THE EFFICACY OF QUANTUM™ PHYTASE IN LAYING HENS FED CORN-SOYBEAN MEAL BASED DIETS

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By

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ABSTRACT

Three experiments were conducted to determine the efficacy of an *Escherichia coli* 6-phytase (Quantum™ phytase) in laying hens fed corn-soybean meal based diets. In experiment 1, the *Escherichia coli* 6-phytase (Quantum™) was evaluated for its efficacy in a 40-wk laying hen production trial. A total of 1080 White Leghorn hens were fed mash corn-soybean meal (CSM) based diets containing 0.35%, 0.25% or 0.15% of non-phytate phosphorus (NPP) with the 0.25% and 0.15% diets containing 200, 400 and 600 U/kg of exogenous phytase. Only minor differences in production characteristics were found between the 0.35% and 0.25% treatments regardless of phytase addition, indicating that 0.25% NPP resulted in P intake that was at or above the hen's requirement. In contrast, the hens fed the 0.15% NPP diet without phytase supplementation had significantly reduced production performance in comparison to the 0.35% treatment. The addition of phytase to the 0.15% diet improved these production characteristics to levels equal to or better than the 0.35% diet. The results indicated that Quantum™ phytase was efficacious in CSM-based diets fed to White Leghorn laying hens and can be used to reduce the need for diet supplementation with inorganic phosphorus.

In experiment 2, the effect of Quantum™ phytase on nutrient digestibility and bone ash in laying hens fed CSM-based diets was investigated. A total of 108 White Leghorn hens were fed CSM-based diets containing 0.35%, 0.25% or 0.15% NPP with the 0.25% and 0.15% diets containing 200, 400 or 600 U/kg of exogenous phytase. A linear reduction in phytate digestibility, ileal protein digestibility and soluble P was reported with increasing levels of exogenous phytase in the 0.25% diet. Tibial bone ash percentage was higher in 61-wk-old hens fed 0.25% diets supplemented with 200 or 400

U/kg phytase. Overall, the Quantum™ phytase was not efficacious in improving nutrient digestibility in laying hens fed CSM-based diets deficient in NPP.

In experiment 3, the impact of dietary Ca and P level on the efficacy of an *E. coli*-derived 6-phytase and the apparent digestibility of various nutrients was investigated in White Leghorn laying hens fed CSM-based diets. A total of 384 White Leghorn hens were fed CSM-based diets containing four levels of Ca (2.5, 3.5, 4.5 or 5.5%), two levels of NPP (0.15 or 0.30%), and two levels of phytase (300 or 600 U/kg feed). Increasing dietary Ca caused a decrease in AMEn, duodenal protein digestibility, Ca and phytate digestibility, percentage soluble P in feces and the percentage of poor quality eggs, while significantly increasing bone ash and hen-housed and hen-day egg production. The higher level of NPP (0.30%) decreased AMEn, fecal protein, Ca and P digestibility, and hen-housed and hen-day egg production, while increasing fecal soluble P and egg specific gravity in comparison to the lower NPP level. The higher level of dietary phytase (600 U/kg feed) significantly increased AMEn, phytate and P digestibility, soluble P in feces, and hen-day and hen-housed egg production, while significantly reducing the percentage of soft shelled, cracked and broken eggs. Overall, dietary phytase, Ca and NPP levels, either as main effects or in an interactive manner, can affect apparent nutrient digestibility and production traits in laying hens fed CSM-based diets

ACKNOWLEDGMENTS

I would like to acknowledge Syngenta Animal Nutrition Inc. for providing Quantum™ phytase and funding support for this project, and the staff at the University of Saskatchewan Poultry Centre for their assistance. The technical assistance of Dawn Abbott is greatly appreciated. I would also like to thank Dr. Classen for taking this 'cow' girl under his wing and teaching me about chickens. Thank you to my thesis committee members – Hank Classen, Murray Drew, Ruurd Zijlstra, John Patience, Fiona Buchanan, Tim Mutsvangwa and my External Examiner Robert Tyler.

DEDICATION

I dedicate my thesis to my family, Mom, Dad, Tara and Kelly, and to my wonderful husband Darryn

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LIST OF ABBREVIATIONS

Abbreviation	
AIA	Acid Insoluble Ash
AME	Apparent Metabolizable Energy
Ca	Calcium
CSM	Corn Soybean Meal
Dig	Digestibility
DM	Dry Matter
E. coli	Escherichia coli
G	Gram
Kg	Kilogram
NC1	Negative Control 1
NC2	Negative Control 2
NPP	Nonphytate Phosphorus
NRC	National Research Council
NS	Non significant
P	Phosphorus
PC	Positive Control
Phy	Phytase
THDP	Total Hen Day Egg Production
THHP	Total Hen Housed Egg Production
Wk	Week

CHAPTER 1
INTRODUCTION AND OBJECTIVES

Phytate, the mixed salts of phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6 hexakis dihydrogen phosphate) is a ubiquitous component of plant-sourced feed ingredients which accounts for approximately two-thirds of the total phosphorus found in plant-based diets (Cosgrove, 1966; Maenz, 2001). The phosphorus that is associated with phytate in feed is poorly utilized by monogastric animals due to low levels of phytase activity in their digestive tracts (Maenz and Classen, 1998) and as a result, inorganic phosphorus is added to feed to facilitate optimal growth and production. The addition of inorganic phosphorus to the diet of a monogastric animal results in a large portion of dietary phosphorus not being utilized by the animal and is excreted in the feces. Phytate also acts as an anti-nutritional factor by binding minerals and rendering them unavailable to the animal (Oatway et al., 2001). The lack of phytase activity within the digestive tract results in phytate phosphorus and other minerals that are bound to it to be poorly digested. The phytase enzyme can be added to the diet of monogastric animals to hydrolyze phytate within the digestive tract, resulting in phytate phosphorus and bound minerals being available for use by the animal and decreasing the need for inorganic phosphorus supplementation (Maenz, 2001). The inclusion of phytase in the diet may also result in the release of phytate-bound nutrients, making them available for use by the animal. These phytate bound nutrients may include protein, amino acids (Ravindran et al., 2000; Rutherfurd et al., 2004), starch and energy (Ravindran et al., 2001; Newkirk and Classen,

2001). The effectiveness of the phytase enzyme in its ability to breakdown the phytate molecule may be affected by the mineral content of the diet that is being fed to the animal (Sandberg et al., 1993). Laying hens have a high dietary calcium requirement in order to sustain egg production. These high dietary calcium levels may affect the efficacy of phytase. Calcium can do this due to its ability to precipitate phytate by forming insoluble Ca-phytate complexes within the digestive tract (Wise, 1983; Nelson and Kirby, 1987). These Ca-phytate complexes are resistant to hydrolysis by the phytase enzyme resulting in decreased phytase efficacy, and in phytate P and bound Ca being unavailable for absorption.

Phytase is the enzyme capable of hydrolyzing phytate within the digestive tract and is produced by feed companies as a commercial feed additive. All phytases produced are not equal. They may vary in temperature stability, pH optimum and ability to function effectively within the digestive tract. Any difference in these characteristics will affect the ability of the phytase enzyme to function effectively and consistently within the digestive tract (Augspurger et al., 2003; Onyango et al., 2005a). Therefore, all phytase enzymes produced must be tested *in vivo* to ensure efficacy before they are introduced to the monogastric feed market.

Numerous studies have been completed on the effect of phytase in both broiler chickens (Simons et al., 1990; Schoner et al., 1991; Denbow et al., 1995; Augspurger and Baker, 2004; Snow et al., 2004) and laying hens (Van Der Klis et al., 1997; Gordon and Roland, 1998; Snow et al., 2003), but little has been completed on the effect of Quantum^{TM} phytase on laying hen nutrient digestibility or the effect of high dietary Ca levels on laying hen nutrient digestibility and production performance. Laying hens have

a high dietary Ca requirement and little research has been completed on its effects on nutrient digestibility and performance when coupled with differing available phosphorus and phytase levels.

The overall objective of this study was to determine the efficacy of an *E. coli*-derived 6-phytase (Quantum™ phytase, Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA) in laying hens fed CSM-based meal diets. To accomplish this objective, three studies were performed.

1. The efficacy of Quantum™ phytase was assessed in a 40-week production trial using White Leghorn laying hens fed CSM-based diets and varying levels of available phosphorus. It was hypothesized that the addition of Quantum™ phytase to diets low in available phosphorus would increase hen production and the level of phosphorus availability to levels that are seen for a diet that is adequate in available phosphorus.

2. The effect of Quantum™ phytase on nutrient digestibility and bone ash in White leghorn hens fed CSM-based diets was investigated. It was hypothesized that the addition of Quantum™ phytase to diets would improve the digestibility of phytate phosphorus, calcium, energy and amino acids.

3. The impact of dietary calcium and phosphorus concentrations on apparent nutrient digestibility and the efficacy of Quantum™ phytase was investigated. It was hypothesized that in laying hens, the efficacy of Quantum™ phytase would be negatively affected by dietary calcium level.

CHAPTER 2
REVIEW OF THE LITERATURE

2.1 Phytate

Phytate is a natural compound that is found in most cereal grains. It serves as the primary storage form of phosphorus in plants, representing approximately two-thirds of the total phosphorus that is found in plant seeds (Cosgrove, 1966, Maenz, 2001). The phosphorus that is associated with phytate is referred to as phytate phosphorus. Phytate phosphorus is organically bound phosphorus and has been considered to be unavailable to monogastric animals (Oatway et al., 2001). Phytate phosphorus cannot be fully utilized by monogastric animals due to very low levels of phytase activity in the brush border membrane of their digestive tracts (Maenz and Classen, 1998). Phytase is the enzyme that has the capability to break down the phytate molecule, releasing phosphorus and other cations that may be bound to it (Cosgrove, 1966). Phytate phosphorus is poorly utilized by monogastric animals, and as a result, their dietary phosphorus requirements are not met by dietary phytate phosphorus alone. Inorganic phosphorus is added to their feed to meet dietary phosphorus requirements and to facilitate optimal growth and production. This practice ultimately leads to a large portion of dietary phosphorus not being utilized by the animal and is excreted in feces. Phytate has also been identified as an anti-nutritional factor. It has the ability to bind minerals, protein and starch, preventing their absorption in the digestive tract and rendering them unavailable to the animal (Cosgrove, 1966; Thompson and Yoon, 1984; Urbano et al., 2000).

Extensive research has been completed on the occurrence of phytate in plant feed ingredients, phytate phosphorus availability and the antinutritional effects of phytate over the past few decades (Eeckhout and De Paepe, 1994; Erdman, 1979; Maga, 1982; Reddy et al., 1982; Ravindran et al., 1995; Nelson, 1967; 1976; Taylor, 1965; Urbano et al., 2000).

2.1.1 Occurrence of Phytate in Plant Seeds

Phytate phosphorus is a common constituent of all plants. It serves as the major portion of total phosphorus in cereals (corn, barley, wheat, oat), grain legumes (field pea, chickpea) and oilseed crops (soybean, canola) (Ravindran et al., 1995; Maenz, 2001; Reddy et al., 1982). On average, about two-thirds of the total phosphorus in these feeds is present in the phytate phosphorus form (Simons et al., 1990).

The seed of the plant is most commonly used as a feed ingredient and is where the majority of the phytate phosphorus is found. Oilseed meals and cereal by-products contain very large amounts of phytate phosphorus as compared to feeds that are not directly derived from the seed, such as leaves, that contain minimal amounts (Ravindran et al., 1994).

Oilseeds contain higher levels of phytate phosphorus than cereal and legume grains (Maenz, 2001). In cereal grains, the phytate phosphorus is not uniformly distributed throughout the kernel and tends to be associated with specific morphological components of the kernel (Oberleas, 1973). Up to 90% of the phytate phosphorus is found in the aleurone layer and the bran of the wheat kernel. Corn differs from other cereal grains in that the majority of the phytate phosphorus, ~90%, is located within the germ or endosperm of the kernel (O'Dell et al., 1972; De Boland et al., 1975). The phytate found in oilseeds is concentrated within subcellular packages, referred to as globoids, which are

distributed throughout the seed (Erdman, 1979). Soybeans are unique in that the phytate is associated with globoids, but is also widely distributed throughout the entire seed (Ravindran et al., 1995).

2.1.2 Properties of Phytate

2.1.2.1 Structure and Chemical Characteristics

The chemical name for phytate is myoinositol 1, 2, 3, 4, 5, 6 hexakis dihydrogen phosphate (Cheryan, 1980). Structurally, phytate consists of six phosphate groups that are attached to a six-carbon molecule (Figure 2-1). This phytate molecule has 12 proton dissociation sites (two proton dissociation sites per phosphate group) (Maenz, 2001). Of the 12 proton dissociation sites on the phytate molecule, six are strongly acidic (pKa values ~ 1.5), three are weakly acidic (pKa = 5.7, 6.8 and 7.6) and the remaining three are very weakly acidic (pKa > 10) (Costello et al., 1976; Martin and Evans, 1986). A proton dissociation site is a site in which a proton (H^+) can be released from the molecule, leaving a negatively-charged site that is free to bind to any positively-charged molecule. In terms of the phytate molecule, proton dissociation is affected by pH and will be discussed in a later section of the review.

2.1.2.2 Phytate-Mineral Interactions

Due to its structure and reactive phosphate groups, phytate has a tendency to bind with cations in the digestive tract, making them unavailable to the animal (Maenz, 2001; Oatway et al., 2001). In the digestive tract this can include minerals (such as calcium, zinc or iron), amino acids, proteins or starch. Phytate is a chelating compound that has the ability to bind cations within a phosphate group, between two phosphate groups on the same phytate molecule or between phosphate groups on different phytate molecules (Cheryan, 1980). A diagram of a phytate molecule chelating with minerals is shown in

Figure 2-2. The binding strength of minerals to the phytate molecule has been identified as $Zn^{2+}>Cu^{2+}>Ni^{2+}>Co^{2+}>Mn^{2+}>Ca^{2+}>Fe^{2+}$ (Cheryan, 1980). Phytate has been shown to decrease calcium (Nelson and Kirby, 1987), copper, magnesium, manganese, and zinc availability in poultry (Nwolko and Bragg, 1977).

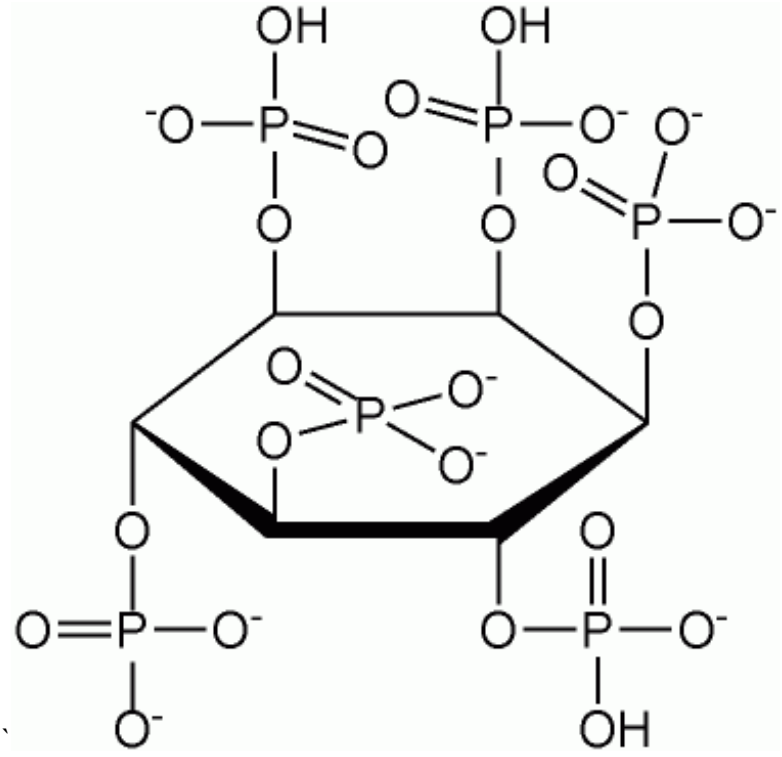

Figure 2-1 The structure of phytate (From Cheryan, 1980).

Phytate can exist in a mineral-free form or as a mineral-phytate complex (Oh et al., 2004). The mineral-phytate complex can be either soluble or insoluble. The form in which the phytate molecule and the mineral-phytate complex exist depends on the pH of the digestive tract and the concentration of minerals within the digestive tract (Cheryan,

1980). When the pH of the digestive tract is acidic, protonation of the phosphate groups in phytate will occur. This protonation will generate the mineral-free phytate molecule. When the pH of the digestive tract is neutral or alkaline, deprotonation of the phosphate groups in phytate will occur. This deprotonation will enhance the affinity of phytate for cations, resulting in the formation of mineral-phytate complexes (Cheryan, 1980; Maenz et al., 1999). When the concentration of minerals is high within the digestive tract, there will be competition for binding sites on the phytate molecule, increasing the formation of mineral-phytate complexes.

The pH of the digestive tract affects the extent to which minerals and phytate bind. When the pH is low (acidic pH, <5) a weak binding of minerals to the phytate molecule can occur, resulting in soluble mineral-phytate complexes. When the pH is higher there will be very strong binding occurring between minerals and the phytate molecule, resulting in insoluble mineral-phytate complexes (Erdman, 1979; Maga, 1982; Reddy et al., 1982).

2.1.3 Phytate Phosphorus Availability to Poultry

In order for phosphorus to be available to the chicken, it must be hydrolyzed to inositol and inorganic phosphorus within the digestive tract (Sandberg, 2002). Research that has been completed on the degree of phytate phosphorus utilization by poultry has given quite variable results. Early research indicated that poultry did not utilize dietary phytate phosphorus at all (Nelson, 1976; Taylor, 1965), while subsequent research has come to the conclusion that dietary phytate phosphorus is utilized by poultry, but the degree to which it is utilized varies considerably. Phytate phosphorus utilization by poultry has been reported to range from 37% (Edwards, 1983) to 50% (Mohammed et al., 1991; Robertson and Edwards, 1994; Perney et al., 1993; Simons et al., 1990). Poultry

are capable of hydrolyzing phytate, but the endogenous phytase activity in the brush border membrane of poultry and the intrinsic phytase activity of poultry feeds are too low to effectively degrade the phytate molecule (Ravindran et al., 1995; Maenz et al., 1999). This is why exogenous phytase is added to the diet to improve the utilization of phytate phosphorus.

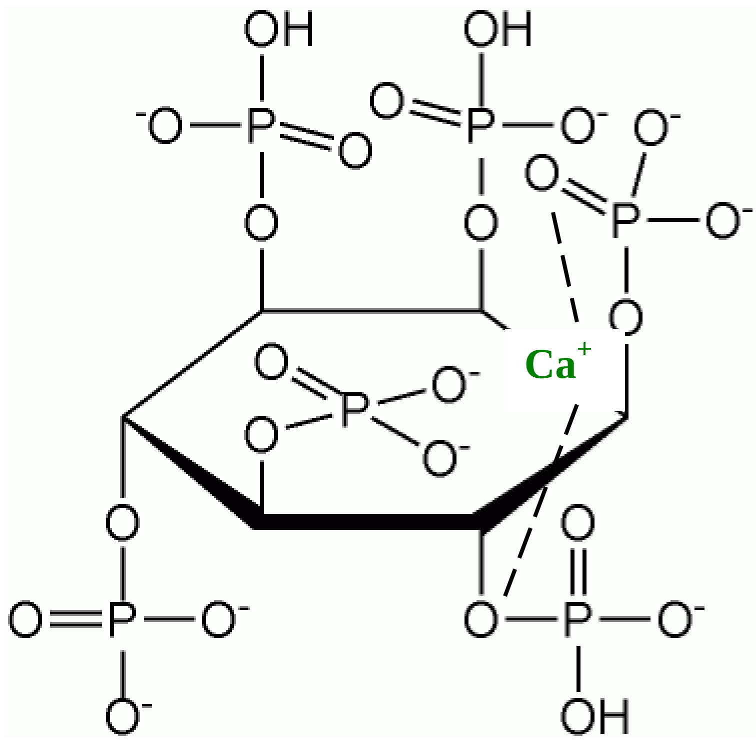

Figure 2-2 The structure of a mineral-phytate complex (From Cheryan, 1980).

2.1.4 Phytate Hydrolysis

Complete hydrolysis of the phytate molecule results in inositol and inorganic phosphorus (Garrett et al., 2004). The breakdown of the phytate molecule within the digestive tract is achieved by phytase activity (Nayni and Markakis, 1986). The phytase

enzyme is responsible for the stepwise removal of inorganic phosphate from the phytate molecule (Sandberg, 2002) and is illustrated in Figure 2-3. There are a number of factors that can affect the extent of phytate hydrolysis in the digestive tract. These include mineral concentrations, pH and the conditions of the digestive tract (temperature, moisture, mixing and digestive tract retention time). For purposes of this paper, minerals and pH will be the main factors that are discussed.

Mineral binding has a negative effect on phytate hydrolysis and has been shown to vary with the mineral content of the diet (Sandberg et al., 1993). Minerals that are attached to the phytate molecule block enzymes from accessing and hydrolyzing the phytate molecule. At neutral pH, Maenz et al. (1999) ranked minerals in order of their potency as inhibitors of phytate hydrolysis as $Zn^{2+}>>Fe^{2+}>Mn^{2+}>Fe^{3+}>Ca^{2+}>Mg^{2+}$. Increasing the concentration of minerals in the diet will result in an increased formation of mineral-phytate complexes within the digestive tract, those of which are resistant to hydrolysis by the phytase enzyme. This is of concern in laying hen diets due to their high dietary calcium requirement. The dietary calcium levels of laying hen diets may affect the efficacy of phytase in hydrolyzing phytate. Research has shown that increasing dietary calcium in poultry diets causes a significant decrease in phytate phosphorus digestibility (Scheideler and Sell, 1987; Mohammed et al., 1991; Nelson and Kirby, 1987; Tamim et al., 2004).

The pH of the digestive tract also has an effect on phytate hydrolysis. Low pH conditions favor the formation of mineral-free phytate, making phytate more susceptible to hydrolysis by enzymes. High pH conditions will favor the formation of mineral bound phytate that is resistant to hydrolysis by enzymes (Maenz, 2001). All phytase enzymes

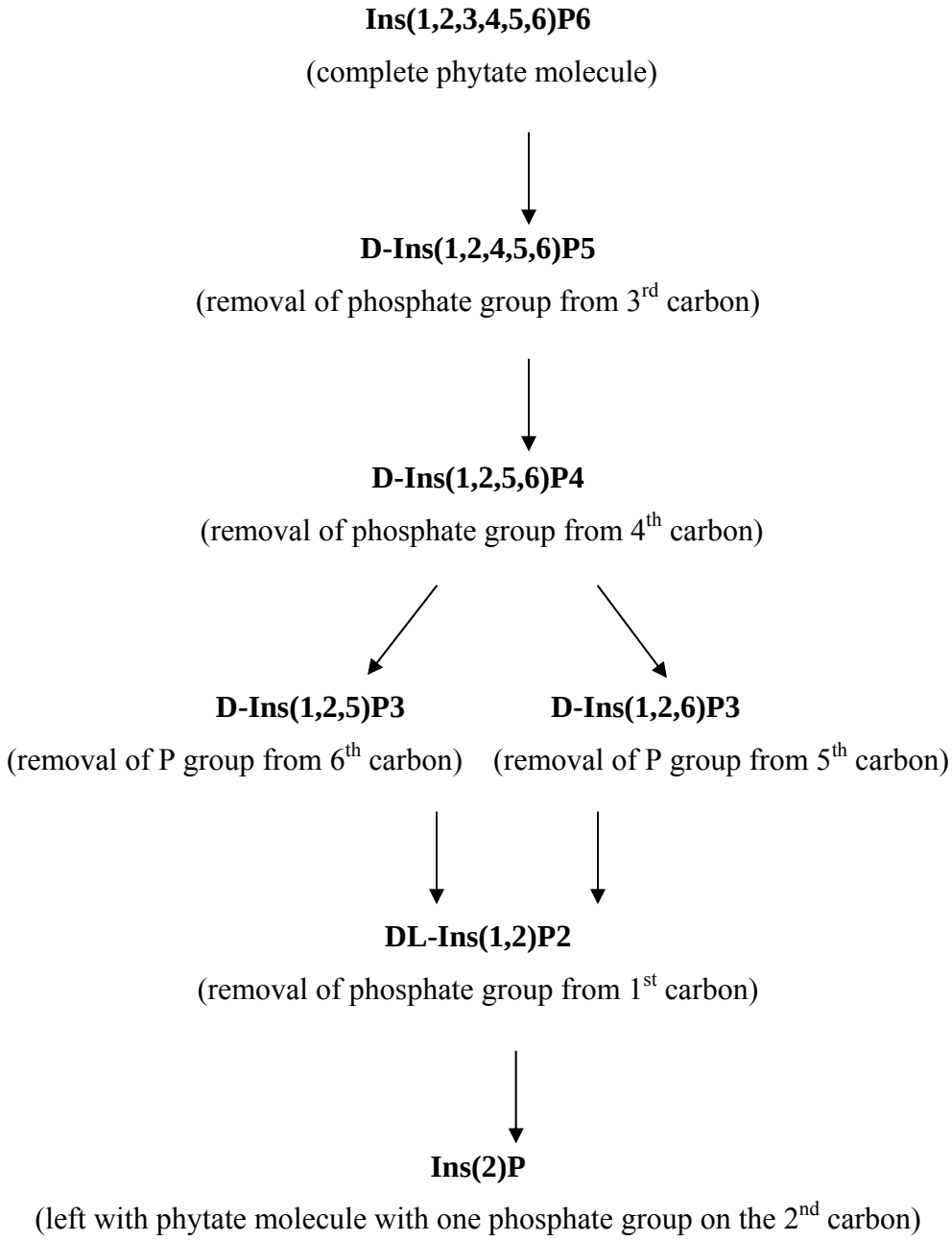

Figure 2-3 The suggested pathway of phytate hydrolysis by microbial phytase (Adapted from Skoglund et al., 1997).

have a pH optimum which is source dependent (phytases coming from different sources have different pH optima) (Oatway et al., 2001). The pH optimum is required in order for the phytase enzyme to work effectively and in an efficient manner. The pH varies

along the length of the digestive tract. As a result, the pH of the digestive tract will determine whether or not the phytase enzyme will be able to work effectively. When the pH of the digestive tract matches the pH optimum of the phytase enzyme, phytase activity will be at its highest and will have the ability to effectively hydrolyze phytate.

2.2 Phytase

Phytase (*myo*-inositol hexaphosphate phosphohydrolase) is an enzyme that is capable of hydrolyzing phytate in the digestive tract to yield lower inositol phosphates and inorganic phosphorus (Liu et al., 1998; Wyss et al., 1999). Extensive research has been completed on the addition of the phytase enzyme to monogastric diets and its ability to improve phytate digestibility (Denbow et al., 1995; Augspurger and Baker, 2004; Snow et al., 2004; Augspurger et al., 2007). Monogastric animals have very low levels of phytase activity in the brush border membrane of their digestive tracts (Maenz and Classen, 1998). Therefore, the phytase enzyme is added to the diet to aid in the hydrolysis of the phytate molecule.

There are two types of phytases, 3-phytase and 6-phytase. Microorganisms such as bacteria, fungi or yeast produce 3-phytase while plants produce 6-phytase. They are classified as 3- and 6-phytase on the basis of the method of hydrolysis of phytate within the digestive tract. Three-phytase will initiate phytate hydrolysis by removing the phosphate group on the third carbon of the phytate molecule and 6-phytase will initiate phytate hydrolysis by removing the phosphate group on the sixth carbon of the phytate molecule (Dvorakova, 1998).

All phytase activities are pH dependent, with the highest activity being observed at a slightly acidic pH. All phytases have pH optima which are source dependent, and as mentioned earlier, this pH optimum will affect the functioning ability of the phytase

enzyme within the digestive tract of the animal (Oatway et al., 2001). Figure 2-4 demonstrates the pH optimum differences between three phytase enzymes. Ronozyme® (DSM Nutritional Products Europe Ltd., Basel, Switzerland) has a very distinct and narrow pH optimum at pH 5.0. Natuphos® (BASF Animal Nutrition, Ludwigshafen, Germany) has two pH optima at pH 2.5 and 5.5. Quantum™ phytase (Syngenta Animal Nutrition, Inc.) has a very broad pH optimum, from pH 2.5-6.0. This broad pH range may give it an advantage in its ability to function more effectively and for a longer period of time within the digestive tract. Quantum™ phytase should have more interaction with phytate in the digestive tract, resulting in more phytate phosphorus and minerals bound to it being more available to the animal.

2.2.1 Phytase Sources

The hydrolysis of phytate within the digestive tract of poultry may be attributed to the action of phytase from one of three possible sources. These sources include phytase of (1) plant – feed ingredients, (2) animal – intrinsic phytase activity, or (3) microbial origin – commercial phytase products (Ravindran et al., 1995).

2.2.1.1 Plant Phytase

Phytase activity has been found in plant feed ingredients such as wheat, rye, barley and soybean. The level of enzyme and its ability to hydrolyze phytate within the seed varies between plants (Eeckhout and De Paepe, 1994). Wheat has been found to have high levels of intrinsic phytase activity, while corn and soybean meal have been found to have low levels (Eeckhout and De Paepe, 1994). The inclusion of high levels of wheat in poultry diets has been shown to have the ability to release phytate phosphorus (Zhu et al., 1990; Barrier-Guillot et al., 1996). The intrinsic phytase activity of poultry feeds is limited by processing procedures such as pelleting. The phytase enzyme is a protein that

is susceptible to denaturation when exposed to high temperatures during feed processing procedures. The pelleting procedure is a common processing method used for poultry diets. The high heat that is used during pelleting may reduce intrinsic plant phytase activity (Liu et al., 1998). Even though plant feed ingredients such as wheat have high levels of intrinsic phytase activity that contribute to phytate digestibility, these levels are not high enough to effectively hydrolyze dietary phytate, and may also be disabled during processing. As a result, exogenous supplementation with phytase or inorganic phosphorus would be required to meet the phosphorus needs of the chicken.

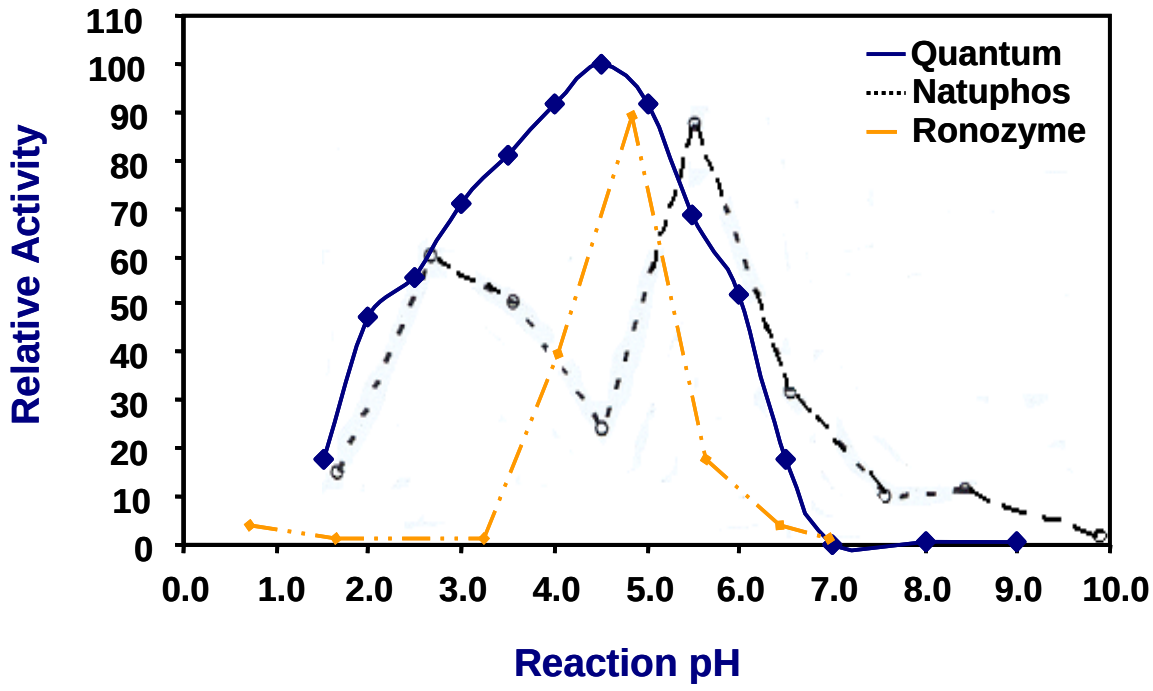

Figure 2-4 The pH optima of three phytase enzymes – Quantum™, Natuphos® and Ronozyme® (Adapted from Palackal et al., 2004).

2.2.1.2 Intrinsic Phytase Activity

Phytase is present in the brush border membrane within the digestive tract of the chicken. Activity is highest in the duodenum and decreases progressively down the

length of the gut (Maenz and Classen, 1998). Although the phytase enzyme is present, it is not present at levels that have the ability to effectively hydrolyze phytate (Maenz and Classen, 1998). The contribution of brush border phytase to phytate phosphorus utilization by animals is not known, but animals are known to utilize a portion of the total dietary phytate phosphorus without supplemental phytase in the diet (Maenz, 2001). Chickens fed phosphorus deficient diets have been shown to have an increase in intestinal phytase activity (Davies and Motzok, 1972; Davies et al., 1970). Intestinal phytase probably does contribute to the utilization of phytate phosphorus, but the activity of the enzyme may be regulated by the mineral status of the animal (Maenz et al., 1995; Maenz, 2001).

2.2.1.3 Commercial Phytase Products

There are several commercial microbial phytase products on the market that can be purchased for use as feed additives. These commercial phytase products can be added to the feed of monogastric animals to effectively hydrolyze phytate within the digestive tract. Phytases from different sources are unique in their appearance, physical and chemical properties, and enzyme activity. Therefore, they may have slightly different effects when used in poultry diets. Commercial phytase products on the market may exhibit slight differences, but they all have the same goal of improving the utilization of dietary phytate phosphorus.

Natuphos® phytase is a 3-phytase produced from a genetically modified *Aspergillus niger* strain produced by BASF Animal Nutrition, Ludwigshafen, Germany. BASF claims that their phytase will improve the digestibility of amino acids and minerals such as Ca and Zn. It has also been claimed that the use of Natuphos® phytase in poultry diets will result in a 30% reduction of phosphorus excretion in the manure. Much research has

been completed on the use of Natuphos® phytase in poultry diets. It has been found that Natuphos® phytase is efficacious in poultry diets and does improve phytate phosphorus utilization (Ravindran et al., 2000; Snow et al., 2003; Wu et al., 2006). Ronozyme® P phytase by DSM Nutritional Products Europe Ltd., Basel, Switzerland is a 6-phytase *Peniophora lycii* which is produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism. It has been claimed that Ronozyme® increases feed intake and Ca and P digestibility, reduces P output in manure, and releases ~50% of the phytate phosphorus that is present in a typical poultry diet. They also claim that their phytase is more heat stable than other phytase products on the market. Research that has been completed with the use of Ronozyme® P phytase in poultry diets has shown that it is efficacious in poultry diets and improves phytate phosphorus and amino acid digestibility (Rutherford et al., 2002; 2004). Quantum™ phytase is a relatively new *E. coli*-derived 6-phytase expressed in *Pichia pastoris*. It has been produced by Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA. It has been claimed that Quantum™ phytase combines heat tolerance, extended digestive tract stability and high activity to offer a more effective and efficient option for releasing phytate phosphorus in poultry diets.

2.2.2 Site of Phytase Activity

The site of action of phytate hydrolysis by the phytase enzyme is mainly in the crop, proventriculus and gizzard of the chicken (Liebert et al., 1993). The majority of the phytase activity is seen in these areas of the digestive tract because of their pH conditions (Yi and Kornegay, 1996). As mentioned earlier, phytases have pH optima and they require that pH in order to function properly. Phytase will function at its peak and will be efficient at hydrolyzing phytate when working under conditions that provide its pH

optimum. The phytase enzyme will become inactivated if put into an environment that has unfavorable conditions for its functioning (Yi and Kornegay, 1996). The pH ranges of the different sections of the digestive tract are demonstrated in Figure 2-5. Areas of the digestive tract with low pH will favor phytase activity. These areas will match the pH optimum of the exogenous phytase enzyme, resulting in peak phytase activity and phytate hydrolysis. The pH of the small intestine is relatively high when compared to the upper digestive tract. As a result, the activity of the phytase enzyme may be quite low or non-existent in this area.

2.2.3 Effect of Phytase Supplementation on Nutrient Utilization

2.2.3.1 Impact of Phytase on Phosphorus Utilization

Research has shown that phytase supplementation can improve phytate phosphorus availability and phosphorus retention and ultimately lead to a reduction in phosphorus excretion (Lei et al., 1993; Broz et al., 1994; Qian et al., 1997; Van Der Klis et al., 1997; Um and Paik, 1999; Zanini and Sazzad, 1999; Ravindran et al., 2000; Jalal and Scheideler, 2001; Dilger et al., 2004; Rutherford et al., 2004; Onyango et al., 2005a; Augspurger et al., 2007).

Phytase supplementation is effective in improving the utilization of phytate phosphorus. The addition of phytase to low phosphorus diets has been shown to significantly increase the availability and retention of phosphorus in chickens (Jalal and Scheideler, 2001; Rutherford et al., 2004; Onyango et al., 2005a) and pigs (Cromwell et al., 1993; 1995; Lei et al., 1993; Li et al., 1998). Van Der Klis et al. (1997) found that phytate degradation without phytase supplementation in laying hens fed a CSM-based diet was 21%; the addition of phytase increased this value to 54-72%.

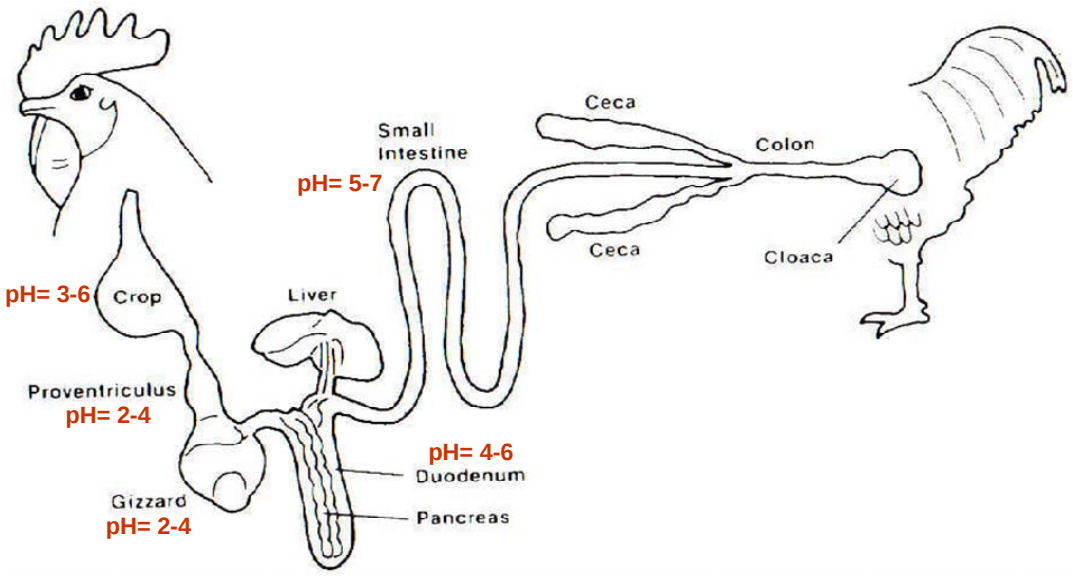

Figure 2-5 The pH of the chicken gastrointestinal tract (Adapted from Applegate, Syngenta Animal Nutrition, Inc., 2005).

When the retention of phosphorus is improved with phytase supplementation, it is quite evident that phosphorus excretion will ultimately be reduced. A decrease in phosphorus excretion due to phytase supplementation has been found by many researchers (Schoner et al., 1991; Kornegay et al., 1999; Ahmad et al., 2000). Phosphorus excretion reductions with the use of supplemental phytase to low phosphorus diets as compared to NRC (National Research Council, 1994) dietary phosphorus recommendations has been reported as 42-51% (Yi et al., 1996b), 32-36% (Kornegay et al., 1999), and 37.5% (Yan et al., 2000). The reduction of phosphorus excretion in manure will result in a reduction in phosphorus pollution when applied to the land. Thus, the use of supplemental phytase in conjunction with reduced dietary phosphorus levels is an effective method of improving phytate phosphorus utilization and decreasing phosphorus excretion in the manure (Waldroup, 1999; Waldroup et al., 2000).

2.2.3.2 Impact of Phytase on Utilization of Other Minerals

As discussed above, the phytate molecule can form insoluble salts with cations at neutral pH, making them unavailable for use by the monogastric animal (Maenz, 2001). The binding strength of minerals to phytate has been identified as $Zn^{2+}>Cu^{2+}>Ni^{2+}>Co^{2+}>Mn^{2+}>Ca^{2+}>Fe^{2+}$ (Cheryan, 1980). When phytate binds with cations, insoluble phytate is formed, making phosphorus and other minerals bound to it unavailable (Nelson and Kirby, 1987). Phytase has the ability to free these minerals from the insoluble phytate molecule and potentially make them available for absorption by monogastric animals.

Research has been conducted on the effect of phytase on mineral availability. Phytase supplementation has been shown to improve Ca availability in both poultry (Ketaren et al., 1993; Mitchell and Edwards, 1996; Zyla et al., 1996; Qian et al., 1997; Ahmad et al., 2000; Tamim et al., 2004; Onyango et al., 2005a) and swine (Lei et al., 1993; Young et al., 1993; Mroz et al., 1994; Li et al., 1998) diets. Simons et al. (1990) indicated that phytase could improve Ca availability in broiler chickens and swine, and Qian et al. (1996) found that Ca retention increased linearly as supplemental phytase was increased in turkey diets. Phytase supplementation has also been shown to improve the availability of Cu and Zn in poultry diets (Sebastian et al., 1996; Zanini and Sazzad, 1999).

2.2.3.3 Impact of Exogenous Phytase on Dietary Energy Availability

Research on this topic is not extensive and the results that have been found are quite variable. Ravindran et al. (2000; 2001), Namkung and Leeson (1999) and Newkirk and Classen (2001) found that phytase addition improved apparent metabolizable energy (AME) values, while Onyango et al. (2004) and Biehl and Baker (1997) found that AME was not improved by phytase supplementation. The breakdown of phytate-nutrient

complexes by the phytase enzyme may result in energy compounds that are available for digestion or absorption (Ravindran et al., 2000). Onyango et al. (2004) indicated that there may be variation in energy retention reported due to the differences in phytase source and dietary ingredients that are used in the studies. This helps explain why there is so much variation in results that have been reported on the effect of phytase on AME to date.

2.2.3.4 Impact of Exogenous Phytase on Protein/Amino Acid Availability

Phytase may also have the ability to free phytate-bound protein for utilization by monogastric animals. Results from previous research on the impact of phytase on amino acid availability are quite variable, but overall, they tend to indicate improvements in protein and amino acid digestibility and availability in chickens (DeRham and Jost, 1979; Yi et al., 1996a; Biehl and Baker, 1997; Sebastian et al., 1997; Ketaren et al., 1993; Namkung and Leeson, 1999; Ravindran et al., 2000; Rutherford et al., 2004). Some amino acids tend to be more available than others with the addition of phytase to the diet. Ravindran et al. (1999) found that phytase addition to a corn-soybean meal diet increased the digestibility of protein and amino acids, with threonine and valine having the highest digestibility. Mroz et al. (1994) found methionine and arginine to have increased digestibility with phytase supplementation and Rutherford et al. (2002) found an increase in isoleucine digestibility in soybean meal with phytase addition. Snow et al. (2003) and Peter and Baker (2001) found that phytase had no significant effect on the digestibility of any amino acid. Similar to AME, I believe that there may be variation in amino acid and protein digestibilities reported due to differences in the phytase source and the dietary ingredients used in the studies.

2.2.4 Factors Affecting Phytase Efficacy

2.2.4.1 Dietary P Level

Supplemental phytase has been proven to be more efficacious in diets that contain low levels of inorganic phosphorus (Denbow et al., 1995; Kornegay et al., 1996; Qian et al., 1997). Increased levels of inorganic phosphorus may inhibit the action of phytase to break down phytate. When inorganic phosphorus is supplied in the diet at levels that meet the needs of the chicken, the functioning of phytase to release phosphorus from phytate may be inhibited or decreased (Wodzinski and Ullah, 1996). The functioning of phytase may be inhibited because more phosphorus is not required – the animal's phosphorus requirements are being met by the supplemental inorganic phosphorus, so more phosphorus is not required from phytate. If the amount of inorganic phosphorus in the diet is low, phytase will be more effective in hydrolyzing phytate. This is because the phosphorus produced by the hydrolysis of phytate will be required by the animal in order to meet its phosphorus requirements.

2.2.4.2 Dietary Ca Level and Ca:P Ratio

Dietary mineral content plays an important role in determining the extent of phytate hydrolysis within the digestive tract of the monogastric animal (Wise, 1983). High Ca levels in the diet can lead to the formation of insoluble Ca-phytate complexes within the digestive tract (Wise, 1983), making the phytate molecule inaccessible by phytase. The extra Ca in the digestive tract can directly decrease phytase activity by competing for the binding sites on the phytate molecule that are designated for the phytase enzyme (McCuaig et al., 1972). Therefore, dietary Ca concentration and the Ca:P ratio are important factors that may influence phytase activity. Research has been completed on the effects of high Ca levels on phytase activity, and it has been found that high levels of

dietary Ca are known to decrease phytase activities in chicks (McCuaig et al., 1972; Applegate et al., 2003; Lim et al., 2003; Tamim et al., 2004) and pigs (Lei et al., 1994). Qian et al. (1997) found that phytase activity was decreased by 4.9 and 7.4%, respectively, when the dietary Ca:P ratio was widened from 1.4:1 to 2:1. This is an indication that the dietary Ca concentration and the overall Ca:P ratio are very important factors that affect the functioning of phytase within the digestive tract. Schoner et al. (1991, 1993) observed that the addition of Ca to the broiler diet caused a decrease in phytase efficacy. In weanling pigs, Lei et al. (1994) found that supplemental phytase had the ability to improve phytate phosphorus utilization more effectively at low levels of dietary Ca (0.4%) than at normally recommended levels (0.8%) for pigs.

It has become well known that high dietary Ca levels can negatively affect the efficacy of phytase in monogastric diets. Most of the research on this topic has been completed with broiler chickens. I believe that the effect of Ca level on the efficacy of phytase would be much greater in laying hen diets due to their high dietary Ca requirements.

2.3 Summary

In summary, the use of microbial phytase products in monogastric diets has become a common practice all over the world. Phytate hydrolysis is very complex and it varies with the ingredient composition of the diet and the conditions of the digestive tract. The mineral levels of the diet also play an important role in phytate hydrolysis due to the formation of mineral-phytate complexes that are resistant to hydrolysis by the phytase enzyme. Laying hens have high dietary calcium and phosphorus requirements and previous research has shown that these high mineral levels may have a negative influence on the efficacy of phytase. The phytase enzyme is capable of hydrolyzing the phytate molecule, but all phytases are not equal. They differ in the source from which they are

derived, their pH optima and their stability within the digestive tract. Differences in phytase products result in variation. Therefore, phytase enzymes need to be tested *in vivo* in order to ensure efficacy. The present study was conducted to assess the efficacy of Quantum™ phytase on production performance and nutrient digestibility in laying hens fed CSM-based diets. The effects of dietary calcium and phosphorus levels on phytase efficacy were also investigated.

CHAPTER 3
THE EFFICACY OF QUANTUM™ PHYTASE IN A 40 WEEK PRODUCTION TRIAL USING WHITE LEGHORN LAYING HENS FED CORN-SOYBEAN MEAL BASED DIETS

3.1 Abstract

Microbial phytase is a prominent feed enzyme used in animal feeds but there is relatively little information on its use in laying hen diets. In this experiment, an *Escherichia coli* 6-phytase (Quantum™) was evaluated for its efficacy in a 40-wk laying hen production trial. A total of 1080 White Leghorn hens (540 each of Shaver and Bovan strains) were fed mash corn-soybean meal (CSM) based diets containing 0.35% (positive control, PC), 0.25% (negative control, NC1) or 0.15% (NC2) non-phytate phosphorus (NPP). Six more diets were manufactured by supplementing the negative control diets with 200, 400 or 600 U/kg of exogenous phytase resulting in a total of 9 treatments. Each dietary treatment x strain subclass was replicated four times with five adjoining cages per replicate (three hens per cage). Production performance was measured from 21 to 61 wk of age. Only minor differences in production characteristics were found between the PC and NC1 treatments regardless of phytase addition, indicating that 0.25% NPP resulted in a P intake that was at or above the hen's requirement. In contrast, the hens fed the 0.15% NPP diet without phytase supplementation had significantly ($P < 0.05$) reduced total hen-housed egg production and body weight at 61 wk of age in comparison to the PC treatment, whereas the incidence of soft shelled, cracked and broken eggs was increased significantly ($P < 0.05$) in hens fed the NC2 diet. Addition of phytase to the

NC2 diet improved these production characteristics to levels equal to or better than the PC diet. The results indicated that Quantum™ phytase was efficacious in CSM-based diets fed to White Leghorn laying hens and can be used to reduce diet supplementation with inorganic phosphorus.

3.2 Introduction

Phytate is the primary storage form of phosphorus found in plant feed ingredients and accounts for approximately two-thirds of the total phosphorus in plant seeds (Cosgrove, 1966; Maenz, 2001). Phytate phosphorus in feed is poorly utilized by monogastric animals despite the presence of phytase activity in the brush border membrane of their digestive tracts (Maenz and Classen 1998). As a consequence, inorganic phosphorus is added to feed to facilitate optimal growth and production. This practice ultimately leads to a large portion of dietary phosphorus not being utilized by the animal and being excreted in feces. Exogenous phytase can be added to diets to hydrolyze phytate within the digestive tract, making more phytate phosphorus available for use by the animal and decreasing the need for dietary inorganic phosphorus supplementation (Van der Klis et al., 1997; Maenz, 2001).

Microbial phytase is now the predominant feed enzyme used in animal diets. Extensive research has been conducted on the use of phytase in broilers (Simons et al., 1990; Broz et al., 1994; Denbow et al., 1995; Rutherfurd et al., 2004), but research on its use in laying hen diets is not extensive.

Punna and Ronald (1999) found that incorporating 300 U/kg phytase into a laying hen diet containing 0.1% non-phytate phosphorus (NPP) resulted in an improvement in egg production and feed intake along with a decrease in mortality. Um and Piak (1999) and

Lim et al. (2003) found that supplementing a low phytate diet with phytase increased egg production in laying hens. Van Der Klis et al. (1997) found that production performance was significantly improved by dietary supplementation, while Jalal and Scheideler (2001) found that phytase supplementation improved feed intake, feed conversion and egg mass. Gordon and Roland (1997) and Francesch et al. (2005) found that laying hens consuming a diet low in NPP with supplementary phytase performed as well as hens that were fed diets containing higher levels of NPP without supplementary phytase. Gordon and Roland (1997) saw an improvement in egg production, feed consumption, egg weight and egg specific gravity, and Francesch et al. (2005) saw an improvement in egg production, hen weight gain and feed consumption in hens that were fed a diet low in NPP with supplementary phytase when compared to hens fed a low NPP diet without supplemental phytase. Gordon and Roland (1997) also indicated that phytase supplementation of diets containing high levels of NPP resulted in no further improvement in hen performance.

It should be remembered that all phytase products are not equal. Phytase enzymes differ in the source from which they are derived. They may differ in characteristics such as pH optimum, thermostability and the ability to resist hydrolysis within the digestive tract. Any difference in these characteristics will affect the ability of the phytase enzyme to function effectively and consistently within the digestive tract (Onyango et al., 2005b). Therefore, all phytase enzymes produced must be tested *in vivo* to ensure efficacy before they are introduced to the monogastric feed market.

Previously, there has been little work done on the effect of an *E. coli*-derived 6-phytase (Quantum™ phytase) supplementation in laying hen diets. The present study was, therefore, conducted to assess the efficacy of Quantum^{TM} phytase in a 40-wk

production trial using White Leghorn laying hens fed corn-soybean meal (CSM) based diets and varying levels of NPP.

3.3 Materials and Methods

The experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan and was performed in accordance with recommendations of the Canadian Council on Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

3.3.1 Animals and Housing

At 17 wk of age, a total of 1080 White Leghorn pullets (540 each of Shaver White and Bovan strains) were housed in laying cages under controlled climate conditions at the Poultry Centre on the University of Saskatchewan campus. Three birds were placed in each cage (cage dimensions 30.5 cm x 46 cm with a height of 52 cm; floor space per hen = 468 cm^2) and each experimental unit consisted of five adjoining cages. At 18 wk of age, the length of the light period was increased from 8L:16D to 14L:10D with a light intensity of 10 lux. The ambient temperature was maintained at a minimum of approximately 20°C throughout the trial. During the pre-experimental period (i.e. up to 21 wk of age) a commercial laying hen diet was offered *ad libitum*, whereas from 21 wk onwards, the hens were randomly assigned to one of the 9 dietary treatments. Each dietary treatment x strain subclass was replicated four times with 5 adjoining cages per replicate (3 hens per cage) in a randomized design.

3.3.2 Experimental Diets

The ingredient and nutrient composition of the experimental diets is shown in Table 3-1. Three isocaloric (2900 Kcal/kg) and isonitrogenous (16.13% CP) CSM-based diets based

on NRC Poultry (1994) recommendations were formulated to contain 3 levels of NPP; 0.35% (positive control, PC), 0.25% (negative control 1, NC1) and 0.15% (negative control 2, NC2). The Ca supply was equal (3.8% of the diet) in all dietary treatments. The PC diet was fed without supplemental phytase. Three levels of phytase (200, 400 and 600 U/kg) were added to each negative control diet. The phytase used was an *E. coli* 6-phytase (Quantum™ phytase, Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA) optimized for improved gastric and thermal tolerance and expressed in *Pichia pastoris*. Experimental diets were fed from 21 to 61 wk of age. Birds had free access to feed and water throughout the experiment. Experimental diets were analyzed for total phosphorus and calcium levels using the method of Zasoski and Barau (1977).

3.3.3 Data Collection

Egg production was recorded on a replication basis five days per wk and was then corrected to a seven days per wk basis. Eggs were classified as normal, broken, cracked, soft-shelled, double yolked and abnormal; abnormal was defined as eggs that were mis-shapen or mini (yolkless). The egg classification was based on all the eggs collected during the experiment. All eggs from one day were collected and weighed on a replication basis every 4 wk. Subsequently, the same eggs were used to assess specific gravity using nine saline solutions ranging from 1.060 to 1.100 with 0.005 increments. Saline solutions were calibrated prior to each test. Feed intake was determined on a replication basis by weighing back all feed every 4 wk. Body weight of all birds was measured at 21, 41 and 61 wk of age. Mortality was recorded on a replication basis. Dead birds were collected, weighed and recorded daily.

Table 3-1. The ingredient and nutrient composition of experimental diets.

Ingredients (%)	Positive Control	Negative Control 1	Negative Control 2
Corn	63.33	63.76	64.20
Soybean meal	22.82	22.75	22.68
Canola oil	2.35	2.22	2.08
Dicalcium phosphate	1.33	0.80	0.26
Limestone	9.03	9.34	9.65
Salt	0.40	0.40	0.40
Vitamin mineral premix [1]	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10
DL-Methionine	0.13	0.13	0.13
Quantum™ phytase[2] (g/kg)	0.00	0.06, 0.11 or 0.17	0.06, 0.11 or 0.17
Calculated nutrients (%)			
AME (kcal/kg)	2900	2900	2900
Crude protein	16.13	16.13	16.13
Calcium	3.80	3.80	3.80
Non-phytate phosphorus	0.35	0.25	0.15
Phytate phosphorus	0.22	0.22	0.22
Total phosphorus	0.57	0.47	0.37
Analyzed minerals, as-is basis (%)			
Calcium	4.48	4.45	4.64
Total phosphorus	0.54	0.44	0.34

[1] Vitamin mineral premix (units per kg of feed) – vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; quinguard M6S, 0.625 mg; calcium carbonate, 500 mg.

[2] Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA; Quantum™ phytase was added at the expense of corn.

3.3.4 Statistical Analyses

The data were analyzed as two separate experiments using PC as the control group with each NC treatment. Each set of experimental data was analyzed as a 5 x 2 factorial arrangement (5 experimental diets x 2 strains) using the Proc GLM procedure of SAS (SAS, 2002). Duncan's Multiple Range Test was used to separate the means when ANOVA was significant and regression analyses were used as appropriate. Differences were considered significant when $P < 0.05$.

3.4 Results

The chemical analysis of the experimental diets is shown in Table 3-1. The calculated and analyzed total P levels were in good conformity. The analyzed Ca level was higher than the planned level, but it was consistently higher across the diets. The high Ca level was due to the fact that the soybean meal contained a much higher level of Ca (0.63%) than was anticipated (0.25%).

3.4.1 PC and NC1 Comparisons

Only minor, non-significant differences were observed between the 0.35% NPP (PC) and 0.25% NPP without phytase supplementation (NC1) treatments for most of the production characteristics studied (Table 3-2). The hens fed the NC1 diet without exogenous phytase supplementation had a comparatively high incidence of abnormal eggs compared to the PC treatment, but the differences were not significant. The addition of phytase to the NC1 diet resulted in production performance values that were similar to those observed for the PC treatment. There were no significant ($P > 0.05$) differences observed for total hen-housed egg production (THHP), mortality, feed to egg mass ratio, feed intake, egg weight, body weight at trial end, incidence of soft shelled, cracked and

broken eggs, or incidence of double yolked eggs when comparing the PC and NC1 treatments, regardless of phytase supplementation. Total hen-day egg production (THDP), body weight at the end of trial and egg specific gravity were significantly ($P < 0.05$) higher for Shaver hens, whereas incidence of soft shelled, cracked and broken eggs, double eggs, abnormal eggs, feed to egg mass ratio, feed intake and egg weight were significantly lower for Shaver hens compared to Bovan hens. There was no dietary treatment x strain interaction observed for any of the variables studied.

3.4.2 PC and NC2 Comparisons

Hens consuming the 0.15% NPP diet (NC2) without phytase supplementation had significantly ($P < 0.05$) reduced THHP and body weight at the end of trial in comparison to the PC treatment, whereas the incidence of soft shelled, cracked and broken eggs was increased significantly in hens fed the NC2 diet (Table 3-3). Although the mortality rate was around 70% higher in NC2 treatment, the difference was not statistically significant. The addition of microbial phytase to the 0.15% NPP diet resulted in an improvement in production characteristics (mainly THDP, THHP, body weight at the trial end, feed to egg mass ratio, and the incidence of soft shelled, cracked and broken eggs) to levels that were equal to or greater than those observed for the PC treatment. There was a linear improvement in THDP, THHP, feed to egg mass ratio and mortality with the addition of exogenous phytase (200, 400 and 600 U/kg diet) to the NC2 diet.

There were no significant ($P > 0.05$) differences observed for egg weight, egg specific gravity, feed intake and the incidence of double yolked and abnormal eggs when comparing the PC and NC2 treatments, regardless of phytase supplementation. The incidence of double eggs, abnormal eggs, feed to egg mass ratio and feed intake were significantly ($P < 0.05$) lower in Shaver hens, whereas body weight at the end of trial and

egg specific gravity were significantly higher for Shaver than the Bovan strain. No dietary treatment x strain interactions were observed in the present experiment, except for body weight at the end of the trial (P = 0.0198).

3.5 Discussion

The results of the current experiment showed that the production performance of laying hens fed the diet containing 0.25% NPP (NC1) was not significantly different from those that were fed the diet containing 0.35% NPP (PC), regardless of phytase addition. The data demonstrate that the 0.25% NPP (NC1) diets provided sufficient phosphorus intake to support maximum hen performance and therefore were at or above the hen's requirement. Our results showed that laying hens can maintain optimal health and production when fed a diet containing 0.25% NPP, provided feed intake is normal. The NRC Poultry (1994) recommends that white-egg layers require 250 mg of NPP per day per hen if the feed intake is 100 g per day in order to maintain optimal health and production. Previous research conducted by Um and Paik (1999) also found that the supplementation of phytase in diets containing 0.24% NPP gave no further improvements in hen performance.

In contrast to the PC and NC1 comparisons, the results showed that feeding the 0.15% NPP diet (NC2) caused significant reductions in hen performance when compared to the PC treatment. This demonstrates that 0.15% NPP was insufficient at providing hens with their daily P requirement. Phytase supplementation completely corrected the significant reductions in hen performance that was caused by a diet deficient in available P. Our results on the effects of phytase supplementation in a 0.15% NPP diet on hen production performance were similar to those found by other workers.

Table 3-2. Effect of diet phosphorus (0.35 and 0.25%) and phytase level on performance characteristics in White Leghorn laying hens fed CSM-based diets.

	Dietary treatments (T)					Strain (S)		T x S	Regression[4]	SEM
Nonphytate P (%)	0.35	0.25	0.25	0.25	0.25	Bovan	Shaver		(Linear or	
Phytase (units per kg)			200	400	600				Quadratic)	
THDP[1] (%)	92.4[ab]	93.2[a]	93.3[a]	91.0[b]	91.9[ab]	91.5[b]	93.2[a]	NS[3]	--------	0.35
THHP[2] (%)	89.7	91.4	91.5	87.3	90.2	88.9	91.2	NS	--------	0.66
SSCBE[5] (%)	0.81	0.91	1.11	0.90	1.01	1.18[a]	0.71[b]	NS	--------	0.095
Double eggs (%)	0.23	0.30	0.17	0.20	0.26	0.34[a]	0.12[b]	NS	--------	0.026
Abnormal eggs (%)	0.06[b]	0.13[ab]	0.19[a]	0.11[ab]	0.10[ab]	0.17[a]	0.06[b]	NS	--------	0.019
Feed to egg mass ratio	1.95	1.93	1.92	1.97	1.96	1.99[a]	1.91[b]	NS	--------	0.010
Egg weight (g)	59.4	59.3	59.3	58.7	58.8	59.5[a]	58.7[b]	NS	--------	0.14
Egg specific gravity	1.083[ab]	1.083[ab]	1.084[a]	1.083[ab]	1.082[b]	1.081[b]	1.085[a]	NS	--------	0.0003
Feed intake (g/h/d)	107.2	106.6	106.4	105.2	105.8	108.1[a]	104.3[b]	NS	--------	0.48
Body wt. at trial end (kg)	1.85	1.82	1.87	1.85	1.85	1.77[b]	1.92[a]	NS	--------	0.016
Mortality (%)	8.33	4.17	5.83	7.50	5.00	7.33	5.00	NS	--------	1.154

[ab] Means within a row (within dietary treatments and strain) with different superscripts are significantly different ($P < 0.05$).
[1] Total hen day egg production.
[2] Total hen housed egg production.
[3] NS - non significant.
[4] Regression analyzed within the NC1 treatment only.
[5] Soft shelled, cracked and broken eggs.

Table 3-3. Effect of diet phosphorus (0.35 and 0.15%) and phytase level on performance characteristics in White Leghorn laying hens fed CSM-based diets.

	Dietary treatments (T)					Strain (S)		T x S	Regression[1]	SEM
Nonphytate P (%)	0.35	0.15	0.15	0.15	0.15	Bovan	Shaver		(Linear or	
Phytase (units per kg)			200	400	600				Quadratic)	
THDP[2] (%)	92.4[ab]	90.7[b]	92.4[ab]	91.9[ab]	93.0[a]	91.6	92.6	NS[3]	Linear	0.28
THHP[4] (%)	89.7[a]	83.6[b]	89.6[a]	88.3[a]	91.5[a]	87.7	89.4	NS	Linear	0.73
SSCBE[5] (%)	0.81[b]	2.76[a]	1.43[b]	1.11[b]	0.91[b]	1.29	1.51	NS	Quadratic	0.156
Double eggs (%)	0.23	0.23	0.24	0.28	0.20	0.35[a]	0.13[b]	NS	--------	0.027
Abnormal eggs (%)	0.06	0.11	0.13	0.11	0.10	0.14[a]	0.06[b]	NS	--------	0.015
Feed to egg mass ratio	1.95[ab]	2.00[a]	1.94[ab]	1.94[b]	1.91[b]	1.99[a]	1.91[b]	NS	Linear	0.012
Egg weight (g)	59.4	59.2	59.1	59.5	59.2	59.5	59.1	NS	--------	0.15
Egg specific gravity	1.083	1.083	1.083	1.083	1.084	1.082[b]	1.085[a]	NS	--------	0.0003
Feed intake (g/h/d)	107.2	108.0	106.2	106.1	105.1	108.6[a]	104.4[b]	NS	--------	0.62
Body wt. at trial end (kg)	1.85[ab]	1.77[c]	1.81[bc]	1.88[a]	1.84[ab]	1.76[b]	1.90[a]	0.0198	--------	0.016
Mortality (%)	8.33[ab]	14.17[a]	6.67[ab]	10.83[a]	2.50[b]	10.00	7.00	NS	Linear	1.217

[ab] Means within a row (within dietary treatments and strain) with different superscripts are significantly different ($P < 0.05$).
[1] Regression analyzed within the NC2 treatment only.
[2] Total hen day egg production.
[3] NS - non significant.
[4] Total hen housed egg production.
[5] Soft shelled, cracked and broken eggs.

Previous research has found that supplementing a diet containing 0.10 – 0.15% NPP with phytase resulted in a significant improvement in laying hen egg production when compared to the same diet that was not supplemented with exogenous phytase (Punna and Roland, 1999; Keshavarz, 2000; Lim et al., 2003; Wu et al., 2006). We observed that there was a linear increase in both hen-day egg production and hen-housed egg production with the addition of phytase to the 0.15% NPP diet. Hens consuming a diet containing reduced levels of NPP without phytase supplementation were least efficient in terms of feed conversion (Jalal and Scheideler, 2001). The addition of phytase to 0.1% (Jalal and Scheideler, 2001) and 0.2% NPP (Scott et al., 2001) diets has the ability to significantly improve feed efficiency. We found that the diet containing 0.15% NPP (NC2) without enzyme addition was the least efficient in terms of feed conversion (feed to egg mass ratio). Lim et al. (2003) documented that phytase supplementation decreased the percentage of broken and soft shelled eggs. There was a quadratic decrease in the percentage of soft shelled, broken and cracked eggs with the addition of phytase to the NC2 diet in the current study.

It has been previously found that the body weight of hens fed a diet containing 0.1% NPP was significantly less than that of hens fed the same diet with the addition of phytase (Gordon and Roland, 1997; Van der Klis et al., 1997). The results of our study are in agreement with these previous studies. The body weight of the hens fed the 0.15% NPP diet was significantly lower than that of the hens fed the same diet with phytase supplementation. Higher mortality in hens consuming diets deficient in NPP have been found by Punna and Roland (1999) and Jalal and Scheideler (2001). Our data showed that there was a linear decrease in mortality with the addition of phytase to the NC2 diet.

A few production characteristics were unaffected by the NPP deficiency or exogenous phytase supplementation. These include egg weight, egg specific gravity, feed intake and the incidence of double yolked and abnormal eggs. Punna and Roland (1999) found that phytase supplementation increased egg weight in hens fed a diet containing 0.1% available phosphorus, but had no effect with 0.2, 0.3 and 0.4% available phosphorus diets. We may have seen a difference in egg weight if the dietary NPP level in this study would have been lower than 0.15%. Boling et al. (2000) also found that there were no significant differences in egg specific gravity, regardless of NPP level or phytase inclusion. It is unknown why we did not see a significant effect of NPP level on egg specific gravity in our study. There was a clear decrease in egg shell quality as shown by the significant increase in soft shelled, cracked and broken eggs when P was removed from the diet. The significant increase in soft shelled, cracked and broken eggs is an indication that egg shell quality has diminished and, therefore, one might also expect a response in egg specific gravity. Contrary to the results of the present study, Gordon and Roland (1997) and Jalal and Scheideler (2001) documented that feed consumption increased in hens fed diets containing 0.1% NPP supplemented with phytase. An increase in feed intake with phytase supplementation may have been seen because of the more severe P deficiency in a 0.1% NPP diet.

The results indicate that the addition of phytase to diets deficient in NPP improved hen production characteristics up to levels seen for hens fed a diet that is adequate in NPP. This shows that Quantum™ phytase is efficacious in corn-soybean meal diets fed to White Leghorn laying hens and can be used to reduce diet supplementation with inorganic P. Therefore, Quantum™ phytase can be added to diets deficient in NPP,

0.25% or 0.15% NPP, to ensure that birds consuming these diets are provided with adequate P for optimal health and production throughout the laying period. Many producers are concerned about production losses caused by diets deficient in NPP. If producers are hesitant to feed 0.15% NPP with phytase supplementation, we recommend that phytase be fed with a 0.25% NPP diet. NRC Poultry (1994) recommends that a hen's daily P requirement is 250 mg. Most producers feed diets high in inorganic P, containing approximately 0.35% NPP to ensure that P requirements are met. In place of inorganic P, we suggest that Quantum™ phytase be fed on top of a 0.25% NPP diet as a safety margin. As a bonus, replacing inorganic P with exogenous phytase has some additional benefits - it is more environmentally friendly due to reduced P excretion, and in the long run, may be more cost effective.

CHAPTER 4
THE EFFICACY OF QUANTUM™ PHYTASE ON NUTRIENT DIGESTIBILITY IN WHITE LEGHORN LAYING HENS FED CORN-SOYBEAN MEAL BASED DIETS

4.1 Abstract

The efficacy of an *Escherichia coli* 6-phytase (Quantum™) on nutrient digestibility and bone ash in laying hens fed corn-soybean meal (CSM) diets was investigated. A total of 108 White Leghorn hens (54 each of Shaver and Bovan strains) were fed CSM diets containing 0.35% (positive control, PC), 0.25% (negative control 1, NC1) or 0.15% (negative control 2, NC2) non-phytate phosphorus (NPP) from 21 to 61 wk of age. Six more diets were manufactured by supplementing the negative control diets with 200, 400 or 600 U/kg of exogenous phytase resulting in a total of 9 treatments. Each dietary treatment x strain subclass was replicated twice with six hens per replication in a randomized complete block design. The digestibility coefficients of ileal and fecal protein were higher ($P < 0.05$), whereas P digestibility was lower, for the unsupplemented negative control treatments compared to the PC. A linear reduction in phytate digestibility, ileal protein digestibility and soluble P was reported with increasing levels of exogenous phytase in the NC1 diet. Supplementation of the NC1 with 200 or 600 U/kg resulted in an improvement in P digestibility, whereas Ca digestibility was reduced significantly ($P < 0.05$) with phytase addition. Phytase addition to the NC1 treatment resulted in a linear decrease in the digestibility of amino acids except for methionine and proline. Microbial phytase supplementation to the NC2 diet resulted in no significant

improvement in digestibility coefficients of phytate, ileal protein, fecal protein and P compared to unsupplemented NC2. Significantly higher phytate and Ca digestibilities were demonstrated with the NC2 treatment containing 400 U/kg Quantum™ phytase compared to the PC. Tibial bone ash percentage was higher ($P < 0.05$) in 61-wk-old hens fed 200 or 400 U/kg phytase supplemented NC2 diets. Significantly higher diet AME and fecal protein digestibility was demonstrated for Shaver hens in comparison to the Bovan hens. Overall, the Quantum™ phytase was not efficacious at improving nutrient digestibility in laying hens fed CSM-based diets deficient in NPP.

4.2 Introduction

Phytate, the mixed salts of phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6 hexakis dihydrogen phosphate) is a ubiquitous component of plant-sourced feed ingredients which accounts for approximately two-thirds of the total P found in plant-based diets. The P that is associated with phytate in feed is poorly utilized by monogastric animals due to low levels of phytase activity in their digestive tracts (Maenz and Classen, 1998). The utilization of phytate P by single-stomached animals has been reported to vary from less than 10 to over 50% (Selle et al., 2000; Ravindran et al., 2001). It is well recognized that phytate reduces the availability of P and other minerals, such as calcium, zinc and copper. Similarly, it is speculated that phytate may also affect the availability of nutrients such as protein, amino acids and energy in poultry feed ingredients. Due to its polyanionic nature, the phytic acid molecule can chelate di- or trivalent cations and interact with carbohydrates and proteins, reducing their availability for poultry (Selle et al., 2000).

Microbial phytase supplementation has the ability to significantly improve phytate P utilization in poultry diets and the results have been well documented by several

researchers (Augspurger and Baker, 2004; Snow et al., 2004). Dietary phytase supplementation may also have the ability to influence protein and amino acid digestibility in broiler chickens (Augspurger and Baker, 2004; Snow et al., 2004) and laying hens (Liebert et al., 2005). The results from previous research on this topic are quite variable, but overall, they tend to indicate that phytase improves protein and amino acid digestibility and availability in poultry diets (Namkung and Leeson, 1999; Rutherford et al., 2004). Improvements in protein and amino acid digestibility due to phytase supplementation would also be expected to result in improved energy utilization in poultry. Published data on the effects of microbial phytase on AME are not extensive and results found by others are quite variable. Ravindran et al. (2001) and Newkirk and Classen (2001) demonstrated an improvement in AME values with phytase supplementation in broiler diets, whereas Onyango et al. (2004) found that AME was not improved by dietary phytase supplementation.

The majority of the research regarding the effects of phytase supplementation on nutrient digestibility has been performed with broiler chickens; data is very limited with laying hens. The efficiency of phytase supplementation in layer diets is still under discussion because of an open debate about the non-phytate phosphorus (NPP) requirement of laying hens and factors influencing phytate degradation by exogenous phytase in the gastrointestinal tract of layers (Liebert et al., 2005). Jalal and Scheideler (2001) documented that supplementation of phytase in normal, CSM-based diets improved Ca and P digestibilities, feed intake, feed conversion and egg mass, and elicited a response in shell quality and egg components at a low level of NPP (0.10%). Snow et al. (2003) observed no phytase effect on ileal amino acid digestibility in molted laying

hens. However, it should be remembered that all phytase products are not equal and may result in variation when looking at their effect on nutrient digestibility and availability. Phytase enzymes differ in the source from which they are derived. They may differ in characteristics such as pH optimum, thermostability and ability to resist hydrolysis within the digestive tract. Any difference in these characteristics will affect the ability of the phytase enzyme to function effectively and consistently within the digestive tract (Onyango et al., 2005a). Therefore, all phytase enzymes produced must be tested *in vivo* to ensure efficacy before they are introduced to the monogastric feed market.

Previously, there has been little or no work done on the effect of an *E. coli*-derived 6-phytase (Quantum™ phytase) supplementation in laying hen diets. The present study was, therefore, conducted to assess the efficacy of Quantum™ phytase on digestibility coefficients of various nutrients (phytate, ileal and fecal protein, P, Ca, amino acids, and diet AME), tibia bone ash and digestive tract characteristics in White Leghorn laying hens fed CSM-based diets containing varying levels of NPP.

4.3 Materials and Methods

The current nutrient digestibility trial was run in conjunction with a 40-wk (21 to 61 wk of age) production trial in order to obtain bone ash data from hens at the middle and end of production (Hughes et al., 2008). The experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan and was performed in accordance with recommendations of the Canadian Council on Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

4.3.1 Animals and Housing

At 17 wk of age, a total of 108 White Leghorn laying hens (54 each of Shaver White and Bovan strains) were housed in cages (cage dimensions 30.5 cm x 46 cm with a height of 52 cm; floor space per hen = 468 cm^2) under controlled climate conditions. Three hens were kept in each cage and an experimental unit consisted of two adjoining cages. Starting at 18 wk of age, the lighting program was changed from 8L:16D to 14L:10D with a light intensity of 10 lux. The ambient temperature was maintained at a minimum of approximately 20°C throughout the trial. During the pre-experimental period (i.e. up to 21 wk of age), a commercial laying hen diet was offered *ad libitum*, whereas from 21 wk onwards, the hens were randomly assigned to one of nine dietary treatments. Each dietary treatment x strain subclass was replicated twice with six hens per replication in a randomized complete block design.

4.3.2 Experimental Diets

The ingredient and nutrient composition of the experimental diets is depicted in Table 4-1. Three isocaloric (2900 Kcal/kg) and isonitrogenous (16.90% CP) CSM-based diets were formulated to contain three levels of NPP: 0.35% (positive control, PC), 0.25% (negative control 1, NC1) and 0.15% (negative control 2, NC2). The Ca supply was equal (3.8% of the diet) for all dietary treatments. The PC diet was fed without supplemental phytase. Each NC diet was prepared as a single batch in a horizontal mixer. Appropriate quantities of each NC diet were selected, and phytase was added prior to remixing with and without 200, 400 or 600 U/kg of supplemental phytase. The phytase is an *E. coli* 6-phytase (Quantum™ phytase, Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA) optimized for improved gastric and thermal tolerance and expressed in *Pichia pastoris*. Celite® (Celite Corporation, Lompoc, California, USA), an indigestible marker

Table 4-1. The ingredient and nutrient composition of experimental diets.

Ingredients (%)	Positive Control	Negative Control 1	Negative Control 2
Corn	57.02	57.51	58.01
Soybean meal	26.15	26.07	25.99
Canola oil	3.72	3.56	3.41
Dicalcium phosphate	1.03	0.56	0.09
Limestone	9.32	9.54	9.75
Common salt	0.41	0.41	0.41
Vitamin mineral premix[1]	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10
DL-Methionine	0.22	0.22	0.22
L-Threonine	0.03	0.03	0.03
Celite®[2]	1.50	1.50	1.50
Quantum™ phytase[3] (g/kg diet)	0.00	0.06, 0.11 or 0.17	0.06, 0.11 or 0.17
Calculated nutrients (%)			
AME (kcal/kg)	2900	2900	2900
Crude protein	16.90	16.90	16.90
Calcium	3.80	3.80	3.80
Nonphytate phosphorus	0.35	0.25	0.15
Phytate phosphorus	0.28	0.28	0.28
Total phosphorus	0.63	0.53	0.43
Analyzed minerals, as-is basis (%)			
Calcium	4.06	4.34	4.38
Total phosphorus	0.55	0.45	0.35

[1] Vitamin mineral premix (units per kg feed) – vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; quinguard M6S, 0.625 mg; calcium carbonate, 500 mg.

[2] Celite Corporation, Lompoc, California, USA

[3] Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA; Quantum™ phytase was added at the expense of corn.

was mixed in experimental diets at a concentration of 1.5%. The diets containing celite® were fed for 7 d from 42 wk onwards i.e. until the end of the digestibility trial. Birds had free access to feed and water throughout the experiment.

4.3.3 Sample Collection and Laboratory Analyses

On d 5 of the experimental week (from wk 42 onwards), trays were placed beneath each cage, and feces were quantitatively collected twice per day for the next two days. Feed consumption was also determined for these two days. Feces were pooled by replication and immediately frozen. They were freeze-dried, mixed and sub-sampled for further analysis. On d 7 of the experiment, all hens were humanely killed by cervical dislocation. The digestive tracts were removed immediately and digesta samples were collected from the terminal ileum (i.e. mid way between Meckel's diverticulum to 2 cm anterior to the ileo-ceacal junction). The digesta samples were also pooled per replication, frozen and then freeze-dried prior to analysis. The empty gut length and weight measurements were also recorded for each section of the small intestine (duodenum, jejunum and ileum). The left tibia of each hen was collected at 42 (middle of 40 wk production trial) and 61 (end of 40 week production trial) wk of age to determine bone ash (AOAC, 1990).

Diet, ileal digesta and fecal samples were analyzed for moisture, crude nitrogen, acid insoluble ash (AIA) and gross energy. Moisture was determined by method 930.15 of the AOAC (1990) while gross energy was determined using an oxygen bomb calorimeter (Model 1281, Parr Instruments, Moline, IL). Crude nitrogen content was determined by the combustion method (984.13; AOAC International, 1995) using a Leco FP-528 protein analyzer (St. Joseph, MI). Amino acid content of the diet and ileal digesta samples was determined by the method described by Llames and Fontaine (1994). The apparent digestibility calculations were based on the use of AIA (Acid Insoluble Ash) as the

indigestible marker which was determined by the procedure of Vogtmann et al. (1975). Diet and freeze-dried fecal samples were analyzed for the determination of total phosphorus and calcium using the method of Zasoski and Burau (1977). Fecal samples were also analyzed for soluble phosphorus content. Phytate (myoinositol hexaphosphate to myoinositol diphosphate; IP6 to IP2) in the diets and ileal digesta was determined by the method described by Newkirk and Classen (1998) using high performance liquid chromatography.

4.3.4 Data Analyses

The data were analyzed as two separate experiments using PC as the control group with each NC treatment. Each set of experimental data was analyzed as a 5 x 2 factorial arrangement (5 experimental diets x 2 strains) using the Proc GLM of SAS (SAS Institute, 2002). Treatment means were compared using Duncan's Multiple Range Test. Regression analyses and apriori contrasts were used as appropriate. Differences were considered significant when $P < 0.05$ and all differences were noted when $P < 0.10$.

4.4 Results

The nutrient compositions of the PC and NC diets are shown in Table 4-1. The calculated and analyzed total P and Ca levels were in good conformity.

4.4.1 Nutrient Digestibility, Tibial Ash and Digestive Tract Characteristics: PC and NC1 Comparisons

The effect of dietary P (0.35 and 0.25%) and phytase level (200, 400 and 600 U/kg diet) on digestibility coefficients of phytate, ileal protein, fecal protein, Ca and P; diet AMEn and tibial ash values is presented in Table 4-2. The digestibility coefficients of ileal and fecal protein were significantly ($P < 0.05$) higher for the NC1 diet (without

Table 4-2. Effect of dietary phosphorus (0.35 and 0.25%) and phytase level on diet AMEn, digestibility coefficients of phytate, ileal protein, fecal protein, Ca and P, and tibial ash values in laying hens fed CSM-based diets.

	Dietary treatments (T)					Strain (S)		SEM	T x S	Regression[1]
Nonphytate P (%)	0.35	0.25	0.25	0.25	0.25	Bovan	Shaver			(Linear or
Phytase (units per kg)			200	400	600					Quadratic)
AMEn (Kcal/kg), 90% DM basis	2667^{ab}	2683^{ab}	2689^{ab}	2719^{a}	2658^{b}	2679	2686	8.4	NS	NS
Phytate Digestibility	0.23^{bc}	0.48^{ab}	0.52^{a}	0.35^{abc}	0.11^{c}	0.37	0.30	0.051	NS	Linear
Ileal Protein Dig	0.71^{b}	0.80^{a}	0.75^{ab}	0.73^{ab}	0.69^{b}	0.74	0.73	0.014	NS	Linear
Fecal Protein Dig	0.30^{c}	0.37^{a}	0.34^{ab}	0.37^{a}	0.31^{bc}	0.33	0.34	0.011	0.0015	NS
Ca Digestibility	0.54^{ab}	0.62^{a}	0.46^{b}	0.46^{b}	0.48^{b}	0.51	0.52	0.024	0.0085	NS
P Digestibility	0.42^{a}	0.10^{b}	0.41^{a}	0.16^{b}	0.45^{a}	0.28	0.33	0.040	NS	NS
Soluble P in feces (%)	0.75^{ab}	0.97^{a}	0.51^{bc}	0.83^{a}	0.41^{c}	0.74	0.65	0.056	NS	Linear
Bone Ash (%), 42 wk	56.1	57.2	57.0	56.4	57.0	56.7	56.8	0.29	0.0166	NS
Bone Ash (%), 61 wk	57.8	57.3	58.5	58.3	58.0	57.4^{b}	58.6^{a}	0.27	NS	NS

^{ab} Means within a row (within dietary treatments and strain) with different letters are significantly different ($P < 0.05$); NS: $P > 0.05$.

[1] Regression analyzed within the NC1 treatment only.

phytase supplementation) compared to the PC, whereas P digestibility was significantly lower for the NC1 than for the PC. There was no difference ($P > 0.05$) in diet AMEn, apparent digestibilities of phytate and Ca, and tibial ash percentage between hens fed either PC or NC1 diet without phytase supplementation.

Supplementation of the NC1 with 200 or 600 U/kg phytase resulted in an improvement in P digestibility compared to the unsupplemented NC1, whereas Ca digestibility was reduced ($P < 0.05$) with exogenous phytase supplementation (Table 4-2). The ileal and fecal protein digestibility coefficients were lower ($P < 0.05$) for the NC1 diet supplemented with 600 U/kg phytase compared to unsupplemented NC1. However, fecal protein digestion was improved for the NC1 diet supplemented with 200 or 400 U/kg phytase compared to the PC. With increasing levels of phytase addition to the NC1 diet, a linear reduction in phytate digestibility, ileal protein digestibility and soluble P was observed. Dietary treatment x strain interactions were observed for fecal protein digestibility ($P = 0.001$) and Ca digestibility ($P = 0.008$). These interactions indicate that both the dietary treatment and bird strain had an effect on fecal protein digestibility and Ca digestibility.

No significant differences were observed in tibial ash percentage of hens at 42 or 61 wk of age fed either the PC or NC1 treatment, regardless of phytase supplementation. The type of bird strain (Bovan or Shaver) had no effect on the digestibility of the various nutrients studied. At 61 wk of age, tibial ash percentage was significantly ($P < 0.05$) higher in Shaver than in Bovan hens. The effect of dietary P and phytase levels on apparent ileal digestibility coefficients of amino acids in laying hens is presented in Table 4-3. The coefficients of digestibility of cysteine, aspartate, alanine and valine were

Table 4-3. Effect of dietary phosphorus (0.35 and 0.25%) and phytase level on apparent ileal digestibility coefficients of amino acids in laying hens fed CSM-based diets.

	Dietary treatments (T)					Strain (S)		SEM	T x S	Regression[1]
Nonphytate P (%)	0.35	0.25	0.25	0.25	0.25	Bovan	Shaver			(Linear or
Phytase (units per kg)			200	400	600					Quadratic)
Arginine	0.82	0.87	0.87	0.83	0.79	0.84	0.84	0.012	NS	Linear
Histidine	0.79	0.86	0.85	0.82	0.78	0.82	0.82	0.012	NS	Linear
Isoleucine	0.78	0.84	0.83	0.79	0.76	0.80	0.79	0.013	NS	Linear
Leucine	0.79	0.85	0.83	0.81	0.77	0.81	0.81	0.012	NS	Linear
Lysine	0.77	0.84	0.82	0.79	0.74	0.80	0.79	0.015	NS	Linear
Methionine	0.84	0.88	0.89	0.85	0.84	0.86	0.86	0.010	NS	NS
Phenylalanine	0.81	0.86	0.86	0.83	0.79	0.83	0.83	0.012	NS	Linear
Threonine	0.68	0.77	0.73	0.71	0.66	0.71	0.71	0.016	NS	Linear
Valine	0.74^{ab}	0.81^{a}	0.81^{a}	0.77^{ab}	0.69^{b}	0.77	0.76	0.016	NS	Linear
Alanine	0.78^{ab}	0.85^{a}	0.83^{ab}	0.80^{ab}	0.76^{b}	0.81	0.80	0.012	NS	Linear
Aspartate	0.77^{ab}	0.84^{a}	0.81^{ab}	0.79^{ab}	0.75^{b}	0.80	0.79	0.012	NS	Linear
Cysteine	0.71^{ab}	0.79^{a}	0.74^{ab}	0.73^{ab}	0.67^{b}	0.74	0.72	0.015	NS	Linear
Glutamine	0.84	0.89	0.88	0.86	0.83	0.86	0.86	0.010	NS	Linear
Glycine	0.72	0.81	0.78	0.75	0.70	0.75	0.75	0.015	NS	Linear
Proline	0.83	0.87	0.87	0.87	0.82	0.86	0.85	0.009	NS	NS
Serine	0.76	0.83	0.79	0.78	0.74	0.78	0.77	0.013	NS	Linear

[ab] Means within a row (within dietary treatments and strain) with different letters are significantly different ($P < 0.05$); NS: $P > 0.05$.
[1] Regression analyzed within the NC1 treatment only.

significantly ($P < 0.05$) reduced for NC1 with 600 U/kg phytase supplementation compared to the unsupplemented NC1. Phytase supplementation of the NC1 treatment resulted in a linear decrease in digestibility of all amino acids except for methionine and proline.

Hens fed unsupplemented NC1 had significantly reduced ileum length in comparison to those who received the PC diet (Table 4-4). The addition of phytase to the NC1 treatment resulted in a linear increase in jejunum and ileum length to levels that were equal to or greater than those observed for the PC hens. The lengths of the jejunum and ileum were increased ($P < 0.05$) in birds fed 600 U/kg phytase-supplemented NC1 diets than those fed unsupplemented NC1. The weights of different intestinal segments were not significantly different between the PC and NC1 treatments, regardless of exogenous phytase addition. However, dietary treatment x strain interactions were observed for duodenum ($P = 0.003$) and jejunum ($P = 0.031$) weights. These interactions indicate that both the dietary treatment and bird strain had an effect on duodenum and jejunum weight.

4.4.2 Nutrient Digestibility, Tibial Ash and Digestive Tract Characteristics: PC and NC2 Comparisons

The effect of dietary P (0.35 and 0.15%) and phytase levels on digestibility coefficients of phytate, ileal protein, fecal protein, Ca and P, diet AMEn and tibial ash values is shown in Table 4-5. The fecal protein digestibility coefficient was significantly ($P < 0.05$) higher for the unsupplemented NC2 treatment compared to the PC, whereas P digestibility was significantly lower for the NC2 treatment when compared to the PC.

Exogenous phytase supplementation at various concentrations of the NC2 diet resulted in no significant improvement in digestibility coefficients of phytate, ileal protein, fecal protein and P digestibility or compared to unsupplemented NC2 (Table 4-5).

Table 4-4. Effect of dietary phosphorus (0.35 and 0.25%) and phytase level on gastrointestinal tract length and weight in laying hens.

	Dietary treatments (T)					Strain (S)		SEM	T x S	Regression[1]
Nonphytate P (%)	0.35	0.25	0.25	0.25	0.25	Bovan	Shaver			(Linear or
Phytase (units per kg)			200	400	600					Quadratic)
Length (cm)										
Duodenum	25.8	24.6	24.5	28.2	25.7	26.3	25.2	0.61	NS	NS
Jejunum	55.7^{ab}	51.6^{b}	51.9^{b}	54.8^{ab}	58.5^{a}	55.5	53.5	0.75	NS	Linear
Ileum	58.9^{a}	53.1^{c}	53.5^{bc}	57.4^{ab}	60.0^{a}	56.9	56.2	0.71	NS	Linear
Weight (g)										
Duodenum	9.4	8.7	9.0	8.5	8.2	8.7	8.8	0.21	0.0038	NS
Jejunum	18.2	16.8	16.8	17.2	17.5	17.3	17.3	0.45	0.0311	NS
Ileum	16.2	14.8	13.3	15.3	15.7	15.1	15.0	0.50	NS	NS

^{ab} Means within a row (within dietary treatments and strain) with different letters are significantly different ($P < 0.05$); NS: $P > 0.05$.

[1] Regression analyzed within the NC1 treatment only.

Table 4-5. Effect of dietary phosphorus (0.35 and 0.15%) and phytase level on diet AMEn, digestibility coefficients of phytate, ileal protein, fecal protein, Ca and P, and tibial ash values in laying hens fed CSM-based diets.

	Dietary treatments (T)					Strain (S)		SEM	T x S	Regression[1]
Nonphytate P (%)	0.35	0.15	0.15	0.15	0.15	Bovan	Shaver			Linear or
Phytase (units per kg)			200	400	600					Quadratic
AMEn (Kcal/kg) 90% DM basis	2667[b]	2661[b]	2736[a]	2626[b]	2653[b]	2651[b]	2686[a]	11.1	NS	NS
Phytate Digestibility	0.23[b]	0.38[ab]	0.53[ab]	0.58[a]	0.35[ab]	0.35	0.48	0.045	NS	NS
Ileal Protein Dig	0.71	0.75	0.73	0.74	0.75	0.72	0.75	0.009	NS	NS
Fecal Protein Dig	0.30[b]	0.35[a]	0.36	0.35[a]	0.35[a]	0.32[b]	0.36	0.009	NS	NS
Ca Digestibility	0.54[b]	0.56[b]	0.60[ab]	0.67[a]	0.54[b]	0.59	0.57	0.016	NS	NS
P Digestibility	0.42[a]	-0.13[b]	0.04[b]	0.05[b]	-0.09[b]	0.01	0.11	0.053	NS	NS
Soluble P in feces (%)	0.75[b]	0.90[ab]	1.01[a]	0.89[ab]	0.81[ab]	0.91	0.84	0.035	NS	NS
Bone Ash (%) 42 wk	56.1	56.8	57.1	55.9	57.1	56.1	57.1	0.40	NS	NS
Bone Ash (%) 61 wk	57.8[a]	55.6[b]	58.0[a]	57.5[a]	57.2[ab]	56.7	57.7	0.28	NS	NS

[ab] Means within a row (within dietary treatments and strain) with different letters are significantly different ($P < 0.05$); NS: $P > 0.05$.

[1] Regression analyzed within the NC2 treatment only.

Significantly ($P < 0.05$) higher phytate and Ca digestibilities were demonstrated with the NC2 treatment containing 400 U/kg exogenous phytase compared to the PC. Diet AMEn was significantly improved with 200 U/kg phytase, whereas the positive effect of phytase supplementation disappeared with higher levels of phytase. Surprisingly, the P digestibility was very low in the NC2 treatment and there was no effect of phytase addition on P digestibility. Significantly higher diet AMEn (1.24% higher) and fecal protein digestibility (13.4% higher) was demonstrated for Shaver hens in comparison to the Bovan strain.

At 61 wk of age, the tibial ash percentage was significantly higher in hens fed the PC diet than those fed the unsupplemented NC2 diet. Bone ash percentage was higher ($P < 0.05$) in 61 wk old hens fed 200 or 400 U/kg phytase supplemented NC2 diets compared to those given unsupplemented NC2. However, at 42 wk of age there was no difference ($P > 0.05$) in tibial ash percentage in birds fed either the PC or the NC2 diet, regardless of phytase supplementation. The effect of dietary P (0.35 and 0.15%) and phytase levels on apparent ileal digestibility coefficients of amino acids are presented in Table 4-6. No significant differences were observed between the PC and NC2 treatments for amino acid digestibility regardless of phytase addition.

The empty weight of the duodenum was lower ($P < 0.05$) in hens receiving the unsupplemented NC2 diet compared to those fed the PC (Table 4-7). No significant differences were observed between the PC and NC2 treatments, regardless of phytase addition, for the lengths and weights of various segments of the digestive tract except that jejunum length increased significantly in hens fed NC2 with 400 U/kg phytase compared to unsupplemented NC2. The duodenum and jejunum were significantly longer in Bovan

Table 4-6. Effect of dietary phosphorus (0.35 and 0.15%) and phytase level on apparent ileal digestibility coefficients of amino acids in laying hens fed CSM-based diets.

	Dietary treatments (T)					Strain (S)		SEM	T x S	Regression[1]
Nonphytate P (%)	0.35	0.15	0.15	0.15	0.15	Bovan	Shaver			(Linear or
Phytase (units per kg)			200	400	600					Quadratic)
Essential amino acids										
Arginine	0.82	0.87	0.83	0.85	0.85	0.84	0.85	0.009	NS	NS
Histidine	0.79	0.85	0.82	0.84	0.83	0.82	0.83	0.009	NS	NS
Isoleucine	0.78	0.83	0.80	0.82	0.81	0.81	0.81	0.011	NS	NS
Leucine	0.79	0.83	0.81	0.83	0.82	0.81	0.82	0.009	NS	NS
Lysine	0.77	0.83	0.77	0.80	0.80	0.79	0.80	0.012	NS	NS
Methionine	0.84	0.87	0.85	0.87	0.85	0.86	0.86	0.009	NS	NS
Phenylalanine	0.81	0.85	0.82	0.84	0.82	0.83	0.83	0.009	NS	NS
Threonine	0.68	0.74	0.70	0.74	0.72	0.71	0.72	0.013	NS	NS
Valine	0.74	0.80	0.77	0.75	0.78	0.78	0.76	0.016	NS	NS
Non essential amino acids										
Alanine	0.78	0.83	0.80	0.82	0.81	0.80	0.81	0.010	NS	NS
Aspartate	0.77	0.82	0.79	0.82	0.80	0.80	0.80	0.010	NS	NS
Cysteine	0.71	0.76	0.72	0.76	0.73	0.73	0.74	0.011	NS	NS
Glutamine	0.84	0.88	0.86	0.87	0.86	0.86	0.86	0.007	NS	NS
Glycine	0.72	0.79	0.75	0.78	0.76	0.75	0.76	0.012	NS	NS
Proline	0.83	0.84	0.85	0.87	0.86	0.85	0.85	0.007	NS	NS
Serine	0.76	0.80	0.77	0.80	0.78	0.78	0.79	0.010	NS	NS

[ab] Means within a row (within dietary treatments and strain) with different letters are significantly different ($P < 0.05$); NS: $P > 0.05$.

[1] Regression analyzed within the NC2 treatment only.

Table 4-7. Effect of dietary phosphorus (0.35 and 0.15%) and phytase level on gastrointestinal tract length and weight in laying hens.

	Dietary treatments					Strain		SEM	T x S	Regression[1]
Nonphytate P (%)	0.35	0.15	0.15	0.15	0.15	Bovan	Shaver			(Linear or
Phytase (units per kg)			200	400	600					Quadratic)
Length (cm)										
Duodenum	25.8^{bc}	26.5^{ab}	24.0^{c}	27.7^{a}	25.0^{bc}	26.7^{a}	24.9^{b}	0.32	NS	NS
Jejunum	55.7^{ab}	54.8^{b}	55.4^{ab}	59.1^{a}	54.6^{b}	58.3^{a}	53.5^{b}	0.66	NS	NS
Ileum	58.9	58.2	55.6	57.9	56.5	58.1	56.8	0.69	NS	NS
Weight (g)										
Duodenum	9.4^{a}	8.3^{b}	8.9^{ab}	8.4^{ab}	8.8^{ab}	8.6	8.9	0.16	0.0161	NS
Jejunum	18.2	18.0	19.0	17.8	17.2	17.6	18.4	0.43	NS	NS
Ileum	16.2	15.7	17.0	14.9	15.0	15.3	16.2	0.48	NS	NS

^{ab} Means within a row (within dietary treatments and strain) with different letters are significantly different ($P < 0.05$); NS: $P > 0.05$.

[1] Regression analyzed within the NC2 treatment only.

hens compared to the Shaver hens. Dietary treatment x strain interactions were also observed for duodenum weight (P = 0.016). These interactions indicate that both the dietary treatment and bird strain had an effect on duodenum weight.

4.5 Discussion

The results of the present study demonstrated that in laying hens, the nutrient digestibility (Ca, P, phytate, protein, amino acids and diet AMEn) of CSM-based diets containing low levels of NPP (0.25 or 0.15%) was comparatively higher than with a higher level of NPP (0.35%). This is an indication that excess levels of P in the diet can actually have a negative impact on the digestion of nutrients in laying hens. Previously, Ravindran et al. (2006) demonstrated that when total P was decreased from 0.67% to 0.53% in broiler diets, the diet AME, ileal P, Ca, protein and amino acid digestibility were increased. Silversides et al. (2004) also reported an increase in diet AME and P digestibility when NPP was decreased from 0.40% to 0.23% in broiler diets. The data presented in this paper showed similar results for most of the nutrients studied, except for P digestibility which was significantly lower in the unsupplemented NC1 and NC2 treatments compared to the PC. When the dietary NPP level is decreased, a major decrease in P digestibility may be observed due to the fact that the majority is bound to phytate, and the excess P that was seen in a diet containing 0.35% NPP is now not available for the animal in a 0.25% or 0.15% NPP diet.

It was speculated that increasing levels of phytase activity would be detected in the ileum, reflecting the increasing amounts of phytase that was included in the experimental diets. However, quite variable, inconsistent and non-significant phytase activity was

reported in ileal digesta in the present study (data not shown). Ileal activity is more likely due to microbial or endogenous phytase than either the added phytase or phytase found in feed ingredients.

Contrary to the results of the current study, Van der Klis et al. (1997) demonstrated an improvement in phytate digestibility of CSM diets containing 0.12% NPP with 250 or 500 U/kg phytase in laying hens. In the present study, phytase supplementation of the NC1 diet was associated with a linear decrease in phytate digestibility. The addition of 200 or 400 U/kg phytase to the NC2 resulted in increased phytate degradation (approximately 39.5 and 52.6%, respectively); however the positive effect of phytase supplementation disappeared with 600 U/kg phytase. It is not known why this decrease in phytate digestibility occurred with increasing levels of phytase addition. It is speculated that phytate is forming complexes with dietary nutrients in the digestive tract, creating phytate-nutrient complexes that are resistant to hydrolysis by the phytase enzyme. This would cause a decrease in phytate digestibility, making phytate P and other nutrients bound to it unavailable to the animal.

The addition of phytase to low P diets has been shown to significantly increase the availability and retention of P in chickens (Rutherfurd et al., 2004; Onyango et al., 2005a). We found that the addition of 400 U/kg phytase to the CSM-based diet containing 0.25% NPP had no significant effect on P digestibility. Also, supplementation of the NC2 diet (0.15% NPP) caused no improvement in P digestibility regardless of the phytase level. This could be due to the fact that all of the P is bound in phytate-nutrient complexes that are resistant to hydrolysis by the phytase enzyme. The phytase enzyme was able to make P more available to the animal in the 0.25% NPP diet, but the low

amount of P in the 0.15% NPP diet may have been completely caught up in phytate-nutrient complexes and that is why we did not see an improvement in P digestibility with the addition of phytase to the NC2 treatment. It was speculated that phytase supplementation would improve P digestibility, leading to less P excretion in the feces. Current data showed that the addition of increasing levels of phytase to the NC1 treatment was associated with a linear decrease in the amount of soluble P in the feces, while there was no significant effect with NC2 diet. Earlier, the use of supplemental phytase in conjunction with reduced dietary P levels has been shown to be an effective method of improving P utilization and decreasing P excretion in the manure (Waldroup et al., 2000).

Dietary phytase supplementation has been shown to improve Ca availability in poultry (Onyango et al., 2005a; Ravindran et al., 2006). Current data with laying hens showed that the addition of phytase to the 0.25% NPP diet resulted in a significant decrease (almost 26%) in Ca digestibility. Supplementation of 400 U/kg phytase in the 0.15% NPP diet significantly increased (almost 20%) Ca digestibility, whereas 200 and 600 U/kg supplemental phytase had no effect. Dietary phytate might form insoluble complexes with Ca within the digestive tract, blocking access by the phytase enzyme and decreasing Ca digestibility in the 0.25% NPP diet. The removal of 0.10% NPP from the NC1 diet allowed phytase to improve Ca digestibility in the NC2 diet, but it is unknown why there was no effect with 600 U/kg phytase.

It was speculated that improvements in protein and amino acid digestibility due to added phytase may result in improved energy utilization in poultry. Previous research on the effects of phytase supplementation on diet AME has produced quite variable results.

Ravindran et al. (2000) demonstrated that adding 400 or 800 U/kg phytase to a diet containing 0.23% NPP resulted in improved AME values, while Liebert et al. (2005) found that 300 U/kg phytase in a laying hen diet containing 0.12% NPP did not improve diet AME. In the present study, no improvement was observed in diet AME with the addition of phytase to the NC1 and NC2 treatments. The variation in the energy retention might be due to the difference in phytase source and dietary ingredients that were used in previous studies. Alternately, microbial phytase used in this study might not have the ability to improve AME due to the formation of insoluble phytate-nutrient complexes which could possibly bind to starch or could inhibit α-amylase activity within the gastrointestinal tract leading to lowered starch digestibility and, in turn, lowered AME values.

Previous research on the effects of exogenous phytase on protein and amino acid digestibility of the diet has produced quite variable results. Some researchers reported an improvement in protein and amino acid digestibility with phytase supplementation (Ravindran et al., 2006; Rutherford et al., 2004), whereas others did not observe any improvement (Zhang et al., 1999). Snow et al. (2003) found that phytase supplementation of 300 U/kg in a CSM-based diet did not improve ileal amino acid digestibility in laying hens. In fact, they found that it decreased amino acid digestibility. On the other hand, Jalal et al. (1999) found that phytase supplementation of 250 or 300 U/kg in a laying hen diet significantly improved the digestibility of four amino acids – methionine, cysteine, alanine and glutamate. It is unknown why ileal protein and amino acid digestibility decreased linearly with the addition of phytase to the diet in the current experiment. Protein-phytate complexes that are resistant to hydrolysis by phytase might be being

formed within the digestive tract, decreasing the availability of protein and amino acids to the animal. This would help explain the decrease in protein and amino acid digestibility, but it does not explain why it continued to decrease with the addition of increasing levels of phytase to the diet.

Similar to the findings of Liebert et al. (2005), our data indicated that phytase supplementation in a diet deficient in NPP significantly improved bone ash up to levels seen for a diet adequate in NPP. The improvement in bone ash level seen with phytase addition was a good indication that phytase was associated with an improved ability of the bird to utilize dietary P and a decrease in the amount of P being removed from the bone, which would result in the long term maintenance of a healthy bird throughout the entire production cycle. Enzyme supplementation accelerates the rate of passage of digesta through the gastrointestinal tract of the chicken, reducing the time available for nutrients to be extracted from the feed within the digestive tract, therefore resulting in shorter gut lengths and lighter gut weights (Lazaro et al., 2003). The current observations did not follow this pattern.

The results of this experiment indicate that the digestibility coefficients of most of the nutrients studied were not improved with exogenous microbial phytase supplementation of the CSM-based diets of laying hens, whereas the removal of NPP from the diet had a positive impact on nutrient digestibility. The addition of phytase to diets deficient in NPP did not improve nutrient digestibility, but was effective in maintaining the P status of the bird and in reducing P excretion in the feces. Overall, Quantum™ phytase was not efficacious at improving nutrient digestibility in laying hens fed CSM-based diets deficient in NPP.

CHAPTER 5
EFFECT OF DIETARY CALCIUM AND NONPHYTATE PHOSPHORUS LEVELS ON THE EFFICACY OF QUANTUM™ PHYTASE IN WHITE LEGHORN LAYING HENS

5.1 Abstract

An experiment was conducted to investigate the impact of dietary Ca and P levels on the efficacy of an *E. coli*-derived 6-phytase (Quantum™ phytase, Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA) and the apparent digestibility of various nutrients in White Leghorn laying hens fed CSM-based diets. A total of 384 White Leghorn hens were fed CSM-based diets containing four levels of Ca (2.5, 3.5, 4.5 and 5.5%), two levels of NPP (0.15 and 0.30%), and two levels of phytase (300 and 600 U/kg feed) resulting in a total of 16 treatments. Each dietary treatment was replicated six times with four hens per replication. Experimental diets were fed *ad libitum* from 58 to 61 wk of age. Digesta samples from duodenum, jejunum and ileum, and feces were collected at 61 wk to determine apparent nutrient digestibility. Phytate hydrolysis increased linearly with increasing levels of dietary Ca and also increased when combined with the lower level of dietary NPP and the higher level of dietary phytase. Increasing dietary Ca caused a linear decrease in AMEn, duodenal protein digestibility, Ca digestibility and percentage soluble P in feces, while increasing bone ash linearly. The higher level of NPP (0.30%) decreased AMEn, and fecal protein, Ca and P digestibility, while increasing fecal soluble P in comparison to the lower NPP level. The higher level of dietary phytase significantly increased AMEn, phytate and P digestibility and soluble

P in the feces. A quadratic increase in hen-day and hen-housed egg production was observed with increasing levels of Ca in the diet. Increasing dietary Ca levels also caused a linear increase in egg specific gravity (59 wk), body weight and feed intake, while decreasing the percentage of soft shelled, cracked and broken eggs in a linear fashion. Hens consuming a diet with higher NPP content had decreased hen-housed and hen-day egg production, with increased egg specific gravity in comparison to those consuming the lower NPP diet. The higher level of dietary phytase supplementation increased hen-day and hen-housed egg production while significantly reducing the percentage of soft shelled, cracked and broken eggs. Significant interactions were noted between treatments for both digestibility and production traits. Overall, dietary phytase, Ca and NPP levels, either as main effects or in an interactive manner, can affect apparent nutrient digestibility and production traits in laying hens fed CSM-based diets.

5.2 Introduction

Phytate (*myo*-inositol 1, 2, 3, 4, 5, 6 hexakis dihydrogen phosphate) accounts for approximately two-thirds of the total phosphorus present in plant-based diets. The phosphorus that is associated with phytate in feed is poorly utilized by monogastric animals due to low levels of phytase activity in their digestive tracts (Maenz and Classen, 1998). To aid in the digestion of phytate, exogenous microbial phytase is added to the diet of monogastric animals. The inclusion of supplemental phytase in the diet of laying hens has been shown to improve the availability of phytate P and other minerals such as Ca and Zn (Jalal and Scheideler, 2001). Similarly, it has been found that phytase supplementation may improve the availability of nutrients such as protein and amino

acids (Ravindran et al., 2000; Rutherfurd et al., 2004) and energy (Ravindran et al., 2000; 2001; Newkirk and Classen, 2001) in poultry.

Although the phytase enzyme has the ability to improve the availability of dietary nutrients to monogastric animals, the mineral content of the diet, especially Ca and P, can affect the efficacy of the phytase enzyme to degrade phytate in the gastrointestinal tract (Sandberg et al., 1993). Calcium has the ability to precipitate phytate by forming insoluble Ca-phytate complexes in the digestive tract (Wise, 1983; Nelson and Kirby, 1987). The Ca-phytate complexes are resistant to hydrolysis by the phytase enzyme, resulting in decreased phytate degradation and thus phytate P and bound Ca being unavailable to the animal. Previous work has shown that high dietary Ca levels decrease the availability of phytate P in laying hens (Scheideler and Sell, 1987). Lei et al. (1994) reported that supplemental phytase has the ability to improve phytate P utilization more effectively at low levels of dietary Ca (0.4%) than at normally recommended levels (0.8%) in weanling pigs. Schoner et al. (1991, 1993) also found that the addition of Ca to a broiler diet caused a decrease in phytase efficacy.

In addition to Ca ions blocking binding sites on the phytate molecule that are required for phytase activity, they may also increase the pH of the digestive tract above the optimum pH required for phytase activity, rendering it unable to function effectively. All phytases have a pH optimum and they require that pH optimum in order to function properly within the digestive tract of the animal (Oatway et al., 2001). Previous work has shown that high levels of dietary Ca decreased digestive tract phytase activity in chicks (McCuaig et al., 1972; Applegate et al., 2003; Tamim et al., 2004) and pigs (Lei et al., 1994). Qian et al. (1997) documented that phytase activity was decreased by 4.9 and

7.4% respectively when the dietary Ca:P ratio was widened from 1.4:1 to 2:1. Published data has shown a clear indication that the dietary Ca concentration and the overall Ca:P ratio are very important factors that affect the functioning of phytase within the digestive tract.

It should be noted that all phytase products are not equal and may result in variation when being studied. Phytase activity can be derived from variable sources, which in turn affect characteristics such as pH optimum, thermal stability and ability to resist hydrolysis within the digestive tract. Any difference in these characteristics will affect the ability of the phytase enzyme to function effectively and consistently within the digestive tract (Augspurger et al., 2003; Onyango et al., 2005a). Therefore, all phytase enzymes produced must be tested *in vivo* to ensure efficacy before they are introduced to the monogastric feed market.

Laying hens have a high dietary Ca requirement in order to sustain egg production and the high dietary Ca level may affect the efficacy of microbial phytase. We hypothesized that the effect of dietary Ca on the efficacy of phytase would be much greater in laying hens due to their high Ca requirements. The objectives of this study were to investigate the impact of dietary Ca and P level on: 1) the efficacy of an *E. coli*-derived 6-phytase (Quantum™ phytase, Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA) and 2) apparent digestibility of various nutrients in White Leghorn laying hens fed CSM-based diets.

5.3 Materials and Methods

The experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan and was performed in accordance with recommendations of

the Canadian Council on Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

5.3.1 Animals and Housing

At 17 wk of age, a total of 384 White Leghorn pullets (Bovan strain) were housed in cages (30.5 cm x 46 cm x 52 cm; floor space per hen = 702 cm^2) under controlled climate conditions. Two hens were kept in each cage and experimental units consisted of two adjoining cages. Starting at 18 wk of age, the lighting program was changed from 8L:16D to 14L:10D with a light intensity of 10 lux. The ambient temperature was maintained at a minimum of approximately 20°C throughout the study. A commercial laying hen diet was offered for *ad libitum* consumption up to 58 wk of age. From 58 wk onwards, the hens were randomly assigned to one of the 16 dietary treatments. Each dietary treatment was replicated six times with four hens (two cages) per replication in a randomized complete block design. Experimental diets were fed *ad libitum* from 58 to 61 wk of age.

5.3.2 Experimental Diets

The ingredient and nutrient composition of the experimental diets is shown in Table 5-1. The diets were isocaloric (2900 Kcal/kg) and formulated to meet or exceed National Research Council (1994) recommendations for laying hens. They were formulated to contain four levels of Ca (2.5, 3.5, 4.5 and 5.5%), two levels of non-phytate P (0.15 and 0.30%), and two levels of phytase (300 and 600 U/kg) resulting in a total of 16 treatments. The phytase used was an *E. coli* 6-phytase (Quantum™ phytase, Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA). Celite® (Celite Corporation, Lompoc, California, USA) was used as an indigestible marker in the experimental diets at a concentration of 1.5%.

Table 5-1. The ingredient and nutrient composition of experimental diets.

NPP[1] (%)	0.30				0.15			
Calcium (%)	2.5	3.5	4.5	5.5	2.5	3.5	4.5	5.5
Ingredients (%)								
Corn	63.89	58.81	53.66	48.51	64.59	59.55	54.40	49.25
Soybean meal	25.06	25.86	26.71	27.56	24.97	25.73	26.59	27.44
Canola oil	1.52	3.15	4.79	6.43	1.29	2.92	4.55	6.19
Dicalcium phosphate	0.77	0.79	0.81	0.83	0.07	0.09	0.11	0.12
Limestone	6.03	8.65	11.26	13.88	6.35	8.97	11.59	14.21
Salt	0.41	0.41	0.40	0.40	0.41	0.41	0.40	0.40
Vit.-min. premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.20	0.21	0.23	0.24	0.20	0.21	0.23	0.24
L-Threonine	0.02	0.03	0.03	0.04	0.02	0.02	0.03	0.04
Celite®[3]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Quantum™ phytase[4] (g/kg)								
Calculated nutrients (%)								
AME (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein	16.89	16.89	16.92	16.95	16.90	16.89	16.92	16.95
Calcium	2.50	3.50	4.50	5.50	2.50	3.50	4.50	5.50
NPP[1]	0.30	0.30	0.30	0.30	0.15	0.15	0.15	0.15
Phytate phosphorus	0.30	0.29	0.27	0.26	0.30	0.29	0.28	0.26
Total phosphorus	0.60	0.59	0.57	0.56	0.45	0.44	0.43	0.41
Analyzed minerals, as is basis (%)								
Calcium	2.83	4.05	4.97	6.18	2.85	3.97	5.15	6.43
Total phosphorus	0.52	0.51	0.49	0.46	0.36	0.35	0.35	0.33

[1] Nonphytate phosphorus.
[2] Vitamin mineral premix (units per kilogram of feed) – vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg, vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; quinguard M6S, 0.625 mg, calcium carbonate, 500 mg.
[3] Celite Corporation, Lompoc CA, USA.
[4] Syngenta Animal Nutrition Inc., Research Triangle Park, NC, USA; Two levels of Quantum™ phytase (300 or 600 U/ kg feed) were used for each of the eight Ca-nonphytate P combinations resulting in a total of 16 treatments. Quantum™ phytase was added at the expense of corn.

5.3.3 Measurements and Laboratory Analyses

The hens were fed their respective diets throughout the 4-wk trial (starting at 58 wk of age) and fecal samples were collected during the final week of the trial. At 61 wk of age, trays were placed beneath each cage, and feces were collected twice per day for the next two days. Feed consumption was also determined for these two days. Feces were pooled by replication, freeze-dried, mixed and sub-sampled for further analysis. At the end of the 4-wk experiment, all hens were humanely killed by cervical dislocation. The digestive tracts were removed and the pH of the digesta was measured immediately in various sections of the small intestine (duodenum, jejunum and ileum) (Rynsburger, unpublished). Digesta samples were then collected separately from each section of the small intestine (duodenum, jejunum and ileum), pooled by replication, and freeze-dried prior to analysis. The left tibia of each hen was also collected at the end of the experiment to determine bone ash (AOAC, 1990).

Samples of diets, intestinal digesta (duodenum, jejunum and ileum) and feces were oven-dried at 100°C for 24 h for dry matter determination (AOAC, 1990). Gross energy was determined using an oxygen bomb calorimeter (Model 1281, Parr Instruments, Moline, IL). Crude nitrogen content was determined by the combustion method (984.13; AOAC International, 1995) using a Leco FP-528 protein analyzer (St. Joseph, MI). Amino acid content of the diet and ileal digesta samples was determined by the method described by Llames and Fontaine (1994). The apparent digestibility calculations were based on the use of acid insoluble ash as the indigestible marker, which was determined by the procedure of Vogtmann et al. (1975). Diet and fecal samples were analyzed for the determination of total phosphorous and calcium using the method of Zasoski and Burau (1977). Fecal samples were also analyzed for soluble phosphorus content.

Phytate (*myo*-inositol hexaphosphate to myoinositol diphosphate; IP6 to IP2) in the diets and ileal digesta was determined as per the method described by Newkirk and Classen (1998) using high performance liquid chromatography.

Egg production data was collected on a replication basis 5 d per wk and was then corrected to a 7 d per wk basis. Eggs were classified as normal, broken, cracked, soft-shelled, double yolked and abnormal; abnormal was defined as eggs that were mis-shapen or mini (yolkless). Egg classification was based on all the eggs collected during the experiment. Egg shell quality as judged by specific gravity was determined on a replication basis on all eggs at 59 and 60 wk of age using nine saline solutions ranging from 1.060 to 1.100 with 0.005 increments. Eggs were left in the same room as solutions overnight to ensure equal temperature. Saline solutions were calibrated prior to each test. Mortality was recorded on a replication basis. Dead birds were collected, weighed and recorded daily.

5.3.4 Statistical Analyses

The data were analyzed as a 4 x 2 x 2 factorial arrangement (4 levels of Ca, 2 levels of non-phytate phosphorus (NPP) and 2 levels of exogenous phytase) using the GLM procedures of SAS (SAS Institute, 2002). Treatment means were compared using Duncan's Multiple Range Test. Regression analyses were used as appropriate. Differences were considered significant when $P < 0.05$.

5.4 Results

The chemical analysis of the experimental diets is shown in Table 5-1. The analyzed Ca level was higher than the planned level, but it was consistently higher across the diets. The high Ca level was due to the fact that the soybean meal contained a much higher level of Ca (0.63%) than the expected level (0.25%). Contrary to Ca values, the analyzed total P levels were lower than expected.

5.4.1 Effect of Diet Ca, NPP and Phytase Level on Nutrient Digestibility

The effects of dietary Ca, NPP and exogenous phytase level on digestibility coefficients of various nutrients and tibial bone ash are presented in Table 5-2. Increasing dietary Ca caused a linear decrease in AMEn, duodenal protein digestibility, fecal Ca digestibility and percentage soluble P in feces, and a linear increase in ileal phytate digestibility and bone ash. The dietary level of Ca had no significant effect on jejunum (data not shown), ileal (data not shown) and fecal protein digestibility, and ileal phytase activity (data not shown).

Feeding a 0.15% NPP diet increased AMEn, fecal protein retention and Ca, P and phytate digestibility, and reduced soluble P in the feces in comparison to hens fed the 0.30% NPP diet (Table 5-2). There was no effect ($P > 0.05$) of NPP level on duodenum, jejunum and ileal protein digestibility, bone ash and ileal phytase activity.

Six hundred U/kg feed of phytase supplementation resulted in a significant ($P < 0.05$) increase in diet AMEn, P and phytate digestibility and soluble P in the feces as compared

Table 5-2. Effect of diet calcium, nonphytate P and exogenous phytase level on nutrient digestibility coefficients in White Leghorn laying hens fed CSM-based diets.

	Calcium (%)				NPP (%)		Phytase (units per kg)		SEM	Interactions[1]	Regression[2]
	2.5	3.5	4.5	5.5	0.15	0.30	300	600			(Linear or Quadratic)
AMEn (Kcal/kg)[3]	2733[a]	2693[b]	2646[c]	2630[c]	2692[a]	2659[b]	2659[b]	2692[a]	8.3	NPP*Phy, Ca*Phy	Linear
Ileal phytate dig.	0.39[c]	0.54[b]	0.49[b]	0.66[a]	0.57[a]	0.47[b]	0.45[b]	0.60[a]	0.022	NPP*Phy	Linear
Duodenal protein dig.	-0.61[a]	-0.69[a]	-1.25[ab]	-1.84[b]	-1.04	-1.15	-1.17	-1.02	0.126	NS	Linear
Fecal protein dig.	0.38	0.39	0.38	0.39	0.40[a]	0.38[b]	0.39	0.38	0.005	NPP*Phy	NS
Ca digestibility	0.55[a]	0.45[b]	0.29[c]	0.27[c]	0.43[a]	0.36[b]	0.38	0.40	0.022	NPP*Phy,	Linear
P digestibility	0.22[a]	0.16[b]	0.17[b]	0.17[b]	0.22[a]	0.13[b]	0.15[b]	0.21[a]	0.012	Ca*NPP*Phy	NS
Percentage soluble P in feces	0.81[a]	0.80[a]	0.68[b]	0.63[b]	0.26[b]	0.90[a]	0.69[b]	0.77[a]	0.024	NS	Linear
Bone ash (%)	56.51[c]	57.50[b]	58.38[ab]	58.59[a]	57.63	57.86	57.79	57.70	0.183	NS	Linear

[ab] Means within a row (within Ca, nonphytate P or phytase level) with different letters are significantly different ($P < 0.05$).

[1] Interaction was significant when $P < 0.05$. NS. Phy stands for phytase, Ca for calcium and NPP for nonphytate phosphorus.

[2] Regression was analyzed within Ca treatments only.

[3] 90% dry matter basis.

to 300 U/kg feed phytase supplementation. The dietary level of phytase had no effect on duodenum, jejunum, ileal and fecal protein digestibility, Ca digestibility, bone ash and ileal phytase activity.

A number of interactions were found between dietary main effects (Tables 5-3 and 5-4). A significant dietary Ca x phytase interaction was observed for diet AMEn (P = 0.0117). Increased dietary phytase (600 vs 300 U/kg) increased AMEn at all dietary Ca levels except for the 4.5% level. The lack of a trend in response suggests that the different response at 4.5% Ca is not a true biological response but rather an artifact of variability. NPP x phytase interactions were significant for phytate digestibility (P = 0.0328), fecal protein digestibility (P = 0.0287) and Ca digestibility (P = 0.0289) and approached significance for AMEn (P = 0.0560). The higher level of dietary phytase increased phytate digestibility at both levels of NPP but the impact was larger for the 0.15% NPP level than for the 0.30% NPP level. AMEn was affected in a similar fashion. For fecal protein and Ca, the 600 U/kg of phytase addition increased digestibility at 0.15% NPP and decreased digestibility at 0.30% NPP. A three way interaction (dietary Ca x NPP x phytase) was found for P digestibility, but biological trends were not obvious and, therefore, data are not presented.

There was no significant effect of diet Ca, NPP and phytase level or their interactions on apparent ileal digestibility of amino acids (data not shown).

5.4.2 Effect of Diet Ca, NPP and Phytase Level on Intestinal pH

A linear decrease in duodenal pH and an increase in ileal pH were observed with increasing levels of diet Ca (Table 5-5). Dietary NPP and phytase level had no effect on intestinal pH.

Table 5-3. The significant interaction between diet Ca and phytase with respect to AMEn in White Leghorn laying hens fed CSM-based diets.

Calcium (%)	Phytase (units per kg)	AMEn (Kcal/kg)
2.5	300	2699
2.5	600	2767
3.5	300	2677
3.5	600	2708
4.5	300	2652
4.5	600	2640
5.5	300	2607
5.5	600	2653
P-value		0.0117

Table 5-4. The significant interactions between diet nonphytate P and phytase with respect to nutrient digestibility in White Leghorn laying hens fed CSM-based diets.

NPP (%)	Phytase (units per kg)	AMEn (Kcal/kg)	Phytate dig.	Fecal protein dig. (%)	Ca dig.
0.15	300	2667	0.46	40.8	0.44
0.15	600	2717	0.69	38.3	0.41
0.30	300	2650	0.43	36.8	0.32
0.30	600	2667	0.51	38.3	0.40
P-value		0.0560	0.0328	0.0287	0.0289

Interaction significant when $P<0.05$.

Table 5-5. Effect of diet calcium, nonphytate P and exogenous phytase level on intestinal pH in White Leghorn laying hens fed CSM-based diets.

	Calcium (%)				NPP (%)		Phytase (units per kg)		SEM	Interactions[1]	Regression[2]
	2.5	3.5	4.5	5.5	0.15	0.30	300	600			(Linear Quadratic)
Duodenum pH	6.14[a]	6.07[ab]	6.08[ab]	6.00[b]	6.08	6.06	6.07	6.07	0.017	NS	Linear
Jejunum pH	6.09	6.14	6.20	6.11	6.11	6.16	6.17	6.10	0.022	NS	NS
Ileum pH	8.03[b]	8.16[a]	8.15[a]	8.17[a]	8.15	8.10	8.15	8.10	0.020	NS	Linear

[ab] Means within a row (within Ca, nonphytate P or phytase level) with different letters are significantly different ($P < 0.05$).

[1] Interaction was significant when $P < 0.05$.

[2] Regression was analyzed within Ca treatments only.

5.4.3 Effect of Diet Ca, NPP and Phytase Level on Performance Characteristics

Total hen-day (THDP) and hen-housed egg (THHP) production increased with increasing levels of calcium in a quadratic fashion (Table 5-6). Essentially, birds fed 2.5% Ca layed fewer eggs than birds on other treatments. Egg specific gravity also increased with increasing dietary calcium, with the response linear at 59 and quadratic at 60 weeks of age. The incidence of obvious poor shelled eggs (soft, cracked or broken) decreased linearly in response to increased dietary Ca, while final body weight and feed intake increased.

Hens receiving a diet containing 0.15% NPP had significantly increased THDP and THHP and reduced egg specific gravity at 60 wk of age as compared to those fed 0.30% NPP diet (Table 5-6). In the present study, the dietary level of NPP had no effect on the percentage of cracked, broken and soft-shelled eggs, double and abnormal eggs (data not shown), egg specific gravity at 59 wk of age, body weight at trial end, feed intake and hen mortality (data not shown).

Six hundred U/kg feed phytase supplementation resulted in a significant increase in THDP, THHP and percentage of abnormal eggs; these hens also had significantly reduced cracked, broken and soft-shelled eggs as compared to those consuming the 300 U/kg phytase-supplemented diet.

Interactions among main effects were also found for production traits (Tables 5-7, 5-8 and 5-9). Dietary Ca x NPP interactions were observed for THDP ($P = 0.0033$), THHP ($P = 0.0023$), percentage cracked, broken and soft shelled eggs ($P = 0.0013$) and egg specific gravity at 59 wk ($P = 0.0476$). In the case of egg production, 0.30% NPP decreased production at 2.5, 4.5 and 5.5% Ca, but increased production at 3.5% Ca. For

Table 5-6. Effect of diet calcium, nonphytate P and exogenous phytase level on performance characteristics in White Leghorn laying hens fed CSM-based diets.

	Calcium (%)				NPP (%)		Phytase (units per kg)		SEM	Interactions[1]	Regression[2]
	2.5	3.5	4.5	5.5	0.15	0.30	300	600			(Linear Quadratic)
THDP[3] (%)	76.0^{b}	85.6^{a}	83.1^{a}	85.3^{a}	83.9^{a}	81.1^{b}	80.6^{b}	84.4^{a}	0.63	Ca*NPP, Ca*NPP*Phy	Quadratic
THHP[4] (%)	75.9^{b}	85.4^{a}	83.1^{a}	85.3^{a}	83.8^{a}	81.0^{b}	80.5^{b}	84.3^{a}	0.64	Ca*NPP, Ca*NPP*Phy	Quadratic
SSCBE[5] (%)	6.07^{a}	3.60^{b}	2.64^{bc}	1.26^{c}	2.91	3.87	4.15^{a}	2.63^{b}	0.352	Ca*NPP, Ca*NPP*Phy	Linear
Egg specific gravity, 59 wk	1.076^{c}	1.080^{b}	1.082^{a} $_{b}$	1.083^{a}	1.080	1.080	1.080	1.080	0.0005	Ca*NPP	Linear
Egg specific gravity, 60 wk	1.078^{b}	1.082^{a}	1.083^{a}	1.084^{a}	1.081^{b}	1.083^{a}	1.082	1.081	0.0005	NS	Quadratic
Body weight at trial end (kg)	1.66^{b}	1.73^{ab}	1.73^{ab}	1.80^{a}	1.72	1.75	1.72	1.74	0.013	NS	Linear
Feed intake (g/h/d)	94.4^{c}	106.7^{b}	105.4^{b}	113.2^{a}	104.1	105.7	104.8	105.0	1.28	Ca*Phy	Linear

[ab] Means within a row (within Ca, nonphytate P or phytase level) with different letters are significantly different ($P < 0.05$).
[1] Interaction was significant when $P < 0.05$. 'Phy' stands for phytase.
[2] Regression was analyzed within Ca treatments only.
[3] Total hen day egg production.
[4] Total hen housed egg production.
[5] Soft shelled, cracked and broken eggs.

Table 5-7. The significant interactions between diet Ca and nonphytate phosphorus with respect to performance characteristics in White Leghorn laying hens fed CSM-based diets.

Calcium (%)	NPP (%)	THDP[1] (%)	THHP[2] (%)	SSCBE[3] (%)	Egg specific gravity[4]
2.5	0.15	78.9	78.9	3.6	1.077
2.5	0.30	73.1	72.9	8.5	1.074
3.5	0.15	83.9	83.4	4.2	1.078
3.5	0.30	87.3	87.3	3.0	1.081
4.5	0.15	86.4	86.4	2.1	1.081
4.5	0.30	79.9	79.9	3.2	1.083
5.5	0.15	86.6	86.6	1.8	1.082
5.5	0.30	84.0	84.0	0.7	1.084
P-value		0.0033	0.0023	0.0013	0.0476

[1] Total hen day egg production
[2] Total hen housed egg production
[3] Soft shelled, cracked and broken eggs.
[4] Egg specific gravity at 59 wk.
Interaction significant when $P<0.05$.

Table 5-8. The significant interaction between diet Ca and phytase with respect to feed intake in White Leghorn laying hens fed CSM-based diets.

Calcium (%)	Phytase (units per kg)	Feed intake (g/h/d)
2.5	300	89.7
2.5	600	99.2
3.5	300	108.6
3.5	600	104.8
4.5	300	106.5
4.5	600	104.3
5.5	300	114.5
5.5	600	111.9
P-value		0.0330

Table 5-9. The significant interactions between diet Ca, nonphytate P and phytase with respect to performance characteristics in White Leghorn laying hens fed CSM-based diets.

Calcium (%)	NPP (%)	Phytase (units per kg)	THDP[1] (%)	THHP[2] (%)	SSCBE (%)
2.5	0.15	300	75.8	75.8	3.6
2.5	0.15	600	81.9	81.9	3.7
2.5	0.30	300	71.1	70.8	11.3
2.5	0.30	600	75.0	75.0	5.8
3.5	0.15	300	83.3	83.3	7.3
3.5	0.15	600	84.4	83.5	1.0
3.5	0.30	300	84.4	84.4	2.4
3.5	0.30	600	90.2	90.2	3.7
4.5	0.15	300	86.9	86.9	1.2
4.5	0.15	600	85.8	85.8	3.0
4.5	0.30	300	74.0	74.0	4.4
4.5	0.30	600	85.8	85.8	2.0
5.5	0.15	300	85.4	85.4	2.7
5.5	0.15	600	87.7	87.7	0.9
5.5	0.30	300	83.5	83.5	0.4
5.5	0.30	600	84.4	84.4	1.0
P-value			0.0388	0.0456	0.0004

[1] Total hen day egg production.
[2] Total hen housed egg production.
[3] Soft shelled, cracked and broken eggs.
Interaction significant when $P<0.05$.

poor shelled eggs (cracked, broken and soft shelled), higher NPP resulted in higher numbers at 2.5 and 4.5% Ca and lower numbers at 3.5 and 5.5% Ca. At 59 weeks of age, 0.30% NPP decreased egg specific gravity at 2.5% Ca, but increased this value at other levels of Ca in comparison to hens fed 0.15% NPP. Dietary Ca x phytase interaction was significant for feed intake ($P = 0.0330$) with a higher feed intake noted for the 600 U / kg level of phytase inclusion for 2.5% Ca diets and a decrease noted for all other Ca levels. The interactions between diet Ca x NPP x phytase were significant for THDP ($P =$

0.0388), THHP (P = 0.0456), and percentage cracked, broken and soft shelled eggs (P = 0.0004), and are shown in Table 5-9.

5.5 Discussion

Dietary Ca levels are known to have an effect on phytate hydrolysis (Wise, 1983), bone ash content and the retention of Ca and P in monogastric animals (Qian et al., 1997). Most of the research completed on the effects of dietary Ca on nutrient digestibility and the efficacy of phytase has used broiler chickens and swine. Little research has been conducted on its effect on laying hen performance. The dietary Ca requirement of laying hens is much greater than that of broilers, so the effect of Ca theoretically could be much greater. The National Research Council (1994) reports normal laying hen dietary Ca requirements to be 3.25% to 3.6% for hens eating 100 g/day of feed, although industry norms are likely higher. The dietary Ca levels used in this experiment ranged from deficient (2.5%) to abundant (5.5%) so that the effect of Ca level on response criteria could be more readily assessed.

Unexpectedly, we found that phytate digestibility increased linearly with increasing levels of dietary Ca. Previous work completed on the effect of dietary Ca level on phytate digestibility does not match our results. Van der Klis et al. (1997) found that 40 g/kg dietary Ca reduced phytate hydrolysis significantly in laying hens compared to those consuming 30 g/kg. Scheideler and Sell (1987) also found that increasing the dietary Ca level from 3% to 4% in laying hens at 50 and 72 wk of age resulted in decreased phytate digestibility. Tamim and Angel (2003) found that adding Ca (5 g/kg) to the diet of broiler chickens reduced phytate hydrolysis. It is unknown why our findings on this matter were the opposite of those found by others, but it is possible that they can be explained by the effect of calcium carbonate in the gastrointestinal tract. Ca is highly

insoluble and has a high buffering capacity (Guinotte et al., 1995); therefore, increased amounts of Ca entering the upper gastrointestinal tract may trigger increased proventricular secretions to make the environment more acidic (Barreras and Donaldson, 1967; Guinotte et al., 1995). The linear decrease in duodenal pH with increasing dietary Ca levels found in this study support this concept, as does an increasingly negative apparent duodenal protein digestibility. A lower pH may increase the time in which the pH in digesta is within the activity spectrum of phytase, and in turn, this may increase phytate digestibility as seen in our work. It is of significance to note that the phytase used in this research is active over a broad pH range and retains 50% of its potential activity at pH 6.0 (Palackal et al., 2004). It is of interest to note that the pH of digesta in the jejunum was unaffected by dietary Ca, but that the pH of ileal digesta increased with dietary Ca. These results reflect both the ability of digestive secretions to increase pH, as well as the impact of calcium carbonate itself.

The results of the present experiment demonstrated that diet AME, Ca and P digestibility and fecal soluble P levels were negatively correlated with dietary Ca level. The impact of Ca level on diet AMEn is unexpected, based on the formulation of diets to be equal in energy content. However, others, including Shafey and McDonald (1991), have found that high dietary Ca levels (24.3 vs 11.8 g/kg) can affect protein digestibility and ME in broiler chickens. This suggests that Ca level can affect nutrient digestibility. Since digestibility of ileal protein and amino acids was unaffected in our research, an effect on starch and/or fat digestibility is likely causing the effect. The formation of indigestible Ca complexes with other nutrients, such as fatty acids, has been shown previously, but the results have been inconsistent (Atteh and Leeson, 1985; Atteh et al.

1989). The linear increase in feed intake we found with increasing levels of dietary Ca corresponds to the lower AMEn associated with this increase. Our results on the effect of Ca level on Ca and P digestibility were as expected and are in agreement with those found by others. Van der Klis et al. (1997) found that the ileal absorption of Ca and P was significantly decreased in laying hens fed 40 g/kg dietary Ca in comparison to those fed 30 g/kg. The adverse effect of dietary Ca level on P absorption is well known (Hurwitz and Bar, 1965; Tamim et al., 2004; Tamim and Angel, 2003) and other research on this topic is in agreement with our findings. The decrease in Ca digestibility that we saw with increasing levels of Ca in the diet makes sense because the hen excretes Ca that is beyond its requirement. Formation of insoluble Ca/P complexes in the gut not only affects absorption of both nutrients but also affects the soluble P content of the feces, which decreased linearly with increasing levels of Ca in the diet.

Dietary Ca level has an important and expected impact on bone ash content, but the continued increase in bone ash as Ca levels increased higher than the recommended requirement was surprising. These results are very interesting due to the age of the hens used in our study. These hens were in the final stages of production, which means that their nutrient reserves may be expended and their nutrient requirements would be heightened. Our results on bone ash and dietary Ca level suggest that hens in later stages of production require more dietary Ca than recommended by NRC Poultry (1994). Previous work has shown that egg shell quality and femur bone ash declines in laying hens as they age (Albatshan et al., 1994), so the addition of more dietary Ca to the laying hen diet in later stages of production could ultimately lead to improved health and welfare of hens due to increased bone mineralization.

The results of the present experiment demonstrated that the nutrient digestibility (Ca, P, phytate, protein and diet AME) of CSM-based diets containing 0.15% NPP was comparatively higher than with 0.30% NPP. This is an indication that excess levels of P in the diet can actually have a negative impact on the digestion of nutrients in laying hens. Previously, Ravindran et al. (2006) demonstrated that when total P was decreased from 0.67% to 0.53% in broiler diets, the diet AME, ileal P, Ca, protein and amino acid digestibility were increased. Silversides et al. (2004) also reported an increase in diet AME and P digestibility when NPP was decreased from 0.40% to 0.23% in broiler diets. The data presented in this paper showed similar results for most of the nutrients studied, except for amino acid digestibility which was not affected by NPP level. The dietary NPP level had no effect on tibial bone ash.

Exogenous microbial phytase is a common ingredient added to monogastric diets to improve availability of phytate P and thus reduce the P outflow into the environment in animal waste (Onyango et al., 2005a). Supplemental microbial phytase has been reported to improve the availability of P and other nutrients in broiler chickens (Simons et al., 1990; Augspurger and Baker, 2004; Rutherfurd et al., 2004; Snow et al., 2004) and laying hens (Jalal and Scheideler, 2001; Liebert et al., 2005). Some previous studies have shown a positive impact on nutrient digestibility in broiler chickens and laying hens with increasing levels of exogenous phytase in the diet. We found that ileal protein, Ca and amino acid digestibility and tibial bone ash were unaffected by the level of phytase supplementation, while diet AME, fecal protein, phytate and P digestibility were significantly improved with the consumption of 600 U/kg Quantum™ phytase in comparison to 300 U/kg. Release of P from phytate is also shown by our finding that

increased phytase supplementation increased the amount of soluble P excreted in the feces.

Our research was relatively short term and primarily designed to investigate the effect of treatments on phytase efficacy and nutrient digestion. However, there were a number of production effects that add to our understanding of dietary treatments and their interactions. The 600 U/kg phytase treatment resulted in higher egg production and a lower level of poor shell quality eggs than did the 300 U/kg phytase treatment. This does not agree with our previous work (Chapter 3), where there were no differences between phytase levels in this range. The previous trial was started at sexual maturity and lasted for 40 weeks, while the current trial started at 58 weeks of age and lasted 4 weeks. It is possible that the response to phytase is different in older hens and that a higher level of phytase is beneficial in these birds.

In the past, numerous studies have been completed on the Ca requirements of laying hens and its effect on performance (for example, Bar et al., 1998, 2002; Roland et al., 1985, 1996; Scott et al., 1999). The data obtained from our experiment indicate that dietary Ca level is an important factor in laying hen performance. We found that increasing the dietary Ca level in laying hens in the late stage of production significantly improved their performance. Previous work has shown that low levels of dietary Ca are associated with reduced egg shell quality and reduced egg production (Bar et al., 1998; Roland et al., 1985; 1996; Scott et al., 1999). Our findings were in agreement, in that hens consuming the 2.5% Ca diet had significantly lower hen-housed and hen-day egg production than those consuming diets higher in Ca. Although the egg production data indicated a Ca requirement of 3.5%, other response criteria showed linear responses with

Ca effects beyond that level. The incidence of broken, cracked and soft-shelled eggs and egg specific gravity were negatively related to Ca level, while a positive linear relationship was seen for 59 week egg specific gravity, body weight and feed intake. The improvement in egg shell quality and bone ash (noted earlier) indicate that higher levels of Ca may be beneficial in older hens. Interactions were found between Ca level and NPP for egg production and poor shell quality eggs, but they are difficult to interpret as they do not exhibit logical trends. An interaction between Ca and NPP for week 59 egg specific gravity demonstrates that at deficient Ca levels, additional NPP reduces shell quality, possibly by binding Ca and exasperating Ca deficiency. In the presence of higher levels of Ca, additional NPP helps meet the hen's P requirement and thereby increases egg shell quality. An interaction was also found between Ca level and phytase for feed intake. Feed intake was low for birds fed the diet with 2.5% Ca and 300 U/kg phytase. When additional phytase was added to the diet, feed intake increased, probably due to an increased release of both Ca and P. At the higher levels of Ca, which were near or above the requirement, feed intakes were higher in general and additional phytase reduced feed intake.

The results of this experiment indicate that higher levels of dietary Ca enhanced the efficacy of phytase in laying hens. The data suggest that this effect is related to increased acid secretion in the proventriculus, which reduces gut pH and extends the time available for phytate hydrolysis. This effect may be particularly important for phytase sources that are active over a broad pH. Our data also confirms that dietary NPP content affects phytase efficacy, with higher levels of NPP adversely affecting phytate hydrolysis by phytase. The data also demonstrate that dietary phytase, Ca and NPP, either as main

effects or in an interactive manner, can affect apparent nutrient digestibility and production traits in laying hens fed CSM-based diets.

CHAPTER 6
DISCUSSION AND CONCLUSIONS

The purpose of this study was to determine the efficacy of an *Escherichia coli* 6-phytase (Quantum™ phytase) in laying hens fed CSM-based diets. We have definitely shown that phytase supplementation has a significant effect in laying hen diets in regards to production performance and nutrient digestibility. The efficacy of the phytase enzyme is also significantly affected by dietary NPP and Ca levels.

The phytase enzyme is known to improve nutrient digestibility in monogastric diets, but little work has been done with laying hens. Laying hen producers ensure that their hens are receiving diets that are adequate in P and Ca (by adding extra P and Ca) in order to meet the high nutritional demands of egg production. Any excess P that is being included in the laying hen diet is wasted and excreted in the feces, leading to an unnecessary amount of P being excreted into the environment as pollution. The phytase enzyme can be added to the laying hen diet to improve the digestibility of P, therefore decreasing the need for such a high P inclusion in the diet. Our data shows that the production performance of hens is most significantly affected by phytase enzyme and dietary P levels. Laying hen producers are concerned about production losses caused by diets deficient in P and may be hesitant to feed diets containing low levels of P combined with phytase supplementation. NRC Poultry (1994) recommends that a hen's daily P requirement is 250 mg. Most producers feed diets high in inorganic P, containing approximately 0.35% NPP to ensure that P requirements are met. In place of inorganic P,

we suggest that Quantum™ phytase be fed on top of a 0.25% NPP diet as a safety margin. This strategy has a bonus - replacing inorganic P with exogenous phytase has some additional benefits. It is more environmentally friendly due to reduced P excretion, and in the long run, may be more cost effective.

We also found that excess levels of P in the diet can have a negative impact on the digestion of nutrients in laying hens. Nutrient digestibility was not increased by simply including phytase in the diet; it was actually improved by the removal of P from the laying hen diet. This is more evidence that producers should feed a diet that is lower in inorganic P with phytase supplementation, instead of just increasing the P level of the diet. The addition of phytase to diets deficient in P was effective in maintaining the P status of the bird and in reducing P excretion in the feces.

High levels of Ca have been studied in broiler diets and found to have a detrimental effect on the efficacy of the phytase enzyme within the digestive tract and hinders its ability to break down the phytate molecule. Laying hens require a very high amount of Ca in their diet in order to sustain egg production throughout the entire laying cycle (40 wks). This excess amount of Ca in the laying hen diet may affect the efficacy of the phytase enzyme. Our data shows that higher levels of dietary Ca actually enhanced the efficacy of phytase in laying hen diets. This effect may be related to increased acid secretion in the proventriculus, which reduces gut pH and extends the time available for phytate hydrolysis. This may be particularly important for phytase sources that are active over a broad pH range, such as the phytase studied – Quantum™ phytase.

The pH of the digestive tract plays a major role in the activity of the phytase enzyme. Phytase is active in an acidic environment. Quantum phytase has a broad pH range of 2.5

to 6.0, which matches the range of the pH's of the upper digestive tract of the chicken (crop, proventriculus, gizzard and duodenum). Phytase will be effective at hydrolyzing phytate when the pH of the digestive tract matches the pH optimum of the phytase enzyme. The ability of the phytase enzyme to mix with the feed and hydrolyze the phytate molecule in the upper areas of the digestive tract will vary and depends on particle size of the feed and digestibility conditions. While the feed passes through the crop, particle size is quite large and hasn't been broken down by digestion. These conditions will not favour high phytase activity even though the pH of the digestive tract matches the pH optimum of the phytase enzyme. Phytase activity will be at its greatest when digestion has significantly decreased particle size and the pH of the digestive tract is still acidic. This should take place in the gizzard and upper duodenum.

Dietary phytase, Ca and P affect nutrient digestibility and production performance in laying hens fed CSM-based diets. Many different interactions between these three parameters were found. The fact that high dietary levels of Ca were found to enhance the efficacy of phytase, and high levels of NPP to adversely affect phytate hydrolysis by the phytase enzyme, is of great interest to the laying hen industry. This shows that the high dietary Ca level in laying hen diets does not hinder the efficacy of Quantum™ phytase; it actually works to help the enzyme function to its full potential. Laying hen producers can enhance their laying hen diets by feeding moderate levels of NPP (0.25%) with supplementation with Quantum™ phytase in order to maintain optimum production performance and decrease the amount of P being excreted into the environment.

Quantum™ phytase is a commercial product that is available to producers for inclusion in laying hen diets. Quantum™ phytase is effective at maintaining optimum

production performance in laying hens (when fed with a diet that has a slight deficiency in NPP – 0.25%) and at maintaining the P status of the bird during the late stages of the production cycle. This is of great importance because of the fact that hens in the late stage of production have expended their P reserves. The inclusion of phytase in the laying hen diet can actually maintain the P status of the laying hen throughout the entire laying cycle, resulting in laying hens that are healthier and hens that have the ability to maintain optimum egg production levels throughout the entire 40 week production cycle. More work needs to be completed on the effect of Quantum^{TM} phytase on nutrient digestibility in laying hens in order to see its overall impact in laying hen diets.

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