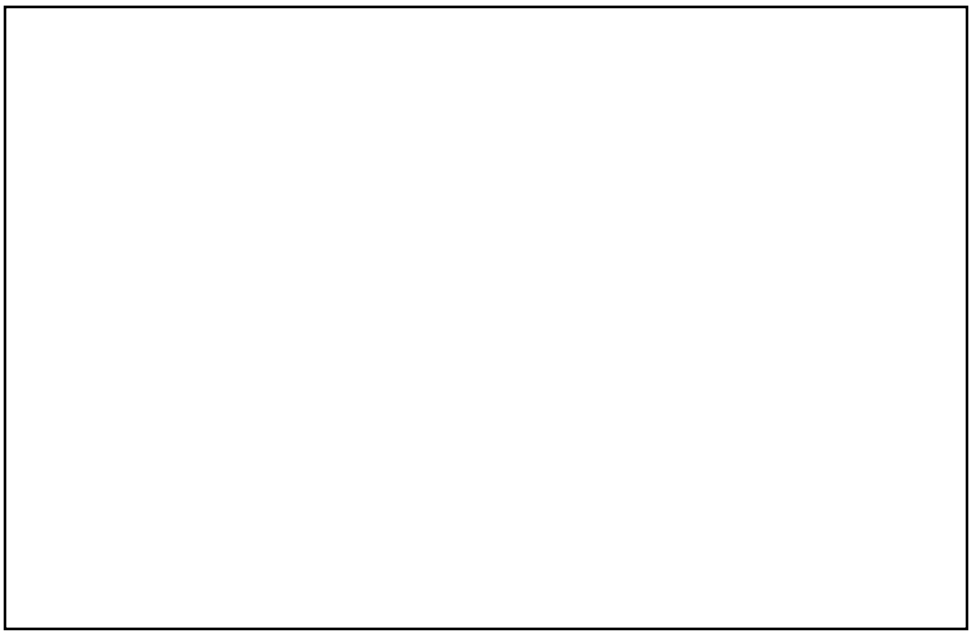

**Figure 1**. A model of inorganic phosphorus transport in the intestine (Source: Carpenter and Drezner, 2007)

## Absorption of P in Poultry

Absorption consists of the processes that result in the passage of small molecules from the lumen of the gastrointestinal tract through the mucosal cells lining the surface of the lumen and into the blood or lymphatic systems (Pond et al., 2005). Phosphorus

needs to be available in the inorganic form (Pi) to be absorbed in the gastrointestinal tract (Gropper et al., 2008; Hill et al., 2008). Inorganic P exists in two forms in the intestinal lumen: the divalent form, $HPO_4^{-2}$, and the monovalent form, $H_2PO_4^-$, and are the basic forms in which P is absorbed (Quamme, 1985). Phosphate can also be absorbed as a structural part of some organic compounds such as phospholipids (Borgström, 1976). Absorption of P is reported to occur along the entire small intestine with the largest fraction being absorbed in the jejunum (Walling, 1977). Intestinal P absorption occurs through both cellular and paracellular pathways. Active absorption occurs at the apical membrane of intestinal tract enterocytes via a type IIb Na/phosphate-cotransporter (Hilfiker et al., 1998). Sodium alters the conformation of the cotransporter, which increases the cotransporter's affinity for phosphate. Once phosphate binds to the cotransporter, a second conformation change occurs, resulting in a transport competent cotransporter conformation like the Na/glucose cotransporter (Danisi and Murer, 1992). Phosphate then enters the cell against the concentration gradient due to the electrical gradient established by Na/K ATPase pump. Once in the enterocyte, phosphate is transported across the basolateral membrane into the blood by a Na independent transporter (Peerce, 1997).

In the past, only three main regulators of phosphate metabolism had been identified: (1) dietary phosphate intake and absorption, (2) calcitriol, which increases phosphate absorption from the gut and bone resorption, and (3) parathyroid hormone (PTH) which directly causes phosphate resorption from bone and decreases its reabsorption in the proximal tubule, and indirectly by stimulating the production of

calcitriol. However, more recent findings have also demonstrated the physiological importance of bone and phosphatonins, such as fibroblast growth factor-23 (FGF-23) in phosphate regulation (Wesseling-Perry, 2010; Alon, 2011; Berndt et al., 2005)

## Factors Affecting P Nutrition

Determination of the bioavailability of P in feedstuffs is a major challenge. There are many factors affecting P bioavailability in birds, including. Some of these factors have been investigated in detail (Angel, 2010) and the major factors are briefly described below.

## Phytic Acid Content

Phytate is the principal storage form of P in plant feedstuffs and is an anti-nutritive factor (Bryden et al., 2007). Phytic acid content in the feed is a major dietary factor determining P availability in feed ingredients for poultry. Phytate has been shown to inhibit activities of some digestive enzymes such as pepsin, α-amylase and trypsin. Phytate may inhibit proteolysis by changing the protein configuration of digestive enzymes (Singh and Krikorian, 1982). Phytic acid is poorly utilized by poultry due to insufficient phytase enzyme activity to hydrolyze phytates in the poultry gastrointestinal tract (Maddaiah et al., 1964; Godoy et al., 2005; Marounek et al., 2010). Phytate carries a strong negative charge and is capable of binding di- and trivalent cations such as calcium, cobalt, copper, iron, magnesium, manganese, nickel and zinc in very stable complexes (Maenz et al., 1999; Wise, 2013), reducing the availability of P as well as these minerals to the animal.

The negative effects associated with phytic acid can be alleviated, in part, by the use of exogenous phytase. Results from several studies presented in Table 2.3.2.3 have shown increased P digestibility and utilization, and hence reduced P excretion into the environment due to phytase addition to poultry diets (Applegate et al., 2003; Penn et al., 2004; Angel et al., 2006; Leytem et al., 2007). Phytate-bound P needs to be hydrolyzed into inorganic phosphates and inositol to make P available for poultry (see Figure 4). It has also been reported that phytate can reduce fat digestibility by forming insoluble Ca-phytate complexes with fatty acids in the gut lumen (Leeson et al., 1993). In its chelated form, the phytate molecule is difficult to hydrolyze by phytase. The pH affects the solubility of phytate.

## Inorganic P (Pi)

A large proportion (about 60 to 80%) of the P in dietary ingredients is bound as the salts of phytic acid (Jongbloed et al., 1993), in the form of phytates. These complexes reduce the bioavailability of P and other essential cations (like Zn, Cu or Mn) for monogastric animals due to the low level of endogenous phytase present in grain and complete absence in the digestive tract of monogastric animals. Thus, high levels of inorganic P need to be supplemented in most of commercial poultry diets. The poultry industry in the United States, and throughout the world, uses millions of tons of high quality feed grade inorganic phosphates each year. Birds fed diets with 3.5 g of nPP/kg consumed significantly more feed compared with birds fed diets containing 2.5 or 4.5 g of nPP/kg, whereas birds fed 2.5 g/kg consumed less feed than those fed diets formulated with 3.5 and 5.5 g/kg. Also, the apparent ileal digestibility of P was reduced

with increasing dietary Ca concentration. Phosphorus digestibility was negatively influenced by feeding diets with 2.5 g of nPP/kg compared with diets with higher nPP (Wilkinson et al., 2014).

The bioavailability of the P in these inorganic sources is usually very high for poultry. However, the exact composition of these commercial sources is often baffling although the guaranteed percentage of P and the range of Ca in these products is known. Some commonly used mineral sources and their respective P composition and retention rate are listed in in Table 2.3.2.2. Dicalcium phosphate supplementation is not only expensive but can also lead to environmental problems by over-supplementation. Excess P from the feces of hens is easy can access ground water, rivers, lakes, and oceans, and lead to mortality of aquatic animals by stimulating algae growth.

The inclusion of high dietary concentrations of Pi has been found to proportionately reduce phytate P hydrolysis (Ballam et al., 1984) and lower the digestibility of P in poultry (Ravindran et al., 2000). These effects were attributed to the inhibition of phytase activity by Pi, the end product of phytate hydrolysis (as reviewed by Selle et al., 2009). The inhibitory effect of Pi (Na2HPO4) on rapeseed phytase activity has also been reported by Mahajan and Dua (1997). This negative effect is greater when dietary Ca and Pi concentrations are both increased which results in increased precipitation of IP6-mineral complexes due to lowered solubility of these minerals and phytate P (Angel et al., 2002). Similarly, reaction of Pi with Ca leading to the flocculent precipitation of $Ca_3(PO_4)^2$ can also make P less available for absorption (Hurwitz and Bar, 1971; Selle et al., 2009).

## Dietary Calcium (Ca)

The most critical factor affecting phytate P availability is the Ca ion concentration in the upper gut where it forms a Ca-phytate precipitate (as reviewed by Selle et al., 2009). Although Ca does not exhibit a strong affinity to chelate with phytate as other inorganic minerals, it readily forms an insoluble complex with phytate due to high dietary inclusions in poultry diets (Angel et al., 2002). High concentrations of dietary Ca, in the form of limestone, have been found to increase the pH of the digesta with negative effects on phytate P hydrolysis (Shafey et al., 1991; Angel et al., 2002). Additionally, high concentrations of dietary Ca increase the size of the IP6-mineral complex reducing the surface area to be attacked during hydrolysis by the phytase (Angel et al., 2002). Therefore, the dietary Ca concentration, and the Ca:P ratio, in poultry diets are critical determinants of P availability.

Past studies have also confirmed the negative relationship between phytate P hydrolysis and dietary Ca concentrations. Al Masri (1995) described a decrease in the availability of feed P from 0.66% to 0.30%, as Ca:P ratios were changed from 1:1 to 2.5:1. The author states that increasing Ca concentration (i.e., from 0.66% to 1.58%) showed a greater effect on P absorption than on P retention, as the animals tended to reduce the endogenous P excretion in trying to conserve the nutrient. Ballam et al. (1984) demonstrated that chicks fed diets with 10 g/kg Ca hydrolyzed less phytate than those fed diets containing 8.5 g/kg Ca. A similar finding was reported by Mohammed et al. (1991) who noted that the utilization of phytate P can be increased by 15% when the dietary Ca content was reduced from 10 to 5 g/kg.

Nelson and Kirby (1987) also reported improved dietary phytate P hydrolysis from 5.6 to 55.0% when dietary Ca concentration was reduced from 5.2 to 1.2 g/kg in broiler diets. The low dietary Ca concentration, however, resulted in poor performance and bone mineralization. The NRC (1994) recommends a Ca: non-phytate P ratio of about 2:1 (weight to weight basis) for broilers. For laying hens, it has been suggested that Ca: P ratio does not have any practical significance because of the higher Ca requirement for egg shell formation. A much wider Ca: non-phytate P ratio of 12:1 is used in layer diets (McDowell, 2003). In recent research, Anwar et al. (2016) reported that increasing dietary Ca concentration from a 6.75 to 11.25g/kg diet, when keeping the dietary P concentration constant (4.5 g/kg), decreased Ca digestibility and Ca retention.

The WPSA working group recommended maintaining a Ca:tP ratio between 1.3:1 and 1.4:1 for “experimental diets for testing P sources of mineral and animal origin” and between 1.4:1 and 1.5:1 for “experimental diets for testing efficacy of supplemented phytase”. The addition of Ca to maintain a constant Ca:tP ratio causes the Ca: NPP ratio to become wider with each additional inclusion of the test feedstuff. The Ca: NPP ratios greater than four resulted in decreased P availability (Smith and Kabaija, 1985; al-Masri, 1995; Qian et al., 1997). Researchers that reported negative EPL were attributed to widening Ca: NPP ratios of titration diets; whereas authors using Ca: NPP ratios of titration diets fixed and below 2.2:1 reported positive EPL (Dilger and Adeola, 2006; Mutucumarana et al., 2014a, b).

## Phytase

Phytases (myo-inositol hexaphosphate hydrolases) are widely distributed in plants, animals and microorganisms, and are capable of hydrolyzing one or more phosphate groups from IP6 yielding Pi and a series of lower phosphoric esters (International Union of Biochemistry, 1979; Hegeman and Grabau, 2001; Angel et al., 2002). Phytase activity varies greatly among species of plants. Some vegetable raw materials, mainly seeds from grains and their by-products, contain significant quantities of natural (endogenous) phytase in the aleurone and scutellum layer of the grain (Oatwayet al., 2001; Humer et al.,2014). The discovery of that as an exogenous source of phytase can cause phytate P degradation which was first reported in 1962 (Wodzinski and Ullah, 1996), but exogenous phytases were not commercially available until 1991 (Selle and Ravindran, 2008). Nelson et al., (1968) demonstrated that the addition of the enzyme phytase to grains and feeds was an effective way to increase phosphorus (P) availability to poultry. Currently used commercial phytases are derived from micro-organisms such as bacteria, yeasts and fungi. Of these, phytases derived from *Aspergillus niger*, which is a 3 phytase, and *Peniophora lycii* and *Escherichia coli*, which are 6-phytases are widely used in the poultry industry (Selle and Ravindran, 2007). Inclusion of microbial phytase in poultry diets has been found to improve utilization of phytate bound P, performance and skeletal strength in broilers (Mroz et al., 1994; Kornegay et al, 1996; Rao et al, 1999; Ravindran et al, 1999; Selle and Ravindran, 2007).

Addition of exogenous phytases to poultry diets has been shown to increase phyate-P retention, thereby reducing the need for expensive inorganic-P sources, as well as

improving the availability of other minerals, energy, and protein. However, the efficacy of both endogenous and exogenous phytase in the small intestine of broilers is reduced by high concentrations of dietary Ca typically used in poultry diets (Wilkinson et al., 2011). The antinutritive effects of phytate for poultry have been well established (Cowieson et al., 2011) as have the additive and interactive effects of high dietary Ca concentrations on phytate hydrolysis and apparent nutrient digestibility (Tamim et al., 2004; Selle et al., 2009). Despite Ca having a relatively lower affinity for phytate than other minerals, due to the high concentrations of Ca used in poultry diets, Ca plays the most defining role in the formation of phytate-mineral complexes (Angel et al., 2002). High concentrations of Ca in poultry diets have been shown to increase the pH of the gizzard contents (Guinotte et al., 1995) and digesta of birds (Shafey, 1999). Therefore, regulating the dietary Ca by reducing its levels in broiler diets may increase nutrient digestibility of poultry diets by decreasing the intestinal pH as well as the formation of Ca-phosphate precipitates and improving pepsin efficacy (Selle et al., 2009; Walk et al., 2012).

## Other Factors Affecting P Nutrition

## Age and Genotype

Older birds efficiently utilize phytate P since more endogenous phytase activity is found in the gastrointestinal tract. Phytate P retention in 16 to 21 day and 42 to 46 day old broilers were 6.8 and 17.3% respectively (Matyka et al., 1990). However, no difference in phytate P retention (0 vs. 3%) was observed by Nelson (1976) in 4 and 9-week-old broilers. Edwards (1983) stated that layer type birds utilize phytate P more

efficiently than meat type birds. Maddaiah et al. (1964), however, observed that the intestinal phytase activity in layers was lower than those in chicks and rats. Similarly, no differences were found in the specific activity of brush-border phytase of broilers and layers in a study conducted by Maenz and Classen (1998). Differences in phytate P utilization have also been observed among different strains of broilers (Edwards, 1983). In contrast, no strain effect was noted for phytate P hydrolysis between Ross 308 and Hubbard x Peterson broilers. The apparent ileal phytate P hydrolysis in 22-day old Ross x Ross broilers and Hubbard x Peterson broilers were found to be 22.0 and 24.1% of the total phytate P at the dietary Ca concentration of 9 g/kg (Applegate et al., 2003).

## Vitamin $D_3$ and metabolites

Numerous studies have demonstrated that vitamin $D_3$ and its metabolites (1,25-dihydroxycholecalciferol and 1α-hydroxycholecalciferol) improved P utilization in broilers (Mohammed et al., 1991; Edwards, 1993; Biehl et al., 1995; Mitchell and Edwards, 1996; Biehl and Baker, 1997; Qian et al., 1997; Applegate et al., 2003; Snow et al., 2004; Rama Rao et al., 2007) and layers (Carlos and Edwards, 1998) fed low P diets. The observed improvement in P utilization may be due to increased (i) synthesis or activity of intestinal phytase and/or alkaline phosphatase (Davies et al., 1970, Biehl et al., 1995; Mitchell and Edwards, 1996); (ii) P absorption (Wasserman and Taylor, 1973); (iii) phytate P hydrolysis (Mohammed et al., 1991); (iv) resorption of P in the kidney (Veum, 2010) and/or (v) accumulation of P in bones (Veum, 2010). Vitamin $D_3$ has been shown to modify P absorption in the jejunum of chickens while enhancing Ca absorption in duodenum which was independent of P absorption (Hurwitz and Bar, 1972). The

enhancing effect of vitamin $D_3$ on P absorption may be the result of primary action of vitamin D and/or secondary to its effect on Ca absorption (Hurwitz and Bar, 1972). Low P and low Ca diets are found to have a stimulatory effect on 25-hydroxyvitamin D3-1α-hydroxylase in chickens (Baxter and DeLuca, 1976), which in turns triggers renal calcitrol (1,25-(OH)2$D_3$) production (Veum, 2010).

Cholecalciferol (vitamin $D_3$) is hydroxylated in the liver and converted into 25-hydroxycholecalceferol (25-(OH) $D_3$) which in turn is converted in the kidney to 1,25-(OH)2$D_3$ in the presence of 25-hydroxyvitamin $D_3$-1α-hydroxylase (Borle, 1974). Parathyroid hormone is found to have a stimulatory effect on the conversion of vitamin D3 to its active hormonal form of 1,25-(OH)2$D_3$ (Borle, 1974; Veum, 2010). Calcitrol regulates Ca absorption by regulating the synthesis of calbindin-D28k, a specialized Ca binding protein found in the intestine and kidneys of chickens, which is like calbindin-D9k found in mammals (Veum, 2010). Receptors for 1,25-(OH)2$D_3$ have been found on the basolateral membrane of chicken intestinal epithelium and the numbers are higher in young birds (Veum, 2010).

## Phosphorus availability

Phosphorus availability can be defined as the amount of P in a feed ingredient that is biologically available to be absorbed and metabolically utilized by the animal (Weremko et al., 1997) and is most often expressed as the relative bioavailability of P (Sands et al., 2003). The slope-ratio assay technique is generally used to assess the available P content in feed ingredients where a low-P diet is supplemented with graded concentrations of P from a reference source (e.g. monosodium phosphate,

monocalcium phosphate) and a test source and the responses (e.g. tibia ash, body weight gain, toe ash) are measured. The available P content of the test ingredient is then calculated by comparing the relative response obtained from the test ingredient with that of the reference material (Plumstead, 2007).

Over the years in various sections of the livestock and poultry industry different definitions of available P have been proposed and several approaches are in use to determine P availability (Shastsk and Rodehustscord, 2013). Different terms are used in nutritional studies to describe different forms of P and confusion exists among the terms currently used (Angel et al., 2002; Applegate and Angel, 2008). This pluralism in definition not only causes confusion in communication, but also makes it almost impossible to compare data that originate from various laboratories. Compiling comprehensive feeding tables, which are needed by the feed and poultry industries, has been hampered by this lack of harmonization leading to an overestimation of true P requirements for poultry resulting in excess P being excreted (WPSA, 2013). Despite the definition confusion, nutritionists have assumed that the NPP fraction of the diet is 100% available, while phytate P (PP) is not available due to insufficient endogenous phytase production in broilers (Nelson, 1976). However, evidence from the literature demonstrates that broilers have an ability to utilize a portion of PP depending on Ca concentrations and experimental feedstuffs (Tamim and Angel, 2003; Leytem et al., 2008; Plumstead et al., 2008).

Presently, total P refers to the 'total amount of P which is determined by atomic absorption spectrophotometry, a colorimetric method or inductively coupled plasma

spectroscopy, following digestion of the given sample'. This fraction includes all forms of P. While, available P (aP) in feedstuffs is generally referred to as NPP (NRC, 1994), which is defined as the portion of P in the feed ingredient that is not bound to the phytate molecule and can be absorbed and fully utilized by an animal. However, the definition of the term 'available P' is known to be affected by different response criteria used in different P evaluation systems (Shastak and Rodehutscord, 2013). These two terms (available P and nonphytate P) are used interchangeably, when expressing the P requirement of animals, although studies have clearly demonstrated that NPP is not totally available and phytate P (PP) is not totally unavailable to the animal (Angel et al., 2002; Coon et al., 2002). Retainable P, on the other hand, refers to the P that is retained in the body and this term is used in the Netherlands as a measure of P availability in feed ingredients. However, increasing dietary concentrations of NPP results in increased levels of plasma inorganic P, and once the physiological threshold is reached, the excess P is eliminated via the urine.

The major difference between available P and non-phytate P is that the term available P includes all absorbed forms of P, both inorganic P (Pi) and organic P (including phytate P) forms. On the other hand, non-phytate P excludes any form of phytate P available to the animal (Angel et al., 2002). Available P is another general criterion used to express the amount of P in feed ingredients that is available to animals and to express P requirements (Plumstead, 2007).

## Phosphorus digestibility and protocols for its determination

The history of traditional biological value assays for P goes back to 1945 and assay procedures have gradually progressed to date. The objective of majority of these studies (Bird et al., 1945; Gillis et al., 1954; Motzok et al., 1956; Dilworth and Day, 1964; Day et al., 1973; Huyghebaert et al., 1980; Potter, 1988; Potter et al., 1995) was to evaluate bioavailability of feed phosphates to poultry. In these assays, the biological value of a test P source is determined by 'slope ratio assay' where chicken are fed a P-deficient basal diet and test diets with different concentrations of the test phosphate during a 2 to 3 week experimental period and the response criteria are compared with those fed a standard phosphate source assumed to be having 100% available (Coon et al., 2002). Multiple response criteria have been used by some authors when computing the relative biological value (Sullivan, 1966) and biological index (Soares et al., 1978). As described by Nelson and Peeler (1961), validation of the selected qualitative measurements of P availability can be achieved by (i) feeding P-deficient diet to the animal, (ii) maintaining the concentration of added P below the animal's requirement, and (iii) using an appropriate standard source to compare with the inorganic P source tested. The most widely used P bioavailability assay was developed by Gillis et al. (1954) by using reagent grade beta-tricalcium phosphate and percent bone ash as the response criteria.

Phosphorus availability data have been reported to vary among common feedstuffs (Shastak and Rodehutscord, 2013) and are likely a result, in part, of differences in experimental methodologies among laboratories (Rodehutscord, 2009).

Historically, in the 1980s and ‘90s, the bioavailability of P was routinely estimated by non-linear bioassays (asymptotic or sigmoidal curves) of body weight and tibia bone or toe ash, using a graded range of P supplements [Potchanacom and Potter, 1987; Potter, 1988; Potter et al., 1995). The values do not define the quantity of bioavailable P and therefore have limited value when formulating diets (Payne, 2005). In an attempt to overcome the difficulties associated with current approaches to P bioavailability determinations, the Nutrition Working Group of the European Federation of Branches of World’s Poultry Science Association (hereafter Rodehutscord and WPSA, 2013) has developed a protocol to determine ileal P digestibility by regression analysis. Two to three diets are formulated for each test P source. A low P basal diet is used, and a minimum of two levels of the P source under test are added to the test diet. At the highest level of inclusion, P supply must not exceed the requirement. With such conditions, the aP of the P source under test can be determined from the slopes of linear regression equations.

However, researchers have reported variability in data for apparent ileal P digestibility (AIPD) of titration diets resulting in regression equations that predicted negative endogenous P losses (EPL) and inconsistent true ileal P digestibility (TIPD) values (Dilger and Adeola, 2006; Iyayi et al., 2013; Liuetal., 2013, 2014; Mutucumarana et al., 2015). Negative EPL are not only physiologically impossible, but also lead to lower estimates of TIPD compared with estimates of TIPD from regression equations that did not predict negative EPL (Mutucumarana et al., 2015). Values for TIPD should, in-fact, always be higher than apparent digestibility values (Fan et al., 2001). Negative EPL were

predicted when titration diet AIPD decreased linearly when the test P source was added to the experimental diets (Liu et al., 2013; Mutucumarana et al., 2014c, d, 2015). However, linear increases in AIPD should occur as the test P source is titrated into the experimental diets, as EPL contribute proportionally less to digesta P output (Fan et al., 2001).

Improved knowledge of P digestibility in feed ingredients will enable the formulation of diets closer to the requirement, improve P utilization and minimize the excretion of P into the environment. A large volume of data on apparent or true digestibility values of P in feed ingredients for pigs are now available (Fan et al., 2001; Shen et al., 2002; Bohlke et al., 2005; Petersen and Stein, 2006; Fang et al., 2007b; Pedersen et al., 2007; Yang et al., 2007; Stein et al., 2008; Akinmusire and Adeola, 2009). Corresponding data for poultry are limited with no precise methodology established to measure digestibility of P in feed ingredients. Although the term 'digestibility' is frequently used synonymously with the term 'availability', these are two distinct terms. To be available, the nutrient must be in a form that can be digested, absorbed and utilized by the animal. Therefore, digestibility does not itself confirm 100% availability of that nutrient to the animal.

## Ileal digestibility in Poultry

In poultry, total tract measurements may yield misleading data if dietary NPP concentrations are above the physiological threshold, and the measurements should therefore be made at the ileal level. The ileum is defined as that portion of the small intestine extending from the vitelline diverticulum to a point 40 mm proximal to the

ileo-caecal junction. Despite the growing consensus that the modifying effects of hindgut microflora should be accounted for in digestibility estimates for poultry and that the ileal digesta approach may be more sensitive than excreta analysis (Ravindran and Bryden, 1999). The determination of ileal digestibility values has become the preferred method for estimating minerals and amino acid availability more specifically for identifying differences in digestibility among dietary ingredients.

On the basis of apparent metabolizable energy assessments, phytase has been shown to consistently enhance energy utilization in poultry (Selle et al., 2007). Hurwitz et al. (1978) measured P ileal digestibility in young turkeys at the lower half of the ileum with the aid of yttrium-91 as a reference substance. The phosphate level in the diets was varied by supplementation with monocalcium phosphate. Grimbergen et al. (1985) and Kornegay et al. (1996) assessed the prececal digestibility of P in various inorganic feed phosphates in broilers. Prececal digestibility is an established criterion for measuring protein quality in poultry. It is preferred because the values are unaffected by post-ileal microbial activity. It also implies that any urinary excretion is excluded. Prececal digestibility can be developed as an alternative to measure P availability, as it has the advantage of being less sensitive to the P level in the diet compared to other approaches (Rodehutscord et al., 2012). This can be an advantage in studies on P availability because urine is the major pathway of regulatory excretion when intake is above requirement, but P excretion with urine is negligibly low below the P requirement. Moreover, the response in P prececal digestibility to increments in dietary P concentration is linear over a wider range of dietary P than the response in P retention

(Rodehutscord et al., 2012), but both approaches provide similar results in evaluation of mineral P sources in poultry (Shastak et al., 2012a).

The effect of urinary P and possible hindgut modification can be overcome by determining the digestibility at the ileal level. The indicator method is used in ileal digestibility studies. An indigestible marker which does not alter nutrient digestibility and which has a high recovery rate of almost 100% is added to the test diet. Titanium dioxide, chromic oxide and acid insoluble ash are the widely used dietary markers in P digestibility studies. The ratio of P and marker in the test diet and the ratio of P and marker ileal digesta are used to calculate the apparent P digestibility as shown below.

$$\text{Apparent P Digestibility (\%)} = \frac{[\,(P/I)\,d - (P/I)\,i\,]}{(P/I)\,d} \times 100$$

Where, (P/I) d = ratio of P and indicator in the diet, and

(P/I) i = ratio of P and indicator in the ileal digesta

Studies investigating P absorption in the gastrointestinal tract of poultry in early 1970's were performed using radio-labelled yttrium (91Y) as a non-absorbed reference material (Hurwitz and Bar, 1970; 1971; Hurwitz et al., 1979). Some studies have evaluated the ileal digestibility of inorganic phosphate sources for broilers (Ketels and De Groote, 1988; Shastak et al., 2012) and young turkeys (Grimbergen et al., 1985; Kornegay et al., 1996).

Ileal digestibility assays are preferred over P retention assays as the values determined are unaffected by hindgut microbial activity and the exclusion of urinary P contribution (Shastak and Rodehutscord, 2013).

## Methods to measure digestibility

The question remains, what is the appropriate response criterion to measure feedstuff P availability. Various approaches are in use by different laboratories for the determination of P availability (Rodehutscord, 2009., 2017). Therefore, the use of an appropriate criterion, preferably based on P digestibility, to assess P availability is needed to enable greater efficiency of utilization of dietary P across the industry. No refined established methodology is currently available to measure the TPD contents in common feed ingredients for poultry.

Several types of both qualitative and quantitative protocols have been utilized to generate P availability values for common feed ingredients, including relative bioavailability (RBA), apparent ileal P digestibility (AIPD), standardized ileal P digestibility, apparent P retention (APR) and standardized P retention (Shastak and Rodehutscord, 2013). Data determined by these methods tend to be widely variable (Rutherfurd et al., 2002; Dilger et al., 2004; Rutherfurd et al., 2004; Dilger and Adeola, 2006a; Selle et al., 2009) see table 2.5.1.1. To have a more efficient P supply to poultry diets and to reduce dietary costs it would be required to transition from formulating diets to meet the NPP requirements and instead, formulating diets using True P utilization (TPU) data. The TPU would be a representative of P availability because both NPP and PP contribute to the value (Fan et al., 2001). Also, TPU values are preferable over apparent as they should not be affected by dietary P content (Ajakaiye et al., 2003).

In pig digestibility studies, three approaches, namely regression analysis (Fan et al., 2001; Shen et al., 2002; Yang et al., 2007; Akinmusire and Adeola, 2009), direct method

(Bohlke et al., 2005; Petersen and Stein, 2006; Almeida and Stein, 2010) and difference or substitution method (Fang et al., 2007b) have been used to estimate P digestibility in feed ingredients. Only limited studies have been conducted to date to measure the digestible P content in feed ingredients for poultry. Dilger and Adeola (2006b) estimated the true ileal P digestibility of soybean meal for broilers using the regression analysis technique where soybean meal was used as the sole dietary source of Ca and P. Iyayi et al. (2013) and Liu et al. (2013) conducted studies to estimate the true P digestibility of black-eyed pea and peanut flour without or with phytase supplementation and to assess the effect of different Ca:P ratios in estimation of true P digestibility of soybean meal, respectively. Wu et al. (2004), using the direct method, determined the apparent ileal digestibility of P in sorghum, wheat, maize and barley.

Only limited attempts have been made to determine the digestible P content in feed ingredients for poultry and two approaches, namely the direct method (Wu et al., 2004; Leytem et al., 2008) and regression method (Dilger and Adeola, 2006), have been used. These experiments were performed using the test ingredient as the sole dietary source of P and Ca.

## Direct method

The direct method consists of a diet in which there is only one P-containing ingredient (the test ingredient); all other ingredients in the diet contain no P. The digestibility of P in the diet equals the digestibility of the P in the test feed ingredient. For example, if the test ingredient is corn it serves as the sole source of P in the test diet. Corn was selected as an experimental P source due to its importance as a common

primary feedstuff in the poultry industry. Calculation of the apparent ileal digestibility coefficient (AIDC) of P in the diet is assumed to represent the P digestibility of the test ingredient. However, as described for amino acids and proteins (Ravindran and Bryden, 1999; Lemme et al., 2004), when the direct method is used to determine the P digestibility in low-P ingredients (e.g. cereals), the AIDC of P can be underestimated due to the relatively greater proportion of endogenous P in the digesta or excreta. For example, apparent ileal P digestibility coefficient of sorghum for broilers determined by Wu et al. (2004) using the direct method was 0.36. Therefore, small differences in the dietary P content of low-P ingredients will result in large changes in P digestibility values between lower and upper limits. The digestibility coefficient of P calculated by the direct method is 'apparent' and this limitation can be overcome by correcting the estimate for endogenous P losses.

## Difference or Substitution (Indirect) method

In the difference method, the basal diet with only one common source of P is mixed in a diet with a second source of P (the test ingredient) at different inclusion levels and P digestibility is determined. The mixture is fed and P-digestibility is determined for this diet. By subtracting the amount of digestible P provided by the basal diet from the total amount of digestible P in the mixture, the digestible amount of P originating from the test feed ingredient can be calculated. By expressing this as a percentage of total P in the test feed, the P digestibility for this source is calculated. However, effects of P digestibility and levels in basal diets on the determination of true ileal digestibility of P in SBM for broiler chickens were not consistent between the studies of Liu et al.

(2014a) and Mutucumarana et al. (2015). The AIDC of P in test ingredients is evaluated by using two diets (a reference diet and a test diet). The reference diet may consist of two or more common feed ingredients (e.g. maize-soybean meal), whereas the test diet consists of a mixture (e.g. 70:30) of predetermined ratios of the reference and the test feed ingredient. The digestibility of P in the test ingredient is determined by using the equation described by Zhou et al. (2004). This method assumes that there is no interaction between the reference diet and the test ingredient, and that the AIDC values are additive (Lemme et al., 2004). A modified method for substitution method has been suggested for P digestibility measurements by Fang et al. (2007b), where a series of test diets were formulated from the test ingredient to contain graded concentrations of P and the true digestibility of P at adjacent dietary P concentrations are calculated, one at a time, and averaged.

## Regression technique

The WPSA, (2013) guidelines clearly states that precaecal digestibility of P of a certain feed should be tested using regression analysis. This implies that a low-P basal diet is used, and a minimum of two levels of the P source under test is supplemented in the test feeds. At the highest level of inclusion, P supply must not exceed the requirement. Such conditions allow for the determination of pcdP of the P source under test from the slopes of linear regressions. This implies that correction for basal endogenous P losses is not necessary. The pcdP (expressed in g/kg of diet) is plotted against tP concentration (g/kg of diet) in a linear regression analysis. The slope of the

regression line, multiplied by 100, gives the percentage prececal digestibility of P from the supplemented source (WPSA, 2013).

In the regression method, a series of semi-purified diets are formulated from the test ingredient to contain graded concentrations of P where the test ingredient is used as the only dietary source for P. The total output of P per dry matter intake (PO-DMI) in the ileal digesta/excreta (g/kg DMI) is plotted against to dietary P contents (PI) on a dry matter (DM) basis. True P indigestibility and endogenous P losses are the slope and intercept, respectively, of the linear regression of PO-DMI on PI. True P indigestibility is an indirect measure of the inefficiency at which dietary P is extracted. True P digestibility is calculated by subtracting percentage true P indigestibility by 100 (Dilger and Adeola, 2006b). Although endogenous P loss and true P digestibility coefficient of the particular feed ingredients are simultaneously determined, the technical complexity of the regression method is a drawback for not gaining wider acceptance in nutritional research. Some regression studies with broilers have reported negative endogenous P losses, resulting in true P digestibility estimates being lower than its corresponding apparent P digestibility coefficients (Iyayi et al., 2013; Liu et al., 2013).

The regression method has been consistently corroborated as a reliable technique to derive the true P digestibility in pigs (Al-Masri and Günther, 1988; Al-Masri, 1995; Fan et al., 2001; Shen et al. 2002; Dilger and Adeola, 2006; Petersen and Stein, 2006; Akinmusire and Adeola, 2009) but is still being researched in poultry (Dilger and Adeola, 2006; Liu et al., 2013; Mutucumarana et al., 2014a, b). In pigs, the combination of the regression method with total collection of feces has proven to be robust (Poulsen,

2007). Bioavailability studies with poultry, yielded a range of relative P bioavailability coefficients from 0.54 to 1.02 (Martinez Amezcua et al., 2004; Lumpkins and Batal, 2005; Amezcua and Parsons, 2007). In these studies, P bioavailability was estimated using $KH_2PO_4$ as the standard. Liu et al. (2013) determined the P digestibility of soybean meal for broilers at different dietary Ca:P ratios (0.8, 1.2, 1.6, and 2.0) and found that apparent ileal P digestibility ranged from 0.64 to 0.90. Apparent total tract digestibility coefficient of P for corn DDGS in pigs has been reported to range from 0.501 (Pedersen et al., 2007) to 0.686 (Almeida and Stein, 2010).

## Apparent and True P digestibility

The term apparent digestible (or absorbed) P is defined as the portion of dietary P intake that is digested and absorbed at the terminal ileal level. The resulting values are corrected for the endogenous P losses to calculate true digestible P values. All of the P present in the digesta or excreta does not only originate from the diet. Some are of endogenous origin such as P derived from salivary, gastric and pancreatic juices, biliary secretions and sloughed mucosal cells (Fan et al., 2001). The digestibility determined therefore is 'apparent' and does not account for endogenous P losses. Fan et al. (2001) identified three major issues relating to the use of apparent P digestibility values in feed formulations. First, reported apparent P digestibility values are highly variable within the same feed ingredient. Second, apparent P digestibility values underestimate the true P utilization and, third, apparent P digestibility values meaured in single ingredients are not always additive when used in diet formulations.

Cowieson et al. (2004) reported that phytate increased the excretion of endogenous Na in broilers, and Woyengo et al. (2009; 2010) subsequently reported that phytic acid increases Na secretion in the jejunum and reduces the AID of Na in piglets. Inconsistent data has been reported with regards to AIPD of titration diets following the WPSA (2013) guidelines (Dilger and Adeola, 2006; Liu et al., 2013; Mutucumarana et al., 2014a, b, 2015a). This is probably due to differences in experimental conditions, especially Ca feeding strategy (Ca concentration and Ca:P ratio), days of adaptation, basal diet protein source, and the evaluated feedstuff.

## Endogenous P Loss (EPL)

Endogenous means originating within the body hence, the basal endogenous loss of P represents the quantities of P that will be lost from the animal regardless of the diet that is being fed. These animal induced P losses are not influenced by the feed ingredient composition per se, but they are strongly influenced by the total dry matter intake (DMI) of the animal (Boisen and Fernández, 1995; Hess and Sève, 1999; Moter and Stein, 2004) and, for this reason, they are expressed in relation to DMI. They may also be affected by the animal's physiological state or by experimental conditions. The EPL may be measured using the regression technique. This technique like others has its advantages and limitations and yield variable estimates compared to other techniques. In pigs, Fan et al. (1999) have successfully determined endogenous P output and true amino acid digestibility by using a regression analysis technique. Fan et al. (2001) and Shen et al. (2002) used this method to determine endogenous P output and true P digestibility (TPD). Zhang (2004) used this method to determine true P and Ca

digestibility. These experiments proved the feasibility of this method which is cheaper, safer and more convenient. Endogenous P determined by Wu et al. (2008) was 1.14 g/kg DMI which is similar to the experimental result (1.08 g/kg DMI) of Zhang (2004) and higher than that of Shen (2002). The endogenous percentage in total P and TDP requirement was 17 and 30.54%, respectively, in the experiment by Wu et al. (2008), compared to 18 and 39.13% reported by Zhang (2004). Fan et al. (2001) reported that the endogenous P output of pigs (body weights were 5 to 20 kg) was 0.31 g/kg DMI, which was 5.8 to 12.8% of the total P requirement and 9.5 to 24.1% of TDP requirement. Later, Shen et al. (2002) determined the value in growing pigs (BW 20 to 45 kg) was 0.67 g/kg DMI which was 12.3% of total P requirement and 26.6% of TDP requirement. There are gender differences and at different periods even in the same gender and period, because endogenous P output can be influenced by factors such as the ratio of Ca to P, P levels and feed management (Wu et al., 2008).

There is a continuous secretion of endogenous P and Ca into the lumen of the intestinal tract of poultry. This endogenous P mixes with dietary P, and are partially digested and absorbed. The unabsorbed fraction left beyond the lower ileum is considered as a loss to the animal. Measurement of these endogenous losses is a primary requirement for the estimation of true digestibility of P. Endogenous P losses have been quantified in pigs (Petersen and Stein, 2006; Pettey et al., 2006; Almeida and Stein, 2010). However, published data on endogenous losses of P in poultry are scant and the values can be affected by assay methodology, animal factors and dietary Ca and non-phytate P concentrations (Al-Masri, 1995; Rodehutscord, 2009). In pigs, regression

analysis and P-free diets have been used to determine endogenous P losses (Pettey et al., 2006; Almeida and Stein, 2010). There appears to be no differences between fecal and ileal endogenous P losses in pigs (Ajakaiye et al., 2003).

Measurement of excreta endogenous P losses, rather than ileal losses, in poultry will result in erroneous results due to the contribution of endogenous urinary P. Minimal P diets (Rutherfurd et al., 2002; 2004), regression analysis (Dilger and Adeola, 2006b) and radioisotope-dilution techniques (Al-Masri, 1995) have been thus far used to estimate endogenous P losses in poultry. The estimates generated for ileal endogenous P are highly variable, ranging from 145 to 446 mg/kg DMI (Rutherfurd et al., 2004; Dilger and Adeola, 2006b), with some negative estimates, ranging from -290 to -864 mg/kg DMI (Iyayi et al., 2013; Liu et al., 2013). Bile is the primary source of endogenous P in poultry. About 90% of the mammalian bile lipids are composed of phospholipids (Cross et al., 1987). Bile phospholipids are found both as vesicles and as mixed micelles conjugated with bile salts (Sklan and Budowski, 1978; Coleman, 1987).

## Excreta digestibility in poultry

Determination of excreta (or total tract) digestibility was the most common method used in nutrient digestibility research in the past. For P, however, excreta digestibility measurements in poultry have two main drawbacks. First, the excreta of poultry contain P from both feces and urine. Therefore, calculations based on poultry excreta are reflective of P retention and it is not accurate to refer to this measurement as digestibility. Second, measuring digestibility at the excreta level includes the possible utilization of P by hindgut microbes (Marounek et al., 2010). However, studies by Vasan

et al. (2008), using intact and caecectomised birds, have shown that the ceca of birds have no effect on P utilization. The effect of renal excretion of P can be overcome by using colostomised birds (Manangi et al., 2007). While in pigs, P digestibility in feed ingredients is usually determined over the total tract (Petersen and Stein, 2006; Fang et al., 2007b; Pedersen et al., 2007; Yang et al., 2007; Stein et al., 2008; Akinmusire and Adeola, 2009) and this approach is workable because fecal samples can be collected without urine contamination and is specifically easier if animals are males.

Retained P refers to the P that is retained in the body and is calculated by subtracting excreta P output from dietary P intake. This retainable P system has been described in detail by van der Klis and Versteegh (1996), and is widely used in the Netherlands where it is popularized as the 'opneembare phosphor system'. Dietary retainable P values, however, are valid only at low dietary P concentrations. For broilers, this threshold is reported to be between 2 to 3 g/kg non-phytate P (Manangi and Coon, 2006). Total tract digestibility or retention of P can be calculated using the following formula.

$$\text{Phosphorous Retention (\%)} = \frac{\text{(Total P Ingested) - (Total P excreted)}}{\text{Total P ingested}} \times 100$$

The digestibility/retention value thus obtained is 'apparent' and must be corrected for endogenous P losses to obtain the 'true' value. Due to this reason, calculations based on poultry excreta thus reflect retainable P values, rather than digestible P, of a particular feed ingredient (van der Klis and Versteegh, 1996).

**Table 2.1.** Calcium and Phosphorus content in the chicken whole body and bones (g/kg).

| | Ca | P |
|---|---|---|
| **Whole body (g/kg)** | | |
| [1]Hatching | 3.4 | 3.3 |
| [1]7 weeks | 6.8 | 5.1 |
| **Tibia content (g/kg DM)** | | |
| [2]Day 35, Male | 168 | 80 |
| [2]Day 35, Female | 165 | 78 |

[1]Larbier and Leclerq, 1992

[2]Venäläinen et al., 2006.

**Table 2.2.1.** Total and phytate P concentrations (g/kg) in common feed ingredients of plant origin

| | **Total P (g/kg)** | **Phytate P (g/kg)** | **Proportion of phytate P to total P (%)** |
|---|---|---|---|
| **Cereals** | | | |
| Barley | 3.21(2.73-3.70) [a] | 1.96 (1.86-2.20) [a] | 61.0 (59-68) [a] |
| Maize | 2.62 (2.30-2.90) | 1.88 (1.70-2.20) | 71.6 (66-85) |
| Sorghum | 3.01 (2.60-3.09) | 2.18 (1.70-2.46) | 72.6 (65-83) |
| Wheat | 3.07 (2.90-4.09) | 2.19 (1.80-2.89) | 71.6 (55-79 |
| **Cereal by-products** | | | |
| Rice bran | 17.82 (13.40-27.19) | 14.17 (7.90-24.20) | 79.5 (42-90) |
| Wheat bran | 10.96 (8.02-13.71) | 8.36 (7.00-9.60) | 76.3 (50-87 |
| **Oilseed meals** | | | |
| Canola meal | 9.72 (8.79-11.50) | 6.45 (4.00-7.78) | 66.4 (36-76) |
| Cottonseed meal | 10.02 (6.40-11.36) | 7.72 (4.9-9.11) | 77.1 (70-80) |
| Soybean meal | 6.49 (5.70-6.94) | 3.88 (3.54-4.53) | 59.9 (53-68) |

Adopted from: Selle and Ravindran (2007)

[a] Range of values

**Table 2.2.1.2**. Total P (TP) (g/kg), retainable P (RP) (% of TP), non-phytate P (NPP) (g/kg), phytate P (PP) (g/kg), PP (% of TP) and calculated PP degradability (%) of some of the feedstuffs in three-week-old broilers

| Feedstuffs | TP (g/kg) | RP (% TP) * | NPP (g/kg) | PP (g/kg) | PP (% TP) | PP Degradability (%) ** |
|---|---|---|---|---|---|---|
| Beans | 4.9 | 52 | 1.3 | 3.6 | 73.5 | 53 |
| Lupin | 3 | 72 | 1.5 | 1.5 | 50 | 80 |
| Maize | 3 | 29 | 0.7 | 2.3 | 76.7 | 16 |
| Peas | 4.1 | 41 | 1.5 | 2.6 | 63.4 | 23 |
| Rapeseed | 10.9 | 33 | 3.8 | 7.1 | 65.1 | 10 |
| Rice Bran | 17.2 | 16 | 3.1 | 14.1 | 82 | 2 |
| Soy bean (Heat treated) | 5.5 | 54 | 2 | 3.5 | 63.6 | 49 |
| SBM | 7.1 | 61 | 2.8 | 4.3 | 60.6 | 61 |
| Sunflower meal | 11.9 | 38 | 4.2 | 7.7 | 64.7 | 19 |
| Wheat | 3.4 | 48 | 0.9 | 2.5 | 73.5 | 46 |
| Wheat Middlings | 10.8 | 36 | 2.8 | 8 | 74.1 | 26 |

Source: Van der Klis and Versteegh, (1999).

* Values were determined in broilers fed diets containing 1.8 g calculated retainable P and 5.0 g Ca per kg diet from 10 days of age onward. P retention was measured in a three-day balance period (from days 21 to 24) in which dietary P intake and excreta P output were measured quantitatively;

** Phytate P degradability was calculated as: 100 ˆ (AP/0.8–NPP)/PP.

**Table 2.2.1.1.** The effect of phytase supplementation on ileal phytate P digestibility in broiler chickens fed corn/SBM-based diets.

| Phytate P content (%) | Phytase (FTU/kg) | Phytate P released (%) | | Difference (%) | Reference |
|---|---|---|---|---|---|
| | | Control | Phytase | | |
| 0.31 | 500 | 0.068 | 0.149 | 0.081 | Camden et al., 2001 |
| 0.28 | 500 | 0.070 | 0.126 | 0.056 | Tamim et al., 2004 |
| 0.30 | 500 | 0.030 | 0.062 | 0.032 | Rutherfund et al., 2004 |
| 0.26 | 1000 | 0.076 | 0.130 | 0.054 | Olukosi et al., 2007 |
| 0.26 | 1000 | 0.008 | 0.057 | 0.049 | Laytem et al., 2008 |
| 0.31 | 600 | 0.077 | 0.109 | 0.032 | Woyengo et al., 2010 |
| 0.29 | | 0.05 | 0.106 | 0.051 | |

**Table 2.2.2.** Phosphorus concentrations (g/kg) in common inorganic feed phosphate sources

| **Feed Phosphate type** | **Total P (g/kg)[1]** |
|---|---|
| Phosphate (defluorinated) | 180 |
| Calcium phosphate (dibasic form) | 187 |
| Calcium phosphate (mono-dibasic) | 210 |
| Sodium phosphate (dibasic form) | 208 |
| Sodium phosphate (monobasic form) | 218 |
| Phosphate (rock curacao, ground) | 140 |

[1]Total P = Non-phytate P.

Source: NRC (1994).

**Table 2.3.** Calcium and P Requirements of Broilers.

| | **FEDNA (2008)** | | **NRC (1994)** | | **INRA (1989)** | |
|---|---|---|---|---|---|---|
| **Age (d)** | **1-15** | **16-37** | **1-21** | **22-42** | **1-15** | **16-35** |
| **Calcium (%)** | 0.95-1.05 | 0.90-1.0 | 1.0 | 0.90 | 1.0-1.1 | 0.90.1.0 |
| **Phosphorous** | | | | | | |
| Total P (%) | 0.65 | 0.60 | - | - | 0.67-0.70 | 0.66-0.69 |
| Available P (%) | 0.45 | 0.43 | - | - | 0.42-0.45 | 0.41-0.44 |
| Non phytic P (%) | - | | 0.45 | 0.35 | - | |
| Ca: NPP | - | - | 2.2 | 2.57 | - | |
| Ca: aP | 2.11-2.33 | 2.09-2.32 | - | | 2.3-2.4:1 | 2.19-2.27 |

**Table 2.3.2.2.** Inorganic feed phosphates and their respective total P (TP) (g/kg) and retainable P (RP) (% of TP)

| **Inorganic P Source** | **TP (g/kg)** | **RP (% TP)*** | **Reference** |
|---|---|---|---|
| Calcium sodium phosphate | 180 | 59 | *Van der Klis and Versteegh, 1999 |
| Dicalcium phosphate | 183 | 83 | Coon, et al., 2007 [1,2] |
| Dicalcium phosphate (anhydrous) | 197 | 55 | * Van der Klis and Versteegh, 1999 |
| Dicalcium phosphate (hydrous) | 181 | 77 | * Van der Klis and Versteegh, 1999 |
| Monocalcium phosphate | 226 | 84 | * Van der Klis and Versteegh, 1999 |
| Monodicalcium phosphate (hydrous) | 213 | 79 | * Van der Klis and Versteegh, 1999 |
| Monodicalcium phosphate | 203 | 77 | Leske and Coon, 2002[1] |
| Monodicalcium phosphate | 200 | 80 | Leske and Coon, 2002[1] |
| Monodicalcium phosphate | 216 | 81 | Leske and Coon, 2002[1] |
| Monosodium phosphate | 224 | 92 | * Van der Klis and Versteegh, 1999 |
| Defluorinated phosphate | 182 | 86 | Coon, et al., 2007 [1,2] |
| Defluorinated phosphate | 179 | 76 | Coon, et al., 2007 [1,2] |

* Values were determined in broilers fed diets containing 1.8 g calculated retainable P and 5.0 g Ca per kg diet from 10 days of age onward. P retention was measured in a three-day balance period (from days 21 to 24) in which dietary P intake and excreta P output were measured quantitatively;

[1] Apparent utilization of P from inorganic sources by broiler as determined under deficiency conditions;

[1,2] Retainable P determined through broken line slope response.

**Table 2.3.2.4:** Phosphorus equivalency values for phytase in some poultry studies (MSP = monosodium phosphate, TCP = tricalcium (defluorinated) phosphate, MCP = mono-calcium phosphate, MDCP = mono-dicalcium phosphate.

| Reference | Species | Diet | Ip Source | Phytase | Responses | P-equivalency, g/kg |
|---|---|---|---|---|---|---|
| Adedokun et al., 2004 | Broilers | Corn-SBM | MSP | *E. coli* | % tibia and toe ash | 1000 FTU = 0.930 to 1.101g |
| Adeola, 2010 | Ducks | Corn-SBM | MSP | *E. coli* | Tibia ash | 500 FTU = 0.453g of iP |
| Jendza et al., 2006 | Broilers | Corn-SBM | MSP | *E. coli* | Weight gain, feed intake, bone ash | 500 FTU/kg = 0.72 g/kg (gain); 1.19 g/kg (ash) |
| Kornegay et al., 1996 | Broilers | Corn-SBM | TCP | *A. niger* | Bone ash, weight gain | 939 FTU = 1 g nPP |
| Onyango et al., 2005 | Broilers | Corn-SBM | MSP | *E. coli* | Bone ash | 500 and 700 FTU = 757 and 1116 mg iP |
| Van der Klis et al., 1997 | Layers | Corn-SBM | MCP | *A. niger* | Ileal P absorption | 250 FTU/kg = 1.3 g iP |
| Van der Klis et al., 1997 | Layers | Corn-SBM | MCP | *A. niger* | Ileal P absorption | 250 FTU/kg = 0.8 g iP |
| Wu et al., 2004 | Broilers | Wheat-SBM | MCP | *A. niger* | Weight gain | 500 FTU/kg = 0.54 % iP |

**Table 2.5.1.1** Variability in methods to determine P availability, data generated on feed ingredient TPU have been found to be highly variable in broilers.

| Publication | Feedstuff | AIPD (%) | APR (%) | TIPD (%) | TPR (%) | EPL (mg/kg) |
|---|---|---|---|---|---|---|
| Mutucumarana et al., 2014a | Corn | 61 to 70 | 45 to 68 | 68 | 63 | 20 to 77 |
| Mutucumarana et al., 2015a | Corn | 40 to 82 | - | 43 to 73 | - | -1,016 to 277 |
| Mutucumarana et al., 2014b | SBM | 14 to 63 | - | 80 | - | 418 |
| Mutucumarana et al., 2015a | SBM | 61 to 81 | - | 52 to 74 | - | -171 to -530 |
| Liu et al., 2013 | SBM | 64 to 89 | 74 to 91 | 46 to 71 | 53 to 58 | -448 to -864 |
| Dilger and Adeola 2006 | SBM | 71 to 88 | 33 to 54 | 94 | 60 | 191 to 209 |
| Mutucumarana et al., 2014a | Canola meal | 51 to 68 | 54 to 70 | 47 | 49 | -464 to -487 |
| Mutucumarana et al., 2014b | Wheat | 38 to 47 | - | 46 | - | 80 |
| Mutucumarana et al., 2014b | Sorghum | 23 to 52 | - | 33 | - | -87 |
| Mutucumarana et al., 2014b | Corn DDGS | 40 to 65 | - | 73 | - | 609 |
| Iyayi et al., 2013 | Black-eyed pea meal | 58 to 78 | 22 to 31 | 29 | 10 | -377 to -843 |
| Iyayi et al., 2013 | Peanut Flour | 76 to 81 | 23 to 43 | 67 | 74 | -290 to 1,104 |
| Adebiyi and Olukosi, 2015 | Wheat DDGS | 57 to 63 | 68 to 79 | 94 | 92 | -476 to -625 |
| Mutucumarana et al., 2015b | Meat and bone meal | 49 to 69 | - | 42 to 69 | - | -370 to 142 |

Source Perryman, 2015.

# CHAPTER III

# EFFECTS OF PHYTASE ON TRUE ILEAL PHOSPHOROUS AND CALCIUM DIGESTIBILITY OF CORN AND SBM INDIVIDUALLY OR IN COMBINATION IN MALE BROILERS


## Abstract

Limited information exists on true P digestibility in corn and SBM in broiler chicks, and how it is affected by microbial phytase. The objectives of the current study were to estimate true P digestibility (TPD) of corn, soybean meal (SBM) and a combination of corn and soybean meal (C-SBM) using the regression method approach, and also to determine the influence of phytase on the TPD values and to determine the additivity of TPD of corn and SBM. Broiler chicks (800 Ross 308 male) were raised on a commercial C-SBM-based starter diet from d 1 until d 15 and then switched to experimental diets until d 23 in accordance to WPSA recommendations. Birds were then fed the experimental diets for a minimum of 7 d before the contents of the posterior half of the ileum were collected. Birds were randomly assigned to each of the 18 treatments with eight replicate pens of five birds each per treatment. Dietary treatments consisted of three graded levels of corn, SBM or corn-SBM with and without 500 U/kg of phytase supplementation, making a total of 18 dietary treatments. Feed intake increased *($P < 0.05$)* in chicks fed various levels of corn and C-SBM with phytase. There was no difference *($P > 0.05$*) among birds fed SBM supplemented with phytase, but there was

an ingredient level effect *(P < 0.05).* Tibia ash was affected by phytase supplementation from an increase (48.62 to 51.70% in corn and C-SBM 49.60 to 52.28%) was seen with increased ingredients levels and phytase supplementation. There was also an increase *(P < 0.0001*) from 47.30 to 51.94% in Tibia Ash % with increasing levels of SBM with and without phytase. The Ileal P digestibility of Corn, SBM, and C-SBM diets were determined by linear regression and varied among increasing ingredient levels and phytase supplementation. TIPD estimate of SBM (52.0%) is more reasonable than the extremely low values obtain for corn (-7.2%) and C-SBM (5.6%). Corn and C-SBM contained inorganic P, whereas SBM diets contained no inorganic P. Phytase improved TIPD in corn (+38 points, to 60.4%; *P < 0.01)* and C-SBM (+14 points, to 19.5%; *P = 0.02*), however it didn't in SBM (+7 points, to 59.9%; *P = 0.55*). AIPD linearly decreased *(P < 0.08)* as the ingredient level increased in corn and SBM, and increased when phytase was supplemented. A significant ingredient level × phytase interaction was found for AIPD in corn-SBM *(P < 0.01).* Apparent Ileal P digestibility was reduced *(P < 0.0001*) significantly by increasing levels of Corn and C-SBM supplemented with phytase but differed *(P > 0.05)* in SBM supplemented with phytase. While there was also an ingredient level effect *(P < 0.05)* on AIPD in SBM diets. Ileal Ca digestibility was significantly reduced *(P < 0.0001*) in diets supplemented with phytase of increasing levels of C-SBM but there was no difference among levels of ingredients *(P > 0.05)* supplemented with phytase on corn and SBM diets. The apparent P retention was reduced *(P < 0.0001*) among all diet levels with and without phytase supplementation, where low level corn supplemented with phytase was significantly higher 83.48 % and

High level of corn without phytase was significantly the least with 61.79 % apparent P retention. In summary, TIPD estimates for SBM and for corn and C-SBM obtained by regression are smaller than AIPD and far from reality possibly due to the use of fixed Ca:P ratio and of inorganic P in corn and C-SBM. Phytase efficiently improved TIPD and AIPD in corn and C-SBM, and AIPD in SBM.

## Introduction

Phosphorus is a critical macronutrient for animals. Corn and SBM dominate the market as common feedstuffs for poultry because of their consistency in nutrient content, and the fact that they are readily available year-round. About two-thirds (60% to 80%) of the total P present in these feedstuffs occur as phytates (myo-inositol hexakisphosphate, InsP6), the salts of phytic acid (Jongbloed et al., 1993; Suttle, 2010). Phytate P (PP) in plants is a mixed calcium-magnesium-potassium salt of phytic acid that is present as a chelate with very low solubility (Pallauf and Rimbach, 1997). Phosphorus in this form is poorly digestible/available for simple-stomached animals (Van Der Klis and Versteegh, 1996), as they have insufficient intestinal enzymes to digest and utilize it effectively. Thus, high levels of inorganic P need to be supplemented in most commercial poultry diets. Phytate P and unused inorganic P is excreted in animal manure which is subsequently spread on farmland, and can contribute to eutrophication of surface waters, particularly in areas of intensive poultry production (Common, 1989; Walsh et al., 1994). The addition of exogenous phytase to diets allows for the breakdown of phytate, and as a result, P and other minerals become available to be absorbed along the small intestine. Nelson et al. (1968) were the first to demonstrate

that the addition of the enzyme phytase to grains and feeds was an effective way to increase P availability to poultry. To minimize the detrimental effect of phytate, phytases have been used by poultry nutritionists in diet formulations for broilers since 1991 (Bedford, 2003). Commercially available phytases are phosphatases that hydrolyzes the phosphate groups from the inositol ring of phytate (Tamim et al., 2004) increasing nutrient availability and digestibility, which results in improved bird performance (Cowieson et al., 2006).

Due to differences in PP concentrations in ingredients, the response of phytase supplementation on available P is expected to be different among different ingredients. Measurement of ileal digestible P has been suggested as the preferable approach to assess P availability in poultry in order to minimize P excretion (Adeola, 1999; Mutucumarana et al., 2014a, b; WPSA, 2013). Digestibility values of P in feed ingredients for pigs are usually determined over the total tract (Stein et al., 2008; Akinmusire and Adeola, 2009), because fecal samples can be collected without urine contamination. In poultry, however, total tract P digestibility measurements will yield misleading data especially if the dietary non-phytate P (NPP) concentrations are above the physiological threshold. Therefore, the digestibility measurements need to be made at the ileum. Additionally, ileal P digestibility measurements reduces errors encountered due to the modifying effects of hindgut microflora (Rodehutscord, 2009).

A number of published reports are available on apparent or true digestibility values of P in common feed ingredients for pigs (Dilger and Adeola, 2006a, Fang et al., 2007a, b; Pedersen et al., 2007; Akinmusire and Adeola, 2009). In these assays, three

approaches, namely regression analysis, a direct method and a substitution method have been used to estimate P digestibility. Corresponding data for poultry, however, are limited. Dilger and Adeola (2006b) estimated the true ileal P digestibility of SBM for broilers using the regression analysis technique where SBM was used as the only dietary source of calcium (Ca) and P. In that study, apparent ileal P digestibility increased (linear and quadratic) from 71 to 88% for conventional SBM, and from 75 to 89% for low phytate SBM. Wu et al. (2004), using the direct method, reported the apparent ileal P digestibility values for P in sorghum, wheat, maize and barley. In that study, apparent ileal digestibility coefficients for phytate P and total P ranged from 0.03 to 0.42 and 0.56 to 0.71, respectively. Diets supplemented with phytase had greater phytate P hydrolysis than unsupplemented diets.

Limited information exists on true P digestibility in corn and SBM, and how it is affected by microbial phytase. Moreover, information on the additivity of ileal P digestibility values from individual ingredients is not available for broilers. Liu et al. (2013) attempted to determine the additivity of digestible P from corn and SBM using P retention but their results were inconclusive. True total-tract P digestibility (TPD) in corn and SBM have been reported to be additive in a corn-soybean meal diet for pigs (Zhai and Adeola, 2013). The objectives of the current study were: 1) to estimate TPD of corn, SBM and a combination of corn and SBM for broilers using the regression method; 2) to determine the influence of phytase on the TPD values; and 3) to determine if TPD in corn and SBM was additive when the two ingredients are fed in combination.

## Materials and methods

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee (ACUC). This study was conducted at the Animal Science Department of the University of Missouri-Columbia.

## Bird husbandry

A total of 800 Ross 308 day-old male broilers was used in this study and were randomly assigned to one of the 18 treatments with eight replicate pens of five birds per treatment. Broilers were weighed, wing banded and randomly assigned to pens in stainless steel batterie, with each pen containing one waterer trough and one trough feeder.

Temperatures were set to 33°C upon placement and were decreased gradually to 27°C by the conclusion of the experiment. Lighting intensity was maintained at 30 lux. Daily observations were made following the animal care and management guidelines according to the standard site practices. The number of mortalities, their weights, and the number of birds per pen were recorded each weigh day. Birds and feed were weighed on a per pen basis on day of arrival from the hatchery (d 0), the beginning of experimental period (d 16), and at the termination of the experimental period (d 23) for the determination of feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR).

## Diets

All birds were fed a standard corn-soybean meal (SBM) starter diet (Table 1) that met all nutrient requirements as indicated by NRC (1994) until 15 d of age. Starter diets were free of feed additives such as enzymes, organic acids, essential oils or medication. The experimental diets for testing efficacy of supplemented phytase met the following requirements. Raw materials of the basal diet were selected to provide sufficient PP (at least 0.23%). No mineral P was added, and intrinsic phytase activity did not exceed 150 FTU/kg of the basal diet. In all diets, the analyzed Ca:tP ratios were between 1.3 to 1.7:1 which was very close to the ratio recommended by WPSA (2013; 1.3:1.0 to 1.4:1.0). All diets were offered as mash. The basal diet was prepared in one batch for all treatments and subsequently divided into the respective treatments. Phytase was then added to each dietary treatment at the desired concentration and diets were mixed again.

The experimental diets consisted of three levels of corn, SBM or a combination of corn-SBM with or without 500 U/kg of phytase, for a total of 18 dietary treatments. The analyzed nutrient composition of corn and SBM is presented in Table 2. Corn and SBM diets were formulated based on the recommendations of WPSA (2013), except that SBM and corn were included at the expense of a 2:1 proportion of cornstarch and sucrose, and potato protein was used as a protein source instead of dried egg albumen (Table 2). The phytase used was a bacterial 6-phytase expressed in *Pseudomonas fluorescens* (CIBENZA® PHYTAVERSE®, Novus International., St. Charles, MO), and was a granulated product. All diets contained $TiO_2$ (0.3%) as an indigestible marker in order to determine

digestibility. The concentrations of total P and PP in the diets ranged from 2.75 to 5.45 and 0.68 to 3.10 g/kg dry matter (DM), respectively.

## Sample collection

Excreta samples were collected for four days (d 20 to d 23) after experimental diets were fed for five days, as recommended by WPSA (2013). Excreta were collected once a day and were bulk-stored for each replicate in a freezer (-20° C). On d 23, all birds in each pen were euthanized with $CO_2$ asphyxiation, and the ileum was then immediately excised, and digesta was collected from the distal two-thirds of the ileum (Ravindran et al. 1999; Rodehutscord et al., 2012), pooled per pen, and stored at -20°C until analysis. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction.

Contents of the posterior ileum were collected by gently flushing with distilled water into plastic containers. Samples of digesta and excreta were dried in a forced-air oven at 55°C for 3 d then ground through a 1 mm screen before being analyzed for Ca, P, and titanium. Right tibias were removed, defleshed (cartilage cap removed), and were pooled per pen. Gizzards from 3 birds per cage were excised and gizzard content pH was measured using a Corning M120 pH meter; Corning Medical and Scientific, Scientific Instrument, Halstead Essex, England CO 92 DX, United Kingdom.

## Chemical analyses

Samples of corn and SBM were analyzed for DM, CP (nitrogen × 6.25), crude fat, ash, total P, phytate P, and Ca (Table 3-2). Samples of the test diets were analyzed for DM, CP (nitrogen × 6.25), total P, Ca, and titanium (Ti), and samples of ileal digesta and

excreta were analyzed for DM, total P, Ca and Ti. Tibias were ashed at 600 °C and ash samples were analyzed for P and Ca content. Dry matter and tibia ash were determined using standard procedures (AOAC International, 2005; methods 930. 15 and 942.05, respectively). Phosphorus (Total P) was determined in the diets and digesta, after samples were hydrolyzed using a nitric-perchloric wet ash procedure ($HNO_3$: $HClO_4$: $H_2O$), using a spectrophotometer, after reaction with ammonium molybdovanadate (*AOAC Official Method 966.01 plant tissue).* Concentrations of Ca were determined by flame atomic absorption spectrometry (FAAS; Varian FS240 AA Varian Inc., Palo Alto, CA). Other minerals were also determined by FAAS (AOAC Official Method 975.03B (b) Metals in Plants and Pet Foods Atomic Absorption Spectrophotometric Method) following nitric-perchloric acid wet ash digestion of samples and a hydrochloric acid sample matrix. Phytate P content was determined by the method referenced in Analytical Biochemistry Vol. 77:536-539 (1977). Crude protein was determined by combustion analysis (AOAC Official Method 990.03, 2006). Crude Fat was determined by Ether Extraction (AOAC Official Method 920.39 (A), and titanium was determined at the University of Missouri Chemical lab using methods previously described procedures (Journal of Animal Science (2004) 82:179-183).

## Calculations

True P and Ca digestibility in corn, SBM and C-SBM were calculated according to the procedure outlined by Dilger and Adeola (2006). Apparent ileal digestibility coefficients (AIDC) and retention coefficients were calculated using the indicator ratio (equation 1).

(1) $AIDC = 1 - [(TI/TO) \times (NO/NI)]$

Where TI is the Ti concentration in the diet, TO is the Ti concentration in the ileal digesta or excreta, NO is the nutrient concentration in the ileal digesta or excreta, and NI is the nutrient concentration in the diet. All analyzed values were expressed as grams per kilogram of DM.

Total output of P or Ca in the ileal digesta or excreta, expressed as g/kg DM intake (DMI), were calculated via the following equation.

(2) NO-DMI (g/kg) = NO-DMO × (TI/TO)

Where NO-DMI and NO-DMO represent the nutrient output (as analyzed in digesta or excreta) on DMI and DM output bases, respectively; TI is the Ti concentration in the diet (g/kg DM); and TO is the Ti concentration in the ileal digesta or excreta (g/kg DM digesta or excreta).

Nutrient outputs were regressed against dietary nutrient contents per 24 pens for each type of diet (corn, SBM or C-SBM) using the following statistical model.

(3) NO-DMI (g/kg) = TNI × NI) + ENL

Where NO-DMI represents the nutrient output concentration on DMI basis (dependent variable), N represents dietary nutrient content on a DM basis (independent variable), TNI, represents true nutrient indigestibility, and ENL represents endogenous nutrient in ileal digesta or excreta on DMI basis. In this equation, TNI and ENL are the slope and intercept, respectively, of a simple linear regression of NO-DMI on NI. True nutrient indigestibility is an indirect measure of the inefficiency at which dietary nutrient is extracted by the bird; therefore, the true ileal digestibility (TIDC) or retention coefficients of P and Ca were calculated as

(4) TIDC = 1 – (TNI)

Where TIDC and TNI represent the true ileal digestibility (or retention) coefficient of P or Ca and true P or Ca indigestible estimates, respectively.

## Statistical analyses

Data were analyzed using the GLM procedure of SAS. Pen served as the experimental unit for all statistical analyses, and differences were considered significant at an alpha level of 0.05. Data from the three inclusion levels of each test ingredient (corn, SBM and C-SBM) were analyzed as a completely randomized design. Data were also analyzed for each ingredient source as a 3 x 2 factorial. Orthogonal polynomial contrasts were used to determine the effects of graded P intake on the different parameters tested. Mean TIDC of P and Ca, and endogenous P and Ca loss (g/kg DMI) estimates were obtained by regressing P or Ca output (g/kg DMI) against dietary P or Ca content (g/kg DM) from samples pooled per pen. Therefore, standard errors for true coefficients were based on a total of 24 observations for each test diet.

## Results

## Dietary analysis

Results of analyzed nutrient composition of the SBM and corn sources used in the study are presented on an as fed basis in Table 3-2.

Ingredient composition, calculated nutrient analysis, and analyzed composition for select nutrients (CP, Ca, Phytate P, and Total P) of experimental diets fed to broilers from 16 to 23 d of age are shown in Table 3-3. Analyzed values were in very good agreement with the calculated values. Based on diet analyses, the Ca:total P ratio in the

diets ranged from 1.3-1.7:1.0, which was very close to the ratio recommended by WPSA (2013; 1.3:1.0 to 1.4:1.0).

## Growth Performance

Birds in all treatments were healthy throughout the entire trial and readily consumed their diets. No mortality occurred when experimental diets were fed to birds from d 16 to 23. Effects of dietary treatments on FI, BWG, and FCR are summarized in Table 3-4.

### Corn

There was no phytase by level interaction *(P > 0.05)* observed for any growth performance variable in birds fed corn diets. Phytase supplementation increased FI (*P = 0.0036)* in birds fed corn, but had no effect on BWG (P = 0.2964) or FCR *(P = 0.0959).* There was a corn level effect, with FI and BWG increasing *(P < 0.0001)* and FCR improving (*P < 0.0001)* with increasing dietary concentrations of corn. The increase in BWG was quadratic (*P = 0.0270*) in birds fed no phytase but linear (*P < 0.0001*) in birds fed phytase. In contrast, the increase in FI and improvement in FCR was linear in birds fed no phytase and those fed phytase.

### SBM

A phytase by level interaction (*P = 0.0196*) was only observed for FI in birds fed SBM diets. The interaction occurred as a result of the difference in FI between birds fed no phytase and those fed phytase. In birds fed no phytase, there was no increase in FI with increasing levels of SBM, whereas in birds fed phytase, FI was higher in birds fed the high level of SBM compared to birds fed the low and medium levels of SBM. Phytase

supplementation of SBM diets increased BWG (*P = 0.0029*) and improved FCR (*P = 0.0339*) compared with birds fed no phytase. There was a SBM level effect, BWG increased (*P < 0.0001*) and FCR improved (*P < 0.0001*) with increasing dietary concentrations of SBM. The increase in BWG and improvement in FCR was linear (*P < 0.0001)* in birds fed no phytase and in those fed phytase.

**C-SBM**

There was no phytase by level interaction *(P > 0.05)* observed for any growth performance variable in birds fed C-SBM diets. Phytase supplementation of C-SBM diets had no effect on BWG (*P = 0.1434*) or FCR (*P = 0.3549*) but increased FI (*P = 0.0049*) compared with birds fed no phytase. There was a C-SBM level effect, with FI and BWG increasing *(P < 0.0001*) and FCR improving (*P < 0.0001*) with increasing dietary concentrations of C-SBM. The increase in FI and BWG and improvement in FCR was linear (*P < 0.0001*) in birds fed no phytase and also in those fed phytase.

## Gizzard pH

There was no effect *(P > 0.05)* of dietary treatments on gizzard pH of birds which averaged 2.419 ± 0.16 (mean ± SEM) and ranged from 1.791 to 3.446 (Table 3-4).

## Tibia ash

**Corn**

No phytase by level interaction was observed for percent tibia ash (*P = 0.1999*) or tibia Ca (*P = 0.0582*). In contrast, a significant phytase by level interaction (*P = 0.0315*) was observed for tibia P. The interaction occurred as a result of differences in tibia P among levels of corn in birds fed phytase compared to those fed no phytase. In birds fed

no phytase there were no differences in tibia P among corn levels, whereas in birds fed phytase, tibia P was higher in birds fed the highest level of corn compared to birds fed low and medium levels of corn. Phytase supplementation had no effect on tibia Ca (*P = 0.4817*) but increased percent tibia ash (P = 0.0085). The increase in percent tibia ash was linear (*P = 0.0092*) in birds fed phytase. There was a significant corn level effect with tibia ash percentage (*P = 0.0475*) and tibia Ca (*P = 0.0007*) increasing with increasing dietary concentrations of corn. The increase in tibia Ca was linear in birds fed phytase (*P = 0.0007*).

**SBM**

There were no phytase by level interactions *(P > 0.05)* observed for percent tibia ash, tibia P or Tibia Ca. Phytase supplementation increased (*P < 0.0001*) percent tibia ash but had no effect on tibia P (*P = 0.3044*) or tibia Ca *(P = 0.5924*). There was no effect of SBM level on tibia P (*P = 0.1788*) or tibia Ca (*P = 0.3249*) but percent tibia ash increased (*P < 0.0001*) with increasing levels of SBM. There was a linear increase (*P < 0.003*) in percent tibia ash with increasing levels of SBM in birds fed phytase and those fed no phytase.

**C-SBM**

No phytase by level interaction was observed for tibia ash *(P = 0.5650*), tibia P (*P = 0.2636*) or tibia Ca (*P = 0.2936*). Phytase supplementation increased (*P=0.0390*) tibia ash and tibia P (*P = 0.0245)* but did not affect tibia Ca *(P = 0.0956).* There was a C-SBM level effect with tibia ash (*P = 0.0144),* tibia P (*P < 0.0001*) and tibia Ca (*P < 0.0001*) all increasing levels of C-SBM. The increase in tibia ash was linear (*P = 0.0104*) in birds fed

no phytase, whereas the increase in tibia P and tibia Ca was quadratic (*P < 0.02*) in birds fed no phytase but linear (*P < 0.05*) in birds fed phytase.

## Digestibility and retention of P and Ca

Effects of dietary treatments on ileal digestibility and retention of P and Ca are summarized in Table 3-5.

**Corn**

There was no phytase by level interaction *(P > 0.05)* observed for ileal DM digestibility, (*P = 0.3709*), P (*P = 0.1751* and Ca digestibility percentage (*P = 0.9005*). Ileal P digestibility was higher (*P < 0.0001*) in birds fed phytase compared to those fed no phytase. There was no phytase effect on ileal DM *(P = 2906*) and Ca digestibility (*P = 0.1107*). In contrast, ileal DM, P, and Ca digestibility all decreased (*P < 0.0001*) with increasing levels of corn. The decrease in ileal DM digestibility with increasing levels of corn was quadratic (*P = 0.0411*) in birds fed no phytase but linear (*P = 0.0035*) in birds fed phytase. The decrease in ileal P and Ca digestibility with increasing levels of corn was linear *(P < 0.01*) in birds fed phytase and those fed no phytase.

Significant phytase by level interactions *(P < 0.003*) were observed for apparent DM, P, and Ca retention. For apparent DM retention, the interaction occurred because DM retention decreased with each increase in corn level in birds fed no phytase, but in birds fed phytase, DM retention was lower in birds fed the high level of corn compared with those fed low and medium levels of corn. For apparent P retention, the interaction occurred because although P retention decreased with each increase in corn level in birds fed no phytase and those fed phytase, the decrease in P retention was much

smaller in birds fed phytase (83.45 to 73.33% vs 80.18 to 61.79%). For apparent Ca retention, the interaction occurred because Ca retention decreased with each increase in corn level in birds fed no phytase but did not change in birds fed phytase.

**SBM**

There was no phytase by level interaction *(P > 0.05)* observed for any Ileal digestibility variables (DM, P and Ca digestibility) in birds fed SBM diets. Ileal DM digestibility was also not affected by phytase supplementation *(P = 0.7868*). However, ileal DM digestibility decreased (*P < 0.0001*) with increasing levels of SBM. The decrease in ileal DM digestibility with increasing levels of SBM was linear (*P < 0.05*) in both birds fed phytase and those not fed phytase. Ileal P digestibility was also not affected by phytase supplementation *(P = 0.0960)* but did decrease *(P = 0.326)* with increasing levels of SBM. Ileal Ca digestibility was lower (*P = 0.0036*) in birds fed phytase compared to birds fed no phytase but was not affected by level of SBM *(P = 0.3442).*

A phytase by level interaction *(P < 0.0001)* was observed for apparent DM retention. Apparent DM retention decreased with increasing levels of SBM in birds no phytase and those fed phytase, however the decrease in DM retention was lower in birds fed medium and high levels of SBM and phytase. A phytase by level interaction *(P < 0.0001)* was also observed for apparent P retention. Apparent P retention decreased with increasing levels of SBM in birds fed no phytase and those fed phytase, however the P retention was higher in birds fed phytase at all levels of SBM. There was no phytase by level interaction (*P = 0.4852*) observed for apparent Ca retention. Apparent Ca retention was greater *(P < 0.0001*) in birds fed phytase compared to those fed no phytase.

Apparent Ca retention was greater *(P < 0.0001)* in birds fed low and high levels of SBM compared to bird fed medium levels of SBM.

**C-SBM**

There was no phytase by level interaction (*P > 0.05*) observed for ileal DM and Ca digestibility in birds fed C-SBM diets but there was a phytase interaction (*P = 0.0062*) observed for ileal P digestibility. This is as a result of ileal P digestibility values for the low level of C-SBM diets without phytase and the medium C-SBM level supplemented with phytase being not different, but apparent P digestibility of the low levels of C-SBM supplemented with phytase was significantly higher than that of all the C-SBM diets not supplemented with phytase. Ileal DM digestibility was not affected (P = 0.3845) by phytase supplementation but decreased *(P < 0.0001*) with increasing levels of C-SBM. The decrease in ileal DM digestibility with increasing levels of C-SBM was linear (*P < 0.0001)* in birds fed no phytase and in those fed phytase. Ileal Ca digestibility was not affected (*P = 0.2497*) by phytase supplementation but decreases *(P < 0.0001*) with increasing levels of C-SBM. The decrease in ileal Ca digestibility with increasing levels of C-SBM was quadratic *(P = 0.0282)* in birds fed no phytase and linear *(P < 0.0001)* in those fed phytase.

There was no phytase by level interaction (*P > 0.05*) observed for apparent Ca retention but interactions were observed for apparent DM (*P = 0.0016*) and P (*P = 0.0012*) retention for birds fed C-SBM diets. For DM retention, the interaction occurred because there were differences in the extent of reduction in DM retention with increasing levels of C-SBM. In birds fed no phytase there was a 6% decrease in DM

retention as the level of C-SBM increased from low to medium, whereas in birds fed phytase there was only a 3.9% reduction in DM retention as the level of C-SBM increased from low to medium. The percentage reduction in DM retention going from medium to high was the same (~ 3%) in birds fed no phytase and those fed phytase. For PM retention, the interaction occurred because there were differences in the extent of reduction in P retention with increasing levels of C-SBM. In birds fed no phytase there was a 20% decrease in P retention as the level of C-SBM increased from low to medium, whereas in birds fed phytase there was only a 16% reduction in P retention as the level of C-SBM increased from low to medium. Similarly, the percentage reduction in P retention going from medium to high was 5% in birds fed no phytase whereas the reduction was 1% in birds fed phytase. Phytase supplementation increased ($P < 0.0001$) Ca retention. Calcium retention increased with increasing levels of C-SBM, and the increase was quadratic ($P = 0.0236$) in birds fed no phytase but linear ($P < 0.0001$) in birds fed phytase.

## Estimation of TPD and EPL of corn, SBM and C-BM

Mean estimates of endogenous P loss (mg/kg of DMI) and true P utilization (%) as determined by feeding broilers with three different inclusion levels of corn, SBM, or c-SBM without or with phytase are presented in Table 3-6.

**Corn**

Phytase significantly influenced true ileal P digestibility (TIPD), with birds fed corn diets supplemented with phytase having significantly higher TIPD (30.4%) than those that were not supplemented with phytase (-7.19%). Phytase also influenced true P

retention with birds fed corn diets supplemented with phytase having a significantly higher P retention (43.5%) than birds that were fed diets without phytase (11.8%). Endogenous loss was negative in birds fed phytase (-196 mg/kg DMI) and those fed phytase (-281 mg/kg DMI) but the value was numerically lower in birds fed phytase.

There was no difference observed in true Ileal Ca digestibility with birds fed phytase having a value of 43.5% and those fed no phytase having a value of 38.6%. However, true Ca retention was significantly higher in birds that were fed corn diets supplemented with phytase (65.9%) compared with birds fed corn diets with no phytase (30.4%). The ileal endogenous loss of Ca for corn diets was not significantly different for birds fed diets without and with phytase supplementation with negative values of -184 and -172 mg/kg DMI, respectively. For true Ca retention, values were also not significantly different among birds that were fed corn diets without and with phytase being -178 and -50 mg/kg DMI, respectively.

**SBM**

True Ileal P digestibility of SBM diets were not significantly affected by phytase, with values of 52.0 and 59.9 %, respectively for birds fed no phytase and those fed phytase. True P retention values were also not significantly affected by phytase, with values of 50.0 and 45.9%, respectively for birds fed no phytase and those fed phytase. Ileal endogenous P loss was estimated to be -67 and -54 mg/kg DMI for birds fed no phytase and those fed phytase, respectively. For P retention, endogenous P losses were estimated to be -66 and -114 mg/kg DMI for birds fed no phytase and those fed phytase, respectively.

True ileal Ca digestibility was significantly affected by phytase supplementation with true Ileal Ca digestibility of diets without phytase supplementation having a digestibility of 76.1% and with phytase supplementation a value of 37.7%. Ileal endogenous Ca losses were not affected by phytase supplementation and averaged 93 and -87 mg/kg DMI for birds fed no phytae and those fed phytase, respectively. True Ca retention was not affected by phytase supplementation and averaged 61.8 and 59.1% respectively, for birds fed no phytase and those fed phytase. Endogenous Ca losses were not affected by phytase and averaged 53 and -11 mg/kg DMI, respectively for birds no phytase and those fed phytase.

**C-SBM**

In C-SBM diets, true ileal P digestibility was significantly affected by phytase with birds fed phytase having a digestibility value of 19.5% compared to a value of 5.58% in birds fed no phytase. Endogenous P loss was not affected by phytase and averaged -238 and -247 mg/kg DMI, respectively in birds fed no phytase and those fed phytase. In C-SBM diet, true P retention was not affected by phytase and averaged 16.6 and 14.4% respectively in birds fed no phytase and those fed phytase. Endogenous P loss (retention) was not affected by phytase and averaged -188 and -222 mg/kg DMI, respectively in birds fed no phytase and those fed phytase.

True ileal Ca digestibility was not affected by phytase and averaged 18.5 and 5.2%, respectively in birds fed no phytase and those fed phytase. Endogenous Ca losses was not affected by phytase and averaged -266 and -365 mg/kg DMI, respectively in birds fed no phytase and those fed phytase. True Ca retention was not affected by phytase

and averaged 24.2 and 21.2% respectively in birds fed no phytase and those fed phytase. Endogenous Ca loss (retention) was not affected by phytase and averaged -232 and -293 mg/kg DMI, respectively in birds fed no phytase and those fed phytase.

## Discussion

The analyzed dietary P concentrations were close to calculated concentrations and were therefore used for the calculation of P digestibility and retention coefficients in the current study. The analyzed concentrations of Ca, on the other hand were in some cases slightly higher than calculated values hence the Ca:tP ratio ranged from 1.3 to 1.7:1 by analysis and 1.4:1 when calculated which was very close to the ratio recommended by WPSA (2013). It was assumed that this would not influence digestibility and retention coefficients. Interestingly, a similar trend between analyzed and calculated Ca values at low dietary Ca concentrations has been previously reported (Driver et al., 2005; Mutucumarana et al., 2014). Since digestive capacity adaptation to Ca and P deficiencies or imbalances can occur within 48 h (Angel et al., 2013), any adaptation to the experimental diets would have already occurred in the present study before samples were taken. However, previous studies have reported that the degree of phytate degradation was highly dependent on the dietary Ca level (Tamim et al., 2004; Plumstead et al., 2008: Selle et al., 2009). In the current study, no mortality or leg problems were observed during the experimental period. However, bone deformities and poor survival rates (33%) were reported in a study conducted by Hayes et al. (1979) when day old chicks were fed corn based semi-purified diet with no added P up to 13 days of age.

In the current study, there was no phytase by level interaction for growth performance values for birds fed corn and C-SBM diets. However, in the SBM diets, FI was similar in birds fed no phytase but was higher in birds fed high SBM compared to those fed low and medium SBM diets in birds fed phytase resulting in a phytase by level interaction. Birds receiving the phytase supplemented corn and C-SBM diets had lower FI compared to those fed no phytase. This reduction in FI is in contrast to previous studies where phytase supplementation of low P diets resulted in increased FI of broiler chickens (Sebastian et al., 1997; Camden et al., 2001; Aureli et al., 2011; Ravindran et al., 2008; Rutherfurd et al., 2012). However, there was a level effect on growth performance, where increasing the inclusion level of ingredients (corn, SBM and C-SBM) also improved growth performance of broilers. This was not surprising since supplementing a P deficient diet with increasing levels of P from increasing levels of the ingredients should improve growth performances in birds. Increasing levels of ingredients (corn, SBM and C-SBM) increased BWG, improved FCR and also caused a high FI, results similar to that reported by Zhang et al. (1999). Similar results were also reported by Mutucumarana et al. (2014) where increasing levels of corn increased BWG and FI.

The lack of a phytase effect on BWG of birds fed corn and C-SBM diets is surprising but fits with the decrease in FI in birds fed phytase compared to those fed no phytase. In contrast to the corn and C-SBM diets, phytase supplementation increased BWG in birds fed SBM. Compared to birds fed corn and C-SBM, birds fed phytase supplemented SBM diets were more efficient in converting feed to gain. In general, phytase

supplementation of low P diets have resulted in improved growth performance of broilers (Selle and Ravindran, 2007; Powell et al., 2011; Paiva et al., 2014). Rutherfurd et al. (2012) reported that dietary inclusion of a novel microbial phytase into a low P diet improved broiler BW gain and FI by approximately 8%. The variable growth performance responses to phytase that occurred with the different grain sources in the current study may be the result of the effects of each grain source or may be due to the length of time the phytase diets were fed. The improvement in growth performance in birds with phytase supplementation reported in previous studies could be attributed to increased feed intake and feed efficiency, which can be due to increased release of P from phytate-mineral complex as reported by Qian et al. (1996) and Sebastian et al. (1996). It could also have been due to increased utilization of inositol (Simons et al., 1990), increased starch digestibility (Knuckles and Betschart, 1987), and increased protein and amino acid digestibility (Ravindran et al., 2000), or overall nutrient utilization (Miles and Nelson, 1974).

In the current study, there was no effect of dietary treatments on gizzard pH In contrast, several studies (Walk et al., 2012; Amerah et al., 2014; Paiva et al., 2014) reported that phytase supplementation caused an increase in gizzard pH in broiler chickens, with digesta pH being higher in all segments of the digestive tract. Walk et al. (2012) attributed this effect to a reduction in the electrolyte balance due to phytate based on the premise that when phytate chelates nutrients from the diet, it creates a more acidic environment in the gastrointestinal tract. Differences between the current

study and those reported previously may be due to the fact that the phytase supplemented diets were only fed for a short period of time.

Rutherfurd et al., (2012) reported that phytase supplementation increased the degradation of PP and absorption of P, Ca, and increased toe ash. In this study, tibia ash increased with phytase supplementation with a linear increase from 48.89 to 51.70% in corn, and 48.92 to 51.94% in SBM but no increase was observed (51.27 to 52.28%) in birds fed C-SBM diets. In birds fed phytase tibia ash increased linearly in birds fed SBM and C-SBM diets, with no increase in birds fed corn diets. These results are relatively consistent with previous reports where phytase has been reported to increase tibia ash as a consequence of phytate degradation and improved P absorption (Pintar et al., 2004; Snow et al., 2004; Angel et al., 2006; Powell et al., 2011; Amerah et al., 2014; Paiva et al., 2014).

There was a phytase by level effect for tibia P observed in birds fed corn diets but not the other ingredients. Birds fed the high level of corn supplemented with phytase had a significantly higher tibia P (0.1451 g/bone) compared to low and medium levels. Whereas birds fed increasing levels of corn without phytase had similar tibia P values. Increasing levels of C-SBM diets also caused a quadratic increase in tibia P in birds fed no phytase but a linear increase in the tibia P of birds fed phytase. Phytase supplementation however, had no effect on tibia P when corn and SBM diets were fed, but linearly increased tibia P in birds fed C-SBM diets. In this study, there was no effect of phytase supplementation on the tibia Ca of birds. Amerah et al. (2014) also reported that phytase supplementation had no effect on Ca digestibility which was similar to the

results in this study. According to Bronner (1987), an increase in dietary Ca concentration resulted in increased Ca absorption required for incorporation of P into bones.

There was no phytase by level interaction observed for ileal DM among birds fed any of the three diets. Phytase supplementation also had no effect on ileal DM digestibility. In contrast, Wu et al. (2004) reported higher ileal DM digestibility for corn, wheat and barley based diets supplemented with phytase. Similarly, Leytem et al. (2008) and Mutucumarana et al. (2014) reported that phytase supplemented diets had the greatest ileal DM digestibility coefficients. There was an ingredient inclusion level effect with Ileal DM digestibility decreasing with each level of ingredient addition, and decrease in ileal DM digestibility was linear both in the absence and addition of phytase. Similarly, Dilger and Adeola (2006) reported that ileal DM digestibility was not affected by replacing highly digestible cornstarch with conventional SBM without phytase. In that study, overall DM retention estimates, however, were shown to decrease from approximately 82 to 74% with consumption of graded levels of each SBM.

There was a phytase by level interaction observed for apparent Ileal P digestibility (AIPD) among birds fed C-SBM diets. The AIPD for birds fed C-SBM supplemented with phytase was significantly higher with a linear reduction in AIPD whereas in those fed no phytase the reduction was both linear and quadratic. Phytase supplementation increased AIPD in birds fed corn and C-SBM diets.

The AIPD estimate for SBM and C-SBM without phytase ranged from 73.80 to 64.79% in the current study as compared with previous studies 49 to 53% (Rutherfurd et al.,

2002; Dilger et al., 2004; Ruthrfurd et al., 2004; Rodehutscord et al., 2017). The values in this study were also within the range of values reported in other studies for similar and other cereal-based diets. Wu et al. (2004) reported ileal P digestibility coefficients for corn-based diets of 70 and 77%, for wheat-based diets 0.58 and 0.70, and for barley-based diets 67 and 77% without and with phytase supplementation, respectively. Leytem et al. (2008) reported AIPD values for corn supplemented with phytase was 86% which agreed with the values in the current study, while Mutucumarana et al. (2014) reported AIPD coefficients for corn supplemented with phytase ranged from 65 to 70% which were in close agreement with Wu et al. (2004). Camden et al. (2001) reported an increase in ileal phytate degradability from 22% for an unsupplemented low-P diet to 45 to 53% after phytase supplementation (250 to 1,000 U/kg). However, as the ingredient level increased there was a reduction in AIPD. This is as a result of the increase in mineral levels in the diets is expected to result in a decrease in absorption of minerals hence, the homeostatic mechanism. Dietary Ca level the probably the major factor influencing phytate P hydrolysis (Ballam et al., 1984; Mohammed et al., 1991; Tamim and Angel, 2003).

Phytase supplementation decreased ileal Ca digestibility in birds fed SBM diets but not in birds fed corn or C-SBM diets. Level of SBM had no effect on Ca digestibility. Paiva et al. (2014) reported that phytase supplementation significantly increased Ca digestibility regardless of Ca and P levels of the diets. Similar to the current study, a reduction in Ileal Ca digestibility in corn and C-SBM based on inclusion level as was also reported by Rodehutscord et al. (2017). The mechanisms involved in the improvement

of Ca and P digestibility when feeding lower levels of Ca in the diet are related to mineral availability in the gastrointestinal system. When Ca and P precipitate, they become unavailable for digestion and absorption (Tamim et al., 2004; Woyengo et al., 2009). This effect is exacerbated in the absence of phytase because excess Ca tends to form mineral-phytate chelates that precipitate in the intestinal environment and become unavailable for the bird (Sebastian et al., 1996; Tamim et al., 2004; Plumstead et al., 2008). Results from that study showed improved P and Ca ileal digestibility due to ingredient inclusion levels particularly in SBM for Ca digestibility. However, in contrast to the current study other researchers reported that the overall Ileal P and Ca digestibility were due to phytase supplementation and supports those earlier reports by Singh et al. (2003), Akyurek et al. (2005), and Jendza et al. (2006) of the efficacy of the phytase used in improving the growth performance of the birds.

Differences in DM retention to phytase supplementation for different levels of the three ingredient sources resulted in significant phytase by level interactions. There was a phytase effect in birds fed corn diets where apparent DM retention was reduced by phytase supplementation. However, phytase did not affect DM retention of birds fed SBM and C-SBM diets. There was a level effect on apparent DM digestibility with apparent DM retention decreasing with increasing levels of all three ingredients by levels. However, birds fed low SBM with and without phytase were significantly higher (77.24 and 77.81%, respectively) compared to other inclusion levels with and without phytase supplementation. The DM retention due to inclusion levels of SBM without phytase was within the range of (82 to 74%) values reported by Dilger and Adeola,

(2006). In birds fed C-SBM diets apparent DM retention decreased with increasing levels of C-SBM hence, birds fed the low level of C-SBM without phytase had a significantly higher DM retention (83.48%) whereas, birds fed the high level of C-SBM diet without phytase had a significantly lower (74.42%) DM retention.

Phytase by level interactions were observed for apparent P retention for all three diets ingredient sources due to differences in P retention in birds fed phytase compared to those fed no phytase. Birds fed diets supplemented with phytase had higher apparent P retention values than those fed diets without phytase. However, apparent P retention of birds fed low inclusion levels (Corn, SBM and C-SBM) with phytase were significantly higher 83.48, 81.92, and 78.95%, respectively, than birds fed high inclusion levels of ingredients. These apparent total tract P retention values for SBM were higher than observed in past research (Perney et al., 1993; Sebastian et al., 1996), ranging from 33 to 54% for conventional SBM and from 31 to 66% for low-phytate SBM diets without phytase supplementation. The data indicate that phytase supplementation in the current study increased P retention in broiler chickens suggesting that phytase supplementation increased phytate degradation and improved P digestibility at all inclusion levels of ingredients. These results are consistent with the report of, Leytem et al. (2008) who in a study with corn, wheat, barley, and oat in broiler chickens reported an increase in the hydrolysis of phytate P, and a 3-fold increase in P retention as a result of addition of 1,000 phytase units to the diets. The apparent P retention of diets without phytase reported in the present study are higher than those reported by Leytem et al. (2008) and Jang et al. (2003) who reported total tract phytate P digestibility coefficients

for barley and corn, which were 39 and 24%, respectively. Juanpere et al. (2004) reported apparent P retention coefficients of 58, 62, and 65% for barley-based diets with high P, low P, or low P + phytase treatments, respectively. Hernández et al. (2005) reported total tract P retention coefficients for corn and wheat in broiler chicks as 0.58 and 0.72. Wu et al. (2004) reported apparent P retention coefficients for wheat with and without phytase addition as 0.51 and 0.40, respectively in broiler chicks.

Other studies have shown that phytase effects on P retention in broiler chickens were reduced at much higher Ca:P ratios (Qian et al., 1997), and that high Ca suppresses phytase ability to hydrolyze phytate P by competing for the active sites of the enzymes. Further, phytate would be less likely to bind Ca and the efficacy of these bacterial phytases would be influenced to a lesser extent by Ca-phytate complexes. Plumstead et al. (2008) reported a linear reduction in ileal phytate P degradation by 71%, when increasing dietary Ca level from 4.7 to 11.6 g/kg in broiler diets. Similar results were reported by Tamim et al. (2004) in both in vitro and in vivo studies. These researchers found that dietary Ca at a level as low as 0.1% reduced phytate-P hydrolysis at pH 6.5 in vitro.

In the current study, a phytase by level interaction was only observed for apparent Ca retention in birds fed corn diets. Birds fed corn diets with phytase and those fed high levels of corn without phytase had similarly higher apparent Ca retention values. Phytase supplementation significantly increased the apparent Ca retention of birds fed all diets although phytate hydrolysis was enhanced by phytase, this did not affect the total tract Ca digestibility (Leytem et al., 2008). However, there was also a level effect

for apparent Ca retention for all diets with, a linear and quadratic decrease in Ca retention in birds fed C-SBM without phytase, a linear decrease in birds fed corn and a quadratic decrease in birds fed SBM diets. Hence, more Ca was retained when phytase was used because phytase increased the amount of Ca absorbed. This observation is also in agreement with results of previous research (Poulsen et al., 2010).

Estimating TPD in cereals, grains and legume seeds is important because they make up a significant proportion of poultry diets. However, P availability from these seeds can be enhanced by phytase supplementation. Using the regression method has been reported to be useful in estimating the digestible P in feed ingredients (Fan et al., 2001; Dilger and Adeola, 2006; Akinmusire and Adeola, 2009). Estimating the TPD is of paramount importance because these feed ingredients are used as the main protein and energy sources in broiler chicken diets. The reported impact of dietary phytase on the availability of phytate P varies markedly across studies. This variation is largely due to differences in experimental design, analytical methods, diet composition and processing, source of phytase and the age and breed of the chickens (Angel et al., 2002). Overall, results of the current study showed that dietary phytase supplementation led to improvements in apparent ileal phytate P and total P digestibility which is consistent with previous reports of by many researchers.

The TIPD estimates are dependent on apparent P digestibility values which were very high as previously indicated. This observation in the current study and previous studies therefore indicates that greater P retention results from lower dietary phytate P content because of limited digestive capacity for phytate-bound P in the chick hence the positive

effects of phytase when supplemented to diets (Bitar and Reinhold, 1972; Maenz and Classen, 1998). Phytase supplementation has been reported to improve the amount of digestible P in plant feedstuffs, and consequently reduce P loss from feed ingredients. Rutherfurd et al. (2004) reported a 10 to 12% increase in TPD at the terminal ileum with phytase supplementation of low P diets containing soybean meal, wheat bran, and rapeseed meal in broiler chickens with a corresponding 10.5% increase in phytate degradation at the terminal ileum. These results show the efficacy of phytase in improving P utilization and confirms previous observations (Sebastian et al., 1997; Rutherfurd et al., 2004; Akyurek et al., 2005; Patras et al., 2006; Akinmusire and Adeola, 2009; Iyayi et al., 2013).

In this study, TPD were estimated by regressing P output against P intake. The TIPD were significantly higher in birds that were fed phytase supplemented corn (30.4%) and C-SBM (19.5%) diets but was also numerically higher in birds fed SBM (59.9%) diets with phytase. Similar TIPD values for SBM were reported by Dilger and Adeola (2006) and were closely in line with what was reported by Rodeshutscord et al. (2017) where ileal P digestibility of SBM was determined by linear regression and varied among stations from 19 to 51%, with significant differences among stations. In the same study the average true ileal digestibility of Ca of the diets across all stations was 57, 51, and 46%, with a similar range among stations as found for TIPD. Mutucumarana et al. (2014b) reported TIPD coefficients for wheat, sorghum, soybean meal, and corn DDGS to be 0.464, 0.331, 0.798, and 0.727, respectively. Mutucumarana et al. (2014a) reported TIPD and true P retention coefficients of corn to be 0.676 and 0.632, and canola meal to be

0.469 and 0.486, respectively. In contrast to the current study, Mutucumarana et al. (2014a) determined true ileal digestibility and total tract retention coefficients for corn and canola meal were not different. The increase in true ileal total P absorption observed in the current study (5 to 19.5%) is similar to the 5 to 21% increase in ileal total P absorption after supplementation with 250 to 1,200 U/kg of phytase reported in several studies (Denbow et al., 1998; Um et al., 2000; Camden et al., 2001; Rutherfurd et al., 2004a; Cowieson and Adeola, 2005; Santos et al., 2008). Aureli et al. (2011) reported a much higher increase in total P utilization with supplementation of the low-P C-SBM diet with the 500 to 2,000 U/kg possibly due to a much lower P content in the low-P diet used by Aureli et al. (2011) as compared with that used by Rutherfurd et al. (2012).

Excreta P output was also significantly different in birds fed corn diets with phytase and without phytase with a 4-fold difference, but no significance difference was observed in birds fed other ingredients but values were noted to be numerically lower when diets were supplemented with phytase. Bougouin et al. (2014) reported that broilers consuming control diets retained 48.4% P, and exogenous phytase supplementation at 1,039 FTU/kg of diet increased P-retention by 8.6 percentage units on average. However, the effect of phytase on P retention across studies were significantly heterogeneous due to differences in Ca content, experiment length, bird age and phytase dose. Due to the different phytate phosphorus (PP) content of ingredients the response of phytase supplementation on available P is expected to be different among different ingredients. Phytase supplementation has also been reported

to improve Ca and P retention in broiler chickens (Lim et al., 2001; Viveros et al., 2002), P utilization in Atlantic salmon (Sajjadi and Carter, 2003), and digestible P in pigs with a reduction in excreted P of 21.5% (Harper et al., 1997). These reports support results in the current study of improved ileal digestible P and a corresponding increase in P retention. The significant effect of phytase addition on apparent P digestibility and retention contributed to TPD. While EPL of ileal and excreta yielded high negative values for birds fed all ingredients with and without phytase supplementation. Such negative estimates for EPL, using the regression technique, have been reported in growing male turkeys (D¨anner et al., 2006) and broiler chickens (Rodehutscord et al., 2012; Shastak et al., 2012; Iyayi et al., 2013; Liu et al., 2013; Mutucumarana et al., 2014a, b; Perryman et al., 2016) and were attributed to a possible consequence of extrapolation of the regression line (Moughan et al., 1998). al-Masri (1995) showed that EPL was negatively correlated with dietary Ca concentrations and wide Ca:total P ratios in broiler chicks. Errors generated by extrapolation are likely a result of variability in the influential first or last data points (Mutucumarana et al., 2014a, b). Moreover, research conducted by Liu et al. (2013, 2014) indicated that the prediction of negative EPL were a result of the differences in P indigestibility between the basal protein source (casein) and the test P ingredient (SBM).

The true Ileal Ca digestibility was numerically higher in corn diets supplemented with phytase while endogenous Ca was significantly higher (65.9%) for diets supplemented with phytase. However, there was a significant linear relationship for true Ileal Ca digestibility with birds fed SBM diets without phytase having significantly higher

digestibility (76.1%) and those supplemented with phytase a much lower (37.7%) true ileal Ca digestibility. Amerah et al. (2014) reported that there is a high efficiency of Ca utilization at lower Ca levels, which may be caused by upregulation of Ca transporters at Ca levels below the bird's requirement (Li et al., 2012). However, large negative endogenous loss Ca values for SBM diets with phytase were observed. A similar association between negative endogenous P estimates and low P digestibility was observed for other feed ingredients in previous studies (Mutucumarana et al., 2014a, b). Those endogenous loss values for Ca for birds fed SBM diets were higher (positive values) in diets that were not supplemented with phytase. Hence, SBM diets without phytase had positive values (93 and 53 mg/kg DMI) for ileal and excreta endogenous loss, respectively. This was not surprising since Ca digestibility was higher in birds fed diets supplemented with phytase compared to those fed no phytase.

In contrast to the current study, Dilger and Adeola (2006) observed, using a regression technique, that the total EPL for 15 to 22 day old male broilers averaged 87.4 mg/d per chick. Rutherfurd et al. (2002, 2004) reported that the estimates of EPL for 28 d-old male broilers at the terminal ileum were 272 ± 108 and 446 ± 59 mg/kg of feed DMI, respectively, which only reflected the P excretion at terminal ileum of chicks. Cowieson et al. (2004) reported that the value of EPL for 42-d-old female broilers was 230 mg/48 h per bird by using a precision-fed assay. On the other hand, ileal endogenous P losses in birds fed wheat, soybean meal, and corn DDGS diets were estimated to be 0.080, 0.609, and 0.418 g/kg DMI, respectively. In birds fed sorghum-

based diets, endogenous P losses were estimated to be negative (–0.087 g/kg DMI) (Mutucumarana et al., 2014a).

Results from the current study show that irrespective of the feed ingredient, there was a similarity in the effect of phytase on P digestibility and retention. However, the TIPD values were low in diets without phytase, but these values increased significantly with addition of phytase. However, values were still very low compared to the high values for AIPD. In the current study, TPD in corn and SBM was not additive when the two ingredients were fed in combination. The variation in P availability of different feed raw materials is high (Shastak and Rodehutscord, 2015), and it is generally accepted that the use of P as a globally finite resource can be optimized by considering the differences that exist in P availability of feed raw materials. Moreover, information on the additivity of ileal P digestibility values from individual ingredients has never been assessed in broilers, only Liu et al. (2013) attempted it using retention P but their results were inconclusive. A possible consequence of regression equations that predict negative EPL is the underestimation of TPU. When negative EPL were predicted, researchers reported lower estimates of TPU compared with the AIPD data that the regression equations were based on (Iyayi et al., 2013; Liu et al., 2013; Mutucumarana et al., 2014a). Estimates for true P digestibility should be higher than apparent P digestibility. Negative EPL are a possible explanation for the lack of significant differences between APD and TIPD data for diets formulated with the fixed Ca:P.

## Conclusion

In conclusion, P output in the excreta is the sum of undigested dietary and endogenous P, P utilized and excreted by the hindgut microflora, and P excreted via urine. Strong linear relationships between digesta and excreta outputs and dietary P intake must be observed for all diets which is a primary requirement for the application of the regression technique (Dilger and Adeola, 2006). This relationship permits the determination of diet-independent theoretical estimates of endogenous P losses (mg/kg DMI) and simultaneous measurements of true digestibility of a particular feed ingredient (Fan et al., 2001; Dilger and Adeola, 2006). The digestibility estimates determined by the regression method are automatically corrected for endogenous losses and represents the true digestibility values. True Ileal P digestibility in birds fed corn, SBM and C-SBM diets supplemented with phytase were determined to be 30.4, 59.9, 19.5%, respectively. In the current study, when Ca: tP ratio is fixed P digestibility goes down, and there was no additivity of P digestibility of corn and SBM. The TIPD estimate of SBM (52.0%) is more reasonable than the extremely low values obtain for corn (-7.2%) and C-SBM (5.6%). Corn and C-SBM contained inorganic P, whereas SBM diets contained no inorganic P. Phytase improved TIPD in corn (+38 points, to 60.4%) and C-SBM (+14 points, to 19.5%), however it didn't in SBM (+7 points, to 59.9%).

The AIPD linearly decreased as the ingredient level increased in corn and SBM, and increased when phytase was supplemented. A significant ingredient level by phytase interaction was found for AIPD in corn-SBM. Phytase also improved AIPD in all ingredient levels however, the response of ingredient level in diets without phytase

(low, med, high: 76.2, 54.7, 45.7%) was quadratic and linear for diets with phytase (low, med, high: 88.1, 76.7, 60.0%). The AIPD coefficients were closely corresponding to the apparent P retention coefficients. This was expected since all diets were formulated below the P requirement. Endogenous losses of P may be different if different methods are used to estimate the losses (Dilger and Adeola, 2006; Almeida and Stein, 2010). In summary, TIPD estimates for corn, SBM, and C-SBM obtained by regression are lowere than AIPD and far from reality, possibly due to the use of a fixed Ca:P ratio and of inorganic P in corn and C-SBM diets. Hence, the higher AIPD values observed and the extremely low true digestibility values are not biologically possible. Overall phytase efficiently improved TIPD and AIPD in corn and C-SBM, and AIPD in SBM. However, unlike the pig where TPD in corn and SBM is additive, in the current study, TPD in corn and SBM was not additive when the two ingredients were fed in combination. Further studies are, needed before definite conclusions can be drawn.

**Table 3-1.** Composition and calculated nutrient analysis of starter diet (NRC 1994)

| **Ingredient** | **%** | **Calculated analysis** | |
|---|---|---|---|
| Corn | 59.3 | ME, kcal/kg | 3040 |
| SBM | 34.41 | CP, % | 22 |
| Soybean oil | 2.14 | Arginine, % | 1.42 |
| Dical P | 1.73 | Gly&Ser, % | 1.97 |
| Limestone | 1.08 | Histidine, % | 0.58 |
| Salt | 0.46 | Isoleucine, % | 0.90 |
| Methionine MHA | 0.35 | Leucine, % | 1.88 |
| Vit premix | 0.25 | Lysine, % | 1.31 |
| L-Lysine | 0.17 | Met&Cys, % | 0.99 |
| L-Threonine | 0.11 | Methionine, % | 0.64 |
| TOTAL | 100 | Phen &Tyr, % | 1.48 |
| | | Phenylalanine, % | 1.03 |
| | | Threonine, % | 0.92 |
| | | Tryptophan, % | 0.29 |
| | | Valine, % | 1.00 |
| | | Linoleic acid, % | 2.56 |
| | | Calcium, % | 0.93 |
| | | Phos avail, % | 0.45 |
| | | Potassium, % | 0.86 |
| | | Sodium, % | 0.20 |
| | | Chloride, % | 0.32 |
| | | Magnesium, mg/kg | 2073.69 |
| | | Manganese, mg/kg | 124.14 |
| | | Zinc, mg/kg | 129.17 |
| | | Iron, mg/kg | 570.12 |
| | | Copper, mg/kg | 18.92 |
| | | Iodine, mg/kg | 1.50 |
| | | Selenium | 0.19 |
| | | Riboflavin | 8.19 |
| | | Pantothenic acid | 14.13 |
| | | Niacin | 49.30 |
| | | Vit $B_{12}$ | 0.01 |
| | | Choline | 1692.49 |
| | | Biotin | 0.18 |
| | | Folacin | 2.16 |

| | | | |
|---|---|---|---|
| | | Thiamin | 4.28 |
| | | Pyridoxine | 7.25 |
| | | Crude fat, % | 4.74 |
| | | Dig Phe + Tyr, % | 1.70 |

**Table 3-2.** Analyzed nutrient composition of the experimental SBM and corn sources.

| **Item (%, as-fed basis)** | **SBM** | **Corn** |
|---|---|---|
| Dry matter | 87.0 | 86.2 |
| Crude Protein[1] | 47.3 | 7.84 |
| Crude Fat[2] | 1.32 | 3.62 |
| Total P[3] | 0.773 | 0.270 |
| Phytate P[4] | 0.493 | 0.213 |
| Non-Phytate P[5] | 0.280 | 0.057 |
| Calcium | 0.349 | 0.000 |

[1]Crude protein determined by combustion analysis, AOAC Official Method 990.03 (2006)

[2]Crude Fat determined by Ether extraction, AOAC Official Method 920.339 (A)

[3]Mineral analyses determined via inductively coupled plasma optical emission spectroscopy, AOAC Official Method 990.08 (2006)

[4]Phytate P content was determined by method references Analytical Biochemistry Vol. 77:536-539 (1977)

[5]Calculated as the difference between total P and phytate P

**Table 3-3.** Ingredient composition, calculated nutrient analysis, and analyzed composition for select nutrients of experimental diets fed to broilers from 16 to 23 d of age.[1]

| | SBM-low | SBM-medium | SBM-high | Corn-low | Corn-medium | Corn-high | C-SBM-low | C-SBM-medium | C-SBM-high |
|---|---|---|---|---|---|---|---|---|---|
| ***Ingredient, % as fed*** | | | | | | | | | |
| Soybean meal (47%) | 40.00 | 51.00 | 62.00 | - | - | - | 10.27 | 20.53 | 30.80 |
| Corn | - | - | - | 22.00 | 46.00 | 72.00 | 20.95 | 41.96 | 62.95 |
| Cornstarch | 36.10 | 29.70 | 22.23 | 33.54 | 18.85 | 2.94 | 31.29 | 15.49 | - |
| Sucrose | 18.05 | 14.85 | 11.11 | 16.77 | 9.43 | 1.47 | 15.65 | 7.74 | - |
| Potato protein[2] | 1.61 | - | - | 23.02 | 20.82 | 18.45 | 16.56 | 8.71 | - |
| Soybean oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.55 | 2.55 | 2.55 |
| Calcium carbonate | 0.740 | 0.947 | 1.160 | 0.925 | 1.151 | 1.396 | 0.420 | 0.733 | 0.983 |
| Vitamin and Mineral premix[3] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Phytase premix[4] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Sodium phosphate, monobasic | - | - | - | 0.700 | 0.700 | 0.700 | - | - | - |
| Dicalcium Phosphate | - | - | - | - | - | - | 0.910 | 0.910 | 0.910 |
| Salt | 0.500 | 0.500 | 0.500 | 0.250 | 0.250 | 0.250 | 0.500 | 0.500 | 0.500 |
| MHA | 0.200 | 0.200 | 0.200 | - | - | - | - | - | - |
| L-Lysine HCl | - | - | - | - | - | - | - | - | 0.170 |
| Threonine | - | - | - | - | - | - | - | - | 0.070 |
| Choline Cl | - | - | - | - | - | - | - | - | 0.070 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Titanium dioxide | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| ***Calculated*** | | | | | | | | | |
| ***analysis*** | | | | | | | | | |
| ME, kcal/kg | 3218 | 3075 | 2930 | 3605 | 3519 | 3427 | 3513 | 3312 | 3099 |
| Crude Protein, % | 20.50 | 24.36 | 29.51 | 20.50 | 20.50 | 20.50 | 20.06 | 20.13 | 19.51 |
| Crude Fat, % | 2.528 | 2.669 | 2.810 | 2.797 | 3.658 | 4.591 | 3.439 | 4.325 | 5.211 |
| Total P, % | 0.315 | 0.397 | 0.482 | 0.259 | 0.319 | 0.385 | 0.328 | 0.453 | 0.577 |
| Non-phytate P, % | 0.112 | 0.143 | 0.174 | 0.181 | 0.194 | 0.209 | 0.209 | 0.250 | 0.291 |
| Phytate P, % | 0.197 | 0.251 | 0.306 | 0.047 | 0.098 | 0.153 | 0.095 | 0.190 | 0.286 |
| Calcium, % | 0.456 | 0.576 | 0.698 | 0.375 | 0.463 | 0.559 | 0.409 | 0.565 | 0.720 |
| Ca:total P ratio | 1.4:1 | 1.4:1 | 1.4:1 | 1.4:1 | 1.4:1 | 1.4:1 | 1.4:1 | 1.4:1 | 1.4:1 |
| ***Analyzed*** | | | | | | | | | |
| ***composition*** | | | | | | | | | |
| CP, % | 22.23 | 24.51 | 28.03 | 21.84 | 20.79 | 19.53 | 20.00 | 19.82 | 19.39 |
| Total P, % | 0.275 | 0.340 | 0.440 | 0.290 | 0.325 | 0.390 | 0.315 | 0.415 | 0.545 |
| Phytate P, % | 0.202 | 0.247 | 0.310 | 0.068 | 0.125 | 0.173 | 0.117 | 0.223 | 0.302 |
| Ca, % | 0.470 | 0.530 | 0.725 | 0.380 | 0.460 | 0.590 | 0.430 | 0.520 | 0.755 |
| Ca:total P ratio | 1.7 | 1.6 | 1.6 | 1.3 | 1.4 | 1.5 | 1.4 | 1.3 | 1.4 |
| Ti, % | 0.248 | 0.244 | 0.251 | 0.238 | 0.239 | 0.236 | 0.232 | 0.245 | 0.235 |

[1]All diets were provided in mash form on an *ad libitum* basis.

[2]Potato protein= 80.46% CP.

[3]Vitamin and Mineral premix includes per kg of diet: 8,000 IU vitamin A, 3,000 IU vitamin $D_3$, 1.1 mg thiamine, 6.6 mg riboflavin, 1.4 mg pyridoxine, 0.7 mg folic acid, 0.03 mg biotin, 0.01 mg vitamin $B_{12}$, 17 IU vitamin E, 0.4 g choline chloride, 28 mg niacin, 6.6 mg calcium-D-panthotenic acid, 0.8 mg menadione, 1.5 mg I, 0.15 mg Se, 100 mg Mn, 11 mg Cu, 100 mg Zn and 50 mg Fe.

[4]58 g of enzyme mixed with 942 g of finely ground corn to contain 800 phytase units (U/g of enzyme premix.

**Table 3-4.** Growth performance, gizzard pH, and tibia characteristics in birds fed corn, SBM or C-SBM without or with phytase supplementation[1]

| | Without phytase | | | With phytase | | | |
|---|---|---|---|---|---|---|---|
| | Low | Medium | High | Low | Medium | High | SEM |
| **Corn** | | | | | | | |
| **BW gain, g/chick** | 236.0 | 263.9 | 369.3 | 208.8 | 264.0 | 367.5 | 11.15 |
| **Feed intake, g/chick** | 517.9 | 510.9 | 577.3 | 448.5 | 496.9 | 559.8 | 13.38 |
| **FCR, g:g** | 2.287 | 1.963 | 1.602 | 2.103 | 1.952 | 1.530 | 0.0636 |
| **Gizzard pH** | 2.070 | 2.041 | 1.791 | 2.073 | 2.344 | 2.139 | 0.1617 |
| **Tibia ash, %** | 48.62 | 49.24 | 49.02 | 48.89 | 50.91 | 51.70 | 0.6808 |
| **Tibia P, g/bone** | 0.1176$^{bc}$ | 0.1173$^{bc}$ | 0.1235$^{b}$ | 0.1069$^{c}$ | 0.1231$^{bc}$ | 0.1451$^{a}$ | 0.0059 |
| **Tibia Ca, g/bone** | 0.2126 | 0.2156 | 0.2298 | 0.1928 | 0.2218 | 0.2615 | 0.0104 |
| **SBM** | | | | | | | |
| **BW gain, g/chick** | 376.3 | 407.2 | 444.5 | 390.3 | 430.1 | 490.4 | 10.70 |
| **Feed intake, g/chick** | 605.8$^{b}$ | 628.2$^{b}$ | 619.2$^{b}$ | 614.7$^{b}$ | 616.1$^{b}$ | 664.0$^{a}$ | 9.794 |
| **FCR, g:g** | 1.614 | 1.545 | 1.396 | 1.578 | 1.436 | 1.362 | 0.0335 |
| **Gizzard pH** | 2.861 | 2.993 | 3.045 | 2.923 | 2.898 | 3.446 | 0.1918 |
| **Tibia ash, %** | 47.30 | 49.67 | 49.97 | 48.92 | 51.61 | 51.94 | 0.512 |
| **Tibia P, g/bone** | 0.1268 | 0.1305 | 0.1293 | 0.1226 | 0.1363 | 0.1445 | 0.0066 |
| **Tibia Ca, g/bone** | 0.2350 | 0.2400 | 0.2366 | 0.2249 | 0.2446 | 0.2575 | 0.0116 |
| **C-SBM** | | | | | | | |
| **BW gain, g/chick** | 359.1 | 405.5 | 455.8 | 337.6 | 391.8 | 456.7 | 9.33 |
| **Feed intake, g/chick** | 575.4 | 634.4 | 670.6 | 567.7 | 601.0 | 653.4 | 8.06 |
| **FCR, g:g** | 1.604 | 1.567 | 1.455 | 1.689 | 1.568 | 1.436 | 0.0291 |
| **Gizzard pH** | 2.026 | 2.105 | 2.074 | 2.048 | 2.298 | 2.380 | 0.1298 |
| **Tibia ash, %** | 49.60 | 51.57 | 51.65 | 51.27 | 52.13 | 52.28 | 0.547 |
| **Tibia P, g/bone** | 0.1140 | 0.1503 | 0.1503 | 0.1339 | 0.1550 | 0.1551 | 0.0051 |
| **Tibia Ca, g/bone** | 0.2074 | 0.2720 | 0.2707 | 0.2373 | 0.2743 | 0.2765 | 0.0090 |

[1]Data are means of 8 replicate cages with 5 birds per cage (16 replicate cages for C-SBM high inclusion diets)

**Table 3-4.1.** P-Value of growth performance, gizzard pH, and tibia characteristics in birds fed corn, SBM or C-SBM without or with phytase supplementation

| | ***P*- value** | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Without phytase | | With phytase | |
| | Phytase | Level | PxL | L | Q | L | Q |
| **Corn** | | | | | | | |
| **BW gain, g/chick** | 0.2964 | <0.0001 | 0.3995 | <0.0001 | 0.0270 | <0.0001 | 0.0939 |
| **Feed intake, g/chick** | 0.0036 | <0.0001 | 0.0798 | 0.0072 | 0.0562 | <0.0001 | 0.7424 |
| **FCR, g:g** | 0.0959 | <0.0001 | 0.4225 | <0.0001 | 0.9550 | <0.0001 | 0.1012 |
| **Gizzard pH** | 0.1072 | 0.3836 | 0.5138 | 0.2431 | 0.6014 | 0.7938 | 0.2352 |
| **Tibia ash, %** | 0.0085 | 0.0475 | 0.1999 | 0.6801 | 0.5954 | 0.0092 | 0.4792 |
| **Tibia P, g/bone** | 0.2480 | 0.0019 | 0.0315 | 0.4357 | 0.6269 | 0.0003 | 0.7878 |
| **Tibia Ca, g/bone** | 0.4817 | 0.0007 | 0.0582 | 0.2182 | 0.6581 | 0.0003 | 0.7712 |
| **SBM** | | | | | | | |
| **BW gain, g/chick** | 0.0029 | <0.0001 | 0.3158 | <0.0001 | 0.7929 | <0.0001 | 0.4703 |
| **Feed intake, g/chick** | 0.0911 | 0.0093 | 0.0196 | 0.3333 | 0.1953 | 0.0022 | 0.0718 |
| **FCR, g:g** | 0.0339 | <0.0001 | 0.4480 | <0.0001 | 0.3197 | 0.0002 | 0.4327 |
| **Gizzard pH** | 0.4385 | 0.1510 | 0.4243 | 0.4694 | 0.8571 | 0.0868 | 0.2686 |
| **Tibia ash, %** | <0.0001 | <0.0001 | 0.9339 | 0.0020 | 0.1306 | 0.0003 | 0.0596 |
| **Tibia P, g/bone** | 0.3044 | 0.1788 | 0.3526 | 0.5993 | 0.5445 | 0.0922 | 0.8048 |
| **Tibia Ca, g/bone** | 0.5924 | 0.3249 | 0.4190 | 0.8544 | 0.5862 | 0.1452 | 0.8557 |
| **C-SBM** | | | | | | | |
| **BW gain, g/chick** | 0.1434 | <0.0001 | 0.4233 | <0.0001 | 0.8659 | <0.0001 | 0.6794 |
| **Feed intake, g/chick** | 0.0049 | <0.0001 | 0.3429 | <0.0001 | 0.3117 | <0.0001 | 0.3359 |
| **FCR, g:g** | 0.3549 | <0.0001 | 0.1818 | <0.0001 | 0.2068 | <0.0001 | 0.8946 |
| **Gizzard pH** | 0.1116 | 0.3030 | 0.5233 | 0.7731 | 0.7323 | 0.0847 | 0.6421 |
| **Tibia ash, %** | 0.0390 | 0.0144 | 0.5650 | 0.0104 | 0.2004 | 0.1798 | 0.6228 |
| **Tibia P, g/bone** | 0.0245 | <0.0001 | 0.2636 | <0.0001 | 0.0150 | 0.0035 | 0.1244 |
| **Tibia Ca, g/bone** | 0.0956 | <0.0001 | 0.2936 | <0.0001 | 0.0137 | 0.0019 | 0.1385 |

**Table 3-5.** Dry matter, phosphorus and calcium digestibility and utilization in birds fed either corn, SBM or C-SBM without or with phytase supplementation[1]

| | Without phytase | | | With phytase | | | |
|---|---|---|---|---|---|---|---|
| | Low | Medium | High | Low | Medium | High | SEM |
| **Corn, g/kg** | | | | | | | |
| **Ileal DM digestibility, %** | 87.62 | 87.01 | 82.15 | 86.24 | 85.82 | 82.73 | 0.7598 |
| **Ileal P digestibility, %** | 81.07 | 70.55 | 58.22 | 91.60 | 85.02 | 75.91 | 1.8876 |
| **Ileal Ca digestibility, %** | 81.46 | 71.87 | 66.53 | 84.44 | 77.38 | 69.88 | 2.9550 |
| **Apparent DM retention, %** | 87.03$^{a}$ | 84.45$^{b}$ | 82.70$^{c}$ | 84.25$^{b}$ | 84.38$^{b}$ | 82.22$^{c}$ | 0.3379 |
| **Apparent P retention, %** | 80.18$^{b}$ | 68.20$^{d}$ | 61.79$^{e}$ | 83.48$^{a}$ | 80.39$^{b}$ | 73.33$^{c}$ | 0.8769 |
| **Apparent Ca retention, %** | 73.87$^{a}$ | 65.84$^{b}$ | 57.74$^{c}$ | 77.82$^{a}$ | 75.35$^{a}$ | 74.56$^{a}$ | 1.8760 |
| **SBM, g/kg** | | | | | | | |
| **Ileal DM digestibility, %** | 80.06 | 74.86 | 73.60 | 78.61 | 77.61 | 71.47 | 1.141 |
| **Ileal P digestibility, %** | 73.80 | 72.23 | 64.79 | 79.62 | 71.86 | 71.93 | 3.012 |
| **Ileal Ca digestibility, %** | 58.20 | 59.92 | 59.30 | 50.32 | 51.01 | 37.16 | 5.107 |
| **Apparent DM retention, %** | 77.24$^{a}$ | 72.81$^{c}$ | 68.05$^{d}$ | 77.81$^{a}$ | 75.02$^{b}$ | 65.42$^{e}$ | 0.438 |
| **Apparent P retention, %** | 72.05$^{c}$ | 67.09$^{d}$ | 63.69$^{e}$ | 81.92$^{a}$ | 78.56$^{b}$ | 68.77$^{d}$ | 0.873 |
| **Apparent Ca retention, %** | 54.40 | 49.38 | 55.73 | 63.33 | 58.36 | 60.53 | 1.986 |
| **C-SBM, g/kg** | | | | | | | |
| **Ileal DM digestibility, %** | 83.88 | 79.42 | 73.18 | 84.44 | 81.39 | 72.91 | 1.040 |
| **Ileal P digestibility, %** | 76.16$^{b}$ | 54.67$^{d}$ | 45.62$^{e}$ | 88.08$^{a}$ | 76.74$^{b}$ | 60.03$^{c}$ | 1.462 |
| **Ileal Ca digestibility, %** | 80.04 | 58.69 | 51.04 | 83.89 | 66.91 | 47.35 | 2.907 |
| **Apparent DM retention, %** | 83.48$^{a}$ | 77.49$^{d}$ | 74.42$^{f}$ | 82.49$^{b}$ | 78.63$^{c}$ | 75.29$^{e}$ | 0.286 |
| **Apparent P retention, %** | 73.95$^{b}$ | 53.14$^{d}$ | 48.22$^{e}$ | 78.95$^{a}$ | 62.20$^{c}$ | 51.51$^{d}$ | 0.773 |
| **Apparent Ca retention, %** | 76.87 | 60.26 | 52.44 | 84.38 | 70.58 | 57.02 | 1.381 |

[1]Data are means of 8 replicate cages with 5 birds per cage (16 replicate cages for C-SBM high inclusion diets)

**Table 3-5.1.** P-Value of Dry matter, phosphorus and calcium digestibility and utilization in birds fed either corn, SBM or C-SBM without or with phytase supplementation

| | ***P*- value** | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Without phytase | | With phytase | |
| | Phytase | Level | PxL | L | Q | L | Q |
| **Corn, g/kg** | | | | | | | |
| **Ileal DM digestibility, %** | 0.2906 | <0.0001 | 0.3709 | <0.0001 | 0.0411 | 0.0035 | 0.1895 |
| **Ileal P digestibility, %** | <0.0001 | <0.0001 | 0.1751 | <0.0001 | 0.883 | <0.0001 | 0.5401 |
| **Ileal Ca digestibility, %** | 0.1107 | <0.0001 | 0.9005 | 0.0053 | 0.5580 | 0.0008 | 0.9831 |
| **Apparent DM retention, %** | 0.0002 | <0.0001 | 0.0005 | <0.0001 | 0.1855 | 0.0010 | 0.0243 |
| **Apparent P retention, %** | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0016 | <0.0001 | 0.1840 |
| **Apparent Ca retention, %** | <0.0001 | <0.0001 | 0.0049 | <0.0001 | 0.8821 | 0.2951 | 0.7443 |
| **SBM, g/kg** | | | | | | | |
| **Ileal DM digestibility, %** | 0.7868 | <0.0001 | 0.1212 | 0.0014 | 0.2093 | 0.0005 | 0.1123 |
| **Ileal P digestibility, %** | 0.0960 | 0.0326 | 0.4121 | 0.0672 | 0.4552 | 0.0709 | 0.2654 |
| **Ileal Ca digestibility, %** | 0.0036 | 0.3442 | 0.3266 | 0.8702 | 0.8335 | 0.1148 | 0.3043 |
| **Apparent DM retention, %** | 0.8831 | <0.0001 | <0.0001 | <0.0001 | 0.7879 | <0.0001 | <0.0001 |
| **Apparent P retention, %** | <0.0001 | <0.0001 | 0.0019 | <0.0001 | 0.5084 | <0.0001 | 0.0033 |
| **Apparent Ca retention, %** | <0.0001 | 0.0361 | 0.4852 | 0.5541 | 0.0073 | 0.4018 | 0.2417 |
| **C-SBM, g/kg** | | | | | | | |
| **Ileal DM digestibility, %** | 0.3845 | <0.0001 | 0.5368 | <0.0001 | 0.3332 | <0.0001 | 0.1184 |
| **Ileal P digestibility, %** | <0.0001 | <0.0001 | 0.0062 | <0.0001 | 0.0020 | <0.0001 | 0.1815 |
| **Ileal Ca digestibility, %** | 0.2497 | <0.0001 | 0.0903 | <0.0001 | 0.0282 | <0.0001 | 0.7741 |
| **Apparent DM retention, %** | 0.1548 | <0.0001 | 0.0016 | <0.0001 | <0.0001 | <0.0001 | 0.5637 |
| **Apparent P retention, %** | <0.0001 | <0.0001 | 0.0012 | <0.0001 | <0.0001 | <0.0001 | 0.0049 |
| **Apparent Ca retention, %** | <0.0001 | <0.0001 | 0.0966 | <0.0001 | 0.0236 | <0.0001 | 0.9442 |

**Table 3-6.** Linear relationships between ileal or excreta P output (g/kg of DMI) vs. dietary P (g/kg of DM) of corn, SBM or C-SBM diets without or with phytase supplementation fed to broilers[1]

| | **Regression of ileal P output vs. P intake** | | **Regression of excreta P output vs. P intake** | |
|---|---|---|---|---|
| | Without phytase | With phytase | Without phytase | **With phytase** |
| **Corn** | | | | |
| **Slope** | 1.07±0.093 | 0.696±0.088 | 0.882±0.050 | **0.565±0.047** |
| **Intercept** | -0.281±0.035 | -0.196±0.033 | -0.212±0.019 | **-0.129±0.018** |
| **$r^2$** | 0.815 | 0.862 | 0.944 | **0.878** |
| **Estimate of true ileal P digestibility or true P retention, %** | -7.19[b] | 30.4 [a] | 11.8[b] | **43.5[a]** |
| **Endogenous P loss (mg/kg DMI)** | -281 | -196 | -212 | **-129** |
| **SBM** | | | | |
| **Slope** | 0.480±0.092 | 0.401±0.092 | 0.500±0.027 | **0.541±0.027** |
| **Intercept** | -0.067±0.036 | -0.054±0.036 | -0.066±0.011 | **-0.114±0.011** |
| **$r^2$** | 0.538 | 0.501 | 0.944 | **0.942** |
| **Estimate of true ileal P digestibility or true P retention, %** | 52.0 | 59.9 | 50.0 | **45.9** |
| **Endogenous P loss (mg/kg DMI)** | -67 | -54 | -66 | **-114** |
| **C-SBM** | | | | |
| **Slope** | 0.944±0.042 | 0.805±0.042 | 0.834±0.025 | **0.856±0.025** |
| **Intercept** | -0.238±0.021 | -0.247±0.021 | -0.188±0.013 | **-0.222±0.013** |
| **$r^2$** | 0.954 | 0.915 | 0.965 | **0.975** |
| **Estimate of true ileal P digestibility or true P retention, %** | 5.58[b] | 19.5[a] | 16.6 | **14.4** |
| **Endogenous P loss (mg/kg DMI)** | -238 | -247 | -188 | **-222** |

[1]Each value represents the mean of 8 replicates of 5 birds/replicate (16 replicate cages for C-SBM high inclusion diets). Regression of ileal or excreta P output (g/kg DMI) against dietary P content (g/kg DM) as determined by feeding broilers with diets containing grades levels of corn, SBM, or C-SBM without or with phytase. The slope represents true P indigestibility, and the intercept represents the endogenous P loss (g/kg DMI).

[a,b]True ileal P digestibility or retention with a common superscript are not different at $P < 0.05$ as compared with 95% CI derived from SE of respective slopes

**Table 3-6.1.** Linear relationships between ileal or excreta Ca output (g/kg of DMI) vs. dietary Ca (g/kg of DM) of corn, SBM or C-SBM diets without or with phytase supplementation fed to broilers

| | **Regression of ileal Ca output vs. Ca intake** | | **Regression of excreta Ca output vs. Ca intake** | |
|---|---|---|---|---|
| | Without phytase | With phytase | Without phytase | **With phytase** |
| **Corn** | | | | |
| **Slope** | 0.614±0.082 | 0.565±0.080 | 0.696±0.067 | **0.341±0.065** |
| **Intercept** | -0.184±0.045 | -0.172±0.042 | -0.178±0.036 | **-0.050±0.035** |
| **$r^2$** | 0.773 | 0.703 | 0.894 | **0.483** |
| **Estimate of true ileal Ca digestibility or true Ca retention, %** | 38.6 | 43.5 | 30.4[b] | **65.9[a]** |
| **Endogenous Ca loss (mg/kg DMI)** | -184 | -172 | -178 | **-50** |
| **SBM** | | | | |
| **Slope** | 0.239±0.140 | 0.623±0.143 | 0.382±0.063 | **0.409±0.066** |
| **Intercept** | 0.093±0.087 | -0.087±0.089 | 0.053±0.041 | **-0.011±0.042** |
| **$r^2$** | 0.265 | 0.497 | 0.714 | **0.505** |
| **Estimate of true ileal Ca digestibility or true Ca retention, %** | 76.1[a] | 37.7[b] | 61.8 | **59.1** |
| **Endogenous Ca loss (mg/kg DMI)** | 93 | -87 | 53 | **-11** |
| **C-SBM** | | | | |
| **Slope** | 0.815±0.071 | 0.948±0.072 | 0.758±0.038 | **0.788±0.038** |
| **Intercept** | -0.266±0.049 | -0.365±0.050 | -0.232±0.026 | **-0.293±0.027** |
| **$r^2$** | 0.863 | 0.789 | 0.919 | **0.947** |
| **Estimate of true ileal Ca digestibility or true Ca retention, %** | 18.5 | 5.2 | 24.2 | **21.2** |
| **Endogenous Ca loss (mg/kg DMI)** | -266 | -365 | -232 | **-293** |

[1]Each value represents the mean of 8 replicates of 5 birds/replicate (16 replicate cages for C-SBM high inclusion diets).

Regression of ileal or excreta Ca output (g/kg DMI) against dietary Ca content (g/kg DM) as determined by feeding broilers with diets containing grades levels of corn, SBM, or C-SBM without or with phytase. The slope represents true Ca indigestibility, and the intercept represents the endogenous Ca loss (g/kg DMI).

[a,b]True ileal P/Ca digestibility or retention with a common superscript are not different at $P < 0.05$ as compared with 95% CI derived from SE of respective slopes

# CHAPTER IV

# PHOSPHORUS ILEAL DIGESTIBILITY OF CORN, SBM AND DICALCIUM PHOSPHATE IS AFFECTED BY DIETARY CALCIUM LEVEL IN BROILERS WHEREAS ENDOGENOUS PHOSPHORUS LOSSES ARE NOT


## Abstract

The measurement of digestible P has been suggested as the preferable approach to assess P availability in feed ingredients for poultry in order to minimize P excretion into the environment (WPSA, 2013). Previous regression analysis estimations of true ileal P digestibility (TIPD) in SBM using a fixed Ca:total P ratio have produced values lower than the apparent ileal digestibility (AIPD) values in broilers. Therefore, the main objective of this current study was to determine if digestibility values of ingredients would be additive when using the direct method approach. Also, to study the effect of Ca (using two fixed Ca levels 0.35 and 0.85%) on AIPD values of corn and dicalcium phosphate, on AIPD and TIPD values of SBM and on the estimations of endogenous P losses. A total of 560 Ross 308 male broiler chicks were fed from d 19 to 22, seven experimental diets that consisted of one level of corn, one level of C-SBM, one level of three levels (20, 40 and 60%; low, medium and high, respectively) of SBM, Dicalcium phosphate and two gelatin-based P-free diets, all diets containing either 0.35 or 0.85% levels of Ca. Birds fed the higher SBM levels thus having higher P content regardless of Ca level grew better and had the best FCR value as P deficiency reduces growth performance primarily due

to reductions in FI. Body weight gain, FCR, and Apparent P digestibility reduced (*P < 0.001)* with high Ca level. Birds fed DCP had a significantly higher (92.64 and 83.55%) AIPD compared to the other diets. Increasing SBM level from low to medium improved AIPD in both Ca concentrations however, increasing SBM from 40 to 60% didn't further increase AIPD.

The level of Ca significantly influenced (*P < 0.001)* the TIPD for broiler chickens with increasing levels of Ca in their diets. The TIPD of SBM with 0.85% Ca was estimated to be 59.3%, that is 23 points lower than the TIPD estimation of SBM with 0.35% Ca (82%). Calcium level didn't affect EPL (*P > 0.4*) in both direct and indirect methodologies. In summary, TIPD values from SBM are greater than AIPD values when a fixed dietary Ca level is employed, however its concentration has a deep impact in both TIPD and AIPD. EPL estimates were not affected in both methodology employed.

## Introduction

Phosphorous is the second most abundant mineral in the body and the third most expensive nutrient for monogastric animals. Phosphorus is an essential inorganic nutrient for all living organisms. It is required as a structural component in nucleic acids and phospholipids, as an element in intermediates in carbon metabolism, and to allow (in) activation of a wide range of enzymes. A portion (about 60 to 80%) of the P in feed ingredients of plant origin is present as the mineral salt of phytic acid, in the form of phytates. Phytate P (PP) is either unavailable to, or poorly utilized by, the chick. As a result, diets are usually supplemented with feed phosphates or phytase or both in order to fulfill the animal's requirement for available P. The poultry industry in the United

States, and throughout the world, uses millions of tons of high quality feed grade inorganic phosphates each year. The bioavailability of the P in these inorganic sources is usually very high for poultry. The Ileal P digestibility of the anhydrous DCP of 59.0%, MDCP of 70.7% observed by Bikker et al. (2016), and monosodium phosphate digestibility 66 to 78% (Van der Klis and Versteegh, 1996). In recent years, there is increasing interest in improving the utilization of dietary P due to concerns over environmental pollution, and depletion of nonrenewable inorganic phosphate reserves, and its high prices (Selle and Ravindran, 2007).

In addition, phytic acid chelates mineral elements, including calcium, and thereby reduces their availability in whole or part. However, the quantitative effect of phytic acid on the total dietary level of any element in a feed has been neglected. It is important, therefore, to know the phytic acid content of feeds in order that adequate amounts of essential minerals may be supplied, particularly P and Ca. Nutritionists look for ingredients that will provide high concentrations of essential nutrients and utilize least cost ration software to compare and choose the ingredients with the highest nutritive composition, consistency and value. However, the variation in P availability between different feed raw materials is high (Shastak and Rodehutscord, 2015), and it is generally accepted that the use of P as a globally finite resource can be optimized by considering the differences that exist in P availability of feed raw materials.

To provide meaningful and useful data to estimate P digestibility in different diets found around the world it is necessary to determine the available P in the main ingredients available for monogastric species that contain significant amounts of PP. The

apparent or TPD values in common feed ingredients for pigs were determined by three approaches, namely regression analysis, the direct method, and the substitution method (Fan et al., 2001; Bohlke et al., 2005; Fang et al., 2007; Stein et al., 2008; Rojas et al., 2013). In poultry, TPD data of feed ingredients are limited, and no-precise method has been developed. Only limited attempts have been made to determine the digestible P content in feed ingredients for poultry using the trial protocol proposed by the World's Poultry Science Association and two approaches, namely the direct method (Wu et al., 2004; Leytem et al., 2008) and regression method (Dilger and Adeola, 2006; Mutucumarana et al., 2014; Rodehutscord et al., 2017) have been used. Corresponding data for other common feed ingredients are still lacking. This current experiment was performed using the test ingredient corn and or SBM as the sole dietary source of P and Ca. Corn and SBM are typically the cereal grains of choice for poultry feeds in the United States. Soybean meal is the dominant protein supplement used in poultry diets and is the standard to which alternative protein sources are compared. Much of the P in SBM and corn grain is bound to phytate, rendering the P in a poorly digested form for monogastric species. Birds do not produce sufficient phytase, which is the enzyme required to break down phytate and release the bound P.

Different response criteria and descriptive terms for available P have been used in the literature over the past seven decades (Shastak and Rodehutscord, 2013). These differences make it difficult to compare results obtained by using different techniques in different laboratories, and to compile comprehensive feedstuff tables needed by the industry. In an attempt to improve this situation, the Working Group No 2: Nutrition of

the European Federation of Branches of the World's Poultry Science Association proposed a standard protocol for the determination of P availability (WPSA, 2013). This protocol is based on using digestibility measured at the terminal ileum of broiler chickens (prececal digestibility of P[pcdP], otherwise referred to as ileal digestibility). The protocol defines assay details relevant for the outcome of the measurement, such as age of birds, minimum number of experimental replicates, diet composition, and P and Ca levels in the diet.

In a recent study, using the trial protocol proposed by the World's Poultry Science Association (WPSA, 2013), true retainable P and TPD contents of corn and canola meal for broilers were measured using the regression approach (Mutucumarana et al., 2014). Rodehutscord et al. (2017) conducted a ring test was conducted with 17 test stations and found that diet, station, and their interaction significantly affected ($P < 0.05$) the prececal digestibility values of P and Ca of the diets. The prececal P digestibility of SBM was determined by linear regression and varied among stations from 19 to 51%, with significant differences among stations. Thus, Rodehutscord et al. (2017) suggested that the WPSA protocol for the determination of digestible P be should extended to include the standardization of the pre-experimental period. No published data that compares the different measurements of P availability in poultry are currently available.

Therefore, the objectives of the current study were to study the effect of Ca on AIPD values of corn and dicalcium phosphate, on AIPD and TPD values of SBM, on the estimation of endogenous P losses, and to determine if TPD of individual ingredients

(e.g. corn and soybean meal) would be additive when ingredients are combined in commercial type poultry diets.

## Materials and method

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee (ACUC). This study was conducted at the Animal Science Department of the University of Missouri-Columbia.

## Bird husbandry

A total of 560 Ross 308 day-old male broilers was used in this study and were randomly assigned to treatments (14 treatments) with eight replicate pens of five birds per treatment. Broilers were weighed, wing banded, and randomly assigned to pens in battery brooders, with each pen containing one waterer trough and one feeder trough. Temperatures were set to 33°C upon placement and were decreased gradually to 27°C by the conclusion of the experiment. Lighting intensity was maintained at 30 lux. Daily observations were made following the animal care and management guidelines according to the standard site practices. The number of mortalities, their weights and, the number of birds per pen were recorded each weigh day. Birds and feed were weighed on a per pen basis on day of arrival from the hatchery (d 0), the beginning of experimental period (d 19), and at the termination of the experimental period (d 22) for the determination of feed intake (FI), body weight gain (BWG), and feed conversion ratio(FCR).

## Facilities

In two rooms of same design and conditions with four stainless steel brooders batteries 112 pens (56 pens/room) were used. Each pen within a battery can house five birds to three weeks of age.

The two rooms are 219 and 220 square feet each. Both rooms have epoxy-coated walls with individual light, temperature and humidity controls, and are supplied with both distilled and tap water.

## Diets

All birds were fed a standard corn-soybean meal (SBM) starter diet (Table 4-1) that met all nutrient requirements as indicated by NRC (1994) from hatch until 19 d of age. Starter diets were free of feed additives such as enzymes, organic acids, essential oils or medication. Ingredients were bought from local sources and were chosen to represent ingredients with low and high P contents, respectively. Representative samples were obtained and analyzed in duplicate for DM, total P, Phytate P, and Ca.

Concentrations of Ca and P in the ingredients were utilized in the formulation of Experimental diets. Vitamin $D_3$ concentration was about 2,500 IU per kg of the diet. Titanium oxide ($TiO_2$) at the rate of 3 g/kg was used as an indigestible marker to calculate P digestibility and retention. Experimental diets and ileal samples were analyzed for Ca, P, $TiO_2$.

On d 19 of the experiment, dietary treatments were fed until the end of the study (d 22) see description of treatments (Table 4-2). The diets were fed in mash form, and

offered ad libitum with water freely available. Major ingredients were analyzed for Ca, P, PP, and Na and the analyzed values were used to formulate the test diets. Experimental diets consisted of a corn-based diet, three diets containing graded levels of SBM (20, 40 and 60%), a corn-SBM-based diet, a P-free diet (gelatin based), and a P-free diet supplemented with dicalcium phosphate (DCP) to provide 0.4% P. Limestone was used to achieve the two targeted dietary Ca levels (0.35 or 0.85%). P-free diets were formulated based on Liu et al. (2012) and contained 20% gelatin. The analyzed nutrient composition of corn and SBM are presented in Table 4-3, and the diet composition is presented in Table 4-4.

## Sample collection and processing

On d 22 post hatching, chicks were euthanized via carbon dioxide ($CO_2$) asphyxiation, and the body cavity then opened and the ileal digesta collected as described by Ravindran et al. (2005). The ileal digesta from the distal two-thirds of the ileum (defined as extending from Meckel's diverticulum to the ileocecal junction) were collected (Rodehutscord et al., 2012). Contents of this segment from three chicks were flushed into a plastic container using distilled water, pooled per pen stored at −20°C, then dried at 55$^{o}$C, ground to pass through a 1mm sieve before chemical analysis.

## Chemical analysis

Samples of corn and SBM were analyzed for DM, CP (nitrogen × 6.25), crude fat, ash, total P, phytate P and Ca. Samples of the test diets were analyzed for DM, CP (nitrogen ×

6.25), total P, Ca and titanium (Ti); samples of ileal digesta and excreta were analyzed for DM, total P, Ca and Ti.

Dry matter and ash were determined using standard procedures (AOAC International, 2005; methods 930.15 and 942.05, respectively). Phosphorus was determined in the diets and digesta, after samples hydrolysis using a nitric-perchloric wet ash procedure ($HNO_3$: $HClO_4$: $H_2O$), using a spectrophotometer after reaction with ammonium molybdovanadate (*AOAC Official Method 966.01 plant tissue).*

Concentration of Ca were determined by the flame atomic absorption spectrometry (Varian FS240 AA Varian Inc., Palo Alto, CA). Other mineral concentrations were determined via flame atomic absorption spectroscopy AOAC Official Method 975.03B(b) Metals in Plants and Pet Foods Atomic Absorption Spectrophotometric Method (applicable to Ca, Cu, K, Mg, Mn, and Zn) after nitric-perchloric wet ash sample preparation with hydrochloric acid sample matrix. Phytate P content was determined by method references Analytical Biochemistry Vol. 77:536-539 (1977).

Crude protein (CP) was determined by combustion analysis, AOAC Official Method 990.03 (2006). Crude Fat was determined by ether extraction, AOAC Official Method 920.39 (A) and titanium was determined at the University of Missouri Chemical lab by a previously described procedure (Journal of Animal Science, 2004, 82:179-183).

## Calculations

Apparent ileal P digestibility (AIPD) was calculated for all diets by the index method using the following equation:

$$AIPD, \% = 1 - [(T_I/T_O) \times (P_O/P_I)]$$

Where TI is the Ti concentration in the diet, $T_O$ is the Ti concentration in the ileal digesta, $P_O$ is the P concentration in the ileal digesta and $P_I$ is the P concentration in the diet. All analyzed values were expressed as grams per kilogram of DM.

True P digestibility (TPD) in SBM was calculated according to the procedure outlined by Dilger and Adeola (2006). Total output of P in the ileal digesta, expressed as g/kg DM intake (DMI), were calculated via the following equation.

$$P_{O\text{-}DMI}\ (g/kg) = P_{O\text{-}DMO} \times (T_I/T_O)$$

Where $P_{O\text{-}DMI}$ and $P_{O\text{-}DMO}$ represent the P output (as analyzed in digesta) on DMI and DM output bases, respectively; $T_I$ is the Ti concentration in the diet (g/kg DM); and $T_O$ is the Ti concentration in the ileal digesta (g/kg DM digesta). Phosphorus output was regressed against dietary phosphorus contents per 24 cages for SBM diets using the following statistical model.

$$P_{O\text{-}DMI}\ (g/kg) = TPI \times P_I) + EPL$$

Where $P_{O\text{-}DMI}$ represents the phosphorus output concentration on DMI basis (dependent variable), $P_I$ represents dietary phosphorus content on a DM basis (independent variable), TPI represents true phosphorus indigestibility, and EPL represents endogenous phosphorus in ileal digesta on DMI basis. In this equation, TPI and EPL are the slope and intercept, respectively, of a simple linear regression of $P_{O\text{-}DMI}$ on $P_{I.}$ True phosphorus indigestibility is an indirect measure of the inefficiency at which dietary nutrient is extracted by the bird; therefore, the true ileal digestibility (TIDC) coefficient of P was calculated as:

TPD = 1 – (TPI)

Where TPD and TPI represent the true phosphorus (ileal) digestibility coefficient and true P indigestible estimate, respectively.

Casein, which is high in P digestibility, to cornstarch-based basal diets does not affect the estimation of EPL or true P digestibility and retention associated with SBM for broiler chickens using the regression method of Liu and coworkers (2014).

## Statistical analyses

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc. (2016). Pen served as the experimental unit for all statistical analyses, and differences were considered significant at an alpha level of 0.05. Data were analyzed by 2-way ANOVA to evaluate the effect of Ca level, diet type, and their interaction. Mean TIDC of P and Ca, and endogenous P and Ca loss (g/kg DMI) estimates were obtained by regressing P or Ca output (g/kg DMI) against dietary P or Ca content (g/kg DM) from samples pooled per cage. Therefore, standard errors for true coefficients were based on a total of 24 observations for each test diet.

## Results

## Dietary analyses

Results of Ca, P, and PP analysis of SBM and corn sources used in the study are presented on an as fed basis Table 4-3.

The analyzed dietary PP level of SBM was higher than the calculated value. The analyzed PP values for corn and SBM were 0.186 and 0.468%, respectively. The

calculated values for PP for corn and SBM were on average 0.19 and 0.25%, respectively. Analyzed Ca was also slightly higher and analyzed P slightly lower than formulated values and would have increased the ratio of Ca:AvP slightly above formulated values. To account for these differences between calculated and analyzed values, nutrient intake, digestibility, and retention values reported herein were calculated utilizing the analyzed nutrient values of each diet. Ingredient composition, calculated nutrient analysis, and analyzed composition for select nutrients (CP, Ca, Phytate P, and Total P) of experimental diets fed to broilers from 19 to 22 d of age are shown in Table 4-4.

## Growth performance

Birds in all treatments were healthy throughout the entire trial and readily consumed their diets. No mortality occurred when experimental diets were fed to birds from d 19 to 22. Effects of dietary treatments containing 0.35 or 0.85% Ca on FI, BWG, and FCR are summarized in Table 4-5.

## Body weight gain (BWG)

There was a diet by Ca level ($P < 0.001$) interaction on BWG. The interaction occurred as a result of differences in BWG amongst diets in birds fed 0.35 and 0.85% Ca. Body weight gain of birds fed high levels of SBM 0.35% Ca (260 g) were significantly higher compared to that of the birds fed 0.85% Ca (187 g). A similar response was observed in birds that were fed C-SBM diets and 0.35% Ca with their BGW (202 g) being much higher than those fed C-SBM and 0.85% Ca (179 g).

Significant Ca and diet effects *(P < 0.001*) among birds were also observed for BWG. Birds fed 0.35% Ca had higher BWG than birds fed 0.85% Ca.

With respect to diet, birds fed the lowest dietary P inclusion, the P-free, and DCP diets had the lowest BWG regardless of Ca level.

## Feed Intake (FI)

A diet by Ca level interaction *(P = 0.190*) was not observed for FI. However, there was a significant diet effect (*P < 0.001)* on FI. Birds fed P-free and DCP diets at both levels of Ca consistently consumed the least amount of feed. While FI for the other diets were higher. There was no Ca level effect (*P = 0.826*) on FI hence, FI of similar diets were similar irrespective of Ca level.

## Feed conversion ratio (FCR)

There was diet by Ca level interaction *(P = 0.033*) observed for FCR. The interaction occurred as a result of differences in FCR amongst diets in birds fed 0.35 and 0.85% Ca. The FCR of birds fed corn (6.34 g:g), SBM-low (6.06 g:g), and P-free (-5.40 g;g) diets was poorer in birds fed 0.85% Ca compared to birds fed 0.35% Ca, 4.62, 4.60, and -3.17 g:g. There was a diet effect (*P < 0.001)* observed for FCR among birds. Birds fed P-free and DCP diets had the poorest feed efficiency with negative FCR values. There was no Ca level effect (P = 0.854) observed for FCR among birds.

## Dry matter and P digestibility

The effects of dietary treatments on DM and apparent P are summarized in Table 4-6.

There was a diet by Ca level interaction (*P < 0.001)* observed for DM digestibility. The interaction occurred due to differences in diets DM digestibility caused by Ca level. In birds fed 0.35% Ca, DM digestibility of SBM-low (84.66%) and C-SBM (71.35%) was lower than that of birds fed 0.85% Ca (87.64% and 68.33%, respectively), whereas DM digestibility of SBM-med was not affected by Ca level.

There was a Ca level effect (*P = 0.040)* observed for DM digestibility in birds that were fed diets at different Ca levels. Birds fed 0.35% Ca have a higher DM digestibility (%) compared to birds fed similar diets with 0.85% Ca.

There was also a diet effect (*P < 0.001)* for DM digestibility (%) among birds. Birds fed DCP (89.03 and 87.07%) and SBM-low (84.66 and 87.64%) had higher DM digestibility compared to birds fed C-SBM (71.35 and 68.33%) and SBM-high (77.01 and 74.14%).

## Apparent P digestibility

There was a significant diet by Ca level interaction (*P < 0.001)* observed for apparent P digestibility (%). The interaction occurred due to differences in diet apparent P digestibility caused by Ca level. For example, the decrease in apparent P digestibility in birds fed C-SBM was much greater (61.74 vs 26.05%) than the decrease observed in birds fed DCP (92.64 vs 83.5%).

There was a Ca level effect (*P < 0.001)* for apparent P digestibility with the apparent P digestibility for birds fed 0.35% Ca diets being significantly higher (P < 0.05) than among birds fed similar diets with 0.85% Ca.

There was a diet effect (*P < 0.001)* observed for apparent P digestibility. Birds fed DCP had higher apparent P digestibility (88.05%) than birds fed other diets (75.6 to 81.5%) except for birds fed the SBM-low diet (86.2%).

## Endogenous P Loss (EPL)

There was no effect (*P = 0.938*) of Ca level on EPL, determined by the regression method, among birds fed different diets (34.8 vs 33.4 mg/kg DMI). Similarly, there was no effect of Ca level *(P = 0.393*) on EPL determined using P-free diets. However, in birds fed 0.85% Ca EPL was numerically higher (52.5%) than in birds fed 0.35% Ca (45.3%).

## True ileal P digestibility

The level of Ca significantly influenced (*P < 0.001)* the TIPD in broiler chickens fed SBM diets. The TIPD of birds fed SBM diets supplemented with 0.35% Ca was significantly higher (82.0%) than TIPD of birds fed diets supplemented with 0.85% Ca (59.3%).

## Discussion

The analyzed dietary P concentrations of corn and SBM (0.26 and 0.66% respectively) were close to calculated concentrations and were therefore used for the calculation of P digestibility coefficients in the current study. Similar analyzed dietary P concentrations of corn and SBM (0.26 and 0.71%, respectively) were reported in by Swine NRC, (2012). The analyzed concentrations of Ca, on the other hand were in some cases slightly lower than calculated values. Therefore, analyzed dietary Ca concentrations were also used to

for calculation of Ca digestibility. Calcium variability between calculated and analyzed values have been previously reported (Driver et al., 2005; Mutucumarana et al., 2014a).

## Growth Performance

As expected birds, fed increasing levels of SBM thus increasing the levels of P, regardless of Ca level grew better and had the best feed conversion ratio. However, overall birds fed the high Ca level had poorer growth performance compared to those fed the lower Ca level.

Phosphorus deficiency reduces growth performance primarily due to reductions in FI (Sullivan, 1999; Mutucumarana et al., 2014a, b; Shastak et al., 2014), as observed for P free and DCP diets. This was expected since P-free diets void of P and lack a realistic Ca:P ratio while DCP diets on the other hand was also consisted primarily of purified ingredients, containing corn starch and gelatin substituted as energy and protein sources. Hence, the weight loss observed was expected in birds fed the lowest dietary P inclusion and in diets consisting of purified ingredients. Diets formulated with purified ingredients have been reported to have low palatability when fed to broilers thus, the reduced FI resulted in limited P intake, which likely influenced the BWG of birds (Perryman et al., 2016). Also, feeding P-deficient diets may also stimulate adaptive mechanisms in the gastrointestinal tract (GIT) to increase P availability to maintain P homeostasis if fed extendedly (Yan et al., 2005).

High Ca levels have been reported to also reduce phytate P availability (high Ca:P ratio), formation of Ca phytate complexes, and exacerbates P deficiency (Shafey and Mcdonald, 1991; Driver et al. 2005; Selle et al. 2009).

## Dry matter digestibility

Dry matter digestibility is the portion of DM in a feed that is digested by animals at a specified level of FI. Digestibility improvements can be obtained by adjusting diet formulation, considering the effect of bird age on the digestibility of nutrients, and energy of the feeds. There was a diet by Ca level interaction observed for DM digestibility for birds fed the two different levels of Ca. The birds fed diets containing 0.35% Ca levels had a higher DM digestibility (%) compared to birds fed similar diets with 0.85% Ca. This was expected since a lower nutrient (Ca) composition of diets reduces the passage rate through the GIT and improves digestibility while excess dietary Ca has been reported to reduce nutrient digestibility (Shafey and M$^{c}$ donald, 1991). This is because low dietary Ca intakes, enhanced the Ca uptake and Ca extrusion (that antiporter membrane protein that removes calcium from cells) activities by the cells through plasma membrane Ca ATPase or sodium/calcium ion exchange (Centeno et al., 2004). Although high Ca intake increases the total amount of Ca absorbed by passive pathway, the overall effect is reduction in the percentage of Ca absorption (Hurwitz and Bar, 1969). Batal and Parsons (2002) further established that feed composition directly affects digestibility, they compared a purified diet (dextrose-casein) to a practical diet

(corn and SBM), and observed increased digestibility of protein fraction components for the practical diet in 2 to 21 d old broilers chcks.

There was diet effect for DM digestibility (%) among birds as well, where birds fed DCP with 0.35% Ca, low level of SBM and DCP with 0.85% Ca, DM digestibility were not different but were significantly higher when compared to the other diets at either Ca levels. This was expected since DCP diets consisted primarily of purified ingredients which are highly digestible and these ingredients are usually inadequate in AA. Previous data have indicated that broilers consume less feed when fed P-deficient diets (Driver et al., 2005). Additionally, diets formulated with purified ingredients have been reported to have low palatability when fed to broilers (Sullivan, 1999; Mutucumarana et al., 2014a, b; Shastak et al., 2014). Moreover, research published by Yan et al., 2005) indicated that broilers may have an ability to adapt to low-P diets to increase P digestibility. Garcia et al. (2007) justified that reduced feed intake can affect the dynamic equilibrium and the accuracy of the estimate of nutrients' digestibility coefficient.

There was a Ca level effect observed for DM digestibility in birds that were fed diets at different Ca levels, this is as a result of a general reduction in DM digestibility at high Ca levels. Upon closer examination, the mean DM digestibility for 0.35% Ca was 80.84% and for 0.85% Ca was 79.92%. Though statistically there was an effect, biologically there were no effect in DM digestibility across Ca levels. However, Zimmerman et al. (1963) and Combs et al. (1966) found that addition of Ca to the diet of baby pigs reduced DM digestibility, especially when the Ca level exceeded 1.0% of the diet.

## Apparent ileal P digestibility (AIPD)

There was a significant diet by Ca level interaction observed for AIPD. The interaction occurred due to differences in diet AIPD caused by Ca level (0.35 vs 0.85%) in each diet across. Where, the decrease in AIPD in birds fed corn was much greater (52.51 vs 33.70%), SBM-low was much greater (59.21 vs 34.68%), SBM-med was much greater (73.32 vs 47.16%), SBM high was much greater (70.36 vs 51.93%), C-SBM was much greater (61.74 vs 26.05%) than the decrease observed in birds fed DCP (92.64 vs 83.5%). The effect of similar Ca concentration on the AIPD of corn titration diets were lower for the 25% corn titration diet [25CTD (1.0%)], intermediate for the 75% corn titration diet [75CTD (19.9%)], and highest for the control diet (50.4%) (Perryman et al., 2016). Mutucumarana et al. (2014a) reported AIPD values of corn ranging from 60.5 to 70.4% which agreed with Wu et al. (2004) who reported a value of 70%, but lower than the value of 86% reported by Leytem et al. (2008). The AIPD values for corn observed in the current study was lower (33.70 to 52.51%) than previous reports but higher than that reported by Perryman et al. (2016). Hence, broilers are better able to utilize phytate P when diets are Ca low (Tamim and Angel, 2003; Tamim et al., 2004; Selle et al., 2009) as observed in the current study (52.52 vs 33.70%). Titration diets used by Mutucumarana et al. (2014a, 2015) had Ca concentrations ranging from 0.03 to 0.48%, which possibly explains why they observed trend in titration diet AIPD. The AIPD values of SBM (34.68 to 73.32%) observed in this study were within similara ranges observed by Mutucumarana et al. (2014b, 2015) 14 to 63% and 61 to 81% and also similar to that

reported by Liu et al. (2013) 64 to 89% but was lower than that reported by Dilger and Adeola, (2006) 71 to 88%. This may be due to difference in experimental conditions, especially Ca feeding strategies (Ca concentration and Ca:P ratio), days of adaptation, basal diet protein source, and or the evaluated feedstuff. In DCP diets the AIPD values ranged from 83.5 to 92.64% this was expected since the dietary P in this diet was in an inorganic state that is easily digestible by the birds also the diet is primarily made up of purified ingredients. Many authors suggested that the P digestibility increased when feeding diets of this nature as a result of the ability of the GIT to adapt to maintain P hemostasis (Yan et al., 2005; Liu et al., 2013; Shastak et al., 2014) and also the increased rate of passage through the GIT these ingredients. Hence it is well documented that the concentration of Ca and P and their respective ratio to each other influence both phytate P and NPP availability (Hurwitz and Bar, 1971; Smith and Kabaija, 1985; Tamim and Angel, 2003; Plumstead et al., 2008; Selle et al., 2009). Similarly, Paiva et al. (2014) reported that Ca and P digestibility was improved when birds were fed lower levels of Ca in the diets.

There was also a diet effect observed for AIPD among birds fed various diet compositions. The DCP diets containing no phytate P, with DCP providing NPP as the primary P source had the highest AIPD (83.5 to 92.64%) compared to other diets. This was not surprising since DCP is an inorganic P source containing no PP, and is easily digestible and absorbed. The values in this study was similar to ileal digestibility values for MCP (72.6 to 88.0 %), MDCP (68.0%), DCP (61.1%), and DFP (45.1%) reported by Bikker et al. (2016). In a study of Van der Klis and Versteegh (1998b), two MDCP sources

were tested resulting in ileal P digestibility values of 72.2 and 81.8%, indicating that substantial differences in MDCP qualities exist.

Lui et al. (2013) reported AIPD for casein ranged 64 to 90% but were higher than that (55.4%) reported for nitrogen-free diet by Perryman et al. (2016). The declining efficiency of intestinal phosphate absorption with the increasing P supply (Anderson, 1991), as one of the P homeostatic regulation mechanisms, and might also have contributed to this trend. In contrast to experiment 1 (73.80 to 64.79%), reported in chapter 3 of this thesis, in the current study SBM AIPD increased with increasing levels of SBM, which was similar to that reported by Liu et al. (2013) where the addition of graded SBM to diets led to a shift toward a higher proportion of the less digestible P. Which suggest that the P content in each lower level of SBM was very low and below the requirement of the birds. As indicated by Rodehutscord et al. (1999), P intake of 0 up to 5 g/kg did not affect the digestibility of P.

There was a Ca level effect for apparent P digestibility among birds fed dietary treatments. The AIPD of birds decreased significantly as Ca level increased (68.3 vs 46.2%). A similar decrease in AIPD due to increased Ca was reported by (Mutucumarana et al., 2014 a, b, 2015; Liu et al., 2013; Dilger and Adeola, 2006). It was reported that the AIPD of low Ca diets may also lead to the overestimation of AIPD because broilers are better able to utilize phytate P when diets are low in Ca (Tamim and Angel, 2003; Tamim et al., 2004; Selle et al., 2009).

## Endogenous P loss (EPL)

Endogenous P loss was measured using the regression procedure (Fan et al., 2001), which is believed to yield total EPL, and compared by using P-Free diet (Petersen and Stein, 2006), which yields basal EPL. The TIPD and EPL estimates were obtained by regressing P output against dietary P content in SBM diets. In pigs, the EPL is known to be highly variable, and is influenced by diet composition, mineral concentration, feeding level, energy supply, age, and growth rate (Jongbloed, 1987). Fan et al. (2001) proposed an approach to quantify EPL by regressing total P output from the animal against dietary P intake. The extrapolation to zero P intake, therefore, provides a theoretical estimate of diet-independent EPL.

On the other hand, the EPL estimates can be obtained, as indicated previously, from P-free diets and measuring P output. In the current study, EPL estimated using three different levels of SBM and the regression procedure was not affected by Ca level (34.8 and 33.4 mg/kg DMI respectively for 0.35% Ca and 0.85% Ca). Similarly, EPL estimates using P-free diets were also not affected by Ca level (45.3 and 52.5 mg/kg DMI, respectively for 0.35 and 0.85% Ca). Purified ingredients have been reported to influence endogenous nutrient losses resulting in lower measures of nutrient digestibility (Ren et al., 2012; Kong and Adeola, 2013; Massey O'Neill et al., 2014). Research conducted by Liu et al. (2012) reported higher EPL for birds fed P-free semipurified diets for 4 h compared with birds fed a similar diet for 72 h. These researchers also suggested that dietary concentrations of AA or other nutrients may

affect EPL. Furthermore, Maneewan and Yamauchi (2004) determined that GIT epithelial tissue was negatively affected by feeding semipurified, AA-deficient diets. In experiment 1 reported in chapter 3 of this thesis the EPL coefficients were negative and evidence of nonlinearity in P output was observed. This was also reported for broiler chicks (Iyayi et al., 2013; Liu et at., 2013; Mutucumarana et al., 2015a)

Endogenous P loss determined in SBM reported by Digler and Adeola. (2006) were higher (191 to 209 mg/kg DMI) and Mutucumarana et al. (2014b; 2015) (418 and -171 to -530 mg/kg DMI). While Mutucumarana et al. (2014) determined EPL in Casein based diet was 438 mg/kg DMI) which was in close agreement with the finding (446 mg/kg DMI) of Rutherfurd et al. (2004) using a minimal P diet. Hence, diet is a key factor influencing the composition and counts of microflora in the gastrointestinal tract (Barnes, 1972). Because microbial cell walls are composed of phospholipids (Cotton, 1972) and high microbial turnover may have contributed to the higher EPL determined in birds fed purified diets. Thus, a markedly higher endogenous P in the excreta of birds fed P-free diet suggests an increase P outut via urine when diets contain little or no Ca. A study by Liu et al. (2013) has shown that Ca deficient diets lead to lower P retention in broiler chicks. Published data on EPL in poultry are not only limited, but also highly variable. It is possible that differences in dietary Ca concentrations could influence EPL, but more research on this topic needs to be conducted to determine if this is the case.

## True ileal P digestibility (TIPD)

According to Dilger and Adeola (2006) differences in dietary Ca concentrations could be responsible for reported variations between TIPD and true P retention (TPR) values of similar feedstuffs amongst laboratories. These researchers also reported negative EPL for both TIPD and TPR regardless of Ca:P ratio. As seen in experiment 1 reported in chapter 3 of this thesis, a negative EPL have been a concern regarding the use of regression to determine true P utilization (TPU) of feedstuffs (D¨anner et al., 2006; Liu et al., 2013; Mutucumarana et al., 2014a, b). Additionally, differences in dietary Ca:P ratios related to Ca concentration have been reported to influence TIPD of SBM (Liu et al., 2013). In the current study, birds fed SBM diets with 0.35% Ca TIPD was significantly higher (82%) than birds fed diets with 0.85% Ca (59.3%). This Ca effect may be attributed to the high Ca levels in the intestine which reduces the absorption of both Ca and P (al-Masri, 1995). The solubility of mineral complexes decreased when Ca and P are supplemented at high levels (Hurwitz and Bar, 1971; Simpson and Wise, 1990; Lonnerdal et al., 1989). High Ca levels can increase ileal pH which could lead to reduction in absorption of both minerals (Shafey,1993). In a recent study, decreasing dietary Ca improve P utilization, while an excess of Ca may cause a P deficiency for ash criteria (Létourneau-Montminy et al., 2008). Browning et al. (2012) show that reducing dietary Ca/available P concentrations were associated with increased efficiency of Ca retention as compared to high Ca/available P diets, which indicates a physiological response by the chicken to overcome a Ca deficiency by up-regulating its nutrient transfer and

deposition infrastructure. The TIPD in SBM estimated in the present study (59.3 to 82.0%) was similar to those reportedby Mutucumarama et al. (2014 b; 2015) for conventional SBM (80%, 52 to 74%) but was lower than what Dilger and Adeola (2006) observed when broilers were fed SBM with low Ca (< 0.20%) diets. On the other hand, the present estimate was considerably greater than the values (0.458 to 0.553) reported by Liu et al. (2013), when measured at dietary Ca:total P ratios ranging from 1.2 to 2.0. However, the estimates in the current study compared closely with the value of 0.708 reported by Liu et al. (2013) at the dietary Ca:total P ratio of 0.8. The negative effect of increased dietary Ca concentration on P availability has been well established (Selle et al., 2009). It has been previously demonstrated that the utilization of dietary P was improved at narrower Ca:P ratios (Qian et al., 1997). High Ca concentrations inhibit both phytate-P digestibility and P absorption (Hurwitz and Bar, 1971; Qian and Kornegay, 1996; Dilger and Adeola, 2006; Liu et al., 2013). Several studies in broilers, pigs, and turkeys have been published supporting the assertion that increasing dietary Ca:P ratio decreased the digestibility of P in the gut (Qian et al., 1996, 1997; Liu et al., 1998; Brady et al., 2002).

Other factors, such as the high acid-binding capacity of limestone, have also been related to significant decreases in the protein and P solubility in the gizzard, and may affect N and P digestibility (Tamim and Angel, 2003; Selle et al., 2009; Walk et al., 2012b). Therefore, different authors have shown that a moderate reduction in dietary Ca had no deleterious effects on broiler performance (down to 0.6%, Driver et al., 2005b; or 0.73%, Ziaei et al., 2008) and bone ash (0.75%, Sing et al., 2013).

The concentrations of P in the diets of this study were below the recommended P requirement (NRC, 1994). Therefore, chicks might have adapted to these low P diets by increasing their P utilization capacity to maintain P homeostasis. This adaptive capacity has been reported when chicks were exposed to P- or Ca deficient diets by Yan et al. (2005). Hence, both Ca and P are essential for bone mineralization (Wasserman, 1960). Calcium has been demonstrated to bind to the phytate molecule and decrease P absorption in the gut. In a recent study, growing White Pekin ducklings fed low P diets had reduced tibia ash concentrations when dietary Ca content was increased (Xie at al., 2009), which shows that the absorption of P was decreased. Studies conducted to examine the interaction between dietary Ca and P have shown that chicks fed diets with a high concentration of Ca and low P had decreased tibia ash and increased incidence of rickets (Driver et al., 2005). However, these negative effects of high dietary Ca concentration were ameliorated by the inclusion of additional P in diets. In the present study, no deficiencies were observed in birds fed low levels of Ca or P-free diets since they were only fed for a short period.

The additivity of true and apparent p digestibility values of feed ingredients in poultry is limited however, pig diets has been evaluated (Fan et al., 2007) where apparent digestibility values have been shown not to be additive, in contrast to standardized and true digestibility values that were additive. Additionally, in pigs it has been demonstrated that the TTTD of P in corn and SBM for growing pigs are additive in corn–SBM diets (Zhai and Adeola 2013a). In the mixed diets in the current study, SBM and corn contributed to the nonadditivity of AIPD. The AIPD of C-SBM at various Ca

levels (0.35 and 0.85%) were 61.74 and 26.05%, respectively. When these two ingredients were combined P additivity (calculated) values for SBM (low, med and High) at 0.35 and 0.85% Ca were 56.66, 64.617, 62.948% and 34.476, 41.505, and 44.191%, respectively. Hence, individual ingredients corn and SBM AIPD values were lower than the additivity (calculated) values of C-SBM. In this case, the combined portions of P that originated from ingredients contributes to the P additivity in C-SBM diets at both Ca levels.

## Conclusions

In conclusion, as Ca level increased body weight decreased. Therefore, decreasing dietary Ca concentrations may improve bird performance, P and nutrient digestibility; however, this should be done with caution as very low Ca levels may also lead to increased leg health problems. The AIPD of birds decreased significantly as Ca level increased. Birds fed DCP with 0.35% Ca had a significantly higher AIPD compared to the other diets at either level of Ca. The TIPD of SBM with 0.85% Ca was 59.3%, which is 23 points lower than the TIPD of SBM with 0.35% Ca (82%). The TIPD values are greater than the AIPD values obtained from the SBM diets supplemented at different inclusion levels. The level of Ca significantly influenced the TIPD for broiler chickens, with lower TIPD observed in birds fed higher levels of Ca. The Ca level had no effect on EPL regardless of methodology used. In summary, TIPD values from SBM are greater than AIPD values when a fixed dietary Ca level is employed, however its concentration has an impact on both AIPD and TIPD. In addition, the additivity of AIPD in C-SBM diets could

be attributed to the intrinsic characteristics of each feed ingredient, especially its P content at various Ca level.

**Table 4-1.** Composition and calculated nutrient analysis of Starter diet

| **Ingredient** | **%** | **Calculated analysis** | |
|---|---|---|---|
| Corn | 59.3 | ME, kcal/kg | 3040 |
| SBM | 34.41 | CP, % | 22 |
| Soybean oil | 2.14 | Arginine, % | 1.42 |
| Dical P | 1.73 | Gly&Ser, % | 1.97 |
| Limestone | 1.08 | Histidine, % | 0.58 |
| Salt | 0.46 | Isoleucine, % | 0.90 |
| Methionine MHA | 0.35 | Leucine, % | 1.88 |
| Vit premix | 0.25 | Lysine, % | 1.31 |
| L-Lysine | 0.17 | Met&Cys, % | 0.99 |
| L-Threonine | 0.11 | Methionine, % | 0.64 |
| TOTAL | 100 | Phen &Tyr, % | 1.48 |
| | | Phenylalanine, % | 1.03 |
| | | Threonine, % | 0.92 |
| | | Tryptophan, % | 0.29 |
| | | Valine, % | 1.00 |
| | | Linoleic acid, % | 2.56 |
| | | Calcium, % | 0.93 |
| | | Phos avail, % | 0.45 |
| | | Potassium, % | 0.86 |
| | | Sodium, % | 0.20 |
| | | Chloride, % | 0.32 |
| | | Magnesium, mg/kg | 2073.69 |
| | | Manganese, mg/kg | 124.14 |
| | | Zinc, mg/kg | 129.17 |
| | | Iron, mg/kg | 570.12 |
| | | Copper, mg/kg | 18.92 |
| | | Iodine, mg/kg | 1.50 |
| | | Selenium | 0.19 |
| | | Riboflavin | 8.19 |
| | | Pantothenic acid | 14.13 |
| | | Niacin | 49.30 |
| | | Vit $B_{12}$ | 0.01 |
| | | Choline | 1692.49 |
| | | Biotin | 0.18 |
| | | Folacin | 2.16 |
| | | Thiamin | 4.28 |
| | | Pyridoxine | 7.25 |
| | | Crude fat, % | 4.74 |
| | | Dig Phe + Tyr, % | 1.70 |

**Table 4-2 –** The description of treatments

| Treat | Method | Ingredient | Inclusion rate | Ca % | NPP % |
|---|---|---|---|---|---|
| A | **Direct method** (ingredient only P source) | Corn | High (97%) | 0.35 | |
| B | | SBM | Low (20%) | | |
| C | | SBM | Med (40%) | | |
| D | | SBM | High (60%) | | |
| E | | C-SBM | (60/30) | | |
| F | | Corn | High (97%) | 0.85 | |
| G | | SBM | Low (20%) | | |
| H | | SBM | Med (40%) | | |
| I | | SBM | High (60%) | | |
| J | | C-SBM | (60/30) | | |
| K | | P-free diet | Almeida et al., 2010 | 0.35 | - |
| L | | P-free diet | | 0.85 | - |
| M | | Dical | | 0.35 | 0.4 |
| N | | Dical | | 0.85 | |

**Table 4-3.** Analyzed nutrient composition of the experimental ingredients[1].

| | Total P, % | Phytate P,[2] % | Non-phytate P,[3] % | Ca, % |
|---|---|---|---|---|
| Corn | 0.26 | 0.186 | 0.074 | 0.12 |
| Soybean meal | 0.66 | 0.468 | 0.192 | 0.30 |
| Limestone | - | - | - | 38.83 |
| Dicalcium phosphate | 18.20 | - | - | 20.20 |

[1]Mineral analyses were conducted by triplicate via inductively coupled plasma optical emission spectroscopy, AOAC Official Method 990.08 (2006)

[2]Phytate P content was determined by method references Analytical Biochemistry Vol. 77:536-539 (1977)

[3]Calculated as the difference between total P and phytate P

**Table 4-4.** Ingredient composition, calculated nutrient analysis, and analyzed composition for select nutrients of experimental diets fed to broilers from 19 to 22 d of age.[1]

| | Low Calcium (0.35%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Corn | SBM-low | SBM-med | SBM-high | Corn-SBM | P-free | Dical |
| ***Ingredient, % as fed*** | | | | | | | |
| Soybean meal (47%) | - | 20.00 | 40.00 | 60.00 | 32.17 | - | - |
| Corn | 96.35 | - | - | - | 64.33 | - | - |
| Soybean oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 4.00 | 4.00 |
| Cornstarch | - | 50.81 | 37.58 | 24.35 | - | 44.91 | 44.15 |
| Sucrose | - | 25.4 | 18.79 | 12.17 | - | 22.45 | 22.07 |
| Gelatin[2] | - | - | - | - | - | 20.00 | 20.00 |
| Solka-floc | - | - | - | - | - | 4.00 | 4.00 |
| Calcium carbonate | 0.601 | 0.739 | 0.584 | 0.429 | 0.449 | 1.750 | 2.027 |
| Dicalcium carbonate | - | - | - | - | - | - | 2.039 |
| Vit. and Min. premix[3] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| AA premix[4] | - | - | - | - | - | 2.69 | 2.69 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Titanium dioxide | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Calculated analysis*** | | | | | | | |
| ME, kcal/kg | 3421 | 3486 | 3242 | 2999 | 3132 | 2971 | 2928 |
| Crude Protein, % | 7.55 | 9.85 | 19.21 | 28.58 | 20.26 | 20.73 | 20.73 |
| Crude Fat, % | 5.47 | 2.27 | 2.53 | 2.78 | 4.73 | 3.99 | 3.99 |
| Total P, % | 0.247 | 0.137 | 0.267 | 0.398 | 0.377 | 0.005 | 0.375 |
| Non-phytate P, % | 0.067 | 0.038 | 0.076 | 0.115 | 0.106 | 0.000 | 0.375 |
| Phytate P, % | 0.179 | 0.094 | 0.187 | 0.281 | 0.270 | 0.000 | 0.000 |
| Calcium, % | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Ca:total P ratio | 1.4 | 2.6 | 1.3 | 0.9 | 0.9 | - | 0.9 |
| ***Analyzed composition*** | | | | | | | |
| Total P, % | 0.254 | 0.138 | 0.255 | 0.388 | 0.384 | 0.000 | 0.279 |
| Phytate P, % | 0.195 | 0.099 | 0.192 | 0.293 | 0.285 | <0.04 | <0.04 |
| Ca, % | 0.387 | 0.402 | 0.276 | 0.404 | 0.305 | 0.303 | 0.398 |
| Ti, % | 0.165 | 0.172 | 0.161 | 0.165 | 0.159 | 0.148 | 0.147 |

[1]All diets were provided in mash form on an *ad libitum* basis.

[2]Obtained from Great Lakes gelatin company, Grayslake, IL. Nitrogen = 16.6%, a 5.55 factor was used to calculate CP content (92.13%).

[3]Vitamin and Mineral premix includes per kg of diet: 8,000 IU vitamin A, 3,000 IU vitamin $D_3$, 1.1 mg thiamine, 6.6 mg riboflavin, 1.4 mg pyridoxine, 0.7 mg folic acid, 0.03 mg biotin, 0.01 mg vitamin $B_{12}$, 17 IU vitamin E, 0.4 g choline chloride, 28 mg niacin, 6.6 mg calcium-D-panthotenic acid, 0.8 mg menadione, 1.5 mg I, 0.15 mg Se, 100 mg Mn, 11 mg Cu, 100 mg Zn and 50 mg Fe.

[4]Amino acid premix (% final diet): Isoleucine (98.5%): 0.483%, Leucine (98.5%): 0.749%, L-Lysine HCl (78.8%): 0.504%, DL-Methionine (99%): 0.311%, Threonine (98%): 0.462%, Tryptophan (98%): 0.184%

**Table 4-4.1.** Ingredient composition, calculated nutrient analysis, and analyzed composition for select nutrients of experimental diets fed to broilers from 19 to 22 d of age.[1]

| | High Calcium (0.85%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Corn | SBM-low | SBM-med | SBM-high | Corn-SBM | P-free | Dical |
| ***Ingredient, % as fed*** | | | | | | | |
| Soybean meal (47%) | - | 20.00 | 40.00 | 60.00 | 31.74 | - | - |
| Corn | 95.06 | - | - | - | 63.47 | - | - |
| Soybean oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 4.00 | 4.00 |
| Cornstarch | - | 49.95 | 36.72 | 23.49 | - | 44.05 | 43.29 |
| Sucrose | - | 24.97 | 18.36 | 11.74 | - | 22.03 | 21.65 |
| Gelatin[2] | - | - | - | - | - | 20.00 | 20.00 |
| Solka-floc | - | - | - | - | - | 4.00 | 4.00 |
| Calcium carbonate | 1.892 | 2.027 | 1.872 | 1.717 | 1.742 | 2.045 | 1.974 |
| Dicalcium carbonate | - | - | - | - | - | - | 2.039 |
| Vit. and Min. premix[3] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| AA premix[4] | - | - | - | - | - | 2.69 | 2.69 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Titanium dioxide | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| ***Calculated analysis*** | | | | | | | |
| ME, kcal/kg | 3378 | 3438 | 3195 | 2951 | 3092 | 2923 | 2881 |
| Crude Protein, % | 7.45 | 9.84 | 19.21 | 28.57 | 19.99 | 20.73 | 20.73 |
| Crude Fat, % | 5.42 | 2.27 | 2.53 | 2.78 | 4.70 | 3.99 | 3.99 |
| Total P, % | 0.243 | 0.137 | 0.267 | 0.398 | 0.372 | 0.005 | 0.375 |
| Non-phytate P, % | 0.066 | 0.038 | 0.076 | 0.115 | 0.105 | 0.000 | 0.375 |
| Phytate P, % | 0.177 | 0.094 | 0.187 | 0.281 | 0.267 | 0.000 | 0.000 |
| Calcium, % | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Ca:total P ratio | 3.5 | 6.2 | 3.2 | 2.1 | 2.3 | - | 2.1 |
| Total P, % | 0.253 | 0.138 | 0.283 | 0.378 | 0.381 | 0.000 | 0.280 |
| Phytate P, % | 0.183 | 0.090 | 0.206 | 0.259 | 0.282 | <0.04 | <0.04 |
| Ca, % | 0.845 | 0.679 | 0.892 | 0.797 | 0.706 | 0.853 | 0.726 |
| Ti, % | 0.162 | 0.171 | 0.163 | 0.181 | 0.160 | 0.154 | 0.164 |

[1]All diets were provided in mash form on an *ad libitum* basis.

[2]Obtained from Great Lakes gelatin company, Grayslake, IL. Nitrogen = 16.6%, a 5.55 factor was used to calculate CP content (92.13%).

[3]Vitamin and Mineral premix includes per kg of diet: 8,000 IU vitamin A, 3,000 IU vitamin $D_3$, 1.1 mg thiamine, 6.6 mg riboflavin, 1.4 mg pyridoxine, 0.7 mg folic acid, 0.03 mg biotin, 0.01 mg vitamin $B_{12}$, 17 IU vitamin E, 0.4 g choline chloride, 28 mg niacin, 6.6 mg calcium-D-panthotenic acid, 0.8 mg menadione, 1.5 mg I, 0.15 mg Se, 100 mg Mn, 11 mg Cu, 100 mg Zn and 50 mg Fe.

[4]Amino acid premix (% final diet): Isoleucine (98.5%): 0.483%, Leucine (98.5%): 0.749%, L-Lysine HCl (78.8%): 0.504%, DL-Methionine (99%): 0.311%, Threonine (98%): 0.462%, Tryptophan (98%): 0.184%

**Table 4-5.** Growth performance of birds fed corn, SBM, C-SBM, P free diet and P-free diet with dicalcium phosphate containing 0.35% or 0.85% Ca from d19 to 22[1]

| Ca, % | Diet | BW gain, g/chick | Feed intake, g/chick | FCR, g:g |
|---|---|---|---|---|
| 0.35 | Corn | 60.6[e] | 260 | 4.62[b] |
| | SBM-low | 55.6[e] | 240 | 4.60[b] |
| | SBM-med | 154.5[d] | 288 | 1.89[c] |
| | SBM-hig | 259.5[a] | 336 | 1.29[c] |
| | C-SBM | 202.3[b] | 314 | 1.76[c] |
| | P-free | -43.2[f] | 111 | -3.14[d] |
| | DCP | -42.6[f] | 101 | -2.59[d] |
| 0.85 | Corn | 42.2[e] | 259 | 6.34[a] |
| | SBM-low | 45.7[e] | 247 | 6.06[ab] |
| | SBM-med | 145.1[d] | 286 | 2.03[c] |
| | SBM-hig | 186.6[bc] | 304 | 1.66[c] |
| | C-SBM | 179.0[c] | 324 | 1.76[c] |
| | P-free | -30.1[f] | 124 | -5.40[e] |
| | DCP | -40.7[f] | 113 | -3.60[de] |
| SEM | | 7.97 | 9.14 | 0.609 |
| *P*-values: | | | | |
| Diet | | <0.001 | <0.001 | <0.001 |
| Ca level | | <0.001 | 0.826 | 0.854 |
| Diet×Ca level | | <0.001 | 0.190 | 0.033 |

[1]Data are means of 8 replicate cages with 5 birds per cage

**Table 4-6.** Dry matter and phosphorus digestibility in birds fed corn, SBM, C-SBM, and a P-free diet with dicalcium phosphate containing 0.35% or 0.85% Ca from d19 to 22[1]

| Ca, % | Diet | DM digestibility, % | P digestibility, % |
|---|---|---|---|
| 0.35 | Corn | 80.44$^{c}$ | 52.51$^{ef}$ |
| | SBM-low | 84.66$^{b}$ | 59.21$^{de}$ |
| | SBM-med | 82.53$^{c}$ | 73.32$^{c}$ |
| | SBM-hig | 77.01$^{d}$ | 70.36$^{c}$ |
| | C-SBM | 71.35$^{f}$ | 61.74$^{d}$ |
| | DCP | 89.03$^{a}$ | 92.64$^{a}$ |
| 0.85 | Corn | 81.83$^{c}$ | 33.70$^{gh}$ |
| | SBM-low | 87.64$^{a}$ | 34.68$^{g}$ |
| | SBM-med | 80.50$^{c}$ | 47.16$^{f}$ |
| | SBM-hig | 74.14$^{e}$ | 51.93$^{ef}$ |
| | C-SBM | 68.33$^{g}$ | 26.05$^{h}$ |
| | DCP | 87.07$^{a}$ | 83.55$^{b}$ |
| SEM | | 0.763 | 2.609 |
| *P*-values: | | | |
| Diet | | <0.001 | <0.001 |
| Ca level | | 0.040 | <0.001 |
| Diet×Ca level | | <0.001 | <0.001 |

[1]Data are means of 8 replicate cages with 5 birds per cage

**Table 4-7.** Linear relationships between ileal P output (g/kg of DMI) vs. dietary P (g/kg of DM) of SBM containing 0.35% or 0.85% Ca in broilers[1]

| | Regression of ileal P output vs. P intake | | |
|---|---|---|---|
| | 0.35% Ca | 0.85% Ca | *P*-value |
| Slope | 0.181±0.043 | 0.407±0.043 | |
| Intercept | 0.035±0.013 | 0.033±0.013 | |
| $r^2$ | 0.457 | 0.807 | |
| Estimate of true ileal P digestibility, % | 82.0[a] | 59.3[b] | <0.001 |
| Endogenous P loss (mg/kg DMI) | 34.8 | 33.4 | 0.938 |

[1]Each value represents the mean of 8 replicates of 5 birds/replicate. Regression of ileal P output (g/kg DMI) against dietary P content (g/kg DM) as determined by feeding broilers with diets containing grades levels of SBM at 0.35 or 0.85% Ca. The slope represents true P indigestibility, and the intercept represents the endogenous P loss (g/kg DMI).

[a,b]True ileal P digestibility with a common superscript are not different at $P < 0.05$ as compared with 95% CI derived from SE of respective slopes.

**Table 4-8.** Endogenous P losses estimates from P-free diets containing 0.35 or 0.85% Ca[1]

| | 0.35% Ca | 0.85% Ca | SEM | *P*-value |
|---|---|---|---|---|
| Endogenous P loss (mg/kg DMI) | 45.3 | 52.5 | 5.71 | 0.393 |

[1]Data are means of 8 replicate cages with 5 birds per cage

# CHAPTER V

# GENERAL SUMMARY AND OVERALL CONCLUSIONS

Two experiments were conducted to determine true digestibility of P in feed ingredients commonly in commercial broiler diets. A second objective was to determine if the true digestible P values of corn and SBM would be additive when the two ingredients are fed in a typical commercial diet and finally how phytase supplementation affects true digestible P values.

In experiment 1, broilers were fed a standard corn-soybean meal diet, that met all of the nutrient requirements (1 to 3 weeks) of broiler chicks (NRC, 1994), from d 1 to 17. Birds, eight replicate pens of five chicks each, were then assigned to each of 18 experimental dietary treatments that included three graded levels of corn, SBM, or combinations of C-SBM with or without phytase supplementation. A fixed Ca:tP ratio was used in the formulation of all diets.

Feed intake increased *(P < 0.05)* for chicks fed various levels of corn and C-SBM with phytase. There was no difference (P > 0.05) among birds supplemented with phytase in SBM, but there was an ingredient level effect *(P < 0.05).* Tibia ash percentage was affected by phytase supplementation from an increase (48.62 to 51.70% in corn and C-SBM 49.60 to 52.28%) was seen with increased ingredients levels and phytase supplementation. There was also an increase *(P < 0.0001*) from 47.30 to 51.94% in Tibia ash percent with increasing levels of SBM with and without phytase. The Ileal P

digestibility of Corn, SBM, and C-SBM diets were determined by linear regression and varied among increasing ingredient levels and phytase supplementation. The TIPD estimate of SBM (52.0%) is more reasonable than the extremely low values obtain for corn (-7.2%) and C-SBM (5.6%). Corn and C-SBM contained inorganic P, whereas SBM diets contained no inorganic P. Phytase improved TIPD in corn (+38 points, to 60.4%; $P < 0.01$) and C-SBM (+14 points, to 19.5%; $P = 0.02$), however it didn't in SBM (+7 points, to 59.9%; $P = 0.55$). AIPD linearly decreased ($P < 0.08$) as the ingredient level increased in corn and SBM, and increased when phytase was supplemented. A significant ingredient level × phytase interaction was found for AIPD in corn-SBM ($P < 0.01$). Apparent Ileal P digestibility was reduced ($P < 0.0001$) significantly by increasing levels of corn and C-SBM supplemented with phytase but differed (P > 0.05) in SBM supplemented with phytase. While there was also an ingredient level effect ($P < 0.05$) on AIPD in SBM diets. Ileal Ca digestibility was significantly reduced ($P < 0.0001$) in diets supplemented with phytase of increasing levels of C-SBM but there was no difference among levels of ingredients (P > 0.05) supplemented with phytase on corn and SBM diets. The apparent P retention was reduced ($P < 0.0001$) among all diet levels with and without phytase supplementation, where low level corn supplemented with phytase was significantly higher 83.48 % and High level of corn without phytase was significantly the least with 61.79 % apparent P retention

In experiment 2, a total of 560 Ross 308 male broiler chicks were fed from d 19 to 22, seven experimental diets that consisted of one level of corn, one level of C-SBM, one level of three levels (20, 40 and 60%; low, medium and high, respectively) of SBM,

dicalcium phosphate and two gelatin-based P-free diets, all diets containing either 0.35 or 0.85% levels of Ca. Birds fed the higher levels of P regardless of Ca level grew better and had the best FCR as P deficiency reduces growth performance primarily due to reductions in FI. However, birds fed the higher Ca levels had poorer growth performance compared to those fed the lower Ca level. Endogenous P loss and TIPD values were obtained by regressing P output against dietary P content in SBM diets. Apparent P digestibility reduced with increasing Ca level. The APD of birds reduced ($P < 0.001$) significantly as Ca level increased. Birds fed DCP had a significantly higher (92.64%) AIPD compared to the other diets. The level of Ca significantly influenced ($P < 0.001$) the TIPD for broiler chickens with increasing levels of Ca in their diets. TIPD of SBM with 0.85% Ca was estimated to be 59.3%, that is 23 points lower than the TIPD estimation of SBM with 0.35% Ca (82%). Increasing SBM level from low to medium improved AIPD in both Ca concentrations however, increasing SBM from 40 to 60% didn't further increase AIPD. Ca level didn't affect EPL ($P > 0.4$) in either methodology employed. In summary, TIPD values from SBM are greater than AIPD values when a fixed dietary Ca level is employed, however its concentration has a deep impact in both TIPD and AIPD. Corn and SBM were additive when combined.

In conclusion, estimates for true P digestibility should be higher than apparent P digestibility data. The negative EPL values in chapter 3 are a possible explanation for the lack of significant differences between APD and TIPD data for diets formulated with the fixed Ca:P as negative EPL. A possible consequence of regression equations that predict negative EPL also is the underestimation of TPU. Based on these findings, it is suggested

that the Ca concentration in broiler diets should be maintained low as realistically as possible to maximize the utilization of other nutrients. However, the information on minimum Ca requirement for optimal skeletal health is scant and further research is warranted. Given that the addition of microbial phytase is currently routine in poultry diets and since the effect of phytase is diet-dependent, measurement of true P digestibility with a background of microbial phytase is of practical relevance. Using the regression approach, dietary phytase supplementation improved the true P digestibility in corn and SBB and a dietary P increases digestibility tend to decrease. However, it is recommended that future research is conducted to determine the additive effect of true digestible P contents determined for individual feed ingredients in compound poultry diets. Also, the duration experimental diets are fed should be looked at closely and probably a longer adaptation period would yield better digestibility values. Hence, more research is recommended to find a good and consistent method to assess P ileal digestibility of ingredients in broilers.

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