EFFECT OF PHYTASE AND GLUCANASE, ALONE OR IN COMBINATION, ON

NUTRITIVE VALUE OF CORN AND SOYBEAN MEAL FED TO BROILERS

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EFFECT OF PHYTASE AND GLUCANASE, ALONE OR IN COMBINATION, ON NUTRITIVE VALUE OF CORN AND SOYBEAN MEAL FED TO BROILERS

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A Dissertation

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Doctor of Philosophy

Auburn, Alabama

December 15, 2006

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December 15, 2006
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DISSERTATION ABSTRACT

EFFECT OF PHYTASE AND GLUCANASE, ALONE OR IN COMBINATION, ON NUTRITIVE VALUE OF CORN AND SOYBEAN MEAL FED TO BROILERS

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Doctor of Philosophy, December 15, 2006
(M.Sc., University of Alberta, 2004)
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131 Typed Pages

Directed by Edwin T. Moran Jr.

In order to efficiently use exogenous enzymes, the effects of these products on nutrient availability must be determined. A series of experiments were designed to evaluate a phytase and a glucanase supplemented to corn-soybean meal diets. The first two experiments were bioassays, using diets with either adequate or low aP fed to broilers between 5 and 10 d of age. AME, ileal digestible energy (IDE), productive energy (PE) and digestibility of minerals and CP were determined. Glucanase supplementation increased IDE and AME in adequate aP diets, but not PE. Phosphorus, Ca and CP digestibility were improved by phytase, but there was no effect of this enzyme on energy parameters. An experiment designed to investigate the effects of phytase and glucanase on IDE of corn and soybean meal separately, and at various ages was performed. Broiler chicks were fed either corn or soybean meal with or without enzyme supplementation, from 7 to 9, 14 to 16, or 21 to 23 days. Glucanase improved the IDE of

both feedsuffs by approximately 100 kcal/kg at all three ages. Phytase did not affect IDE at any age. The location and extent of phytate degradation, and the effects of phytase and day length were investigated. Broilers were fed complete diets with or without phytase, and were exposed to either 12 or 24 hours of light per day. The experiment was performed between 20 and 24 d of age. The degree of phytate degradation was determined through analysis of digesta and excreta samples for the products of degradation (IP5 through IP2). Degradation occurred primarily in the crop, gizzard and proventriculus. Phytase decreased IP6 and increased IP5 concentrations. Shorter day length generally increased phytate degradation. Phytase supplementation increased ileal IP6 digestibility from 41 to 60%. A final experiment was designed to confirm the results seen in previous experiments in a practical trail. A positive control (PC) was formulated to meet all nutrient requirements. A negative control was formulated by reducing the energy, aP and Ca level in the positive control by 90 kcal/kg, 0.15% and 0.20% respectively. Phytase, glucanase and both enzymes together were supplemented to the NC to create 5 diets. Phytase supplementation improved the performance of birds fed the NC to the level of those fed the PC diets for all response variables. Glucanase supplementation failed to improve the NC diet for any response variable. These experiments show that the phytase used can replace 0.15% aP and 0.20% Ca in corn soybean meal diets. While there was no energy response in the bioassay experiments, the practical experiment suggests that there is an energetic response to phytase supplementation. Glucanase supplementation did not result in an energy response in practical situations.

ACKNOWLEDGEMENTS

Thanks to Syngenta for their financial support of this work, and the Poultry Science Department of Auburn University, and Novus International for providing personal financial support during my stay here in Auburn. Dr. Edwin Moran provided not only academic support, but advice and patience when necessary, and made this experience more enjoyable and valuable. And my committee, Dr. Bedford, Dr. Hess, and Dr. Blake, provided advice and support when needed. Thank you all.

Without the tireless support of Jin Fung Chen, this work would not have been possible. Jin Fung never once complained, even when things didn't go as planned, and was always was watching out for me. Thank you.

Numerous students helped out with the dissections involved in these experiments. Without their help, and the use of equipment from Dr. McKee's lab, I would still be in the lab analyzing samples today.

And of course, the moral support of Nancy Joseph, my family and friends, I would have failed to maintain my sanity. Thank you for being there.

Style manual or Journal used: Journal of Poultry Science

Computer software used: Microsoft Word 2003

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1.0 LITERATURE REVIEW

1.1 INTRODUCTION

During the past 20 years, advances in biotechnology and microbiology have had a substantial impact on animal agriculture. The ability to produce enzymes in large quantities at an affordable cost has increased the use of marginal feed ingredients. Of the enzymes that have been investigated and developed, phytase has been the most commercially successful. This is likely a combined result of pollution concerns and the cost of supplementing inorganic phosphorus. Fiber digesting enzymes have also enjoyed some success, particularly in areas of the world that use wheat and barley as their primary grain source in poultry feed.

The research described in the accompanying chapters describes the evaluation of a phytase and glucanase enzymes in poultry diets. The following sections of this chapter are intended to provide the background required to understand the effects of these enzymes.

1.2 PHYTIN AND PHYTASE

Occurrence and Location of Phytin

Phytin is a storage form of phosphorus (P) in plants and is particularly abundant in seeds. Phytin is composed of a six carbon myo-inositol ring with a phosphate group

attached to each carbon. The phosphate groups attached to carbons 1, 3, 4, 5, and 6 are typically in the equatorial position while the phosphate on carbon 2 is in the axial position (Reddy et al., 1982). As a result, the phosphate group on carbon 2 is the most difficult to remove enzymatically. Nomenclature for this molecule varies in the literature. It is referred to as phytin or phytic acid, which is the molecule alone, it's scientific name, myo-inositol 1,2,3,4,5,6 hexakis phosphate, or phytate, refers to the salt form of phytin.

The prevalence of phytin in cereals and legumes was reviewed in detail by Reddy et al. (1982). In brief, as the bulk of poultry feed is composed of plant material, the amount of phytin in these feeds can be high. While phytin levels in each feedstuff can vary by the growing conditions that prevail, the average phytin content in some feedstuffs is: 0.89% in corn, 0.62 to 1.35% in wheat, 0.97 to 1.16% in barley, and 1.40 to 1.60% in soybean meal (Reddy et al., 1982). Thus the total phytin content of a poultry diet could exceed 1%. This amounts to approximately 0.28% of dietary P that is unavailable to the bird.

Not only does phytin level differ between feedstuffs, its location also varies. The largest portion (88%) of phytate in corn is located within the germ (Reddy et al., 1982), while 87% of the phytin in wheat resides in the aleurone layer. This is significant because the germ is generally highly digestible whereas the contents within the aleurone remain mostly undisturbed because they are protected by a thick fibrous cell wall. As a result, the phytin in corn is more likely to pose a threat and cause anti-nutritional effects than in wheat. To understand the potential anti-nutritional effects of phytin, it is important to first discuss some of its chemical characteristics.

Chemical Characteristics of Phytin

The interaction between phytin and other nutrients in the intestinal lumen is a function of net negative charge on the phytin molecule. The solubility of negatively charged molecules differs with pH. The uncharged phytin molecule is polar, due to the phosphate groups, and thus soluble in water. In solution, phytin can only be uncharged at very low pH levels. As there are six phosphate groups on the phytin molecule, there are twelve dissociable hydrogen atoms (Reddy et al., 1982). Of these, six are strongly dissociable with a low pK, around 1.8. This means at pH lower than 1.8, all hydrogen atoms will be attached to the phytin molecule, while at pH values above 1.8, these hydrogen atoms will dissociate supplying 6 negative charges on the molecule. Two other hydrogens are weakly acidic, with a pK of 6.3, and the remaining four are very weakly acidic with pK values that cannot be accurately measured. The pH range found in the gastrointestinal tract of the chicken ranges from approximately 4.5 in the crop, 2.5 or lower in the gizzard, and up to 5.7 to 6.8 in the small intestine (Leeson and Summers, 2001). At these pH levels, phytin has at least 6 negative charges throughout the digestive tract (Reddy et al., 1982). In the crop, proventriculus, and gizzard, there are likely only six negative charges, while in the small intestine there would be eight.

Several researchers have investigated the solubility of phytin at various pH levels and in the presence of various minerals (Kaufman and Kleinberg, 1971; Evans and Pierce, 1981; Cheryan et al., 1983). Phytin is generally more soluble at lower pH levels. This is in large part due to the capacity of phytin to chelate minerals. As the pH drops, the number of charged phosphate groups decreases and the ability of phytin to chelate minerals decreases. Several of the minerals form strong ionic bonds with phytin and

precipitate. Divalent cations are more likely to form bonds with phytin, with Zn and Ca together being the least soluble. While phytin has a high affinity for Zn, Ca is generally present in the larger quantities. The formation of phytate salts in the digestive tract results in precipitation of the compound.

As well as minerals, positively charged amino acids can also interact with phytin (Reddy et al., 1982). This interaction differs slightly, as it occurs at lower pH levels than with minerals. At the pH found in the gizzard and proventriculus, lysine and arginine residues are positively charged because the epsilon amino group has a pK of 10.8 to 12.5. Thus the single positive charge forms an ionic bond with one of the six negatively charged phosphate groups of phytin. The result is a reduction in the solubility and digestibility of the protein, although this has not been conclusively shown in vivo. The mechanism involved in mineral chelation requires two adjacent negative charges to balance the two positive charges on the cation. Thus, this reaction occurs more readily in the small intestine where there are eight negative charges on the phytin molecule. Studies have also shown that a particularly stable precipitate is formed when protein, Zn and Ca are involved together (Evans and Pierce, 1981; Prattley et al., 1982; Pallauf et al., 1994).

Anti-nutritive Effects of Phytin

Much of the work that demonstrates the anti-nutritive effects of phytin has been accomplished by adding phytase to a diet high in phytin and eliciting a response. This approach is necessary to achieve concentrations of phytin similar to those found in commercial feed, and to avoid the costs associated with using large amounts of purified

phytin. However, occasionally it is difficult to determine the precise effect of phytase; that is which nutrient it is affecting. These earlier studies will be discussed in further sections, but it is worth mentioning some of the more important examples here as well.

Phytin reduces the availability of P to the animal. Since it is largely indigestible and is the principal storage form of P in plant feedstuffs, poultry diets must be supplemented with inorganic P. This is expensive and inefficient as excess P is excreted, which contributes to environmental pollution. Also, when phytin chelates with other minerals, particularly Zn and Ca, it reduces their availability, which makes over-supplementation necessary for these minerals as well. Protein digestibility may also be reduced, leading to excessive nitrogen excretion. This effect has been inconsistent in the literature thus far.

In addition to reducing nutrient digestibility, Cowieson et al. (2004) have shown that phytin contributes to increased endogenous losses in poultry. That is, phytin can also reduce the ability of the bird to absorb proteins and minerals that it secretes into the small intestine during the digestive process. Enzymes, minerals and sloughed cells that enter the lumen of the small intestine can precipitate with phytate and become unavailable for re-absorption. This effect increases the energy cost to the bird associated with digestion of the feed. Other researchers have shown in vitro that phytate can actually reduce the activity of some digestive enzymes. Singh and Krikorian (1982) described a reduction of trypsin activity in solutions of phytate, and Knuckles and Betschart (1987) described a similar effect on amylase activity. The mechanism behind this inhibition has not been determined, and may include direct association with the enzyme (precipitation),

association with the substrate (blocking the enzyme substrate complex from forming), or association with stabilizing mineral co-factors (e.g. amylase requires Ca for stability).

As a result of these anti-nutritional effects, phytate in poultry feeds can reduce the availability of minerals and protein, reduce the efficiency of digestion, and increase the energy and protein costs of digestion. Thus phytase enzymes have the potential to impact the nutritive value of poultry feedstuffs in many areas.

Phytase Enzymes

Phytase is a generic term that refers to a range of enzymes with different specificities and modes of action (Mullaney and Ullah, 2003). Generally, in animal and poultry nutrition, phytase refers to histidine acid phosphatases (EC 3.1.3.8). The production, characteristics, and manipulation of thermo-tolerance and gastric digestion resistance of phytases is beyond the scope of this paper but has been described in detail elsewhere (Ha et al., 2000; Pandey et al., 2001; Lei and Porres, 2003; Vohra and Satyanarana, 2003; Garrett et al., 2004). Phytases commonly used in animal and poultry feeds are obtained from fungal, or bacterial sources with the DNA code spliced into yeast for mass production. Alterations are made in the genetic sequence to slightly alter the amino acid composition at strategic locations. This improves thermo-tolerance, which allows the enzyme to survive pelleting.

Phytases fall into one of two categories, either 3-phytase or 6-phytase depending on where dephosphorylation begins. A 3-phytase begins by removing the phosphate in the carbon 3 position, while a 6-phytase begins with the phosphate in the carbon 6

position. Both types of phytase are capable of removing all of the phosphate groups, with the exception of the axial phosphate in the second position.

Generally, phytases are active at the pH found in the crop, proventriculus, and gizzard, and lose activity in the small intestine. While this coincides with the peak solubility of phytin in the upper digestive tract, it limits the amount of time phytase can act on its substrate. The activity of phytase is defined at pH 5.5 and 37°C, with 1 unit phytase activity defined as liberating 1 µmol inorganic phosphate per minute under those conditions. Depending on the pH and temperature optimums of the enzyme, actual performance in the bird will vary.

Phytase in Animal Feeds

In the past 15 years, there have been numerous experiments performed in chickens and swine using phytase supplemented feed. Most of this work focused on determining the ability of phytase to replace inorganic P supplementation. Response variables have included body weight gain, feed intake, feed conversion (Lim et al., 2001; Ravindran et al., 2001), tibia, femur or toe breaking strength and ash content (Perney et al., 1993; Qian et al., 1996; Pintar et al., 2004), and egg production parameters in layers (Nahashon et al., 1994; Jalal and Schnideler, 2001; Wu et al., 2006). Most trials use diets deficient in available phosphorus (aP), and results consistently show improvements in these traits by phytase supplementation.

The effect of phytase on the digestibility of other minerals, such as Ca, Zn, Mg, and Fe is less consistent than on P digestibility (Sebastian et al., 1996a; 1996b). Most experiments do not use deficient levels of these minerals in the diets making the results

variable. As a result, a portion of the response in mineral retention can be attributed to an improvement in bone mineralization, rather than an improvement in bioavailability. As well, the requirements of most minerals have been determined or estimated using diets that contain phytin in the absence of phytase. Therefore, current supplementation of these minerals accounts for any reduced availability that may be caused by phytin. Trials involving Ca digestibility have found that Ca supplementation can be reduced without a negative response when phytase is added. In fact, reduced Ca levels can improve bone mineralization when phytase is supplemented (Sebastian et al., 1996b).

The response in amino acid or protein digestibility with phytase supplementation is also inconsistent. Several studies have tried to find an amino acid or nitrogen digestibility response, some have been successful (Biehl and Baker, 1996; Yi et al., 1996; Rutherford et al., 2002; Ravindran et al., 2006), while others have found no response (Biehl and Baker, 1997; Ravindran et al., 1999a; Augspurger and Baker, 2004; Martinez-Amezcua et al., 2006). Ravindran et al. (2001) demonstrated that phytase supplementation to lysine deficient diets improved the digestibility of lysine. The authors calculated that phytase supplementation in that experiment was the equivalent to adding 0.074% lysine to the diet. As phytate associated primarily with lysine and arginine, this response was not surprising. Selle et al. (2003a) described a similar experiment in pigs with no lysine response to phytase supplementation. It seems further work is required in order to accurately apply a protein or amino acid value to phytase.

Further evidence shows that, under certain circumstances, phytase supplementation can increase the AME value of the test feed. The most positive effects are found in wheat based diets (Ravindran et al., 2001). The mechanism for an increase

in AME is unclear, but may relate to a reduction in endogenous losses, or improved luminal enzyme efficiency. In order to efficiently use phytase in poultry diets, the efficacy of phytase under specific conditions needs to be determined. That is, the feeding conditions that result in improvements in AME and CP digestibility need to be clearly defined. Currently it is unclear if these responses are due to differences in feedstuff usage, or the nutrient interactions within the feed.

Phytase Interactions

The effect of other nutritional compounds on the efficacy of phytase has been investigated. Studies by Qian et al. (1997) and Driver et al. (2005) have found that vitamin D_3 can optimize phytase function. This nutrient may enhance the endogenous phytase activity of the bird. Maenz and Classen (1998) suggested that brush border phytase activity may be regulated by vitamin D_3, which would account for the greater availability of phytate P in the previous two studies (Qian et al., 1997; Driver et al., 2005). Alternately, greater dephosphorylation may have occurred as a result of reduced Ca in the intestinal lumen and therefore greater solubility of phytate.

Boling et al. (2000) showed an improvement in phytate P digestibility as a result of citric acid supplementation, with or without phytase supplementation in chicks. A reason was not discovered, but they stated that the crop conditions more favorable to phytin degradation. Martinez-Amezcua et al. (2006) showed that 1000 units of phytase increased phytate P digestibility slightly more than adding 3% citric acid. In pigs, P availability increased with phytase and an organic acid simultaneously, compared to phytase alone (Omogbenigun et al., 2003). Citric acid, vitamin D_3, and phytase all

increased phytate phosphorus digestibility in an additive manner, but there was no synergistic effect between phytase and the other two compounds in broilers (Snow et al., 2004) or pigs (Radcliffe et al., 1998).

Fibrolytic enzymes may also improve the animals response to phytase supplementation by providing access to its substrate or through a reduction in gut viscosity that allows greater diffusion of the enzyme and substrate in the digesta (Peng et al., 2003; Selle et al., 2003b; Wu et al., 2004; Juanpere et al., 2005). The influence of fiber and fibrolytic enzymes in poultry feed is covered in the next section.

1.3 FIBERS AND FIBROLYTIC ENZYMES

Fiber poses a number of obstacles in poultry feed. As plant feedstuffs are composed of several different cell types, with different cell wall compositions, each feedstuff creates a different challenge. This section will briefly describe the organization of various cell components, the fiber types involved, and the influence they have on nutrient utilization. As well, enzymatic solutions to these problems will be discussed. For more in depth information on cell wall organization, the reader is directed to Fincher and Stone (1986).

Types of Fiber

Quantitatively, cellulose, arabinoxylans, and beta-glucans comprise most of the fiber in cereal grains fed to poultry (Bach Knudsen, 1997). Cellulose, an insoluble fiber composed of beta (1-4) linked glucose molecules, is considered to be the major structural component of cell walls. Arabinoxylans are composed of a linear backbone of beta (1-4)

linked xylose units with side chains of arabinose and other sugars linked to carbons 2, 3, or 5 on xylose. The degree of branching varies between cereals, with a higher degree of polymerization and molecular weight found in wheat and barley than in corn. Beta-glucans are beta (1-3, 1-4) linked glucose molecules. While there are side chains on beta-glucan molecules, the major differences between grains involve the ratio of 1-4 to 1-3 bonds and the molecular weight of the polymer.

Cellulose, as an un-branched linear molecule, is highly insoluble in water. Arabinonxylans and beta-glucans, however, are soluble in water. Generally, xylan solubility is directly related to the molecular weight and degree of branching. Beta-glucan solubility is related to both molecular size and the types of bonds in the polymer. Luchsinger (1965) showed that beta-glucans that are water soluble had fewer long sequences of beta (1-4) bonds before interruption with a beta (1-3) bond. The longer the sequence of beta (1-4) bonds, the more it behaved like cellulose.

Grain differences in hemicellulose content and characteristics result in substantial nutritive effects when fed to poultry (Fincher and Stone, 1986). In comparison to other grains, wheat contains larger amounts of high molecular weight arabinoxylans, particularly in the endosperm and aleurone layers. Since these molecules are soluble in water, and only loosely attached to the cell walls, they form a viscous solution in the digestive tract. Similarly, in barley, the endosperm cell wall contains large amounts of beta-glucans with a high ratio of beta (1-3) to beta (1-4) bonds. The gels formed when these two grains are fed reduce nutrient digestibility and availability. Non-viscous grains, such as corn, have cell walls made up primarily of low molecular weight arabinoxylans and small amounts of beta-glucans, which do not cause viscosity problems.

Along with cereal grains, most poultry feeds contain a large amount of soybean meal. Soybeans contain some xylans and beta-glucans as structural components of cell walls, but their levels are relatively low (Bach Knudsen, 1997). There are higher levels of the oligosaccharides stachyose and raffinose, along with pectin. Stachyose is composed of two galactose molecules bound to a glucose and a fructose molecule, while raffinose is composed of one galactose bound to a glucose and a fructose molecule. Poultry do not have endogenous galactosidase activity so these oligosaccharides are indigestible. The pectins found in soybean meal are composed of a backbone of galacturonic acids with side chains containing rhamnose, galactose, arabinose, xylose, and fructose. Pectins are thought to associate with cellulose in the cell wall, and may become soluble in the GIT.

Impact of Fiber on Nutrition

While most of the fiber in poultry feed has no measurable anti-nutritional effect, it can be costly in that it is indigestible and provides little or no value to the bird. However, there are some polymers found in common feed ingredients that do pose nutritional problems. The most obvious is the gel forming properties of soluble non-starch polysaccharides (NSPs) such as arabinoxylans and beta-glucans. It has been conclusively shown that addition of wheat and barley to the diet at high levels, without supplementation of an appropriate enzyme, leads to a reduction in AME, and sticky droppings in broilers (Maisonnier et al., 2001). The reduction in AME, as well as decreases in the digestibility and availability of other nutrients, is caused by a decrease in movement of the gut contents (Scott et al., 1998; Cowieson et al., 2005). The gel

formation reduces the likelihood of endogenous enzymes contacting appropriate substrates, thus reducing digestibility. As well, nutrients are less likely to come in contact with the brush border, reducing the likelihood of nutrient absorption. Further, gel formation increases the proliferation of microbes in the small intestine, which are competing with the bird for the nutrients present (Jensen and Jorgensen, 1994; Choct, 2000; Hubener et al., 2002). Although to a much smaller degree, it has been suggested that pectins in soybean meal may act similarly to arabinoxylans and beta-glucans (Langhout et al., 2000; Malathi and Devegowda, 2001).

As the bird can not digest many of the components of the plant cell wall, it is also likely that intact plant cells would pass through the digestive tract. While grinding, pelleting, and physical digestion of the feed in the gizzard function to reduce particle size and improve digestibility, it has been shown that some 7% of the starch in a corn-soybean meal diet escapes digestion (Carre, 2004). This encapsulation, and the viscosity effects of soluble fibers, have lead to a surge in research into the effects of fibrolytic enzymes in poultry feed.

Fibrolytic Enzymes in Animal Feeds

The addition of fibrolytic enzymes to animal feed has met some success, particularly in wheat- and barley-based diets. The use of xylanases in wheat and beta-glucanases in barley diets has successfully reduced the viscosity of the digesta by 30% (Mathlouthi et al., 2002; Wu et al., 2004) to 50% (Steenfeldt et al., 1998) in wheat and by over 300% in barley-based diets (Juanpere et al., 2005). Reduced viscosity leads to

improvements in AME, protein digestibility, body weight gain, feed consumption, and feed conversion.

In corn-based diets, the application of fibrolytic enzymes has focused on improving starch digestibility and protein by reducing encapsulation of these substrates. Experiments have tested xylanases, mannanases, and beta-glucanases, often in combination with each other as well as microbial proteases and amylases (Ouhida et al., 2002; Pan et al., 2002; Mathlouthi et al., 2003; Juanpere et al., 2005; Meng et al., 2005). Several experiments have shown increases in AME (Ouhida et al., 2002) and nutrient digestibility (Mathlouthi et al., 2003; Juanpere et al., 2005). Improvements are generally small compared to those seen in feeds containing viscous grains such as wheat and barley.

Recently, studies were conducted to increase the energy value of soybean meal. As poultry cannot digest raffinose and stachyose, the energy value of soybean meal for poultry is poor compared to that for swine, which can utilize those fibers. Thus, Ghazi et al. (2003) attempted to improve soybean meal's energy value by supplementing the enzyme lacking in poultry, alpha-galactosidase. The results show that alpha-galactosidase and a protease supplemented to soybean meal improved the TME value of the feedstuff by 17%

In non-viscous grains, the application of fibrolytic enzymes is thought to improve the nutritive value of the feedstuff by disrupting intact cells and allowing endogenous enzymes access to their substrates. The products of cell wall degradation themselves are likely present in such small quantities that they would not significantly improve the energy value of the feedstuff. As such, there has been some limited research

investigating the potential interaction between fibrolytic enzymes with phytases (Peng et al., 2003; Selle et al., 2003b; Wu et al., 2004; Silversides et al., 2006). While these studies were performed in wheat based diets, results showed that the combination of enzymes performed additively. Ravindran et al. (1999b) showed a synergistic effect of phytase and fibrolytic enzymes in poor quality wheat, increasing the AME of the sample to that of average quality wheat. Further experiments in the same report resulted in additive effects. Cowieson and Adeola (2005) reported additive energetic effect in corn-based diets formulated to marginal nutritional levels. Further research is warranted in this area in order to optimize the use of both enzymes.

1.4 OBJECTIVES

The experiments described here were designed to evaluate a phytase and a glucanase, alone or in combination, and to determine their influence on corn soybean meal diets fed to broilers. In order to properly evaluate these products, it is necessary to first develop matrix values that can be applied to corn and soybean meal, and then to test those values in a practical experiment.

The second chapter in this dissertation describes two experiments that were designed to test the phytase and glucanase in broilers fed corn and soybean meal diets. The experiments are bioassays, measuring the AME, and ileal and excreta digestibilities of several minerals, protein and energy.

Chapter three describes three experiments designed to test the effects of the phytase and glucanase on corn and soybean meal separately. In order to separate the enzyme effects on the two feedstuffs, a short bioassay was performed where diets used

were composed only of corn or soybean meal, the insoluble ash marker, and the enzymes. The experiment was repeated at three different ages in order to determine if the effect of the enzymes used were affected by digestive tract development. The primary measurement of interest was ileal digestible energy.

The fourth chapter describes an experiment designed to test the effect of day length on the degradation of phytate by phytase. The degradation products of phytate were measured in various portions of the digestive tract in birds exposed to 24h or 12h of continuous light per day and fed complete diets with either 0 or 500 units of phytase activity per kg diet.

The final experimental chapter describes an 8 week grow out trial designed to test the matrix values determined in the previous experiments. Diets were formulated using the energy, Ca digestibility, and aP values found in chapters 2, 3 and 4, as compared to a positive control, formulated with standard values. The variables of interest were BW gain, feed conversion, feed intake, processing yields, and femur breaking strength and ash content.

1.5 REFERENCES

Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82: 1100-1107.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feed. Anim. Feed Sci. Tech. 67: 319-338.

Biehl, R. R., and D. H. Baker. 1996. Efficacy of supplemental 1-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus- or amino acid-deficient corn-soybean meal diets. J. Anim. Sci. 74: 2960-2966.

Biehl, R. R., and D. H. Baker. 1997. Microbial phytase improves amino acid utilization in young chicks fed diets based on soybean meal but not diets based on peanut meal. Poult. Sci. 76: 355-360.

Bolings, S. D., D. M. Webel, I. Mavromichalis, C. M. Parsons, and D. H. Baker. 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. J. Anim. Sci. 78: 682-689.

Carre, B., J. Gomez, and A. M. Chagneau. 1995. Contribution of oligosaccharide and polysaccharide digestion, and excreta losses of lactic acid and short chain fatty acids, to dietary metablizable energy values in broiler chickens and adult cockerels. Br. Poult. Sci. 36: 611-629.

Cheryan, M. F., F. W. Anderson, and F. Grynspan. 1983. Magnesium-phytate complexes: Effect of pH and molar ratio on solubility characteristics. Cereal Chem. 60: 235-237.

Choct, M. 2000. Enzyme supplementation of poultry diets based on viscous cereals. Pages 145-160 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G. G. Partridge eds. CABI Publishing, Oxon, UK.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effect of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45: 101-108.

Cowieson, A. J. and O. Adeola. 2005. Carbohydrases, proteases, and phytase have an additive beneficial effect in nnutritionally marginal diets for broiler chicks. Poult. Sci. 84: 1860-1867.

Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards Jr. 2005. Phytase and 1-alpha-hydroxycholecalciferol supplementation of broiler chickens during the starting and growing/finishing phases. Poult. Sci. 84: 1616-1628.

Evans, W. J., and A. G. Pierce. 1981. Calcium–phytate complex formation studies. J. Am. Oil Chem. Soc. 58: 850-851.

Fincher, G. B. and B. A. Stone. 1986. Cell walls and their components in cereal grain technology. Pages 207-296 in Advances in Cereal Science and Technology. American Association of Cereal Chemists, St. Paul, MN.

Garrett, J. B., K. A. Kretz, E. O'Donoghue, J. Kerovuo, W. Kim, N. R. Barton, G. P. Hazlewood, J. M. Short, D. E. Robertson, and K. A. Gray. 2004. Enhancing the thermal tolerance and gastric performance of a microbial phytase for use as a phosphate-mobilizing monogastric-feed supplement. Appl. Environ. Microbiol. 70: 3041-3046.

Ghazi, S., J. A. Rooke, and H. Galbraith. 2003. Improvement of the nutritive value of soybean meal by protease and alpha-galactosidase treatment in broiler cockerels and broiler chicks. Br. Poult. Sci. 44: 410-418.

Ha, N. C., B. C. Oh, S. Shin, H. J. Kim, T. K. Oh, Y. O. Kim, K. Y. Choi, and B. H. Oh. 2000. Crystal structures of a novel, thermostable phytase in partially and fully calcium loaded states. Nat. Struct. Biol. 7: 147-153.

Hubener, K., W. Vahjen, and O. Simon. 2002. Bacterial response to different dietary cereal types and xylanase supplementation in the intestine of broiler chickens. Arch. Tierernahr. 56: 167-187.

Jalal, M. A., and S. E. Schnideler. 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci. 80: 1463-1471.

Jensen, B. B., and H. Jorgensen. 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60: 1897-1904.

Juanpere, J., A. M. Perez-Vendrell, E. Angulo, and J. Brufau. 2005. Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poult. Sci. 84: 571-580.

Knuckles, B. E., and A. A. Betschart. 1987. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J. Food Sci. 52: 719-721.

Kaufman, H. W., and I. Kleinberg. 1971. Effect of pH on calcium binding by phytic acid and it's inositol phosphoric acid derivatives and on the solubility of their calcium salts. Arch. Oral Biol. 16: 445-460.

Langhout, D. J., J. B. Schutte, J. de Jong, H. Sloethes, M. W. Verstegen, and S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83: 533-540.

Lei, X. G., and J. M. Porres. 2003. Phytase enzymology, applications, and technology. Biotechnol. Lett. 25: 1787-1794.

Leeson, S. and J. D. Summer. 2001. Scott's Nutrition of the Chicken. 4th Edition. University Books. Guelph, ON.

Lim, H. S., H. Namkung, J. S. Um, K. R. Kang, B. S. Kim, and I. K. Paik. 2001. The effects of phytase supplementation on the performance of broiler chickens fed diets with different levels of non-phytate phosphorus. Asian-Australian Journal of Anim. Sci. 14: 250-257.

Luchsinger, W. W., S. C. Chen, and A. W. Richards. 1965. Mechanisms of action of malt beta-glucanases 9. The structure of barley beta-D-glucan and the specificity of A11-endo-beta-glucanase. Arch. Biochem. Biophys. 112: 531-536.

Maenz, D. D., and H. L. Classen. 1998. Phytase activity in the small intestinal brush border membrane of the chicken. Poult. Sci. 77: 557-563.

Maisonnier, S., J. Gomes, and B. Carre. 2001. Nutrient digestibility and intestinal viscosity in broiler chickens fed on wheat diets, compared to maize diets with added guar gum. Br. Poult. Sci. 42: 102-110.

Malathi, V., and G. Devegowda. 2001. In vitro evaluation of nonstarch polysaccharide digestibility of feed ingredients by enzymes. Poult. Sci. 80: 302-305.

Mathlouthi, N., L. Saulnier, B. Quemener, and M. Larbier. 2002. Xylanase, beta-glucanase, and other side enzymatic activities have greater effects on the viscosity of several feedstuffs than xylanase and beta-glucanase used alone or in combination. J. Agric. Food Chem.50: 5121-5127.

Mathlouthi, N., M. A. Mohamed, and M. Larbier. 2003. Effect of enzyme preparation containing xylanase and beta-glucanase on performance of laying hens fed wheat/barley of maize/soybean meal based diets. Br. Poult. Sci. 44: 60-66.

Martinez-Amezcua, C., C. M. Parsons, and D. H. Baker. 2006. Effect of microbial phytase and citric acid on phosphorus bioavailability, apparent metabolizable energy, and amino acid digestibility in distillers dried grains with solubles in chicks. Poult. Sci. 85: 470-475.

Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell, and W. Guenter. 2005. Degradation of cell wall polysaccharides by combination of carbohydrase enzymes and their effect on nutrient utilization and broiler performance. Poult. Sci. 84: 37-47.

Mullaney, E. J., and A. H. Ullah. 2003. The term phytase comprises several different classes of enzyme. Biochem. Biophys. Res. Commun. 312: 179-184.

Nahashon, S. N., H. S. Nakaue, and L. W. Mirosh. 1994. Phytase activity, phosphorus and calcium retention, and performance of single comb white leghorn layers fed diets containing two levels of available phosphorus and supplemented with direct-fed microbials. Poult. Sci. 73: 1552-1562.

Omogbenigun, F. O., C. M. Nyachoti, and B. A. Slominski. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci. 81: 1806-1813.

Ouhida, I., J. F. Perez, M. Anguita, and J. Gasa. 2002. Influence of beta-mannase on broiler performance, digestibility, and intestinal function. J. Appl. Poult. Res. 11: 1-6.

Pallauf, J., G. Rimbach, S. Pippig, B. Schindler, and E. Most. 1994. Effect of phytase supplementation to a phytate-rich diet based on wheat, barley and soya on the

bioavailability of dietary phosphorus, calcium, magnesium, zinc, and protein in piglets. Agribiol. Res. 47: 39-48.

Pan, B., D. Li, X. Piao, L. Zhang, and L. Guo. 2002. Effect of dietary supplementation with alpha-galactosidase preparation and stachyose on growth performance, nutrient digestibility and intestinal bacterial populations of piglets. Arch. Anim. Nutri. 56: 327-337.

Pandey, A., G. Szakacs, C. R. Soccol, J. A. Rodrigues-leon, and V. T. Soccol. 2001. Production, purification and properties of microbial phytases. Bioresour. Technol. 77: 203-214.

Prattley, C. A., D. W. Stanley, T. K. Smith, and F. R. Van De Voort. 1982. Protein-phytate interactions in soybeans. III. The effect of protein-phytate complexes on zinc bioavailability. J. Food Biochem. 6: 273-282.

Peng, Y. L., Y. M. Guo, and J. M. Yuan. 2003. Effects of microbial phytase replacing partial inorganic phosphorus supplementation and xylanase on growth and nutrient digestibility in broilers fed wheat-based diets. Asian-Australas. J. Anim. Sci. 16: 239-247.

Perney, K. M., A. H. Cantor, M. L. Straw, and K. L. Herkelman. 1993. The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. Poult. Sci. 72: 2106-2114.

Pintar, J., B. Homen, K. Grbesa, M. Sikiric, and T. Cerny. 2004. Effects of supplemental phytase on performance and tibia ash of broilers fed different cereal based diets. Czech. J. Anim. Sci. 49: 542-548.

Qian, H., E. T. Kornegay, and H. P. Veit. 1996. Effects of supplemental phytase and phosphorus on histilogical, mechanical and chemical traits of tibia and performance of turkeys fed on soybean-meal-based semi-purified diets high in phytase phosphorus. Br. J. Nutr. 76: 263-272.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytase phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76: 37-46.

Radcliffe, J. S., Z. Zhang, and E. T. Kornegay. 1998. The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. J. Anim. Sci. 76: 1880-1886.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999a. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78: 699-706.

Ravindran, V., P. H. Selle, and W. L. Bryden. 1999b. Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. Poult. Sci. 78: 1588-1595.

Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80: 338-344.

Ravindran, V., P. C. H. Morel, G. G. Partridge, M. Hruby, and J. S. Sands. 2006. Influence of an Escherichia coli-derived phytase on nutrient utilization in broiler

starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85: 82-89.

Reddy, N. R., S. K. Sathe, and D. K. Salunke. 1982. Phytates in legumes and cereals. Adv. Food Res. 28: 1-92.

Rutherford, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 44: 598-606.

Scott, T. A., F. G. Silversides, H. L. Classen, M. L. Swift, and M. R. Bedford. 1998. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. Poult. Sci. 77:456-463.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996a. The effect of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, and zinc in broiler chickens fed corn-soybean diets. Poult. Sci. 75: 729-736.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996b. Efficacy of supplemental microbial phytase at different levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 75: 1516-1523.

Selle, P. H., D. J. Cadogan, and W. L. Bryden. 2003a. Effects of phytase supplementation of phosphorus-adequate, lysine-deficient, wheat-based diets on growth performance of weaner pigs. Aust. J. Agric. Res. 54: 323-330.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003b. Influence of phytase and xylanase supplementation on growth performance and

nutrient utilization of broilers offered wheat-based diets. Asian-Australas. J. of Anim. Sci. 16: 394-402.

Silversides., F. G., T. A. Scott, D. R. Korver, M. Afsharmanesh, and M. Hruby. 2006. A study on the interaction of xylanase and phytase enzyme in wheat-based diets fed to commercial white and brown laying hens. Poult. Sci. 85: 297-305.

Singh, M., and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30: 799-800.

Snow, J. L., D. H. Baker, and C. M. Parsons. 2004. Phaytase, citric acid, and 1-alpha-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poult. Sci. 83: 1187-1192.

Steenfeldt, S., A. Mullertz, and J. F. Jensen. 1998. Enzyme supplementation of wheat-based diets for broilers 1. Effect on growth performance and intestinal viscosity. Anim. Feed Sci. Tech. 75: 27-43.

Vohra, A., and T. Satyanarana. 2003. Phytases: Microbial sources, production, purification, and potential biotechnological application. Crit.l Rev. Biotechnol. 23: 29-60.

Wu, G., Z. Liu, M. M. Bryant, and D. A. Roland Sr. 2006. Comparison of Natuphos and Phyzyme as phytase sources for commercial layers fed corn-soy diet. Poult. Sci. 85: 64-69.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually of in combination, on performance, apparent metabolizable energy, digestive tract measurements and

gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br. Poult. Sci. 45: 76-84.

Yi, Z., E. T. Kornegay, V. Ravindran, and D. M. Denbow. 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. Poult. Sci. 75: 240-249.

2.0 THE EFFECT OF PHYTASE AND GLUCANASE, ALONE AND IN COMBINATION, ON THE ENERGY, PROTEIN AND MINERAL VALUE OF CORN SOYBEAN MEAL DIETS FED TO BROILERS

2.1 INTRODUCTION

The focus of the experiments presented here were to determine the effect of a phytase and glucanase, alone or in combination, on AME, nutrient digestibility and retention of broiler chicks when fed corn-soybean meal diets with either adequate or deficient aP levels. It was well established that supplementation of poultry and swine feeds with exogenous phytase increased phosphorus availability from plant sources by hydrolyzing phytate (Cromwell et al., 1993; Broz et al., 1994; Cromwell et al., 1995a, 1995b; Ibrahim et al., 1999; Juanpere et al., 2005). Other nutritional effects of phytase in poultry included greater micromineral and Ca availability and improved protein digestibility by increasing mineral and protein solubility. Further, it has been suggested that phytate hydrolysis increases AME, either by destabilizing plant cell walls (Frolich, 1990) or reducing endogenous energy and protein costs to the bird (Cowieson et al., 2004).

Studies involving fibrolytic enzymes, including glucanase, have focused primarily on wheat- and barley-based diets due to their ability to decrease digesta viscosity in these

highly soluble nonstarch polysaccharide (NSP) feedstuffs (Scott et al., 1998a, 1998b). Another beneficial effect of hemicellulose digesting enzymes that is not exclusive to high-NSP feeds, these enzymes can degrade cell walls thereby increasing the digestibility of fibrous components and providing access to substrates for endogenous enzymes in the gut (Classen, 1996). Consequently, there is some research on the effect of fibrolytic enzymes in corn-soybean meal diets, which are inherently low in soluble NSPs and do not suffer from the effects of high digesta viscosity.

2.2 MATERIALS AND METHODS

Stock and Management

All procedures were reviewed and approved by the Auburn University Institutional Animal Care and Use Committee. Initially, a total of 740 day-old Ross x Ross 308 chicks were obtained from a commercial hatchery and housed in battery cages. Feed and water were available at all times, except when feed withdrawal was required to facilitate clearance of the digestive tract. Chicks were subjected to 24-hour light in temperature controlled rooms. On day 5, feed was withdrawn for 8 hours and chicks were weighed and re-distributed among cages to achieve equal mean body weight and variance between cages. Birds were distributed into two rooms, according to the experiment to which they were assigned.

Experiment 1

Experiment 1 was a balance study designed to investigate the effect of phytase and glucanase, alone and in combination, on the nutrient availability in corn soybean meal diets either adequate or deficient in aP. The experiment was a 2x2x2 factorial, with six replicates of 10 birds per treatment. Eight diets were formulated to contain either 0.25 or 0.45% aP, with either 0 or 500 units of phytase per kg of feed and either 0 or 50 units of glucanase per kg of feed (Table 2-1). Chicks were assigned to one of 48 cages. An additional 60 chicks (representing six replicates of 10 birds) were euthanized by CO_2 asphyxiation at the onset to estimate the initial body composition prior to experimentation. A commercial type starter diet was fed from 0-5 d of age, and experimental diets were provided from 5 to 10 days of age. Trays lining each cage were employed to ensure total collection of excreta material. On day 10, feed was removed from the cages for 8 hours to facilitate clearance of the digestive tract. Following feed withdrawal, birds were euthanized by CO_2 asphyxiation, weighed, and frozen for body composition analysis. Fecal collection trays were weighed and a sub-sample was frozen for further analysis. Frozen carcasses were ground using a meat grinder, the samples pooled within each pen, and both carcass and fecal samples were freeze-dried. All samples were weighed and re-ground after freeze-drying in order to calculate DM and ensure a homogeneous sample. Samples were analyzed for Ca, P, Zn, Cu, Mg, Fe using an inductively coupled plasma source optical emission spectrometer[1], N using a

[1] Model Vista, Varian Inc, Palo Alto California

combustion nitrogen analyzer[2] and gross energy composition using a bomb calorimeter[3]. Crude protein was calculated as 6.25 multiplied by the nitrogen content of the sample.

Experiment 2

Experiment 2 was designed to determine the ileal digestibility of nutrients as affected by phytase and glucanase supplementation, alone and in combination in an adequate aP diet. The diets used were the same as diets 1 through 4 in Experiment 1 (Table 2-1). Two hundred and forty chicks were assigned to 24 cages with six replicates of 10 chicks per treatment. As before, experimental diets were provided from 5 to 10 days of age and trays were employed again for excreta collection. To collect ileal contents, feed was not withdrawn prior to euthanization. On day 10, the birds were euthanized via CO_2 asphyxiation and ileal contents were removed. Fecal samples were also collected as in Experiment 1. Feed, ileal, and fecal samples were analyzed for gross energy, Ca, P, Zn, Cu, Mg, Fe, N and acid insoluble ash as described in Experiment 1. Acid insoluble ash content of the feed, digesta, and excreta were determined using the method described by Scott et al. (1998a, b).

Feed, excreta and ileal samples pooled across pens within each treatment were prepared for microscopic analysis (Olympus BX-50), and stained with tulidine blue to accentuate cell walls, protein, and phytate, or light green and iodine to emphasize protein, amylose, and amylopectin. Samples were photographed and the relative degradation of cell walls, starch, and phytate was noted.

[2] Model CNS-2000, Leco Corp, St. Josephs Michigan
[3] Model 1266, Parr Inc, Moline Illinois

Data Analysis

Data collected from both experiments were used to calculate the productive energy (energy gain of the carcass as a percentage of energy consumed) as well as ileal digestible energy (IDE) and excreta apparent metabolizable energy (AME). Ileal digestibility data from Experiment 2 was calculated according to the following equation (Selle et al., 2003):

$$\% \text{ Digestibility} = \frac{(NT/AIA)_d - (NT/AIA)_{i/e}}{(NT/AIA)_d} \times 100$$

where NT was the nutrient concentration, AIA was the acid insoluble ash concentration, d indicated the dietary contents whereas i/e indicated the ileal or excreta contents. Nitrogen retention in Experiment 2 was calculated by the following equation (Scott et al., 1998a):

$$\text{N Retention} = 100 - \left[100 \times \left(\frac{\% \text{ AIA}_d}{\% \text{ AIA}_{i/e}} \right) \times \left(\frac{\% \text{ N}_{i/e}}{\% \text{ N}_d} \right) \right]$$

AME and IDE were calculated using the following two formulas, using the total excreta collection and AIA methods, respectively:

$$\text{AME (kcal/kg diet)} = (GE_d \times \text{DM feed intake}) - (GE_e \times \text{Total fecal DM})$$

$$\text{AME or IDE (kcal/kg diet)} = GE_d \left[GE_{i/e} \times \left(\frac{AIA_d}{AIA_{i/e}} \right) \right]$$

where GE_d is the gross energy (kcal) of the diet, and GE_e is the gross energy (kcal) of the excreta.

Statistical analyses in both experiments were conducted according to a one-way ANOVA using the GLM procedure of SAS® (SAS Institute, 2001), with the cage as the experimental unit. Comparisons between means were made using the Tukeys test.

2.3 RESULTS

Experiment 1

Growth performance in Experiment 1 was positively affected by phytase and glucanase supplementation (Table 2-2). The addition of phytase to the diets significantly increased both feed intake and BW gain, with no improvement in feed conversion. Both feed intake and BW gain showed a significant interaction between aP level and phytase, where intake and gain increased in phytase-supplemented diets deficient in aP (Table 2-3). DM digestibility increased with phytase, while AME and PE were not affected. Glucanase supplementation reduced feed intake, with no change in BW gain (Table 2-2). As a result, an improvement in feed conversion approached significance (P=0.06). Glucanase did not influence DM digestibility, AME, or PE. However, glucanase in the adequate aP diet increased AME compared to the other glucanase and aP level treatments (Table 2-3). The aP content of the feed influenced several productive traits. Diets deficient in aP resulted in reduced feed intake, gain, AME, and PE.

Mineral retention in this experiment was estimated from carcass analysis (Table 2-4). Phytase supplementation increased retention of Ca, P, and improved CP digestibility. Phytase increased P retention in diets deficient in aP. No other minerals showed a response to phytase. Glucanase inclusion in the diet increased Ca retention,

with no change in other minerals or CP digestibility. The level of aP fed did not affect mineral retention or CP digestibility.

Experiment 2

Similar to Experiment 1, phytase increased feed intake and BW gain in Experiment 2 (Table 2-5). Unlike the first experiment, phytase lowered feed conversion and did not affect DM digestibility. IDE and AMEs were not affected by phytase. Glucanase supplementation had no affect on feed intake or BW gain, but reduced feed conversion. While excreta AME values were not influenced by the addition of glucanase, IDE increased. DM digestibility was not affected by glucanase supplementation.

Mineral and CP digestibility and retention were calculated based upon both ileal (Table 2-6) and excreta (Table 2-7) analysis, respectively using an acid insoluble ash marker. Ileal analysis indicated that phytase supplementation improved the digestibility of P and CP, while excreta analysis showed a greater retention of P and Fe, but not CP or any other mineral. Glucanase supplementation improved the ileal digestibility of Zn, Cu, and Mg, while Fe digestibility approached significance. The excreta analysis found no effects of glucanase on mineral retention values.

2.4 DISCUSSION

Both experiments were designed to evaluate the influence of phytase and glucanase on the energy availability of corn soybean meal diets. No effect of phytase was seen in either experiment on AME, IDE, or PE. Energy responses to supplemental phytase have been noted by some authors (Ravindran et al., 2001; Wu et al., 2003;

Silversides et al., 2004), however these authors used primarily wheat soybean meal diets. Wu et al. (2003) also failed to find evidence of an energetic response to phytase in corn soybean meal diets. The mechanism responsible for increasing AME in wheat based diets is unclear. However, the degradation of phytate imbedded in the cell walls of the wheat kernel has been suggested (Frolich 1990; Wu et al., 2003).

The use of exogenous hemicellulose digesting enzymes, such as beta-glucanase (Wu et al., 2004) and xylanase (Von Wettstein et al., 2003), have been extensively used in poultry and swine diets to improve nutrient digestibility and digesta characteristics. The suggested mechanism of this improvement in barley and wheat based diets are primarily explained by a reduction in digesta viscosity (Scott et al., 1998a; 1998b), a subsequent increase in the efficacy of endogenous enzymes, and greater contact between the nutrients and the brush border of the small intestine (Classen, 1996). Unlike soluble hemicellulose fractions that dissolve to form a gel-like consistency in the lumen of the intestine, insoluble hemicellulose fractions are a structural part of the plant cell wall (Classen, 1996). Consequently, digestion of these structural hemicelluloses allows endogenous enzymes to access substrates that would otherwise be encased in the cell (Classen, 1996). Additional energy may be obtained through absorption of the digested hemicellulose products (Marsman et al., 1997). While intestinal viscosity is not considered a problem in corn soybean meal diets, the addition of fibrolytic enzymes may prove beneficial because of this mechanism.

Inclusion of glucanase in the diet increased IDE values in Experiment 2 and AME in adequate aP diets in Experiment 1. The improvement in IDE suggested that glucanase, in degrading cell wall material in the feed, provided increased access to the starch within

the feed. The lack of a consistent effect on excreta AME between Experiments 1 and 2 was puzzling, and most likely stems from differences in the methodology used. Experiment 1 used a total excreta collection technique to estimate the AME value of the feed, while Experiment 2 used an indigestible marker to estimate the same value. The ileal collection in Experiment 2 made total fecal collection impossible, as the digestive tract was full at the time of ileal sampling. The SEM values shown in Tables 2-2 and 2-3 (Experiment 1) and Table 2-5 (Experiment 2) clearly show that the total collection method is a more sensitive test. However, in order to understand the effects of the colonic and cecal microflora, IDE values must be employed. As shown in Table 4, IDE values are much lower than AME values due in large part to the digestion of fiber by the microbial population of the ceca and large intestine. However, only a portion of the energy liberated by the microbes is actually utilized by the bird, the remainder is used by the microbes for growth and maintenance. The lack of difference in AME value due to glucanase supplementation found in Experiment 2 (Table 4) may be a result of the microflora utilizing the substrates that glucanase digests (ie. fiber) or makes available to the bird (ie. starch within structurally intact cells), thereby reducing the apparent difference in energy utilization.

Figures 2-1 and 2-2 show micrographs of the ileal digesta and excreta collected from Experiment 2 respectively. These figures subjectively support this theory, as the starch content of the ileal contents appears to be reduced with the addition of both phytase and glucanase. The excreta contents, however, have less starch compared to the ileal samples regardless of enzyme supplementation, suggesting the cecal microflora may be involved. As well, the lack of an effect on PE in Experiment 1 (Table 2-2) as a result

of glucanase supplementation may be due to the partial utilization of the products of microbial degradation (e.g. volatile fatty acids) by the bird.

In this study, dietary aP was reduced while Ca was maintained at commercial levels to determine if the addition of phytase to the low aP diet would improve performance and AME to levels comparable to the adequate aP diet. In Experiment 1, aP level affected both excreta AME and PE. While this response agreed with Nelson and Miles (1972) who found an increase in AME with increased aP, it was contrary to other reports that found the opposite effect (Ravindran et al., 2000; 2001) or no effect at all (Wu et al., 2003; Silversides et al., 2004). Sibbald and Price (1977) and Atteh and Leeson (1984) showed that fatty acids, especially those that were saturated, formed insoluble soaps in the small intestine with Ca, rendering both Ca and the fatty acid indigestible thus lowering AME. Ravindran et al. (2000; 2001) reduced Ca in their diets to maintain a normal Ca to P ratio, which they believed increased fatty acid digestibility by decreasing soap formation. In the current study, since dietary Ca level was not changed to maintain the proper Ca to aP ratio, no increase in AME due to fatty acid digestibility was expected. Rather, it was possible that the high Ca to aP ratio in the low aP diets decreased AME. Nelson et al. (1981) showed a decrease in AME of similar degree when the Ca to aP ratio was increased and attributed the response to an excess of cations in proportion to anions in the feed.

Sibbald (1975) showed that feed intake directly influenced the AME estimate of a feedstuff. The explanation was that as feed intake decreased, the amount of obligatory endogenous energy losses as a proportion of the excreta increased. Since AME was calculated by subtracting energy excreted from energy intake, endogenous losses were

not compensated for. The AME estimate from lower feed intakes resulted then in lower AME values. Feed intake in this experiment was significantly lower for birds fed the low aP diet than for those fed the adequate aP diet. While this type of experimentation is important to determine the efficacy of phytase under conditions similar to those used in the poultry industry, the potential interference in AME estimation from either the cation-anion balance of the diet or feed intake in the low aP treatment should be taken into account. Future studies should compensate for the anions removed as P is decreased in the diet to determine the influence of aP itself on AME.

Productive traits measured in these experiments were used to determine if the addition of phytase to a low aP diet would support similar feed intake and BW gain levels as an adequate aP diet. Positive results from Experiment 1 on feed intake and gain suggested that phytase addition to the corn soybean meal diets replaced as much as 0.2 % aP. Supplementation of the adequate aP diet with phytase further increased feed intake in Experiment 1 and feed intake, BW gain, and feed conversion in Experiment 2. Glucanase decreased feed intake, without any affect on BW gain (Experiment 1). The expected decrease in feed conversion approached significance in Experiment 1, and was statistically significant in Experiment 2. The influence of glucanase on productive traits was explained largely by the effect of this enzyme on the AME value of the feed.

The influence of dietary phytase supplementation on the digestibility and retention of minerals has been the focus of much research. The beneficial effects on Ca and P digestibility was well documented (Ketaren et al., 1993; Lei et al., 1993; Ravindran et al., 2000; Rutherford et al., 2002; Wu et al., 2003; Augspurger and Baker, 2004; Dilger et al., 2004; Johnston et al., 2004; Silversides et al., 2004), and were again demonstrated

in the present study. In Experiment 1, phytase improved Ca retention in all diets, P retention in diets deficient in aP, and improved digestibility of aP in Experiment 2. Retention of Ca was also increased by glucanase supplementation in Experiment 1, probably due to the increase in BW gain associated with the enzyme and consequent increase in required bone mass (Haag, 1939). Less consistent results with phytase supplementation have been seen with the other minerals perhaps because of their binding capacity to phytate. For example, Zn was shown to form a very strong bond and insoluble compound with phytin (Prattley et al., 1982). In this experiment, glucanase supplementation increased Zn, Cu and Mg digestibility; however, retention values of these minerals were not affected. This was likely due to the adequate inclusion in the diet of all minerals except aP. Minerals in excess of the animal's requirements are excreted, maintaining the same retention value regardless of digestibility.

The effects of phytase supplementation on amino acid and crude protein digestibility have also been extensively investigated (Prattley et al., 1982; Ravindran et al., 2000; 2001; Rutherford et al., 2002; Wu et al., 2003; Augspurger and Baker, 2004; Dilger et al., 2004; Johnston et al., 2004; Silversides et al., 2004). It was thought that degradation of phytate would improve protein digestion by freeing proteins from the insoluble complex formed with phytin (Prattley et al., 1982). Reports on phytase and amino acid digestibility have varied (Ravindran et al., 2000; 2001; Rutherford et al., 2002; Dilger et al., 2004; Johnston et al., 2004), probably due to the relatively small amount of any single amino acid released for digestion. Crude protein digestibility has been shown to increase more consistently with the use of phytase (Ravindran et al., 2000; 2001; Wu et al., 2003; Silversides et al., 2004). The current trials provide more evidence

that CP digestibility is improved by phytase supplementation. Ileal digestibility values from Experiment 2, and retention values from Experiment 1, show substantially higher nitrogen utilization with phytase-supplemented diets. Neither glucanase supplementation nor aP level affected the utilization of nitrogen.

In summary, the supplementation of phytase to corn soybean meal diets in these experiments improved productive traits and the digestibility of several nutrients, including Ca, P, and CP. The energy value of the feed was not affected by phytase inclusion. Glucanase supplementation increased the digestibility of several minerals, and improved the IDE value of the feed. While there was no synergistic effect of the two enzymes together, there were also no negative interactions. These enzymes can be used together in a product, and are most effective in diets that are formulated to take advantage of the improvements in nutrient availability.

2.5 REFERENCES

Atteh, J. O., and S. Leeson. 1984. Effects of dietary saturated or unsaturated fatty acids and calcium levels on performance and mineral metabolism of broiler chicks. Poult. Sci. 63: 2252-2260.

Augspurger, N. R. and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82: 1100-1107.

Broz, J., P. Oldale, A. H. Perrin-voltz, G. Rychen, J. Schulze, and C. Simoes Nunes. 1994. Effects of supplemental phytase on performance and phosphorous

utilization in broiler chickens fed a low phosphorous diet without addition of inorganic phosphates. Br. Poult. Sci. 35: 273-280.

Classen, H. L. 1996. Cereal grain starch and exogenous enzymes in poultry diets. Anim. Feed Sci. Tech. 62: 21-27.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45: 101-108.

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1993. Efficacy of phytase in improving the bioavailability of phosphorous in soybean meal and corn-soybean meal diets for pigs. J. Anim. Sci. 71: 1831-1840.

Cromwell, G. L., R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1995a. Efficacy of low-activity, microbial phytase in improving the bioavailability of phosphorous in corn-soybean meal diets for pigs. J. Anim. Sci. 73: 449-456.

Cromwell, G. L., R. D. Coffey, G. R. Parker, H. J. Monegue, and J. H. Randolph. 1995b. Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorous in corn-soybean meal diets for pigs. J. Anim. Sci. 73: 2000-2008.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O Adeola. 2004. Evaluation of microbial phytase in broiler diets. Poult. Sci. 83: 962-970.

Frolich,W. 1990. New developments in dietary fibre, Furda, I. and Brine, C. J. (Eds) pp. 83-93 (New York, Plenum press).

Hagg, J. R. 1939. The calcium and phosphorous contents of chickens of various ages. Poult. Sci. 18: 279-281.

Ibrahim, S. J. P. Jacob, and R. Blair. 1999. Phytase supplementation to reduce phosphorous excretion of broilers. J. Appl. Poult. Res. 8:414-425.

Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effects of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. J. Anim. Sci. 82: 705-714.

Juanpere, J., A. M. Perez-vendrell, E. Angulo, and J. Brufau. 2005. Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poult. Sci. 84: 571-580.

Ketaren, P. P., E. S. Batterham, and E. B. Dettmann. 1993. Phosphorus studies in pigs 3. Effect of phytase supplementation on the digestibility and availability of phosphorus in soya-bean meal for grower pigs. Br. J. Nutr. 70: 289-311.

Lei, X. G., P. K. Ku, E. R. Miller, and M. T. Yokoyama. 1993. Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. J. Anim. Sci. 71: 3359-3367.

Marsman, G. J. P., H. Gruppen, A. F. B. Van Der Poel, R. P. Kwakkal, M. W. A. Verstegen, and A. G. J. Voragen. 1997. The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibilities, and chime characteristics in broiler chicks. Poult. Sci. 76: 864-872.

Nelson, T. S., L. K. Kirby, and Z. B. Johnson. 1981 The effects of altering cation-anion content with calcium and phosphorus on the digestion of dry matter and amino acids on energy utilization. Poult. Sci. 60: 786-789.

Nelson, T. S., and R. D. Miles. 1972. Effect of calcium and phosphorous on energy utilization by chicks. Poult. Sci. 51: 1536-1540.

Prattley, C. A., D. W. Stanley, T. K. Smith, and F. R. Van De Voort. 1982. Protein-phytate interaction in soybeans. III The effects of protein-phytate complexes on zinc bioavailability. J. Food Biochem. 6: 273-282.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous: II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41: 193-200.

Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80: 338-344.

Rutherford, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in broiler chicken. Br. Poult. Sci. 44: 598-606.

SAS Institute Inc., 2001. The SAS System for Windows, Release 8.02. SAS Institute Inc., Cary, NC, 27315.

Scott, T. A., F. G. Silversides, H. L. Classen, M. L. Swift, M. R. Bedford. 1998a. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. Poult. Sci. 77: 456-463.

Scott, T. A., F. G. Silversides, H. L. Classen, M. L. Swift, M. R. Bedford, and J. W. Hall. 1998b. A broiler chick bioassay for measuring the feeding value of wheat and barley in complete diets. Poult. Sci. 77: 449-455.

Sibbald, I. R., and K. Price. 1977. The effects of level of dietary inclusion and of calcium on the true metabolizable energy values of fats. Poult. Sci. 56: 2070-2078.

Sibbald, I. R. 1975. The effect of level of feed intake on metabolizable energy values measured with adult roosters. Poult. Sci. 54: 1990-1997.

Silversides, F. G., T. A. Scott, and M. R. Bedford. 2004. The effect of phytase enzyme and level on nutrient extraction by broilers. Poult. Sci. 83: 985-989.

Von Wettstein, D., J. Warner, and C. G. Kannangara. 2003. Supplementation of transgenic malt of grain containing (1,3-1,4)-beta-glucanase increase the nutritive value of barley-base broiler diets to that of maize. Br. Poult. Sci. 44: 438-449.

Wu, Y. B., V. Ravindran, and W. H. Hendriks. 2003. Effects of microbial phytase, produced by solid-state fermentation, on the performance and nutrient utilization of broilers fed maize- and wheat-based diest. Br. Pout. Sci. 44: 710-718.

Wu., Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. Influence of phytase and xylanase, individually or in combination,on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate levels of phosphorous. Br. Poult. Sci. 45: 76-84.

TABLE 2-1. Ingredient and calculated nutrient compositions of diets 1 through 8 in Experiment 1 and 1 through 4 in Experiment 2 fed to broiler chicks from 5 to 10 d of age

	Diet							
Ingredient	1	2	3	4	5	6	7	8
	% of diet as-fed							
Ground Yellow Corn	49.55	49.55	49.55	49.55	50.00	50.00	50.00	50.00
Soybean Meal (48% CP)	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Poultry Fat	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	1.60	1.60	1.60	1.60	2.05	2.05	2.05	2.05
Dicalium Phosphate (21.5% Ca, 18.5% P)	1.50	1.50	1.50	1.50	0.60	0.60	0.60	0.60
Vit/Min premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Celite	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Phytase[2]	0	500	0	500	0	500	0	500
Glucanase[2]	0	0	50	50	0	0	50	50
Calculated composition								
Metabolizable energy, cal/g	3,026	3,026	3,026	3,026	3,026	3,026	3,026	3,026
Crude Protein, %	23.30	23.30	23.30	23.30	23.30	23.30	23.30	23.30
Calcium, %	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Available Phosphorus, %	0.45	0.45	0.45	0.45	0.26	0.26	0.26	0.26
Phytate P, % (analyzed)	0.23	0.21	0.19	0.28	0.25	0.15	0.31	0.27

[1] The vitamin and mineral premix provided the following per kg of diet: 7500 IU Vitamin A, 2500 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 37 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.1 mg biotin, 500 mg choline, 125 mg ethoxyquin, 66 mg Mn, 55 mg Zn, 6 mg Fe, 6 mg Cu, 0.15 mg Se, 1 mg I.

[2] Ingredients expressed as units of activity per kg diet on an “as is” basis. Supplied by Syngenta Animal Nutrition.

TABLE 2-2. Experiment 1: Effect of phytase and glucanase on broiler feed intake, body weight gain, feed conversion, AME, productive energy, and dry matter digestibility from 5 to 10 d of age

Treatment	Feed intake (g)	Gain (g)	Feed conversion (g feed/g gain)	AME[1] (cal/g feed)	Productive energy (cal/g feed)	DM digestibility (%)
Phytase[2]						
0	2015[b]	1374[b]	1.47	2885	1067	72.0[b]
500	2087[a]	1439[a]	1.46	2935	1087	74.4[a]
Glucanase[2]						
0	2074[a]	1410	1.49	2906	1061	73.7
50	2028[b]	1412	1.44	2913	1093	73.7
SEM	13.5	17.1	0.018	21.0	13.8	0.54
Probability						
Phytase (Phy)	0.0006	0.01	NS	NS	NS	0.003
Glucanase (Glu)	0.02	NS	0.06	NS	NS	NS
Phy × Glu	NS	NS	NS	NS	NS	NS

[1] Apparent metabolizable energy (AME) as determined by total excreta collection.
[2]Enzyme treatments are expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 2-3. Experiment 1: Effect of phosphorous level and phytase or glucanase on broiler feed intake, body weight gain, feed conversion, AME, productive energy and dry matter digestibility from 5 to 10 d of age

Treatment		Feed intake (g)	Gain (g)	Feed conversion (g feed/g gain)	AME[1] (cal/g feed)	Productive energy (cal/g feed)	DM digestibility (%)
aP Level							
0.26 %		1985[b]	1363[b]	1.46	2854[b]	1050[b]	72.63
0.45 %		2117[a]	1450[a]	1.47	2966[a]	1105[a]	73.70
SEM		13.5	17.1	0.018	21.0	13.8	0.540
aP Level	Phytase[2]						
0.26 %	0	1923[c]	1308[b]	1.47	2852	1040	71.57
	500	2048[b]	1418[a]	1.45	2855	1059	73.69
0.45 %	0	2108[ab]	1440[a]	1.47	2917	1094	72.37
	500	2126[a]	1460[a]	1.46	3015	1116	75.02
aP Level	Glucanase[2]						
0.26 %	0	2023	1375	1.48	2903[b]	1041	72.61
	50	1948	1352	1.44	2804[b]	1059	72.63
0.45 %	0	2125	1427	1.49	2910[b]	1082	72.71
	50	2109	1473	1.44	3022[a]	1127	74.68
SEM		19.1	24.2	0.025	29.8	19.6	0.763
Probability							
aP Level		0.0001	0.001	NS	0.0005	0.008	NS
aP × Phytase (Phy)		0.008	0.06	NS	NS	NS	NS
aP × Glucanase (Glu)		NS	NS	NS	0.01	NS	NS
aP × Phy × Glu		NS	NS	NS	NS	NS	NS

[1] AME as determined by total excreta collection.
[2]Enzyme treatments are expressed as units of activity per kg of feed.
[a-c]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 2-4. Experiment 1: Mineral retention calculated from carcass composition data and crude protein digestibility.

Treatment		Ca	P	Zn	Cu	Mg	Fe	CP
		---------- % retained ----------						
aP Level								
0.26 %		20.9	46.6	18.1	9.8	11.7	16.0	76.6
0.45 %		20.2	42.2	13.7	9.4	11.2	16.2	76.2
Phytase[1]								
0		17.1[b]	39.6[b]	17.5	10.2	12.0	10.9	74.9[b]
500		24.1[a]	49.2[a]	14.3	9.0	11.0	11.4	77.9[a]
Glucanase[1]								
0		17.9[b]	43.5	13.2	7.6	10.5	11.4	75.6
50		23.3[a]	45.3	18.7	11.5	12.5	20.9	77.2
SEM		1.52	2.21	3.82	3.19	2.11	4.08	0.80
aP Level	Phytase							
0.26 %	0	15.7	35.5 b	20.9	11.1	12.6	20.5	74.6
	500	26.2	57.6 a	15.2	8.4	10.9	11.5	78.6
0.45 %	0	18.4	43.6 b	14.0	9.2	11.3	21.2	75.2
	500	22.1	40.8 b	13.5	9.6	11.1	11.2	77.2
aP Level	Glucanase							
0.26 %	0	18.4	47.4	12.9	4.4	9.2	11.1	76.8
	50	23.5	45.7	23.3	15.2	14.3	21.0	76.4
0.45 %	0	17.4	39.5	13.5	10.9	11.8	11.7	74.4
	50	23.1	44.9	14.0	7.91	10.6	20.8	77.9
Phytase	Glucanase							
0	0	15.9	41.5	12.5	7.3	9.6	13.3	74.0
	50	18.3	37.6	22.5	7.3	14.3	28.5	77.2
500	0	19.9	45.5	13.9	7.9	11.3	9.5	75.8
	50	28.3	52.9	14.8	10.1	10.6	13.3	78.6
SEM		2.15	3.13	5.40	4.51	2.99	5.77	1.13
Probability								
aP Level		NS	NS	NS	NS	NS	NS	NS
Phytase (Phy)		0.002	0.004	NS	NS	NS	NS	0.01
Glucanase (Glu)		0.02	NS	NS	NS	NS	NS	NS
aP × Phy		NS	0.0003	NS	NS	NS	NS	NS
aP × Glu		NS	NS	NS	NS	NS	NS	NS
Phy × Glu		NS	0.08	NS	NS	NS	NS	NS

[1]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 2-5. Experiment 2: Effect of phytase and glucanase on broiler feed intake, body weight gain, feed conversion and DM digestibility of diets with adequate available phosphorous from 5 to 10 d of age

Treatment	Feed intake (g)	Gain (g)	Feed conversion	DM digestibility (%)	Ileal Digestible Energy[1] (cal/g feed)	AME[1] (cal/g feed)
Phytase[2]						
0	1858[b]	1475[b]	1.28[a]	73.2	2536	2958
500	1939[a]	1572[a]	1.23[b]	75.2	2590	2992
Glucanase[2]						
0	1914	1504	1.27[a]	72.5	2419[b]	2896
50	1883	1543	1.23[b]	75.1	2707[a]	3054
SEM	25.16	16.83	0.008	1.87	63.0	66.2
Probability						
Phytase (Phy)	0.04	0.0005	0.002	NS	NS	NS
Glucanase (Glu)	NS	NS	0.003	NS	0.004	NS
Phy × Glu	NS	NS	NS	NS	NS	NS

[1]AME and IDE as determined by acid insoluble ash marker.
[2]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 2-6. Experiment 2: Ileal digestibility values for minerals and CP

Treatment	Ca	P	Zn	Cu	Mg	Fe	CP
	---------- % ----------						
Phytase[1]							
0	46.5	13.5[b]	20.1	60.2	(6.7)[2]	(23.8)	70.8[b]
500	37.9	31.5[a]	24.0	49.5	6.0	(12.5)	76.3[a]
Glucanase[1]							
0	38.7	18.7	13.0[b]	44.3[b]	(10.4)[b]	(32.2)	72.5
50	45.7	26.3	31.1[a]	65.3[a]	9.7[a]	(7.5)	74.7
SEM	3.49	4.55	5.68	4.45	5.82	9.44	1.94
Probability							
Phytase (Phy)	NS	0.01	NS	NS	NS	NS	0.05
Glucanase (Glu)	NS	NS	0.03	0.03	0.02	0.07	NS
Phy × Glu	NS	NS	NS	NS	NS	NS	NS

[1]Enzyme treatments expressed as units of activity per kg of feed.
[2]Brackets indicate negative values.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 2-7. Experiment 2: Mineral and CP retention values calculated from feed and excreta composition using an acid insoluble ash marker

Treatment	Ca	P	Zn	Cu	Mg	Fe	CP
	---------- % ----------						
Phytase[1]							
0	30.2	24.3[b]	(35.7)[2]	(7.9)	(16.2)	(57.2)[b]	68.2
500	41.9	42.2[a]	(12.4)	(4.1)	(2.2)	(14.3)[a]	66.8
Glucanase[1]							
0	36.8	33.3	(27.6)	(8.4)	(12.5)	(41.3)	66.2
50	35.4	33.1	(20.6)	(3.6)	(5.9)	(30.3)	68.8
SEM	5.42	5.54	10.69	8.95	8.24	12.41	2.02
Probability							
Phytase (Phy)	NS	0.03	NS	NS	NS	0.02	NS
Glucanase (Glu)	NS	NS	NS	NS	NS	NS	NS
Phy × Glu	NS	NS	NS	NS	NS	NS	NS

[1]Enzyme treatments expressed as units of activity per kg of feed.
[2]Brackets indicate negative values.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

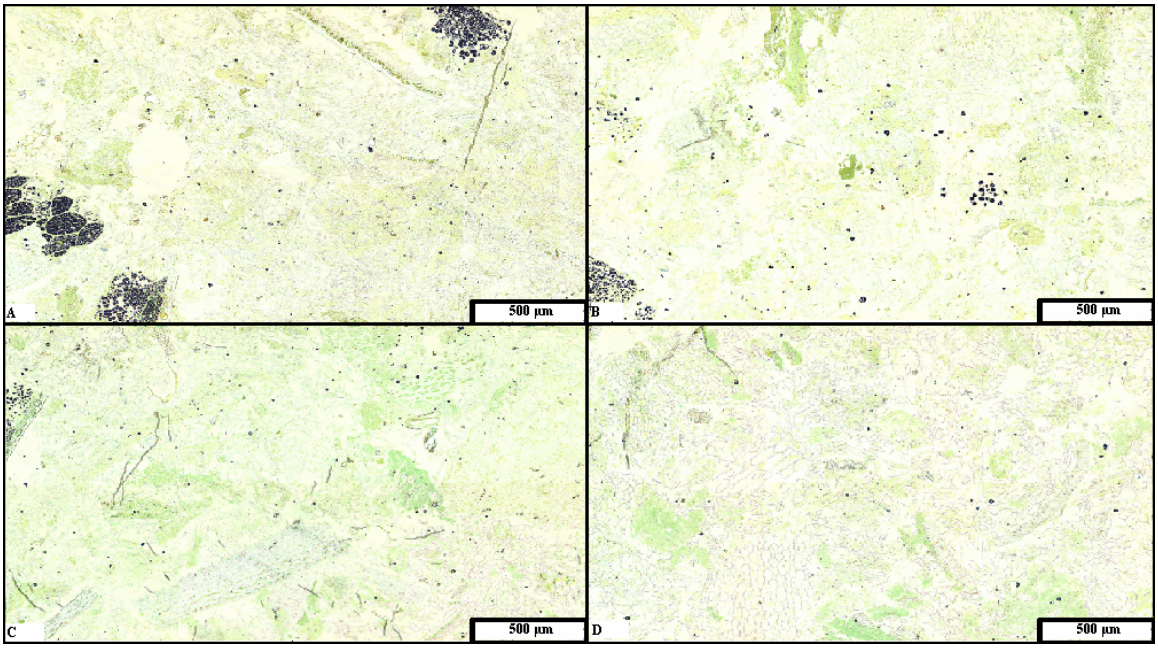

FIGURE 2-1. The ileal digesta of broilers fed corn soybean meal without supplementary enzymes (A), or supplemented with 500 FTU (B), 500 units glucanase (C) or 500 FTU plus 50 units glucanase. Staining was performed with light green and iodine in order to extenuate undigested starch granules (purple) and protein (dark green). In the photomicrographa from the unsupplemented and phytase supplemented treatments (A and B), there appears to be more undigested starch in the ileal digesta than there is in the treatments that received glucanase.

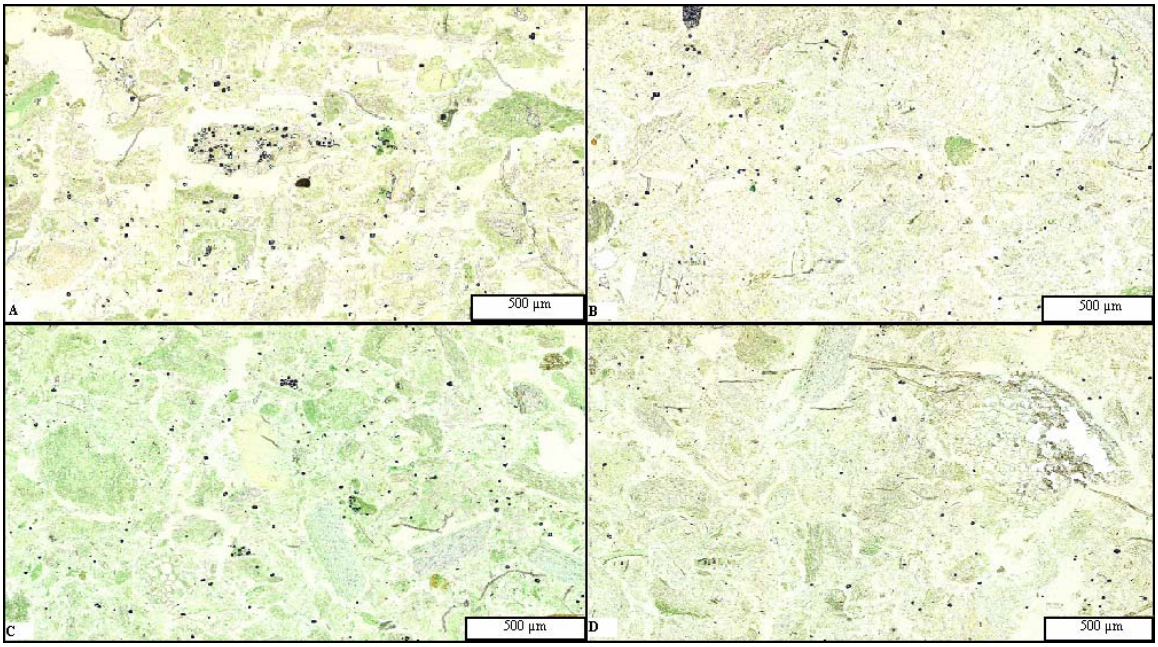

FIGURE 2-2: The excreta of broilers fed corn soybean meal without supplementary enzymes (A), or supplemented with 500 FTU (B), 500 units glucanase (C) or 500 FTU plus 50 units glucanase. Staining was performed with light green and iodine in order to extenuate undigested starch granules (purple) and protein (green). The photomicrographs show small amounts of undigested starch in the excreta from all treatments, with no large differences apparent between treatments.

3.0 THE EFFECT OF PHYTASE AND GLUCANASE ON THE ILEAL DIGESTIBLE ENERGY OF CORN AND SOYBEAN MEAL FED TO BROILERS

3.1 INTRODUCTION

Exogenous enzymes are added to poultry diets in order to manipulate conditions in the digestive tract and improve the nutrient value of feedstuffs (Meng et al., 2005; Classen, 1996). Numerous studies on the effects of phytase show that the enzyme increases phosphorus availability by hydrolyzing phytate and increases mineral and protein solubility, thus improving protein digestibility (Shirley and Edwards, 2003; Igbasan et al., 2001; Onyango et al., 2005; Sebastian et al., 1996; Kies et al., 2001). In wheat and barley diets, phytate hydrolysis also increases AME values (Kies et al., 2001; Ravindran et al., 2000; 2001). Fibrolytic enzymes have been used extensively in wheat and barley based diets, in order to reduce viscosity in the small intestine through the cleavage of soluble NSPs. Additionally, these enzymes degrade cell walls and increase digestibility and absorption of sugars from hemicellulose (Meng et al., 2005). In doing so, substrates (i.e. starch) within cell walls become available for degradation by endogenous enzymes (Classen, 1996). To date, research on these enzymes has not extensively examined their effect on corn or soybean meal individually. Moreover, the potential for different responses based upon the age and physiological development of the digestive tract is often ignored.

The feeding value of commercially available enzymes is often based upon young chicks less than 2 wk of age (Murakami et al., 1994; 1995; Meng and Slominski, 2005) or prime-age roosters (Yaghobfar and Boldaji, 2003). In newly-hatched chicks, the enterocyte is poorly developed, limiting the bird's abilities in digestion and absorption (Iji et al., 2001a; 2001b; 2001c). During this maturation period, the gut lacks the competency to fully digest feedstuffs and absorb smaller molecules, due to a lack of brush-border enzymes, inadequate maintenance of absorptive mechanisms, and low surface area due to immature villus height (Van Leeuwen et al., 2004). As the GI tract develops, it is able to take advantage of fibrolytic enzyme effects. Prior to this however, the pancreatic enzymes needed to initiate digestion in the intestinal lumen are limited in both volume and activity (Noy and Sklan, 1995). Thus, they may be unable to utilize substrates made available by a fibrolytic enzyme. In addition, phytate has been shown to reduce both amylase and trypsin activity in vitro (Deshpande and Cheryan, 1984; Thompson and Yoon, 1984; Knuckles and Betschart, 1987). In vivo studies have not yet been performed.

The objectives of this experiment were to determine the influence of phytase and glucanase on the energy digestibility of corn and soybean meal independently and to investigate the effect of age on the response to these enzymes. To eliminate the influence of cecal microflora, ileal digestibility was used to quantify energy availability. As an indirect test of phytate effect on endogenous enzyme activity, digesta and pancreatic enzyme levels were also determined.

3.2 MATERIALS AND METHODS

Experimental Design

All procedures were reviewed and approved by the Auburn University Institutional Animal Care and Use Committee. A population of day-old Ross x Ross 308 broiler chicks were obtained from a commercial hatchery. The chicks were housed in battery cages in three rooms (48 cages per room) and provided a standard corn-soy diet and water *ad libitum*. Each room represented a different age at which the experiment would start, with chicks in one room fed the experimental rations from 7 to 9 days, another from 14 to 16 days and the third from 21 to 23 days. All birds received a commercial-type complete starter diet (3050 kcal/kg ME, 23% CP, 1.00% Ca, 0.45% aP) until the initiation of the experiment.

The experimental diets consisted of either corn or soybean meal, supplemented with 0 or 500 units of phytase (FTU), and 0 or 500 units of glucanase per kg of diet. Each feedstuff was mixed with Celite,® an acid insoluble ash marker (AIA), pelleted and crumbled. Enzymes were added post-pelleting to prevent pelleting losses that could interfere with the experiment.

The procedure performed was the same for each of the age ranges tested. Eight hours prior to the onset of each experimental period, birds were weighed and feed was withdrawn. Birds were then fed the experimental diets for 48 h, weighed, and euthanized via asphyxiation with CO_2 gas. The pancreas and the contents of the duodenum and jejunum (pooled) and the ileum were removed and immediately frozen at –20 °C for subsequent analysis. Duodenum-jejunum and pancreas samples were homogenized in phosphate buffer and centrifuged at 3,000 rpm for enzymatic analysis. Ileal samples

were freeze-dried, ground, and analyzed for acid insoluble ash (AIA) content (Scott and Hall, 1998) and gross energy content[1].

Enzyme Analysis

Pancreatic samples and duodenal-jejunal contents were analyzed for amylase activity (Rick and Stegbauer, 1974), proteolytic activity (Rick, 1974), and protein content via the Coomassie dye binding procedure[3]. Briefly, to determine amylase activity, 3 µl of homogenate was combined in a test tube with phosphate buffer (pH 6.9) and a solution of potato starch (1% starch w/v), and incubated at 35°C for 10 min. A solution containing 3,5-dinitrosalysilic acid was added to the test tube and incubated again at 100°C for 5 min to stop the reaction. After the samples cooled, the absorbance was read using a UV-1601[2] spectrophotometer at 246 nm and compared against a maltose standard curve.

Proteolytic activity was determined using casein as a substrate (Rick, 1974). Three microliters of homogenate was incubated in a phosphate buffer (pH 7.6) with 1 ml of 0.5% casein and incubated at 35°C for 10 min. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid to precipitate all protein in the solution. Samples were centrifuged for 10 min at 13,000 rpm. The supernatant was removed and absorbance read on a spectrophotometer at 540 nm. Samples were compared against a standard curve generated using porcine trypsin of known activity.

The amount of protein in the homogenate was determined using the Coomassie dye-binding procedure[3]. Briefly, 3 µl of homogenate was mixed with coomassie dye,

[1] Parr 6300 Calorimeter, Parr Instrument Company, Moline, IL, USA
[2] Model UV-1601 UV-Visible Spectrophotometer, Shimadzu Corp, Kyoto, Japan.
[3] Coomassie Bradford Protein Assay Kit, Pierce, 3747 N. Meridian Road, Rockford IL, 61105.

allowed to equilibrate, and read on a spectrophotometer at an absorbance of 595 nm. The samples were compared against a known standard of bovine albumen. Amylase and proteolytic activities were then expressed as units of activity per mg of protein in the sample.

Statistical Analysis

The experiment was a 2 × 2 × 2 factorial with feedstuff, phytase level, and glucanase levels as the main effects. Birds were placed 10 per cage, with 6 replicates of each treatment described above. All measurements were taken using the pen as the experimental unit. Data were analyzed by three-way analysis of variance (ANOVA) using the General Linear Models procedure of SAS® (SAS Institute, 2001). Tukey's Honestly Significant Difference was used to separate treatment means at $P < 0.05$.

3.3 RESULTS AND DISCUSSION

Diets used in these experiments were not supplemented with calcium, phosphorus, micro-minerals or vitamins, and were not balanced for protein or energy. Therefore, interpretation of the live performance data was of somewhat limited value. The assumption was that the short timeline of the trial would prevent any symptoms of deficiency from interfering with the results (Sullivan et al., 1974; Ceccarelli et al., 1975; Barrett and Keely, 2000). Minerals essential to enzyme function (e.g. calcium and chloride for amylase activity) and the digestive tract (e.g. sodium for active transport) were believed to be of sufficient quantity both in the feedstuffs and endogenously, to maintain normal enzyme and absorptive function (Sullivan et al., 1974; Ceccarelli et al.,

1975; Barrett and Keely, 2000). Since all diets were deficient in either energy or protein, feed intake data was useful in determining the effect of an energy or protein imbalance on enzyme activity (Swennen et al., 2004).

Neither phytase nor glucanase significantly affected any live performance parameter (Table 3-1). Essential amino acid deficiencies likely increased feed intake and feed conversion in corn versus SBM at all three ages tested, and lowered BW gain at 7 to 9 and 14 to 16 d of age. The effect of phytase and feedstuffs on live performance was not consistent. The addition of phytase to SBM decreased feed intake at 7 to 9 d of age compared to SBM alone with no subsequent effect at other ages. Phytase lowered feed conversion of birds fed soybean meal from 14 to 16 d of age. Phytase can improve protein digestibility (Knuckles and Betschart, 1987; Camden et al., 2001), and since SBM is a high protein feedstuff, an increase in protein digestibility would likely reduce feed intake because it creates a greater imbalance between energy and protein (Swennen et al., 2004; Rosebrough and Steele 1985; Rosebrough et al., 1996). However, an increase in protein digestibility of SBM should have increased feed conversion because of the metabolic cost of catabolizing and excreting the excess nitrogen (Swennen et al., 2004). Alternatively, corn supplemented with phytase increased feed intake at 14 to 16 d of age with no differences at the other two ages tested. The converse occurs with corn, more protein digestibility would increase feed intake because of an improvement in the energy to protein ratio (Swennen et al., 2004).

Glucanase decreased feed intake in birds fed corn compared to SBM alone, and reduced the feed conversion of birds fed SBM from 21 to 23 d of age. Glucanase supplementation is thought to increase energy availability by liberating substrates like

starch obstructed by fibrous structures and utilizing otherwise indigestible carbohydrates (Classen, 1996). Therefore, glucanase may have exacerbated the energy to protein ratio in corn causing a decrease in feed intake, while improving that ratio in SBM thus lowering feed conversion.

The digestible energy and DM digestibility of corn and SBM are presented in Tables 2 and 3, respectively. From 7 to 9 and 14 to 16 d of age, neither feedstuff was influenced by phytase supplementation. These results agree with previous experiments that show phytase does not affect AME in corn soybean meal diets (Chapter 2). Between 21 and 23 d of age the DM digestibility of corn was 78.5%, with phytase it increased to 79.3%. These results agree with those found in wheat-based, but not corn-based diets. In wheat-based diets, phytate is thought to be integrated into the cell wall (Frolich, 1990), and as it is degraded, holes are left in that wall through which endogenous enzymes can enter (Classen, 1996). This results in degradation of encapsulated substrates, improving DM digestibility and AME. In corn, there is no evidence that phytate is incorporated into cell walls, and no similar effect would be expected.

Glucanase increased both the IDE and the DM digestibility of both corn and IDE of soybean meal at all three ages tested (Tables 3-2 and 3-3). The degree of improvement in IDE due to glucanase supplementation was similar across all age groups, and similar between feedstuffs. An increase in the IDE value of corn with increasing age was also seen, while no comparable increase was noted for soybean meal. The immature gastrointestinal tract of young chickens is characterized by a small and unstable microbial population and low endogenous enzyme activity (Iji et al., 2001a). As the digestive tract

develops, endogenous enzyme activity increases (Noy and Sklan, 1995). However, fiber digestion remains insubstantial as a stable microflora is yet to be established (Josefiak et al., 2004). As the chicken is unable to digest and utilize many of the complex carbohydrates found in soybean meal, their ability to utilize energy from the feedstuff may increase with a stable microflora while enzyme activities have little influence. As the measurements discussed here were taken in the ileum, the largest portion of the microfloral influence was omitted. However, microbial populations in the crop and small intestine may contribute to fiber digestion in older birds. The main source of energy in corn is from starch, so increases in amylase activity are likely responsible for the increase in IDE over time.

The effect of glucanase on the IDE value of corn was likely due to an increase in amylase access to starch granules within the cells of the endosperm. Fibrolytic enzymes are thought to degrade portions of the cell wall, allowing endogenous enzymes to have access to the cell contents. The mode of action of glucanase on soybean meal is less obvious due to differences in fiber structure and starch content of the feedstuff. The glucanase used in this experiment has a low substrate specificity, and may contribute to the degradation of hemicelluloses other than glucans.

Amylase and proteolytic activities in the pancreas and digesta were examined because in vitro studies suggested that phytate inhibited both amylase and trypsin (Deshpande and Cheryan, 1984; Thompson and Yoon, 1984; Knuckles and Betschart, 1987; Singh and Kirkorian, 1982). Two mechanisms are thought to be involved in the inhibition of amylase. As calcium is required for the proper conformation and proteolytic inhibition of amylase, chelation of calcium ions by the phytate molecule may reduce

activity by making the molecule susceptible to degradation. Another potential mechanism suggested by Deshpande and Cheryan (1984) and Knuckles and Betchart (1987) is that a calcium-amylase-phytate complex may form that reduces amylase solubility and decreases the enzymes activity. The mechanism by which proteolytic activity is inhibited is unknown, although it may involve precipitation of the enzyme with phytate in the small intestine's less acidic environment. Alternately, phytate may bind to the lysine and arginine R-groups that are targeted by trypsin, reducing the ability of the enzyme to bind to its substrate.

In the current study, if the presence of phytate in the intestinal lumen reduced amylase or trypsin activity, the hypothesized result was decreased activity in the digesta coinciding with increased pancreatic activity to compensate (Simoes-Nunes and Corring, 1979; Nitsan and Madar, 1978). The supplementation of phytase would be expected to alleviate these responses. As the activity of both enzymes were measured in vitro using a substrate that was not exposed to phytate in the digestive tract, this experiment would only detect differences if the phytate molecule interfered directly with the enzyme.

Results for corn diets showed that phytase had no effect on the activity of either enzyme in the digesta or pancreas (Tables 3-4 and 3-5), suggesting that any effect phytate may have on amylase or proteolytic enzymes is indirect, through interference with the substrate. In SBM diets, phytase supplementation increased the activity of pancreatic proteases at 7-9 and 14-16 days (Table 3-6), and the activity of digesta amylase at 21-23 days (Table 3-7). The data also showed that the activity of those enzymes was highly variable, as the SEM values were as high as 20% of the mean. Pancreatic enzyme

activity was particularly variable. Comparisons in activity between corn and SBM diets were not made because the activities were expressed on a unit protein basis.

The aim of this study was to determine the effect of phytase and glucanase on corn and soybean meal separately. While there was no effect of phytase on digestible energy, glucanase improved the energy value of both corn and SBM at all ages. Corn's response to glucanase supplementation was highest between 14 and 16 d of age, while the greatest response with SBM was seen at 21 to 23 d of age. These data suggests that the age related competency of the digestive tract must be taken into account when assessing the energy value of fibrolytic enzymes in poultry feed.

3.4 REFERENCES

Barrett, K. E., and S. J. Kelly. 2000. Chloride secretion by the intestinal epithelium: molecular basis and regulatory aspects. Annu. Rev. Physiol. 62: 535-572.

Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedfors. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. Anim. Sci. 73: 289-297.

Ceccarelli, B., F. Clemente, and J. Meldolesi. 1975. Secretion of calcium in pancreatic juice. J. Physiol. 245: 617-638.

Classen, H. L. 1996. Cereal grain starch and exogenous enzymes in poultry diets. Anim. Feed Sci. Tech. 62: 21-27.

Deshpande, S. S., and M. Cheryan. 1984. Effects of phytic acid, divalent cations, and their interactions on α-amylase activity. J. Food Sci. 49: 516-524.

Frolich, W. 1990. Chelating properties of dietary fiber and phytate: the role for mineral availability. Pages 83-93 in New developments in dietary fibre. I. Furda & C. J. Brine (Eds) . Plenum Press, New York.

Igbasan, F. A., O. Simon, G. Miksch, and K. Manner. 2001. The effectiveness of an Escherichia coli phytase in improving phosphorus and calcium bioavailabilities in poultry and young pigs. Arch. Tierernahr. 54: 117-126.

Iji, P. A., A. Saki, and D. R. Tivey. 2001a. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci. 42: 505-513.

Iji, P. A., A. Saki, and D. R. Tivey. 2001b. Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. Br. Poult. Sci. 42: 514-522.

Iji, P. A., A. Saki, and D. R. Tivey. 2001c. Body and intestinal growth of broiler chicks on a commercial starter diet. 3. Development and characteristics of tryptophan transport. Br. Poult. Sci. 42: 523-529.

Josefiak, D., A. Rutkowski, and S. A. Martin. 2004. Carbohydrate fermentation in the avian ceca: a review. Anim. Feed Sci. Tech. 113: 1-15.

Kies, A. K., K. H. F. Van Hemert, and W. C. Sauer. 2001. Effect of phytase on protein and amino acid digestibility and energy utilization. World Poult. Sci. J. 57: 109-125.

Knuckles, B. E., and A. A. Betschart. 1987. Effect of phytate and other myo-inositol phosphate esters on α-amylase digestion of starch. J. Food Sci. 52: 719-724.

Meng, X., and B. A. Slominski. 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poult. Sci. 84: 1242-1251.

Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell, and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poult. Sci. 84: 37-47.

Murakami, H., Y. Akiba, and M. Horiguchi. 1994. A modified bioassay for energy utilization in newly hatched chicks. 1. Determination of optimum durations for feed withdrawal and excreta collection. Poult. Sci. 73: 1094-1106.

Murakami, H., Y. Akiba, and M. Horiguchi. 1995. A modified bioassay for energy utilization in newly hatched chicks. 2. Determination of feed input and procedures to estimate endogenous loss. Poult. Sci. 74: 343-351.

Nitsan, Z., and Z. Madar. 1978. The level and origin of amylase (EC 3.2.1.1) in the digestive tract of chicks receiving trypsin inhibitors in their diet. Br. J. Nutr. 40: 235-242.

Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. Poult. Sci. 74: 366-373.

Onyango, I. G., M. R. Bedford, and O Adeola. 2005. Efficacy of an evolved Escherichia coli phytase in diets of broiler chicks. Poult Sci. 84: 248-255.

Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy,

and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80: 338-344.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41: 193-200.

Rick, W. 1974. Trypsin: Measurment using Casein as a substrate. Pages 1013 to 1024 in Methods of Enzymatic Analysis, 2nd ed., Vol 2. H. U. Bergmeyer, ed. Weinham, Fla, USA.

Rick, W., and H. P. Stegbauer. 1974. Measurement of reducing groups. Pages 885-889 in Methods of Enzymatic Analysis, 2nd ed.,Vol 2. H. U. Bergmeyer, Ed. Academic Press, New York.

Roseborough, R. W., and N. C. Steele. 1985. Energy and protein relationships in the broiler. 1. Effect of protein levels and feeding regimens on growth, body composition, and in vitro lipogenesis of broiler chicks. Poult. Sci. 64: 119-126.

Roseborough, R. W., A. D. Mitchell, and J. P. McMertry. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin-like growth factor I concentrations in broiler chickens. J. Nutr. 126: 2888-2898.

SAS Institute Inc., 2001. The SAS System for Windows, Release 8.02. SAS Institute Inc., Cary, NC, 27315.

Scott, T. A., and J. W. Hall. 1998. Using acid insoluble ash marker ratios (diet:digesta) to predict digestibility of wheat and barley metabolizable energy and nitrogen retention in broiler chicks. Poult. Sci. 77: 674-679.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. Efficacy of supplemental microbial phytase at different calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 75: 1516-1523.

Shirley, R. B., and H. M. Edwards Jr. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci. 82: 671-680.

Simoes-Nunes, C., and T. Corring. 1979. Pancreatic exocrine secretion in the pig following test meals of different composition and intra-duodenal loads of glucose and maltose. Horm. Metab. Res. 5: 323-378.

Singh, M., and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30:799-800.

Sullivan, J. F., R. E. Burch, and D. F. Magee. 1974. Enzymatic activity and divalent cation content of pancreatic juice. Am. J. Physiol. 226: 1420-1423.

Swennen, Q., G. P. J. Janssens, E. Decuypere, and J. Buyse. 2004. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: energy and protein metabolism and diet-induced thermogenesis. Poult. Sci. 83: 1997-2004.

Thompson, L. U., and J. H. Yoon. 1984. Starch digestibility as affected by polyphenols and phytic acid. J. Food Sci. 49: 1228-1229.

Van Leeuwen, P., J. M. Mouwen, J. D. van der Klis, and M. W. Verstegen. 2004. Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. Br. Poult. Sci. 45: 41-48.

Yaghobfar, A., and F Boldaji. 2002. Influence of level of feed input and procedure on metabolisable energy and endogenous energy loss (EEL) with adult cockerels. Br. Poult. Sci. 43: 696-704.

TABLE 3-1. Body weight (BW) gain, feed intake and feed to gain ratio of broilers fed diets composed of either corn or soybean meal supplemented with phytase and glucanase

		7 to 9 d of age			14 to 16 d of age			21 to 23 d of age		
Treatment		BW Gain (g)	Feed intake (g)	F:G	BW Gain (g)	Feed intake (g)	F:G	BW Gain (g)	Feed intake (g)	F:G
Phytase[1]										
0		12	53	6.22	18	105	6.06	23	174	12.46
500		11	51	5.97	19	108	5.96	27	177	10.45
Glucanase[1]										
0		12	52	5.71	19	105	5.97	28	179	11.35
500		10	52	6.49	19	108	6.05	23	172	11.56
Grain										
Corn		8[b]	55[a]	8.79[a]	17[b]	118[a]	7.16[a]	30	195[a]	14.03[a]
SBM		15[a]	49[b]	3.40[b]	20[a]	94[b]	4.85[b]	20	155[b]	8.88[b]
SEM		0.8	0.80	0.683	0.9	1.7	0.245	5.6	2.9	1.910
Phytase	Glucanase									
0	0	10	51	6.03	20	107	5.95	27	173	13.26
0	500	12	51	6.41	19	108	5.96	27	180	11.66
500	0	11	52	5.39	18	102	6.14	18	170	9.43
500	500	12	53	6.56	19	107	5.98	28	177	11.46
Phytase	Grain									
0	Corn	8	54[a]	8.59	18	113[b]	6.82[a]	29	193	14.78
0	SBM	15	51[c]	3.85	19	97[c]	5.30[b]	17	154	10.14
500	Corn	7	56[a]	8.99	17	123[a]	7.51[a]	31	198	13.27
500	SBM	15	47[d]	2.96	22	97[c]	4.41[c]	23	155	7.62
Glucanase	Grain									
0	Corn	8	55	8.16	18	118	6.88	40	204[a]	11.20[ab]
0	SBM	16	50	3.27	20	98	5.06	15	153[c]	11.50[ab]
500	Corn	7	55	9.43	17	119	7.45	20	187[b]	16.86[a]
500	SBM	13	48	3.54	21	91	4.64	25	156[c]	6.26[b]
SEM		1.1	1.1	0.990	1.2	2.5	0.346	8.0	4.1	2.841
Probability										
Phytase (Phy)		NS	NS	NS	NS	NS	NS	NS	NS	NS
Glucanase (Glu)		0.08	NS	NS	NS	NS	NS	NS	0.09	NS
Grain (Gr)		0.0001	0.0001	0.0001	0.02	0.0001	0.0001	NS	0.0001	0.05
Phy × Glu		NS	NS	NS	NS	NS	NS	NS	NS	NS
Phy × Gr		NS	0.01	NS	0.09	0.004	0.03	NS	NS	NS
Glu × Gr		NS	NS	NS	NS	NS	NS	0.07	0.03	0.04
Phy × Glu × Gr		NS	NS	NS	0.04	0.009	NS	NS	0.02	NS

[1]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 3-2. Ileal digestible energy (IDE) and DM digestibility of corn supplemented with phytase and glucanase

		7 to 9 d of age		14 to 16 d of age		21 to 23 d of age	
Treatment		IDE[1] (kcal/kg)	DM digestibility (%)	IDE (kcal/kg)	DM digestibility (%)	IDE (kcal/kg)	DM digestibility (%)
Phytase[2]							
0		3043	75.8	3119	77.9	3177	78.5[b]
500		3070	76.8	3130	79.1	3170	79.3[a]
Glucanase[2]							
0		3004[b]	74.7[b]	3058[b]	76.7[b]	3136[b]	77.7[b]
500		3108[a]	77.9[a]	3191[a]	80.3[a]	3210[a]	80.1[a]
SEM		36.1	0.85	20.8	0.45	11.2	0.23
Phytase	Glucanase						
0	0	2990	73.8	3048	75.6	3148	77.0
0	500	3095	77.7	3190	80.3	3206	79.9
500	0	3018	75.5	3068	77.8	3124	78.3
500	500	3121	78.0	3192	80.3	3215	80.2
SEM		53.4	1.25	29.3	0.64	16.5	0.34
Probability							
Phytase (Phy)		NS	NS	NS	NS	NS	0.02
Glucanase (Glu)		0.05	0.01	0.0002	0.0001	0.0001	0.0001
Phy × Glu		NS	NS	NS	NS	NS	NS

[1]IDE as determined by acid insoluble ash marker.
[2]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 3-3. Ileal digestible energy (IDE) and DM digestibility values of SBM supplemented with phytase and glucanase

		7 to 9 d of age		14 to 16 d of age		21 to 23 d of age	
Treatment		IDE[1] (kcal/kg)	DM digestibility (%)	IDE (kcal/kg)	DM digestibility (%)	IDE (kcal/kg)	DM digestibility (%)
Phytase[2]							
0		2400	52.6	2416	54.9	2467	55.4
500		2426	55.1	2387	55.0	2489	56.8
Glucanase[2]							
0		2346[b]	53.1	2321[b]	53.4[b]	2391[b]	54.7
500		2480[a]	54.6	2482[a]	56.4[a]	2564[a]	57.5
SEM		45.6	1.01	24.5	0.60	54.3	1.17
Phytase	Glucanase						
0	0	2353	52.4	2335	53.7	2359	53.5
0	500	2446	52.8	2498	56.1	2574	57.2
500	0	2340	53.8	2308	53.2	2423	55.8
500	500	2513	56.4	2467	56.8	2554	57.9
SEM		64.4	1.42	34.6	0.85	76.8	1.66
Probability							
Phytase (Phy)		NS	NS	NS	NS	NS	NS
Glucanase (Glu)		0.05	NS	0.002	0.002	0.04	NS
Phy × Glu		NS	NS	NS	NS	NS	NS

[1]IDE as determined by acid insoluble ash marker.
[2]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 3-4. The amount of α-amylase[1] in the duodenal-jejunal contents and pancreas of broilers fed corn supplemented with phytase and glucanase

		7 to 9 d of age		14 to 16 d of age		21 to 23 d ofage	
Treatment		Digesta	Pancreatic	Digesta	Pancreatic	Digesta	Pancreatic
Phytase[2]							
0		435	892	677	1529	732	2171
500		394	1301	655	1534	691	1870
Glucanase[2]							
0		427	1039	653	1653	786[a]	1961
500		402	1154	679	1410	638[b]	2080
SEM		45.7	267	78.0	156.2	49.4	233.9
Phytase	Glucanase						
0	0	406	832	690	1617	839	2259
0	500	463	952	663	1442	625	2082
500	0	447	1247	615	1690	732	1663
500	500	340	1355	695	1378	651	2078
SEM		67.7	397.1	93.4	244.3	73.0	333.1
Probability							
Phytase (Phy)		NS	NS	NS	NS	NS	NS
Glucanase (Glu)		NS	NS	NS	NS	0.05	NS
Phy × Glu		NS	NS	NS	NS	NS	NS

[1]Amount of amylase expressed as units of activity per mg of protein.
[2]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 3-5. The amount of protease activity[1] in the duodenal-jejunal contents and pancreas of broilers fed corn supplemented with phytase and glucanase

		7 to 9 d of age		14 to 16 d of age		21 to 23 d of age	
Treatment		Digesta	Pancreatic	Digesta	Pancreatic	Digesta	Pancreatic
Phytase[2]							
0		290	211	549	216.5	979	323
500		305	233	522	210.0	1028	284
Glucanase[2]							
0		323	221	503	222.6	960	362[a]
500		272	223	567	204.0	1046	245[b]
SEM		60.5	42.4	74.5	20.22	92.7	41.1
Phytase	Glucanase						
0	0	230	215	566	206.5	954	378
0	500	349	206	532	226.5	1004	269
500	0	415	226	441	238.7	967	347
500	500	195	239	603	181.4	1089	220
SEM		92.1	63.1	91.2	28.74	92.7	60.9
Probability							
Phytase		NS	NS	NS	NS	NS	NS
Glucanase		NS	NS	NS	NS	NS	0.05
Phytase x Glucanase		NS	NS	NS	NS	NS	NS

[1]Amount of protease activity expressed as units of per mg of protein.
[2]Enzyme treatments are expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 3-6. The amount of protease activity[1] in the duodenal-jejunal contents and pancreas of broilers fed SBM supplemented with phytase and glucanase

		7 to 9 d of age		14 to 16 d of age		21 to 23 d of age	
Treatment		Digesta	Pancreatic	Digesta	Pancreatic	Digesta	Pancreatic
Phytase[2]							
0		160	140[b]	581	313[b]	493	388
500		218	273[a]	590	658[a]	570	328
Glucanase[2]							
0		144	224	652[a]	471	480	436
500		234	189	520[b]	500	583	280
SEM		46.1	46.6	35.7	55.8	49.6	60.7
Phytase	Glucanase						
0	0	134	127	673	278	421	491
0	500	187	153	490	349	564	286
500	0	154	321	631	665	538	382
500	500	281	225	549	652	601	274
SEM		65.2	74.2	52.9	83.1	70.1	95.1
Probability							
Phytase		NS	0.05	NS	0.0002	NS	NS
Glucanase		NS	NS	0.01	NS	NS	NS
Phytase x Glucanase		NS	NS	NS	NS	NS	NS

[1]Amount of protease activity expressed as units per mg of protein.
[2]Enzyme treatments are expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

TABLE 3-7. The amount of α-amylase[1] in the duodenal-jejunal contents and pancreas of broilers fed SBM supplemented with phytase and glucanase

		7 to 9 d of age		14 to 16 d of age		21 to 23 d of age	
Treatment		Digesta	Pancreatic	Digesta	Pancreatic	Digesta	Pancreatic
Phytase[2]							
0		226	999	454	1556	250[b]	1603
500		251	645	456	1758	312[a]	2271
Glucanase[2]							
0		248	936	463	1652	251[b]	2092
500		228	707	449	1661	311[a]	1781
SEM		27.0	188.3	35.6	190.4	20.1	380.6
Phytase	Glucanase						
0	0	267	1299	475	1516	229	1874
0	500	184	698	434	1596	272	1332
500	0	229	574	450	1789	273	2310
500	500	272	717	463	1727	351	2231
SEM		38.2	280.0	50.4	270.5	28.4	568.2
Probability							
Phytase (Phy)		NS	NS	NS	NS	0.04	NS
Glucanase (Glu)		NS	NS	NS	NS	0.04	NS
Phy × Glu		NS	NS	NS	NS	NS	NS

[1]Amount of amylase expressed as units of activity per mg of protein.
[2]Enzyme treatments expressed as units of activity per kg of feed.
[a,b]Means within a column and main effect with no common superscript differ significantly.
NS, $P > 0.05$.

4.0 EFFECT OF PHYTASE AND DAY LENGTH ON EXTENT AND LOCATION OF PHYTATE DEGRADATION IN THE DIGESTIVE TRACT

4.1 INTRODUCTION

Phytate is the storage form of phosphorus in plant feedstuffs, and consists of an inositol ring with 6 phosphate groups. Phytases are enzymes that cleave each phosphate group from the ring, usually beginning with the 3- or 6- position, and are generally capable of removing all phosphate groups except the 2- position because of its axial configuration (Barrientos and Murthy, 1996). Exogenous microbial or plant source phytase is commonly used in poultry and swine rations because it can hydrolyze or degrade phytate. This increases phosphorus availability in the feed, thus reducing the need for inorganic phosphorus supplementation and the amount of phosphorus excretion (Ravindran et al., 2000; Wu et al., 2003). In addition to exogenous sources, many animals, including poultry, have some endogenous phytase activity (Maenz and Classen, 1998). Specific endogenous phytase activity was found in the small intestine of layers and broilers, with the highest activity in the duodenum (Maenz and Classen, 1998). The microflora of the small intestine, ceca, and large intestine may be capable of phytate degradation; however, there would be little opportunity for absorption by the bird (Hurwitz and Bar, 1970).

Schlemmer et al. (2001) studied phytate degradation in pigs. The extent of phytate hydrolysis was the same, with or without phytase supplementation; however, the location within the gut differed. With exogenous phytase, gastric degradation of phytate to its lower inositol phosphate (IP) forms was high (58%). Without exogenous phytase, gastric degradation was very low, though degradation in the colon and large intestine was high. The limitation is that phosphate groups liberated in the large intestine can not be absorbed (Hurwitz and Bar, 1970). Moreover, phytate degradation in the chicken is less complete than in swine, probably because of low transit time. It is possible that reduced day length may improve phytate degradation and facilitate the actions of phytase.

The objectives of the present experiment were to determine the extent and location of phytate degradation to lower IPs. As well, the solubility of IPs in various parts of the digestive tract, and the effect of day length on phytate degradation were investigated.

4.2 MATERIALS AND METHODS

All experimental procedures were approved by Auburn University Institutional Animal Care and Use Committee. Two hundred forty male Ross 308 chicks were obtained from a commercial hatchery and immediately placed in 24 Petersime battery brooders. The battery cages were located in four rooms, with six cages per room, and ten chicks per cage (0.73 sq. ft. per bird). All birds were provided with a commercial starter diet and water ad libitum from placement to the beginning of the trial (Table 4-1). For the first 20 days, the chicks were reared under 24 h of light per day.

The experiment was a 2 × 2 factorial with two levels of phytase (0 or 500 FTU per kg diet) and two day lengths (12 h of light or 24 h of light per day), with two replicate rooms for each lighting program, and three replicates of each phytase treatment per room. Feeds used were complete corn soybean meals diets (Table 4-1), formulated for 0.45% aP, differing only in level of phytase. Treatments were applied between 20 and 24 d of age. On day 24, all birds were euthanized via CO_2 asphyxiation, and various parts of the digestive tract removed. Crop, proventriculus and gizzard (pooled; gastric composite), duodenum and jejunum (pooled), and ileum contents and an excreta sample were collected from each bird and pooled within each cage. Samples were immediately frozen and freeze-dried. Dry samples were analyzed for acid insoluble ash content and level of inositol phosphates as described below.

Extraction of inositol phosphates (IP) was performed for each sample by two methods to obtain both total and water soluble phytate and phytate degradation products. Water soluble IPs were taken as an indication of the amount of substrate available for degradation by phytase, as the substrate-enzyme interaction is most efficient when both molecules are in solution. The two procedures were identical except that 2N HCl was used for total IP extraction while distilled water was used to determine water soluble IPs. Depending on the procedure, an aliquot of 2.5 ml acid or 2.0 ml of water was added to approximately 0.25 g of digesta or excreta. The sample was vortexed, then agitated vigorously on a shaker for 3 h. One ml of 5% chloroform was added to remove any lipid followed by centrifugation at 5,000 rpm for 10 min. The supernatant was poured into an empty test tube. To maintain consistent pH and volume, 0.5 ml of 10N HCl was added to the water soluble IP samples after centrifugation. The supernatant was analyzed for IP6

through IP2 content using the HPLC method as described by Newkirk and Classen (1998). Each IP level was adjusted using the acid insoluble ash content to relate it back to the feed levels. The feed used was analyzed for IP6, and found to contain 0.98% phytate and an "as is" basis, amounting to approximately 0.28% total phosphorus in the form of phytate.

Statistical analysis was performed using the General Linear Models Procedure of SAS® (SAS Institute, 2001). The experiment was analyzed as a 2 × 2 factorial arrangement in a randomized complete block design, with the main effects of phytase level and day length and room as the block. Means were separated using a Tukey's Honestly Significant Difference test and significance implied $P<0.05$. During shipping, some samples were lost, and statistical analysis reflects this as the SEMs for main effects are not the same in all cases. Concentrations of IP in each sample were adjusted using the acid insoluble ash content in order to avoid increasing IP concentrations due to disappearance of DM.

4.3 RESULTS

Phytase supplementation significantly increased feed intake (Table 4-2). It did not affect BW gain; consequently, feed to gain ratio was higher than the control diet. As well, both ileal and total intestinal tract DM digestibility was lower in the phytase diet. The effects of day length were similar to that of phytase, continuous light (24 h per day) increased feed intake and feed to gain ratio compared to 12 h of light. Increased day length reduced ileal DM digestibility but did not affect total tract DM digestibility.

The phytase-supplemented diet when fed under 24 h of light resulted in the highest feed consumption compared to the other treatments. This led to an increase in feed to gain ratio for the same treatment. The ileal DM digestibility for the control diet and 12 h light treatment was higher than all other treatments.

It was of interest to determine the effect of phytase on the extent and location of phytate degradation in the gut. The total and water soluble IP levels for the crop, gastric composite, duodenum-jejunum, ileum, and excreta are presented in Tables 4-3 through 4-7, respectively. Phytase supplementation reduced total phytate (IP6) concentrations beginning in the crop all the way through to the excreta. Total IP5 through IP3 concentrations were higher with phytase than without in the duodenum-jejunum through to the excreta. Water soluble IPs were found in the crop, gastric composite, and duodenum-jejunum samples. Water soluble phytate levels in the crop, gastric composite and IP5 in the crop were higher in the control diet than in the phytase diet.

Day length did not affect total or water soluble IP concentrations in the crop and duodenum-jejunum samples. Birds reared under 24 h light had higher total phytate concentrations in the gastric composite (Table 4-4), and lower concentrations of IP5 through IP2 in the ileum compared to 12 h light (Table 4-6). In the ileal samples, phytase supplementation combined with 12 h light increased the concentration of lower IP forms. However, in the excreta samples, phytase combined with 24 h light had the highest IP3 concentrations.

4.4 DISCUSSION

Previous experiments found that phytase supplementation increased feed consumption and lowered feed conversion in poultry (Huyghebaert, 1996; Camden et al., 2001) and other species (Beers and Jongbloed, 1992; Young et al., 1993). These findings were attributed to an increase in phosphorus availability (aP). In the current study, since diets contained adequate aP levels, increased feed consumption with phytase resulted in a faster passage rate through the gut and decreased the digestibility of the diet. Feed conversion increased compared to the control diet, presumably because the additional aP was not needed by the bird (i.e. it did not alleviate a deficiency). A similar effect was seen with day length treatments, 24 h light increased passage rate compared to the 12 h day length treatment. The interaction further demonstrated that birds fed phytase-supplemented diets and reared under a long day length consumed more feed but had a higher feed conversion than the other treatments.

There has been little research in poultry species that has determined the location and extent of phytate degradation through the digestive tract. One report used Western blots to determine phytase activity along the digestive tract (Yu et al., 2004). The experiment found that phytase survived digestion through to the jejunum but was absent in the ileum. As well, the enzyme was in its active form as far as the duodenum (Yu et al., 2004). Sooncharernying and Edwards (1993) demonstrated that phytate degradation was influenced by bird age and aP level in the diet. That is, 3 wk old birds digested phytate more effectively than 2 wk old birds. Those fed 0.42% aP hydrolyzed more phytate than those fed 0.27% aP. None of the diets in that study included phytase

(Sooncharernying and Edwards, 1993). There is some debate as to whether endogenous phytase activity actually arises from the bird itself. The possibility exists that phytase present in the feedstuffs (eg. wheat, Brearley and Hanke, 1996) or produced by microflora may be responsible for endogenous phytate degradation. Nelson (1976) found no phytate hydrolysis in chicks fed corn-based diets, which are considered to be low in phytase activity. Savage et al. (1964) and Wise and Gilburt (1982) observed that phytate was not hydrolyzed in germ-free chicks and rats, respectively. However, phytase activity was found in preparations of intestinal brush border membrane vesicles with activity between pH 5 and 6.5 (Maenz and Classen, 1998).

This experiment was designed to determine the effect of exogenous phytase and day length on the degradation of phytate in the small intestine. An adequate aP level in both diets served to reduce endogenous phytase expression in either the microflora (Hidayat et al., 2006) or small intestine (Maenz and Classen, 1998), and thus reduce variability in the results.

The effects of supplemental phytase were seen primarily in the crop and proventriculus-gizzard. This was expected as the optimal pH of the phytase used was approximately 5.5. There was nearly twice as much phytate (IP6) in the crop contents of the control birds compared to the phytase-supplemented group. There was also more water soluble IP6 and IP5 in the crop contents from the control birds than the phytase birds. This suggested that exogenous phytase digested the soluble IP6. A similar trend was seen in the gastric composite. The amount of phytate detected in these samples was similar to the crop. In the small intestine, water soluble IP6 and its products were negligible, likely because they precipitated with minerals (Kaufman and Kleinberg,

1971). The duodenal-jejunal and ileal results suggest that very little phytate degradation occurred in these, as the relative values across both the control and phytase diets remained fairly constant. The higher levels of lower IP forms seen in the duodenum-jejunum compared to the upper digestive tract may have resulted from an unequal transit rate for Celite® (the acid insoluble ash marker) and the larger particles in the diet. That is, Celite® could travel faster through the gizzard than larger particles, which would be retained for physical digestion.

It has been shown previously that birds reared on shorter day lengths consumed large amounts of feed prior to commencement of the dark period to maintain a full digestive tract through the night (Buyse et al., 1993). This "cropping up" behavior leads to feed being retained longer in the digestive tract than would occur if birds were allowed to feed 24 h per day. As the current study indicated, a substantial portion of phytate digestion occurs in the crop. Therefore, it was expected that a shorter day length would increase phytate degradation in all birds compared to continuous light, but to a greater extent in those fed phytase.

This data showed that, as with most nutrients, phytate digestibility increased with slower passage rate. This effect was not evident in the crop or gastric composite, but appeared in the duodenal-jejunal and ileal samples. The timing of dissection may have caused these results. Birds were euthanized in the morning, after the lights came on. The birds exposed to the short day length rapidly consumed feed once the lights were on; consequently, there were no differences in phytate degradation from the upper digestive tract samples. The interaction between phytase supplementation and day length seen in the ileal digesta and excreta suggest that slowing the feed passage rate by reducing day

length can improve the efficacy of exogenous phytase. This effect was not seen in the gastric composite because of high variation in IP6 concentrations.

An experiment with pigs found that, regardless of phytase supplementation, phytate degradation was near complete through the digestive tract (Schlemmer et al., 2001). Without phytase, degradation occurred largely in the colon, with little absorption of the liberated phosphate. With phytase, degradation occurred largely in the stomach increasing phosphorus availability (Schlemmer et al., 2001). The results from the present study show that phytate degradation in the chicken is incomplete, even with phytase supplementation (Table 4-7). The discrepancy between these two species may be attributed to transit time of the digesta or the degree of microbial digestion in the ceca and colon. In poultry, transit time is approximately 4 h, depending on numerous dietary factors (Lazaro et al., 2003). In swine, transit time is much longer, approximately 24 h (Hennessy et al., 2000). There is less time for microbial or exogenous phytase to act on its substrate in poultry than in swine. As well, Carre (2004) suggests that particle size can also limit the extent of cecal digestion. Digesta that consist of more than two plant cells may be too large for fermentation. Thus, if phytate is present in plant structures that have survived through the small intestine, they are excluded from the ceca and remain undigested (Carre, 2004). Studies have shown that supplementation of phytase at rates higher than those commonly used in industry are more effective at phytate hydrolysis (Shirley and Edwards, 2003). These data supports the hypothesis that transit time limits phytate degradation.

Phytase improved phytate degradation in the acidic portions of the digestive tract, but had little influence in the small intestine. Reduced day length increased phytate

digestion, and improved the ability of phytase to dephosphorylate phytate. The degree of dephosphorylation of the phytate molecule was low, suggesting that higher levels of phytase would improve phosphorus availability. The results of this experiment should be taken into account when using phytase in conjunction with a lighting program.

4.5 REFERENCES

Barrientos, L. G., and P. P. N. Murthy. 1996. Conformational studies of myo-inositol phosphates. Carbohydrate Research 296: 39-54.

Beers, S., and A. W. Jongbloed. 1992. Effect of supplementary Aspergillus niger phytase in diets for piglets on their performance and apparent digestibility of phosphorus. Anim. Prod. 55: 425-430.

Brearley, C. A., and D. E. Hanke. 1996. Inositol phosphates in barley (Hordeum vulgare L.) aleurone tissue are steriochemically similar to the products of breakdown of InsP6 in vitro by wheat-bran phytase. Biochem. J. 318: 279-286.

Buyse, J., D. S. Adelsohn, E. Decuypere, and C. G. Scanes. 1993. Diurnal-nocturnal changes in food intake, gut storage of ingesta, food transit time and metabolism in growing broiler chickens: a model for temporal control of energy balance. Br. Poult. Sci. 34: 699-709.

Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailability of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. Anim. Sci. 73: 289-297.

Carre, B. 2004. Causes for variation in digestibility of starch among feedstuffs. Worlds Poultry Science Journal. 60: 76-89.

Hidayat, B. J., N. T. Eriksen, and M. G. Wiebe. 2006. Acid phosphatase production by Aspergillus niger N402A in continuous flow culture. FEMS Microbiol. Lett. 254: 324-331.

Hennessy, D. R., J. Praslicka, and H. Bjorn. 2000. The disposition of pyrantel in the gastrointestinal tract and effect of digesta flow rate on the kinetic behaviour of pyrantel in the pig. Vet. Parasitol. 92: 277-285.

Huyghebaert, G. 1996. The response of broiler chicks to phase feeding for P, Ca and phytase. Arch. Geflugelk. 60: 132-141.

Hurwitz, S., and A. Bar. 1970. The sites of calcium and phosphate absorption in the chick. Poult. Sci. 49: 324-325.

Kaufman, H. W., and I. Kleinberg. 1971. Effect of pH on calcium binding by phytic acid and its inositol phosphoric acid derivatives and on the solubility of their calcium salts. Archs Oral Biol. 16: 445-460

Lazaro, R., M. Garcia, P. Medel, and G. G. Mateos. 2003. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. Poult. Sci. 82: 132-140.

Maenz, D. D., and H. L. Classen. 1998. Phytase activity in the small intestinal brush border membrane of the chicken. Poult. Sci. 77: 557-563.

Nelson, T. S. 1976. The hydrolysis of phytate by chicks and laying hens. Poult. Sci. 55: 2262-2264.

Newkirk, R. W., and H. L. Classen. 1998. In vitro hydrolysis of phytate in canola meal with purified and crude sources of phytase. Anim. Feed Sci. Tech. 72: 315-327.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41: 193-200.

SAS Institute Inc., 2001. The SAS System for Windows, Release 8.02. SAS Institute Inc., Cary, NC.

Savage, J. E., J. M. Yohe, E. E. Pickett, and B. L. O'Dell. 1964. Zinc metabolism in the growing chick. Tissue concentrations and effect of phytate on absorption. Poult. Sci. 43: 420-426.

Schlemmer, U., K. D. Jany, A. Berk, E. Schultz, and G. Rechkemmer. 2001. Degradation of phytate in the gut of pigs- Pathway of gastrointestinal inositol phosphate hydrolysis and enzymes involved. Arch. Anim. Nutr. 55: 255-280.

Shirley, R. B., and H. M. Edwards Jr. 2003. Graded levels of phytase past industry standards improve broiler performance. Poult. Sci. 82: 671-680.

Sooncharernying, S., and H. H. Edwards. 1993. Phytate content of excreta and phytate retention in the gastrointestinal tract of young chickens. Poult. Sci. 72: 1906-1916.

Wise, A., and D. J. Gilburt. 1982. Phytate hydrolysis in germfree and conventional rats. Applied and Environmental Microbiology. 43: 753-756.

Wu, Y. B., V. Ravindran, and W. H. Hendriks. 2003. Effects of microbial phytase, produced by solid-state fermentation, on the performance and nutrient utilization of broilers fed maize- and wheat- based diets. Br. Poult. Sci. 44: 710-718.

Young, L. G., M. Leunissen, and J. L. Atkinson. 1993. Addition of microbial phytase to diets of young pigs. J. Anim. Sci. 71: 2147-2150.

Yu, B., Y. C. Yan, T. K. Chung, T. T. Lee, and P. W. S. Chiou. 2004. Exogenous phytase activity in the gastrointestinal tact of broiler chickens. Anim. Feed Sci. Tech. 117: 295-303.

TABLE 4-1. Ingredient and calculated nutrient composition of a commercial starter diet fed from 0 to 20 days and experimental diets fed from 20 to 24 days of age

Ingredients (%)	Commercial Starter	Diet 1	Diet 2
Ground Yellow Corn	53.85	53.35	53.35
Soybean Meal (48% CP)	36.50	36.50	36.50
Poultry Oil	5.00	5.00	5.00
Dicalcium Phosphate (21.5% Ca, 18.5% P)	1.90	1.90	1.90
Limestone	1.35	1.35	1.35
L-Lysine HCl	0.10	0.10	0.10
DL-Methionine	0.35	0.35	0.35
Salt	0.45	0.45	0.45
Vitamin Premix[1]	0.25	0.25	0.25
Mineral Premix[1]	0.25	0.25	0.25
Celite	0	0.50	0.50
Phytase (Units per kg)[2]	0	0	500
Calculated Composition			
ME (kcal/kg)	3072	3072	3072
CP (%)	22.5	22.5	22.5
Lysine (%)	1.30	1.30	1.30
Methionine (%)	0.68	0.68	0.68
TSAA (%)	1.04	1.04	1.04
aP(%)	0.47	0.47	0.47
Analyzed Composition			
Phytate (%)	0.98	0.98	0.98

[1] The vitamin and mineral premix provided the following per kg of diet: 7500 IU Vitamin A, 2500 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 37 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.1 mg biotin, 500 mg choline, 125 mg ethoxyquin, 66 mg Mn, 55 mg Zn, 6 mg Fe, 6 mg Cu, 0.15 mg Se, 1 mg I.
[2] Ingredients expressed as units of activity per kg diet on an "as is" basis.

TABLE 4-2. Effect of phytase supplementation and day length on live performance of broilers fed a corn soybean meal diet from 20 to 24 d of age

		Feed Intake	BW Gain (g/bird)	Feed Conversion	Ileal DM Digestibility	Total Tract DM Digestibility (%)
Phytase	0	515[b]	303	1.71[b]	77.3[a]	81.9[a]
	500	558[a]	291	1.92[a]	75.2[b]	79.3[b]
	SEM	6.8	8.1	0.044	0.46	0.39
Day Length	12h light	495[b]	289	1.73[b]	77.1[a]	80.1
	24h light	578[a]	306	1.91[a]	75.4[b]	81.1
	SEM	6.8	8.1	0.044	0.46	0.39
Phytase x Day Length						
0	12h light	485[c]	289	1.70[b]	79.7[a]	81.3
0	24h light	545[b]	318	1.71[b]	74.8[b]	82.5
500	12h light	505[c]	288	1.75[b]	74.4[b]	78.9
500	24h light	611[a]	294	2.10[a]	76.0[b]	79.6
	SEM	9.6	11.5	0.062	0.65	0.54
		----------P Value----------				
Phytase		0.0002	NS	0.02	0.004	0.0001
Day Length		0.0001	NS	0.01	0.02	0.08
P x D		0.03	NS	0.01	0.0001	NS

[a,b] Means within columns with different superscripts are statistically different ($P < 0.05$).

TABLE 4-3. Total and water soluble inositol phosphates isolated from crop contents of broilers fed a diet with or without phytase and subject to 12 or 24 h of light per day

		Total Inositol Phosphates[1]					Water Soluble Inositol Phosphates[1]				
		IP6	IP5	IP4	IP3	IP2	IP6	IP5	IP4	IP3	IP2
Phytase	0	0.51[a]	0.09	0.00[b]	0.01[b]	0.00	0.22[a]	0.05[a]	0.00	0.00	0.00
	500	0.27[b]	0.07	0.17[a]	0.04[a]	0.00	0.00[b]	0.00[b]	0.00	0.00	0.00
	SEM[2]	0.045	0.011	0.015	0.010	0.000	0.018	0.009	0.003	0.000	0.000
Day Length	12h light	0.38	0.07	0.09	0.01	0.00	0.11	0.03	0.00	0.00	0.00
	24h light	0.39	0.08	0.09	0.04	0.00	0.11	0.02	0.00	0.00	0.00
	SEM	0.045	0.011	0.015	0.010	0.00	0.018	0.009	0.003	0.000	0.000
Phytase x Day Length											
0	12h light	0.45	0.08	0.00	0.00	0.00	0.22	0.06	0.01	0.00	0.00
0	24h light	0.56	0.10	0.00	0.01	0.00	0.23	0.05	0.00	0.00	0.00
500	12h light	0.32	0.07	0.17	0.03	0.00	0.00	0.00	0.00	0.00	0.00
500	24h light	0.23	0.07	0.18	0.06	0.00	0.00	0.00	0.00	0.00	0.00
	SEM	0.063	0.016	0.021	0.015	0.000	0.026	0.012	0.004	0.000	0.000
		P value					P value				
Phytase		0.001	NS	0.0001	0.02	NS	0.0001	0.0006	NS	NS	NS
Day Length		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P x D		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Inositol phosphate concentrations where adjusted using the acid insoluble ash content of the feed and digesta, and represent a % of the feed.
[2] SEMs for main effects are different due to missing values, as a consequence of damage incurred while samples were in transit.
[a,b] Means within columns with different superscripts are statistically different ($P < 0.05$)

TABLE 4-4. Total and water soluble inositol phosphates isolated from the gizzard and proventriculus contents of broilers fed a diet with or without phytase and subject to 12 or 24 h of light per day

		Total Inositol Phosphates[1]					Water Soluble Inositol Phosphates[1]				
		IP6	IP5	IP4	IP3	IP2	IP6	IP5	IP4	IP3	IP2
Phytase	0	0.54[a]	0.01	0.00	0.00[b]	0.00	0.13[a]	0.00	0.00	0.00	0.00
	500	0.21[b]	0.00	0.16	0.14[a]	0.00	0.01[b]	0.00	0.00	0.00	0.00
	SEM[2]	0.067	0.010	0.034	0.031	0.000	0.022	0.000	0.000	0.000	0.000
Day Length	12h light	0.21[b]	0.00	0.03	0.03	0.00	0.07	0.00	0.00	0.00	0.00
	24h light	0.54[a]	0.01	0.13	0.11	0.00	0.07	0.00	0.00	0.00	0.00
	SEM	0.067	0.010	0.034	0.031	0.000	0.021	0.000	0.000	0.000	0.000
Phytase x Day Length											
0	12h light	0.34	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00
0	24h light	0.74	0.03	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00
500	12h light	0.07	0.00	0.06	0.05	0.00	0.00	0.00	0.00	0.00	0.00
500	24h light	0.35	0.00	0.25	0.23	0.00	0.03	0.00	0.00	0.00	0.00
	SEM	0.095	0.014	0.048	0.044	0.000	0.030	0.000	0.000	0.000	0.000
		----------P value----------					----------P value----------				
Phytasee		0.002	NS	0.004	0.005	NS	0.001	NS	NS	NS	NS
Day Length		0.002	NS	0.06	0.06	NS	NS	NS	NS	NS	NS
P x D		NS	NS	0.06	0.06	NS	NS	NS	NS	NS	NS

[1] Inositol phosphate concentrations where adjusted using the acid insoluble ash content of the feed and digesta, and represent a % of the feed.
[2] SEMs for main effects are different due to missing values, as a consequence of damage incurred while samples were in transit.
[a,b] Means within columns with different superscripts are statistically different ($P < 0.05$)

TABLE 4-5. Total and water soluble inositol phosphates isolated from duodenum and jejunum contents of broilers fed a diet with or without phytase and subject to 12 or 24 h of light per day

		Total Inositol Phosphates[1]					Water Soluble Inositol Phosphates[1]				
		IP6	IP5	IP4	IP3	IP2	IP6	IP5	IP4	IP3	IP2
Phytase	0	0.74	0.10[b]	0.02[b]	0.04[b]	0.00	0.02	0.00	0.00	0.00	0.00
	500	0.55	0.22[a]	0.17[a]	0.09[a]	0.00	0.03	0.00	0.00	0.00	0.00
	SEM[2]	0.075	0.032	0.023	0.011	0.000	0.022	0.000	0.000	0.000	0.000
Day Length	12h light	0.62	0.18	0.12	0.07	0.00	0.00	0.00	0.00	0.00	0.00
	24h light	0.68	0.14	0.07	0.06	0.00	0.05	0.00	0.00	0.00	0.00
	SEM	0.075	0.033	0.024	0.011	0.000	0.022	0.000	0.000	0.000	0.000
Phytase x Day Length											
0	12h light	0.66	0.10	0.00[c]	0.04	0.00	0.00	0.00	0.00	0.00	0.00
0	24h light	0.82	0.10	0.03[bc]	0.05	0.00	0.04	0.00	0.00	0.00	0.00
500	12h light	0.57	0.27	0.24[a]	0.10	0.00	0.01	0.00	0.00	0.00	0.00
500	24h light	0.53	0.18	0.11[b]	0.07	0.00	0.05	0.00	0.00	0.00	0.00
	SEM	0.111	0.049	0.036	0.016	0.000	0.034	0.000	0.000	0.000	0.000
		----------P value----------									
Phytase		0.09	0.01	0.0001	0.008	NS	NS	NS	NS	NS	NS
Day Length		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P x D		NS	NS	0.03	NS	NS	NS	NS	NS	NS	NS

[1] Inositol phosphate concentrations where adjusted using the acid insoluble ash content of the feed and digesta, and represent a % of the feed.
[2] SEMs for main effects are different due to missing values, as a consequence of damage incurred while samples were in transit.
[a,b] Means within columns with different superscripts are statistically different ($P < 0.05$)

TABLE 4-6. Total and water soluble inositol phosphates isolated from the ileum contents of broilers fed a diet with or without phytase and subject to 12 or 24 h of light per day

		Total Inositol Phosphates[1]					Water Soluble Inositol Phosphates[1]				
		IP6	IP5	IP4	IP3	IP2	IP6	IP5	IP4	IP3	IP2
Phytase	0	0.58[a]	0.12[b]	0.00[b]	0.00[b]	0.00	0.00	0.00	0.00	0.00	0.00
	500	0.37[b]	0.23[a]	0.22[a]	0.04[a]	0.00	0.01	0.00	0.00	0.00	0.00
	SEM[2]	0.052	0.022	0.024	0.005	0.000	0.009	0.000	0.000	0.000	0.000
Day Length	12h light	0.51	0.22[a]	0.17[a]	0.04[a]	0.00	0.01	0.00	0.00	0.00	0.00
	24h light	0.44	0.13[b]	0.05[b]	0.01[b]	0.00	0.00	0.00	0.00	0.00	0.00
	SEM	0.052	0.022	0.024	0.005	0.000	0.001	0.000	0.000	0.000	0.000
Phytase x Day Length											
0	12h light	0.57	0.13[b]	0.00[c]	0.00[b]	0.00	0.00	0.00	0.00	0.00	0.00
0	24h light	0.59	0.12[b]	0.01[bc]	0.01[b]	0.00	0.00	0.00	0.00	0.00	0.00
500	12h light	0.44	0.31[a]	0.34[a]	0.07[a]	0.00	0.03	0.00	0.00	0.00	0.00
500	24h light	0.29	0.14[b]	0.10[b]	0.02[b]	0.00	0.00	0.00	0.00	0.00	0.00
	SEM	0.073	0.030	0.033	0.007	0.000	0.012	0.000	0.000	0.000	0.000
			----------P value----------								
Phytase		0.008	0.002	0.0001	0.0001	NS	NS	NS	NS	NS	NS
Day Length		NS	0.009	0.002	0.002	NS	NS	NS	NS	NS	NS
P x D		NS	0.02	0.001	0.0003	NS	NS	NS	NS	NS	NS

[1] Inositol phosphate concentrations where adjusted using the acid insoluble ash content of the feed and digesta, and represent a % of the feed.
[2] SEMs for main effects are different due to missing values, as a consequence of damage incurred while samples were in transit.
[a,b] Means within columns with different superscripts are statistically different ($P < 0.05$)

TABLE 4-7. Total and water soluble inositol phosphates isolated from the excreta of broilers fed a diet with or without phytase and subject to 12 or 24 h of light per day

		Total Inositol Phosphates[1]					Water Soluble Inositol Phosphates[1]				
		IP6	IP5	IP4	IP3	IP2	IP6	IP5	IP4	IP3	IP2
Phytase	0	0.45 [a]	0.08 [b]	0.00 [b]	0.00 [b]	0.00	0.00	0.00	0.00	0.00	0.00
	500	0.33 [b]	0.15 [a]	0.10 [a]	0.02 [a]	0.00	0.00	0.00	0.00	0.00	0.00
	SEM[2]	0.014	0.006	0.005	0.003	0.000	0.000	0.000	0.000	0.000	0.000
Day Length	12h light	0.42	0.13	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	24h light	0.35	0.10	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	SEM	0.024	0.010	0.009	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Phytase x Day Length											
0	12h light	0.45 [a]	0.09	0.00 [b]	0.00 [b]	0.00	0.00	0.00	0.00	0.00	0.00
0	24h light	0.44 [ab]	0.06	0.00 [b]	0.00 [b]	0.00	0.00	0.00	0.00	0.00	0.00
500	12h light	0.39 [b]	0.16	0.09 [a]	0.01 [b]	0.00	0.00	0.00	0.00	0.00	0.00
500	24h light	0.27 [c]	0.13	0.12 [a]	0.04 [a]	0.00	0.00	0.00	0.00	0.00	0.00
	SEM	0.029	0.012	0.011	0.007	0.000	0.000	0.000	0.000	0.000	0.000
			----------P value----------								
Phytase		0.0001	0.0001	0.0001	0.0002	NS	NS	NS	NS	NS	NS
Day Length		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P x D		0.02	NS	0.01	NS	NS	NS	NS	NS	NS	NS

[1] Inositol phosphate concentrations where adjusted using the acid insoluble ash content of the feed and digesta, and represent a % of the feed.
[2] SEMs for main effects are different due to missing values, as a consequence of damage incurred while samples were in transit.
[a,b] Means within columns with different superscripts are statistically different ($P < 0.05$)

5.0 THE EFFECT OF PHYTASE AND GLUCANASE IN CORN SOYBEAN MEAL DIETS ON BROILERS FROM 0 TO 54 D OF AGE

5.1 INTRODUCTION

The addition of exogenous enzymes to broiler diets has been extensively researched, with the goals of providing a more complete digestion of feed and lowering the cost of production. Numerous commercially available phytases are used in poultry feed as a result of consistently demonstrated improvements in phosphorus digestibility (Parks et al., 1999; Ravindran et al., 2000; Pintar et al., 2005), and a reduction in costly inorganic P supplementation and excretion. In wheat and barley-based diets, xylanases and glucanases are routinely used to reduce digesta viscosity, increase nutrient utilization and litter quality (Mathlouthi et al., 2002; Mathlouthi et al., 2003; Wu et al., 2004).

Recent research has focused on the application of these fibrolytic enzymes to corn and soybean meal diets, which lack soluble NSPs (Ouhida et al., 2002; Mathlouthi et al., 2003). The reason is, while most nutrients in corn are considered to be highly digestible, there is some evidence that the ME value varies with bird age (Mahagna et al., 1995). For example, only 82% of the starch is digested when fed to young birds (Noy and Sklan, 1995). This is thought to be a function of the neonate's low enzyme activity and relative surface area in the small intestine available for nutrient absorption (Iji et al., 2001a; Iji et al., 2001b). As well, the undigested portion may remain encapsulated within endosperm

cells (Classen, 1996). Thus, fibrolytic enzymes designed to digest cell wall components can be of value in corn and soybean meal diets.

A series of experiments was designed to evaluate a phytase (Quantum™ 2500D)[1], and a novel glucanase (Leslie, Dissertation, Chapters 2 to 4) in their ability to improve the nutrient digestibility of corn-soybean meal diets for broilers. These experiments demonstrated that phytase increased the digestibility and retention of phytate P and Ca, and impacted CP digestibility. Glucanase increased the ileal digestible energy of corn and soybean meal by approximately 100 kcal/kg in birds ranging from 7 to 23 d of age. These experiments were conducted in battery cages over a short duration. The evaluation of exogenous enzymes requires determination of matrix values for formulation and a test of those values in practical situations. The objective of this study was to determine if phytase and glucanase could improve live performance and further processed yields of broiler chickens when added to marginally deficient diets.

5.2 MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee at Auburn University. Seven hundred and fifty male Ross 708 broiler chicks were obtained from a commercial hatchery. Twenty five chicks were randomly selected, weighed, and placed in one of 30 floor pens, providing 1.4 sq. ft. per bird. Chicks were given access to feed and water at all times, and were subject to a continuous lighting program. Temperature was controlled based on industry standards, and adjusted according to bird behavior.

[1] Quantum™ Phytase, Zymetrics Inc, Minneapolis, MN

The experiment was a randomized complete block design, with five treatments, and six replicates per treatment. Diet 1 was a positive control (PC), designed to meet or exceed NRC requirements for all nutrients (NRC, 1994). Diet 2 was a negative control (NC), designed to reduce the nutrient density in accordance with the expected feeding value of the enzymes (Table 5-1). Energy was reduced by adding 100 kcal/kg to the matrix values of corn and soybean meal, and reformulating to the same requirement levels. Thus, the ME level of the NC was approximately 90 kcal/kg lower than the positive control. The aP and Ca content of the feed was reduced by approximately 0.15% and 0.2%, respectively, corresponding to values found in previous experiments using Quantum™ 2500D Phytase (Leslie, Dissertation, Chapters 2 and 3). The NC was formulated to the same essential amino acid and crude protein levels as the PC. Diets 3 to 5 were composed of the NC diet with 500 FTU/kg (Diet 3), 50 units/kg of glucanase (Diet 4), or 500 FTU and 50 units/kg glucanase (Diet 5), respectively. Starter diets were fed from 0 to 20 d, grower diets from 20 to 33 d, and finisher diets from 33 to 54 d of age.

On d 20, the birds were group weighed by pen and feed consumption to date was recorded. At 33 d, birds were individually weighed to facilitate carcass yield calculations. After weighing each bird, one-half were randomly selected from each pen and placed in coops, while the remainder were fed finisher diet. The following morning, cooped birds were slaughtered in the Auburn University pilot processing plant. After 4 h of static chilling in slush-ice water, eviscerated carcass and fat pad weights were recorded. Carcasses were held on ice for 24 h and further processed by experienced personnel using stationary cones to determine fillet, tender, wing, drum, frame, and

boneless thigh weights. Both femurs were frozen at -20 °C for analysis of breaking strength using a TA.XT Plus model Texture Analyzer.[2] The femurs were placed in a drying oven for 24 h and then weighed. Bone ash was determined by ashing at 600°C for 24 h and calculated as a percentage of dry weight. At 54 d, the remaining birds were weighed, and processed in the same way described above.

Statistical Analysis

The experimental unit in this study was individual floor pen. Pens for each treatment were blocked by location in the rearing facility. The data were analyzed by one-way analysis of variance for dietary treatment using the General Linear Models procedure in SAS® (SAS Institute, 2001). Differences between treatment means were separated using the Tukey's honestly significant difference test. Significance implied a P-value equal to or less than 0.05.

5.3 RESULTS

Dietary Restrictions

This experiment determined whether a phytase and a glucanase increased the availability of phosphorus, calcium, and energy in a diet marginally deficient in all three. The positive control diet served as a benchmark by which the other four diets could be compared. The PC diet resulted in period BW gains of 703, 1686, and 3273 g at 20, 33, and 54 d of age, respectively (Table 5-2). This exceeded the goals described in the Ross 708 Management Guide of 845, 1038, and 2124 g for the same ages (Aviagen, 2004).

[2] Texture Technologies Corp, Scarsdale, NY

The feed conversion ratio in each period was comparable to the Management Guide, with statistical differences only evident in the 0-20 day period where the PC outperformed the NC. After processing at 33 and 54 d of age, the carcass and all further processed parts mirrored the BW results for these diet (Tables 5-3 and 5-4). In contrast, the negative control diet significantly impaired BW gains by 191, 290 and 513 g at 20, 33 and 54 days of age measured and carcass weight at by 309 and 739 g at 33 and 54 d of age respectively. Carcass yield was also reduced by 2.4% at 54 d of age but wing and drum stick yields were higher than the PC diet. In addition, fat pad weights were reduced in response to the NC diet at both processing ages, demonstrating that the energy differences between the two diets were substantial. The severity of the aP and Ca deficiencies in the negative control diet was evident as bone breaking strength and percent ash (DM basis) were lower than the positive control diet (Table 5-5).

Phytase and Dietary Restriction

Despite a 90 kcal/kg reduction in ME value, adding phytase to the NC diet increased BW gain and feed intake to the level of the PC diet at all ages (Table 2). This diet also exceeded the Ross 708 Management Guide's performance standards. From 0 to 20 d of age, phytase also reduced feed conversion to the level of the PC diet. At 33 and 54 d of age, phytase increased the absolute weight of the carcass and parts. Fillet yield at 33 d and fat pad yield at both ages were increased to the level of the PC diet. Femur breaking strength and ash content were increased because of phytase supplementation at 33 and 54 d.

Glucanase and Dietary Restriction

The NC diet plus glucanase did not elicit an improvement in BW gain, feed intake, or feed conversion at any age, compared to the negative control alone. The enzyme did not affect any processing parameter measured, femur breaking strength, or femur ash content. The NC diet with phytase and glucanase showed no improvement in any trait measured when compared to the NC plus phytase treatment.

5.4 DISCUSSION

This experiment sought to determine whether phytase could increase the aP level of a corn soybean meal diet by approximately 0.15% and to improve Ca digestibility by approximately 0.2%. These values were derived from bioassay experiments that examined the effect of phytase and glucanase on P and Ca digestibility and retention (Leslie, Dissertation, Chapters 2 and 3). The results from the present study suggest that phytase (Quantum™ 2500D) can be used to replace inorganic P and Ca supplementation at the derived rates without negatively impacting performance. The same bioassay experiments tested the ability of phytase to improve the energy value of corn soybean meal diets, using AME, PE, and IDE as measures of energy availability. None of those experiments found an energetic response to phytase supplementation. In the absence of an energetic response the NC plus phytase diet should have shown poorer live performance, and smaller fat pad than the positive control. This response was not seen, suggesting that there was an energetic effect of phytase in this trial.

Previous research with fibrolytic enzymes has shown that certain enzymes and combinations of enzymes improve the energy value of corn soybean meal diets

(Mathlouthi et al., 2003; Juanpere et al., 2005). Juanpere et al. (2005) supplemented broiler diets with alpha-galactosidase, and saw a decrease in P excretion as a result of the enzyme. The authors attributed the response to a reduction in gut viscosity achieved through digestion of raffinose and stachyose. Mathlouthi et al. (2003) found that an enzyme cocktail (xylanase and beta-glucanase) improved egg production and feed conversion when fed to laying hens. As viscosity in corn soybean meal diets is thought to be attributed to pectin, the glucanase used in this experiment was unlikely to affect viscosity. The energy response seen in previous experiment (Leslie, Dissertation, Chapter 2 and 3) is thought to result from improved access of endogenous enzyme to encapsulated substrates within the feed.

In a recent review, Carre (2004) described three reasons for incomplete starch digestion: structure of the starch granule, anti-nutritional factors, and access to the starch granule. Age-dependant development of the digestive tract may also play a role, as α-amylase activity is limited in newly-hatched chicks (Café et al., 2002). Coarse particles in ground corn may obstruct access to starch granules. While the concentration of glucans in corn is low, approximately 0.1% on a DM basis (Knudsen, 1997), digestion of these compounds may weaken the integrity of the cell wall and allow digestive enzymes access to the cell contents. Previous results measuring IDE (Leslie, Dissertation, Chapter 2 and 3) and microscopic investigation (Leslie, Dissertation, Chapter 2) suggest this is the case. Other experiments using fibrolytic enzymes in wheat- (Peng et al., 2003; Selle et al., 2003; Wu et al., 2004) and corn-based (Mathlouthi et al., 2003; Juanpere et al., 2005) diets have shown an improvement in productive traits resulting from an improvement in energy availability. However, the AME and PE results from a previous experiment

(Leslie, Dissertation, Chapter 2) and the current trial suggest that the energy response obtained by supplementing glucanase was not present at a productive level.

One possible explanation of the current results is that the cells containing undigested starch granules were fermented in the ceca, and a portion of the energy absorbed by the bird in the form of VFAs, reducing the net energetic effect of the enzyme. However, Carre (2004) also stated that the ceca play only a minor role in starch digestion, as only very small particles and liquids can enter the ceca for fermentation.

The potential interaction between phytase and glucanase was of interest as well in this experiment. Other authors have described experiments where fibrolytic enzymes improve the efficacy of phytase due either to a decrease in intestinal viscosity or substrate access (Peng et al., 2003; Sell 2003; Wu et al., 2004). Previous experiments using the current enzymes have failed to find an interaction (Leslie, Dissertation Chapters 2 and 3). As corn is not a viscous grain, it was expected that improved substrate access may result in an improvement in P digestibility that would be evident in the bone traits described in Table 5. This was not the case, and again no interaction was seen between phytase and glucanase for any trait measured. These results suggest that phytate is not encapsulated in intact cells in corn. Reddy et al. (1982) reported that 87% of the phytate in corn was located within the germ, which is not thought to pose a substantial barrier to digestion.

The results of the current experiment support the previous findings that Quantum™ 2500D Phytase can be used to replace 0.15% aP and 0.2% Ca from inorganic sources in broiler diets without negatively impacting performance. The glucanase had no positive effect in any of the traits measured, and the two enzymes together did not have a synergistic effect.

5.6 REFERENCES

Aviagen. 2004. Subject: Ross x Ross 708 North American Broiler Performance Objectives. www.aviagen.com. Accessed May 9, 2006.

Café, M. B., C. A. Borges, C. A. Fritts, and P. W. Waldroup. 2002. Avizyme improves performance of broilers fed corn soybean meal-based diets. J. Appl. Poult. Res. 11:29-33.

Carre, B. 2004. Causes for variation in digestibility of starch amoung feedstuffs. Worlds Poult. Sci. J. 60:76-89.

Classen, H. L. 1996. Cereal grain starch and exogenous enzymes in poultry diets. Anim. Feed Sci. Tech. 62: 21-27.

Iji, P., A. S. Saki, and D. R. Tivey. 2001a. Body and intestinal growth of broiler chicks on commercial starter diets. 1. Intestinal weights and mucosal development. Br. Poult. Sci. 42: 505-513.

Iji, P., A. S. Saki, and D. R. Tivey. 2001b. Body and intestinal growth of broiler chicks on commercial starter diets. 1. Develioment of characteristics of intestinal enzymes. Br. Poult. Sci. 42: 514-522.

Juanpere, J., A. M. Perez-Vendrell, E. Angulo, and J. Brufau. 2005. Assessment of potential interactions between phytase and glycosidase enzymes supplementation on nutrient digestibility in broilers. Poult. Sci. 84: 571-580.

Knudsen, K. E. B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Tech. 67: 319-338.

Mahagna, M., N. Said, I. Nir, and Z. Nitsan. 1995. The development of digestibility of some nutrients and of energy utilization in young broiler chickens. World's Poultry Science Association Proceedings, 10th European Symposium on Poultry Nutrition 70: 2329-2334.

Mathlouthi, N., M. A. Mohamed, and M. Larbier. 2003. Effect of enzyme preparation containing xylanase and beta-glucanase on performance of laying hens fed wheat/barley or maize/soybean meal based diets. Br. Poult. Sci. 44: 60-66.

NRC. 1994. Nutrient Requirements of Poultry (9th Ed.). National Academy Press, Washington, DC.

Noy, Y., and D. Sklan. 1995. Digestion and absorption in the young chick. Poult. Sci. 74: 366-373.

Ouhida, I., J. F. Perez, M. Anguita, and J. Gasa. 2002. Influence of β-mannase on broiler performance, digestibility, and intestinal fermentation. J. Appl. Poult. Res. 11: 1-6.

Parks, S. C., Y. W. Choi, and T. K. Oh. 1999. Comparative enzymatic hydrolysis in various animal feedstuffs with two different phytases. J. Vet. Med. Sci. 61: 1257-1259.

Peng, Y. L., Y. M. Guo, and J. M. Yuan. 2003. Effects of microbial phytase replacing partial inorganic phosphorus supplementation and xylanase on the growth performance and nutrient digestibility in broilers fed wheat-based diets. Asian-Australas. J. Anim. Sci. 16: 239-247.

Pintar, J., B. Homen, K. Gazic, Z. Janjecic, M. Sikiric, and T. Cerny. 2005. Effects of supplemental phytase on nutrient excretion and retention in broilers fed different cereal based diets. Czech. J. Anim. Sci. 50: 40-46.

Ravindran, V. S., G. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influencd by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41: 193-200.

Reddy, N. R., S. K. Sathe, and D. K. Salunke. 1982. Phytates in legumes and cereals. Adv. Food Res. 28: 1-92.

SAS Institute Inc., 2001. The SAS system for Windows, Release 8.02. SAS Institute Inc., Cary, NC, 27315.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Australas. Anim. Sci. 16: 394-402.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate levels of phosphorus. Br. Poult. Sci. 45: 76-84.

TABLE 5-1. Composition of the starter, grower and finisher diets fed from 0 to 20 d of age, including a positive control (Diet 1), a negative control (Diet 2)

	Starter (0-20d)		Grower (20-33d)		Finisher (33-54d)	
	PC (Diet 1)	NC (Diet 2)	PC (Diet 1)	NC (Diet 2)	PC (Diet 1)	NC (Diet 2)
Ingredient (%)						
Corn	51.32	55.87	61.13	64.97	66.75	71.12
Soybean Meal (48% CP)	40.00	39.00	32.10	31.50	27.00	26.00
Poultry Fat	3.80	1.10	2.80	0.20	2.70	0.10
L-Lysine HCl	0.05	0.20	0.07	0.16	0.05	0.15
DL-Methionine	0.43	0.43	0.25	0.25	0.30	0.30
L-Tryptophan	0.05	0.05	0.00	0.03	0.00	0.02
L-Threonine	0.15	0.15	0.05	0.09	0.05	0.10
Biocox[2]	0.05	0.05	0.05	0.05	0.05	0.05
Dicalcium Phosphate (21.5% Ca, 18.5% P)	1.70	0.90	1.15	0.40	1.00	0.50
Limestone	1.50	1.30	1.45	1.40	1.35	1.15
Salt	0.45	0.45	0.45	0.45	0.45	0.45
Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
Phytase[4]	0	0	0	0	0	0
Glucanase[4]	0	0	0	0	0	0
Calculated Composition						
ME (kcal/kg)	3054	2962	3100	3002	3162	3072
CP (%)	23.20	23.20	20.00	20.10	18.00	18.00
Lysine (%)	1.35	1.45	1.15	1.22	1.00	1.06
Methionine (%)	0.78	0.78	0.57	0.57	0.59	0.59
TSAA (%)	1.16	1.17	0.89	0.89	0.88	0.88
Ca (%)	1.04	0.79	0.89	0.7	0.80	0.62
aP(%)	0.45	0.31	0.34	0.20	0.31	0.22

[1] Diets 3, 4, and 5 were composed of the NC (diet 2) plus 500 FTU, 50 units glucanase, and 500 FTU plus 50 units glucanase per kg respectively.
[2]Alpharma, New Jersey. Provided 60 ppm salinomycin in the finished diet.
[3] The vitamin and mineral premix provided the following per kg of diet: 7500 IU Vitamin A, 2500 IU Vitamin D_3, 8 IU Vitamin E, 2 mg Vitamin K_2, 0.02 mg Vitamin B_{12}, 5.5 mg riboflavin, 37 mg niacin, 13 mg d-pantothenic acid, 0.5 mg folic acid, 2.2 mg pyridoxine, 1 mg thiamine, 0.1 mg biotin, 500 mg choline, 125 mg ethoxyquin, 66 mg Mn, 55 mg Zn, 6 mg Fe, 6 mg Cu, 0.15 mg Se, 1 mg I.
[4] Ingredients expressed as units of activity per kg diet on an "as is" basis.

TABLE 5-2. Live performance of broilers fed diets supplemented with phytase and/or glucanase from 0 to 20 d, 20 to 33 d and 33 to 54 d of age

	BW Gain[1]	Feed Intake	Feed to Gain Ratio	Total Mortality	Cumulative Feed to Gain Ratio	Final BW
	(g)	(g)		(%)		(g)
	----------0 to 20 days----------					
Positive Control	845[a]	1085[a]	1.29[b]	0.0[b]		884 [a]
Negative Control	654[b]	911[b]	1.39[a]	1.3[ab]		687 [b]
NC + Phytase[2]	812[a]	1060[a]	1.31[b]	4.7[a]		842 [a]
NC + Glucanase[3]	663[b]	885[b]	1.34[ab]	1.3[ab]		698 [b]
NC + Phytase + Glucanase	796[a]	1062[a]	1.34[ab]	2.0[ab]		829 [a]
SEM	13.5	17.8	0.016	0.04		15.0
	----------P Value----------					
Treatment	0.0001	0.0001	0.0008	NS		0.0001
	----------20 to 33 days----------					
Positive Control	1038[a]	1766[a]	1.70	1.3	1.514	1922 [a]
Negative Control	748[b]	1316[b]	1.76	1.3	1.576	1440 [b]
NC + Phytase	1009[a]	1698[a]	1.68	0.7	1.516	1859 [a]
NC + Glucanase	784[b]	1348[b]	1.77	2.7	1.545	1485 [b]
NC + Phytase + Glucanase	1040[a]	1772[a]	1.71	0.7	1.550	1874 [a]
SEM	37.7	43.1	0.047	0.89	0.0235	38.4
	----------P Value----------					
Treatment	0.0001	0.0001	NS	NS	NS	0.0001
	----------33 to 54 days----------					
Positive Control	2124[a]	4398[a]	2.07	1.3	1.816	4046 [a]
Negative Control	1611[b]	3156[b]	1.97	6.7	1.784	3051 [b]
NC + Phytase	2078[a]	4212[a]	2.03	2.0	1.788	3938 [a]
NC + Glucanase	1653[b]	3239[b]	1.98	8.7	1.763	3138 [b]
NC + Phytase + Glucanase	2137[a]	4326[a]	2.03	0.7	1.805	4011 [a]
SEM	63.4	98.8	0.0547	1.80	0.0254	64.6
	----------P Value----------					
Treatment	0.0001	0.0001	NS	0.005	NS	0.0001

[a-b] Means within a column with a common superscript do not differ (P > 0.05)

[1] Mean in initial BW was 38.8 g, and was not different between treatments

[2] Phytase supplemented at 500 FTU per kg diet on an "as is" basis

[3] Glucanase supplemented at 50 units per kg diet on an "as is" basis

TABLE 5-3. Carcass and abdominal fat yields of broilers fed corn soybean meal diets supplemented with phytase and/or glucanase from 0 to 33 and 0 to 54 d

	Carcass without fat[1]		Abdominal fat[2]	
	(g)	(%)	(g)	(%)
	---------- 33 d----------			
Positive Control	1232 [a]	63.8	19.0 [a]	1.49 [a]
Negative Control	923 [b]	63.4	11.3 [b]	1.16 [ab]
NC + Phytase[3]	1186 [a]	63.7	17.2 [a]	1.41 [a]
NC + Glucanase[4]	920 [b]	64.0	9.0 [b]	0.93 [b]
NC + Phytase + Glucanase	1203 [a]	63.9	15.4 [ab]	1.26 [a]
SEM	14.4	0.26	0.26	0.09
	----------P Value----------			
Treatment	0.0001	NS	0.0001	0.0001
	----------54 d----------			
Positive Control	2821 [a]	69.8 [a]	43.9 [a]	1.55 [a]
Negative Control	2082 [b]	67.4 [b]	19.9 [b]	0.91 [b]
NC + Phytase	2750 [a]	70.3 [a]	43.3 [a]	1.56 [a]
NC + Glucanase	2096 [b]	67.0 [b]	21.4 [b]	0.98 [b]
NC + Phytase + Glucanase	2780 [a]	69.3 [a]	47.5 [a]	1.69 [a]
SEM	42.0	0.41	1.85	0.07
	----------P Value----------			
Treatment	0.0001	0.0001	0.0001	0.0001

[a,b] Means within a column with a common superscript do not differ (P > 0.05).
[1] Carcass without neck and giblets after 4 hr of slush-ice chilling followed by removal of abdominal fat, expressed on an absolute basis and relative to the full-fed live weight.
[2] Depot fat removed from the abdominal cavity, expressed on an absolute basis and relative to the chilled carcass.
[3] Phytase supplemented at 500 FTU per kg diet on an “as is” basis.
[4] Glucanase supplemented at 50 units per kg diet on an “as is” basis.

TABLE 5-4. Further processing yields for broilers fed corn soybean meal diets supplmented with phytase and/or glucanase from 0 to 33 d or 0 to 54 d of age[1]

	Fillets		Tenders		Wings		Drums		Thighs		Trim	
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
	33 Days of Age											
Positive Control	304 [a]	24.7 [ab]	66 [a]	5.3	142 [a]	11.6	169 [a]	13.8	174 [a]	13.8	108 [a]	8.8
Negative Control	216 [b]	23.5 [b]	47 [b]	5.1	113 [b]	12.4	129 [b]	14.1	121 [b]	14.1	88 [ab]	9.1
NC + Phytase[2]	294 [a]	24.8 [a]	64 [a]	5.4	138 [a]	11.7	163 [a]	13.8	168 [a]	13.8	97 [ab]	8.2
NC + Glucanase[3]	220 [b]	23.6 [b]	46 [b]	5.4	124 [ab]	14.2	125 [b]	13.7	118 [b]	13.7	77 [b]	7.9
NC + Phytase and Glucanase	296 [a]	24.6 [ab]	66 [a]	5.5	137 [a]	11.5	165 [a]	13.8	173 [a]	13.8	110 [ab]	9.1
SEM	4.7	0.27	1.1	0.13	5.2	0.75	2.3	0.15	2.7	0.15	8.1	0.99
	P Value											
Treatment	0.0001	0.0003	0.0001	NS	0.0001	NS	0.0001	NS	0.0001	0.0001	0.03	NS
	54 Days of Age											
Positive Control	757 [a]	26.8	171 [a]	6.0	297 [a]	10.6 [b]	368 [a]	13.1 [b]	405 [a]	14.4	277 [a]	9.8 [a]
Negative Control	565 [b]	27.0	120 [b]	5.7	237 [b]	11.5 [a]	283 [b]	13.7 [a]	280 [b]	13.4	187 [b]	8.9 [ab]
NC + Phytase	737 [a]	26.7	163 [a]	5.9	290 [a]	10.6 [b]	367 [a]	13.4 [ab]	398 [a]	14.5	249 [a]	9.0 [ab]
NC + Glucanase	565 [b]	26.7	125 [b]	5.9	237 [b]	11.5 [a]	285 [b]	13.6 [a]	286 [b]	13.5	185 [b]	8.8 [ab]
NC + Phytase and Glucanase	760 [a]	27.4	168 [a]	6.1	292 [a]	10.5 [b]	369 [a]	13.3 [ab]	415 [a]	14.9	241 [a]	8.6 [b]
SEM	13.9	0.26	3.77	0.11	4.1	0.10	5.8	0.13	8.0	0.18	8.5	0.28
	P Value											
Treatment	0.0001	NS	0.0001	NS	0.0001	0.0001	0.0001	0.003	0.0001	NS	0.0001	0.01

[a,b] Means within a column with a common superscript do not differ (P > 0.05).

[1] Yields are expressed on an absolute basis and as a percentage of the chilled carcass.

[2] Phytase supplemented at 500 FTU per kg diet on an “as is” basis.

[3] Glucanase supplemented at 50 units per kg diet on an “as is” basis.

TABLE 5-5. Femur breaking strength and ash content from broilers fed corn soybean meal diets from 0 to 33 d or 0 to 54 d of age

		Breaking Strength	Ash Content
		(kg)	(%)
		----------33 Days----------	
Positive Control		25.0 [ab]	29.7 [a]
Negative Control		10.3 [c]	24.8 [b]
NC + Phytase[1]		26.0 [a]	29.8 [a]
NC + Glucanase[2]		11.0 [c]	25.5 [b]
NC + Phytase + Glucanase		23.8 [b]	29.9 [a]
	SEM	0.42	0.39
		----------P Value----------	
		0.0001	0.0001
		----------54 Days----------	
Positive Control		41.1 [a]	26.4 [ab]
Negative Control		22.1 [b]	25.0 [bc]
NC + Phytase		43.4 [a]	27.5 [a]
NC + Glucanase		24.5 [b]	24.5 [c]
NC + Phytase + Glucanase		42.1 [a]	27.4 [a]
	SEM	0.68	0.46
		----------P Value----------	
Treatment		0.0001	0.0001

[a-c] Means within a column with a common superscript do not differ (P > 0.05)

[1] Phytase supplemented at 500 FTU per kg diet on an "as is" basis

[2] Glucanase supplemented at 50 units per kg diet on an "as is" basis

6.0 CONCLUSIONS

The opening chapter described in some detail the negative effects phytate and dietary fiber can have on the digestion and utilization of poultry feedstuffs. It has been well established in the literature that phytate not only prevents the absorption of plant source phosphorus (Reddy et al., 1982; Ballam et al., 1984; Qian et al., 1997), but also reduces the availability of other minerals and protein in poultry diets (Evans and Pierce, 1981; Cheryan et al., 1983; Pallauf et al., 1994). As well, it has been shown that phytate can increase the endogenous losses associated with digestion and absorption, thereby reducing the feeding value of a diet (Cowieson et al., 2004). In corn-based diets, dietary fibers impede digestion primarily through encapsulation of nutrients within indigestible cell walls, preventing utilization of the cell contents (Classen, 1996; Carre, 2004). Previous experiments have tested fibrolytic enzymes, alone and in combination with phytase, and found a beneficial effect on AME and live production parameters (Peng et al., 2003; Selle et al., 2003; Juanpere et al., 2005; Meng et al., 2005). The goal of the experiments described in the preceding chapters was to determine the effect of phytase and a glucanase, alone and in combination, in corn soybean meal diets fed to broilers.

Experiments described in Chapters 2 and 3 were primarily concerned with measuring the effects of phytase and glucanase on energy digestion and utilization. In the first set of experiments, glucanase was shown to improve the ileal digestible energy

value of corn soybean meal diets by close to 300 kcal/kg, with no effect on productive energy. There was a significant AME advantage as well, but only in diets containing adequate aP. It was thought that cecal fermentation in the ceca may have reduced the treatment effects on the energy content of the digesta to the degree that differences were not detectable in the excreta (Carre et al., 1995).

As the error associated with AME values determined through use of an acid insoluble ash marker (Experiment 2, Chapter 2) was double that of the error associated with the total collection method (Experiment 1, Chapter 2), it seemed probable that differences between treatments were simply within the error of the experiment. Likewise, the error associated with PE was high, and likely obscured any treatment effect. To further investigate the effect of glucanase on IDE, another bioassay experiment was performed using corn and soybean meal separately. This experiment was repeated at three different ages in order to determine if the effects were consistent over the various stages of GIT development. This experiment showed that glucanase improved the IDE of both corn and soybean meal. While the IDE values of the feedstuffs appeared to change between the age groups, the effect of glucanase seemed relatively consistent for each group. The effects of phytase in these experiments were also consistent, in that no energy effect was seen in any of the experiments. There was a transient increase in DM digestibility of the feed as a result of phytase supplementation, but this did not give rise to an improvement in IDE, AME or PE.

In an attempt to explain improvements in CP digestibility seen in Chapter 2, the impact of phytase on luminal and pancreatic activities of amylase and total proteases was measured. Previous research has suggested that phytate may interfere with the activities

of amylase and trypsin, possibly by direct association with the enzymes themselves (Singh and Krikorian, 1982; Deshpande and Cheryan, 1984; Knuckles and Betschart, 1987). The results showed no consistent effect of either exogenous enzyme on the activities of amylase or proteases. It is possible that phytate may reduce the activity of these enzymes through either chelation of Ca, necessary for amylase activity or association with the substrate. As trypsin cleaves proteins at bonds adjacent to lysine and arginine, phytate bound to these same amino acids may inhibit trypsin's action.

Also measured were the mineral and CP digestibility values of feeds supplemented with glucanase and phytase. Phytase was shown to improve ileal digestibility of P from 13.5% to 31.5% in adequate aP diets, and the retention of P from 35.5% to 57.6% in low aP diets. As well, the retention of calcium, and the digestibility and retention of CP were improved by phytase supplementation. Glucanase improved the digestibility of Zn, Cu, and Mg, and improved the retention of Ca, with no effect on CP or other minerals. Somewhat surprising was the lack of any interaction between glucanase and phytase for any trait measured in Chapters 2 and 3.

In Chapter 4, an experiment designed to provide information on the action of phytase through the digestive tract of broilers was performed. This experiment used the appearance of the products of phytate degradation to determine the location and extent of IP6 digestion through the digestive tract. As well, the effect of day length was investigated through the application of 24 or 12 hours light per day. As expected by the pH of the digestive tract, the majority of digestion of IP6 occurred in the crop, proventriculus and gizzard. As well, decreased day length improved the extent of phytate degradation. Phytase improved total ileal IP6 digestibility from 40.8% to 62.2%.

The final experiment was performed in order to confirm the observations made in the previous experiments. A positive control (PC) and negative control (NC) diet were formulated, with the NC containing about 0.15% less aP and 0.20% less calcium. The diet was also lower in energy, as it was formulated using specifications for corn and soybean meal that were 100 kcal/kg higher than the positive control. These reductions were in line with improvements in digestibility seen in the previous experiments. The results showed that phytase supplementation of the NC improved live performance, meat yields and bone breaking strength to the same level as the PC. Glucanase supplementation did not improve any of the traits measured compared to the negative control, suggesting an energetic effect from this enzyme was not realized in a practical setting. Again, no interaction between phytase and glucanase was observed.

The lack of response in the final experiment with regard to glucanase was somewhat puzzling, as its effect in previous experiments on IDE was fairly consistent. In a review by Carre (2004), one of the man factors affecting starch digestibility was said to be access of endogenous enzymes to the starch granule. Encapsulation can occur, where the cell was is not disrupted by grinding, pelleting or physical digestion, and chemical digestion is incapable of digesting the cellulose and hemicellulose that composes the cell wall. It is thought that improvements in the feeding value of non-viscous grains seen as a result of supplementing fibrolytic enzymes arises through disruption of these cell walls, providing access for endogenous enzymes (Classen, 1996). The cell wall need not be completely digested, rather a loosening of the structure is thought to provide enough access to permit digestion. The IDE results indicate that the glucanase used had this effect, and resulted in an improvement in starch digestibility. It is unlikely that digestion

of fiber alone accounted for the improvement in IDE, as the quantity of glucan in corn and soybean meal is low (Bach Knudsen, 1997).

The fact that the improvement in energy digestibility was not seen on a productive level suggests that the net effect of the enzyme is reduced by the microbial population of the ceca. While endogenous enzymes found in poultry cannot digest the cell walls, the microflora of the ceca can (Crittenden et al., 2002). Fermentation of the cell walls and the encapsulated starch, and subsequent absorption of the resulting VFAs may account for the results seen in the final chapter. Muller et al. (1989) suggest that the energy obtained through VFA absorption can amount to 70% of that obtained through directly absorbing the sugars. A study by Carre et al. (1995) suggested that, accounting for losses of VFAs in the excreta, broilers likely obtain 50% of the energy present in the sugars through fermentation and VFA absorption. It is possible that this mechanism reduced the net effect of glucanase. This potential explanation is supported subjectively by the micrographs found at the end of Chapter 2. These pictures show the starch granules, stained in black, in the ileum and excreta of birds with and without supplemental glucanase. Unsupplemented diets appear to have a greater quantity of starch in the ileal digesta than glucanase supplemented diets, while excreta in both treatments contains little starch. In order to confirm this possible explanation, more experiments are required.

It was thought that the supplementation both phytase and glucanase would result in a synergistic effect. Much as glucanase can improve access of endogenous enzymes to their substrates, it was thought the enzyme might improve the access of phytase to phytate. However, no interaction was seen between the two enzymes in any of the experiments performed. This perhaps is a result of the location of phytate within the corn

kernel. The bulk of the phytate, as much as 87%, is located within the germ (Reddy et al., 1982). Encapsulation of nutrients is thought to occur primarily in the endosperm, rather than the germ. As little phytate is located in the endosperm, it is possible that phytate in corn is not encapsulated to any great degree. Other experiments using fibrolytic enzymes in combination with phytase have found interactions between the two (Peng et al., 2003; Selle et al., 2003; Juanpere et al., 2005). These experiments, however, have predominantly been performed in wheat based diets. The interaction in this case may result from an increase in diffusion of phytase in the digestive tract, and disruption of the aleurone layer of the wheat seed. In contrast to corn, the bulk of phytate in wheat is located within the aleurone layer, which provides substantial potential for encapsulation.

These experiments show that Quantum™ 2500D phytase can be used in corn soybean meal diets to replace 0.15% aP and 0.20% Ca. The glucanase used requires additional research to determine conditions for successful use in poultry rations.

6.1 REFERENCES

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Tech. 67: 319-338.

Ballam, G. C., T. S. Nelson, and L. K. Kirby. 1984. Effect of fiber and phytate source and of calcium and phosphorus level on phytate hydrolysis in the chick. Poult. Sci. 63: 333-338.

Carre, B. 2004. Causes for variation in digestibility of starch among feedstuffs. Worlds Poult. Sci. J. 60: 76-89.

Carre, B., J. Gomez, and A. M. Chagneau. 1995. Contribution of oligosaccharide and polysaccharide digestion, and excreta losses of lactic acid and short chain fatty acids, to dietary metabolizable energy values in broiler chickens and adult cockerels. Br. Poult. Sci. 36: 611-629.

Cheryan, M., F. W. Anderson, and F. Grynspan. 1983. Magnesium-phytate complexes: Effects of pH and molar ratio on solubility characteristics. Cereal Chem. 60: 235-237.

Classen, H. L. 1996. Cereal grain starch and exogenous enzymes in poultry diets. Anim. Feed Sci. Tech. 62: 21-27.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effect of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci, 45: 101-108.

Crittenden, R., S. Karppinen, S. Ojanen, M. Tenkanen, R. Fagerstrom, J. Matto, T. Mattila-Sandholm, and K. Poutanen. 2002. In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. J. Sci. Food Agric. 82: 781-789.

Deshpande, S. S., and M. Cheryan. 1984. Effects of phytic acid, divalent cations, and their interactions on alpha-amylase activity. J. Food Sci. 49: 516-520.

Evans, W. J., and A. G. Pierce. 1981. Calcium-phytate complex formation studies. J. Am. Oil Chem. Soc. 58: 850-851.

Juanpere, J., A. M. Perez-Vendrell, E. Angulo, and J. Brufau. 2005. Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. Poult. Sci. 84: 571-580.

Knuckles, B. E., and A. A. Betschart. 1987. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J. Food Sci. 52: 719-721.

Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell, and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase and their effect on nutrient utilization and broiler performance. Poult. Sci. 84: 37-47.

Muller, H. L., M. Kiechgessner, and F. X. Roth. 1989. Energy utilization of intracaecally infused carbohydrates and casein in sows. Pages 123-126 in Energy Metabolism of Farm Animals. ed. Y. van der Honing and W. H. Close, Wageningen, The Netherlands.

Pallauf, J., G. Rimbach, S. Pippg, B. Schindler, and E. Most. 1994. Effect of phytase supplementation to a phytate-rich diet based on wheat, barley and soya on the bioavailability of dietary phosphorus, calcium, magnesium, zinc, and protein in piglets. Agribiol. Res. 47: 39-48.

Peng, Y. L., Y. M. Guo, and J. M. Yuan. 2003. Effects of microbial phytase replacing partial inorganic phosphorus supplementation and xylanase on growth and nutrient digestibility in broilers fed wheat-based diets. Asian-Australas. J. Anim. Sci. 16:239-247.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium;total phosphorus ratio in broiler diets. Poult. Sci. 76: 37-46.

Reddy, N. R., S. K. Sathe, and D. K. Salunke. 1982. Phytates in legumes and cereals. Adv. Food Res. 28: 1-92.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilization of broilers offered wheat-based diets. Asian-Austrailas. J. Anim. Sci. 16: 394-402.

Singh, M., and A. D. Krikorian. 1982. Inhibition of trypsin activity in vitro by phytate. J. Agric. Food Chem. 30: 799-800.