FEED ENZYMES AND WHOLE WHEAT IN POULTRY DIETS

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ABSTRACT

Seven studies were undertaken to examine the effects of microbial phytase, glycanases, and whole wheat feeding in broiler diets. The major focus of this doctoral research was to investigate the effects of a microbial phytase produced by solid state fermentation in broiler diets.

1. The aim of the first study was to examine the effects of microbial phytase on the performance, apparent ileal digestibility of phosphorus (P), phytate P and nitrogen, and utilisation of nutrients in male and female broilers fed wheat-soy diets from 1 to 42 day of age. There were eight dietary treatments. Diets 1 to 4 were supplemented with inorganic phosphorus to contain 0.30, 0.36, 0.42 and 0.48% of non-phytate P (nP), respectively in the starter phase (1-21 day) and 0.20, 0.26, 0.32 and 0.48% in the finisher phase (22-42 day), respectively. Diets 5 to 8 were based on diet 1 and supplemented with phytase to contain 500, 1000, 1500 and 2000 PU/kg diet, respectively. Within sex, each of the eight dietary treatments was assigned to five pens of eight birds each. In both sexes, weight gain (P<0.05 to 0.001), feed efficiency (P<0.05 to 0.001) and toe ash contents (P<0.001) were increased as the P or phytase were added to low-P diet (diet 1). The magnitude of increments in performance and toe ash contents parameters were greatest with the first addition of P or phytase and then tended to plateau with further additions. Feed efficiency of birds fed phytase-supplemented diets was superior to those fed adequate-P diets. Toe ash contents of birds fed low-P diet with 500 PU/kg diet were comparable to those fed adequate-P diets. Addition of 500 PU/kg phytase to the low-P diet increased the nitrogen digestibility by 3.1 and 5.3% in males and females, respectively. Addition of phytase increased the apparent metabolisable energy (AME) of wheat-soy diets during the starter and finisher phases, but the increments were greater in the finisher diets. Based on weight gain responses to graded additions of supplemental non-phytate P and phytase, estimates were obtained for P equivalency of the microbial phytase. These estimates translate into P release values of 72 to 131% from phytate and are apparently spurious. These unexpectedly high equivalency estimates may be due, partly, to the secondary enzymes activities present in the phytase product evaluated.

2. Selected data from the first study were analysed to compare the influence of sex on the performance, toe ash contents, phytate P release, AME and digestibility of nutrient

in broilers fed diets containing low and adequate dietary levels of phosphorus. Sex of broilers had no effect on AME values determined during week 3. During week 6, the AME values for male broilers were higher ($P<0.01$) than those for the females. Female broilers tended ($P<0.10$) have a higher ileal nitrogen digestibility than the males. Apparent ileal phytate-P degradation values in males were higher than those in females (0.282 vs 0.234), but the differences were not significant ($P>0.05$). A significant interaction ($P<0.05$) between nP level x sex was observed for apparent ileal P digestibility. Increasing dietary nP levels increased apparent ileal P digestibility in both males and females, but the improvements were higher in females (13.4 vs 6.1 percentage units).

3. The aim of the second study was to examine the influence of microbial phytase addition on the performance, toe ash contents and nutrient utilisation of male broilers fed diets based on corn and wheat. The experiment was conducted as a 2 x 2 x 2 factorial arrangement of treatments. Within the factorial, two diet types (corn-soy or wheat-soy) containing two levels of non-phytate P (0.30 or 0.45%) were evaluated and each level of non-phytate P was supplemented with 0 or 500 PU phytase/kg diet. The results showed that microbial phytase was effective in both corn-based and wheat-based diets and that with supplemental 500 PU phytase/kg, dietary P level can be lowered by 0.15% to reduce excreta P output by 35% and still maintain comparable growth performance and bone mineralisation to birds fed a diet containing adequate levels of P. Phytase addition improved the AME values of wheat-based diets, but had little effect on the AME of corn-based diets. Phytase improved ileal nitrogen digestibility in both diet types, but the responses to added phytase tended to be higher in wheat-based diets, as shown by a diet type x phytase interaction ($P<0.10$).

4. The aim of the third study was to examine the influence of phytase and glycanases, individually or in combination, on the AME and nutrient digestibility of sorghum, corn, wheat and barley using 4-week-old broilers. Microbial phytase improved ($P<0.05$) apparent ileal phosphorus digestibility in all cereals. Phytase supplementation improved ($P<0.05$) the AME of corn and barley, and numerically improved the AME in sorghum and wheat. Further improvements ($P<0.05$) in the AME of wheat and barley were observed when the phytase was combined with glycanases. The observed improvements in AME were not always associated with enhanced digestibility of protein and starch.

5. In the fourth experiment, potential beneficial effects from the side activities present in

a microbial phytase produced by the solid state fermentation were examined by comparing the release of phosphorus, reducing sugars and α-amino nitrogen by two other phytase preparations in wheat- and corn-based diets using an *in vitro* digestion model. Microbial phytase produced by solid state fermentation released more ($P<0.05$) phytate-bound P (11.0% and 7.8% in wheat- and corn-based diets, respectively) and α-amino nitrogen (1.7% and 6.2% for wheat- and corn-based diets, respectively) than a phytase produced by submerged liquid fermentation without detectable side activities. Phytase produced by solid state fermentation also released 2.9% more reducing sugars in wheat-based diets and 6.2% α-amino nitrogen in corn-based diets. The superiority of microbial phytase produced by solid state fermentation in releasing nutrients in both types of diets is likely to be due to the presence of other enzyme activities.

6. In the fifth experiment, the influence of microbial phytase and xylanase, individually or in combination, on the performance, AME, digesta viscosity, digestive tract measurements and gut morphology in broilers fed wheat-soy diets containing adequate P levels were examined. The experimental diets were formulated by supplementing the basal diet with xylanase (1000 XU/kg), phytase (500 PU/kg) or combination of phytase and xylanase. The results showed that microbial phytase was as effective as xylanase in improving the performance of broilers. This may be due to the phytase product used in the study was produced by solid state fermentation and contained relatively high levels of β-glucanase, xylanase and protease. Supplemental phytase improved ($P<0.05$) the weight gains and feed efficiency by 17.5 and 2.9%, respectively. Corresponding improvements due to the addition of xylanase were 16.5 and 4.9%, respectively. Combination of phytase and xylanase had no further effects. The improved performance by supplemental phytase or xylanase was associated with reduced digesta viscosity, improved AME, and reduced relative weight and length of small intestine. Phytase and xylanase supplementation had no effect ($P>0.05$) on villus height, crypt depth, goblet cell number, epithelium thickness, and ratio of crypt depth to villus height in duodenum, jejunum and ileum. The only exception was that addition of phytase increased ($P<0.05$) villus height in the duodenum and decreased ($P<0.05$) the number of goblet cells in the jejunum compared to those in the unsupplemented basal diet. Interestingly, xylanase supplementation tended ($P<0.10$) to increase goblet cell numbers in the duodenum and decreased ($P<0.05$) crypt depth in the jejunum.

7. Whole grain feeding for broilers has received attention in recent years due to

associated economic benefits. The aim of the sixth experiment was to examine the influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digesta viscosity, and digestive tract measurements of broilers fed wheat-based diets. A 3 x 2 of factorial arrangement of treatments was used with three diet forms (64.8% ground wheat [GW], GW replaced with 20% of whole wheat before [WW1] or after cold-pelleting [WW2]) and two enzyme doses (0 and 1000 XU/kg diet). The results demonstrated the beneficial effects of whole wheat inclusion and xylanase supplementation in broiler diets. Birds fed diets containing whole wheat had improved (P<0.05 to 0.001) weight gain (2.1-3.9%), feed efficiency (4.1-5.8%) and AME (3.6-6.0%) compared to those fed diets containing ground wheat. The relative gizzard weights of birds fed WW2 diets were higher (P<0.05) than those fed GW and WW1 diets. Pre-pelleting inclusion of whole wheat had no effect (P>0.05) on the relative gizzard weights. Post-pelleting inclusion of whole wheat resulted in greater improvements (P<0.05 to 0.001) in feed efficiency, AME and relative gizzard weights compared to the pre-pelleting treatment. Improved performance with post-pelleting inclusion of whole wheat was probably due to the development of gizzard and to improved AME. However, it is difficult to propose a mechanism for the improvements observed with pre-pelleting inclusion of whole wheat. Improvements in bird performance by xylanase supplementation were associated with reduced digesta viscosity and improved AME. Neither xylanase supplementation nor whole wheat inclusion influenced (P>0.05) the relative weights and length of the duodenum, jejunum, ileum or total small intestine.

8. The aim of the seventh study was to examine the influence of post-pelleting inclusion of whole wheat and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broilers fed wheat-soy diets from 1 to 35 days of age. There were five dietary treatments. Diet 1 was based on corn and soybean meal. Diets 2 and 3 were based on ground wheat (GW) and soybean meal without and with added xylanase at a level of 1000 XU/kg, respectively. Diets 4 and 5 were whole wheat (WW) replacing GW (10 and 20% whole wheat replacing GW during 1-21 and 22-35 day, respectively) without and with added xylanase at a level of 1000 XU/kg, respectively. Post-pelleting inclusion of whole wheat reduced (P<0.10) weight gains, but improved (P<0.05) the feed efficiency over the 35-day experimental period. Improved feed efficiency with whole wheat inclusion was associated with the

development of gizzard. Xylanase supplementation improved the performance of broilers fed both ground wheat and whole wheat diets. Interestingly, feed efficiency of birds fed diets with whole wheat and supplemental xylanase were comparable to those fed corn-based diets. The 'apparent additivity of the combination of whole wheat and xylanase suggests that the mechanisms involved are different. Neither whole wheat inclusion nor xylanase supplementation influenced ($P>0.05$) the relative weight and length of the small intestine, carcass recovery and relative weights of breast muscle and abdominal fat pad.

PUBLICATIONS

Studies completed during candidature, some of which are reported in this thesis have been presented in the following conference proceedings:

Ravindran, V.; Wu, Y.B.; Thomas, D.V.; Camden, B.J.; Morel, P.C.H. and Hendriks, W.H. (2001) Improving phosphorus availability in broiler diets based on wheat-soybean meal using microbial phytase produced in solid state fermentation. In: *Biotechnology in the Feed Industry, Proceedings of Alltech's 17th Annual Symposium* (Lyons, T.P. and Jacques, K.A.; editors), pp. 461-490. Nottingham University Press, Nottingham, United Kingdom.

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LIST OF ABBREVIATIONS

AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
AIND	Apparent ileal nitrogen digestibility
AIPD	Apparent ileal phosphorus digestibility
AISD	Apparent ileal starch digestibility
Arg	Arginine
BW	Body weight
Ca	Calcium
CB	Corn-based diet
Co	Cobalt
Cu	Copper
Cys	Cysteine
DCP	Dicalcium phosphate
DFP	Defluorinated phosphate
DM	Dry matter
EGP	Egg production
EP	Experimental period
FCR	Feed conversion ratio
Fe	Iron
GE	Gross energy
GMD	Geometric mean diameter
His	Histidine
Hrs	Hours
I	Iodine
Ile	Isoleucine
Lys	Lysine
Leu	Leucine
Mg	Magnesium
MCP	Monocalcium phosphate
Met	Methinonine
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
nP	Non-phytate phosphorus
NSP	Non-starch polysaccharides
P	Phosphorus
Phe	Phenylalanine
SAA	Sulphur amino acids
Se	Selenium
Ser	Serine
SSF	Solid state fermentation
1,25-$(OH)_2D_3$	1,25-dihydroxycholecalciferol
Thre	Threonine
Val	Valine
WB	Wheat-based diet
Zn	Zinc

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

GENERAL INTRODUCTION

A large portion of poultry diets consists of plant-derived ingredients that contain a number of anti-nutritional factors. Phytate and non-soluble polysaccharides (NSP) are two anti-nutritional factors found in common feedstuffs. Nutritionally significant amounts of NSP are found in viscous grains such as wheat, oats, barley and rye, and in legume seeds, while phytate is found in all plant-derived ingredients. Both NSP and phytate have negative effects on the digestion of nutrients, resulting in poor bird performance (Ravindran *et al.*, 1995; Choct, 1997; Kornegay, 1999).

With advances in biotechnology and fermentation processes, cost of production of feed enzymes has dramatically reduced, and the use of feed enzymes in poultry diets has become popular. The increased use of feed enzymes during the past decade is also due to a better understanding of the structure of substrates and the mode of action of the enzymes. One or more of the following mechanisms contribute to the beneficial effects of enzymes on bird performance (Bedford and Schulze, 1998): (i) degradation of specific bonds in ingredients not usually degraded by endogenous enzymes, (ii) degradation of anti-nutritional factors that lower nutrient digestibility (iii) disruption of endosperm cell wall integrity and release of nutrients that are bound to or entrapped by the anti-nutritional factors, and/or (iv) supplementation of endogenous digestive enzymes, which are insufficient or absent in young animals.

Glycanases (xylanase and β-glucanase) are widely used by the poultry industry to overcome the negative effects on birds fed diets based on wheat or barley. The effects of glycanases in improving nutrient utilisation and bird performance are well established (Annison and Choct, 1991; McNab, 1992; Bedford and Schulze, 1998). More recently, microbial phytases that target the phytate-complexes in plant-derived ingredients have also become commercially available. This group of enzyme has attracted attention because of its ability to release phytate-bound phosphorus (P), thereby reducing P output in the manure from intensive livestock operations which is a major problem in many parts of the world.

Two types of fermentation technology are available for the commercial production of phytase. One involves sub-merged liquid fermentation and the other solid

state fermentation. Most of the currently available commercial phytases are produced by the former technology, but a microbial phytase produced by solid state fermentation has recently become available. Because of the fermentation technology used, the phytase produced by solid state fermentation contains side activities of several enzymes including protease, β-glucanase and xylanase. The effectiveness of microbial phytases produced by submerged liquid fermentation in poultry diets in improving P bioavailability in poultry diets and minimising P excretion in the manure is now well documented (Coelho and Kornegay, 1999). However, limited studies have been done to evaluate the efficacy of microbial phytase produced by the solid state fermentation in poultry diets.

The major focus of this thesis is on the evaluation of a microbial phytase product, produced by solid state fermentation, in poultry diets. The effects of glycanases and whole wheat in poultry diets were also evaluated.

This thesis consists of ten chapters. The first two chapters have been constructed to form a framework for the experimental research with Chapter 1 providing the rationale for the focus of the research. A review of current literature pertaining to various aspects of the use of microbial phytase in poultry diets is presented in Chapter 2 which form the basis for Chapters 3 to 10 by highlighting the issues requiring further experimental research. Chapters 3 through 10 present the research experiments. Each chapter contains the research aims, abstract, introduction, materials and methods, results and discussion. The chapters include,

i. The effects of microbial phytase on the performance, toe ash contents, digestibility and utilisation of nutrients in male and female of broilers fed wheat-soy diets (Chapter 3).
ii. The influence of sex on nutrient utilisation in broilers (Chapter 4).
iii. The effects of microbial phytase on the performance and nutrient utilisation of broilers as influenced by diet type (Chapter 5).
iv. The influence of microbial phytase and glycanases, individually or in combination, on energy utilisation and nutrient digestibility of cereals for broilers (Chapter 6).
v. Comparison of *in vitro* nutrient release by three phytase preparations in wheat- and corn-based diets (Chapter 7).

vi. The influence of microbial phytase and xylanase, individually or in combination, on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate P levels (Chapter 8).

vii. The influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digesta viscosity and digestive tract measurements of broilers (Chapter 9).

viii. The influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broilers (Chapter 10).

Chapter 11 is a general discussion of the results of each of the experiments. This chapter concludes the research, addresses the major findings and suggests areas for future research.

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Chapter 2

Review of the Literature

INTRODUCTION

A large portion of poultry diets consists of plant-derived ingredients that contain a number of anti-nutritional factors. Phytate and non-soluble polysaccharides (NSP) are two anti-nutritional factors found in common feedstuffs. Nutritionally significant amounts of NSP are found in viscous grains such as wheat, oats, barley and rye, and in legume seeds, while phytate is found in all plant-derived ingredients. Both NSP and phytate have negative effects on the digestion of nutrients, resulting in poor bird performance (Ravindran *et al.*, 1995a; Choct, 1997; Kornegay, 1999).

The use of feed enzymes in poultry diets has been extensively researched during the past decade (Bedford and Schulze, 1998; Coelho and Kornegay, 1999). Different types of feed enzymes have been developed to target different substrates. A typical example is the use of glycanases that target NSP in wheat- or barley-based diets. Another example is the use of microbial phytase to release phytate-bound phosphorus (P) and lower the excretion of P in poultry manure.

The mechanisms by which feed enzymes improve animal performance remain largely unknown. However, one or more of the following mechanisms are thought to be involved (Bedford and Schulze, 1998): (i) Degradation of specific bonds in ingredients not usually degraded by endogenous enzymes, (ii) degradation of anti-nutritional factors that lower nutrient digestibility, and improve feed passage rate, (iii) disruption of endosperm cell wall integrity in ingredients and the release of nutrients that are bound to or entrapped by the anti-nutritional factors, or (iv) supplementation of endogenous digestive enzymes, which are insufficient or absent in young animals.

With advances in biotechnology, the cost of production of feed enzymes has been dramatically reduced, and the use of feed enzymes has become popular. The major focus of this thesis is on the microbial phytase that has attracted attention in recent years as a mean of lowering P output from intensive animal operations. The first section of this literature review describes early research on feed enzymes, different types of feed enzymes, and their modes of action. The following sections describe phytic acid in plant-derived ingredients with emphasis on their chemical structure and contents, phytase activity in plants, types of phytase. Factors that affect phytate P utilisation are also discussed. The effects of supplemental microbial phytase on the availability of P, amino acids, energy and minerals in poultry diets are discussed.

Finally published data on the use of glycanases, combination of phytase with glycanases and the use of whole wheat feeding in poultry diets are reviewed.

HISTORY OF FEED ENZYMES

Early Research on the Use of Feed Enzyme

Enzymes are proteins, naturally occurring and are produced by all living organisms. More than 3000 different enzymes are known to exist and they have been used by humans for thousands of years. The classic examples are the use of enzymes in cheese making and brewing. Today, enzymes are widely used in many industries from detergent, paper production, leather and textile processing to the food industry (Sheppy, 2001). With advances in biotechnology assisting in the production of enzymes, the cost of producing enzymes has been lowered. There is also a better understanding of the chemical structure of substrates. These advances have made it possible to commercially use feed enzymes in poultry diets (Choct, 2001a).

The first report on the use of feed enzymes in poultry diets appeared during the 1920's (Clickner and Follwell, 1925). The enzyme used in the study was protozyme, which was produced from *Aspergillus orizae*. An improved bird performance was observed, when this enzyme was added to the diet. Since then, reports on the use of enzymes in poultry diets have appeared only sporadically (Choct, 2001b). For example, various preparations of amylase have been evaluated to overcome the poor performance of birds fed barley-based diets by increasing the starch availability (Fry *et al.*, 1957; Moran *et al.*, 1968).

Burnett (1966) was the first researcher to identify β-glucan as the factor responsible for the poor nutritive value of barley and to elucidate the effect of viscosity on nutrient digestion in the gut (Choct, 2001b). During the mid to late 1980's, the use of xylanase in wheat (Pettersson and Åman, 1989) or β-glucanase in barley-based diets (Campbell *et al.*, 1989) was evaluated. Application of these enzymes in poultry diets has been extensively investigated in the past decade (Bedford, 1995; Bedford and Schulze, 1998; Bedford, 2000).

Microbial phytase was initially introduced to poultry diets to reduce P content in manure in the Netherlands in early 1990's in response to increasing concerns about P pollution (Jongbloed *et al.*, 2000). The first report on the use of microbial phytase,

however, was more than three decades ago. Nelson *et al.* (1968) showed that the phytate P in ingredients such as soybean meal could be hydrolysed by a crude phytase preparation produced by *Aspergillum ficuum.* Rojas and Scott (1969) reported that phytase from *A. ficuum* almost completely hydrolysed the phytate in cottonseed meal. The large scale use of microbial phytase in poultry and pig production began in late 1990's. Since then, the use of phytase to release phytate-bound nutrients (P, minerals, protein/amino acids, starch) has been extensively researched and the enzyme has been shown to be effective in releasing phytate-bound P in diets based on a range of plant derived feedstuffs for utilisation by poultry (Coelho and Kornegay, 1999).

Available Feed Enzymes

Five types of feed enzymes have been used in the feed industry: NSP-degrading enzymes, phytate-degrading enzymes, protein-degrading enzymes, starch-degrading enzymes, and lipid-degrading enzymes (Table 1). The following section provides a brief overview of each type of enzyme in terms of their target substrate and mode of action.

NSP-degrading enzymes

Poultry have a limited ability to digest NSP, because of a lack of digestive enzymes. In poultry diets based on ingredients such as wheat, barley, rye or triticale, a large proportion of the NSP consist of arabinoxylan and β-glucan. NSP-degrading enzymes (xylanase, β-glucanase and α-galactosidases) have been developed to break down this type of fibre.

The mode of action of NSP-degrading enzymes remains largely unknown. One or more of the following mechanisms are thought to be involved (Bedford and Schulze, 1998): (i) Degradation of the NSP in the cell wall matrix of the ingredients with, the release of encapsulated nutrients, (ii) lowered viscosity of digesta caused by soluble NSP and improved the rate of diffusion between substrates, enzymes, and digestion end products, (iii) increased accessibility of nutrients to endogenous digestive enzymes, (iv) stimulation of intestinal motility and improved feed passage rate, and (v) supplementation of the enzyme capacity of young animals.

Table 1. Type of feed enzymes and their practical use.

Type of enzymes	Enzymes	Substrate	Feedstuffs	Function	Practical use
NSP-degrading enzyme	β-glucanases	β-glucan	Barley, oats and rye	Viscosity reduction	Enhanced digestion and utilisation of nutrients
	Xylanases	Arabinoxylans	Wheat, rye, triticale and barley	Viscosity reduction	As above
	α-galactosidases	Oligosaccharides	Grain legumes, lupins	Viscosity reduction	Improved energy availability
Phytate-degrading enzymes	Phytases	Phytic acid	Plant ingredients	Release of phytate-bound P	Reduced need for inorganic phosphorus
Protein-degrading enzymes	Proteases	Proteins	Plant proteins e.g. soybean meal	Hydrolysis of proteins and peptide	Improved protein digestibility and lower nitrogen excretion
Starch-degrading enzyme	Amylase	Starch	Cereal grains	Hydrolysis of starch	Improved starch digestibility
Lipid-degrading enzyme	Lipases	Fats	Animal and vegetable fats	Hydrolysis of fats	Improved fat digestibility and enhanced energy retention

Modified from Marquardt (1997).

Phytate-degrading enzymes

Phosphorus is stored as phytate (phytic acid) in most plants and therefore is present in all plant-derived ingredients. About two-thirds of the phytic acid in plant-derived ingredients is not digested or absorbed by monogastric animals. Phytate can also bind with proteins and minerals to form phytate-protein complexes (Ravindran, 2001).

Phytase can be produced by many species of bacteria, yeast and fungi as well as plants. However, commercial phytases are commonly produced by *Aspergillus niger*. Phytase hydrolyses phytic acid, and liberates phytate-bound P and decreases the need for inorganic P that is usually added to poultry diets. The effects of microbial phytase on P, protein, apparent metabolisable energy (AME), and mineral utilisation in poultry and its mode of action are discussed in the relevant sections of this chapter.

Protein-degrading enzymes

Vegetable proteins contain various anti-nutritional factors. Most legumes contain lectins and protease inhibitors. Protein inhibitors can cause a reduction in chymotrypsin activity and impair digestion, while lectins can damage gut wall, impair immune response and increase endogenous nitrogen loss. Protease works by hydrolysing proteins or peptides, and thus improving protein digestibility (Thorpe and Beal, 2001).

Starch-degrading enzymes

The addition of amylase in poultry diets complements endogenous enzymes, especially in young animals. Amylase can degrade cereal starch to dextrins and sugars, thereby improving energy availability.

Lipid-degrading enzymes

Use of lipase in broiler diets containing animal and vegetable fats can help the birds to hydrolyse fats. Thus lipase can improve fat digestibility and enhance energy utilisation in birds.

Phytate and Phytase

Chemical Structure of Phytate

Phytic acid [*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate] is the major storage form of P in plants. Phytate P consists of 60-80% of the total P in grains and their by-products (Ravindran *et al.*, 1995a). The chemical structure of phytic acid is shown in Figure 1. This structure was initially proposed by Anderson (1914) and is generally accepted by the scientific community, since physiochemical properties, interactions and nutritional effects of the compound can be explained by this model (Ravindran *et al.*, 1995a).

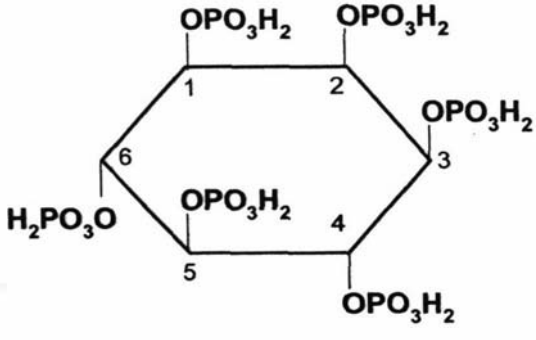

Figure 1. Structure of phytic acid (Modified from Erdman, 1979)

As seen in Figure 1, phytate bears six P groups on one 6-carbon molecule. At neutral pH the phosphate groups in phytic acid have either one or two negatively charged oxygen atoms (Reddy *et al.*, 1982). Therefore, various cations can chelate strongly between two phosphate groups or weakly with a single phosphate group. As a result, phytic acid can bind mineral elements and amino acids, and reduce their bio-availability.

Phytate Phosphorus Concentration in Plant-Derived Ingredients

The levels of phytate P in common feedstuffs have been reported by a number of workers (Nelson *et al.*, 1968; Reddy *et al.*, 1978; Eeckhout and de Paepe *et al.*, 1994;

Ravindran *et al.*, 1994). Compilation of phytate P in common feedstuffs are also available (Reddy *et al.*, 1982; Ravindran *et al.*, 1995a). Typical levels of phytate P of common feedstuffs are shown in Table 2.

Table 2. Phytate P content in various feed ingredients.

Ingredient	Phytate P (g/100g dry matter)	Phytate P (as % of total P)
Cereals		
Barley	0.27	64
Corn	0.24	72
Rice (unpolished)	0.27	77
Sorghum	0.24	66
Wheat	0.27	69
Cereal by-products		
Rice bran	1.03	80
Rice polishings	2.04	89
Wheat bran	0.92	71
Grain legumes		
Field peas	0.24	50
Oilseed meals		
Rapeseed meal	0.70	59
Sesame meal	1.02	81
Soybean meal	0.39	60
Sunflower meal	0.89	77

Modified from Ravindran *et al.* (1995a).

The level of phytate P in a feedstuff generally depends on the part of the plant from which it is derived. In general, oilseeds meals and cereal by-products contain large amounts of phytate P, whereas cereals and grain legumes contain only moderate amounts (Ravindran *et al.* 1995a). The proportion of phytate P varies from 60-80% of the total P in seeds of cereals, grain legumes and oil-bearing plants. In most cereals, phytic acid is not uniformly distributed within the kernel, but associated with specific morphological components of the seed (Oberleas, 1973). Phytate concentration in plant materials depend on several factors including the stage of maturity, degree of processing, cultivar, climate, water availability, soil, geographical location and year (Reddy *et al.*, 1982; Ravindran, 1999b).

Phytases

The phytate can be hydrolysed by phytases. There are three sources of phytase namely, plant phytase, intestinal phytase and microbial phytase.

Plant Phytase

Endogenous phytase activity in feedstuffs is variable (Table 3). The highest activities are reported in rye, wheat and wheat bran (Ravindran *et al.*, 1995a). In contrast, corn, sorghum and oilseeds have very little endogenous phytase activity. Published data on the effects of plant phytase activity on animal performance is limited.

Intestinal Phytase Activity

The presence of intestinal phytase activity in poultry is controversial. Liebert *et al.* (1993) reported that the phytase activity in the contents of the crop, stomach and small intestine of chickens is negligible. Kornegay (1999) stated that the significance of phytase produced by microorganisms residing in the intestinal tract is negligible. Maenz and Classen (1998), however, reported that intestinal brush border alkaline phosphatase could contribute to degradation of phytate P. The specific and total activities of alkaline phosphatase in intestinal brush border were highest in the duodenum and declined in the jejunum and ileum. When 4-wk-old broilers and laying hens were compared, the specific activity of alkaline phosphatase were comparable, but the hens had a 35% higher total activity of alkaline phosphatase in the brush border.

Microbial Phytase

Microbial phytase can be found in numerous bacteria, yeast and fungi. *Aspergillus* is the most widely used fungi in the commercial production of microbial phytase. Two types of commercial phytase products are available. One is derived from submerged liquid fermentation that uses genetically manipulated organisms to achieve maximum enzyme production. Examples of phytases produced by this type of production method include, Natuphos® (Gist Brocades BSD V.V., Delft, The Netherlands) and Ronozyme® P (F. Hoffmann-La Roche, Switzerland and Novo Nordisk, Denmark). Finnfeed phytase is produced by *Bacillus subtilis* fermentation (Finnfeed International Ltd., UK). The other type of product is based on solid-state fermentation (SSF) that uses normal organisms for enzyme production. Allzyme® SSF (Alltech Inc., Nocholasville,

is Novo phytase (Novo, Nordisk, Denmark). One unit of phytase (U) has been defined as amount of enzyme that liberates 1 μmol inorganic P per minute from 0.0015 mol/L sodium phytate at pH 5.5 at 37 °C (Camden *et al.*, 2001). An example of a commercial phytase using this measurement unit is Finnfeed phytase (Finnfeed International Ltd., UK). One unit of phytase activity (PU) is defined as the amount of enzyme required to liberate 1 μmol of inorganic orthophosphate from 0.0051 mol/L sodium phytate per min at 37 °C and pH 5.5 (Engelen *et al.*, 1994) with minor modifications. An example of a commercial phytase using this measurement unit is Allzyme® SSF (Alltech Inc., Nicholasville, Kentucky, USA).

Optimum pH, Temperature and Stability of Phytase

The optimum pH for the maximal biological activity of microbial and plant phytases are different. Microbial phytase has two optimal pH, one at pH 2.5 and the other at pH 5.5, while plant phytase from wheat has an optimal pH at 5.2. The optimal temperature for microbial phytases from bacteria, fungi and yeast, and plant phytases ranges from 45 to 77 °C (Liu *et al.*, 1998).

The influence of pelleting temperature on the survival of microbial phytase is of practical interest and has been investigated in several studies. Simons *et al.* (1990) found that a pelleting temperature of 78 or 81 °C had no adverse effect on phytase activity in the feed, but only 46% activity remained, when the diets were pelleted at a temperature of 87 °C. Wyss *et al.* (1998) compared the stability of two sources of phytase (*Aspergillus fumigatus* and *Aspergillus niger*) in commercial diets pelleted at 75 or 85 °C and found that both phytases are stable after pelleting at 75 °C, but pelleting at 85 °C resulted in a greater inactivation of the enzyme from *A. niger* than that from *A. fumigatus*. Eeckhout *et al.* (1995) reported that temperatures between 69 and 74 °C destroyed 50-65% of the phytase activity from *A. niger*. In general, it appears that microbial phytases are stable at pelleting temperatures of less than 75 °C, but temperatures over 85 °C result in a substantial loss of enzyme activity.

Several approaches have been used or suggested to reduce the negative effects of high pelleting temperatures including protecting the enzymes from steam penetration by encapsulation or granulation, using heat-resistant enzymes or simply adding enzymes as a liquid after processing (Bedford *et al*, 2001). Of the three options, post-pelleting

application of phytase either in the form of liquid or powder (Edens *et al.*, 2002) is the more realistic approach.

FACTORS THAT AFFECT PHYTATE P UTILISATION

The ability (or inability) of chickens to utilise phytate P has been reviewed by Taylor (1965), Nelson (1967), and Ravindran *et al.* (1995a). Phytate P utilisation by poultry is influenced by several factors including dietary levels of calcium (Ca), P, ratio of Ca to total P, levels of vitamin D_3, age and genotype of the animal, citric acid, dietary fibre and size of feed particles. Phytate P utilisation in poultry has been shown to range from 10 to 87% depending on dietary levels of Ca, P, vitamin D_3, microbial phytase, and diet type (Edwards, 1993). The following sections provide an overview of the above-mentioned factors.

Ca, P and Ratio of Ca to Total P

Dietary levels of Ca, P, and the ratio of Ca to total P are the major factors that affect phytate P utilisation, with the effects of dietary Ca being much greater (Ravindran *et al.*, 1995a; Kornegay *et al.*, 1999b). Of the factors named above have been demonstrated in several studies with poultry (Ballam *et al.*, 1984b; Mohammed, 1991; Mitchell and Edwards, 1996a, b; Qian *et al.*, 1996, 1997). Mitchell and Edwards (1996a) showed that phytate P utilisation is decreased with increased levels of dietary Ca in young broilers fed corn-soy diets. The phytate P retention decreased from 54 to 28% when dietary Ca was increased from 0.77 to 1.07%. Similarly, Ballam *et al* (1984b) reported that phytate P retention was increased by 11% on average in tested ingredients including corn, sorghum, dehulled soybean and wheat, when dietary levels of calcium were decreased from 1.10 to 0.75%. Mohammed *et al.* (1991) reported that phytate P utilisation was increased by 15% when dietary Ca levels were reduced from 1.0 to 0.5%. Two possible mechanisms for the decreased phytate P utilisation at high dietary Ca levels have been proposed (Kornegay, 1999b): (i) the precipitation of phytate by Ca through the formation of extremely insoluble Ca-phytate complexes that are less accessible to phytase, (ii) the direct depression of phytase activity resulting from extra Ca competing for the active sites of phytase.

In addition to dietary Ca and P levels, the ratio of Ca to total P plays an important role in phytate P utilisation. Vandepopuliere *et al* (1961) reported that responses, as measured by percentage bone ash, of chickens fed a diet where the dietary ratio of Ca to total P was 2:1 were poorer than those fed a diet where the ratio was 1:1. In a study with turkeys, increasing the Ca to total P ratio from 1:1 to 2:1 in a diet supplemented with phytase was shown to decrease the P retention by an average of 7.8 percentage units (Qian *et al.*, 1996). Rao *et al.* (1999) reported that an increase in the Ca to total P ratio from 1.47 to 1.97 in broilers diets with added phytase at a level of 500 PU/kg resulted in a decrease in the P retention by 11.5 percentage units (from 65.2 to 53.7%). A Ca to total P ratio between 1.1 to 1.4 appears to be optimal for phytase action (Qian *et al.*, 1997). This was further confirmed by Zyla *et al.* (2000) who reported that weight gain, feed intake, and toe ash of broilers fed diets supplemented with phytase were negatively affected when the dietary ratio of Ca to total P was increased from 1.44 to 1.93. These adverse effects were attributed to the formation of Ca-phytate complexes that are resistant to phytase action.

Vitamin D_3

The interaction of dietary vitamin D_3 with both dietary Ca and P levels can affect phytate P utilisation. When birds are fed diets marginal or deficient in vitamin D_3, phytate P utilisation is depressed (Ewing, 1963). In contrast, increasing the dietary level of vitamin D_3 enhances phytate utilisation even in the absence of phytase addition (Edwards, 1991, 1992, 1993; Mohammed *et al.*, 1991; Fisher, 1992; Mitchell and Edwards, 1996a, b; Biehl and Baker, 1997a and b; Carlos and Edwards, 1998; Applegate *et al.*, 2000). Edwards (1993) showed that addition of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) at 5 or 10 µg/kg of diet increased the amount of phytate P utilisation from 31 to 50% in the basal ration to 68 to 87% in vitamin D_3 supplemented diets. Mitchell and Edwards (1996b) also demonstrated that phytate P utilisation is influenced by dietary levels of 1,25-$(OH)_2D_3$ in young broilers fed corn-soy diets. The retention of phytate P increased with added 1,25-$(OH)_2D_3$ at every level of dietary total P tested (0.52, 0.62, and 0.72%). In diets containing 0.52% total P, phytate P retention was increased from 45 to 58% with the addition of 1,25-$(OH)_2D_3$ (5 µg/kg diet). Applegate *et al.* (2000) reported that apparent ileal phytate P digestibility was increased from 42.9 to 64.0% by 1,25-$(OH)_2D_3$ supplementation. Studies with

layers (Carlos and Edward, 1998) showed that the addition of 5 μg/kg 1,25-$(OH)_2D_3$ in the layer diets increased phytate P retention by 10 percentage units (from 32.2 to 42.1%).

Phytate P utilisation in response to vitamin D_3 supplementation is related to dietary levels of Ca and P. Mohammed *et al.* (1991) reported that a decrease in dietary calcium improves phytate digestibility in broilers. Phytate P digestibility was 50% when the diet contained 1.0% calcium, 0.69% total P, and 12.5 μg/kg 1,25-$(OH)_2D_3$. The utilisation increased to 77% when both levels of Ca and total P in the diet were decreased to 0.5%. Mitchell and Edwards (1996b) similarly showed that the phytate P retention in broilers during the starter phase (1-21 d) increased from 28 to 58% when dietary levels of total P decreased from 0.72 to 0.52% in the presence of 5 μg/kg 1,25-$(OH)_2D_3$. It is relevant to note that phytate P utilisation was influenced not only by the amount of vitamin D_3 but also the source of vitamin D_3 (Biehl and Baker, 1997a).

The mechanisms of improved phytate P in response to vitamin D_3 supplementation are unknown. However, one or more of the following may be involved (Ravindran *et al.*, 1995a): (i) increased synthesis or activity of intestinal phytase, (ii) increased phytate hydrolysis by stimulation of calcium absorption, thus rendering the phytate more soluble and available for utilisation, or (iii) enhanced absorption of P.

Age and Genotype of Birds

It is generally accepted that older birds hydrolyse more phytate P than chicks, as there is more dephosphorilating activity present in the gastrointestinal tract of older birds (Ravindran *et al.*, 1995a). The ability of poultry to utilise phytate P also increases with age. Edwards and Palo (1989) reported that 21-day-old broilers utilised phytate P much better than 7 and 14- day old broilers. The phytate P retention at 7, 14, 21 day-old broilers was 35, 47, and 59%, respectively. A significant effect of sex was also observed with males utilising 20.6 percentage units more phytate P than the females (38.7 vs 18.1%). Matyka *et al.* (1990) reported that phytate P utilisation was 5.3 and 17.3%, respectively, in 16- and 42- old broilers fed wheat-soy diets containing calcium (1.1%) and non-phytate P (0.52%). Nelson (1976) found that phytate P utilisation of 9-wk-old broilers fed corn-soy diets was increased by 3 percentage units (from 0 to 3%) compared to 4-wk-old birds fed the same diets. When wheat was substituted for half of

the corn, phytate P hydrolysis was 8 and 13% for 4- and 9-wk-old birds, respectively. In contrasts, Scheideler and Sell (1987) reported that the ability of layers to utilise phytate P declined with age. The retention of phytate P was high at 34 weeks of age, averaging 46.7%, but decreased at 50 and 72 weeks of age to 9.1 and 16.5%, respectively.

Some studies (Edwards, 1983; Zhang *et al.*, 1998) have shown that there may be breed and strain differences in the utilisation of phytate P by poultry. Edwards (1983) found that Leghorn chickens utilised phytate P much better than meat-type birds. Zhang *et al.* (1998) studied the differences of utilising dietary phytate P in birds from 45 sire and 180 dam families and found that there was variation between different lines and among families. These results imply that genetic selection for improved utilisation of dietary phytate P may prove effective.

Citric Acid

The effect of citric acid on phytate P utilisation in poultry has been examined in several studies. The effect of citric acid in improving apparent activity of phytase and subsequent phytate P utilisation and performance has been demonstrated in broilers (Zyla *et al.*, 1996; Boling *et al.*, 1998; Maenz *et al.*, 1999; Qian *et al.*, 1999; Boling *et al.*, 2000a; Zyla *et al.*, 2000) and pigs (Han *et al.*, 1998; Li *et al.*, 1998; Boling *et al.*, 2000c). Boling *et al.* (2000a, c) found that the addition of citric acid to a diet containing supplemented phytase caused further improvements in weight gain and weight of bone ash in broilers, but responses in pigs were smaller. However, such positive responses were not observed in layers fed corn-soy diets (Boling *et al.*, 2000b). These improvements in broilers and pigs are attributed to the ability of citric acid to remove Ca from forming complexes with phytase.

Fibre

There is limited information available on the effect of type of dietary fibre on phytate P utilisation. Ballam *et al.* (1984a) reported that rats given a wheat bran diet hydrolysed more phytate than those given a cottonseed husk diet. Ballam *et al.* (1984b) investigated effects of fibre, phytate source and dietary Ca and P level on phytate hydrolysis in broilers and found that birds fed diets containing 10% cellulose

hydrolysed more phytate than those fed diets containing other sources of fibre (15% rice bran, 15% wheat bran, 15% alfalfa meal and 15% cottonseed hulls). Moreover, the effect of the type of dietary fibre was associated with dietary levels of Ca and P. Ballam *et al.* (1984b) reported that birds fed a lower level of Ca (0.85%) and non-phytate P (0.42%) in diets containing alfalfa meal and cellulose, hydrolysed more phytate than those fed diets containing other sources of fibres. The effect of fibrous feedstuffs on phytate P utilisation warrants further investigations.

Size of Feed Particles

Particle size has been reported to influence phytate P utilisation. Kasim and Edwards (2000) showed that phytate P retention was significantly influenced by the corn particle size, measured as geometric mean diameter (GMD), in corn-soy diets in broilers. Phytate P retention of birds fed diets containing a large corn particle size (GMD: 894 μm) was improved by 8.6 percentage units (from 38.9 to 47.5%) when compared to those birds fed the same diets containing a fine corn particle size (GMD: 484 μm). However, improvements in phytate P utilisation were not reflected in body weight or feed efficiency. The improved phytate P retention was attributed to a longer retention time of the diet in the foregut, as the crop and proventriculus are the main sites of phytase activity in poultry (Kornegay, 1999).

MICROBIAL PHYTASE AND P UTILISATION IN POULTRY

Effect of Microbial Phytase on Bird Performance and Bone Mineralisation

Published literature on the effects of microbial phytase on the performance, bone mineralisation and P utilisation in various poultry species are summarised in Tables 4, 5, and 6. The addition of microbial phytase (500-750 FTU/kg) to low-P corn-based diets have been found to improve weight gain and feed intake of broilers by 5.9-99.7%, and 4.1-59.1%, respectively (Table 4). Feed conversion ratio (FCR) values can be lowered by 1.9-16.2%. Published data on the effect of microbial phytase in broilers fed wheat-soy and other types of diets is limited (Farrell *et al.*, 1993; Cabahug *et al.*, 1999; Ravindran *et al.*, 1999a and 2001; Selle *et al.*, 2001; Zyla *et al.*, 1999 and 2000). Addition of microbial phytase (400-800 FTU/kg) to low-P wheat- or sorghum-based diets improved weight gains and feed efficiency of broilers by 0.9-26.9%, and 2.9-9.9%,

respectively. One report examined the effects of two doses of microbial phytase in broilers fed wheat-based diets (Cabahug *et al.*, 1999). These authors reported improvements in weight gain and feed efficiency (18.4 and 5.9%, respectively) when a low-P wheat-based diet was supplemented with phytase at a level of 400 FTU/kg. No further responses were observed when 800 FTU/kg phytase was added. Overall, there were improved weight gains and feed efficiency following the addition of microbial phytase (500-1500 FTU/kg) to the diets of ducks and turkeys (Table 5). The addition of microbial phytase (750-1500 FTU/kg) to low-P corn- or sorghum-based diets improved weight gain and feed efficiency of ducks by 3.5-13.6% and 1.0-8.5%, respectively. Similarly, improvements in weight gain and feed efficiency by addition of microbial phytase (500-750 FTU/kg) to corn-based diets in turkeys were 3.9-67.3%, and 3.0-33.4%, respectively.

Supplementation of microbial phytase (200-600 FTU/kg diet) to a low-P (0.10-0.24% non-phytate P) corn-soy diet had consistent positive effects on production parameters in layers in terms of improving egg production (0.4-44.9%), egg weight (0.3-12.0%), and feed efficiency (1.2-7.9%) (Table 6), compared to those fed low-P diets. Gordon and Roland, (1997 and 1998) reported that addition of microbial phytase to low-P diets improved bone mineral content and bone density by 6.1-11% and 5.8-8.5%, respectively. Boiling *et al.* (2000b) reported that the addition of microbial phytase as low as 100 FTU/kg to corn-soy diets containing 0.10% non-phytate P resulted in egg production performance that was not significantly different from that of hens fed a corn-soy diet containing 0.45% non-phytate P. However, data on the effect of microbial phytase in layers fed wheat-based diets is inconsistent (Peter and Jeroch, 1993; Usayran and Balnave, 1995; Scott *et al.*, 2000). Scott *et al.* (2000) reported a positive effect of phytase addition on egg production and feed efficiency after week 55 in layers fed wheat-based diets. In contrast, adverse effects of phytase on egg production in hens fed wheat-soy diets have been found in other studies (Peter and Jeroch, 1993; Usayran and Balnave, 1995). It was suggested that the variable responses of layers to phytase addition may be related to the endogenous phytase activity in wheat. More work is needed to determine the effects of adding phytase to wheat-based diets in layers.

Table 4. Effect of microbial phytase on bird performance, bone mineralisation, and phosphorus (P) utilisation in broilers.

References	EP (day)	Diet type	Phytase (FTU/kg)	nP (%)	Ratio[1]	Weight gain[2] (%)	Feed intake[2] (%)	FCR[2] (%)	Toe ash[2] (% units)	P retention[2] (% units)	P excretion[3] (%)
Simons *et al.* (1990)	1-24	Corn-soy	500	0.15	1.3	+84.3	-	-15.7	-	+9.8	-57.0
			750	0.15	1.3	+99.7	-	-16.2	-	+9.8	-57.0
Perney *et al* (1993)	4--10	Corn-soy	500	0.32	1.9	+12.5	+8.6	-3.8	+1.2	+8.0	-19.9
Denbow *et al.* (1995)	1-21	Corn-soy	600	0.20	2.0	+70.9	+59.1	-10.9	+2.3	-	-
			600	0.27	2.0	+36.9	+39.0	+2.0	+1.6	-	-
			600	0.30	2.0	+13.6	+13.1	-	+1.1	-	-
Kornegay *et al.* (1996)	1-21	Corn-soy	600	0.20	2.0	+37.9	+40.2	-2.3	+1.6	+3.1	-46.7
				0.27	2.0	+10.4	+10.7	-	+1.5	+8.7	-42.1
				0.34	2.0	+5.9	+4.1	-1.9	+0.5	+6.3	-28.5
Sebastian *et al.* (1996a)	1-21	Corn-soy	600	0.30	1.7	+9.1	+56.5	-4.7	-	+4.0	-
Sebastian *et al.* (1996b)	1-21	Corn-soy	600	0.33	2.6	+13.3	+13.5	-	-	+12.5	-
Yi *et al.* (1996a)	1-21	Corn-soy	700	0.27	2.0	+20.1	+25.3	+3.5	+1.0	+6.2	-36.8
Yi *et al.* (1996b)	1-21	Corn-soy	600	0.45	1.5	+11.0	+18.9	+6.3	+0.7	-	-
Camden *et al.* (2001)	1-21	Corn-soy	500	0.28	1.4	+12.1	+8.0	-3.4	+0.8	+4.6	-
Ravindran *et al.* (1999a)	7-28	Wheat-soy	600	0.45	1.3	+0.9	+0.7	-	-	-	-
Zyla *et al.* (1999)	1-21	Wheat-soy	600	0.15	1.4	+9.6	+5.0	-4.4	+0.6	-	-
Zyla *et al.* (2000)	1-21	Wheat-soy	500	0.17	1.4	+13.4	+7.4	-5.1	+1.4	-	-
Selle *et al.* (2001)	7-29	Wheat-soy	600	0.25	1.2	+3.8	-3.0	-6.9	-	-	-
Cabahug *et al.* (1999)	7-25	Wheat-sorghum-soy	400	0.23	1.6	+18.4	+10.3	-5.9	+3.4	+6.4	-
			800	0.23	1.6	+19.1	+7.7	-9.9	+3.4	+6.6	-
Ravindran *et al.* (2001)	7-28	Wheat-sorghum-soy	500	0.45	1.3	+5.0	+1.5	-3.3	+0.3	-	-
Farrell *et al.* (1993)	1-18	Sorghum-soy	750	0.17	-	+26.9	+14.8	-9.6	+5.4[4]	+16.0	-
				0.21	-	+7.0	+3.9	-2.9	+3.4[4]	+7.1	-

[1] Ratio of Ca to total P; [2] Percentage or percentage units changes for phytase addition diets over the low-P basal diets; [3] Percentage reductions for phytase addition diets over the adequate-P (0.45-0.47% non-phytate P) diets. [4] Tibia ash; EP, experimental period; nP, non-phytate P.

Table 5. Effect of microbial phytase on bird performance, bone mineralisation, and phosphorus (P) utilisation in ducks and turkeys.

References	Species	EP (day)	Diet type	Phytase (FTU/kg)	nP[1] (%)	Ratio[2]	Weight gain[3] (%)	Feed intake[3] (%)	FCR[3] (%)	Toe ash[3] (% units)	P retention[3] (% units)
Farrell *et al.* (1993)	Ducks	2-17	Sorghum-soy	850	0.14	-	+13.6	+15.7	-1.9	+9.0	+9.0
					0.17	-	+6.3	+7.5	-1.1	+7.0	+6.0
					0.21	-	+6.4	+7.5	-1.0	+4.0	+6.0
Martin and Farrell (1994)	Ducks	19-40	Sorghum-soy	1000	0.21	1.2	+9.2	-	-8.5	+1.4[5]	+10.3
Farrell and Martin (1998)	Ducks	19-40	Sorghum-soy	1000	0.21	1.3	+9.2	-	-8.5	+0.8[5]	+8.4
Martin *et al.* (1998)	Ducks[4]	4--23	Sorghum-soy	1000-1500	0.39	1.4	+3.5	-	-2.3	-	+14.7
Orban *et al.* (1999)	Ducks	22-42	Corn-soy	750	0.18	1.8	+11.8	+4.9	-6.1	+0.31	-
Ravindran *et al.* (1995c)	Turkeys	1-21	Corn-soy	600	0.27	2.0	+67.3	+11.4	-33.4	+3.2	-
				600	0.36	2.0	+6.5	+6.5	-	+2.8	-
				600	0.45	2.0	+3.9	-2.3	-5.9	-	-
Zyla *et al.* (1995)	Turkeys	7-21	Corn-soy	500	0.19	2.6	+54.9	+41.3	-8.8	+2.1[5]	-
Qian *et al.* (1996)	Turkeys	1-21	Corn-soy	600	0.27	1.4	+11.3	+11.7	-7.2	+1.6	+8.0
					0.36	1.4	+14.5	+4.4	-8.8	+1.0	+3.9
Yi *et al.* (1996c)	Turkeys	1-20	Corn-soy[7]	750	0.45	1.2	+11.1	+1.5	-7.8	+1.4	-
Kornegay *et al.* (1999b)	Turkeys[6]	1-21	Corn-soy	500	0.56	0.7	+9.5	+6.3	-3.0	-	+3.0

[1] nP, non-phytate P.

[2] Ratio of Ca to total P.

[3] Percentage or percentage units changes for phytase addition diets over the basal diets.

[4] Sexed equally mixed.

[5] Tibia ash.

[6] Female birds.

[7] Containing 28% crude protein; EP, experimental period; FCR, feed conversion ratio.

Table 6. Effect of microbial phytase on production parameters and phosphorus (P) utilisation in layers.

References	EP (wk)	Diet-type	Phytase (FTU/kg)	nP[1] (%)	Ratio[2]	Wt[3] (%)	FI[3] (%)	FCR[3] (%)	EGP[3] (%)	EW[3] (%)	ESW[3] (%)	P retention (% units)	P excretion[4] (%)
Peter and Jeroch (1993)	29-45	Corn-soy	500	0.12	-	-	+19.3	-1.2	+27.1	+3.1	-	-	-
Gordon and Roland (1997)	21-38	Corn-soy	300	0.10	12.1	-	+2.8	-	+3.6	+0.3	+0.4	-	-
Van der Klis *et al.* (1997)	20-68	Corn-soy	200	0.10	11.1	-	+5.8	-1.5	+4.8	+3.2	-	-	-
Carlos and Edward (1998)	56-65	Corn-soy	600	0.24	9.1	-	-	-	+15.6	-	-	-	-
Gordon and Roland (1998)	58-64	Corn-soy	300	0.10	4.9	+1.4	+9.4	-	+24.2	+0.8	+6.0	-	-
Rao *et al.* (1999)	47-55	Corn-soy	250	0.11	10.8	-	+12.2	-	+44.9	+5.3	+9.6	-	-
Scott *et al.* (1999)	55-67	Corn-soy	250	0.20	7.7	-	+3.6	-7.9	+15.2	+12.0	+0.1	-	-
UM and Paik (1999)	21-40	Corn-soy	500	0.12	10.1	-	+2.4	-	+0.4	+2.4	+2.1	+24.0[4]	-40.9[4]
UM *et al.* (1999)	48-56	Corn-soy	250	0.16	8.3	-	+1.4	-1.7	+2.1	+0.7	-	+21.0[4]	-32.7[4]
Boling *et al.* (2000c)	20-70	Corn-soy	300	0.15	-	-	-	-	+6.8	+9.3	-	-	-
Jalal and Scheideler (2001)	40-60	Corn-soy	300	0.15	10.6	1.3	-	-	-	+0.7	+1.2	-	-
Tangendjaja *et al.* (2002)	23-48	Corn-soy	300	0.19	5.5	-	-	-	+0.2	-	-	-	-
Peter and Jeroch (1993)	29-45	Wheat-soy	500	0.12	-	-	+4.6	+9.3	-3.1	-0.8	-	-	-
Usayran and Balnave (1995)	59-70	Wheat-soy	500	0.12	-	-	+3.9	-	-4.0	-	+0.1	-	-
Scott *et al.* (2000)	55-67	Wheat-soy	250	0.20	10.0	-	+0.8	-4.7	+6.0	+5.4	-	-	-

[1] nP, non-phytate P.

[2] Ratio of Ca to total P.

[3] Percentage changes for phytase addition diets over the low-P (0.10-0.24% non-phytate P) basal diets.

[4] Percentage units or percentage changes for phytase addition diets over the adequate-P (0.26-0.37% non-phytate P) diets.

Wt, body weight; FI, feed intake; FCR, feed consumed per kilogram of egg production (kg:kg); EGP, egg production; EP, experimental period; EW, egg weight; ESW, egg shell weight.

Improvements in weight gain with added phytase is generally attributed to increased feed intake, resulting from the release of P from phytate-bound P by microbial phytase. Improvements in feed efficiency with phytase addition have been reported in a number of studies but not in others (Denbow *et al.*, 1995; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996b; Yi et al., 1996a, b; Ravindran *et al.*, 1999a). The improvements in feed efficiency by adding microbial phytase reflect a greater improvement in weight gain compared to feed intake, which may be related, in part, to improved energy and protein utilisation.

The influence of microbial phytase on bone mineralisation has been consistent in both corn- and wheat-based diets. Addition of phytase to low-P diets improved bone mineralisation to levels comparable to those of adequate-P diets in growing birds (Tables 4 and 5) and layers (Usayran and Balnave, 1995; van der Klis *et al.*, 1997; Carlos and Edwards, 1997, 1998; Gordon and Roland, 1998; Um *et al.*, 1999).

Effect of Microbial Phytase on P Digestibility and Utilisation

The beneficial effect of supplemental phytase on P availability, measured as bone ash, in broilers was first reported by Nelson (1971). The effectiveness of microbial phytase in improving phytate-bound P availability, P retention and reducing P excretion has been demonstrated in studies with broilers (Table 4), ducks and turkeys (Table 5). Addition of microbial phytase to broiler diets (500-750 FTU/kg) increased apparent P retention by 3.1-16.0 percentage units. When compared to adequate-P (0.45-0.47% non-phytate P) diets, addition of microbial phytase to low-P (0.15-0.37% non-phytate P) broiler diets reduces P excretion by 20-57% (Table 4). Similarly, addition of microbial phytase to duck diets (750-1500 FTU/kg) and turkey diets (500-750 FTU/kg) increases apparent P retention by 6.0-14.7 and 3.0-8.0 percentage units, respectively (Table 5).

Published data on the effect of microbial phytase on P digestibility and utilisation in layers are limited. Van der Klis *et al.* (1997) reported that addition of microbial phytase (250 FTU/kg) to low-P (0.08% non-phytate P) corn-soy diets improved apparent ileal digestibility of P by 21.5 percentage units (from 26.2 to 47.7%) and 24.3 percentage units (from 12.5 to 36.8%) for 24- and 36-wk old layers, respectively. Improvement in P retention by addition of microbial phytase (250-500 FTU/kg) to corn-soy diets in layers

was 21-24 percentage units. Compared to the adequate-P diets (0.26-0.35% non-phytate P), addition of phytase to low-P diets reduced P excretion in layers by 32.7-40.9% (Table 6).

The amount of P excreted in the manure is determined by the amount of dietary non-phytate P and the level of supplemental microbial phytase. Kornegay (1999) summarised the data from 23 experiments and generated equations for the calculation of improvements in P digestibility and percentage reduction in P excretion with the addition of phytase. Based on the P digestibility equations, expected improvement in P digestibility by addition of 500 FTU/kg was calculated to be 7.8 percentage units. Based on the P retention equation, P excretion can be reduced by 32% when 500 FTU phytase/kg is added to a low-P diet compared to an adequate-P diet.

Phosphorus Replacement Value

P replacement value is defined as the amount of inorganic P that can be removed from the diet by a given amount of added phytase (Kornegay, 1999). This value is commercially relevant, as inorganic P is the most expensive mineral source used in poultry diets.

Procedures for the calculation of P replacement value for microbial phytase have been described by Denbow *et al.* (1995) and Yi *et al.* (1996b). Birds are offered diets containing graded levels of non-phytate P and microbial phytase, and the data on various response variables (e.g. weight gain, FCR and toe ash) are obtained. Non-linear: $Y = a[1 - be^{(-kX)}]$ and linear: $Y = a + bX$ model that best fit the data are derived for non-phytate P levels and for phytase levels, where, Y= response measurements e.g. weight gains, feed intake and toe ash, X = non-phytate P (percentage) or phytase added (units/ kg diet). Within each of responses measurement, the equation with highest R^2 value for the added non-phytate P and for the added phytase are set equal and solved, and the P replacement value is calculated.

Widely ranging P replacement values have been reported depending on the response criteria used, and dietary Ca and P levels. Published data on P replacement values for microbial phytase in broilers and layers are summarised in Tables 7 and 8, respectively. All values have been generated with corn-soy diets, as no reported values are available for other diet types. Depending on the response criteria used, around 550-1000 and 250-300

Table 7. Phosphorus (P) replacement values reported for microbial phytase in broiler diets.

Reference	Species	EP (day)	Diet type	Ratio[1]	Response criteria	Amount & source of inorganic P	P Replacement values (FTU/kg diet)
Schöner *et al.* (1991)	Broilers	1-14	Corn-soy	-	Crude ash	1 g of MCP	762
Schöner *et al.* (1993)	Broilers	1-14	Corn-soy	-	Weight gain	1 g of MCP	570
				-	P retention	1 g of MCP	1050
Denbow *et al.* (1995)	Broilers	1-21	Corn-soy	2.0	Weight gain and toe ash	1 g of DFP	821
Ravindran *et al.* (1995c)	Turkeys	1-21	Corn-soy	2.0	Weight gain and toe ash	1 g of DFP	652
Kornegay *et al.* (1996)	Broilers	1-21	Corn-soy	2.0	Weight gain and toe ash	1 g of DFP	939
Yi *et al.* (1996a)	Broilers	1-21	Corn-soy	2.0	Weight gain and toe ash	1 g of DFP	785
Orban *et al.* (1999)	Ducks	22-42	Corn-soy	1.8	Plasma P concentration	0.5 g of DFP	750
					Weight gain	0.7 g of DFP	750

[1]Ratio of Ca to total P.

DCP, dicalcium phosphate; DFP, defluorinated phosphate; EP, experimental period; MCP, monocalcium phosphate.

Table 8. Phosphorus (P) replacement values reported for microbial phytase in layers.

Reference	EP (wk)	Diet type	Response criteria	Amount & source of inorganic P	P replacement values (FTU/kg diet)
Simons *et al.* (1992)	-	Corn-soy	Egg production, egg weight, FCR	1 g of MCP	200
Peter and Jeroch (1993)	29-46	Corn-soy	Egg production, egg weight, FCR	1 g of MCP	500
Gordon and Roland (1997)	-	Corn-soy	Egg production, egg weight, FCR,	1 g of DCP	300
Van der Klis *et al.* (1997)	20-24	Corn-soy	Ileal P absorption	1 g of MCP	250

[1]Ratio of Ca to total P.

DCP, dicalcium phosphate; EP, experimental period; FCR, feed conversion ratio; MCP, monocalcium phosphate.

FTU phytase/kg diet is required to replace 1 gram (g) of P in the form of defluorinated phosphate (DFP) or mono-calcium phosphate (MCP) for broiler and laying hens, respectively. It is generally observed that the response to supplemental phytase on P retention is non-linear and that the magnitude of response is much greater at lower non-phytate P levels (Kornegay, 1999). The efficiency of phytase appears to be greater for layers than for growing birds, as suggested by the lower P replacement values. Phytase supplementation to layer diets is much easier to carry out, as layer diets are usually fed in a mash form and thus heat stability of phytase associated with pelleting is therefore not a problem.

The response criteria used in generating the equation to calculate P bioavailability include weight gain, tibia ash and toe ash (De Groote, 1983; Potchanakorn and Potter, 1987; Potter, 1988; Sullivan and Doughlas, 1990). These criteria have been used for assessing the influence of phytase on P utilisation in broilers (Denbow *et al.*, 1995; Ravindran *et al.*, 1995b, c; Kornegay *et al.*, 1996; Yi *et al.*, 1996a). It is generally accepted that weight gain, tibia ash, and toe ash are sensitive response criteria for assessing P bioavailability. For these criteria to be accurate and sensitive, a low-P basal diet (0.25-0.30% of non-phytate P) must be fed. In addition the birds need to have limited P stores, or be growing rapidly so that P requirements are high (Kornegay and Yi, 1999). It should be noted, however, that the use of weight gain as an response may overestimate the P replacement value under certain circumstances. For example, when a microbial phytase providing with side-enzyme activities is added to a diet, part of the weight gain may be due to the action of other enzymes.

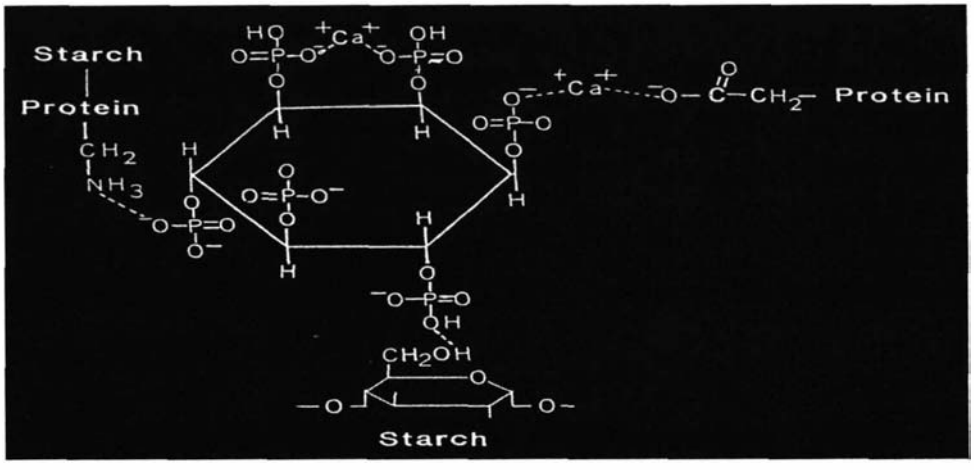

Figure 2. Phytate-protein complex (after Thompson, 1988).

EFFECT OF MICROBIAL PHYTASE ON THE UTILISATION OF NUTRIENTS OTHER THAN P

Phytate-Protein Complexes

Phytic acid is generally considered as a factor primarily limiting P availability from plant derived materials. However, phytate can also form phytate complex with protein and a range of minerals (Figure 2).

Three situations may be relevant to phytate-protein complexes. First, native protein-phytate complexes exist in plant material. Secondly, protein-phytate complexes can form in the gastrointestinal tract and finally, phytate can bind digestive enzymes, which themselves are proteins (Ravindran, 2001).

In the highly acidic stomach region, amino acids, in particular lysine (Lys), methionine (Met), arginine (Arg) and histidine (His) can bind directly to phytate P creating insoluble phytate-protein complexes. In the less acidic region of the intestine, mineral cations (Ca, Mg, Zn, Fe) act as a bridge between phytate P and protein, resulting in protein-mineral-phytate complexes. Phytate-protein complexes are insoluble and less subject to attack by proteolytic enzymes than the same protein alone (Anderson, 1985).

In vitro studies have shown that phytate may form complexes with proteins and free amino acids. Jongbloed *et al.* (1997) reported that soluble proteins in casein, corn, rice polishings, soybean meal and sunflower meal were substantially precipitated in the presence of phytic acid. Incubation of feedstuffs with microbial phytase largely prevented the precipitation. Rutherfurd *et al.* (1997) incubated lysine monochloride with rice polishing as a source of phytic acid with and without added phytase. Incubation without phytase reduced the recovery of free lysine to 78%, but this was increased to 91% by the addition of phytase. It has been suggested that the complexing with amino acids may occur either during mixing, storage or in the animal's gut post-feeding (Ravindran, 2001).

Phytate can inhibit a number of digestive enzymes such as pepsin, α-amylase and trypsin (Ravindran, 2001). The inhibition of digestive enzymes by phytate appears to involve non-specific protein binding. Inhibition may also result from the chelation of calcium ions that are essential for the activity of trypsin and α-amylase, or possibly from an interaction with the substrates used by these enzymes (Ravindran, 2001).

Effect of Microbial Phytase on Protein Utilisation

The effects of microbial phytase on protein and energy utilisation have been recently reviewed (Selle *et al.*, 2000; Kies *et al.*, 2001). Influence of microbial phytase on protein/amino acid digestibility in broiler chickens (Farrell *et al.*, 1993; Yi *et al.*, 1996c; Biehl and Baker, 1997c; Sebastian *et al.*, 1997; Ledoux *et al.*, 1999; Namkung and Leeson, 1999; Zhang *et al.*, 1999a; Ravindran *et al.*, 2000; Camden *et al.*, 2001; Ravindran *et al.*, 2001), laying hens (Van der Klis and Versteegh, 1991) and ducklings (Martin *et al.*, 1998) has extensively been examined. Improvements in digestibility have been reported in most studies, although the responses have been variables. Some reports, however, found that microbial phytase has no effect on protein utilisation (Newkirk and Classen, 1995; Peter and Baker, 2001; Zhang *et al.*, 1999b).

In general, the improvement in nitrogen digestibility by addition of microbial phytase in poultry diets is small, ranging from 0.9 to 4.9% (Table 9). Van der Klis and Versteegh (1991) reported that the addition of phytase (250-300 FTU/kg) to layer diets improved apparent ileal nitrogen absorption from 79.3 to 80.9%. Similarly, Farrell *et al.* (1993) reported that the addition of 750 FTU/kg phytase to sorghum-soy diets improved nitrogen utilisation from 56.0 to 57.6%. Martin and Farrell (1994) investigated the effect of added phytase (1000 U/kg) on nutrient digestibility in sorghum-soy diets containing various levels of rice bran. When compared to unsupplemented diets, improvements in apparent nitrogen digestibility with added phytase in diets containing 0, 300 and 600 g/kg diet rice bran were 14.1, 7.8 and 9.7%, respectively.

Reported improvements in amino acid digestibility range from 0.3-7.2% depending on the individual amino acids measured and dietary levels of non-phytate P (Table 9). Yi *et al.* (1996c) reported that there was a 1-2% improvement in amino acid digestibility when 750 FTU/kg phytase was added to a corn-soy diet for female turkeys. Biehl and Baker (1997c) also found that there was a 2% increase in true amino acid digestibility values for nine essential amino acids and cysteine (Cys) when phytase (1200 FTU/kg) was fed to caecectomised roosters on soyabean meal-dextrose diets. Sebastian *et al.* (1997) showed phytase addition improved the apparent ileal digestibility of all amino acids, except lysine, methionine and phenylalanine (Phe), in 28-d old female broilers fed a corn-soy diet. However, there were no phytase effects observed in

male broiler chickens except in the digestibility of protein ($P<0.07$), lysine ($P<0.07$) and methionine ($P<0.05$).

Namkung and Leeson (1999) reported that the ileal digestibility of nitrogen and amino acids for chickens fed a corn-soy diet supplemented with phytase (1200 U/kg) increased by 2%. In studies with caecectomised roosters, Zhang *et al.* (1999a) demonstrated that added phytase increased the digestibility of most of the amino acids in barley, canola meal, and a barley-canola meal blend. The magnitude of the improvements was greater for lysine, arginine, cysteine (Cys), serine (Ser) and threonine (Thre). However, the addition of phytase did not affect the digestibility of protein or amino acids in ducklings fed diets containing various levels of rice bran (Martin *et al.*, 1998). Ledoux *et al.* (1999) found that phytase addition significantly ($P<0.05$) increased digestibility of nitrogen and amino acids in turkey poults fed corn-soy diets. Ravindran *et al.* (2000) evaluated the effect of three dietary levels of phytic acid (1.04, 1.32, and 1.57%), two dietary level of non-phytate P (0.23 and 0.45%) and three levels of phytase addition (0, 400, 800 FTU/kg) on ileal amino acid digestibility in broilers fed wheat-sorghum-soy diets. The magnitude of response was greater in diets containing low-P (0.23% non-phytate P) compared to those fed adequate-P (0.45% non-phytate P) diets with the same dietary levels of phytic acid. The percentage increase in digestibility in diet containing low non-phytate P diets with phytase (400 FTU/kg) was 2.0, 3.2, 7.2, 3.7, 5.0, 4.7, 7.0, 5.3 and 5.8% for Met, Lys, Thre, Arg, His, isoleucine (Ile), leucine (Leu), Phe and valine (Val), respectively. The percentage increase in digestibility in diet containing the adequate-P diets was 0.4, 1.4, 2.4, 1.3, 1.5, 1.9, 2.1, 2.1 and 1.9% for Met, Lys, Thre, Arg, His, Ile, Leu, Phe and Val, respectively. No further improvements were observed when 800 FTU/kg was added (Ravindran *et al.*, 2000).

Effect of Microbial Phytase on Energy Utilisation

An " energy effect " for phytase addition to broiler diets was first reported by Rojas and Scott (1969). In this study, the AME contents for chickens fed cottonseed meal and soybean meal following treatment with a crude phytase preparation from *Aspergillus ficuum* were significantly improved. Using a similar crude enzyme product, Miles and Nelson (1974) also observed that there was a significant improvement in the AME content for chickens fed phytase-treated cottonseed meal (7.1 vs 8.5 MJ/kg) and wheat bran (4.6 vs 7.0 MJ/kg), but not for soybean meal (11.5 vs. 10.7 MJ/kg). It is, however,

Table 9. The effect of microbial phytase on apparent ileal digestibility of nitrogen (N) and amino acids in poultry.

References	Species	Sex	EP	Phytase	nP[1]	Ratio[2]	Dietary CP	Improvements[3] (%)										
			(day)	(FTU/kg)	(%)		(%)	N	Met	Cys	Lys	Thre	Arg	His	Ile	Leu	Phe	Val
Yi *et al.* (1996c)	Turkeys	F	1-29	750	0.45	1.3	22.5	0.9	0.7	0.9	0.7	0.9	0.7	0.4	0.8	0.9	0.9	0.9
							28.0	2.0	0.4	3.8	1.4	1.7	1.2	1.3	2.0	1.5	1.6	1.9
Biehl and Baker (1997c)	Roosters	M	21wk	600	-	-	-	-	3.3	0.2	1.7	0.2	1.6	0.2	0.6	0.7	0.5	0.7
				1200	-	-	-	-	2.3	5.0	2.9	1.0	2.7	1.7	1.5	1.6	0.5	1.6
Sebastian *et al.* (1997)	Broilers	F	1-28	600	0.31	1.3	22.4	4.9	0.3	-	0.7	5.7	2.0	2.7	3.9	2.2	1.3	3.0
Namkung and Leeson (1999)	Broilers	M	1-15	1200	0.35	2.2	-	3.2	-	-	2.0	0.8	2.4	2.1	5.2	2.3	2.8	4.5
Ravindran *et al.* (2000)	Broilers	M	7-25	400[4]	0.23	1.5-1.7	21.8	3.6	2.0	-	3.2	7.2	3.7	5.0	4.7	7.0	5.3	5.8
				800[4]	0.23	1.5-1.7	21.8	3.0	1.7	-	2.6	6.4	3.7	4.3	4.2	5.9	4.9	5.1
Ravindran *et al.* (2000)	Broilers	M	7-25	400[4]	0.45	1.5-1.7	21.8	2.2	0.4	-	1.4	2.4	1.3	1.5	1.9	2.1	2.1	1.9
				800[4]	0.45	1.5-1.7	21.8	2.4	0.7	-	1.3	3.6	2.2	2.2	2.9	3.6	3.3	2.8
Camden *et al.* (2001)	Broilers	M	1-21	500	0.30	1.38	21.4	2.3	2.3	-	2.7	3.6	1.2	3.1	2.3	1.4	1.8	2.5
Ravindran *et al.* (2001)	Broilers	M	7-28	500	0.45	1.4	19.6	4.0	-	-	4.5	4.8	4.3	3.5	4.5	3.7	4.8	5.1
				750	0.45	1.4	19.6	3.7	-	-	5.0	4.0	3.8	4.8	4.2	4.2	4.0	4.0

[1] non-phytate P; [2] Ratio of Ca to total P; [3] Percentage improvements for phytase addition diets over the unsupplemented basal diets.
[4] Mean values from diets containing three levels of phytic acid. CP, crude protein; EP, experimental period; F, female; M, male.

possible that the energy responses observed in these studies may have been due partly to the presence of other enzymes in the crude preparations.

The effect of microbial phytase in improving AME in poultry fed corn-, wheat- and sorghum-based diets has been demonstrated in a number of recent studies in broilers (Ledoux *et al.*, 1999; Namkung and Leeson, 1999; Selle *et al.*, 1999; Ravindran *et al.*, 2000) and ducks (Martin and Farrell, 1994). Overall, the addition of phytase to poultry diets increased the AME by 1.1-6.3% depending on dietary level of non-phytate P and diet type (Table 10).

The mode of action underlying the effect of microbial phytase on energy utilisation is not fully understood. Several possible mechanisms have been proposed (Ravindran, 1999a). Firstly, improvements in protein and amino acid utilisation with added phytase may contribute, at least in part, to the observed energy effects. Secondly, a wide ratio of Ca to total P leads to the formation of insoluble Ca-phytate complexes. The latter complexes can further react with fatty acids in the gut lumen to form insoluble metabolic soaps thereby lowering fat digestibility. Microbial phytase may prevent the formation of the insoluble Ca-phytate complexes by hydrolysing the phytate. It is also possible that phytase may reduce the adverse effects of phytic acid on starch digestion and endogenous losses.

Effect of Microbial Phytase on Mineral Utilisation

Effect of Microbial Phytase on Ca Utilisation

Supplementation of microbial phytase in poultry diets has been shown to improve Ca availability and retention in broilers (Simons *et al.*, 1990; Schöner *et al*, 1991, 1994; Sebastian *et al.*, 1996b; Kornegay *et al.*, 1999a; Zanini and Sazzad, 1999) and turkeys (Qian *et al.*, 1996; Kornegay *et al.*, 1999b). Simons *et al.* (1990) reported that the addition of 500 FTU/kg microbial phytase to a low-P diet (0.15% non-phytate P) improved apparent faecal availability of Ca by 12% compared to an adequate-P diet (0.45% non-phytate P). Sebastian *et al.* (1996b) reported that the addition of phytase to low-P diets significantly increased Ca retention by 12.2% in male broilers but not in females. Qian *et al.* (1996, 1997) reported that phytase supplementation improved Ca retention at various levels of non-phytate P and at varying dietary Ca to total P ratios. Calcium retention increased linearly as the amount of supplemental phytase increased,

Table 10. The effect of microbial phytase on apparent metabolisable energy (AME; MJ/kg dry matter) in poultry.

References	Species	EP (day)	Diet type	Phytase (FTU/kg)	nP (%)	AME -	AME +	Improvement (%)
Martin and Farrell (1994)	Ducks	1-23	Sorghum-soy	1000-1500	0.56-0.71	11.7	12.3	4.7
						12.4	12.8	4.7
Biehl and Baker (1997c)	Roosters	20 wks	Corn-soy	1200	-	10.0	10.1	1.1
Namkung and Leeson (1999)	Broilers	15	Corn-soy	1200	0.35	11.9	12.1	2.7
Selle *et al.* (1999)	Broilers	7-25	Sorghum-soy	600	0.50	12.6	12.9	2.6
Selle *et al.* (1999)	Broilers	7-25	Sorghum-soy	600	0.39	12.4	12.9	4.0
Ravindran *et al.* (2000)	Broilers	7-25	Wheat-sorghum-soy	400	0.23	13.1	13.8	5.3
				800	0.23	13.1	13.3	1.5
Ravindran *et al.* (2000)	Broilers	7-25	Wheat-sorghum-soy	400	0.45	12.6	13.1	3.9
				800	0.45	12.6	13.4	6.3
Ravindran *et al.* (2001)	Broilers	7-28	Wheat-sorghum-soy	500	0.45	14.2	14.6	2.8
				750	0.45	14.2	14.7	3.5
Selle *et al.* (2001)	Broilers	7-29	Wheat-soy	600	0.25	14.2	14.1	-

EP, experimental period; nP; non-phytate P.

and decreased as the Ca to total P ratios became wider. Zanini and Sazzad (1999) reported that supplemental phytase (500 PU/kg) improved apparent faecal Ca utilisation by 16%.

Calcium replacement values for microbial phytase have been reported for broilers (Schöner *et al.*, 1994) and turkeys (Kornegay *et al.*, 1999b). Schöner *et al.* (1994) reported that 500 FTU/kg microbial phytase was equivalent to 0.35 g Ca, as measured by weight gain, and 0.56 g Ca as measured by tibia ash. Kornegay *et al.* (1999b) studied Ca replacement values for microbial phytase in turkeys fed corn-soy diets using weight gain and gain per feed as response variables (Table 11). The Ca replacement values for microbial phytase at a level of 500 FTU/kg was calculated to be 1.2 g and 0.7 g for weight gain and gain per feed, respectively.

Table 11. Calcium replacement values for microbial phytase in poultry.

Reference	Species	Diet type	Phytase (FTU/kg)	Ratio[1]	Response variables	Value[2] (g of Ca)
Schöner *et al.* (1994)	Broilers	Corn-soy	500	-	Weight gain	0.35
				-	Tibia ash	0.56
Kornegay *et al.* (1999b)	Turkeys	Corn-soy	500	2.0	Weight gain	1.20
				2.0	Gain per feed	0.70

[1] Ratio of Ca to total P. [2] Ca replacement value.

Effect of Microbial Phytase on Zn Utilisation

Microbial phytase has also been reported to improve the availability and retention of zinc (Zn) in poultry (Thiel *et al.*, 1993; Roberson and Edward, 1994; Biehl *et al.*, 1995; Sebastian *et al.*, 1996b; Mohanna and Nys, 1999; Zanini and Sazzad, 1999). Thiel *et al.* (1993) reported that the Zn content of femurs from chickens fed a diet containing 30 ppm Zn plus 700 U phytase/kg was comparable to that of chickens fed a diet containing 39 ppm Zn without phytase. Roberson and Edward (1994) reported that supplemented phytase increased the tibial Zn concentration but did not improve apparent Zn retention in broilers. Similarly, Biehl *et al.* (1995) found that the addition of 1200 U/kg phytase to diet based on glucose-soybean concentrate increased growth rate of broilers by 40% and total tibial Zn by 107%. Sebastian *et al.* (1996b) found that addition of phytase to the low-P (0.33% non-phytate P) diets increased the relative Zn retention by 27.2% and

62.3% at 10 and 17 days of age, respectively. Zanini and Sazzad (1999) reported that the addition of microbial phytase (500 PU/kg) improved apparent Zn utilisation by 24%. Mohanna and Nys (1999) reported that the addition of phytase improved tibia Zn levels by 5.9% when dietary Zn was low (14 mg/kg) but tibia Zn was unaffected at high dietary level of Zn (35 mg/kg). In contrast, Sebastian *et al.* (1996a) failed to demonstrate any beneficial effect of phytase supplementation on the retention of Zn, magnesium (Mg), iron (Fe), and manganese (Mn).

Zn replacement value for microbial phytase has been reported in broilers (Yi *et al.*, 1996b). Approximately 0.9 mg of Zn was released per 100 U/kg phytase over the range of 150 to 600 U of phytase in poultry diets based on corn-soy diets.

Effect of Microbial Phytase on Utilisation of Copper and Iron

Published reports on the effect of supplemental phytase in improving the availability and retention of copper (Cu) in poultry diets are inconsistent. Sebastian *et al.* (1996b) found that the addition of phytase to low-P diets increased the relative Cu retention by 19.3%. In another study by the same group, phytase supplementation had no effect on relative retention of Cu and Fe, but increased plasma Cu levels (Sebastian *et al.*, 1996a). Um *et al.* (2000) reported that phytase supplementation had no effect on the relative retention of Mg, Zn, Fe and Cu. Biehl *et al.* (1997) also reported that microbial phytase had no effect on availability of Fe in broilers fed corn-soy diets.

USE OF GLYCANASES IN POULTRY DIETS

The use of glycanases (xylanases in wheat-based and β-glucanases in barley-based diets) has now become commonplace in the poultry industry. Use of glycanases to improve the nutritive value of poultry feeds has been discussed in numerous reports (Annison and Choct, 1991; Classen and Bedford, 1991; Campbell and Bedford, 1992; Chesson, 1993; Bedford, 1995; Bedford and Morgan, 1996; Bedford and Schulze, 1998; Bedford, 2000). Overall, responses to the enzyme supplementation in growing birds are variable. Factors affecting enzyme responses in wheat-based diets include variability in added enzymes, and quality of ingredient, breed and age of birds (Bedford, 1997). Some evidence also suggests that the enzyme response may be related to gut microflora of birds and microbial interactions (Choct *et al.*, 1996; Bedford and Apajalahti, 2001).

The mechanisms causing the improvements in nutrient utilisation by glycanases supplementation, however, are not fully understood. The major mode of action appears to involve degradation of non-starch polysaccharides in cell wall matrix (Bedford and Schulze, 1998), thereby releasing the nutrients encapsulated within the cell and lowering digesta viscosity, thus improving the rate of diffusion among substrates, enzymes, and digestion of end products. The principal factor controlling the magnitude of response to enzymes is intestinal viscosity especially when the viscosity in wheat is larger than 10 mPa.s (Bedford, 1997; Bedford and Schulze, 1998).

Published data on the addition of exogenous enzymes to corn-based broiler diets is limited. Zanella *et al.* (1999) reported that addition of commercial enzyme preparations containing amylase (2000 U/g), xylanase (800 U/g), and protease (6000 U/g) activity to corn-soy diets improved weight gains, feed efficiency, AME (2.5%) and digestibility of crude protein (2.9%), starch (1.8%) and fat (1.6%). In a study with corn-soy diets, Café *et al.* (2002) reported that the addition of an enzyme preparation containing xylanase, protease and α-amylase activity resulted in improvements in weight gain by 1.6% at both 35 and 49 days of age, but had no effect on the weight gain at 42 days of age. However, feed efficiency was not improved by the enzyme supplementation. The enzyme addition had no effect on yield of breast meat at 35, 42 and 49 days of age and relative weights of abdominal fat pad at 35 days of age, but significantly increased the abdominal fat pad at 42 and 49 days of age.

Limited studies have examined the effects of xylanase supplementation on protein digestibility in poultry diets. Hew *et al.* (1998) reported that addition of xylanase to wheat-based diets in broilers improved AME values by 12.6-18.6% and ileal nitrogen digestibility by 7.0-7.4%. Ileal digestibility of Thre, Val, Met, Lys, and Arg in the enzyme supplemented diets were improved by 8.9, 6.7, 4.9, 8.5 and 6.7%, respectively compared to those in the diets without the added enzyme. However, Zanella *et al.* (1999) reported that addition of commercial enzyme preparations containing xylanase (800 U/g), amylase (2000 U/g), and protease (6000 U/g) activity to corn-soy diets had no influence on the digestibility of Lys, Met and Arg, but improved digestibility of Val and Thre.

The effects of glycanase supplementation on relative weight and length of the gastro-intestinal tract and other organs in wheat- or barley-based diets have been reported in broilers (Brenes *et al.*, 1993; Viveros *et al.*, 1994) and layers (Jaroni *et al.*, 1999). Brenes *et al.* (1993) reported that the addition of a commercial enzyme

preparation containing mainly β-glucanase (8000 BGU/g) and also xylanase (300 XU/g) activity to the barley-based diets reduced the relative weights of the proventriculus, pancreas, liver, duodenum, jejunum, ileum and colon, but the same enzyme preparation had no effect on the organ size of birds when included in wheat-based diets. Viveros *et al.* (1994) reported that supplementation of a crude enzyme preparation containing β-glucanase, xylanase, hemicellulase, α-amylase, cellulase and protease activity to a barley-based broiler diet reduced the relative length of the duodenum, jejunum, ileum and caeca. Jaroni *et al.* (1999) reported that addition of xylanase and protease to corn-wheat middling-based diets tended to reduce the relative weights of the gizzard and pancreas by 6.4 and 6.1%, respectively in 60-wk old layers compared to those fed the unsupplemented basal diet. The reduction in the size of the digestive tract with supplemented glycanases in wheat or barley-based diets is attributed to a decreased intestinal viscosity and improved feed passage rate and consequently decreased microbial activity associated with stimulation of intestinal growth (Brenes *et al.*, 2002).

The effects of enzyme supplementation to wheat- or barley-based diets on gut morphology have been reported in broilers (Viveros, *et al.*, 1994; Iji *et al.*, 2001), turkeys (Ritz *et al.*, 1995) and layers (Scheideler *et al.*, 1998; Jaroni *et al.*, 1999). Viveros *et al.* (1994) reported that the jejunum of birds fed a diet containing 60% barley without added β-glucanase showed shortening, thickening and atrophy of the villi and an increased number of goblet cells compared to those birds fed a corn-soy diet. In a study in laying hens, Jaroni *et al.* (1999) observed similar abnormal villi in the jejunum of laying hens fed wheat middling-based diets without xylanase addition, but these adverse effects were reversed with the enzyme addition in both studies. However, Iji *et al.* (2001) reported that supplementation of xylanase to wheat-based diets had no effect on villus height, crypt depth and villus surface area in the duodenum, jejunum and ileum of male and female broilers at 28 d of age.

The mechanisms for the morphological changes in the intestinal tract of birds fed diets containing high-viscous grains such as wheat and barley with added glycanases are unclear. It has been suggested that the increased digesta viscosity, caused by high levels of soluble NSP may stimulate the growth of anaerobic microflora and their interaction with nutrients. The anaerobic microflora that are generally found in large numbers in the caeca, also tend to migrate to the small intestine where most nutrient absorption takes place (Campbell and Bedford, 1992). Those unbeneficial bacteria can

irritate the gut lining and result in a thicker lining with damaged microvilli. It has been shown that enzyme supplementation appears to reduce the microbial population in the intestinal tract (Choct *et al.*, 1996 and 1999; Sinlae and Choct, 2000) and thus the negative effects of NSP on intestinal villi. Choct *et al.* (1999) reported that supplementation of xylanase to wheat-based diets reduced the microbial activity in the ileal digesta as indicated by reduced concentration of volatile fatty acids. Sinlae and Choct (2000) reported that addition of xylanase to a wheat-based diet reduced the number of undesirable organisms such as *clostridium perfringens* in the caeca.

COMBINATION OF PHYTASE AND GLYCANASES IN POULTRY DIETS

Several recent studies have demonstrated beneficial effects of combining phytase with glycanases in improving bird performance, AME and nutrient utilisation in broilers (Piao *et al.*, 1998; Ravindran *et al.*, 1999a; Zyla *et al.*, 1999, 2000), and turkeys (Zyla *et al.*, 1996). Overall, improvements in weight gains and feed efficiency of birds fed diets supplemented with enzyme combinations were 5-13, and 1.2-6.4%, respectively compared to those in the phytase group (Table 12).

Ravindran *et al.* (1999a) evaluated the effects of an enzyme combination on the AME and ileal protein digestibility in two cereals grains (wheat and barley) and a wheat-based diet in broilers. The results showed that individual addition of xylanase and phytase increased the AME values of a low-AME wheat by 9.7 and 5.3%, respectively. The combination of the two enzymes significantly increased the AME values of wheat by 19.0%. Individual additions of phytase and xylanase in chickens fed a wheat-casein diet improved the apparent ileal nitrogen digestibility by 3.3%. When phytase and xylanase were added together, further improvements in nitrogen digestibility were observed. Individual additions of phytase improved the AME values of barley by 2.7%. When phytase and glucanase were added together, further improvements in AME were observed. Simbaya *et al.* (1996) reported that the use of phytase in combination with protease and carbohydrase in wheat-canola diets improved weight gain and feed efficiency of broilers by 5.3 and 4.7%, respectively. Zyla *et al.* (1996) reported additional improvements in the apparent retention of P and calcium (11 and 20%, respectively) in growing turkeys fed corn-soy diets supplemented with an enzyme cocktail containing phytase, acid phosphatase, protease and pectinase compared to those fed the phytase-supplemented diets.

Table 12. The effect of combination of microbial phytase with other enzymes on the performance and apparent metabolisable energy (AME) in broilers.

Reference	Treatments	EP (day)	Diet type	Weigh gain[1] (%)	Feed intake[1] (%)	Feed/gain[1] (%)	AME[1] (%)
Simbaya *et al.* (1996)	B + phytase	4-18	Wheat-canola meal	+1.0	-	-2.6	-
	B + phytase+ carbohydrase + protease			+6.3	-	-7.2	-
Ravindran *et al.* (1999a)	B + phytase	7- 28	Wheat-soy	-	-	-	+4.5
	B + phytase + xylanase			+5.0	+1.6	-3.2	+6.6
Zyla *et al.* (1999)	B + phytase	1-14	Wheat-soy	+9.6	+5.0	-4.4	-
	B + phytase + xylanase			+21.5	+8.3	-10.8	-
Zyla *et al.* (2000)	B + phytase	1-21	Wheat-soy	+13.4	+7.4	-5.1	-
	B + phytase + xylanase			+26.4	+18.1	-6.3	-
Selle *et al.* (2001)	B + phytase	7-29	Wheat-soy	+3.8	-3.0	-6.9	-
	B + phytase + xylanase			+6.7	-3.0	-9.4	+2.8

[1] Percentage changes over the low-P unsupplemented basal diet.

B, basal diet; EP, experimental period.

USE OF WHOLE WHEAT FEEDING IN POULTRY DIETS

Whole wheat feeding for broilers has received attention in recent years due to the associated economic benefits. The use of whole wheat in poultry diets was initially reported in growing birds (McIntosh *et al.*, 1962b) and layers (McIntosh *et al.*, 1962a; O'Neil, 1964). Both reports showed the beneficial effects of the use of whole wheat with no or little loss in bird performance compared to those fed ground wheat, but feed costs were greatly reduced. The effects of whole wheat feeding in poultry diets have been reviewed (Hughes, 1984; Rose and Kyriazakis, 1991; Forbes and Covasa, 1995) and the results are contradictory.

A number of recent reports (Preston *et al.*, 2000; Nahas and Lefrancois, 2001; Svihus and Hetland, 2001; Hetland *et al.*, 2002; Plavnik *et al.*, 2002) have shown that there are beneficial effects of using whole wheat feeding in broilers. Preston *et al.* (2000) reported that feed efficiency of birds fed diets containing 33% whole wheat was improved by 5.2% at 28 days of age compared to those birds fed wheat-based diets. Relative weights of gizzard and abdominal fat pad of birds fed diets containing whole wheat were increased by 47 and 5.2%, respectively. Nahas and Lefrancois (2001) examined effect of inclusion of whole wheat in corn-soy diets on the performance in broilers and found that daily weight gain and final body weight of birds fed whole wheat (10-20 and 20-35% replacing corn during 7-21 d and 22-38 d, respectively) was significantly increased by 1.4-5.1 and 3.5-5.3%, respectively compared to those fed corn-soy diets. However, feed efficiency was not affected by inclusion of whole wheat. The relative weight of abdominal fat pad was improved by 10-20% when 10-35% of whole wheat was included. Weight of organs (crop, pancreas, proventriculus, liver and heart) including gizzard, was not affected by the inclusion of whole wheat. Svihus and Hetland (2001) reported that weight gain and feed efficiency of birds fed wheat-based diets containing whole wheat (38.5% whole wheat replacing ground wheat during 10-21 d) were numerically improved by 5.1 and 3.1%, respectively, compared to those fed diets containing ground wheat. Relative gizzard weights were 42% heavier than those fed diets containing the ground wheat. In another study by the same group in which broilers were fed wheat-based diets, Hetland *et al.* (2002) reported that weight gain of birds fed diets containing moderate to high levels of whole wheat (12.5-33 and 30-44% during 10-24 and 25-38 d, respectively) was numerically reduced, but feed efficiency was significantly improved by 5.1-6.7% over the experimental period (10-38 d)

compared to those fed diets containing ground wheat. Relative gizzard weights of birds fed diets containing moderate to high levels of whole wheat at 24 and 38 d of age were increased by 56-86% and 36-100%, respectively. Inclusion of whole wheat also improved apparent ileal starch digestibility by 2.0 (from 97 to 99%) and 3.0 percentage units (from 94 to 97%) during 10-24 d and 25-38 d, respectively. Plavnik *et al.* (2002) reported that inclusion of whole wheat (10-20% replacing ground wheat) in corn-wheat-soy diets improved weight gain and feed efficiency by 2.6-3.8 and 5.7-5.8%, respectively, due to the lower feed intake in the whole wheat diets. Inclusion 20% whole wheat caused a numerical improvement in gizzard weight compared to those fed ground wheat, but the effects were not significant in the 10% whole wheat diets. In a subsequent trial, inclusion of whole wheat (5-15%) did not affect weight gain, but improved feed efficiency by 2.9-5.1%. Relative gizzard weights of male and female broilers fed diets containing 15% whole wheat were increased by 25 and 35%, respectively.

Several reports (Munt *et al.*, 1995; Uddin *et al.*, 1996; Taylor and Jones, 2001; Bennett *et al.*, 2002), however, have shown that there are no benefits of whole wheat inclusion in broiler diets. Munt *et al.* (1995) reported that feed efficiency of birds fed diets containing whole wheat during the experimental period (22-42 d) was not significantly affected (1.887 vs 1.818), but final live weight was decreased compared to those birds fed the pelleted diets. The gizzard weight of birds fed diets containing whole wheat were 39% heavier than those fed the pelleted ground wheat-based diets. However, economic analysis of the data showed that using whole wheat feeding in broiler diets generated 33.4% more profits compared to pelleted ground wheat diets. Uddin *et al.* (1996) reported that the weight gain of birds fed diets containing 15-30% of whole wheat (15 and 30% during 24-33 d and 33-42 d, respectively) was reduced by 2.4%, while feed efficiency was improved by 2.1% compared to those fed diets containing ground wheat. The effects, however, were not significant for weight gain and feed efficiency. Taylor and Jones (2001) reported that weight gain and feed efficiency of birds were not influenced by inclusion of 20% whole wheat in wheat or sorghum-based diets. Relative gizzard weights were 7.8-10.7% heavier compared to those fed diets containing ground wheat. Taylor and Jones (2001) indicated that lack of whole wheat effect was caused by the poor quality of wheat in the study. Wheat form had no effects on the weight or length of the duodenum, jejunum and ileum. However, weight of the abdominal fat pad was increased in birds fed the whole wheat containing diets.

Bennett *et al.* (2002) examined the effects of the inclusion levels of whole wheat at different ages (0, 7, 14, 28 d) on bird performance and found no beneficial effects on growth rate or feed efficiency due to the inclusion of whole wheat. Replacing ground wheat with whole wheat in wheat-soy diets at different ages (5, 20 and 35-65% whole wheat during 0-6, 6-13, 27-48 d) had no influence on weight gain. Feed efficiency was reduced by inclusion of 20% whole wheat compared to those fed diets without whole wheat. Feed efficiency was not affected during finisher phase (27-48 d) even when up to 35-50% of whole wheat in the diet were included. Inclusion of whole wheat significantly ($P<0.001$) improved gizzard weights by 18-37%, but had no effect on weights of other organs (crop, proventriculus, gizzard, small intestine), carcass yield and abdominal fat pad.

The effects of whole wheat inclusion in broilers diets on energy utilisation have also been evaluated. McIntosh *et al.* (1962b) reported that whole wheat resulted in approximately 10% more metabolisable energy compared to ground wheat (13.21 vs 11.96 MJ/kg) in broilers. Preston *et al.* (2000) reported that inclusion of 33% whole wheat in wheat-based diets resulted in a numerical improvement in AME values at 38 d of age compared to those fed ground wheat. Uddin *et al.* (1996), however, found that the nitrogen-corrected AME of diets containing whole wheat when fed from 19 to 27 d of age was not affected by the inclusion level of whole wheat (10-40%).

The mechanism of the beneficial effect of whole wheat inclusion in the diet is not clear. It is probably due to a more developed gizzard and increased grinding activity of the gizzard, resulting in a greater release of nutrients and improved AME (Preston *et al.*, 2000). This is supported by two studies (Svihus and Hetland, 2001; Hetland *et al.*, 2002) where it was found that ileal starch digestibility of birds fed diet containing whole wheat was significantly ($P<0.05$) improved compared to those fed ground wheat.

The health and welfare of birds may be improved by the use of whole cereals due to increased gizzard weight resulting a more efficient grinding activity, and due to more effective antimicrobial effects of hydrochloric acid (Cumming, 1992). The effects of whole wheat feeding on coccidiosis in broilers have been reported, but results are conflicting. Several reports (Cumming, 1992, 1994) showed that offering broilers a choice of whole wheat greatly reduced the excretion of oocysts following infection with Eimeria. However, such anti-coccidiosis effects of whole wheat feeding were not observed in other reports (Waldenstedt *et al.*, 1998; Banfield and Forbes, 2001; Banfield *et al.*, 2002).

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Chapter 3

Effects of Microbial Phytase on the Performance, Toe Ash Contents and Nutrient Utilisation of Male and Female Broilers Fed Wheat-Soy Diets

Microbial phytase in poultry diets has become popular worldwide in recent years due to the ability of phytase to release phytate-bound phosphorus (P) and thereby reducing P output in the manure, which is a major problem in many parts of the world. The effectiveness of microbial phytase produced by submerged liquid fermentation in improving P bioavailability in poultry diets is now well documented (Coelho and Kornegay, 1999). However, no studies have been conducted to examine the effects of microbial phytase produced by solid state fermentation on the performance and nutrient utilisation in poultry diets. The aim of the present study was to examine effects of microbial phytase produced by solid state fermentation on the performance, toe ash contents, phytate P degradation, digestibility and utilisation of nutrients (P and nitrogen) of male and female broilers fed wheat-soy diets.

ABSTRACT

The aim of the present study was to examine the effects of microbial phytase produced by solid state fermentation on the performance, apparent ileal digestibility of phosphorus (P), phytate P and nitrogen, and utilisation of nutrients (P and nitrogen) of male and female broilers fed wheat-soy diets from 1 to 42 days of age. There were eight dietary treatments. Diets 1 to 4 were based on wheat and soybean meal and supplemented with inorganic phosphate to contain 0.30, 0.36, 0.42 and 0.48% of non-phytate P, respectively, during the starter phase (1-21 d) and 0.20, 0.26, 0.32 and 0.48%, respectively, during the finisher phase (22-42 d). Diets 5 to 8 were based on diet 1 and supplemented with phytase to contain 500, 1000, 1500 and 2000 PU/kg diet, respectively. Within sex, each of the eight dietary treatments was assigned to five pens of eight birds each. The results showed that the response in performance and toe ash content beyond the first addition of inorganic P or phytase were minimal. The greatest responses were noted with the first addition and reached a plateau with further additions. In the males, over the 42-day trial period, compared to the low-P diet (containing 0.30% non-phytate P during 1-21 d and 0.20% non-phytate P during 22-42 d), addition of 500, 1000, 1500 and 2000 PU phytase/kg diet increased weight gain by 7.7, 9.5, 10.2 and 13.4% and lowered feed/gain by 7.1, 8.2, 9.5 and 8.9%, respectively. In the females, the corresponding increments in weight gain were 8.9, 10.6, 12.0 and 10.9%, and reductions in feed/gain were 4.5, 7.1, 4.7 and 8.4%, respectively. Phytase supplementation of the low-P diet increased toe ash content to a level comparable to those in the adequate-P diets. In male broilers, the addition of 500, 1000, 1500 and 2000 PU phytase/kg to the low-P diet increased toe ash content by 10.5, 12.3, 11.8 and 13.2%, respectively. The corresponding increases in females were 11.0, 12.6, 14.0 and 14.3%, respectively. The addition of phytase to the low-P diet resulted in marked increases in ileal degradation of phytate. The apparent phytate degradation in the wheat-soy diet, averaged across male and female broilers, was 27.6% and this was increased by 28.0, 32.0, 42.7 and 47.7 percentage units, respectively, with the addition of 500, 1000, 1500 and 2000 PU phytase/kg.

Microbial phytase significantly ($P<0.05$) improved the apparent ileal nitrogen digestibility. Addition of 500 PU/kg phytase to the low-P diet increased the nitrogen digestibility by 2.5 and 4.2 percentage units in males and females, respectively.

Addition of phytase increased the AME of wheat-soy diets during both starter and finisher phases, but the increments were greater in the finisher diets. Addition of 500 PU/kg phytase to the low-P diet improved the AME by 0.18 to 0.28 MJ/kg dry matter during the starter phase and by 0.33 to 0.87 MJ/kg dry matter during the finisher phase.

Reductions in excreta P and nitrogen contents and improvements in apparent retention of P and nitrogen with the addition of phytase were consistent with the improvements observed in the ileal phytate degradation and ileal digestibility of P and nitrogen. Phosphorus contents in the excreta were markedly increased when dietary P needs were supplied in the form of inorganic P. Compared to the adequate-P diet, the excreta P level in the low-P diet containing 500 PU/kg phytase was lowered by approximately 35% in male and female broilers, while excreta nitrogen contents were lowered by 2.4 and 3.3-6.4% for male and female broiler, respectively.

An additional aim of the present study was to calculate the P replacement values for microbial phytase based on weight gain response. The amount of non-phytate P equivalent to 500 PU/kg diet during the starter phase for male and female broilers were 0.54 and 0.53%, respectively. The amount of non-phytate P equivalent to 500 PU/kg diet during the finisher phase for male and female broilers were 0.42 and 0.39%, respectively. Based on the weight gain responses, the estimates obtained for P equivalency of the microbial phytase (500-2000 PTU/kg) translate into P release values of 72 to 131% from phytate P and are apparently spurious. Possible reasons for the observed high P replacement values are discussed.

INTRODUCTION

Phytic acid is a ubiquitous compound that is abundant in all seeds (cereals, grain legumes and oilseeds) serving as the chief storage form of phosphorus (P). The phytic acid molecule has a high P content (28.2%) and, since the majority poultry and pig diets consists of plant-derived ingredients, P from the phytic acid may have considerable nutritional significance.

The ability of poultry and pigs to utilise phytate P is generally assumed to be poor (Nelson, 1967; Ravindran *et al.*, 1995; Kornegay, 1999) due to insufficient quantities of intestinal phytase. This inability of poultry and pigs to utilise phytate P results in the excretion of large amounts of P in the manure, posing an environmental concern especially in areas of intensive animal production. This issue has become a major concern in many parts of the world in recent years.

In order for P to be utilised by monogastric animals, inorganic P from phytate must be released. The dephosphorylation of phytic acid requires phytases, a class of enzymes that catalyse the removal of the six inorganic P molecules from phytic acid in a stepwise manner. Commercial microbial phytases capable of hydrolysing phytic acid and releasing phytate-bound P are now available. Two distinct phytase products are commercially available – one derived from submerged liquid fermentation that uses genetically manipulated organisms to achieve maximum enzyme production and the other based on solid state fermentation that uses non-genetically manipulated organisms for enzyme production. Phytase evaluated in the present study belongs to the latter group and is produced by growing *Aspergillus niger* on water-insoluble substrates in the presence of minimal free-flowing water.

The aim of the present study was to investigate the effects of microbial phytase produced by solid state fermentation on the performance, phytate P release, retention and excretion of nutrients (P and nitrogen) in wheat-soy diets for broiler chickens. An additional aim was to calculate the P replacement values for this microbial phytase product based on performance responses.

MATERIALS AND METHODS

All experimental procedures were approved by the Massey University Animal Ethics Committee (Anonymous, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Enzymes

The phytase (Allzyme SSF; Alltech, Inc., Nicholasville, KY, USA) contained 1000 PU/g phytase activity. One unit of phytase (PU) was defined as the amount of enzyme required to liberate 1 μmol of inorganic phosphate from 0.0051 mol/L sodium phytate in 1 min at pH 5.5 and 37 °C.

Experimental Design and Diets

There were eight dietary treatments. The dietary treatments and enzyme incorporation rates in broiler starter (1-21 d) and finisher (22-42 d) diets are shown in Tables 1 and 2, respectively.

Table 1. Dietary treatments and enzyme incorporation rates in broiler starters (1-21 d).

Diet no.	Non-phytate P level (%)	Added phytase (PU/kg diet)
1	0.30	0
2	0.36	0
3	0.42	0
4	0.48	0
5	0.30	500
6	0.30	1000
7	0.30	1500
8	0.30	2000

The basal diet was based on wheat, soybean meal and canola meal. Two sets of basal diets were used: one for broiler starters and the other for broiler finishers. The basal diet for broiler starters was formulated to meet or exceed recommended specifications for all nutrients, except P and calcium. Non-phytate P level was maintained at 0.30%. This level of non-phytate P was selected to maintain the dietary available P below current NRC (1994) recommendations and to ensure responses with inorganic phosphate and phytase additions. The basal diet was supplemented with monocalcium phosphate (MCP) to provide three levels of non-phytate P (0.36, 0.42 and

0.48%) or with four levels of phytase (500, 1000, 1500 and 2000 PU/kg diet). The composition of the diets is shown in Table 3.

Phytase was added and replaced corn starch while sand was used to replace monocalcium phosphate and limestone. The Ca to total P ratio was maintained at 1.4:1 in all diets. The wheat used in the study was first steam pelleted at 90 ^{0}C to reduce the intrinsic phytase activity and then ground prior to mixing into feed.

Table 2. Dietary treatments and enzyme incorporation rates in broiler finishers (22-42 d).

Diet no.	Non-phytate P Level (%)	Added phytase (PU/kg diet)
1	0.20	0
2	0.26	0
3	0.32	0
4	0.38	0
5	0.20	500
6	0.20	1000
7	0.20	1500
8	0.20	2000

For the broiler finisher phase, the basal diet was formulated to meet or exceed recommended specifications for all nutrients, except P and calcium (Table 4). Non-phytate P was maintained at 0.20% and, was supplemented with MCP to provide three levels of non-phytate P (0.26, 0.32 and 0.38%) or with four levels of phytase (500, 1000, 15000 and 2000 PU/kg diet). Titanium oxide (0.05%) was included in all finisher diets as a dietary marker. The Ca: total P ratio was maintained at 1.4:1. The enzyme product was first mixed into the premix and then into the diet. After mixing, the diets were cold pelleted (65-70 °C).

General Experimental Procedures

Day-old broilers (Ross) were obtained from a commercial hatchery and 640 chicks (320 males and 320 females) of uniform body weights were randomly assigned to 80 pens (8 birds/ pen) in 3-tier electrically heated battery brooders. Within each sex (female or male), the eight dietary treatments were randomly assigned to five pens of eight birds each. The birds were transferred to colony cages in an environmentally controlled room on day 14. Room temperature was maintained at 32 ± 1 ^{0}C during the first week and gradually decreased to 21 ^{0}C by the end of the sixth week. Ventilation was controlled by mechanical fans in the walls.

Table 3. Ingredient composition and calculated analysis of diets for broiler starters.

Ingredients	0.30% non-phytate P[1]	0.36% non-phytate P	0.42% non-phytate P	0.48% non-phytate P
			%	
Wheat	67.17	67.17	67.17	67.17
Soyabean meal	20.01	20.01	20.01	20.01
Canola meal	5.00	5.00	5.00	5.00
Vegetable oil	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.73	1.00	1.30	1.58
Limestone	1.53	1.66	1.73	1.84
Sand	1.16	0.76	0.39	0.00
Maize starch	0.20	0.20	0.20	0.20
Lysine·HCl	0.35	0.35	0.35	0.35
DL-methionine	0.40	0.40	0.40	0.40
Salt	0.25	0.25	0.25	0.25
Trace mineral premix[2]	0.15	0.15	0.15	0.15
Vitamin premix[3]	0.05	0.05	0.05	0.05
Calculated analysis				
AME (MJ/kg)	13.0	13.0	13.0	13.0
Lysine, %	1.15 (1.17)[4]	1.15 (1.14)	1.15 (1.17)	1.15 (1.13)
Methionine + cysteine, %	0.94 (0.92)	0.94 (0.94)	0.94 (0.93)	0.94 (0.91)
Calcium, %	0.81 (0.83)	0.90 (0.93)	0.98 (1.02)	1.06 (1.10)
Total P, %	0.58 (0.57)	0.64 (0.65)	0.70 (0.70)	0.76 (0.75)
Phytate P, %	0.28 (0.29)	0.28 (0.29)	0.28 (0.29)	0.28 (0.29)
Non-phytate P, %[5]	0.30 (0.28)	0.36 (0.36)	0.40 (0.41)	0.48 (0.46)

[1] 500, 1000, 1500 or 2000 PU of phytase was added to the negative control diet containing 0.30% non-phytate P.
[2] Poultry mineral 4 (Tegel Foods Ltd, Auckland, New Zealand). Supplied per kilogram diet: choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.
[3] Broiler starter vitamin (Tegel Foods Ltd, Auckland, New Zealand). Supplied per kilogram diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg. [4] Values in parentheses refer to analysed values. [5] Non-phytate P =Total P – phytate P.

Table 4. Ingredient composition and calculated analysis of diets for broiler finishers.

Ingredients	0.20% non-phytate P[1]	0.26% non-phytate P	0.32% non-phytate P	0.38% non-phytate P
			%	
Wheat	71.82	71.82	71.82	71.82
Soyabean meal	16.33	16.33	16.33	16.33
Canola meal	5.00	5.00	5.00	5.00
Vegetable oil	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.28	0.55	0.83	1.04
Limestone	1.36	1.47	1.57	1.64
Sand	0.20	0.20	0.20	0.20
Maize starch	1.04	0.66	0.28	0.00
Lysine·HCl	0.34	0.34	0.34	0.34
DL-methionine	0.23	0.23	0.23	0.23
Salt	0.20	0.20	0.20	0.20
Trace mineral premix[2]	0.15	0.15	0.15	0.15
Vitamin premix[2]	0.05	0.05	0.05	0.05
Calculated analysis				
AME (MJ/kg)	13.1	13.1	13.1	13.1
Lysine, %	1.05 (1.08)[3]	1.05 (1.06)	1.05 (1.06)	1.05 (1.08)
Methionine + cystine, %	0.74 (0.76)	0.74 (0.73)	0.74 (0.72)	0.74 (0.76)
Calcium, %	0.67 (0.68)	0.75 (0.77)	0.83 (0.85)	0.89 (0.92)
Total P, %	0.48 (0.47)	0.54 (0.52)	0.60 (0.59)	0.64 (0.64)
Phytate P, %	0.28 (0.29)	0.28 (0.29)	0.28 (0.30)	0.28 (0.29)
Non-phytate P, %[4]	0.20 (0.18)	0.26 (0.23)	0.32 (0.29)	0.36 (0.35)

[1] 500, 1000, 1500 or 2000 PU of phytase was added to the negative control diet containing 0.20% non-phytate P.
[2] See Table 1.
[3] Values in parentheses refer to determined values.
[4] Non-phytate P =Total P – phytate P.

Body weight and feed intake were recorded on a pen basis at weekly intervals. Feed was offered *ad libitum* and water was freely available at all times. Mortality was recorded daily. Any bird that died was weighed and these weights were used to correct feed/gain values.

Collection and Processing of Samples

During the third week (day 18-21) and sixth week (day 38-41), feed intake and excreta outputs were measured quantitatively per pen over four consecutive days. Excreta were collected daily, weighed and pooled within a pen. Pooled excreta were mixed well into slurry using a blender and two representative samples per pen were obtained and freeze-dried. Dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at - 4 ^{0}C until chemical analyses. Dry matter (DM), gross energy (GE), nitrogen and P contents of the diets and excreta were determined.

Toe ash samples were obtained, by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left and right middle toes of the birds in each pen were pooled separately to yield two toe samples. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 550 °C for 16 h (Potter, 1988).

On day 42, all surviving birds from each pen were sacrificed by intravenous injection of sodium pentobarbitone. The small intestine was immediately exposed and digesta contents were collected from the terminal ileum. Digesta were pooled within a pen, lyophilised, ground to pass through a 0.5-mm sieve and stored at -4 °C in air-tight containers until laboratory analysis. Samples of diets and ileal digesta were assayed for nitrogen, P, phytate P, gross energy, and titanium (Ti).

Chemical Analysis

Dry matter contents were determined using standard procedures (AOAC, 1990). Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid. Total P and nitrogen were determined following Kjeldahl digestion by colorimetric autoanalyses (Twine and Williams, 1971; Technicon, 1973).

Phytate P was analysed by the colorimetric procedure of Caldwell (1992). In this method, phytate was extracted using hydrochloric acid/sodium sulphate solution and precipitated as ferric phytate. The precipitate was hydrolysed and the P content was determined colorimetrically using phosphomolybdate method. Calcium was determined using the atomic absorption spectrophotometry. The diets were also analysed for phytase activity according to the procedures of Engelen *et al.* (1994) with minor modifications.

Calculations

The AME contents of the diets were calculated using the following formula. Appropriate corrections were made for differences in DM content.

$$\text{AME (MJ/kg diet)} = [(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})] / \text{Feed intake}$$

Correction for zero nitrogen retention was made using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958) and nitrogen-corrected AME (AME_n) values were calculated.

Apparent ileal nutrient digestibility/degradation were calculated, using titanium as the indigestible marker, as shown below.

$$\text{Apparent ileal nutrient digestibility} = \frac{(Nt/Ti)_d - (Nt/Ti)_i}{(Nt/Ti)_d}$$

where, $(Nt/Ti)_d$ = ratio of nutrient to titanium in diet, and

$(Nt/Ti)_i$ = ratio of nutrient to titanium in ileal digesta.

Data Analysis

The data were analysed by the General Linear Models procedure of the SAS® (SAS Institute, 1997) with pen means as the experimental unit. Since sex effects were not of interest, data from male and female broilers were analysed separately. Linear and quadratic effects of inorganic P from MCP (Diets 1 to 4) and supplemental phytase (Diet 1 and Diets 5 to 8) on the performance, toe ash contents and nutrient utilisation parameters were tested using orthogonal polynomial contrast. Differences are

considered significant at $P<0.05$. If the data suggest a trend, P values up to $P<0.10$ are shown in the text.

Linear and non-linear response functions of body weight gain, feed intake and feed efficiency during the starter (1-21 d) and finisher (22-42 d) phases that best fitted the data were derived for inorganic P levels (Diets 1 to 4) and for phytase levels (Diet 1 and Diets 5 to 8). The following models were used:

Linear function $Y = a + bX$

Non-linear function $Y = a\,(1\text{-}be^{-kX})$ or $Y = a + b_1X + b_2X^2$

Where Y = the response measurement, X = non-phytate P (percentage) or phytase added (units per kilogram of diet).

The non-linear or linear response equations with the higher R^2 value for added inorganic P and the equations for added phytase were set equal and solved using the procedures described by Yi *et al.* (1996). The resulting values were used to determine the amount of P released and to calculate the P equivalency values.

RESULTS

The determined phytase activity in the eight dietary treatments is summarised in Table 5. The recovery of the enzyme in cold-pelleted diets was high, ranging from 77 to 112%.

Table 5. Analysed phytase activity (PU/kg diet) in the experimental diets.

Diet no.	Starter diet		Finisher diet		Target
	Analysed phytase activity	Microbial phytase activity[1]	Analysed phytase activity	Microbial Phytase activity[1]	Value
1	138	-	63	-	-
2	122	-	53	-	-
3	126	-	68	-	-
4	112	-	62	-	-
5	579	454	615	553	500
6	980	855	1155	1093	1000
7	1372	1247	1746	1684	1500
8	1674	1549	2141	2079	2000

[1] Microbial phytase activity = Analysed phytase activity minus average ingredient derived phytase activity (average of diets 1, 2, 3 and 4).

Mortality at 42 days of age in this study was higher than expected (5.6%). Most of the deaths occurred between day 15 and 18, and appear to have been exacerbated by the stress of moving the birds from the brooder to colony cages on day 14. Post-mortem examination of the dead birds revealed no abnormalities in the gross pathology of major organs. Mortality, however, was not related to dietary levels of non-phytate P (nP) or phytase. The mortality, averaged across sexes, for dietary treatments 1 to 8 were 5.0, 2.5, 3.8, 8.8, 6.3, 7.5, 3.8 and 7.5%, respectively. Statistical analysis of mortality data, after transformation of percentage values to $\sqrt{n+1}$, showed no treatment effects (P=0.65).

Table 6. Weight gain, feed intake, feed/gain of male broilers (1 to 21 day of age)[1] as influenced by dietary non-phytate P level and phytase.

Treatment		Weight gain (g)	Feed intake (g)	Feed/gain (g/g)
Non-phytate p (%)	Added phytase (PU/kg diet)			
0.30	-	737	1143	1.557
0.36	-	812	1194	1.473
0.42	-	834	1235	1.485
0.48	-	811	1203	1.498
0.30	500	811	1170	1.463
0.30	1000	844	1213	1.461
0.30	1500	827	1201	1.454
0.30	2000	868	1222	1.426
	Pooled SEM	12.5	19.5	0.017
Significance[2]				
Phosphorus effect				
	Linear	**	†	NS
	Quadratic	**	†	*
Phytase effect				
	Linear	***	*	***
	Quadratic	**	NS	**

[1] Each mean represents five pens (8 birds per pen). Feed per gain values were corrected for mortality.
[2] NS, not significant; † P<0.10; * P<0.05; ** P<0.01; *** P<0.001.

Performance Data

Weight gain, feed intake, feed/gain of male and female broilers (1 to 21 day of age) as influenced by dietary P level and phytase are shown in Tables 6 and 7, respectively. In

males, increasing dietary nP levels improved (P<0.10 to 0.001) weight gain, feed intake and feed/gain, but an inexplicable quadratic effect (P<0.10 to 0.01) was observed at 0.48% nP. Weight gain and feed/gain were improved quadratically (P<0.01) and feed intake linearly (P<0.05) with increasing dietary additions of phytase.

In females, weight gain and feed/gain improved linearly (P<0.05 to 0.001) as the level of nP in the diet increased. Dietary nP had no effect on feed intake. Increasing additions of phytase quadratically (P<0.05 to 0.001) improved weight gain and feed/gain, and linearly (P<0.001) increased feed intake.

In both sexes, the performance of birds fed 0.30% nP diets with 500 PU phytase/kg were similar (P>0.10) to those fed the 0.48% nP diet.

Table 7. Weight gain, feed intake, feed/gain of female broilers (1 to 21 day of age)[1] as influenced by dietary non-phytate P level and phytase.

Treatment		Weight gain (g)	Feed intake (g)	Feed/gain (g/g)
Non-phytate P (%)	Added phytase (PU/kg diet)			
0.30	-	683	1072	1.581
0.36	-	702	1075	1.531
0.42	-	728	1107	1.523
0.48	-	748	1106	1.505
0.30	500	763	1142	1.507
0.30	1000	786	1161	1.484
0.30	1500	803	1192	1.514
0.30	2000	776	1136	1.471
	Pooled SEM	16.3	23.6	0.011
Significance[2]				
Phosphorus effect				
	Linear	*	NS	***
	Quadratic	NS	NS	NS
Phytase effect				
	Linear	***	***	***
	Quadratic	*	NS	***

[1] Each mean represents five pens (8 birds per pen). Feed per gain values were corrected for mortality.
[2] NS, not significant; * P<0.05; *** P<0.001.

Weight gain, feed intake, feed/gain of male and female broilers (22 to 42 day of age) as influenced by dietary P level and phytase are shown in Tables 8 and 9,

respectively. In males, increasing dietary nP levels quadratically ($P<0.05$) increased feed intake and, tended ($P=0.11$) to improve weight gain and feed/gain (Table 8). Phytase additions had no effect on feed intake, but caused linear ($P<0.001$) increases in weight gains and quadratic ($P<0.05$) responses in feed/gain. In females, dietary nP linearly ($P<0.05$) improved weight gain and tended ($P=0.11$) to improve feed/gain (Table 9). Increasing additions of phytase quadratically improved weight gain ($P<0.10$) and feed/gain ($P<0.001$). Feed intake was unaffected by phytase levels.

In both sexes, performance of birds fed diets containing 0.20% nP with 500 PU phytase/kg were similar ($P>0.10$) to those fed diets containing 0.38% nP.

Table 8. Weight gain, feed intake, feed/gain of male broilers (22 to 42 day of age)[1] as influenced by dietary non-phytate P level and phytase.

Treatment		Weight gain (g)	Feed intake (g)	Feed/gain (g/g)
Non-phytate P (%)	Added phytase (PU/kg diet)			
0.20	-	1715	3483	2.034
0.26	-	1774	3650	2.057
0.32	-	1765	3633	2.060
0.38	-	1827	3545	1.948
0.20	500	1829	3420	1.869
0.20	1000	1840	3422	1.859
0.20	1500	1828	3383	1.852
0.20	2000	1912	3520	1.841
	Pooled SEM	34.7	50.8	0.034
Significance[2]				
Phosphorus effect				
	Linear	NS	NS	NS
	Quadratic	NS	*	NS
Phytase effect				
	Linear	**	NS	***
	Quadratic	*	NS	*

[1] Each mean represents five pens (8 birds per pen). Feed per gain values were corrected for mortality.
[2] NS, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Weight gain, feed intake, feed/gain and toe ash of male and female broilers (1 to 42 day of age) as influenced by dietary P level and phytase are shown in Tables 10 and 11, respectively. In males, increasing nP levels had a linear effect ($P<0.05$) on weight

gain and feed/gain, and a quadratic effect ($P<0.05$) on feed intake. Weight gain and feed intake improved quadratically ($P<0.001$) with increasing additions of phytase in the diet. Phytase had no effect on feed intake. In females, increasing nP levels linearly improved weight gain ($P<0.01$) and feed/gain ($P<0.05$). Feed intake was unaffected by dietary nP levels. Increasing levels of phytase quadratically improved weight gain ($P<0.05$) and feed/gain ($P<0.001$), and linearly ($P<0.05$) increased feed intake.

Table 9. Weight gain, feed intake, feed/gain of female broilers (22 to 42 day of age)[1] as influenced by dietary non-phytate P level and phytase.

Treatment		Weight gain (g)	Feed intake (g)	Feed/gain (g/g)
Non-phytate P (%)	Added phytase (PU/kg diet)			
0.20	-	1456	3069	2.107
0.26	-	1514	3120	2.062
0.32	-	1553	3208	2.065
0.38	-	1576	3182	2.020
0.20	500	1566	3158	2.017
0.20	1000	1579	3078	1.950
0.20	1500	1592	3194	2.007
0.20	2000	1596	3046	1.909
	Pooled SEM	29.6	68.4	0.027
Significance[2]				
Phosphorus effect				
	Linear	*	NS	NS
	Quadratic	NS	NS	NS
Phytase effect				
	Linear	**	NS	***
	Quadratic	†	NS	***

[1] Each mean represents five pens (8 birds per pen). Feed per gain values were corrected for mortality.
[2] NS, not significant; † $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

In both sexes, the magnitude of improvement to added enzyme was greatest at 500 PU/kg of diet and tended to plateau with further additions. It is also noteworthy that the weight gain and feed/gain of birds fed phytase-supplemented diets were numerically superior to those fed diets supplemented with MCP. The birds receiving the low-P diets with 500 PU phytase/kg had a similar weight gain and better ($P<0.05$) feed efficiency compared to those receiving P-adequate diets.

No signs of leg weakness were observed in any of the dietary treatments during the trial. Increasing dietary levels of nP (linear, $P<0.01$; quadratic, $P<0.10$) and phytase (linear, $P<0.001$; quadratic, $P<0.05$) increased the ash percentage of dry toes in male broilers (Table 10). Toe ash contents in female broilers increased as the levels of nP (linear, $P<0.001$) and phytase (linear, $P<0.001$; quadratic, $P<0.01$) in the diet increased (Table 11). In both sexes, toe ash contents of birds fed the low-P diet with 500 PU phytase/ kg of diet were comparable to those of birds fed adequate-P diets.

Table 10. Weight gain, feed intake, feed/gain and toe ash of male broilers (1 to 42 d of age)[1] as influenced by dietary non-phytate P level and phytase.

Treatment		Weight gain (g)	Feed intake (g)	Feed/gain (g/g)	Toe ash (% dry matter)
Non-phytate P[2] (%)	Added phytase (PU/kg diet)				
0.30/0.20	-	2452	4626	1.911	9.68
0.36/0.26	-	2586	4844	1.873	10.57
0.42/0.32	-	2600	4869	1.880	10.67
0.48/0.38	-	2627	4741	1.832	10.69
0.30/0.20	500	2640	4590	1.775	10.70
0.30/0.20	1000	2684	4637	1.754	10.87
0.30/0.20	1500	2701	4584	1.730	10.82
0.30/0.20	2000	2781	4743	1.740	10.96
	Pooled SEM	34.9	61.4	0.019	0.21
Significance[2]					
Phosphorus effect					
	Linear	*	NS	*	**
	Quadratic	NS	*	NS	†
Phytase effect					
	Linear	***	NS	***	***
	Quadratic	***	NS	***	**

[1] Each mean represents five pens (8 birds per pen). Feed per gain values were corrected for mortality.
[2] Non-phytate P sequence used for 1-21 d and 22-42 d, respectively.
[3] NS, not significant; † $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Apparent Ileal Digestibility of Phytate P, Phosphorus, Nitrogen and Apparent Metabolisable Energy

Apparent ileal digestibility of phytate P, phosphorus and nitrogen, and AME of male and female broilers fed wheat-soy diets as influenced by dietary non-phytate P level and

phytase are summarised in Tables 12 and 13, respectively. Apparent ileal degradation of phytate in the basal diet was around 0.270. Phytate degradation was unaffected by nP levels, but increased (linear effect, $P<0.001$) with the addition of phytase. Phytate degradation in males fed diets with 0, 500, 1000, 1500 and 2000 PU phytase/kg were 0.280, 0.593, 0.635, 0.697 and 0.791, respectively. Corresponding values in the females were 0.272, 0.519, 0.556, 0.709 and 0.715, respectively.

Table 11. Weight gain, feed intake, feed/gain and toe ash of female broilers (1 to 42 days of age)[1] as influenced by dietary non-phytate P level and phytase.

Treatment		Weight gain (g)	Feed intake (g)	Feed/gain (g/g)	Toe ash (% dry matter)
Non-phytate P (%)	Added phytase (PU/kg diet)				
0.30/0.20	-	2139	4142	1.944	9.71
0.36/0.26	-	2216	4195	1.895	10.35
0.42/0.32	-	2280	4315	1.892	11.23
0.48/0.38	-	2324	4287	1.885	11.16
0.30/0.20	500	2329	4300	1.857	10.77
0.30/0.20	1000	2365	4242	1.806	10.93
0.30/0.20	1500	2395	4390	1.853	11.07
0.30/0.20	2000	2372	4182	1.780	11.10
	Pooled SEM	40.6	86.6	0.014	0.17
Significance[2]					
Phosphorus effect					
	Linear	**	NS	*	***
	Quadratic	NS	NS	NS	NS
Phytase effect					
	Linear	***	*	***	***
	Quadratic	*	NS	***	**

[1] Each mean represents five pens (8 birds per pen). Feed per gain values were corrected for mortality.
[2] Non-phytate phosphorus sequence used for 1 to 21 and 22 to 42 days of age, respectively.
[3] NS, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Apparent ileal P digestibility was improved by the addition of nP and phytase (Tables 12 and 13), but the increments were greater with phytase additions. Increasing levels of nP increased P digestibility both in males (Table 12; linear effect, $P<0.001$; quadratic effect, $P<0.06$) and females (Table 13; linear effect, $P<0.001$; quadratic effect, $P<0.06$). Addition of phytase to the low-P diet improved the ileal P digestibility in both males and females (linear effect, $P<0.001$; quadratic effect, $P<0.001$), but the

Table 12. Apparent ileal digestibility of phytate P, phosphorus (AIPD), nitrogen (AIND), apparent metabolisable energy (AME; MJ/kg dry matter) and N-corrected AME (AMEn; MJ/kg dry matter) of male broilers fed wheat-soy diets as influenced by dietary non-phytate P level and phytase.

Treatments		Phytate P	AIPD	AIND	AME		AMEn	
Non-phytate P[1] (%)	Phytase (PU/kg diet)				18-21 d	38-41 d	18-21 d	38-41 d
0.30/0.20	-	0.280	0.433	0.794	13.32	13.45	12.58	12.82
0.36/0.26	-	0.195	0.457	0.788	13.39	13.28	12.64	12.66
0.42/0.32	-	0.282	0.508	0.786	13.04	13.10	12.31	12.48
0.48/0.38	-	0.283	0.494	0.777	13.05	13.08	12.33	12.48
0.30/0.20	500	0.593	0.609	0.819	13.51	13.77	12.73	13.15
0.30/0.20	1000	0.635	0.660	0.841	13.62	13.90	12.84	13.26
0.30/0.20	1500	0.697	0.677	0.831	13.72	13.93	12.94	13.31
0.30/0.20	2000	0.791	0.735	0.844	13.84	14.07	13.05	13.40
	Pooled SEM	0.0275	0.0163	0.0068	0.127	0.101	0.117	0.097
Significance[2]								
Phosphorus effect	Linear	NS	***	†	NS	†	NS	NS
	Quadratic	NS	†	NS	NS	NS	NS	NS
Phytase effect								
	Linear	***	***	***	**	**	***	**
	Quadratic	NS	***	*	NS	NS	NS	NS

[1] Non-phytate P sequence used during days 1-21 and 22-42, respectively.
[2] NS, not significant; † $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 13. Apparent ileal digestibility of phytate P, phosphorus (AIPD), nitrogen (AIND), apparent metabolisable energy (AME; MJ/kg dry matter) and N-corrected AME (AMEn; MJ/kg dry matter) of female broilers fed wheat-soy diets as influenced by dietary non-phytate P level and phytase.

Treatment		Phytate P	AIPD	AIND	AME		AMEn	
Non-phytate P[1] (%)	Phytase (PU/kg diet)				18-21 d	38-41 d	18-21 d	38-41 d
0.30/0.20[1]	-	0.272	0.354	0.788	13.34	12.91	12.57	12.25
0.36/0.26	-	0.228	0.430	0.798	13.49	13.18	12.74	12.60
0.42/0.32	-	0.219	0.499	0.801	13.40	13.26	12.68	12.67
0.48/0.38	-	0.195	0.488	0.801	13.27	12.91	12.53	12.33
0.30/0.20	500	0.519	0.588	0.830	13.62	13.78	12.83	13.13
0.30/0.20	1000	0.556	0.600	0.836	13.81	13.84	13.02	13.21
0.30/0.20	1500	0.709	0.687	0.834	13.69	13.72	12.91	13.11
0.30/0.20	2000	0.715	0.674	0.833	14.99	14.02	13.20	13.40
	Pooled SEM	0.0307	0.0119	0.0052	0.078	0.098	0.078	0.089
Significance [2]								
Phosphorus effect								
	Linear	NS	***	*	NS	NS	NS	NS
	Quadratic	NS	**	NS	NS	NS	NS	NS
Phytase effect								
	Linear	***	***	***	***	***	*	***
	Quadratic	NS	***	***	*	***	*	***

[1] Non-phytate phosphorus sequence used during days 1-21 and 22-42, respectively.
[2] NS, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

increments were greater with added phytase. The ileal digestibility coefficients of P in males fed the low-P diet, adequate-P diet and low-P plus 500 PU phytase/kg were 0.433, 0.494 and 0.609, respectively. The corresponding values in the females were 0.354, 0.488 and 0.588, respectively.

Increasing dietary nP levels resulted in small, but significant (Table 12; linear effect, $P<0.10$) reductions in the apparent ileal digestibility of nitrogen in male broilers, while the reverse situation was observed in the females. In female broilers, increasing dietary nP levels resulted in small, but significant (Table 13; linear effect, $P<0.05$) increases in the apparent ileal digestibility of nitrogen. Addition of phytase increased the digestibility of nitrogen (linear effect, $P<0.001$; quadratic effect, $P<0.05$ to 0.001) in males and females. The increases between the low-P diet and the phytase diets ranged from 2.5-5.0 percentage units in the males and from 4.2-4.5 percentage units in the females.

The AME value during the starter phase (18-21 d) in both males and females was unaffected by increasing dietary nP levels (Tables 12 and 13). Addition of graded levels of phytase improved the AME of diets in the males (linear effect, $P<0.01$) and females (linear effect, $P<0.001$; quadratic effect, $P<0.05$). Addition of 500 PU phytase/kg to the low-P diet increased the AME by 0.19 and 0.28 MJ/kg dry matter in the males and females, respectively.

Increasing dietary nP levels tended to linearly ($P<0.10$) decrease the AME content during the finisher phase (38-41 d) in male broilers, but had no influence in female broilers (Tables 12 and 13). Added phytase improved the AME contents in both male (linear effect, $P<0.01$) and female (linear effect, $P<0.001$; quadratic effect, $P<0.001$) birds. Increments in AME during the finisher phase were higher compared to those in the starter phase (Tables 12 and 13). Addition of 500 PU phytase/kg to the low-P diet increased the AME by 0.32 and 0.87 MJ/kg dry matter in the males and females, respectively.

Correction for zero-nitrogen retention had little effect on the trends. The differences in AME between treatments remained the same after correction to zero-nitrogen retention.

Apparent Dry Matter Retention, Excreta Phosphorus Contents, and Utilisation (Retention and Excretion) of Phosphorus (18-21 d)

Apparent retention of dry matter, excreta P contents and utilisation (retention and excretion) of phosphorus in male and female broilers fed wheat-soy diets as influenced by dietary levels of non-phytate P and phytase are summarised in Tables 14 and 15, respectively. In both males and females, dry matter retention was not influenced by dietary nP levels. Addition of 500 PU/kg phytase to the low-P diet caused linear (P<0.05 to 0.01) increases in dry matter retention in males and females by 1.6 and 0.7 percentage units, respectively.

Excreta P contents were increased linearly (P<0.001) with increasing levels of dietary nP (Tables 14 and 15). The excreta P content was increased by approximately 40% when the dietary nP level was increased from 0.30 to 0.48%. Addition of phytase to the low-P diet linearly (P<0.05) decreased excreta P content in males and females. Compared to the adequate-P diet, the excreta P level was lowered by approximately 35% in the low-P diet containing 500 PU phytase/kg.

Apparent P retention, expressed as % of intake, decreased linearly (P<0.001) when dietary nP level was increased from 0.30 to 0.48% (Tables 14 and 15). P retention in the males and females fed diets containing 0.30 and 0.48% nP was 0.442 and 0.364, and 0.421 and 0.357, respectively. P retention, expressed as g/kg DM intake, was not influenced by dietary nP levels.

P retention, expressed as % of intake and as g/kg DM intake, increased linearly (P<0.10 to 0.01) when phytase was added to the low-P diet. Addition of 500 PU/kg phytase improved the retention of P in males and females by 2.5 and 4.4 percentage units, respectively.

In both males and females, apparent P excretion, when expressed as % of intake or g/kg DM intake, was increased linearly (P<0.001) when dietary nP level was increased from 0.30 to 0.48% (Tables 14 and 15). P excretion in the males and females fed diets containing 0.30 and 0.48% nP was 0.558 and 0.636, and 0.579 and 0.643, respectively.

P excretion, when expressed as % of intake or g/kg DM intake, decreased linearly (P<0.05 to 0.01) when phytase was added to the low-P diet. Compared to the adequate-P diets, addition of 500 PU/kg phytase lowered P excretion in males and females by 10.3 and 9.8 percentage units, respectively.

Table 14. Apparent retention of dry matter (DM), excreta phosphorus (P) contents and utilisation (retention and excretion) of phosphorus by male broilers fed wheat-soy diets (18-21 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		DM retention	Excreta P	P retention	P retention	P excretion	P excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% of intake)	(% DM)	(% of intake)	(g/kg DM intake)	(% of intake)	(g/kg DM intake)
0.30	-	0.667	1.01	0.442	2.54	0.558	3.20
0.36	-	0.672	1.11	0.438	2.83	0.562	3.63
0.42	-	0.656	1.34	0.336	2.34	0.664	4.61
0.48	-	0.662	1.42	0.364	2.74	0.636	4.80
0.30	500	0.678	0.95	0.467	2.68	0.533	3.06
0.30	1000	0.679	0.96	0.464	2.66	0.536	3.08
0.30	1500	0.685	0.95	0.479	2.75	0.521	3.99
0.30	2000	0.689	0.95	0.486	2.79	0.514	2.95
	Pooled SEM	0.007	0.018	0.012	0.074	0.012	0.074
Significance[2]							
Phosphorus effect							
	Linear	NS	***	***	NS	***	***
	Quadratic	NS	NS	NS	NS	NS	NS
Phytase effect							
	Linear	*	NS	†	†	*	*
	Quadratic	NS	NS	NS	NS	NS	NS

[1] Non-phytate P sequence used during days 1-21.
[2] NS, not significant; † $P<0.10$; * $P<0.05$; *** $P<0.001$.

Table 15. Apparent retention of dry matter (DM), excreta phosphorus (P) contents and utilisation (retention and excretion) of phosphorus by female broilers fed wheat-soy diets (18-21 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		DM retention	Excreta P	P retention	P retention	P excretion	P excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% of intake)	(% DM)	(% of intake)	(g/kg DM intake)	(% of intake)	(g/kg DM intake)
0.30	-	0.675	1.03	0.421	2.42	0.579	3.33
0.36	-	0.677	1.15	0.425	2.75	0.575	3.71
0.42	-	0.674	1.42	0.335	2.33	0.665	4.62
0.48	-	0.678	1.50	0.357	2.70	0.643	4.85
0.30	500	0.685	0.99	0.455	2.61	0.545	3.13
0.30	1000	0.694	0.99	0.475	2.72	0.525	3.02
0.30	1500	0.678	0.95	0.471	2.70	0.529	3.04
0.30	2000	0.700	0.97	0.493	2.83	0.507	2.91
	Pooled SEM	0.004	0.026	0.013	0.081	0.013	0.081
Significance[2]							
Phosphorus effect							
	Linear	NS	***	***	NS	***	***
	Quadratic	NS	NS	NS	NS	NS	NS
Phytase effect							
	Linear	**	*	**	**	**	**
	Quadratic	NS	NS	NS	NS	NS	NS

[1] Non-phytate P sequence used during days 1-21.
[2] NS, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Excreta Nitrogen Contents and Apparent Utilisation (Retention and Excretion) of Nitrogen (18-21 d)

Excreta nitrogen content, retention and excretion of nitrogen in male and female broilers fed wheat-soy diets as influenced by levels of non-phytate P and phytase are shown in Tables 16 and 17, respectively. Excreta nitrogen contents were decreased quadratically ($P<0.001$) in males, but increased linearly ($P<0.10$) in females with increasing levels of dietary nP. Phytase had no effect ($P>0.05$) on the excreta N contents in both the males and females.

In male broilers, dietary nP levels had no effect ($P>0.05$) on apparent nitrogen retention when expressed as % of intake or g/kg DM intake (Tables 16 and 17). Phytase addition to the low-P diet, on the other hand, increased (linear effect, $P<0.01$; quadratic effect, $P<0.10$) nitrogen retention. In female broilers, nitrogen retention, both as % of intake and g/kg DM intake, was lowered (linear effect, $P<0.05$ to 0.001; quadratic effect, $P<0.10$ to 0.05) by increasing nP levels. Added phytase improved the retention, but an unexplainable quadratic effect ($P<0.06$) was observed.

Increasing levels of nP had no effect in males, but increased ($P<0.05$) in females on nitrogen excretion when expressed as % of intake (Tables 16 and 17). In both males and females, dietary nP levels had no effect on the nitrogen retention when expressed as g/kg DM intake. Nitrogen excretion, both as % of intake and as g/kg DM intake, was decreased (linear effect, $P<0.05$; quadratic effect, $P<0.10$) when phytase was added to the low-P diet. Compared to the adequate-P diets, addition of 500 PU/kg phytase lowered nitrogen excretion in males and females by 3.8 and 3.2 percentage units, respectively.

Apparent Dry Matter Retention, Excreta Phosphorus Contents, and Utilisation (Retention and Excretion) of Phosphorus (38-41 d)

Apparent retention of dry matter, excreta phosphorus contents and utilisation (retention and excretion) of phosphorus by male and female broilers fed wheat-soy diets as influenced by dietary levels of non-phytate P and phytase are summarised in Tables 18 and 19, respectively. In both males and females, dry matter retention, when expressed as % of intake, was not influenced by dietary nP levels. Addition of 500 PU/kg phytase

Table 16. Excreta nitrogen (N) contents, apparent retention and excretion of nitrogen by male broilers fed wheat-soy diets (18-21 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		Excreta N	N retention	N retention	N excretion	N excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% DM)	(% of intake)	(g/kg DM of intake)	(% of intake)	(g/kg DM of intake)
0.30	-	4.49	0.576	20.32	0.424	14.97
0.36	-	4.38	0.590	20.72	0.410	14.37
0.42	-	4.27	0.577	20.00	0.423	14.68
0.48	-	4.42	0.569	19.73	0.431	14.92
0.30	500	4.31	0.607	21.40	0.393	13.88
0.30	1000	4.29	0.610	21.51	0.390	13.77
0.30	1500	4.41	0.606	21.37	0.394	13.91
0.30	2000	4.41	0.611	21.54	0.389	13.74
	Pooled SEM	0.053	0.010	0.336	0.010	0.336
Significance[2]						
Phosphorus effect						
	Linear	NS	NS	NS	NS	NS
	Quadratic	***	NS	NS	NS	NS
Phytase effect						
	Linear	NS	*	*	*	*
	Quadratic	NS	†	†	†	†

[1] Non-phytate P sequence used during days 1-21.
[2] NS, not significant; † $P<0.10$; * $P<0.05$; *** $P<0.001$.

Table 17. Excreta nitrogen (N) contents, apparent retention and excretion of nitrogen by female broilers fed wheat-soy diets (18-21 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		Excreta N	N retention	N retention	N excretion	N excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% DM)	(% of intake)	(g/kg DM intake)	(% of intake)	(g/kg DM intake)
0.30	-	4.40	0.595	21.01	0.405	14.28
0.36	-	4.49	0.586	20.58	0.414	14.51
0.42	-	4.63	0.565	19.60	0.435	15.08
0.48	-	4.52	0.579	20.06	0.421	14.59
0.30	500	4.37	0.611	21.55	0.389	13.73
0.30	1000	4.44	0.617	21.75	0.383	13.53
0.30	1500	4.36	0.602	21.25	0.398	14.03
0.30	2000	4.55	0.613	21.63	0.387	13.65
	Pooled SEM	0.085	0.007	0.259	0.007	0.259
Significance[2]						
phosphorus effect						
	Linear	†	*	***	*	NS
	Quadratic	NS	†	*	NS	NS
Phytase effect						
	Linear	NS	NS	NS	NS	NS
	Quadratic	NS	†	†	†	†

[1] Non-phytate P sequence used during days 1-21.
[2] NS, not significant; † $P<0.10$; * $P<0.05$; *** $P<0.001$.

to the low-P diet increased (linear effect, $P<0.05$ to 0.001; quadratic effect, $P<0.10$) dry matter retention in both sexes (0.9 and 2.2 percentage units, respectively).

Phosphorus content in the excreta increased linearly ($P<0.001$) with increasing levels of dietary nP (Tables 18 and 19). The excreta P content was increased by approximately 40% when the dietary nP level was increased from 0.20 to 0.38%. Addition of phytase to the low-P diet decreased the excreta P content in the males (linear effect, $P<0.05$) and females (linear effect, $P<0.001$; quadratic effect, $P<0.001$). Compared to the adequate-P diet, the excreta P level was lowered by around 35% in the low-P diet containing 500 PU phytase/kg.

Apparent P retention, when expressed as % of intake, decreased linearly ($P<0.01$) with increasing dietary nP levels in male broilers (Table 18), but was unaffected in the females (Table 19). On the other hand, P retention, when expressed as g/kg DM intake, was unaffected by dietary nP levels in the males, but decreased (linear effect, $P<0.01$) in females. Phosphorus retention in the males and females fed diets containing 0.20 and 0.38% nP was 0.382 and 0.306, and 0.278 and 0.276, respectively. Phytase addition improved (linear effect, $P<0.001$; quadratic effect, $P<0.05$ to 0.001) P retention in both sexes.

In both males and females, P excretion, when expressed as % of intake or g/kg DM intake, increased linearly ($P<0.05$ to 0.001) when dietary nP level was increased from 0.30 to 0.48% (Tables 18 and 19). The only exception was that in the females dietary nP levels had no effect ($P>0.05$) on apparent P excretion, when expressed as % of intake. Phosphorus excretion expressed as % of intake in males and females fed diets containing 0.30 and 0.48% nP were 0.618 and 0.694, and 0.714 and 0.724, respectively. In males and females P excretion, when expressed as % of intake or as g/kg DM intake, decreased linearly ($P<0.05$) when phytase was added to the low-P diet. Compared to adequate-P diets, addition of 500 PU/kg phytase lowered P excretion in males and females by 12.0 and 16.4 percentage units, respectively.

Excreta Nitrogen Contents and Apparent Utilisation (Retention and Excretion) of Nitrogen (38-41 d)

Excreta nitrogen content, and apparent retention and excretion of nitrogen by male broilers fed wheat-soy diets (38-41 d) as influenced by dietary levels of non-phytate P and phytase are shown in Tables 20 and 21, respectively. In both sexes, excreta N

Table 18. Apparent retention of dry matter (DM), excreta phosphorus (P) contents and utilisation (retention and excretion) of phosphorus by male broilers fed wheat-soy diets (38-41 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		DM retention	Excreta P	P retention	P retention	P excretion	P excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% of intake)	(% DM)	(% of intake)	(g/kg DM intake)	(% of intake)	(g/kg DM intake)
0.30/0.20	-	0.702	0.98	0.382	1.81	0.618	2.92
0.36/0.26	-	0.702	1.16	0.338	1.77	0.662	3.46
0.42/0.32	-	0.700	1.34	0.317	1.86	0.683	4.01
0.48/0.38	-	0.697	1.46	0.306	1.96	0.694	4.43
0.30/0.20	500	0.711	0.94	0.426	2.01	0.574	2.71
0.30/0.20	1000	0.718	0.92	0.453	2.14	0.547	2.58
0.30/0.20	1500	0.712	0.93	0.450	2.13	0.550	2.60
0.30/0.20	2000	0.728	0.89	0.489	2.31	0.511	2.42
	Pooled SEM	0.005	0.022	0.014	0.081	0.014	0.081
Significance [2]							
Phosphorus effect							
	Linear	NS	***	**	NS	**	***
	Quadratic	NS	NS	NS	NS	NS	NS
Phytase effect							
	Linear	**	*	***	***	***	***
	Quadratic	NS	NS	*	*	NS	NS

[1] Non-phytate P sequence used during days 1-21 and 22-42, respectively.
[2] NS, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 19. Apparent retention of dry matter (DM), excreta phosphorus (P) contents and utilisation (retention and excretion) of phosphorus by female broilers fed wheat-soy diets (38-41 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		DM retention	Excreta P	P retention	P retention	P excretion	P excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% of intake)	(% DM)	(% of intake)	(g/kg DM intake)	(% of intake)	(g/kg DM intake)
0.30/0.20	-	0.687	1.082	0.286	1.348	0.714	3.372
0.36/0.26	-	0.696	1.170	0.319	1.668	0.681	3.562
0.42/0.32	-	0.693	1.360	0.290	1.703	0.710	4.167
0.48/0.38	-	0.692	1.498	0.276	1.761	0.724	4.614
0.30/0.20	500	0.709	0.910	0.440	2.074	0.560	2.646
0.30/0.20	1000	0.715	0.972	0.414	1.952	0.586	2.768
0.30/0.20	1500	0.707	0.923	0.424	2.003	0.576	2.717
0.30/0.20	2000	0.721	0.930	0.450	2.127	0.550	2.593
	Pooled SEM	0.004	0.0218	0.015	0.078	0.015	0.078
Significance [2]							
Phosphorus effect							
	Linear	NS	***	NS	**	NS	***
	Quadratic	NS	NS	NS	NS	NS	NS
Phytase effect							
	Linear	***	***	***	***	***	***
	Quadratic	†	***	***	***	***	***

[1] Non-phytate P sequence used during days 1-21 and 22-42, respectively.
[2] NS, not significant; † $P<0.10$; ** $P<0.01$; *** $P<0.001$.

Table 20. Excreta nitrogen (N) contents, apparent retention and excretion of nitrogen by male broilers fed wheat-soy diets (38-41 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		Excreta N	N retention	N retention	N excretion	N excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% DM)	(% of intake)	(g/kg DM intake)	(% of intake)	(g/kg DM intake)
0.30/0.20	-	4.22	0.576	17.08	0.424	12.56
0.36/0.26	-	4.28	0.573	17.06	0.427	12.73
0.42/0.32	-	4.27	0.570	16.91	0.430	12.79
0.48/0.38	-	4.33	0.555	16.35	0.445	13.11
0.30/0.20	500	4.44	0.569	16.86	0.431	12.78
0.30/0.20	1000	4.35	0.587	17.39	0.413	12.25
0.30/0.20	1500	4.44	0.579	17.17	0.421	12.47
0.30/0.20	2000	4.21	0.614	18.20	0.386	11.44
	Pooled SEM	0.076	0.009	0.258	0.009	0.258
Significance [2]						
Phosphorus effect						
	Linear	NS	NS	†	NS	NS
	Quadratic	NS	NS	NS	NS	NS
Phytase effect						
	Linear	NS	**	**	**	**
	Quadratic	**	*	*	*	*

[1] Non-phytate P sequence used during days 1-21 and 22-42, respectively.
[2] NS, not significant; † $P<0.10$; * $P<0.05$; ** $P<0.01$.

Table 21. Excreta nitrogen (N) contents, apparent retention and excretion of nitrogen by female broilers fed wheat-soy diets (38-41 d) as influenced by dietary levels of non-phytate P and phytase.

Treatment		Excreta N	N retention	N retention	N excretion	N excretion
Non-phytate P[1] (%)	Added phytase (PU/kg diet)	(% DM)	(% of intake)	(g/kg DM intake)	(% intake)	(g/kg DM intake)
0.30/0.20	-	4.67	0.514	15.24	0.486	14.40
0.36/0.26	-	4.54	0.536	15.96	0.464	13.83
0.42/0.32	-	4.39	0.545	16.19	0.455	13.51
0.48/0.38	-	4.40	0.540	15.90	0.460	13.56
0.30/0.20	500	4.12	0.596	17.67	0.404	11.97
0.30/0.20	1000	4.31	0.585	17.35	0.415	12.29
0.30/0.20	1500	4.35	0.569	16.87	0.431	12.77
0.30/0.20	2000	4.50	0.576	17.08	0.424	12.56
	Pooled SEM	0.137	0.017	0.500	0.017	0.500
Significance [2]						
Phosphorus effect						
	Linear	NS	NS	NS	NS	NS
	Quadratic	NS	NS	NS	NS	NS
Phytase effect						
	Linear	NS	*	*	*	*
	Quadratic	*	**	**	**	**

[1] Non-phytate P sequence used during days 1-21 and 22-42, respectively.
[2] NS, not significant; * $P<0.05$; ** $P<0.01$.

contents were not affected by dietary levels of nP. Addition of phytase to the low-P diet caused quadratic (P<0.05 to 0.01) increase in males and decrease in females in excreta N content. Compared to the adequate-P diet, the excreta N content was lowered by approximately 0.28 percentage units in the low-P diet containing 500 PU phytase/kg in the females.

In both sexes, apparent nitrogen retention, expressed as % of intake, was unaffected by increasing dietary nP levels, but increased (linear effect, P<0.05 to 0.01; quadratic effect, P<0.05 to 0.01) with phytase addition. When the nitrogen retention was expressed as g/kg DM intake, phosphorus effect tended to be significant (P<0.09) only in the males and phytase effects (linear effect, P<0.05 to 0.01; quadratic effect, P<0.05 to 0.01) were seen in males and females.

In both sexes, dietary nP levels had no effect on apparent nitrogen excretion when expressed as % of intake or g/kg DM intake (Tables 20 and 21). N excretion, when expressed as % of intake or as g/kg DM intake, was decreased (linear effect, P<0.05 to 0.01; quadratic effect, P<0.05 to 0.01) when phytase was added to the low-P diet. Compared to the adequate-P diets, addition of 500 PU/kg phytase lowered N excretion in males and females by 1.4 and 5.6 percentage units, respectively.

Phosphorus replacement value for microbial phytase

Linear and non-linear response functions of body weight gain, feed intake and feed efficiency that best fit the data were derived for inorganic P and phytase levels in both sexes, and were used to determine the amount of P released. However, equations with moderate or high R^2 values could not be consistently generated for feed intake and feed per gain data during the finisher phase, although the probability values were significant.

Based on equations for weight gain (Table 22), the amounts of nP equivalent to additions of 500, 1000, 1500 and 2000 PU phytase/ kg diet in male and female broilers during the starter and finisher phases were calculated and the results are summarised in Table 23. Using the formula, released nP = P equivalency estimate – nP in the basal diet (0.30 or 0.20%), the weight gain responses from phytase additions were determined to be equivalent to 0.24-0.26 and 0.21-0.38% release nP during the starter and finisher phase, respectively. The above estimates translate into P release values of 72 to 131% from phytate P.

Table 22. Linear and non-linear equations for weight gain responses of male and female broilers fed wheat-soy diets containing four non-phytate P (nP) and five phytase levels from 1 to 42 day of age.

	nP effects		Phytase effects	
Item	Equation	R^2	Equation	R^2
Males				
1-21 d	$Y = -367.0 + 5730X - 6800X^2$	0.66	$Y = 856.9 (1- 0.14e^{-0.00029X})$	0.71
22-42 d	$Y = 1557.1 + 547X$	0.55	$Y = 1746.0 + 0.079X$	0.49
Females				
1-21 d	$Y = 572.8 + 365.3X$	0.51	$Y = 789.9 (1- 0.135e^{-0.003X})$	0.67
22-42 d	$Y = 1265.6 + 664X$	0.51	$Y = 1496.5 + 0.061X$	0.66

Table 23. Equivalency values of inorganic phosphorus for microbial phytase in broilers fed wheat-soy diets from 1 to 42 day of age.

	Phytase (PU/kg diet)			
Item	500	1000	1500	2000
Equivalent of nP, %				
Starter phase (1-21 d)				
Males	0.53	0.52	0.52	0.51
Females	0.53	0.58	0.59	0.59
Finisher phase (22 -42 d)				
Males	0.42	0.49	0.56	0.63
Females	0.39	0.44	0.49	0.53
Mean equivalent of nP, %				
Starter phase (1-21 d)	0.53	0.55	0.56	0.55
Finisher phase (22 -42 d)	0.41	0.47	0.53	0.58
Released P, %				
Starter phase[1]	0.24	0.25	0.26	0.25
Finisher phase[2]	0.21	0.26	0.32	0.38
Released P, as % of phytate P[3]	72 - 79	86 - 90	90 - 110	86 - 131

[1] Released P (%) = equivalent of nP (%) – 0.30%.
[2] Released P (%) = equivalent of nP (%) – 0.20%.
[3] Released P (%) = released P (%)/ dietary level of phytate P (0.29%).

DISCUSSION

The conditions necessary for the study, that the growth and toe ash contents in broilers fed the low-P diet would be low in order to respond to added inorganic P or phytase, were met. There were, however, no visible leg problems or excessive mortality in the present study. Phytase supplementation of the low-P diet caused significant improvements in growth in both sexes. Increased weight gain was due both to an increase in feed intake and improvement in feed efficiency. In general, the treatment

effects on parameters tested were similar in both sexes. The responses in growth and toe ash beyond the first addition of inorganic phosphate or the enzyme were minimal. The greatest responses were noted with the first addition and reached a plateau with further additions. In males, over the 42-day trial, compared to the low-P diet (containing 0.30% non-phytate P during the first 21 days and 0.20% non-phytate P during the last 21 days), 500, 1000, 1500 and 2000 PU phytase/kg diet increased weight gain by 7.7, 9.5, 10.2 and 13.4%; and lowered feed/gain by 7.1, 8.2, 9.5 and 8.9%, respectively. In females, the corresponding increments in weight gain were 8.9, 10.6, 12.0 and 10.9%, and reductions in feed/gain were 4.5, 7.1, 4.7 and 8.4%, respectively. It is noteworthy that birds receiving the low-P diet plus 500 PU phytase/kg had a similar weight gain but 5 to 6 point advantage in feed efficiency compared to those fed adequate-P diets.

Toe ash content is a good indicator of the improvements in P availability following the use of phytase (Potter, 1988). Toe ash data provided evidence for the action of phytase on the release of phytate-bound P and demonstrates the effectiveness of phytase in improving P availability in wheat-soybean meal-based diets for broiler chickens. Phytase supplementation of the low-P diet increased toe ash content to a level comparable to those in the adequate-P diets. In male broilers, compared to the low-P treatment, the addition of 500, 1000, 1500 and 2000 PU phytase/kg to the low-P diet increased toe ash content by 10.5, 12.3, 11.8 and 13.2%, respectively. The corresponding increases in females were 11.0, 12.6, 14.0 and 14.3%, respectively.

Published data on the influence of microbial phytase on ileal phytate degradation of birds fed wheat-based diets is scanty, since most phytase evaluation studies have used toe ash or total tract P retention measurements as measures of improvements in P availability (Ravindran *et al.*, 2000). The addition of phytase to the low-P diet resulted in marked increases in ileal degradation of phytate. The apparent phytate degradation in the unsupplemented wheat-soy diet, averaged across male and female broilers, was 27.6% and this was increased by 28.0, 32.0, 42.7 and 47.7 percentage units, respectively, with the addition of 500, 1000, 1500 and 2000 PU phytase/kg. These results are consistent with other reports in wheat or wheat-based diets (Leske and Coon, 1999; Ravindran *et al.*, 2000) and in corn-based diets (Leske and Coon, 1999; Camden *et al.*, 2001). Leske and Coon (1999) reported that the addition of 600 FTU/kg phytase improved faecal phytate P digestibility of wheat and corn for broilers by 16.1

percentage units (from 30.7 to 46.8%) and by 28.4 percentage units (30.8 to 59.2%), respectively. Ravindran *et al.* (2000) reported that the addition of 400 FTU phytase to low-P wheat-sorghum-soy diets increased the ileal P digestibility in diets containing 1.04, 1.32 and 1.57% phytic acid by 21.8, 28.9 and 27.8%, respectively. Based on the assumption that these increases resulted from the in release of P from phytate, the improvements in phytate degradation with phytase addition in their study were calculated to be 40.3, 58.9 and 44.1%, respectively, in low, medium and high phytic acid diets. Camden *et al.* (2001) reported that the addition of 500 PU/kg phytase to corn-based diets improved the ileal phytate degradability by 28.8 percentage units (from 19.3 to 48.1%).

In the present study, 500, 1000, 1500 and 2000 PU phytase/kg diet increased the ileal P digestibility in males by 17.6, 22.7, 24.4 and 30.2 percentage units, respectively. In females, the corresponding increments in ileal P digestibility were 23.5, 24.7, 33.4 and 32.1 percentage units, respectively. The improvements observed with phytase addition in ileal P digestibility are largely a reflection of increased degradation of phytate.

Significant improvements in the AIND were observed with supplemental phytase. Addition of 500 PU/kg phytase to the low-P diet increased the nitrogen digestibility by 3.1% (from 0.794 to 0.819) and 5.3% (from 0.788 to 0.830) for male and female broilers, respectively. The observed improvements are higher than the 2.2 to 3.6% increases reported in broilers fed wheat-based diets supplemented with a phytase produced by submerged liquid fermentation (Ravindran *et al.*, 1999; Ravindran *et al.*, 2000, 2001). Ravindran *et al.* (1999) showed that addition of 600 FTU/kg phytase to a wheat-casein diet increased the AIND by 3.2% (from 0.843 to 0.870). Ravindran *et al.* (2000) reported that the addition of 400 FTU/kg phytase to low-P (0.23% non-phytate P) wheat-based diets improved the AIND by 3.6% (from 0.804 to 0.833). Corresponding improvements in the AIND in the adequate P (0.45% non-phytate P) diets were 2.2% (from 0.806 to 0.823). Ravindran *et al.* (2001) similarly reported that the addition of 500 FTU/kg phytase improved the AIND by 3.1% (from 0.781 to 0.812) in broilers fed wheat-sorghum diets containing 1.0% lysine and 0.45% non-phytate P.

Published data on the effects of microbial phytase on the ileal nitrogen digestibility in the female broilers is scanty. In the present study, greater responses in the AIND were observed with 500 PU/kg phytase addition in the females compared to the males (5.3 vs 3.1%). These results are consistent with the report of Sebastian *et al.*

(1997) who found that the addition of 600 FTU/kg phytase to corn-based diets increased the ileal nitrogen digestibility in female broilers by 1.6% (from 0.819 to 0.832), but had no effect in the males.

The basis for the protein digestibility responses with phytase addition is not well understood, but appears to be related to the capacity of phytic acid to bind protein/amino acids and to the ability of the enzyme to release these bound nutrients by hydrolysing phytic acid (Selle *et al.*, 2000). In the case of the phytase product evaluated in the present study, it is also likely that part of the improvements in protein digestion may have been caused by the presence of side activities by especially proteases.

The energy effects of microbial phytase, especially in wheat-based diets, are also being increasingly recognised (Selle *et al.*, 2000). Addition of phytase increased the AME of wheat-soy diets during both starter and finisher phases, but the increments were greater in the finisher diets. Addition of 500 PU/kg phytase to the low-P diet improved the AME by 1.4-2.1% during the starter phase and by 2.4-6.7% during the finisher phase. Improvements in the AME value of wheat-based diets by phytase addition were consistent with other reports in the wheat or wheat-based diets (Ravindran *et al.*, 1999, 2000, 2001). Ravindran *et al.* (1999) reported that the addition of 600 FTU/kg phytase improved the AME of wheat for broilers by 4.5% (from 13.55 to 14.16 MJ/kg dry matter). Ravindran *et al.* (2000) reported that 400 FTU/kg phytase supplementation increased the AME of low-P wheat-sorghum diets by 1.3% (from 13.36 to 13.54 MJ/kg dry matter). The corresponding improvement in the AME of adequate-P (0.45% non-phytate P) diets was 5.7% (from 12.66 to 13.38 MJ/kg dry matter). Ravindran *et al.* (2001) observed a 2.3% improvement (from 14.22 to 14.54 MJ/kg dry matter) in the AME of wheat-sorghum-soy diets containing 0.45% non-phytate P with addition of 500 FTU/kg phytase. Selle *et al.* (2001), however, reported that the AME of low-P (0.25% non-phytate P) wheat-soy diets was not affected by addition of 600 FTU/kg phytase.

Three explanations may be proposed for the improved energy utilisation in wheat-based diets with supplemental phytase. First, this finding is likely to be reflective, in part, of the increased protein digestibility. It is also possible that added phytase may improve the digestibility of starch by releasing starch from phytate-complexes (Sharma *et al.*, 1978; Thompson, 1988). The overall influence may therefore be a multifaceted effect resulting from small, and possibly additive, improvements in protein and carbohydrate digestion. Second, it is possible that

supplemental phytase may be acting in a manner similar to that of exogenous xylanases in wheat-based diets, by disrupting the cell wall matrix and enhancing the contact between digestive enzymes and cell contents (Ravindran *et al.*, 1999). Finally, and most importantly, the presence of several side enzyme activities, including protease, amylase, cellulase, xylanase and β-glucanase, may have contributed to improved nutrient digestion. In agreement with previous studies (Ravindran *et al.*, 1999; Zyla *et al.*, 1999), these data suggest that enzyme products with multiple activities may provide a useful approach to improve the nutrient utilisation of wheat-based poultry diets.

Reductions in excreta P contents and improvements in apparent retention of P with the addition of phytase are consistent with the improvements observed in the ileal phytate degradation and ileal P digestibility. Phosphorus contents in the excreta were markedly increased when dietary P needs are supplied in the form of inorganic phosphate. In contrast, addition of phytase to the low-P diet caused a 4 to 16% reduction in excreta P content. Compared to the adequate-P diet, the excreta P level in the low-P diet containing 500 PU/kg phytase was lowered by approximately 35%. The magnitude of reductions in the excreta P contents were comparable to those reported with microbial phytase produced by submerged liquid fermentation in corn-based diets (Waldroup *et al.*, 2000; Yan *et al.*, 2000). Waldroup *et al.* (2000) similarly recorded a 28% reduction in excreta P content, compared to those fed adequate-P (0.45% non-phytate P) diets when 800 FTU/kg phytase was added to corn-soy starter diets. Yan *et al.* (2000) found that lowering dietary P levels in corn-soy starters diets to 0.15% below NRC recommendations along with phytase supplementation resulted in a 36% reduction in excreta P level.

Improved retention of nitrogen in phytase-supplemented diets is consistent with the responses observed in ileal nitrogen digestibility and suggests that nitrogen excretion in the manure can also be lowered by phytase addition. Compared to the adequate-P diets, excreta nitrogen contents in the low-P diet containing 500 PU/kg phytase were lowered by 2.4 and 3.3-6.4% in male and female broilers, respectively. These findings highlight the potential value of microbial phytase to reduce the overall nutrient load in the manure.

The estimates obtained for P equivalency of the microbial phytase in the current study translate into P release values of 72 to 131% from phytate P. The procedures employed in the calculations are based on the assumption (Kornegay *et al.*, 1996; Yi *et al.*, 1996) that the weight gain and feed/gain responses are due solely to increments in

available P levels resulting from the addition of inorganic P or release of phytate-bound P with added phytase. While this assumption holds true for inorganic P additions, it is clear that factors unrelated to P availability are also responsible for the responses observed with added phytase. The finding that, averaged across inclusion levels, the feed efficiency of birds fed phytase-supplemented diets were superior to those fed adequate-P diets without supplemental phytase during the 42-d trial period lends support to this view. Among the possible reasons for the pronounced positive effect of the phytase product evaluated, three are worth considering. First, this phytase, being a preparation produced by solid-state fermentation, contains several side-enzyme activities besides phytase. In the present study, although it is routine to add exogenous xylanases in wheat-based diets, the use of xylanases was intentionally avoided. It is likely therefore that the secondary enzymes may have played a part in improving nutrient availability from wheat. Second, it has been suggested that supplemental phytase may directly improve energy utilisation in wheat (Ravindran *et al.*, 1999) by acting in a manner similar to that of exogenous xylanases, by disrupting cell walls and enhancing contact between digestive enzymes and cell contents in wheat. This thesis is based on the report by Frolich (1990) that phytate is an integral component of the cell wall matrix in wheat. In an enzyme product with phytase and xylanase activities, the combined effects of the two enzymes may be expected to result in synergistic responses. Third, the influence of microbial phytase on the availability of nutrients other than P is now well recognised (Ravindran *et al.*, 2000). The observed performance responses, in part, may therefore reflect improved amino acid and energy availability by the added phytase. Overall, these findings suggest that the strategy of using phytase products with multiple enzyme activities may be advantageous in wheat-based poultry diets.

CONCLUSIONS

It is concluded, based on the bird performance, toe ash, phytate P degradation and P retention data, that phytase produced by solid state fermentation is effective in improving P bioavailability in wheat-soy diets for broiler chickens. The performance and bone mineralisation of broilers fed wheat-soy diets were significantly affected when dietary non-phytate P levels were lowered from 0.45% to 0.30%. These negative effects were reversed by the addition of 500 PU phytase/kg suggesting that the phytase was effective in releasing phytate-bound P and improving P availability in wheat-soy diets

for broilers. The better performance of birds fed phytase-supplemented diets compared to those fed diets supplemented with adequate levels of inorganic P may, in part, be attributed to side-enzyme activities present in the phytase evaluated. The present data, along with previous reports (Ravindran *et al.*, 1999; Zyla *et al.*, 1999), suggest that preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilisation in wheat-based diets for poultry.

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Chapter 4

Influence of Sex on Nutrient Utilisation in Broilers Fed Diets With Low and Adequate Dietary Phosphorus Levels

The aim of the study reported in Chapter 3 was to examine the effects of microbial phytase on the performance, toe ash contents and digestibility and utilisation of nutrients in male and female broilers fed wheat-soy diets from day 1 to 42 of age. Selected data from this study were analysed to examine the influence of sex on the performance, toe ash contents, phytate P release, apparent metabolisable energy (AME) and digestibility of phosphorus and nitrogen in broilers fed diets containing low and adequate dietary levels of phosphorus.

ABSTRACT

Energy utilisation and apparent ileal digestibility of nitrogen, phosphorus (P) and phytate P in male and female broilers fed diets deficient or adequate levels of P were compared. Sex of broilers had no effect on the apparent metabolisable energy values determined during week 3. During week 6, the apparent metabolisable energy values for male broilers were higher ($P<0.01$) than those for the females. An interaction ($P<0.05$) between non-phytate P (nP) level x sex was also observed. Apparent metabolisable energy value determined with male broilers was lower in the adequate-P diet, whereas no effect of nP level was observed in females. Female broilers tended to ($P<0.10$) have a higher ileal nitrogen digestibility than the males, but a significant ($P<0.01$) nP level x sex interaction was observed. Males receiving the adequate-P diet had a lower nitrogen digestibility than those receiving the P-deficient diet, whereas the opposite was true in the females.

Apparent ileal phytate P degradation in males was higher than in females (0.282 vs 0.234), but the differences were not significant. A significant interaction ($P<0.05$) between nP level x sex was also observed for apparent ileal P digestibility. Increasing dietary nP levels increased apparent ileal P digestibility in males and females, but the improvements were greater in the females.

INTRODUCTION

Nutrient utilisation in broiler chickens is influenced by a number of factors that are related to the bird, feed, environment and husbandry. The bird-related factors that are relevant include genotype, age, sex and physiological status. Limited published data are available on the influence of sex on the nutrient utilisation in broiler chickens, but there is an increasing interest in this topic (Hughes, 2001; Hughes *et al.*, 2001). Hughes (2001), in studies with 21-day old broiler chickens, found that the females were superior in their ability to utilise energy. It was proposed that sex-related differences in gut morphology and gut microflora may be responsible for this superiority. In the present chapter, selected data from the study reported in Chapter 3 were used to compare the apparent metabolisable energy (AME), phytate phosphorus (P) degradation and nutrient (P and nitrogen) digestibility in male and female broiler chickens fed diets containing deficient or adequate levels of P.

MATERIALS AND METHODS

Details of experimental procedures have been presented in Chapter 3. Of the eight dietary treatments, only the data from two, namely the low-P (diet 1) and adequate-P (diet 4) diets, were used. Briefly, day-old broiler (Ross) birds, females and males, were randomly assigned to 20 pens (8 birds/ pen) in 3-tier electrically heated battery brooders. Within each sex (female or male), the two dietary treatments (low or adequate P levels) were randomly assigned to five pens of eight birds each.

For the broiler starter phase (1-21 d), P-deficient diet was formulated to meet or exceed recommended specifications for all nutrients, except P and calcium (Table 1). The non-phytate P (nP) levels in the deficient and adequate diets were maintained at 0.30 and 0.48%, respectively. During the broiler finisher phase (22-42 d), the corresponding non-phytate P levels were 0.20 and 0.38%, respectively. Titanium oxide was included in finisher diets as a dietary marker.

Feed was offered *ad libitum* and water was freely available at all times during the 42-day trial period. Body weights and feed intake were recorded on a pen basis at weekly intervals. During the third (day 18-21) and sixth week (day 38-41), total collection of excreta was carried out for the determination of AME and nitrogen

retention. On day 42, all surviving birds were euthanased and digesta contents from the lower half of the ileum were collected. Toe samples were also obtained for toe ash measurements. Apparent ileal digestibility of P (AIPD), nitrogen (AIND) and phytate P (AIPPD) were calculated using the ratio of marker in the diet and digesta as described in Chapter 3. The nitrogen-corrected AME (AME_n) values were calculated using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958).

Table 1. Ingredient composition and calculated analysis of broiler starter and finisher diets.

Ingredient	Starter diets		Finisher diets	
	Low-P	Adequate-P	Low-P	Adequate-P
			%	
Wheat	67.17	67.17	71.82	71.82
Soyabean meal	20.01	20.01	16.33	16.33
Canola meal	5.00	5.00	5.00	5.00
Monocalcium phosphate	0.73	1.58	0.28	1.04
Limestone	1.53	1.84	1.36	1.64
Vegetable oil	3.00	3.00	3.00	3.00
Corn starch	0.20	0.20	1.04	0.00
Lysine·HCl	0.35	0.35	0.34	0.34
DL-methionine	0.40	0.40	0.23	0.23
Salt	0.25	0.25	0.20	0.20
Vitamin-trace mineral premix[1]	0.20	0.20	0.20	0.20
Sand	1.16	0.00	0.20	0.20
Calculated analysis				
AME (MJ/kg)	13.0	13.0	13.1	13.1
Lysine, %	1.15	1.15	1.05	1.05
Methionine + cysteine, %	0.94	0.94	0.74	0.74
Calcium, %	0.81	1.06	0.67	0.89
Total P, %	0.58	0.76	0.48	0.64
Non-phytate P, %	0.30	0.48	0.20	0.36

[1] Supplied per kilogram diet: see chapter 3, Table 5.

Data Analysis

Pen means served as the experimental unit for statistical analysis. Data were subject to two-way analysis of variance using the General Linear Models procedure of the SAS® (SAS Institute, 1997) to determine the main effects (nP level and sex) and their

interactions. Differences are considered significant at $P<0.05$. If the data suggest a trend, P values up to $P<0.10$ are shown in the text.

RESULTS AND DISCUSSION

Dietary nP levels influenced broiler performance and toe ash contents (Table 2). As expected, weight gains and toe ash contents of birds fed the adequate-P diet were higher ($P<0.001$) and the feed/gain were lower ($P<0.01$) than those fed the P-deficient diet. Weight gains were higher ($P<0.001$) and feed/gain was lower ($P<0.05$) in male broilers compared to the females. Toe ash content, which is a good indicator of bone mineralisation (Potter, 1988), was not significant ($P>0.05$) different between the sexes. nP level x sex interactions were not significant ($P>0.05$) for performance parameters or toe ash contents.

Though both diets were formulated to contain similar levels of AME, the determined values during week 3 showed that the AME of the P-adequate diet for broilers was lower ($P<0.05$) than that of P-deficient diet (Table 3). It is unclear why increasing the dietary nP level lowered energy utilisation, but similar observations have been previously reported (Ravindran *et al.*, 1999). Perhaps the high molar ratio of calcium to phytate in adequate-P diets leads to the formation of insoluble calcium-phytate complexes, thereby contributing to the observed effects, but how the calcium-phytate complex lowers AME is difficult to explain. Ravindran *et al.* (1999) postulated that calcium-phytate may complex with fatty acids in the gut lumen to form insoluble soaps, thereby lowering fat digestibility and AME. The dietary nP level had no effect on the AME values determined during week 6.

Sex of broilers had no effect on AME values determined during week 3. During week 6, the AME values for female broilers were lower ($P<0.01$) than those for the males. This finding is in contrast to previous reports where females were shown to utilise feed energy better than the males. Ten Doeschate *et al.* (1993) reported that female broilers showed a small, but significantly, higher energy metabolisability than those of males (0.74 *vs* 0.73). Hughes *et al.* (2001) similarly reported that the energy value of wheat-based diets is influenced by sex, with female broilers showing significantly higher AME values (14.6 *vs* 14.9 MJ/kg DM). On the other hand, Wallis and Balnave (1984) found that sex had no major effect on the metabolisable energy of a wheat-based diet for broiler chickens from 30 to 50 days of age. Guirguis (1975, 1976)

found that sex had no significant effect on the AME of a range of feedstuffs, except for oats, tallow and fish meal where AME values were higher for females. In the present study, an interaction ($P<0.10$) between nP level x sex was also observed; the AME values in male broilers tended ($P<0.10$) to decrease when dietary nP level was increased, whereas no effect was observed in females.

Table 2. Weight gain, feed/gain and toe ash contents of male and female broilers (1-42 days post-hatching) fed diets containing deficient or adequate levels of phosphorus (P).

Treatment	Weight gain (g)	Feed/gain (g/g)	Toe ash (%, dry basis)
Males			
P-deficient diet	2452	1.911	9.68
P-adequate diet	2627	1.832	10.70
Females			
P-deficient diet	2139	1.944	9.71
P-adequate diet	2324	1.885	11.16
Pooled SEM	41.4	0.018	0.20
Source of variation			
nP level	***	**	***
Sex	***	*	NS
nP level x sex	NS	NS	NS

NS Not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Correction for zero-nitrogen retention was carried out to exclude the possibility that the observed sex differences in energy utilisation during week 6 were not the result of differences in nitrogen retention. Nitrogen correction had little effect on the magnitude of differences between the males and females, with differences ($P<0.01$) in favour of males remaining.

Overall female broilers had a slightly better ($P<0.10$) ileal nitrogen digestibility than the males. These findings are in agreement with those reported by Ten Doeschate *et al.* (1993) who found that female broilers showed nitrogen digestibility coefficients that were, in general, 3% higher than those of male birds. However, Wallis and Balnave (1984) reported that amino acid digestibilities were not influenced by the sex of broilers. The significant ($P<0.01$) nP level x sex interaction indicated that the AIND differed between sexes at each nP level. Males receiving the adequate-P diet had a

lower AIND than those receiving the P-deficient diet, whereas the reverse was true in the females.

Table 3. Apparent metabolisable energy (AME; MJ/kg dry matter), nitrogen-corrected AME (AME_n; MJ/kg dry matter) and apparent ileal digestibility of nitrogen (AIND), phytate P (AIPPD) and phosphorus (AIPD) of diets containing deficient or adequate levels of P for male and female broilers.

Treatment	AME		AMEn		AIND	AIPPD	AIPD
	18-21 d	38-41 d	18-21 d	38-41 d			
Males							
P-deficient diet	13.32	13.45	12.58	12.82	0.794	0.280	0.433
P-adequate diet	13.05	13.08	12.33	12.48	0.777	0.283	0.494
Females							
P-deficient diet	13.34	12.81	12.57	12.27	0.788	0.273	0.354
P-adequate diet	13.27	12.91	12.53	12.33	0.801	0.195	0.488
Pooled SEM	0.08	0.13	0.07	0.12	0.005	0.029	0.013
Source of variation							
nP level	*	NS	†	NS	NS	NS	***
Sex	NS	**	NS	*	†	NS	**
nP level x sex	NS	†	NS	NS	**	NS	*

NS Not significant; † $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Apparent ileal phytate P degradation in males was higher than that in females (0.282 vs 0.234), but the differences were not significant (Table 3). These results are in disagreement with reports by Edwards and Palo (1989) who found that there was a significant effect of sex with males utilising 20.6 percentage units more phytate P than the females (0.387 vs 0.181). Main effects of nP level and sex were observed for apparent ileal P digestibility ($P<0.05$ to 0.001). A significant nP level x sex interaction ($P<0.05$) was also observed. Increasing dietary nP levels increased apparent ileal P digestibility in males and females, but the improvements were higher in the females than those in the males (13.4 vs 6.1 percentage units). These findings are in contrast to the report of Ravindran *et al.* (2000) who observed that increasing dietary nP levels decreased the apparent ileal P digestibility in male broilers.

CONCLUSIONS

The present data, when considered along with previous published data, suggest that the effect of sex on energy utilisation and nutrient digestibility in broiler chickens are inconsistent and inconclusive. In the present study, AME values were similar between sexes during week 3, but favoured the males during week 6. Apparent ileal nitrogen digestibility, on the other hand, tended to favour the females.

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Chapter 5

Effects of Microbial Phytase on the Performance and Nutrient Utilisation of Broilers as Influenced by Diet Type

There have been numerous studies evaluating the efficacy of microbial phytase in broilers fed corn- or wheat-based diets, but none in which the effects on the two diet types were examined in the same study. The aim of the present study was to examine the influence of phytase addition on the performance, toe ash contents and nutrient utilisation of broilers fed diets based on corn or wheat and containing low or adequate levels of phosphorus.

ABSTRACT

The influence of microbial phytase, produced by solid-state fermentation, on the performance, toe ash contents and nutrient utilisation of male broilers fed diets based on corn and wheat was investigated. The experiment was conducted as a 2 x 2 x 2 factorial arrangement of treatments. Within the factorial, two diet types (corn-soy or wheat-soy) containing two levels of non-phytate phosphorus (0.30 or 0.45%) were evaluated and each level of non-phytate phosphorus (P) was supplemented with 0 or 500 PU phytase/kg diet.

Main effects of diet type and phytase were observed for all parameters. Main effect of non-phytate P was significant ($P<0.05$ to 0.001) only for feed/gain and toe ash contents. Phytase addition improved ($P<0.001$) weight gains irrespective of diet type or non-phytate P level, but the magnitude of improvement was greater in the P-deficient wheat-soy diet resulting in a diet type x non-phytate P interaction ($P<0.05$). Responses in toe ash contents were noted only in P-deficient diets, as indicated by a non-phytate P x phytase interaction ($P<0.001$).

Phytase addition improved ($P<0.01$) the apparent metabolisable energy (AME) of wheat-based diets, but had little effect on the AME of corn-based diets as shown by a diet type x phytase interaction ($P<0.10$). The AME values were not influenced by dietary levels of non-phytate P. Phytase improved ($P<0.001$) ileal nitrogen digestibility in both diet types, but the responses to added phytase tended to be higher in wheat-based diets, as shown by a diet type x phytase interaction ($P<0.10$).

Increasing the dietary non-phytate P level lowered ($P<0.05$) ileal P digestibility and increased ($P<0.001$) excreta P content. Addition of phytase improved ($P< 0.001$) P digestibility, but the increments were higher in low-P diets resulting in a non-phytate P x phytase interaction ($P<0.001$). Phytase addition tended ($P<0.10$) to lower excreta P contents, but the effects were greater in birds fed low-P diets, as shown by a non-phytate P x phytase interaction ($P<0.01$). Compared to those in the adequate-P diets, 500 PU/kg phytase addition to the low-P diets resulted in reductions of excreta P and nitrogen contents (around 35% and 0.9-1.6%, respectively) in both diet types.

INTRODUCTION

Performance responses in broiler chickens to microbial phytase addition are influenced, *inter alia*, by dietary levels of calcium, non-phytate phosphorus (P) and vitamin D_3 (Ravindran *et al.*, 1995). It is likely that the efficacy of microbial phytase is also influenced by diet type, due to inherent variations in the concentration and structural or chemical properties of phytic acid in feed ingredients. This aspect, however, has not been investigated. There have been numerous studies evaluating the efficacy of microbial phytase for broilers fed corn- and wheat-based diets (Coelho and Kornegay, 1999), but none in which the effects on the two diet types were examined in the same study. In the present study, the influence of phytase addition on the performance, toe ash contents and nutrient utilisation of broilers fed diets based on corn or wheat and containing low or adequate levels of P was examined.

MATERIALS AND METHODS

Experimental procedures were approved by the Massey University Animal Ethics Committee (Anonymous, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Enzyme

Phytase (Allzyme® SSF; Alltech Inc., Nicholasville, KY, USA) produced by solid-state fermentation was used and a sample was analysed to contain 1147 PU/g. One unit of phytase (PU) is defined as the quantity of enzyme that releases 1 μmol of inorganic phosphorus/min from 0.0051 mol/L sodium phytate at pH 5.5 at 37 ^{0}C. Because of the fermentation technology employed, this phytase product also contained several side enzyme activities, including protease, amylase, cellulase, xylanase and β-glucanase.

Dietary Treatments

The experiment was conducted as 2 x 2 x 2 factorial arrangement of treatments. Two diet types (corn-soy or wheat-soy) containing two levels of non-phytate P (0.30 or

0.45%) were evaluated (Table 1) and each level of non-phytate P was supplemented with either 0 or 500 PU phytase/kg diet. The increases in non-phytate P level were achieved by the addition of inorganic phosphate. All diets were formulated to contain NRC (1994) recommendations for major nutrients except the low-P diets, which had lower levels of calcium and non-phytate P. Phytase was added in place of corn starch and sand was used to replace monocalcium phosphate and limestone. The enzyme product was first mixed into the premix and then into the diets. The Ca to total P ratio was maintained at around 1.45 to 1 in all diets. Titanium oxide (0.5%) was included in all diets as a dietary marker. After mixing, the diets were cold pelleted (65-70 ^{0}C).

Birds and Conduct of the Trial

Day-old male broiler (Ross) chicks were obtained from a commercial hatchery and randomly assigned to 48 pens (8 chicks/pen) in electrically heated, raised wire-floored starting batteries in an environmentally controlled room. The eight dietary treatments were randomly assigned to six pens of eight chicks each. The diets were fed from day 1 to 21. Diets were offered *ad libitum* and water was available at all times. Body weights and food intake were recorded on a pen basis at weekly intervals. Mortality was recorded daily. Feed conversion ratios, adjusted for mortality, were calculated by dividing total feed intake by weight of live plus dead birds.

During the third week, food intake and excreta outputs were measured quantitatively per pen over four consecutive days (day 18-21). Pooled excreta were mixed well into a slurry (using a blender), representative samples (two samples per pen) were obtained and lyophilised for dry matter (DM) determination. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at -4 ^{0}C until chemical analyses. Samples of diets and excreta were analysed for dry matter, gross energy, nitrogen and P.

On day 21, all surviving chicks were sacrificed by intravenous injection of sodium pentobarbitone. The small intestine was immediately exposed and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from vitelline diverticulum to a point 5cm proximal to the ileo-caecal junction. Digesta were pooled within a pen, lyophilised, ground to pass through a 0.5-

Table 1. Ingredient composition and calculated analysis of experimental diets.

Ingredients	Wheat-soy diet[1]		Corn-soy diet[1]	
	Low-P	Adequate-P	Low-P	Adequate-P
		%		
Wheat	67.85	67.85	0.00	0.00
Corn	0.00	0.00	59.00	59.00
Soyabean meal	24.01	24.01	33.10	33.10
Vegetable oil	3.00	3.00	3.00	3.00
Monocalcium phosphate	0.75	1.46	0.84	1.55
Limestone	1.55	1.77	1.48	1.70
Sand	0.93	0.00	0.93	0.00
Corn starch	0.20	0.20	0.20	0.20
Lysine.HCl	0.33	0.33	0.10	0.10
DL-methionine	0.43	0.43	0.40	0.40
Salt	0.25	0.25	0.25	0.25
Trace mineral premix[2]	0.15	0.15	0.15	0.15
Vitamin premix[3]	0.05	0.05	0.05	0.05
Titanium oxide	0.50	0.50	0.50	0.50
Calculated analysis				
AME (MJ/kg)	12.90	12.90	12.90	12.90
Lysine, %	1.15	1.15	1.15	1.15
Methionine + cysteine, %	0.94	0.94	0.94	0.94
Calcium, %	0.80	1.00	0.80	1.00
Total P, %	0.55	0.70	0.54	0.69
Phytate P, %	0.25	0.25	0.24	0.24
Non-phytate P, %	0.30	0.45	0.30	0.45

[1] 0 or 500 PU of phytase was added to each of the diets.

[2, 3] See Chapter 3, Table 5 for composition of the premixes.

mm sieve and stored at - 4 ^{0}C in airtight containers until chemical analysis. Samples of diets and digesta were assayed for nitrogen, P and titanium.

Toe samples were also obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left and right middle toes of the birds were pooled separately to yield two samples of toes per pen. The composite samples were dried to a constant weight at 100 ^{0}C and then ashed in a muffle furnace at 550 ^{0}C for 16 h (Potter, 1988).

Chemical Analysis

Dry matter content was determined using standard procedures (AOAC, 1990). Gross energy (GE) was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardized with benzoic acid. Total P and nitrogen were determined following Kjeldahl digestion and colorimetric autoanalyses (Twine and Williams, 1971; Technicon, 1973). The titanium content was measured on a UV spectrophotometer following the method of Short *et al.* (1996). The diets were also analysed for phytase activity according to the procedures of Engelen *et al.* (1994) with minor modifications.

Calculations

The AME values were calculated using the following formula. Appropriate corrections were made for differences in dry matter contents.

$$\text{AME (MJ/kg diet)} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

Nitrogen-corrected AME values were calculated using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958).

Apparent ileal nutrient digestibility coefficients were calculated, using titanium as the indigestible marker, as shown below.

$$\text{Apparent ileal nutrient digestibility} = \frac{(Nt / Ti)_d - (Nt / Ti)_i}{(Nt / Ti)_d}$$

where, $(Nt / Ti)_d$ = ratio of nutrient to titanium in diet, and $(Nt / Ti)_i$ = ratio of nutrient to titanium in ileal digesta.

Data Analysis

Pen means served as the experimental unit for statistical analysis. All data were subject to three-way analysis of variance using the General Linear Models procedure of the SAS® (SAS Institute, 1997) to determine the main effects (diet type, non-phytate P level and phytase) and their interactions. Differences were considered significant at $P<0.05$. If the data suggest a trend, probability values up to $P<0.10$ are shown in the text.

RESULTS

The determined phytase activity in the eight dietary treatments is summarised in Table 2. The phytase activity in unsupplemented corn-soy diet was 52 PU/kg diet. In the unsupplemented wheat-soy diet, the phytase activity was high, with an average of 540 PU/kg diet. This high value is reflective of endogenous phytase activity in wheat. The recovery of the enzyme in treatment diets ranged from 73 to 112%.

Table 2. Analysed phytase activity (PU/kg diet) in the experimental diets.

Dietary nP, %	Phytase	Determined phytase activity	Microbial phytase activity[1]	Target value
Corn-soy diet				
0.30	-	52	-	-
	+	460	408	500
0.45	-	59	-	-
	+	426	367	500
Wheat-soy diet				
0.30	-	560	-	-
	+	1054	559	500
0.45	-	522	-	-
	+	935	413	500

[1] Phytase activity in the supplemented diet minus phytase activity in the unsupplemented diet.

Significant ($P<0.05$ to 0.001) effects of diet type and phytase were observed for weight gain, feed intake and feed/gain of broilers, but the effect of non-phytate P was significant ($P<0.05$) only for feed/gain (Table 3). Significant ($P<0.05$ to 0.001) two-way interactions were also observed, but the three-way interaction was not significant ($P>0.05$) for any trait.

Table 3. Response of broiler starters (1-21 days post-hatching) to phytase addition[1] as influenced by diet type (corn-soy or wheat-soy) and dietary levels of non-phytate P (nP)(0.30 or 0.45%)[2].

Dietary nP, %	Phytase	Weight gain (g/bird)	Feed intake (g/bird)	Feed/gain (g/g)	Toe ash (%, dry basis)
Corn-soy diet					
0.30	-	855	1132	1.335	10.52
	+	899	1186	1.320	11.55
0.45	-	883	1151	1.304	11.72
	+	914	1164	1.302	11.99
Wheat-soy diet					
0.30	-	793	1173	1.489	11.02
	+	858	1254	1.472	11.94
0.45	-	796	1168	1.489	12.06
	+	805	1171	1.460	12.21
Pooled SEM		13.4	18.9	0.0107	0.129
Main effect means[3]					
Diet type	Corn	888	1158	1.315	11.44
	Wheat	813	1192	1.478	11.81
nP level	0.30	852	1186	1.404	11.26
	0.45	850	1164	1.389	11.99
Phytase	-	832	1156	1.404	11.33
	+	869	1194	1.389	11.92
ANOVA					
Probability, *P*=	df				
Diet type	1	<0.001	0.02	<0.001	0.001
nP level	1	0.83	0.11	0.05	<0.001
Phytase	1	0.001	0.01	0.05	<0.001
Diet x nP level	1	0.02	0.12	0.25	0.37
Diet x phytase	1	0.98	0.74	0.35	0.54
nP level x phytase	1	0.08	0.04	0.98	<0.001
Diet type x nP level x phytase	1	0.25	0.51	0.40	0.98

[1] Without or with the addition of 500 PU/ kg diet.
[2] Means are based on six pens of eight birds each per treatment group.
[3] Each main effect mean is based on 24 pens of eight birds each.

Birds fed corn-based diets grew faster ($P<0.001$), consumed less feed ($P<0.05$) and had a lower ($P<0.001$) feed/gain than those fed wheat-based diets. Feed/gain was influenced ($P<0.05$) by dietary non-phytate P level, with an increase in dietary P level lowering feed/gain. Weight gain ($P<0.001$), feed intake ($P<0.01$) and feed/gain ($P<0.05$) were influenced by phytase addition. Supplementation of 500 PU/kg diet increased weight gain and feed intake, and lowered feed/gain.

A significant ($P<0.05$) diet type x non-phytate P interaction was observed for weight gain. This was due to an increase in weight gain with increasing non-phytate P levels on the corn-based diets, but the opposite response was seen on the wheat-based diets. Phytase addition resulted in improvements in weight gain and feed intake in the low-P diet, but had little effect in the adequate-P diet, resulting in a non-phytate P x phytase ($P<0.10$ to 0.05) interaction.

Toe ash content of broilers was increased ($P<0.001$) by dietary P level and added phytase (Table 3). The response to phytase addition, however, was only seen in the low-P diets, which resulted in a significant ($P<0.001$) non-phytate P x phytase interaction. Interestingly, toe ash content was influenced ($P<0.001$) by diet type. Birds fed wheat-based diets had higher toe ash contents compared to those fed corn-based diets (11.8 vs 11.4% dry basis).

Influence of phytase addition on AME and apparent ileal digestibility of phosphorus (AIPD) and nitrogen (AIND) in broilers fed corn-soy or wheat-soy diets containing deficient or adequate levels of non-phytate P levels are shown in Table 4. The main effects of diet type ($P<0.001$) and phytase ($P<0.01$) were significant for AME. However, a diet type x phytase interaction ($P<0.06$) was observed. Phytase addition improved the AME of wheat-based diets, but had little effect on the AME of corn-based diets. The AME_n was not influenced by dietary non-phytate P level. The treatments observed for AMEn were similar to those observed for AME.

Apparent ileal P digestibility was influenced by diet type, non-phytate P and phytase (Table 4). Phosphorus in wheat-based diets was more ($P<0.001$) digestible than that in corn-based diets (0.522 vs 0.496). Increasing the dietary P level resulted in reductions ($P<0.05$) in P digestibility. Addition of phytase improved ($P<0.001$) P digestibility, but the increments were higher in low-P diets resulting in a non-phytate P x phytase interaction ($P<0.001$). A tendency for a greater phytase response in wheat-based diets was also observed, as shown by diet type x phytase interaction ($P<0.10$).

Apparent ileal digestibility of nitrogen (AIND) was affected by diet type and phytase (Table 4). Nitrogen in corn-based diets was more ($P<0.001$) digestible than that in wheat-based diets (0.812 vs 0.761). Phytase improved AIND in both diet types, but the responses to added phytase tended to be higher in wheat-based diets, as shown by a diet type x phytase interaction ($P<0.10$).

The influence of phytase addition on dry matter retention and retention and excretion of phosphorus in broilers fed corn-soy or wheat-soy diets containing deficient or adequate levels of non-phytate P is shown in Table 5. Dry mater retention was influenced ($P<0.05$ to 0.001) by diet type, non-phytate P level and phytase. Dry matter retention of birds fed corn-based diets was significantly ($P<0.001$) higher than those in birds fed wheat-based diets (0.748 vs 0.721). Increasing dietary P levels (0.30 to 0.45%) and addition of phytase increased ($P<0.001$) the dry matter retention.

Table 4. Influence of phytase addition[1] on the apparent metabolisable energy (AME; MJ/kg dry matter), nitrogen-corrected AME (AMEn; MJ/kg dry matter) and apparent ileal digestibility of phosphorus (AIPD) and nitrogen (AIND) coefficients in broilers fed corn-soy or wheat-soy diets containing deficient or adequate levels of non-phytate P (nP)[2].

Dietary nP, %	Phytase	AME	AME_n	AIPD	AIND
Corn-soy diet					
0.30	-	14.57	13.68	0.473	0.809
	+	14.64	13.72	0.541	0.826
0.45	-	14.53	13.61	0.470	0.814
	+	14.55	13.63	0.498	0.822
Wheat-soy diet					
0.30	-	13.62	12.86	0.472	0.740
	+	13.81	13.01	0.584	0.775
0.45	-	13.66	12.85	0.498	0.753
	+	13.93	13.10	0.535	0.778
Pooled SEM		0.063	0.058	0.010	0.007
Main effect means[3]					
Diet type	Corn	14.57	13.66	0.496	0.812
	Wheat	13.75	12.96	0.522	0.761
nP level	0.30	14.16	13.31	0.517	0.787
	0.45	14.17	13.30	0.500	0.792
Phytase	-	14.09	13.25	0.478	0.779
	+	14.23	13.37	0.540	0.800
ANOVA					
Probability, *P*=	df				
Diet type	1	<0.001	<0.001	0.001	<0.001
nP level	1	0.87	0.61	0.02	0.35
Phytase	1	0.01	0.01	<0.001	<0.001
Diet x nP level	1	0.12	0.19	0.37	0.51
Diet x phytase	1	0.06	0.05	0.07	0.07
nP level x phytase	1	0.81	0.64	0.001	0.29
Diet type x nP level x phytase	1	0.45	0.51	0.22	0.94

[1] Without or with the addition of 500 PU/ kg diet.
[2] Means are based on six pens of eight birds each per treatment group.
[3] Each main effect mean is based on 24 pens of eight birds each.

Excreta P content was influenced by diet type, non-phytate P level and phytase (Table 5). Phosphorus content in the excreta of birds fed wheat-based diets was significantly (P<0.001), higher than in the excreta of those fed corn-based diets (1.26 vs 1.19%). Increasing dietary P levels, by the addition of inorganic phosphate, increased (P<0.001) the excreta P content from 0.98 to 1.43% dry matter. Phytase addition tended (P<0.10) to lower the excreta P content, but the effects were greater in birds fed low-P diets, as shown by a non-phytate P x phytase interaction (P<0.01).

The P retention and excretion data, as % of intake and g/kg dry matter intake, are presented in Table 5. The main effects of diet type, non-phytate P level and phytase were significant for P retention and excretion in broilers. Birds fed corn-based diets excreted less (P<0.001) P and retained more P (P<0.001) in their body than those fed

wheat-based diets. However, significant (P<0.05 to 0.001) diet type x non-phytate P interactions were observed for the retention and excretion of P. Increasing P level lowered P retention in birds fed corn-based diets, but had no effect on those fed wheat-based diets. P excretion was increased with increasing P level in both diet types, but the magnitude of the increment was higher in wheat-based diets.

Table 5. Influence of phytase addition[1] on dry matter (DM) retention, excreta phosphorus (P) content, retention and excretion of P in broilers fed corn-soy or wheat-soy diets containing deficient or adequate levels of non-phytate P (nP)[2].

Dietary nP, %	Phytase	DM retention (% of intake)	Excreta P content (% DM)	P retention		P excretion	
				% of intake	g/kg DM intake	% of intake	g/kg DM intake
Corn-soy diet							
0.30	-	0.743	0.98	0.578	3.72	0.423	2.72
	+	0.747	0.92	0.613	3.95	0.387	2.49
0.45	-	0.750	1.43	0.486	3.63	0.514	3.84
	+	0.750	1.43	0.487	3.63	0.513	3.83
Wheat-soy diet							
0.30	-	0.712	1.02	0.502	3.19	0.498	3.16
	+	0.719	0.98	0.537	3.41	0.463	2.94
0.45	-	0.720	1.51	0.422	3.31	0.578	4.52
	+	0.734	1.53	0.441	3.45	0.559	4.38
Pooled SEM		0.004	0.016	0.006	0.044	0.006	0.044
Main effect means[3]							
Diet type	Corn	0.748	1.19	0.541	3.73	0.459	3.22
	Wheat	0.721	1.26	0.476	3.34	0.524	3.75
nP level	0.30	0.730	0.97	0.557	3.57	0.443	2.83
	0.45	0.738	1.47	0.459	3.51	0.541	4.14
Phytase	-	0.731	1.23	0.497	3.46	0.503	3.56
	+	0.738	1.21	0.520	3.61	0.480	3.41
ANOVA							
Probability, P=	df						
Diet type	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
nP level	1	0.001	<0.001	<0.001	0.06	<0.001	<0.001
Phytase	1	0.05	0.09	<0.001	<0.001	<0.001	<0.001
Diet x nP level	1	0.23	0.09	0.02	<0.001	0.02	0.01
Diet x phytase	1	0.12	0.50	0.31	0.28	0.31	0.28
nP level x phytase	1	0.75	0.01	0.01	0.03	0.01	0.03
Diet type x nP level x phytase	1	0.38	0.95	0.29	0.25	0.29	0.24

[1] Without or with the addition of 500 PU/ kg diet.
[2] Means are based on six pens of eight birds each per treatment group.
[3] Each main effect mean is based on 24 pens of eight birds each.

Phytase addition improved (P<0.001) P retention and lowered (P<0.001) excretion (Table 5). The phytase effects on P retention and excretion were influenced

by dietary P level, as indicated by non-phytate P x phytase interactions ($P<0.03$). The improvements in retention and the reductions in excretion were greater in low-P diets compared to adequate-P diets.

The influence of phytase addition on excreta nitrogen content and retention and excretion of nitrogen in broilers fed corn-soy or wheat-soy diets containing deficient or adequate levels of non-phytate P levels is shown in Table 6. Excreta nitrogen content was influenced by diet type and phytase. Nitrogen content in the excreta of birds fed corn-based diets was higher ($P<0.001$) than in the excreta of those fed wheat-based diets (4.51 vs 4.35% dry matter). Phytase addition lowered ($P<0.05$) the excreta nitrogen content, but the effects were seen only in birds fed corn-based diets, as shown by a diet type x phytase interaction ($P<0.10$).

The main effects of diet type and phytase were significant ($P<0.05$ to 0.001) for excreta N contents (Table 6). Phytase addition to low-P diets reduced the excreta nitrogen contents in both diet types, but the reductions were greater in corn-based diet than in wheat-base diets (4.3 vs 1.6%) as indicated by a significant ($P<0.10$) diet type x phytase interaction. Compared to the adequate-P diets, 500 PU/kg phytase addition lowered the excreta nitrogen content by 1.6 and 0.9% for the corn- and wheat-based diets, respectively.

The main effects of diet type, non-phytate P level and phytase were significant for nitrogen retention and excretion (Table 6). Birds fed corn-based diets excreted less ($P<0.001$) nitrogen and retained more ($P<0.001$) in their body than those fed wheat-based diets. Increasing P level caused improvements ($P<0.001$) in nitrogen retention and reduced ($P<0.01$) N excretion. The significant ($P<0.001$) diet type x non-phytate P interaction observed for the retention of nitrogen was the result of increasing nP level which increased the retention in birds fed the wheat-based diets, but had little effect on those fed the corn-based diets. The addition of phytase resulted in significant improvements ($P<0.001$) in nitrogen retention and lowered ($P<0.001$) N excretion.

Table 6. Influence of phytase addition[1] on excreta nitrogen (N) content, retention and excretion of N in broilers fed corn-soy or wheat-soy diets containing deficient or adequate levels of non-phytate P (nP)[2].

Dietary nP, %	Phytase	Excreta N content (% DM)	N retention		N excretion	
			% of intake	g/kg DM intake	% of intake	g/kg DM intake
Corn-soy diet						
0.30	-	4.64	0.654	24.45	0.346	12.91
	+	4.44	0.677	25.28	0.323	12.08
0.45	-	4.51	0.674	25.09	0.326	12.14
	+	4.44	0.676	25.19	0.324	12.04
Wheat-soy diet						
0.30	-	4.38	0.607	20.93	0.393	13.53
	+	4.31	0.624	21.49	0.377	12.97
0.45	-	4.35	0.630	22.23	0.370	13.05
	+	4.37	0.646	22.78	0.355	12.50
Pooled SEM		0.047	0.005	0.191	0.005	0.191
Main effect means[3]						
Diet type	Corn	4.51	0.670	25.0	0.330	12.3
	Wheat	4.35	0.627	21.9	0.373	13.0
nP level	0.30	4.44	0.641	23.0	0.360	12.8
	0.45	4.41	0.657	23.8	0.343	12.4
Phytase	-	4.47	0.641	23.2	0.359	12.9
	+	4.39	0.656	23.6	0.344	12.4
ANOVA						
Probability, *P*=	df					
Diet type	1	<0.001	<0.001	<0.001	<0.001	<0.001
nP level	1	0.32	0.001	<0.001	0.001	0.01
Phytase	1	0.03	0.001	<0.001	<0.001	0.001
Diet x nP level	1	0.46	0.10	<0.001	0.10	0.79
Diet x phytase	1	0.08	0.67	0.75	0.67	0.74
nP level x phytase	1	0.42	0.17	0.17	0.17	0.17
Diet type x nP level x phytase	1	0.81	0.21	0.19	0.21	0.19

[1] Without or with the addition of 500 PU/ kg diet.
[2] Means are based on six pens of eight birds each per treatment group.
[3] Each main effect mean is based on 24 pens of eight birds each.

DISCUSSION

The main aim of the current study was to test the proposition that broilers fed different diet types (corn- or wheat-based) would respond similarly, in terms of performance and nutrient utilisation parameters, to phytase addition. The lack of significant diet type x phytase interaction for most parameters indicates that the efficacy of microbial phytase was similar in both diet types.

To our knowledge, this is the first study wherein the effects of microbial phytase on broiler performance and nutrient utilisation in wheat-based and corn-based diets have been evaluated in the same study. In the present study, phytase addition to wheat-based diets improved AME value by 1.4% (from 13.62 to 13.81 MJ/kg dry matter), but had no effect on the AME of corn-based diets. Improvements in the AME value of wheat-based diets by phytase addition were consistent with the study reported in Chapter 3 and other reports (Ravindran *et al.*, 2000, 2001). The study in Chapter 3 showed that the addition of 500 PU/kg phytase to low-P diets improved the AME for male broilers by 1.4% (from 13.32 to 13.51 MJ/kg dry matter) during starter phase and 2.4% (from 13.45 to 13.77 MJ/kg dry matter) during finisher phase. Similarly, Ravindran *et al.* (2000) reported that the addition of 400 FTU/kg phytase improved the AME of wheat-soy diets by 1.3 to 5.7% depending on the dietary level of non-phytate P. Ravindran *et al.* (2001) recorded a 2.3% improvement in the AME (from 14.22 to 14.54 MJ/kg dry matter) of wheat-sorghum-soy diets containing 0.45% non-phytate P with the addition of 500 FTU/kg phytase. Selle *et al.* (2001), however, reported that AME value of broilers fed low-P wheat-soy diets was not affected by the addition of 600 FTU/kg phytase.

In the present study, AME of corn-based diets was not influenced by phytase addition. These results were in contrast to other reports (Namkung and Leeson, 1999; Rostagno *et al.*, 2000; Camden *et al.*, 2001) where AME responses have been observed in corn-based diets. Namkung and Leeson (1999) reported that the addition of 1200 FTU/kg phytase improved the AMEn of corn-soy diets by 2.3% (from 11.9 to 12.1 MJ/kg diet). Similarly, Rostagno *et al.* (2000) also reported that AME values in broilers fed low-P (0.32% non-phytate P) corn-soy diets was improved by 3.9% by 1000 PU/kg phytase addition. Camden *et al.* (2001) showed that addition of 500 U/kg phytase to corn-soy diets improved the AME by 5.6% (from 14.50 to 15.31 MJ/kg dry matter) for broilers.

In the present study, supplemental phytase improved the AIND in both diet types, but the improvements in the AIND with added phytase tended to be higher in the wheat-based diets compared to those in the corn-based diets (4.7 vs 2.1%). Addition of 500 PU/kg phytase to wheat-based diets improved the AIND by 4.7% (from 0.740 to 0.775). The magnitude of responses in the wheat-based diets was higher than the 3.1% improvement (from 0.794 to 0.819) observed in the study reported in Chapter 3 for male broilers. Ravindran *et al.* (1999a) showed that addition of 600 FTU phytase to a wheat-casein diet improved the AIND by 3.2% (from 0.843 to 0.870). Ravindran *et al.* (2000)

recorded an improvement in the AIND by 2.2 to 3.6%, depending on the dietary level of non-phytate P, when 400 FTU/kg phytase was added to wheat-sorghum-soy diets for broilers. Ravindran *et al.* (2001) also reported that the addition of 500 FTU/kg phytase improved the AIND by 3.1% (from 78.1 to 81.2%) for broilers fed wheat-sorghum diets containing adequate level of P.

In the present study, improvement in the AIND when phytase was added to low-P corn-based diets was 2.1% (from 0.809 to 0.826). These results are consistent with several reports (Yi *et al.*, 1996b; Namkung and Leeson, 1999; Ravindran *et al.*, 1999b; Camden *et al.*, 2001), but in disagreement with other reports (Sebastian *et al.*, 1997; Zhang *et al.*, 1999) in broilers fed corn-based diets. Yi *et al.* (1996b) reported that the addition of 750 FTU/kg phytase to corn-based diets (0.45% non-phytate P) improved the AIND by 1.5% (from 89.6 to 91.1%) for turkeys. Similarly, Namkung and Leeson (1999) showed that ileal nitrogen digestibility in broiler chickens fed corn-soy diets supplemented with phytase (1200 U/kg) was increased by 2.0%. Ravindran *et al.* (1999b) also reported that the addition of 1200 FTU/kg phytase increased the AIND of corn in broilers by 4.2% (from 74.4 to 77.5%). Camden *et al.* (2001) showed that the addition of 500 U/kg phytase to corn-based diets improved the AIND by 2.7% (from 84.4 to 86.3%). Sebastian *et al.* (1997), however, reported that there were no effects of microbial phytase on the AIND in male broilers fed corn-based diets. Zhang *et al.* (1999) reported that the addition of 600 FTU/kg phytase to corn-based diets had no effect on the AIND.

As discussed above, the AME and ileal nitrogen digestibility data showed wheat-based diets to be more responsive to phytase supplementation than corn-based diets. Possible mechanisms involved in phytase effects on protein responses have been recently reviewed (Selle *et al.*, 2000). The mechanisms causing the energy effect are poorly understood, but improved digestibility of protein and starch may be responsible, at least in part, for these responses. In the case of the phytase product evaluated in the present study, it is also likely that part of the improvements in AME and nitrogen digestibility may have been caused by the presence of side activities of protease, amylase, cellulase, xylanase and β-glucanase. Moreover, based on the observation that phytate is an integral component of the cell wall matrix in wheat (Frolich, 1990), it has also been postulated that microbial phytase may be acting on wheat in a manner similar to that of exogenous xylanase, by disrupting cell wall matrix and enhancing contact between digestive enzymes and cell contents (Ravindran *et al.*, 1999a).

Birds fed the corn-based diets grew faster and were more efficient in utilising feed than those fed the wheat-based diets. Supplementation of wheat-based diets with exogenous xylanase was intentionally avoided in the present study and, hence, this finding was an expected result. This approach was taken to overcome any confounding effects of combining phytase and xylanase, since previous studies have shown that combination of these two enzymes may have synergistic effects on the performance and nutrient utilisation by poultry (Ravindran *et al.*, 1999a; Zyla *et al.*, 1999). As anticipated, the AME and apparent ileal nitrogen digestibility of corn-based diets were higher than those of wheat-based diets. The P in wheat-based diets was better digested probably due to the presence of endogenous phytase activity in wheat.

Lowering the dietary P level caused depressions in weight gain and feed intake of birds fed corn-based diets, but not in those fed wheat-based diets. Despite being formulated to contain 0.15% less non-phytate P than the adequate-P diet, no depression in weight gain or feed intake was seen in birds fed low-P wheat-based diets. This lack of a P effect was unexpected. In a previous study evaluating the same phytase product (See Chapter 3), the growth performance in broilers fed a wheat-based diet was markedly reduced at low P levels. The wheat used in that study reported in Chapter 3 was steam-pelleted and ground prior to incorporation into the diets to lower the endogenous phytase activity present in the wheat. In the present study, no processing was done to destroy the endogenous phytase in wheat and the unsupplemented diet was analysed and contained a high endogenous phytase activity (540 PU/kg diet). However, it is relevant to note that the toe ash content was lower in birds fed the low-P wheat-based diet and that toe ash content and ileal digestibility of P both responded to phytase addition. These observations may provide some indication of the relative efficacies of microbial and plant phytases. Even though, under the conditions of this study, the plant phytase activity was sufficient to support growth in broilers, it appeared to be inadequate to supply the P required for bone mineralisation. The data showed that microbial phytase was more effective in degrading the phytate than endogenous wheat phytase and as a result released more P for bone mineralisation.

Reductions in excreta P content, P excretion, and improvements in apparent retention of P with the addition of phytase are consistent with the improvements observed in ileal P digestibility. Compared to those fed adequate-P diets, the excreta P contents in birds fed phytase-supplemented low-P diets were 35% lower and the reductions were observed in both diet types in the present study. Similar reductions in

corn-based diets with the addition of phytase, produced by submerged liquid fermentation, have been previously reported (Waldroup *et al.*, 2000; Yan *et al.*, 2000). These reductions resulted in birds consuming low-P diets supplemented with phytase, excreting less P in the manure. Waldroup *et al.* (2000) recorded a 28% reduction in excreta P contents (from 1.21 to 0.87%), compared to those in the adequate-P diets (0.45% non-phytate P), when 800 FTU/kg phytase was added to corn-based starter diets. Similarly, Yan *et al.* (2000) reported that the addition of 1000 FTU/kg phytase to low-P (0.30% non-phytate P) corn-based diets reduced the excreta P contents at 21 days of age by 36% compared to those in the adequate-P (0.45% non-phytate P) diets. Compared to the adequate-P diets, phosphorus excretion was reduced by approximately 35% in both wheat- and corn-based diets in the present study. These results are consistent with data reported in Chapter 3 for wheat-based diets and previous reports for corn-based diets (Simons *et al.*, 1990; Yi *et al.*, 1996a; Kornegay *et al.*, 1996). The study reported in Chapter 3 showed that P excretion in broiler starters was reduced by 36% (from 4.8 to 3.06 g/kg dry matter intake) when 500 PU/kg phytase was added to low-P (0.30% non-phytate P) wheat-based diets compared to adequate-P (0.48% non-phytate P) diets. Simons *et al.* (1990) reported that, P excretion of birds fed low-P (0.27% non-phytate P) corn-based diets with added phytase (500 FTU/kg), was reduced by 57% (from 4.9 to 2.1 g/kg dry matter intake). Kornegay *et al.* (1996), similarly, reported that the addition of 600 FTU/kg phytase to low-P (0.27% non-phytate P) corn-based diets reduced P excretion by 42% (from 3.75 to 2.17 g/kg DM intake). Yi *et al.* (1996a) also recorded a reduction in the P excretion by 37% (from 3.75 to 2.37 g/kg dry matter intake) by the addition of 750 FTU/kg phytase to low-P (0.27% non-phytate P) corn-based diets for broilers.

Another interesting observation in the present study was the small, but significant, reductions in the excreta nitrogen content and nitrogen excretion with phytase addition. Compared to those in adequate-P diets, excreta nitrogen contents of birds fed phytase-supplemented low-P diets were reduced by 1.6 and 0.9% for corn- and wheat-based diets, respectively. These findings highlight the role that microbial phytase supplementation can play in nutritional strategies to lower overall nutrient (P and nitrogen) output in manure from poultry operations.

CONCLUSIONS

In conclusion, the data showed that phytase by the solid state fermentation was effective in both corn- and wheat-based diets. The current data also demonstrated that, with supplementation of 500 PU microbial phytase/kg diet, dietary P level in broiler diets can be lowered by 0.15% to reduce excreta P output by 35% and still maintain comparable growth performance and bone mineralisation to birds fed a diet containing recommended levels of P. The improvements in AME and ileal nitrogen digestibility demonstrate that the inclusion of phytase would permit formulation of commercial diets with reduced levels of not only P, but also of energy and protein/amino acids.

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Chapter 6

Influence of Microbial Phytase and Glycanases, Individually or in Combination, on Energy Utilisation and Nutrient Digestibility of Cereals for Broilers

Results reported in Chapter 5 have shown that broiler responses to microbial phytase addition were influenced by diet type and dietary levels of phosphorus. Sorghum, corn, wheat and barley are the common energy grains used in poultry diets. The objective of the study reported in Chapter 6 was to examine the influence of microbial phytase and/or glycanases on energy utilisation and nutrient digestibility of these cereals.

ABSTRACT

The influence of phytase and glycanases, individually or in combination, on the apparent metabolisable energy (AME) and nutrient digestibility of sorghum, corn, wheat and barley was examined using 4-week-old broilers. Microbial phytase improved ($P<0.05$) apparent ileal phosphorus digestibility in all cereals. Phytase supplementation significantly ($P<0.05$) improved the AME of corn (2.6%) and barley (7.9%) and numerically improved the AME in sorghum (1.9%) and wheat (2.2%). Further improvements ($P<0.05$) in the AME of wheat and barley were observed when the phytase was combined with glycanases. The observed improvements in AME, however, were not always associated with enhanced digestibility of protein and starch.

INTRODUCTION

The primary objective of adding exogenous enzymes to poultry feeds is to improve the utilisation of nutrients in raw materials. This is achieved by one or more of the following mechanisms: (i) degradation of specific bonds in ingredients not usually degraded by endogenous digestive enzymes, (ii) degradation of anti-nutritive factors that lower the availability of nutrients, (iii) increased accessibility of nutrients to endogenous digestive enzymes, and/or (iv) supplementation of the enzyme capacity of young animals (Bedford and Schulze, 1998). The enzymes widely used by the industry are the glycanases (xylanase and β-glucanase) that cleave the non-starch polysaccharides in some cereals and, more recently, microbial phytase that target the phytate-complexes in plant-derived ingredients. The effects of glycanases on nutrient utilisation are well known (Annison and Choct, 1991; Bedford and Schulze, 1998), but corresponding information on the effects of phytase is limited. The objective of the present study was to examine the influence of phytase and/or glycanases (xylanase or β-glucanase) on the apparent metabolisable energy (AME) and nutrient digestibility in sorghum, corn, wheat and barley using 4-week-old broilers.

MATERIALS AND METHODS

The experimental procedures were approved by the Massey University Animal Ethics Committee (Anonymous, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Enzymes

The following enzymes (supplied by Alltech, Inc., Nicholasville, Kentucky, USA) were used: microbial phytase (Allzyme SSF; activity, 1080 phytase units (PU)/g), xylanase (Allzyme PT; activity, 1700 xylanase units (XU)/g) and β-glucanase (Allzyme PG; activity, 800 β-glucanase units (BGU)/g). One unit of phytase (PU) is defined as the quantity of enzyme that releases 1 μmol of inorganic phosphorus/min from 0.0051 mol/L sodium phytate at pH 5.5 at 37 ^{0}C. One unit of xylanase (XU) is defined as that amount of enzyme that liberates 1 μmol of xylose in one minute at pH 5.3 at 50 °C.

One unit of glucanase (BGU) is defined as that quantity of enzyme that liberates 1 μmol of reducing sugars (expressed as glucose) in one minute at pH 5.0 at 30 °C. The influence of phytase addition was evaluated in all cereals. In wheat and barley diets, the effects of adding glycanases, individually and in combination with phytase, were also evaluated. All three enzymes were added at twice the recommended levels to ensure the expression of nutrient release by the enzymes. Granular formulations of the enzymes were used. The microbial phytase used was produced by the solid state fermentation and, because of the technology employed, it also contained several side enzyme activities, including protease, amylase, cellulase and β-glucanase.

Experimental Diets

Assay diets contained 99.0% of the test cereal, supplemented with salt, mineral and vitamin premixes (Table 1). Titanium oxide was included as an inert marker for the estimation of nutrient digestibility. The grains served as the sole source of nitrogen in the assay diets. The diets were given in mash form and the birds had free access to diets and water.

Table 1. Ingredient composition of the assay diets.

Ingredient	Amount (%)			
Sorghum	99.0	-	-	-
Corn	-	99.0	-	-
Wheat	-	-	99.0	-
Barley	-	-	-	99.0
Salt	0.20	0.20	0.20	0.20
Vitamin-trace mineral premix[1]	0.30	0.30	0.30	0.30
Titanium oxide	0.50	0.50	0.50	0.50

[1]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

Birds and Conduct of Trials

Day-old male broiler chicks (Ross) were obtained from a commercial hatchery and reared on litter to 14 days of age on a commercial starter diet (23% crude protein). The birds were transferred to colony cages on day 14 and continued to be fed on the starter diet until day 28. On day 28, birds were weighed individually and birds with relatively high or low body weights were discarded. A total of 196 birds ranging in body weight from 1332 to 1668 g (mean weight, 1511 g) were chosen and assigned to treatments on the basis of body weight. Each assay diet was fed to four replicate pens (four male broilers per pen) from day 28 to 35 post-hatching. Feed intake and excreta outputs were measured quantitatively per pen over four consecutive days (day 32-35) to determine AME. Pooled excreta were mixed well into slurry using a blender and representative samples (two samples per pen) were obtained and lyophilised. Dried excreta samples were ground to pass through a 0.5-mm sieve and stored in airtight plastic containers at - 4 ^{0}C until chemical analyses. Diets and excreta samples were analysed for dry matter, gross energy and nitrogen.

On day 35, the birds were euthanased by an intracardial injection of sodium pentobarbitone, and the contents of the lower half of the ileum were collected into plastic containers by gently flushing with distilled water. Digesta were pooled within a pen, lyophilised, ground to pass through a 0.5-mm sieve, and stored at - 4 ^{0}C in airtight containers until chemical analysis. Samples of diets and digesta were assayed for nitrogen, starch, phosphorus and titanium (Ti).

Chemical Analysis

Dry matter content was determined using standard procedures (AOAC, 1990). The gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid. Total P and nitrogen were determined following Kjeldahl digestion by colorimetric autoanalyses (Twine and Williams, 1971; Technicon, 1973). Starch content was measured using an assay kit (Megazyme, Boronia, VIC, Australia) based on the use of thermostable α-amylase and amyloglucosidase (McCleary *et al.* 1997). The titanium content was measured on a UV spectrophotometer following the method of Short *et al.* (1996).

Calculations

The AME of the diets were calculated using the following formula:

$$\text{AME (MJ/kg)} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

The AME values of the ingredients were calculated as follows:

$$AME_{grain} = \frac{AME_{diet}}{0.99}$$

Appropriate corrections were made for differences in moisture contents. The nitrogen-corrected AME (AME_n) values were calculated by correcting for nitrogen equilibrium (zero retention) by using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958).

Apparent ileal digestibility of nitrogen and starch was calculated, using the ratio of titanium in the diet and digesta.

$$\text{Apparent ileal nutrient digestibility} = \frac{(Nt / Ti)_d - (Nt / Ti)_i}{(Nt / Ti)_d}$$

where, $(Nt / Ti)_d$ = ratio of nutrient to titanium in diet, and

$(Nt / Ti)_I$ = ratio of nutrient to titanium in ileal digesta.

The nutrient (P and nitrogen) retention were calculated using the following formula: Nutrient retention

$$= \frac{(\text{Feed intake} \times \text{Nutrient content}_{diet}) - (\text{Excreta output} \times \text{Nutrient content}_{excreta})}{\text{Feed intake} \times \text{Nutrient content}_{diet}}$$

Data Analysis

The data for each cereal were considered separately. For each cereal, the data were statistically analysed using one-way analysis of variance using the General Linear

Models procedure (SAS, 1997) to determine the treatment effects. Means with a significant F ratio were separated by the least significant difference test. Differences were considered to be significant at $P<0.05$. If the data suggested a trend, the values at $P<0.10$ were shown in the text.

RESULTS

Effects of phytase on the various nutrient response parameters of sorghum for broilers are summarised in Table 2. Addition of phytase improved the AME and AMEn of sorghum by 1.9 and 1.7%, respectively. However, the differences were not significant ($P>0.05$). Phytase had no effect ($P>0.05$) on apparent ileal digestibility of nitrogen (AIND) and starch (AISD).

Apparent ileal P digestibility (AIPD) was increased ($P<0.05$) from 36.2 to 51.7% with phytase addition. A similar trend was observed for P retention. Retention of dry matter and nitrogen were not influenced by added phytase.

Table 2. Effect of phytase on various nutrient utilisation parameters of sorghum for broilers.

Parameters[1]	Sorghum	Sorghum + phytase[2]	Pooled SEM
AME (MJ/kg DM)	15.03	15.32 (1.9)[3]	0.20
AMEn (MJ/kg DM)	14.74	14.99 (1.7)	0.19
Apparent ileal nitrogen digestibility	0.79	0.79 (0.4)	0.008
Apparent ileal P digestibility	0.362[a]	0.517[b] (42.8)	0.028
Apparent ileal starch digestibility	0.920	0.945 (2.8)	0.009
Dry matter retention (% of intake)	0.791	0.806 (1.9)	0.012
Nitrogen retention (% of intake)	0.396	0.459 (1.6)	0.021
Phosphorus retention (% of intake)	0.330[a]	0.657[b] (99)	0.045

[a,b] Means in a row with different superscripts differ ($P<0.05$).

[1] Average of four replicates.

[2] 1000 PU/kg diet.

[3] Values in parentheses refer to percentage improvements from the phytase addition.

Effects of phytase on various nutrient response parameters of corn for broilers are presented in Table 3. The addition of phytase significantly ($P<0.05$) improved the AME and AMEn of corn by 2.6 and 2.2%, respectively. A similar trend was observed

for the retention of dry matter, P, and nitrogen (Table 3). Phytase had no effect (P>0.05) on AIND. The AIPD in the unsupplemental diets was unexpectedly high and increased (P<0.05) by the phytase addition. Phytase had on effect (P>0.05) on apparent ileal starch digestibility.

Table 3. Effects of phytase on nutrient response parameters of corn for broilers.

Parameters[1]	Corn	Corn + phytase[2]	Pooled SEM
AME (MJ/kg DM)	14.98[a]	15.37[b] (2.6)[3]	0.07
AMEn (MJ/kg DM)	14.80[a]	15.12[b] (2.2)	0.07
Apparent ileal nitrogen digestibility	0.778	0.778	0.013
Apparent ileal P digestibility	0.701[a]	0.772[b] (10.1)	0.012
Apparent ileal starch digestibility	0.946	0.959 (1.3)	0.007
Dry matter retention (% of intake)	0.793[a]	0.813[b] (2.5)	0.003
Nitrogen retention (% of intake)	0.325[a]	0.442[b] (35.9)	0.010
Phosphorus retention (% of intake)	0.087[a]	0.301[b] (244)	0.024

[a,b] Means in a row with different superscripts differ (P<0.05).

[1] Average of four replicates.

[2] 1000 PU/kg diet.

[3] Values in parentheses refer to percentage improvements from the phytase addition.

Effects of phytase and xylanase, individually or in combination, on nutrient utilisation parameters of wheat for broilers are presented in Table 4. The addition of phytase increased the AMEn of wheat by 1.8% (Table 3), but the difference was not statistically significant (P>0.05). The AMEn tended (P<0.10) to increase with added xylanase and increased significantly (P<0.05) with the combination of the two enzymes by 4.3 and 7.1%, respectively. A similar trend was observed for dry matter retention.

Ileal nitrogen digestibility tended (P<0.10) to improve with enzyme addition. Ileal digestibility of starch was improved by 4.6 to 6.3%, but the differences were not significant (P>0.05). The AIPD in the unsupplemented wheat diets was 51.0%. Individual additions of phytase and xylanase improved apparent ileal P digestibility to 60.4 and 57.9%, respectively, but the differences were not significant. When added together, the enzymes improved (P<0.05) the ileal P digestibility to 70.2%.

Addition of phytase and xylanase increased (P<0.05) the retention of nitrogen and P. No further improvements (P>0.05) were observed with the enzyme combination.

Table 4. Effects of phytase and xylanase, individually or in combination, on nutrient utilisation parameters of wheat for broilers.

Parameters[1]	Wheat	Wheat + phytase[2]	Wheat + xylanase[3]	Wheat + phytase + xylanase	Pooled SEM
AME (MJ/kg DM)	13.58[a]	13.87[a] (2.2)[4]	14.22[b] (4.7)[5]	14.61[b] (7.6)	0.230
AMEn (MJ/kg DM)	13.33[a]	13.56[a] (1.8)	13.90[b] (4.3)[5]	14.27[b] (7.1)	0.230
Apparent ileal nitrogen digestibility[5]	0.757[a]	0.774 (2.2)	0.806(6.5)	0.819[b] (8.2)	0.023
Apparent ileal P digestibility	0.510[a]	0.604[ab] (19)	0.579[ab] (14)	0.702[b] (38)	0.059
Apparent ileal starch digestibility	0.871	0.911 (4.6)	0.913(4.8)	0.926 (6.3)	0.023
Dry matter retention (% of intake)	0.725[a]	0.740[a] (2.0)	0.760[b] (4.8)[5]	0.783[b] (7.9)	0.013
Nitrogen retention (% of intake)	0.332[a]	0.404[b] (22)	0.424[b] (28)	0.438[b] (34)	0.020
Phosphorus retention (% of intake)	0.059[a]	0.349[b] (492)	0.297[b] (404)	0.486[b] (724)	0.082

[a,b] Means in a row with different superscripts differ (P<0.05).

[1] Average of four replicates.

[2] 1000 PU /kg diet was added.

[3] 2000 XU /kg diet was added.

[4] Values in parentheses refer to percentage improvements from the addition of enzyme(s) over the basal diets.

[5] P<0.10.

Effects of phytase and glucanase, individually and in combination, on nutrient utilisation parameters of barley for broilers are summarised in Table 5. The AMEn, apparent ileal digestibility of nitrogen and starch of barley were markedly improved (P<0.05) with the individual addition of phytase or glucanase, with no further improvements being observed when the enzymes were combined compared to those in the glucanase group. Individual additions of phytase and xylanase improved (P<0.05) apparent ileal P digestibility to 0.75 and 0.67, respectively. When added together, the enzymes further improved (P<0.05) the ileal P digestibility to 0.77 compared to those in the glucanase group.

The dry matter retention of barley was improved (P<0.05) by the addition of phytase and glucanase with no further improvements being observed when the enzymes were combined. A similar trend was observed for nitrogen retention. The P retention was numerically improved by the individual addition of phytase or glucanase, but significantly (P<0.05) increased with the enzyme combination.

Table 5. Effects of phytase and glucanase, individually or in combination, on nutrient utilisation parameters of barley for broilers.

Parameters[1]	Barley	Barley + phytase[2]	Barley + glucanase[3]	Barley + phytase + glucanase	Pooled SEM
AME (MJ/kg DM)	12.11[a]	13.06[b] (7.9)[4]	13.18[bc] (8.9)	13.71[c] (13.3)	0.210
AMEn (MJ/kg DM)	11.83[a]	12.70[b] (7.4)	12.85[bc] (8.6)	13.36[c] (12.9)	0.210
Apparent ileal nitrogen digestibility	0.647[a]	0.715[b] (10.4)	0.737[b] (13.8)	0.737[b] (13.9)	0.014
Apparent ileal P digestibility	0.611[a]	0.745[c] (22.0)	0.669[b] (9.5)	0.770[c] (26.2)	0.011
Apparent ileal starch digestibility	0.886[a]	0.937[b] (5.8)	0.970[c] (9.5)	0.980[c] (10.7)	0.009
Dry matter retention (% of intake)	0.647[a]	0.701[b] (8.4)	0.707[b] (9.2)	0.732[b] (13.1)	0.010
Nitrogen retention (% of intake)	0.368[a]	0.473[b] (28.6)	0.444[b] (20.6)	0.480[b] (30.4)	0.019
Phosphorus retention (% of intake)	0.239[a]	0.377[ab] (57.9)	0.340[ab] (42.4)	0.505[b] (111.5)	0.062

[a,b] Means within a row with different superscripts differ ($P<0.05$).

[1] Average of four replicates.

[2] 1000 PU/kg diet.

[3] 2000 BGU/kg diet.

[4] Values in parentheses refer to percentage improvements from the addition of enzyme(s) over the basal diets.

DISCUSSION

The AME value of sorghum (15.0 MJ/kg dry matter) evaluated in the present study is within the range of 14.9 to 15.1 reported for Australian sorghums (Choct *et al.*, 2001). In the present study, small non-significant improvements in the AME and AMEn values of sorghum (0.29 and 0.25 MJ/kg dry matter, respectively) with phytase addition were observed. The magnitude of the increment was comparable to those reported by Selle *et al.* (1999), but lower than those reported by Farrell *et al.* (1992) in sorghum-based diets. Farrell *et al.* (1992) reported that the addition of 750 FTU/kg phytase to sorghum-soy broiler diets improved AMEn by 0.54 MJ/kg diet (from 12.46 to 13.00 MJ/kg diet). Selle *et al.* (1999) reported improvements in AME of 0.33 MJ/kg diet (from 12.55 to 12.88 MJ/kg diet) in broilers fed sorghum-soy diets with the addition of 600 FTU/kg phytase.

In the present study, phytase addition increased the ileal P digestibility of sorghum from 36.2 to 42.8%. Numerical improvements in the retention of P with phytase addition were consistent with improvements in ileal P digestibility. Improvements in P and nitrogen retention are in agreement with the report of Farrell *et al.* (1992) who found that addition of phytase (750 FTU/kg) to sorghum-soy diets

improved the retention of P and nitrogen by 53.4% (from 0.402 to 0.617) and 9.0% (from 0.520 to 0.567), respectively.

In the present study, significant improvements in AME and AMEn values of corn (2.3 and 2.6%, respectively) by the phytase addition were observed. The magnitude of improvements was comparable to those reported by Ledoux *et al.* (1999) and Namkung and Leeson (1999). The latter authors reported that the addition of phytase improved AMEn values of corn-soy diets by 2.3%.

Apparent ileal P digestibility of corn was increased by the phytase addition. However, the ileal P digestibility determined in the unsupplemented corn diet was unexpectedly high (70.1%). In the present study, assay diets contained low levels of Ca and P, which may have led to high level of hydrolysis of phytate P in the corn. The effects of lower dietary levels of Ca and P in enhancing phytate utilisation in broilers fed corn-based diets have been reported (Ballam *et al.*, 1984; Mohammed *et al.*, 1991; Coelho and Kornegay, 1999). Ballam *et al.* (1984) reported that phytate P utilisation in broilers fed corn-based diets containing 0.49% non-phytate P was enhanced by 13.1 percentage units (from 0.095 to 0.226) when dietary levels of Ca were reduced from 1.0 to 0.85%. Similarly, Mohammed *et al.* (1991) reported that phytate P digestibility in broilers fed corn-based diets was increased by 26.4 percentage units (from 0.501 to 0.765) when dietary Ca was reduced from 1.0 to 0.50%.

Supplemental phytase had no effect on the ileal nitrogen digestibility of corn in the present study. These results are in agreement with previous reports (Sebastian *et al.*, 1997; Zhang *et al.*, 1999; Peter and Baker, 2001), but were in contrast to the study reported in Chapter 5 of this thesis and other reports (Namkung and Leeson, 1999; Ravindran *et al.*, 1999b). Zhang *et al.* (1999) reported that the addition of 600 FTU/kg to corn-based diets containing 15.0 to 15.5% crude protein did not significantly improve the apparent ileal nitrogen digestibility. Sebastian *et al.* (1997) also observed no effect of microbial phytase on the AIND in male broilers chicken fed corn-based diets. Peter and Baker (2001) showed that microbial phytase (1200 FTU/kg) did not improve nitrogen utilisation of soybean meal in broilers. However, Namkung and Leeson (1999) reported that ileal nitrogen digestibility in chickens fed corn-soy diets supplemented with phytase (1200 U/kg) was increased by 2.0%. Ravindran *et al.* (1999b) also showed that the addition of 1200 FTU/kg phytase increased the ileal protein digestibility of corn in broilers by 4.2% (from 0.744 to 0.775). The study reported in Chapter 5 of this thesis

showed that the addition of 500 PU/kg phytase to low P (0.30% non-phytate P) corn-based diets improved ileal nitrogen digestibility by 2.1% (from 0.809 to 0.826).

The determined AME of wheat (13.57 MJ/kg dry matter) in the present study was within the range of 10.20 to 15.95 MJ/kg dry matter reported for wheat grown in New Zealand (Ravindran *et al.*, 2001). In the present study, an improvement of 0.30 MJ/kg dry matter was observed with phytase addition and these results are consistent with those reported in Chapter 3 and 5 of this thesis and other reports (Ravindran, 1999a; Ravindran *et al.*, 2000) in wheat or wheat-based diets. The results reported in Chapter 3 of this thesis showed that the addition of 500 PU/kg to low P diets (0.30% non-phytate P) improved the AME by 0.19 and 0.32 MJ/kg dry matter during starter and finisher phases, respectively. Ravindran *et al.* (2000) reported that the addition of 400 FTU/kg phytase improved the AME of wheat-soy diets by 1.3-5.7% depending on the dietary level of non-phytate P.

As expected, apparent ileal P digesibilty of wheat was increased by phytase addition. The high ileal P digestibility in unsupplemented wheat may be related to high level of endogenous plant activity in wheat. As discussed earlier, it is probable that low level of Ca in the assay diets may also have increased the hydrolysis of phytate P in wheat.

Phytase addition numerically improved the ileal nitrogen digestibility of wheat by 2.2% (from 0.757 to 0.772). These results are in contrast with the results observed with wheat-based diets in the Chapter 3 and 5 where significant improvements of 3.3 to 4.0% were recorded.

Improvements in the P retention in broiler fed corn and wheat diets with added phytase are consistent with the improvements observed in the ileal P digestibility and are in agreement with the results reported in Chapter 5 of this thesis. However, P retention in the unsupplemented diet of the two ingredients was extremely low. Large excretion of P in urine may partly explain these extremely low P retention values.

The effects of xylanase supplementation in improving AME values of wheat and wheat-based diets are well documented (Annison and Choct, 1991; Bedford and Schulz, 1998). The magnitude of the response is generally related to the initial AME of wheat. The responses in AME are greater in low-AME wheats compared to normal wheats (Choct *et al.*, 1995). In the present study, xylanase supplementation improved ($P<0.10$) the AME value of wheat by 4.7% (from 13.58 to 14.22 MJ/kg dry matter). Choct *et al.* (1995) reported an improvement in the AME of normal wheat by 2.1% (from 14.52 to

14.83 MJ/kg dry matter) by the xylanase supplementation, whereas improvements in the range of 12.6-18.6% were reported by Hew *et al.* (1998).

In the present study, ileal nitrogen digestibility in wheat was improved by 6.5% with xylanase addition. These results are consistent with the previous report of Hew *et al.* (1998) who found that the ileal nitrogen digestibility of wheat was improved by 7.0-7.4% by addition of xylanase.

In the present study, responses in AME and nitrogen digestibility were maximised by the xylanase addition, with no further improvement observed when combined with phytase. These results are in contrast to previous reports (Ravindran *et al.*, 1999a; Selle *et al.*, 2000) where synergistic effects of combining phytase and xylanase on energy utilisation were reported. Ravindran *et al.* (1999a) found that individual additions of phytase and xylanase improved AME of wheat by 9.7 and 5.3%, respectively. When the wheat was supplemented with a combination of the two enzymes, AME was improved by 19.0%.

The AME of barley (12.1 MJ/kg) evaluated in the present study was within the range of 11.6 to 13.8 MJ/kg DM reported by Choct *et al.* (2001) for Australian barleys. The AME of barley was markedly improved by the addition of phytase or glucanase (7.9 and 8.9%, respectively) with further improvements when these enzymes were combined compared to those in the phytase group. Enzyme (β-glucanase or phytase) addition and the combination of enzyme increased apparent ileal nitrogen and starch digestibility in barley. In the present study, β-glucanase improved apparent ileal digestibility of P, nitrogen and starch of barley by 9.5 13.8, and 9.5%, respectively. The benefits of supplemental β-glucanase in improving the AME and nutrient digestibility of barley-based diets are consistent with previous reports (Hesselman and Åman *et al.*, 1986; Viveros *et al.*, 1994; Perttilä *et al.*, 2001). Hesselman and Åman *et al.* (1986) reported that the addition of β-glucanase to barley-based diets improved the ileal digestibility of nitrogen and starch by 19.2 and 8.8%, respectively. Viveros *et al.* (1994) showed that the addition of β-glucanase to barley-based diets improved the ileal starch digestibility by 10.3% (from 0.884 to 0.975). Perttilä *et al.* (2001) reported that the addition of β-glucanase to barley-based diets improved the AMEn by 0.60 MJ/kg dry mater (from 11.1 to 11.7 MJ/kg DM) and retention of nitrogen and P (14.0 and 12.0%, respectively).

In the present study, phytase increased the apparent ileal P digestibility in barley from 0.610 to 0.745. Phytase addition improved apparent ileal digestibility of nitrogen and starch of barley by 10.4 and 5.8%, respectively. The finding that phytase was as effective as exogenous glucanase was surprising. Two reasons may be responsible for this. First, the phytase used in the present study was produced by solid state fermentation and contained relatively high levels of β-glucanase and xylanase. Second, microbial phytase may be acting on barley in a manner to that of exogenous glucanase, perhaps by disrupting the cell walls and enhancing the contact between digestive enzymes and cell contents (Ravindran *et al.*, 1999a).

The beneficial effects of glucanase and phytase combinations on the AME of barley are in agreement with previous reports. Ravindran *et al.* (1999a) reported that individual addition of phytase improved AME of barley 2.7%. When the diets were supplemented with a combination of the two enzymes, AME was improved by 5.9%.

The mechanism of the improved energy utilisation by supplemented phytase remains unclear. It has been proposed that improved digestibility of protein and starch may explain, in part, the energy responses (Selle *et al.*, 2000). In the present study, the improvements in AME were, however, not always associated with enhanced digestibility of protein and starch.

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Chapter 7

Comparison of *In Vitro* Nutrient Release by Three Phytase Preparations in Wheat- and Corn-Based Diets

The results from studies reported in Chapter 3, 5 and 6 of this thesis have shown that microbial phytase produced by the solid state fermentation was effective in enhancing the utilisation of nutrients in a range of diet types for broiler chickens and these responses were, in part, attributed to the presence of side-enzyme activities in this phytase product. The trial designs used in these studies, however, did not permit a definite conclusion on the benefits of the side activities. The objective of the study reported in this chapter was to examine possible beneficial effects from the side activities of microbial phytase produced by solid state fermentation by comparing *in vitro* nutrient release in wheat- and corn-based diets with microbial phytase produced by submerged liquid fermentation with no detectable side activities.

ABSTRACT

Potential beneficial effects from the side activities in a microbial phytase produced by solid state fermentation was examined by comparing the release of phosphorus, reducing sugars and α-amino nitrogen by two other phytase preparations in wheat- and corn-based diets using an *in vitro* model. Phytase produced by solid state fermentation released more ($P<0.05$) phytate-bound phosphorus (11.0% and 7.8% in wheat- and corn-based diets, respectively) and α-amino nitrogen (1.7% and 6.2% in wheat- and corn-based diets, respectively) than a phytase produced by submerged liquid fermentation with no detectable side activities. Phytase produced by solid state fermentation also released 2.9% more reducing sugars in the wheat-based diet and 6.2% more α-amino nitrogen in corn-based diets. The superiority of this phytase product in releasing nutrients in both types of diets is likely to be due to activities of other enzymes present, but these results need to be confirmed in well designed *in vivo* studies.

INTRODUCTION

The usefulness of microbial phytase in releasing phytate-bound phosphorus (P) and improving P availability in poultry and pig diets is now well documented. Several commercial microbial phytase products are currently available and two distinct fermentation technologies are used to produce these products - one involving submerged liquid fermentation and the other based on solid state fermentation. Because of the technology employed, the phytase produced by solid state fermentation also contains several side enzyme activities, including protease, amylase, cellulase, xylanase and β-glucanase. Studies reported in Chapters 3, 5 and 6 have shown that phytase produced by solid state fermentation is effective in enhancing the utilisation of nutrients in a range of diet types for broiler chickens and these responses were, in part, attributed to the presence of side enzyme activities. The trial designs used in these studies, however, did not permit any definite conclusion on the benefits of the side activities.

In vitro simulation models have been recently developed and successfully used to predict the release of nutrients by exogenous enzymes for turkeys (Zyla *et al.*, 1995) and broilers (Zyla *et al.*, 1999a; 2000). The objective of the present study was to examine whether or not there are beneficial effects from the other enzymes present in the phytase produced in solid state fermentation by determining *in vitro* nutrient release in wheat- and corn-based diets.

MATERIALS AND METHODS

Enzymes

Phytase A (Allzyme® SSF; supplied by Alltech, Inc., Nicholasville, KY, USA) was determined to contain the following enzyme activities: phytase, fungal protease, fungal amylase, cellulase, xylanase, and β-glucanase. Phytase activity in the product exceeded product guarantees of 1216 PU/g. Phytase B was formulated, using pure sources of enzymes, to contain similar enzyme profile to Phytase A. Phytase C was Natuphos® 5000 (Gist Brocades BSD V.V., Delft, The Netherlands) which was determined to contain 3645 PU/g phytase activity and had no detectable side enzyme activities.

Experimental Design

There were eight treatments (Table 1). Treatments 1 to 5 were phytase A supplying five levels of phytase (0, 250, 500, 750 and 1000 PU/kg diet). Treatments 6 and 7 were Phytase B supplying two levels of phytase (500 and 750 PU/kg). Treatment 8 was Phytase C supplemented at a level of 500 PU/kg.

Table 1. Dietary treatments and dosage of enzymes tested.

Treatment	Source of enzyme	Dosage (PU/kg)
1	Phytase A	0
2	Phytase A	250
3	Phytase A	500
4	Phytase A	750
5	Phytase A	1000
6	Phytase B	500
7	Phytase B	750
8	Phytase C	500

The influence of the enzyme treatments were tested with two diet types. The composition of the diets are shown in Table 2.

In Vitro Procedures Designed for the Release of Phosphorus, Reducing Sugars, and α-amino Nitrogen

The *in vitro* digestion model that was developed by Zyla *et al.* (1999a) to study the release of P, reducing sugars, and α-amino nitrogen in broiler chickens was used, with minor modifications (Figure 1). The modifications involved pH values in the crop, gizzard, duodenum and small intestine, which were obtained in a pilot trial from birds fed corn- or wheat- based diets. pH values used in the *in vitro* digestion model were adjusted to 5.7, 2.7 and 5.9 for the three incubation periods simulating the three sections (crop, gizzard and duodenum, respectively) of the digestive tract of chickens fed wheat-based diets. Corresponding pH values in corn-based diets was 5.9, 2.9 and 6.1, respectively.

Table 2. Ingredient composition and calculated analysis of the two diets.

Ingredient	Wheat-based diet	Corn-based diet
	%	
Wheat	67.45	0.00
Corn	0.00	60.01
Soybean meal	25.46	33.10
Corn oil	4.00	4.00
Dicalcium phosphate	0.50	0.60
Limestone	1.28	1.20
Lysine	0.29	0.09
Methionine	0.42	0.40
Salt	0.30	0.30
Vitamin-mineral premix [1]	0.30	0.30
Calculated analysis		
AME (MJ/kg diet)	13.52	13.51
Crude protein, %	21	21
Lysine, %	1.15	1.15
Methionine + Cysteine, %	0.94	0.94
Calcium, %	0.69	0.69
Total P, %	0.49	0.48
Phytate P, %	0.26	0.25
Non-phytate P, %	0.25	0.25

[1] Supplied per kilogram of diets: biotin, 0.22 mg; cholecalciferol, 528 IU; cyanocobalamin, 0.03 mg; folic acid, 1.1 mg; menadione, 2.8 mg; niacin, 55 mg; pantothenate, 17.6 mg; pyridoxine, 4.9 mg; *trans*-retinol, 11025 IU; riboflavin, 7.7 mg; thiamine, 2.2 mg; dl-α-tocopheryl acetate, 33 IU; choline chloride, 479 mg; Cu, 10 mg; Fe, 40 mg; I, 1.9 mg; Mn, 64 mg; Se, 300 µg; Zn, 75 mg.

Representative diet samples (corn- or wheat-based diets) were obtained and ground using a laboratory-scale grinder. The first step was to simulate the digestion in the crop. One gram (1 ± 0.0001) of the sample (wheat- or corn-based diet) was weighed (in triplicate) into a 10 ml plastic syringe without Luer-locks. The samples were hydrated with 1.5 ml distilled water or enzyme dilution solution. Contents of the syringe were vortexed and incubated in a water bath at 40 °C for 30 min. After 30 min incubation, 1.5M HCl (0.46 and 0.49 ml for the wheat- and corn-based diet, respectively) was added and vortexed. Half a ml of pepsin solution (6000 Units/ml; P-7012, Sigma Chemical Co, Saint Louis, MO, USA) was then added and the syringe sealed with parafilm and incubated for a further 45 min at 40 °C. This step simulated digestion in the gizzard.

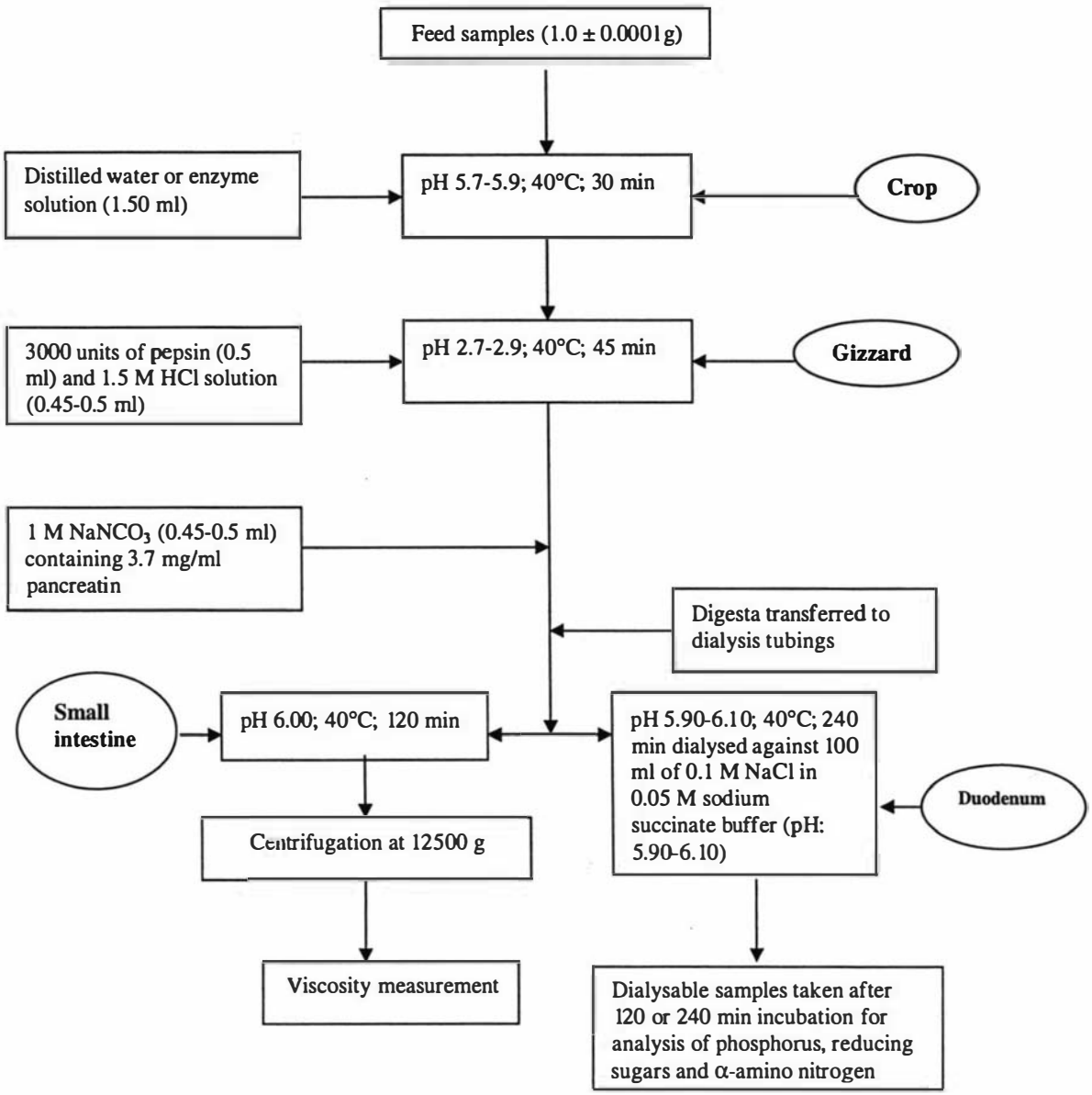

Figure 1. Flow chart of the *in vitro* procedure used to study the release of phosphorus, reducing sugars, α-amino nitrogen and *in vitro* viscosity (modified from Zyla *et al.*, 1999a)

The third step was to simulate the digestion in the duodenum of chickens. 0.45 to 0.50 ml of 1M $NaHCO_3$ containing 3.7 mg pancreatin per ml solution (P-3292, Sigma Chemical Co, Saint Louis, MO, USA) was added to each syringe under constant stirring. The digesta slurry was then transferred to segments of dialysis tubing (molecular weight cutoff, 12000-14000 Da diameter 18.0 mm; Sigma Chemical Co., Saint Louis, MO, USA). The dialysis tubings were placed in a 250 ml flask containing 100 ml of 0.1M NaCl in a 0.05 M sodium succinate buffer (pH 5.90 and 6.10 for wheat-

and corn-based diets, respectively) and incubated in a shaker within an incubator. The temperature of incubator was maintained at 40 °C. Six milliliters of the dialysate samples were taken at second and fourth hour of the incubation. The dialysate was analysed for inorganic phosphate (Shieh *et al.*, 1969), reducing sugars (Miller, 1960) and α-amino nitrogen using the ninhydrin method (Moore, 1954).

In Vitro Viscosity Measurement

Samples for viscosity measurements in wheat-based diets were obtained using the *in vitro* procedures described above, with the only difference being that centrifuge tubes were used. Simulation of the digestion in the small intestine was performed with addition of 0.46 ml of 1M $NaHCO_3$ containing 3.7 mg per ml pancreatin solution (Figure 1). After 120 min of digestion, the samples were centrifuged at 12500 x g and supernatant viscosity was determined at 40 °C using a capillary viscometer (Brookfield Engineer Ltd, Middleboro, MA, USA) according to the method described by Almirall *et al.* (1995).

Data Analysis

The experimental data were collected in three replicates for the all response variables and analysed by the General Linear Model (GLM) procedure (SAS, 1997). Linear and quadratic effects on dialysable phosphorus, reducing sugars, and α-amino nitrogen were tested with five levels of phytase for Phytase A using the contrast statement. The linear and quadratic equations for P, reducing sugars and α-amino nitrogen against five levels of phytase for Phytase A were generated by ProcReg statement using the SAS package. Comparison between treatment 3 and 6 (or 8), and between treatment 4 and 7, were made using non-orthogonal contrasts. Significant differences were considered at $P<0.05$.

RESULTS

Although dialysable samples were taken at both second and fourth hours, only the data taken on the fourth hour are reported (Table 3). Data from the two samplings followed a similar trend.

Dialysable P levels were quadraticaly increased with increasing levels of phytase A in wheat-based diets (R^2 = 0.99, P<0.0001) (Figure 2) and corn-based diets (R^2 = 0.99, P<0.0001) (Figure 3).

Compared to the Phytase C, Phytase A at 500 PU /kg diet released (P<0.05) more phytate-bound P (11.0 and 7.8% for wheat- and corn-based diets, respectively). Compared to the Phytase B, Phytase A released (P<0.05) more P at both 500 and 750 PU/kg diet in both types of diets.

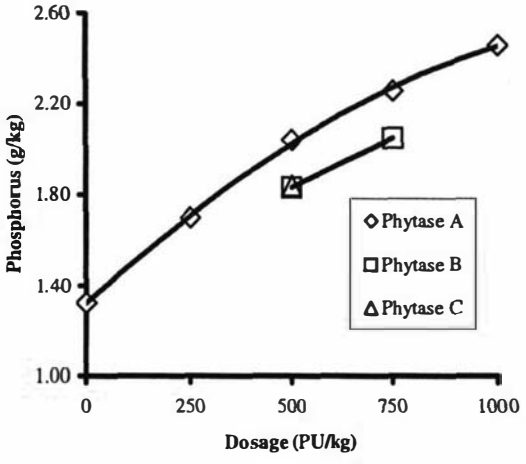

Figure 2. Effect of source and concentration of phytase on dialysable phosphorus in the wheat-soy diet.

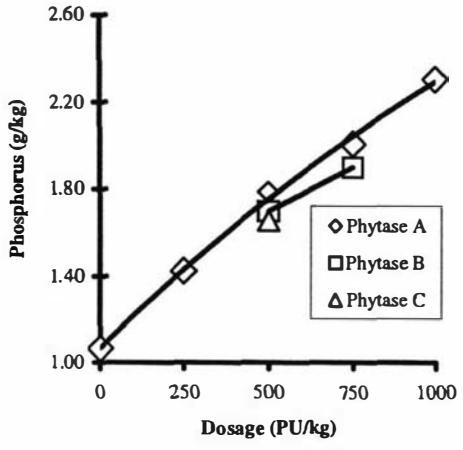

Figure 3. Effect of source and concentration of phytase on dialysable phosphorus in the corn-soy diet.

Dialysable reducing sugar levels were linearly (R^2 = 0.85, P<0.0001) increased with increasing levels of Phytase A in wheat-based diets (Figure 4). Phytase A supplemented at 500 PU/kg diet released significantly (P<0.05) more reducing sugars (2.9%) than Phytase C supplemented at the similar activity. There were no differences (P>0.05) between Phytase A and B at 500 PU/kg diet. Phytase B released more reducing sugars than Phytase A at 750 PU/kg diet, but the differences were not statistically significant (P > 0.05).

Table 3. Effects of source (Phytase A, B and C) and concentration of phytase on *in vitro* dialysable phosphorus (g/kg), reducing sugars (g/kg), α-amino nitrogen (g/kg) and *in vitro* viscosity (mPa.s) in wheat- and corn-based diets [1].

Treatment	Enzyme	Dose (PU/kg diet)	Phosphorus[2]		Reducing sugars[2]		α-amino nitrogen[2]		Viscosity[2]
			WB	CB	WB	CB	WB	CB	WB
1	Phytase A	0	1.325	1.065	43.42	116.23	1.194	0.625	1.93
2	Phytase A	250	1.699	1.424	43.90	114.62	1.195	0.647	1.85
3	Phytase A	500	2.041	1.786	44.73	117.10	1.224	0.665	1.84
4	Phytase A	750	2.257	2.007	45.53	121.91	1.217	0.682	1.81
5	Phytase A	1000	2.457	2.305	46.23	113.20	1.218	0.683	1.77
6	Phytase B	500	1.832	1.699	44.65	117.17	1.199	0.655	1.44
7	Phytase B	750	2.050	1.899	46.05	121.17	1.212	0.668	1.36
8	Phytase C	500	1.839	1.656	43.45	117.75	1.204	0.626	1.80
Pooled SEM			0.0285	0.0253	0.348	2.492	0.0094	0.0063	0.0195
Probabilities[3]									
Phytase A effect									
Linear			***	***	***	NS	NS	***	***
Quadratic			***	*	NS	NS	NS	NS	NS
Contrasts									
Treatment 3 vs 8			***	**	*	NS	NS	*	NS
Treatment 3 vs 6			***	*	NS	NS	NS	NS	*
Treatment 4 vs 7			***	**	NS	NS	NS	NS	*

WB, wheat-based diet; CB, corn-based diet.

[1] Details of *in vitro* procedures, see materials and methods.

[2] Mean value from three replicate samples.

[3] NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

In the corn-based diet, increasing levels of Phytase A had no effect (P>0.05) on reducing sugar levels and no differences (P>0.05) were observed between the three phytases at 500 PU/ kg diet (Figure 5).

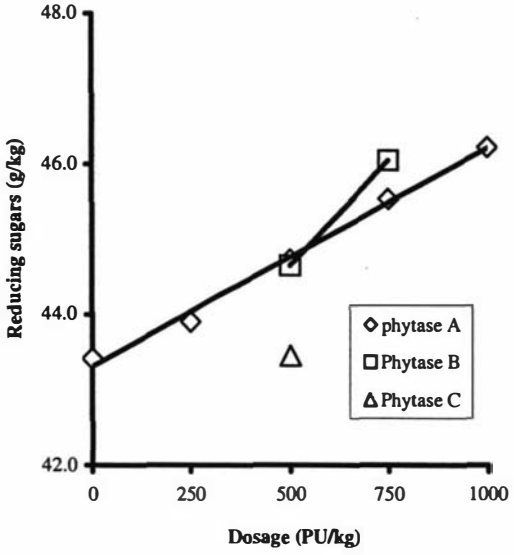

Figure 4. Effect of source and concentration of phytase on dialysable reducing sugars in the wheat-based diet.

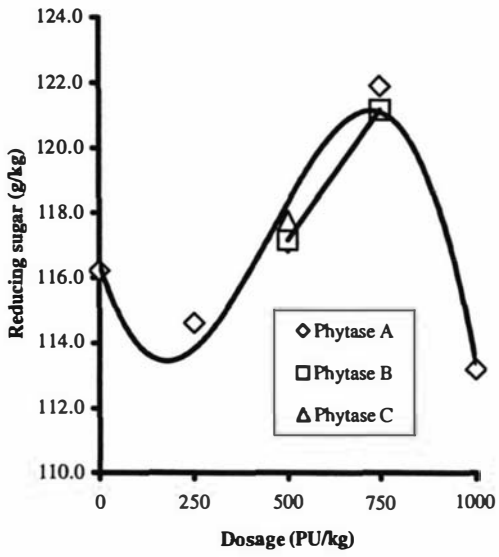

Figure 5. Effect of source and oncentration of phytase on dialysable reducing sugars in the corn-based diet.

Increasing levels of Phytase A had no effect (P>0.05) on α-amino nitrogen levels (expressed as glycine per kg diet) in the dialysate of the wheat-based diet (Figure 6), but quadratically (R^2 = 0.71; P<0.001; Figure 7) increased α-amino nitrogen levels in the dialysate of the corn-based diet. Compared to Phytase C, Phytase A at 500 PU/kg diet released 1.7% more α-amino nitrogen in the wheat-based diet, but the differences were not significant (P>0.05). In the corn-based diet, Phytase A at 500 PU/kg diet released 6.2% more (P<0.05) α-amino nitrogen than Phytase C. Phytase A released more α-amino nitrogen than Phytase B at 500 and 750 PU kg diet in both types of diets (Table 3). However, the differences were not significant (P>0.05).

In vitro viscosity value was highest in the unsupplemented wheat-based diet. The viscosity value decreased (P<0.001) with increasing levels of Phytase A. There were no differences between Phytases A and B at 500 PU/kg diet. Interestingly, the viscosity value for the diet supplemented with Phytase B was lower (P<0.05) than those supplemented with Phytases A and C (Table 3).

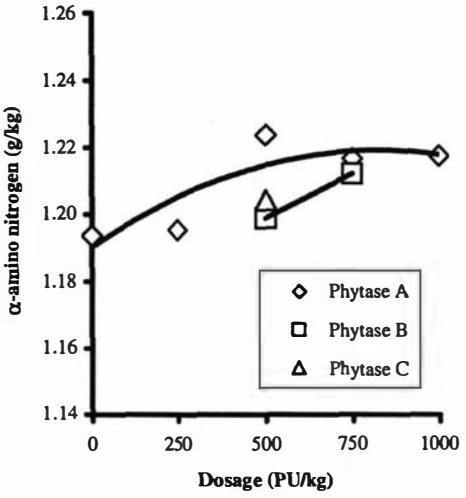

Figure 6. Effect of source and concentration of phytase on dialysable α-amino nitrogen in the wheat-soy diet.

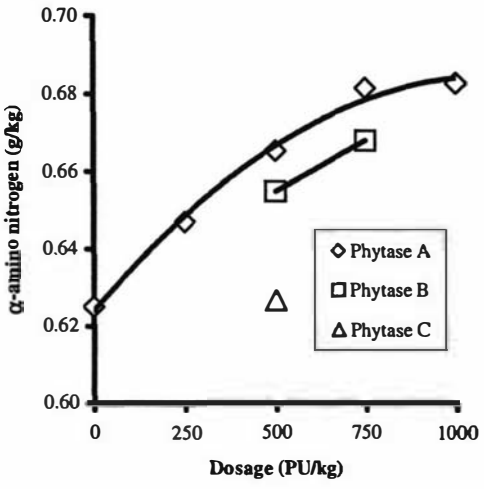

Figure 7. Effect of source and concentration of phytase on dialysable α-amino nitrogen in the corn-soy diet.

DISCUSSION

The objective of the present study was to examine whether or not there were beneficial effects from the side activities present in Phytase A, which was produced by solid state fermentation. In order to ensure P release responses, the diets were formulated to contain 0.25% non-phytate P, which is 0.20% lower than the NRC (1994) recommendation for broiler starters, as the *in vivo* responses are known to be greater in low-P diets (Kornegay *et al.*, 1996).

Dialysable P levels for phytase were increased with increasing levels of phytase in both diet types. These results are consistent with previous reports (Zyla *et al.*, 1999a, b; 2000) with several commercial phytase products, including Phytase C, in wheat-based diets. Compared to Phytase C produced by submerged liquid fermentation with no detectable side activities, Phytase A significantly ($P<0.05$) released more phytate-bound P (11.0% and 7.8% in wheat-based and corn-based diets, respectively).

Phytase A was determined to release more phytate-bound P than the Phytase B at 500 and 750 PU /kg diet in both wheat-based and corn-based diets. This finding is difficult to explain since Phytase B was blended to contain similar enzyme activities as Phytase A.

Phytase A at 500 PU/kg diet released ($P<0.05$) more reducing sugars (2.9%) than Phytase C in wheat-based diet. The lacks of responses in corn-based diet are in general agreement with the observation from the *in vivo* studies reported in Chapter 6 of this thesis.

Increasing levels of Phytase A had no effect ($P>0.05$) on the α-amino nitrogen levels in the dialysate in the wheat-based diet, but quadratically increased the α-amino nitrogen levels in the corn-based diet. The lack of response in the wheat-based diet was unexpected and is difficult to explain. Compared to Phytase C, Phytase A at 500 PU/kg diet released 1.7% more α-amino nitrogen in the wheat-based diet, but the differences were not significant. In the corn-based diet, Phytase A at 500 PU/kg diet released 6.2% more α-amino nitrogen than Phytase C.

As expected, *in vitro* viscosity value was highest in the unsupplemented wheat-based diet. The viscosity values in the diet supplemented with the Phytase B (500 and 750 PU/kg) were significantly lower than those in Phytase A. These results are difficult to explain since Phytase B was blended to contain similar enzyme activities as Phytase A.

CONCLUSIONS

Phytase A, produced by the solid state fermentation, released more phytate-bound P in both diet types, and more energy in the wheat-based diet as indicated by the release of reducing sugars. Phytase A also released more protein than the Phytase C in corn-based diets as indicated by *in vitro* α-amino nitrogen. The results of this *in vitro* study showed that Phytase A, a product with side enzyme activities, produced better response in terms of nutrient release than Phytase C, a source of pure phytase. It should be, however, noted that these *in vitro* results may not be directly applicable to *in vivo* situations and therefore should be considered only as crude indicators of the relative efficacy of the enzymes evaluated.

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Chapter 8

Influence of Microbial Phytase and Xylanase, Individually or in Combination, on the Performance, Apparent Metabolisable Energy, Digestive Tract Measurements and Gut Morphology in Broilers Fed Wheat-Based Diets Containing Adequate Phosphorus Levels

The study reported in Chapter 6 has demonstrated the beneficial effects of adding microbial phytase, individually or in combination, with xylanase on improving the apparent metabolisable energy and apparent ileal digestibility of nitrogen and starch in wheat for broilers. However, the mechanisms by which the enzymes improve nutrient utilisation remain largely unclear. The aim of the present study was to examine the influence of microbial phytase and xylanase, individually or in combination, on the performance, apparent metabolisable energy, digesta viscosity, digestive tract measurements and gut morphology of broilers fed wheat-soy diets formulated to contain adequate phosphorus levels.

ABSTRACT

The aim of the present study was to examine the influence of microbial phytase and xylanase, individually or in combination, on the performance, apparent metabolisable energy, digesta viscosity, digestive tract measurements and gut morphology in broilers fed wheat-soy diets containing adequate phosphorus (P) levels. The basal diet was based on wheat and soybean meal and formulated to contain 0.45% non-phytate P. The experimental diets were formulated by supplementing the basal diet with xylanase (1000 XU/kg), phytase (500 PU/kg) or a combination of phytase and xylanase. Supplemental phytase improved ($P<0.05$) weight gains and feed efficiency by 17.5 and 2.9%, respectively. Corresponding improvements due to the addition of xylanase were 16.5 and 4.9%, respectively. Combination of phytase and xylanase improved ($P<0.05$) weight gains by 19.8% and lowered ($P<0.05$) feed/gain by 5.4% compared to those fed the basal diet. Individual additions of xylanase or phytase resulted in numerical improvements in apparent metabolisable energy, but the differences were not significant ($P>0.05$). The combination of the two enzymes improved ($P<0.05$) the apparent metabolisable energy. Addition of xylanase and the combination of the two enzymes reduced ($P<0.05$) the viscosity of digesta in the duodenum, jejunum and ileum. Interestingly, phytase supplementation also reduced digesta viscosity in the duodenum and ileum.

Enzyme supplementation lowered ($P<0.05$) the relative weight and length of small intestine. Additions of xylanase and phytase reduced the relative weight of the small intestine by 15.5 and 11.4%, respectively, while the corresponding reductions in relative length of small intestine were 16.5 and 14.1%, respectively. The combination of phytase and xylanase had no further effects on the relative weight and length of the small intestine compared to the xylanase group.

Neither xylanase nor phytase supplementation had effect ($P>0.05$) on villus height, crypt depth, epithelial thickness, goblet cell number, and the ratio of crypt depth to villus height in the duodenum, jejunum and ileum. The exceptions were that xylanase supplementation tended ($P<0.10$) to increase goblet cell numbers in duodenum and decreased ($P<0.05$) crypt depth in jejunum. The addition of phytase increased ($P<0.05$) villus height in the duodenum and decreased ($P<0.05$) the number of goblet cell in the jejunum compared to those fed the unsupplemented basal diet. The

combination of phytase and xylanase increased ($P<0.05$) villus height in the ileum and crypt depth in the jejunum and ileum, but had no effects on duodenal and jejunal villus height or the number of goblet cell in the duodenum, jejunum and ileum compared to those fed the supplemental phytase group.

INTRODUCTION

The enzymes widely used in the poultry feed industry are the glycanases (xylanases and β-glucanases) that cleave the non-starch polysaccharides (NSP) in cereal grains such as wheat and barley and microbial phytase that target phytate-complexes in plant-derived ingredients. Several mechanisms have been proposed to explain the beneficial effects of glycanases in improving energy and nutrient utilisation of wheat-based diets (Bedford and Schulze, 1998) and these include, (i) degradation of NSP in the cell wall matrix and the release of encapsulated nutrients, (ii) lowered digesta viscosity in the intestinal tract and improvements in the rate of diffusion between substrates, enzymes, and digestion end products, (iii) increased accessibility of nutrients to endogenous digestive enzymes, (iv) stimulation of intestinal motility and improved feed passage rate, and/or (v) supplementation of the enzyme capacity of young animals.

The mode of action of glycanases may also involve overcoming the negative effects of NSP on the microscopic structure of gut (Viveros *et al.*, 1994), but published data on this aspect are limited and controversial. Viveros *et al.* (1994) reported that the jejunum of birds fed a diet containing 60% barley showed shortening, thickening and atrophy of the villi and an increased number of goblet cells compared to those fed a corn-soy diet. Addition of β-glucanase counteracted these effects. Iji *et al.* (2001) found that the supplementation of xylanase to wheat-based diets had no effect on villus height, crypt depth and villus surface area in the duodenum, jejunum and ileum of broilers. No published data are available on the effects of microbial phytase on the gut morphology in broiler chickens.

The beneficial effects of phytase and xylanase individually, or in combination, in improving energy utilisation and nutrient digestibility in wheat were demonstrated in the studies reported in Chapter 6. Similar improvements by combining phytase and xylanase have been reported previously (Ravindran *et al.*, 1999; Zyla *et al.*, 1999). The aim of the present study was to examine the influence of phytase and xylanase, individually or in combination, on the performance, apparent metabolisable energy (AME), digesta viscosity, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus (P).

MATERIALS AND METHODS

Experimental procedures were approved by the Massey University Animal Ethics Committee (Anonymous, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Enzymes

The following enzymes (supplied by Alltech, Inc., Nicholasville, KY, USA) were used: phytase (Allzyme SSF; activity, 1080 PU/g), and xylanase (Allzyme PT; activity, 1700 XU/g). One unit of phytase (PU) is defined as the quantity of enzyme that releases 1 μmol of inorganic phosphorus/min from 0.0051 mol/L sodium phytate at pH 5.5 at 37 °C. One unit of xylanase (XU) is defined as that amount of enzyme that liberates 1 μmol of xylose in one minute at pH 5.3 at 50 °C. Both enzymes were added at the levels recommended by the manufacturer. Granular formulations of the enzymes were used.

Experimental Design and Diets

Details of the dietary treatments are shown in Table 1. The basal diet (Treatment 1) was based on wheat, and soybean meal (Table 2) and formulated to contain adequate levels of non-phytate P (0.45%) for broiler starters as recommended by the NRC (1994). Treatments 2, 3 and 4 were formulated by supplementing the basal diet with xylanase (1000 XU/kg), phytase (500 PU/kg) or a combination of phytase and xylanase, respectively. The enzymes were added in place of corn starch. The Ca to total P ratio was maintained at 1.4:1. All diets were cold-pelleted (65-70 °C).

General Procedures

Day-old male broiler (Ross) chicks were obtained from a commercial hatchery and randomly assigned to 20 pens (8 birds/ pen) in 3-tier electrically heated battery brooders. Each of the four dietary treatments was randomly assigned to five pens. The brooders were housed in an environmentally controlled room with 24-hour fluorescent

lighting. The birds were transferred to colony cages in an environmentally controlled room on day 14. Room temperature was maintained at 32 ± 1 °C during the first week and gradually decreased to 24 °C by the end of the third week.

Body weights and feed intake were recorded on a pen basis at weekly intervals. Feed was offered *ad libitum* and water was freely available at all times during the 21-day trial period. Mortality was recorded daily and feed per gain values were corrected for mortality.

Table 1. Dietary treatments tested.

Treatment	Non-phytate P (%)	Xylanase[1]	Phytase[2]
1	0.45	-	-
2	0.45	+	-
3	0.45	-	+
4	0.45	+	+

[1] 1000 XU/kg diet.

[2] 500 PU/ kg diet.

Collection and Processing of Samples

During the third week (day 17-21), total collection of excreta was carried out for the determination of AME. Feed intake and excreta output were measured quantitatively per pen over four consecutive days. Excreta were pooled within a pen, mixed well using a blender and two representative samples per pen were taken. The samples were freeze-dried, dried samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at - 4 ^{0}C until chemical analyses.

On day 21, two birds from each pen (close to the mean pen body weight) were selected and weighed. The birds were killed by cervical dislocation and the digestive tract, from the proventriculus to caeca, was carefully excised. After removing the intestinal contents, the empty weights and lengths of duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileocaecal junction) and caeca were recorded. About 5 cm length of samples of duodenum (middle point of the pancreatic loop), jejunum and ileum (5 cm before and after Meckel's diverticulum, respectively) were taken for gut morphological measurements. The intestinal samples were flushed with ice-cold saline and

immediately placed in Bouin's fluid. The samples were then transferred into 70% ethanol after 24 hours.

Table 2. Ingredient composition and calculated analysis of the basal diet.

Ingredient	Amount (%)
Wheat	67.25
Soybean meal	25.51
Vegetable oil	3.00
Monocalcium phosphate	1.45
Limestone	1.78
Corn starch	0.05
L-Lysine	0.29
DL-Methionine	0.42
Salt	0.25
Vitamin-mineral premix[1]	0.30
Calculated analysis	
AME (MJ/kg diet)	12.91
Crude protein, %	21
Lysine, %	1.15
Methionine + cysteine, %	0.94
Calcium, %	1.00
Total P, %	0.71
Phytate P, %	0.26
Non phytate P, %	0.45

[1]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

Viscosity Measurement

Viscosity of gut digesta was determined according to the method described by Almirall *et al.* (1995) with minor modifications. Fresh digesta was obtained from each section of intestine (duodenum, jejunum and ileum) from one bird per pen. Digesta were immediately placed in a centrifuge tube, centrifuged at 3000 x g for 15 min. Supernatant (0.5 ml) was withdrawn and the viscosity was determined using a Brookfield viscometer (model LVDVII+CP, Brookfield engineering Laboratories,

Stoughton, MA) maintained at 40 °C with a CP40 cone with shear rates between 5-500 per second. The reading was taken after one min.

Toe Ash

Toe samples were obtained from the remaining birds (5 birds/pen) by severing the middle toe through the joint between the second and third tarsal bones for toe ash measurements. The left and right middle toe of the birds were pooled separately to yield two samples of toes per pen. The composite samples were dried to a constant weight at 105 °C and then ashed in a muffle furnace at 550 °C for 16 hrs (Potter, 1988).

Histological Examination

Histological examination was carried out according to the method described by Iji *et al.* (2001) with minor modifications. Intestinal samples from each section were immersed in formaldehyde, fixed in Bouin's solution, paraffin-embedded, sectioned at a thickness of 7 μm, stained with the alcian blue/hematoxylin and eosin, and examined by light microscopy. Visual measurements of villus height, crypt depth, goblet cell number and epithelium thickness were made at 100 to 400 x magnification using computer software (Sigma Scan, Jandel Scientific, San Rafael, California, USA). The slides were viewed on a Zeiss Axiophot microscope and digitised using computer software, Image Pro Plus (Version 4.1.0.9, Media Cybernetics, Silver Spring, Maryland, USA).

Chemical Analysis

Dry matter content was determined using standard procedures (AOAC, 1990). Gross energy (GE) was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid.

Calculations

The AME values were calculated using the following formula. Appropriate corrections were made for differences in dry matter content.

$$AME = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

Data Analysis

For performance, AME, and viscosity measurements, pen means served as the experimental unit for statistical analysis. For digestive tract measurements and gut morphology, individual birds were considered as the experimental unit. All data were statistically analysed using one-way analysis of variance using the General Linear Model (GLM) procedure (SAS, 1997) to determine the treatment effects. Means with a significant F ratio ($P<0.05$) were separated by the Least Significant Difference test. The differences were considered to be significant at $P<0.05$. If the data suggest a trend, the values at $P<0.10$ were also shown in the text.

RESULTS

Effects of phytase and xylanase, individually or in combination, on the performance, toe ash contents and AME of broiler chickens fed wheat-based diets are presented in Table 3. Individual additions of xylanase and phytase significantly ($P<0.01$) improved weight gains by 16.5 and 17.5%, respectively. Combination of phytase and xylanase did not cause further improvements in weight gains. A similar trend was observed for feed intake. Feed intakes of birds fed the diets supplemented with xylanase or phytase were significantly ($P<0.01$) increased by 10.8 and 14.0%, respectively.

The feed per gain value of birds fed the basal diet with added xylanase or phytase were lowered ($P<0.05$) by 4.9 and 2.9%, respectively, compared to those fed the unsupplemented basal diet. Combination of xylanase and phytase resulted in further improvements ($P<0.05$) compared to those in the supplemental phytase group.

Addition of xylanase or phytase had no effect on toe ash contents (Table 3). Additions of xylanase and phytase individually resulted in numerical improvements in AME, but the differences were not significant ($P>0.05$). Combination of the two enzymes significantly ($P<0.05$) improved the AME over the unsupplemented group.

Table 3. Effects of phytase and xylanase, individually or in combination, on weight gain (g/bird), feed intake (g/bird), feed/gain (g/g), toe ash (% dry matter) and apparent metabolisable energy (AME; MJ/kg dry matter) of broiler chickens fed a wheat-based diet containing adequate phosphorus levels[1].

	Basal	Basal + xylanase[2]	Basal + phytase[3]	Basal + xylanase + phytase	Pooled SEM
Weight gain	726[a]	847[b] (+16.5)[4]	853[b] (+17.5)	871[b] (+19.8)	24.8
Feed intake	1101[a]	1220[b] (+10.8)	1254[b] (+14.0)	1249[b] (+13.5)	35.6
Feed/gain	1.516[a]	1.442[bc] (-4.9)	1.472[b] (-2.9)	1.434[c] (-5.4)	0.0105
Toe ash	12.28	11.94	12.08	12.20	0.221
AME	14.19[a]	14.43[ab](+1.7)	14.39[ab](+1.4)	14.68[b](+3.5)	0.116

[a,b,] Means in a row with different superscripts differ ($P<0.05$).

[1] Values are means of five replicates.

[2] 1000 XU xylanase/kg diet.

[3] 500 PU/kg diet.

[4] Values in parentheses refer to percentage changes from the addition of enzyme over the basal diet.

Effects of phytase and xylanase, individually or in combination, on the viscosity of digesta from different sections of the small intestine of broilers fed the wheat-based diet are shown in Table 4. The viscosity of digesta from the duodenum of birds fed the basal diet was highest and this was lowered ($P<0.05$) with the additions of xylanase and phytase to the basal diet.

Table 4. Effects of phytase and xylanase, individually or in combination, on the viscosity of digesta of broiler chickens fed a wheat-based diet [1].

	Basal	Basal + xylanase[2]	Basal + phytase[3]	Basal + xylanase + phytase	Pooled SEM
Viscosity (mPa.s)					
Duodenum	2.27[a]	1.62[b](-28.6)[4]	1.89[b](-16.7)	1.69[b](-25.6)	0.126
Jejunum	2.80[a]	2.17[b](-22.5)	2.64[ab](-5.7)	2.02[bc](-27.9)	0.162
Ileum	3.58[a]	2.20[b](-38.5)	2.70[b](-24.6)	2.10[b](-41.3)	0.240

[a,b] Means in a row with different superscripts differ ($P<0.05$).

[1] Values are means of five birds.

[2] 1000 XU xylanase/kg diet.

[3] 500 PU/kg diet.

[4] Values in parentheses refer to percentage reductions from the addition of enzyme over the basal diet.

Combination of the two enzymes caused no further reductions in digesta viscosity. Similar trends were observed for the viscosity of ileal digesta. In the

jejunum, addition of xylanase and the enzyme combination reduced (P<0.05) digesta viscosity. The jejunal digesta viscosity was not influenced by the addition of phytase.

Effects of phytase and xylanase, individually or in combination, on the relative weight and length of different sections of the intestine of birds fed a wheat-based diet are presented in Table 5. The treatments had no effect (P>0.05) on the relative weight of the proventriculus plus gizzard.

Table 5. Effects of phytase and xylanase, individually or in combination, on relative weight and length of different sections of the intestine of birds fed a wheat-based diet [1].

	Basal	Basal + xylanase[2]	Basal + phytase[3]	Basal + xylanase + phytase	Pooled SEM
Relative weights			g/100g body weight		
Proventriculus + gizzard	1.48	1.40(-5.4)[4]	1.33(-10.1)	1.37(-7.4)	0.064
Duodenum	0.62[a]	0.51[b](-17.8)	0.51[b](-17.7)	0.59[ab](-4.8)	0.026
Jejunum	1.33[a]	1.12[b](-15.8)	1.20[b](-9.8)	1.27[ab](-4.5)	0.037
Ileum	1.03[a]	0.88[b](-14.6)	0.92[ab](-10.7)	0.95[ab](-7.8)	0.043
Small intestine[5]	2.97[a]	2.51[b](-15.5)	2.63[bc](-11.4)	2.80[ac](-5.7)	0.074
Caeca	0.34	0.38	0.37	0.36	0.016
Relative lengths			cm/100g body weight		
Duodenum	3.16[a]	2.48[b](-21.5)[4]	2.55[b](-19.3)	2.59[b](-18.0)	0.165
Jejunum	7.87[a]	6.63[b](-15.8)	6.85[b](-13.0)	6.78[b](-13.9)	0.228
Ileum	7.74[a]	6.56[b](-15.2)	6.72[b](-13.2)	6.65[b](-14.1)	0.262
Small intestine[5]	18.77[a]	15.67[b](-16.5)	16.12[b](-14.1)	16.02[b](-14.7)	0.575
Caeca	1.63	1.52(-6.7)	1.55(-4.9)	1.48(-9.2)	0.054

[a,b] Means in a row with different superscripts differ (P<0.05).

[1] Values are means of ten birds.

[2] 1000 XU xylanase/kg diet.

[3] 500 PU/kg diet.

[4] Values in parentheses refer to percentage reductions from the addition of enzyme over the basal diet.

[5] Small intestine = duodenum + jejunum + ileum.

Addition of xylanase significantly (P<0.05) reduced the relative weight of the duodenum, jejunum, ileum and small intestine by 17.8, 15.8, 14.6 and 15.5%, respectively (Table 5). The corresponding reductions with supplemented phytase were 17.7, 9.8, 10.7 and 11.4%, respectively. The relative weights of the ileum of birds fed the diet supplemented with phytase were not significantly (P>0.05) different from those

fed the basal diet. Combination of the phytase and xylanase had no further effects on any of the digestive tract measurements. Relative weights of the caeca were unaffected ($P>0.05$) by treatments.

Individual additions of phytase and xylanase significantly ($P<0.05$) reduced the relative length of the duodenum, jejunum, ileum and small intestine. Combination of xylanase and phytase had no further effect (Table 5). Individual additions of phytase, xylanase and the two enzymes combination reduced the relative length of the caeca, but the differences were not significant ($P>0.05$).

Effects of phytase and xylanase, individually or in combination, on villus height, epithelial thickness, goblet cell numbers, crypt depth and the ratio of crypt depth to villus height of different sections of the small intestine of birds fed a wheat-based diet are shown in Table 6. Xylanase supplementation had no effects ($P>0.05$) on villus height, crypt depth, epithelial thickness, goblet cell numbers and the ratio of crypt depth to villus height in the duodenum, jejunum and ileum. The only exception was that xylanase supplementation tended to increase ($P<0.10$) goblet cell numbers in the duodenum and decreased ($P<0.05$) crypt depth in the jejunum.

Table 6. Effects of phytase and xylanase, individually or in combination, on villus height (μm), epithelial thickness (μm), goblet cell number (per 100μm villus height) and the ratio of crypt depth to villus height in different sections of the small intestine of birds fed a wheat-based diet [1].

	Basal	Basal + xylanase[2]	Basal + phytase[3]	Basal + xylanase + phytase	Pooled SEM
Duodenum					
Villus height	1860.4[a]	1845.8[a]	2031.3[b]	1705.6[c]	40.91
Epithelial thickness	51.7	49.1	48.1	51.2	2.58
Goblet cell number[4]	9.5[a]	11.7[b]	8.5[ac]	7.3[c]	0.93
Crypt depth	147.1	154.9	151.7	157.6	5.37
Ratio[5]	0.080[a]	0.085[ac]	0.075[ab]	0.092[c]	0.0032
Jejunum					
Villus height	871.7	842.9	844.5	900.2	27.22
Epithelial thickness	36.1	40.2	31.6	34.8	2.25
Goblet cell number	13.9[a]	12.9[a]	8.7[b]	8.6[b]	1.02
Crypt depth	119.0[a]	103.4[b]	105.7[b]	133.0[c]	4.47
Ratio[5]	0.138	0.124	0.128	0.151	0.0057
Ileum					
Villus height	784.1[a]	831.7[a]	790.6[a]	915.4[b]	30.3
Epithelial thickness	36.4	35.3	36.8	34.3	1.97
Goblet cell number[4]	11.4[a]	10.0[ab]	10.6[ab]	8.3[b]	1.29
Crypt depth	101.0[a]	102.2[a]	99.7[a]	131.5[b]	3.79
Ratio[5]	0.129[a]	0.130[a]	0.129[a]	0.147[b]	0.0060

[a,b] Means in a row with different superscripts differ ($P<0.05$).

[1] Values are means of 25 observations.

[2] 1000 XU xylanase/kg diet.

[3] 500 PU/kg diet.

[4] $P<0.10$

[5] Ratio of crypt depth to villus height.

Villi in the duodenum of birds fed the unsupplemented wheat-based diet were observed to be shortened and relatively thickened (Figure 1). Addition of phytase significantly ($P<0.05$) increased the duodenal villus height (Figure 2). Interestingly, however, the duodenal villus heights were decreased ($P<0.05$) by the combination of phytase and xylanase compared to those in the unsupplemental basal diet.

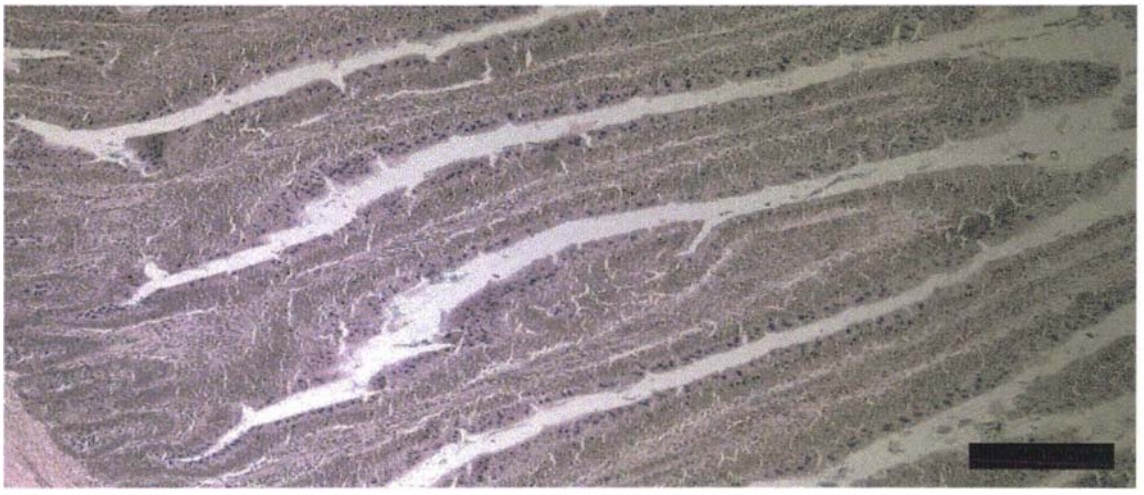

Figure 1. Light micrography of the duodenum of a bird fed the unsupplemented wheat-soy diet showing shortening and thickening of the villi (Alcian blue H&E stain, magnification x100; Bar = 100μm).

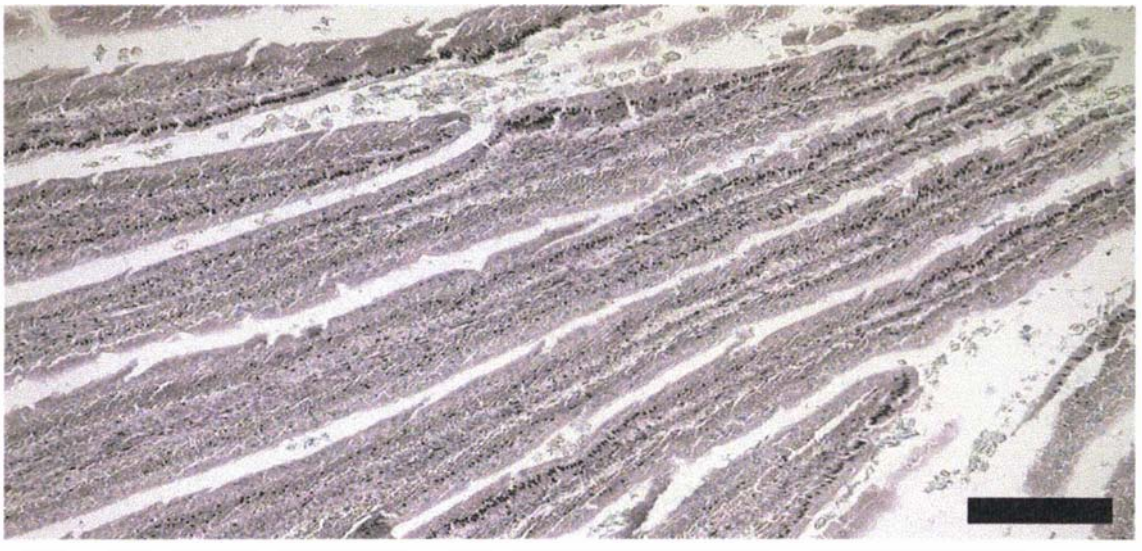

Figure 2. Light micrography of the duodenum of a bird fed phytase-supplemented diet showing long and normal epithelial thickness of the villi (Alcian blue H&E stain, magnification x100; Bar = 100μm).

The addition of phytase and the enzyme combination had no effect ($P>0.05$) on villus height, epithelial thickness and the ratio of crypt depth to villus height in the jejunum (Table 6). The goblet cell numbers in the villi of the jejunum of birds fed the unsupplemental basal diet (Figure 3) and diets with xylanase were higher than those in birds fed diets with phytase and enzymes combinations (Figure 4). Interestingly, the jejunal crypt depths of birds fed diets with individual additions of phytase and xylanase were lower ($P<0.05$) those fed the unsupplemented basal diet. However, combinations of the two enzymes increased ($P<0.05$) crypt depth compared to those in the unsupplemented basal diet (Figure 4).

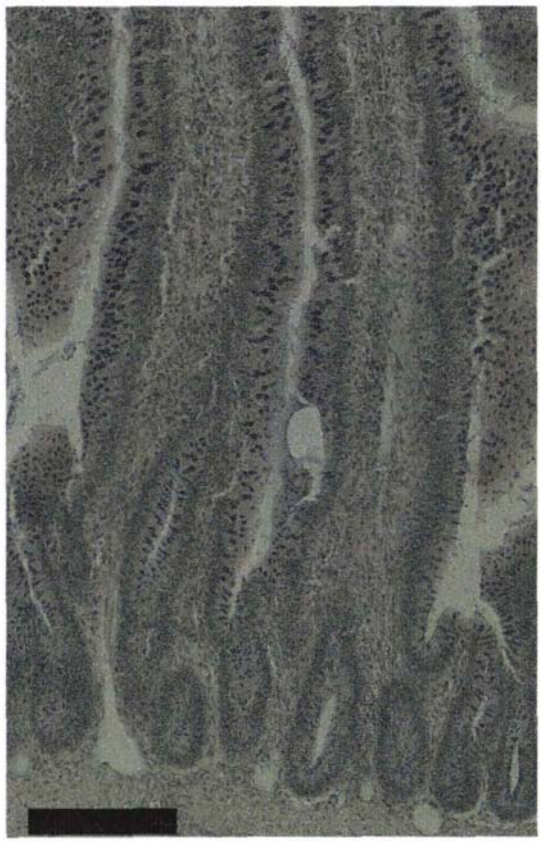

Figure 3. Light micrography of the jejunum of a bird fed the unsupplemented wheat-based diet showing increased goblet cell numbers at the base of the villi (Alcian blue H&E stain, magnification x 200; Bar = 50µm).

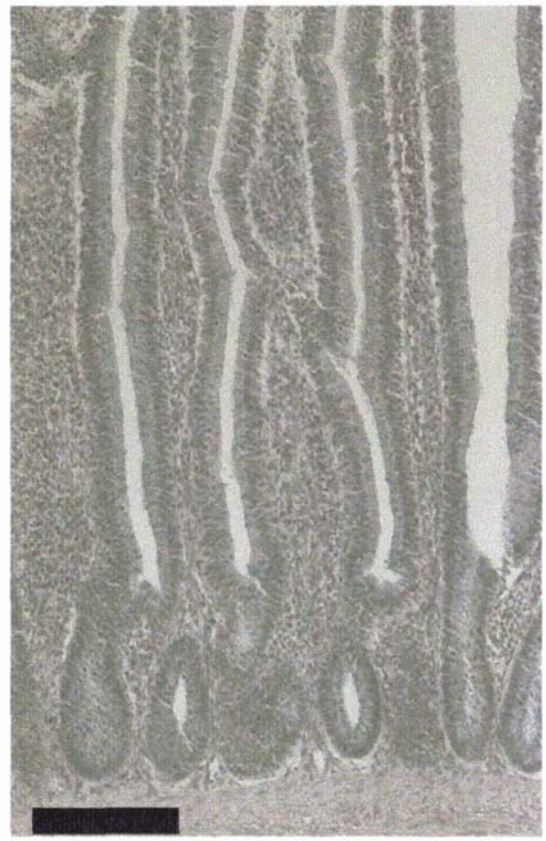

Figure 4. Light of micrography of the jejunum of a bird fed diets with combination of phytase and xylanase showing normal numbers of goblet cell and higher crypt depths at the base of the villi (Alcian blue H&E stain, magnification x 200; Bar = 50µm).

Shortening of villi and increased goblet cell numbers were also observed in ileum of birds fed the unsupplemented basal diet (Figures 5 and 7). Ileal villus height was not affected by phytase supplementation, but increased ($P<0.05$) by the combination of phytase and xylanase (Figure 6). Goblet cell numbers in the villi of the ileum were not affected ($P>0.05$) by the phytase supplementation, but decreased ($P<0.05$) when the two enzymes were added together (Figure 8).

Figure 5. Light micrography of the ileum of a bird fed the unsupplemented diet showing shortening of the villi (Alcian blue H&E stain, magnification x 100; Bar = 100µm).

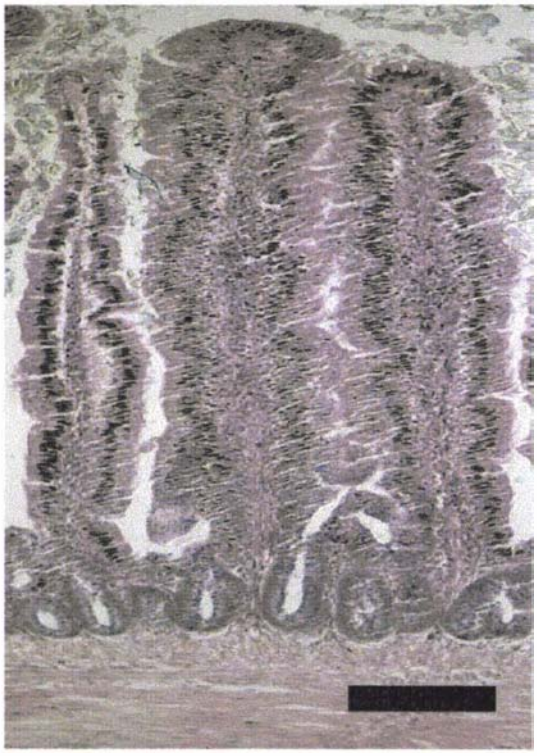

Figure 6. Light micrography of the ileum of a bird fed the diet with a phytase and xylanase combination showing the normal villi (Alcian blue H&E stain, magnification x 100; Bar = 100µm).

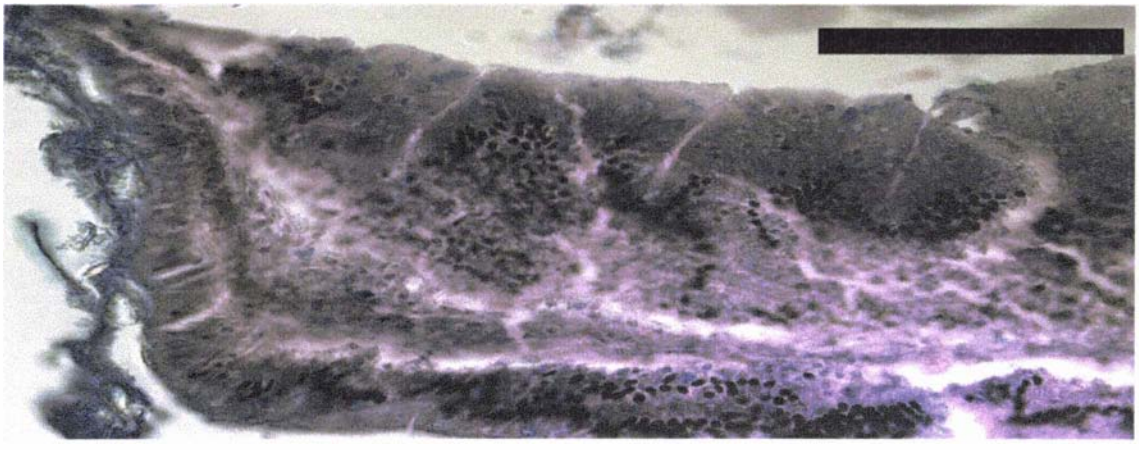

Figure 7. Light micrography of the ileum of a bird fed the unsupplemented diet showing increased numbers of goblet cells at the tip of the abnormal villi (Alcian blue H&E stain, magnification x 400; Bar = 50µm).

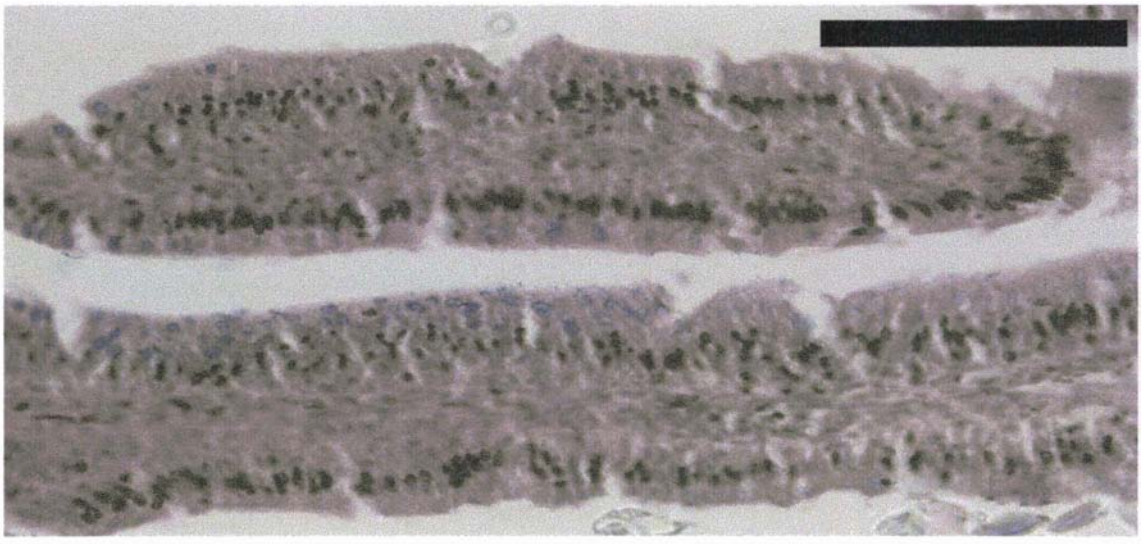

Figure 8. Light micrography of the ileum of a bird fed the diet with a phytase and xylanase combination showing normal characteristics of goblet cells at the tip of the villi (Alcian blue H&E stain, magnification x 400; Bar = 50µm).

DISCUSSION

The objective of the present study was to examine the influence of phytase and xylanase, individually or in combination, on the performance, AME, digesta viscosity, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of P. Individual additions of phytase and xylanase to wheat-based diets improved the weight gains by 18 and 17%, respectively. Feed per gain values were lowered by 2.9, 4.9, and 5.4% for phytase, xylanase and the enzyme combination, respectively. The improvement in bird performance with supplemented xylanase in wheat-based diets was anticipated and is consistent with previous reports (Annison and Choct, 1991; Bedford and Classen, 1992; Steenfeldt *et al.*, 1998; Steenfeldt and Petterson, 2001). The finding that phytase was as effective as exogenous xylanase, especially in diets containing adequate levels of P, was unexpected. This may be due to the fact that the phytase product used in the present study was produced by solid state fermentation and contained relatively high levels of β-glucanase (900 BGU/g), xylanase (400 XU/g) and protease (7600 HUT/g). This suggestion may also explain why the combination of phytase and xylanase had no further beneficial effects on broiler performance in the present study.

As expected, birds fed the unsupplemented basal diet had the lowest AME and the AME was only numerically improved by addition of phytase and xylanase.

Combination of phytase and xylanase, however, significantly improved the AME. These results confirm the beneficial effects of the enzyme combination on energy utilisation in wheat-based diets, which was reported in Chapter 6 of this thesis. The synergistic effect of combination of xylanase and phytase on energy utilisation in wheat-based diets is consistent with previous reports (Ravindran *et al.*, 1999; Selle *et al.*, 2001). Ravindran *et al.* (1999) found that individual addition of phytase and xylanase improved AME of wheat by 9.7 and 5.3%, respectively. When the diets were supplemented with a combination of the two enzymes, AME was improved by 19.0%.

Treatments had no effect on toe ash contents, since dietary P level used in the present study was adequate (0.45%). These findings suggest that the observed performance responses from supplemental phytase are not related to a P effect, but likely to reflect the enzyme effects of other nutrients.

The improvements in weight gain and feed efficiency in birds given wheat-based diets with individual additions of xylanase or phytase also paralleled the reductions in the viscosity of digesta in the duodenum, jejunum and ileum. The viscosity in duodenal, jejunal and ileal contents of birds fed diets with supplemental xylanase were reduced by 29, 22 and 38%, respectively, These results are in agreement with other reports (Bedford and Classen, 1992; Iji *et al.*, 2001; Steenfeldt and Pettersson, 2001). The negative effects of NSP on viscosity and bird performance, and the influence of exogenous NSP enzymes in counteracting these adverse effects are well known (Choct and Annison, 1992; Choct *et al.*, 1994; Bedford and Schulze, 1998). Interestingly, the viscosity of the digesta in the duodenum and ileum were significantly reduced by dietary supplementation of phytase. Two reasons may be proposed to explain these findings. First, phytase product used in the present study contained relatively high levels of β-glucanase and xylanase activity. Second, it is possible that microbial phytase may act in a similar manner as that of exogenous xylanase in disrupting the cell wall matrix of wheat (Ravindran *et al.*, 1999). This action may degrade NSP and lower the viscosity.

The relative heavier intestine of birds fed the unsupplemented wheat-based diet is probably due to the increased viscosity caused by the presence of water-soluble NSP in the wheat, reduced intestinal mobility and consequently increased microbial activity that stimulate intestinal growth (Brenes *et al.*, 2002). Increased viscosity is known to stimulate the growth of anaerobic microflora (Choct *et al.*, 1999; Sinlae and Choct, 2000). Choct *et al.* (1999) reported that the supplementation of xylanase to wheat-based

diets reduced the microbial activity in ileal digesta as indicated by reduced concentrations of volatile fatty acids. Sinlae and Choct (2000) reported that the addition of xylanase to a wheat-based diet reduced the number of undesirable organisms such as *clostridium perfringens* in the caeca. In the present study, xylanase supplementation lowered the viscosity which may have a reduced size of the intestine. The addition of xylanase reduced the relative weight and length of small intestine by 15.5 and 16.5%, respectively. These results are in contrast to the report of Brenes *et al.* (1993) who found that the addition of xylanase to wheat-based diets had no effect on the relative weight of the proventriculus, pancreas, liver and small intestine in broilers. The finding that phytase was as effective as exogenous xylanase in reducing the size of the intestine is noteworthy. Phytase reduced the relative weight and length of the small intestine by 11.4 and 14.1%, respectively. As previously discussed, the microbial phytase product used in the present study contained relatively high levels of β-glucanase and xylanase and this may be responsible for the observed effects.

In the present study, the addition of xylanase to wheat-based diets had no effect ($P>0.05$) on villus height and crypt depth in the duodenum, jejunum and ileum. These results are consistent with the report by Iji *et al.* (2001) who found that supplementation of xylanase to wheat-based diets had no effect on villus height, crypt depth and villus surface area in the duodenum, jejunum and ileum of broilers fed wheat-based diets. Jaroni *et al.* (1999), however, found shortening, thickening and atrophy of the villi in the jejunum of laying hens fed diets based on wheat middlings, but these adverse effects were reversed with xylanase addition. The present data showed that xylanase supplementation to a wheat-based diet had no effect on epithelial thickness and the ratio of crypt depth to villus height in the duodenum, jejunum and ileum. Xylanase supplementation increased goblet cell numbers in the duodenum and decreased crypt depth in the jejunum. These results are unexpected and difficult to explain.

No published data are available on the effects of microbial phytase on gut morphology of broiler chickens. In the present study, microbial phytase increased villus height in the duodenum of birds fed wheat-based diets, but had no effect in the jejunum and ileum. Epithelial thickness and the ratio of crypt depth to villus height in the duodenum, jejunum and ileum were unaffected by supplemental phytase. Crypt depths in the jejunum of birds fed diets with supplemental phytase were lower ($P<0.05$) than those fed the unsupplemented basal diets. These results are unexpected and difficult to explain.

Increased numbers of goblet cells were observed in the villi of the duodenum, jejunum and ileum of birds fed the wheat-based diet. The goblet cell numbers were, however, reduced ($P<0.05$) by phytase addition in the villi of the jejunum, but no effects were seen in the duodenum and ileum. Combination of phytase and xylanase decreased ($P<0.10$ to 0.05) goblet cell numbers in the villi of the duodenum, jejunum and ileum compared to those fed the unsupplemental basal diet. These results are consistent with the report of Viveros *et al.* (1994) who found that the addition of β-glucanase to barley-based diets decreased the number of goblet cells compared to those birds fed the corn-soy diet. The microbial phytase product used in the present study contained high levels of β-glucanase and xylanase. This may be responsible for the observed effects.

CONCLUSIONS

As expected, supplemental xylanase improved the performance of broilers fed a wheat-based diet containing adequate levels of P. Interestingly, microbial phytase was as effective as xylanase in improving bird performance. Combination of phytase and xylanase caused no further improvements on bird performance. Improved performance with enzyme supplementation was associated with reduced digesta viscosity, improved AME, reduced relative weight and length of small intestine.

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Chapter 9

Influence of Method of Whole Wheat Inclusion and Xylanase Supplementation on the Performance, Apparent Metabolisable Energy, Digesta Viscosity and Digestive Tract Measurements of Broilers

Whole grain feeding for poultry has received attention in recent years due to associated economic benefits. The aim of the study reported in Chapter 9 was to examine the influence of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digesta viscosity, and digestive tract measurements of broilers fed wheat-based diets. The influence of the method of whole wheat inclusion (pre- or post-pelleting) was also compared.

ABSTRACT

The aim of the present study was to examine the influence of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digesta viscosity, and digestive tract measurements of broilers fed wheat-based diets. The influence of the method of whole wheat inclusion (pre- or post-pelleting) was also compared. A 3 x 2 factorial arrangement of treatments was used with three diet forms (64.8% ground wheat [GW], GW replaced by 20% of whole wheat before [WW1] or after cold-pelleting [WW2]) and two enzyme (xylanase) doses (0 and 1000 XU/kg diet). Significant (P<0.05 to 0.001) main effects of whole wheat and xylanase supplementation were observed for weight gain, feed per gain, AME and relative weights of the gizzard and pancreas. There were no significant (P>0.05) interactions between diet form and enzyme for any of these response variables. Birds fed diets containing whole wheat had improved (P<0.05 to 0.001) weight gains (2.1-3.9%), feed efficiency (4.1-5.8%) and AME (3.6-6.0%) compared to those fed diets containing ground wheat. The relative gizzard weight of birds fed WW2 diets were higher (P<0.05) than those fed GW and WW1 diets. Pre-pelleting inclusion of whole wheat had no effect (P>0.05) on the relative gizzard weight. Post-pelleting inclusion of whole wheat resulted in greater improvements (P<0.05 to 0.001) in feed efficiency, AME and relative weight of the gizzard compared to the pre-pelleting treatment. Improved performance by post-pelleting inclusion of whole wheat was probably due to the development of the gizzard, and to improved AME. These results showed that there are beneficial effects of whole wheat inclusion in broiler fed wheat-based diets.

Xylanase supplementation significantly (P<0.05) improved weight gains (2.6%), feed efficiency (1.5%) and AME (1.1%), irrespective of the wheat form used. Viscosity of the digesta in the duodenum, jejunum and ileum were reduced (P<0.001 to 0.01) with xylanase addition by 11.3, 20.9 and 18.1%, respectively. Xylanase supplementation reduced (P<0.10 to 0.01) the relative weight of the gizzard and pancreas by 8.4 and 10.3%, respectively. Neither the xylanase supplementation nor whole wheat inclusion influenced (P>0.05) the relative weight and length of the small intestine.

INTRODUCTION

The use of exogenous xylanase in wheat-based diets has been used primarily to reduce the anti-nutritive effects of non-starch polysaccharides, which increase the viscosity of the digesta and limit nutrient utilisation (Bedford, 1997). The beneficial effects of exogenous xylanase supplementation on the performance of birds fed wheat-based diets have been demonstrated in the previous chapter.

Whole grain feeding for broilers has attracted attention in recent years due to its economic benefits. Beneficial effects of whole wheat feeding on the performance of broiler have been demonstrated in several recent reports (Preston *et al.*, 2000; Nahas and Lefrancois, 2001; Svihus and Hetland, 2001; Hetland *et al.*, 2002; Plavnik *et al.*, 2002). Other reports (Munt *et al.*, 1995; Uddin *et al.*, 1996; Jones and Taylor, 2001; Taylor and Jones, 2001; Bennett *et al.*, 2002), however, have failed to show any advantage of including whole wheat in broiler diets.

The coarse fibrous nature of whole wheat may enhance development of the gastrointestinal system allowing improved nutrient absorption and digesta motility (Williams *et al.*, 1997) and therefore may reduce the use of exogenous enzymes in broilers diets (Jones and Taylor, 2001). Published data on the combined effects of whole wheat inclusion and xylanase supplementation in wheat-based broiler diets are limited. It is of considerable practical interest to evaluate whether or not whole wheat inclusion will modify the responses to dietary xylanase addition. In most published reports, whole wheat has been included post-pelleting of diets. Only one report (Taylor and Jones, 2001) has examined the effects of pre-pelleting inclusion of whole wheat on the performance of broilers fed wheat-based diets. No studies have been conducted to compare the influence of the method of whole wheat inclusion (pre- or post-pelleting) on the performance of broilers fed wheat-based diets. The aim of the present study was to examine the influence of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digesta viscosity, and digestive tract measurements of broilers fed wheat-based diets. The influence of the method of whole wheat inclusion (pre- or post-pelleting) was also compared.

MATERIALS AND METHODS

Experimental procedures were approved by the Massey University Animal Ethics Committee (Anonymous, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Enzymes

The xylanase (Allzyme® PT; Alltech, Inc., Nicholasville, KY, USA) contained 1218 XU per gram of sample. One unit of xylanase (XU) is defined as that amount of enzyme that liberates 1 μmol of xylose in one minute at pH 5.3 at 50 °C.

Experimental Design and Diets

The experimental design was a randomised complete block with a 3 x 2 factorial arrangement of treatments (Table 1), which included three diet forms (ground wheat [GW], 20% whole wheat replacing GW before pelleting [WW1], and 20% whole wheat replacing GW after pelleting [WW2]) and two enzyme doses (0 and 1000 XU/kg of diet).

Table 1. Dietary treatments evaluated.

Treatment	Diet Form [1]	Xylanase[2]
1	GW	-
2	GW	+
3	WW1	-
4	WW1	+
5	WW2	-
6	WW2	+

[1] GW, ground wheat; whole wheat [20% whole wheat replacing GW prior to (WW1) or post pelleting of diets (WW2)].

[2] Inclusion rate at 1000 XU/kg diet.

The basal diet was based on wheat and soybean meal (Table 2) and formulated to contain 0.45% non-phytate P. All diets were cold-pelleted (65-70 °C). Diets were offered *ad libitum* and water was available at all times.

Table 2. Ingredient composition and calculated analysis of the basal diet.

Ingredient	Amount (%)
Wheat[1]	64.80
Soybean meal	29.50
Vegetable oil	1.31
Dicalcium phosphate	1.62
Limestone	1.65
Lysine·HCL	0.19
DL-methionine	0.38
Salt	0.25
Vitamin-mineral premix [2]	0.30
Calculated analysis	
AME (MJ/kg)	12.38
Crude protein, %	22.6
Lysine, %	1.15
Methionine + Cysteine, %	0.94
Calcium, %	1.09
Non-phytate P, %	0.45

[1] Ground wheat; in experimental diets, 20% whole wheat replaced ground wheat prior to or post pelleting.

[2] Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

General Procedures

Day-old male broiler (Ross) chicks were obtained from a commercial hatchery and randomly assigned to 36 pens (8 birds/ pen) in 3-tier electrically heated battery brooders. Each of the six dietary treatments was randomly assigned to six pens of eight chicks each. The brooders were housed in an environmentally controlled room with 24-hour fluorescent lighting. The birds were transferred to colony cages in an environmentally controlled room on day 14. Room temperature was maintained at 32 ± 1 ^{0}C during the first week and gradually decreased to 24 ^{0}C by the end of the third week.

Body weights and feed intake were recorded on a pen basis at weekly intervals. Mortality was recorded daily. Any bird that died was weighed and the weight was used to adjust feed/ gain. The trial lasted three weeks.

Determination of Feed Passage Rate

On Day 15, feed was withdrawn for two hours and diets containing chromic oxide (0.1%) were offered for 15 min. The rate of passage was determined as the time from the introduction of the diets to the first appearance of green-coloured excreta.

Collection and Processing of Samples

During the third week (day 17-21), total collection of excreta was carried out for the determination of AME. Feed intake and excreta output was measured quantitatively per pen over four consecutive days. Excreta were pooled within a pen, mixed well using a blender and two representative samples per pen were taken. The samples were freeze-dried. Dried samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at - 4 ^{0}C until chemical analyses.

On day 20, excreta were scored for consistency on a scale of 1 to 5, with a score of 1 representing normally formed excreta and 5 representing pasty and very sticky excreta. On day 21, two birds from each pen (close to the mean pen body weight) were selected and weighed. The birds were killed by cervical dislocation and the digestive tract, from the proventriculus to the caeca, were carefully excised. Empty weights of gizzard and weight of the pancreas were recorded. After removing the intestinal contents, the empty weights and lengths of the duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileocaecal junction), and caeca were recorded.

In addition, two birds from each pen were killed by intravenous injection of sodium pentobarbitone. The contents from different sections of intestine (duodenum, jejunum, and ileum) were obtained and centrifuged. The supernatant was transferred into a 2 ml Eppendorf tube and frozen at -20 °C for viscosity measurements.

Viscosity Measurement

Viscosity measurements were conducted as described in the Material and Methods section in Chapter 8.

Chemical Analysis

The gross energy (GE) determination was as described in the Material and Methods section in Chapter 8.

Calculation

The calculation of AME was as described in the Material and Methods section in Chapter 8.

Data Analysis

For viscosity and digestive tract measurements, individual birds were considered as the experimental unit. For performance and AME data, pen means served as the experimental unit. Data were subjected to two-way analysis of variance using the General Linear Models (GLM) procedure of the SAS® (SAS Institute, 1997) to determine the main effects (diet form and enzyme) and their interactions. Comparison between ground wheat (diet GW) and whole wheat (diet WW1 and WW2), and between diet WW1 and WW2 were made using non-orthogonal contrasts. Differences were considered to be significant at $P<0.05$. If the data suggested a trend, the values at $P<0.10$ were also shown in the text.

RESULTS

Effects of whole wheat inclusion and xylanase supplementation on the performance and AME of broilers fed wheat-based diets are presented in Table 3. Birds fed diets containing 20% whole wheat had higher ($P<0.05$) weight gains and lower ($P<0.001$) feed per gain than those fed diets containing ground wheat, but feed intake was not influenced ($P>0.05$).

Table 3. Effects of whole wheat inclusion and xylanase supplementation on weight gain, feed intake, feed/gain and apparent metabolisable energy (AME) of broilers fed wheat-based diets[1].

Treatment		Weigh gain	Feed intake	Feed/gain	AME
Diet Form[2]	Xylanase[3]	(g/bird)	(g/bird)	(g/g)	(MJ/kg DM)
GW	-	785	1252	1.595	12.29
	+	816	1282	1.584	12.47
WW1	-	821	1259	1.536	12.78
	+	841	1270	1.513	12.88
WW2	-	810	1225	1.513	13.06
	+	825	1209	1.479	13.19
Pooled SEM		13.2	18.8	0.0125	0.065
Main effects					
Diet form					
GW		800	1267	1.589	12.38
WW1		831(+3.9)[4]	1265 (-0.2)	1.524 (-4.1)	12.83(+3.6)
WW2		817(+2.1)	1217 (-3.8)	1.496 (-5.8)	13.12(+6.0)
Xylanase					
-		806	1245	1.548	12.71
+		827(+2.6)[4]	1253 (+0.6)	1.525(-1.5)	12.85(+1.1)
Probabilities (*P*<)					
Diet form		0.10	0.05	0.001	0.001
Xylanase		0.05	NS	0.05	0.05
Diet form * xylanase		NS	NS	NS	NS
Contrast diet GW vs WW1, WW2		0.05	NS	0.001	0.001
Contrast diet WW1 vs WW2		NS	0.05	0.05	0.001

[1] Values are means of six replicate pens (8 birds/pen).

[2] GW, ground wheat; whole wheat [20% whole wheat replacing GW prior to (WW1) or post pelleting of diets (WW2)].

[3] 1000 XU/kg diet.

[4] Values in the parentheses represent percentage increase or decrease relative to birds fed the GW diet or the unsupplemented diet.

The method of inclusion of whole wheat had no effect (P>0.05) on the weight gain of birds. When 20% whole wheat was included post pelleting, feed intake was lowered (P<0.05) compared to those fed the GW and WW1 diets. Pre-pelleting

inclusion of whole wheat had no effect ($P>0.05$) on feed intake. Feed per gain value of birds fed WW2 diets was lower ($P<0.05$) than those fed WW1 diets.

Xylanase supplementation improved ($P<0.05$) weight gains and feed efficiency irrespective of wheat form used (Table 3), but had no effect ($P>0.05$) on feed intake. Two-way interactions between diet form and enzyme were not significant ($P>0.05$) for any of the performance parameters.

Inclusion of whole wheat improved ($P<0.001$) the AME values (Table 3). Post-pelleting inclusion of whole wheat caused greater ($P<0.001$) improvements in AME compared to pre-pelleting inclusion of whole wheat. Xylanase supplementation improved ($P<0.05$) AME irrespective of the wheat form used.

Significant main effects of diet form ($P<0.10$ to 0.05) and enzyme supplementation ($P<0.001$ to 0.01) were observed for intestinal digesta viscosity (duodenum, jejunum and ileum) and excreta scores (Table 4). The only exception was the lack of an effect ($P>0.05$) of diet form on ileal digesta viscosity. Inclusion of whole wheat increased ($P<0.10$ to 0.05) the duodenal and jejunal digesta viscosity by 5.2-14.5 and 7.5-11.4%, respectively. Compared to those fed WW1 diets, birds fed WW2 diets tended to have higher ($P<0.10$) duodenal viscosity and lower ($P<0.05$) excreta scores.

Xylanase supplementation significantly ($P<0.01$ to 0.001) reduced digesta viscosity in the duodenum, jejunum and ileum by 11.3, 20.9 and 18.1%, respectively. Xylanase supplementation and whole wheat inclusion had no effect ($P>0.05$) on passage rate (Table 4), but a significant ($P<0.05$) interaction was observed. Xylanase supplementation had no effect ($P>0.05$) on feed passage rate in birds fed diets containing whole wheat, but increased ($P<0.05$) feed passage rate in the GW diets.

Effects of whole wheat inclusion and xylanase supplementation on the relative weight of organs and relative weight and length of different sections of the small intestine of broilers are shown in Tables 5 and 6. Two-way interactions were not significant ($P>0.05$) for any of the parameters. There were significant ($P<0.10$ to 0.001) main effects of whole wheat inclusion and xylanase supplementation on the relative weight of the gizzard and pancreas (Table 5).

Table 4. Effects of whole wheat inclusion and xylanase supplementation on digesta viscosity in different sections of the small intestine, excreta score and feed passage rate of broilers fed wheat-based diets[1].

Treatment		Relative viscosity (mPa.s)			Excreta	Feed passage
Diet Form[2]	Xylanase[3]	Duodenum	Jejunum	Ileum	score	Rate (min)
GW	-	1.81	2.17	2.43	3.2	112
	+	1.63	1.85	2.22	2.1	126
WW1	-	1.88	2.34	2.61	3.1	133
	+	1.73	1.98	2.11	2.3	121
WW2	-	2.12	2.65	2.74	2.1	119
	+	1.81	1.84	2.04	1.6	127
Pooled SEM		0.083	0.104	0.133	0.31	4.1
Main effects						
Diet form						
GW		1.72	2.01	2.32	2.64	119
WW1		1.81(+5.2)[4]	2.16(+7.5)	2.36(+1.7)	2.67(+1.1)	127(+6.7)
WW2		1.97(+14.5)	2.24(+11.4)	2.39(+3.0)	1.83(-30.7)	123(+3.4)
Xylanase						
-		1.94	2.39	2.59	2.79	121
+		1.72(-11.3)[4]	1.89(-20.9)	2.12(-18.1)	1.97(-29.4)	125 (+3.3)
Probabilities (*P*<)						
Diet form		0.05	0.10	NS	0.05	NS
Xylanase		0.01	0.001	0.001	0.01	NS
Diet form * xylanase		NS	0.05	NS	NS	0.05
Contrast diet GW vs WW1, WW2		0.05	0.05	NS	NS	0.10
Contrast diet WW1 vs WW2		0.10	NS	NS	0.05	NS

[1] Values are means of six replicate pens (8 birds/pen).

[2] GW, ground wheat; whole wheat [20% whole wheat replacing GW prior to (WW1) or post pelleting of diets (WW2)].

[3] 1000 XU/kg diet.

[4] Values in parentheses represent percentage increase or decrease relative to chickens fed the GW diet or the unsupplemented diet.

Post-pelleting inclusion of 20% whole wheat increased (P<0.001) the relative gizzard weight by 73%, whereas pre-pelleting inclusion had no effect (Table 5). Post-pelleting inclusion of 20% whole wheat increased (P<0.001) the relative pancreas weights by 20%, but was unaffected in the pre-pelleting treatment. Xylanase supplementation reduced (P<0.10 to 0.01) the relative weights of gizzard and pancreas by 8.4 and 10.3%, respectively, irrespective of the wheat form used.

Table 5. Effects of whole wheat inclusion and xylanase supplementation on the relative weight of organs and different sections of the intestine of broilers fed wheat-based diets[1].

Treatment		Relative length (g /100g body weight)						
Diet Form[2]	Xylanase[3]	Gizzard	Pancreas	Duodenum	Jejunum	Ileum	Small intestine[4]	Caeca
GW	-	1.00	0.27	0.64	1.39	1.16	3.19	0.41
	+	1.01	0.23	0.66	1.38	1.17	3.21	0.37
WW1	-	1.04	0.27	0.61	1.33	1.04	2.97	0.32
	+	0.98	0.25	0.60	1.37	1.09	3.06	0.36
WW2	-	1.88	0.31	0.60	1.37	1.08	3.05	0.39
	+	1.62	0.28	0.62	1.37	1.04	3.03	0.36
Pooled SEM		0.064	0.012	0.026	0.056	0.046	0.110	0.020
Main effects								
Diet form								
GW		1.01	0.25	0.65	1.39	1.16	3.20	0.39
WW1		1.01	0.26(+4.0)	0.61(–6.2)	1.35(-1.4)	1.06(-8.6)	3.01(-5.9)	0.34(-12.8)
WW2		1.75(+73.2)[5]	0.30(+20.0)	0.61(-6.2)	1.37(-0.7)	1.06(-8.6)	3.04(-5.0)	0.38(-2.6)
Xylanase								
-		1.31	0.29	0.62	1.36	1.09	3.07	0.37
+		1.20(-8.4)[5]	0.26(-10.3)	0.63(+1.6)	1.37(+0.7)	1.10(+0.9)	3.10(+1.0)	0.36(-2.7)
Probabilities (*P*<)								
Diet form		0.001	0.001	NS	NS	NS	NS	NS
Xylanase		0.10	0.01	NS	NS	NS	NS	NS
Diet form * xylanase		NS	NS	NS	NS	NS	NS	NS
Contrast diet GW vs WW1, WW2		0.001	0.05	0.10	NS	0.05	0.10	0.05
Contrast diet WW1 vs WW2		0.001	0.01	NS	NS	NS	NS	0.10

[1] Values are means of 12 birds per treatment.

[2] GW, ground wheat; whole wheat [20% whole wheat replacing GW prior to (WW1) or post pelleting of diets (WW2)].

[3] 1000 XU/kg diet.

[4] Small intestine = duodenum + jejunum + ileum.

[5] Values in parentheses represent percentage increase or decrease relative to birds fed the GW diet or the unsupplemented diet.

Table 6. Effects of whole wheat inclusion and xylanase supplementation on the relative length of different sections of gastrointestinal tract of broilers fed wheat-based diets[1].

Treatment		Relative length (cm/100g body weight)				
Diet Form[2]	Xylanase[3]	Duodenum	Jejunum	Ileum	Small intestine[4]	Caeca
GW	-	3.02	7.52	7.81	18.35	1.74
	+	3.03	7.29	7.48	17.81	1.70
WW1	-	2.96	7.36	7.61	17.93	1.63
	+	2.95	7.08	7.37	17.39	1.60
WW2	-	3.18	7.73	8.13	19.04	1.81
	+	2.85	7.35	7.28	17.47	1.59
Pooled SEM		0.106	0.229	0.238	0.514	0.059
Main effects						
Diet form						
GW		3.03	7.40	7.65	18.08	1.72
WW1		2.96(-2.3)[5]	7.22(-2.6)	7.49(-2.1)	17.66(-2.3)	1.61(-6.4)
WW2		3.01(-0.7)	7.54(+1.8)	7.70(-0.7)	18.26(+1.0)	1.70(-1.2)
Xylanase						
-		3.06	7.54	7.85	18.44	1.72
+		2.94(-3.9)[5]	7.24(-4.0)	7.38(-6.0)	17.56(-4.7)	1.63(-5.2)
Probabilities (*P*<)						
Diet form		NS	NS	NS	NS	NS
Xylanase		NS	NS	NS	NS	NS
Diet form * xylanase		NS	NS	NS	NS	NS
Contrast diet GW vs WW1, WW2		NS	NS	NS	NS	NS
Contrast diet WW1 vs WW2		NS	NS	NS	NS	NS

[1] Values are means of 12 birds per treatment.

[2] GW, ground wheat; whole wheat [20% whole wheat replacing GW prior to (WW1) or post pelleting of diets (WW2)].

[3] 1000 XU/kg diet.

[4] Small intestine = duodenum + jejunum + ileum.

[5] Values in parentheses represent percentage increase or decrease relative to birds fed the GW diet or the unsupplemented diet.

There were no significant (P>0.05) main effects of whole wheat inclusion and xylanase supplementation on relative weight or length of different sections of the intestine (Tables 5 and 6). Relative lengths of duodenum, jejunum, ileum and small intestine of birds fed diets with added xylanase were reduced by 3.9, 4.0, 6.0 and 4.7%, respectively, compared to those fed unsupplemented diets, but the differences were not statistically significant (P>0.05; Table 6).

DISCUSSION

The aim of the present study was to examine the beneficial effects of whole wheat inclusion and xylanase supplementation in broilers fed wheat-based diets. Birds fed diets containing 20% whole wheat, irrespective of whether the grain was incorporated pre- or post-pelleting, had higher weight gains (2.1-3.9%) and better feed efficiencies (4.1-5.8%) than those fed diets containing ground wheat. This is in general agreement with a number of recent reports (Preston *et al.*, 2000; Nahas and Lefrancois, 2001; Svihus and Hetland, 2001; Hetland *et al.*, 2002; Plavnik *et al.*, 2002), where inclusion of whole wheat was shown to be beneficial.

In most published reports, whole wheat was included into diets after pelleting. Only a limited number of studies have examined the effects of pre-pelleting inclusion of whole wheat on the performance of broilers fed wheat-based diets. In the present study, pre-pelleting inclusion of 20% whole wheat improved weight gains and feed efficiency, but had no effect on relative gizzard weights. Improvements in feed efficiency were greater in birds fed diets containing 20% whole wheat post-pelleting compared to those fed diets containing whole wheat prior to pelleting. The lack of a response in the gizzard weight of birds fed the diet containing whole wheat prior to pelleting was in contrast to the report of Taylor and Jones (2001). This observation may be due to the whole wheat in the WW1 diet being crushed during pelleting. Taylor and Jones (2001) reported that pre-pelleting inclusion of whole wheat had no effects on weight gains and feed efficiency, but increased relative gizzard weights by 7.8% compared to those fed diets containing ground wheat.

In the present study, improvements in feed efficiency of birds given 20% whole wheat post-pelleting paralleled the increases in relative gizzard weights and AME compared to those fed diets containing ground wheat. Birds fed diets containing 20% whole wheat post-pelleting had 73% heavier gizzards compared to those fed diets containing ground wheat. These results are in agreement with a number of reports (Preston *et al.*, 2000; Svihus and Hetland, 2001; Hetland *et al.*, 2002; Plavnik *et al.*, 2002) where whole wheat was included into diets after pelleting. Preston *et al.* (2000) observed numerical improvements (3%) in feed per gain and 50% greater gizzard weights in birds given 33% whole wheat compared to those fed diets containing ground wheat. Svihus and Hetland (2001) similarly reported that the weight gain and feed

efficiency of birds fed wheat-based diets containing whole wheat (38.5% whole wheat replacing ground wheat during 10-21 d) were improved by 5.1 and 3.1%, respectively, compared to those fed diets containing ground wheat. Relative gizzard weights of birds fed diets containing whole wheat were 42% heavier than those fed diets containing ground wheat. Hetland *et al.* (2002) reported that the weight gains of birds fed diets containing moderate to high levels of whole wheat (12.5-33 and 30-44% during 10-24 and 25-38 d, respectively) were numerically reduced, but feed efficiency was significantly improved by 5.1-6.7% over the experimental period (10-38 d). Relative gizzard weights of birds at 24 and 38 d of age were increased by 56-86% and 36-100%, respectively. In this study, inclusion of whole wheat also improved the apparent ileal starch digestibility by 2-3 percentage units.

No published data are available on the effects of the method of whole wheat inclusion (pre- or post-pelleting) on the AME in broilers. Greater responses in AME were observed in birds fed diets containing whole wheat post-pelleting (6.0 vs 3.6%), compared to those fed diets containing whole wheat prior to pelleting. The improved energy utilisation observed with whole wheat feeding in the present study is in agreement with previous reports. McIntosh *et al.* (1962) reported that diets containing whole wheat yielded about 10% more metabolisable energy than the ground wheat diets (13.21 vs 11.96 MJ/kg dry matter). Similarly, Preston *et al.* (2000) reported that the AME was improved by 3% in birds fed diets containing whole wheat compared to those fed diets containing ground wheat. Uddin *et al.* (1996), however, found that nitrogen-corrected AME of birds fed wheat-based diets from 19-27 d of age was not affected by the inclusion of whole wheat (10-40%).

The mechanisms contributing to the beneficial effects of whole wheat are not well understood. It may be related to a more developed gizzard and increased grinding activity of gizzard, resulting in greater release and digestion of nutrients and improved AME (Preston *et al.*, 2000). Improved ileal starch digestibility in birds fed diets containing whole wheat compared to those fed diets containing ground wheat has been reported by Svihus and Hetland (2001) and Hetland *et al.* (2002). The mode of action through the development of the gizzard is relevant for the post-pelleting inclusion of whole wheat in the present study, but not for pre-pelleting inclusion. Pre-pelleting inclusion of whole wheat improved AME, but had no effect on the relative gizzard weight. Other factors are clearly involved and these need to be elucidated.

The viscosity of digesta in the duodenum and jejunum of birds fed diets containing 20% whole wheat were increased by 5.2-14.5%, irrespective of the method of whole wheat inclusion, compared to those fed diets containing ground wheat. Increased digesta viscosity, however, had no adverse effects on bird performance. Viscosity is not the only limiting factor determining the performance responses of birds fed wheat-based diets, especially if the digesta viscosity is less than 10 mPa.s (Bedford and Schulze, 1998). In the present study, the digesta viscosity values ranged from 1.2-4.0 mPa.s.

Whole wheat inclusion had no effect on the relative weight and length of the intestine compared to those birds given ground wheat. These results are consistent with other reports (Preston *et al.*, 2000; Taylor and Jones; 2001; Banfield *et al.*, 2002) in wheat-based diets. This may be due to the fact that birds had well developed gizzards and were able to process the whole wheat efficiently, removing the need for any further physiological adaptation relating to nutrient digestion or absorption (Banfield *et al.*, 2002).

Observed improvements in weight gains (1.1%) and feed efficiency (1.5%) in birds given wheat-based diets with added xylanase paralleled the reductions in the digesta viscosity in the duodenum, jejunum and ileum and improvements in the AME. Apparent metabolisable energy was improved by xylanase supplementation, irrespective of the wheat form used. These results confirm the results of the study reported in Chapter 8 that xylanase was effective in improving bird performance fed wheat-based diets and that the improved bird performance was associated with reduced viscosity and improved AME.

Published data on the combined effects of whole wheat inclusion and xylanase supplementation in wheat-based broiler diets are limited. The present data has shown that economic advantage of whole wheat inclusion were further improved by xylanase supplementation. In the present study, inclusion of whole wheat improved feed efficiency by 4.1-5.8% compared to those fed diets containing ground wheat. Feed efficiency was further improved by 1.5-2.2% when xylanase was added to the diets containing whole wheat.

Excreta moisture, as indicated by excreta scores, was lowered by the xylanase supplementation. This was an expected result, since the action of xylanase in degrading arabinoxylans and lowering water binding capacity is well known (Choct and Annison, 1992).

Whole wheat inclusion or xylanase supplementation had no effect on feed passage rate and these results are consistent with the findings of Svihus *et al.* (2002). In the present study, a significant interaction between diet form and xylanase was observed for feed passage rate. Xylanase supplementation had no effect on feed passage rate through the digestive tract of birds fed diets containing whole wheat, but increased the passage rate of birds fed diets containing ground wheat.

Enzyme supplementation had no effect on the relative weight of the intestine. Relative length of the duodenum, jejunum, ileum and small intestine of birds fed diets with added xylanase were numerically reduced by 3.9, 4.0, 6.0 and 4.7%, respectively, compared to those fed the unsupplemented enzyme diets, but the differences were not significant. These results are in general agreement with the report of Brenes *et al.* (1993) and Taylor and Jones (2001), but in contrast to the data reported in Chapter 8. Brenes *et al.* (1993) reported that the addition of xylanase to wheat-based diets had no effects on the relative weights of the proventriculus, pancreas, liver, duodenum, jejunum and ileum. Similarly, Taylor and Jones (2001) found that xylanase supplementation had no effect on the relative weights of the duodenum, jejunum and ileum. The study reported in Chapter 8, however, showed that xylanase supplementation reduced the relative weights and lengths of the duodenum, jejunum, ileum and small intestine. The lack of the response on gastro-intestinal measurements in the present study may be related to the quality of wheat used.

CONCLUSIONS

Whole wheat inclusion and xylanase supplementation were effective in improving the performance of birds fed wheat-based diets. Improved bird performance by post-inclusion of whole wheat may be related to a well developed gizzard with increased grinding activity and to the improved AME. On the other hand, it is difficult to propose a mechanism for the improvements observed with pre-pelleting inclusion of whole wheat. Improved performance by xylanase supplementation was associated with reduced viscosity and improved AME. Neither whole wheat inclusion nor xylanase supplementation had any effect on the relative weight and length of the small intestine.

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Chapter 10

Influence of Whole Wheat inclusion and Xylanase Supplementation on the Performance, Digestive Tract Measurements and Carcass Characteristics of Broilers

Results from the 21-day study reported in Chapter 9 showed that the inclusion of 20% whole wheat in wheat-based diets improved the performance of broilers. Performance responses observed with post-pelleting inclusion of whole wheat were related to a well developed gizzard and to improved AME. Improvements in the feed efficiency of broilers were greater when whole wheat was included post-pelleting compared to pre-pelleting inclusion. The aim of the present study was to examine the influence of post-pelleting inclusion of whole wheat and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broilers fed wheat-based diets from 1 to 35 days of age.

ABSTRACT

The aim of the present study was to examine the influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broilers fed wheat-soy diets from 1 to 35 days of age. There were five dietary treatments. Diet 1 was based on corn and soybean meal. Diets 2 and 3 were based on ground wheat (GW) and soybean meal without and with added xylanase at a level of 1000 XU/kg, respectively. Diets 4 and 5 were whole wheat (WW) replacing GW (10 and 20% whole wheat replacing GW during 1-21 d and 22-35 d, respectively) without and with added xylanase at a level of 1000 XU/kg, respectively. Each of the five dietary treatments was fed to four replicate pens (46 birds/pen). During the starter (1-21 d) phase, birds fed diets containing 10% whole wheat had comparable (P>0.05) weight gains and feed/gain compared to those fed diets containing the ground wheat. During the finisher (22-35 d) phase, however, 20% whole wheat inclusion reduced (P<0.05 to 0.001) weight gains and feed intake, but lowered (P<0.05) feed/gain compared to those fed the diets containing ground wheat. Trends observed for weight gains (P=0.07), feed intake (P<0.001) and feed/gain (P<0.05) over the experimental period (1-35 d) were similar to those observed during the finisher phase.

During the starter phase (1-21 d), overall xylanase supplementation improved (P<0.05 to 0.001) weight gains and feed intake. The improvements were attributed to the significant improvements in ground wheat diets. Xylanase supplementation lowered (P<0.05) feed/gain in both ground wheat and whole wheat diets, but the differences were not significant (P>0.05) in ground wheat diets. Similar trends were observed for weight gains (P<0.05 to 0.01), feed intake (P<0.05) and feed/gain (P<0.05) during 22-35 d and over the 35-day experimental period. The only exception was that xylanase supplementation had no effect (P>0.05) on feed intake during 22-35 d. Interestingly, birds fed diets with a combination of whole wheat and xylanase had comparable (P>0.05) feed/gain compared to those fed the corn-based diet.

Whole wheat inclusion increased (P<0.05 to 0.001) the relative weight of the gizzard and pancreas, and had no effects (P>0.05) on the relative weight of the crop, proventriculus, and the small intestine, but interestingly decreased the relative weight of the liver and heart at 35 days of age. Xylanase supplementation reduced (P<0.05) the relative weight of the small intestine at 21 days of age, but had no effects (P>0.05) on

the relative weight and length of the duodenum, jejunum, ileum, caeca and small intestine at 35 days of age.

The dietary treatments had no effects ($P>0.05$) on carcass recovery, breast muscle yield, and abdominal fat pad weight. Birds fed diets containing whole wheat had a lower ($P<0.05$) relative weight of the abdominal fat pad, compared to those fed the corn-based diet (0.88 vs 1.20 g/100g body weight).

INTRODUCTION

Beneficial effects of whole wheat inclusion in wheat-based diets for broilers were demonstrated in the study reported in Chapter 9. Greater responses in feed efficiency were observed when 20% whole wheat was included post-pelleting compared to pre-pelleting.

Results from the study reported in Chapter 8 showed that there were beneficial effects of xylanase supplementation in reducing the relative weight and length of the small intestine in broilers fed wheat-soy diets, which may translate into greater yields of carcass (Brenes *et al.*, 1993). The objective of the present study was to examine the influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics in broilers fed wheat-soy diets. For comparative purposes, a corn-soy diet was also included.

MATERIALS AND METHODS

Experimental procedures were approved by the Massey University Animal Ethics Committee (Anonymous, 1992) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Enzymes

Xylanase (Allzyme PT; activity, 1218 xylanase units/g; supplied by Alltech, Inc., Nicholasville, Kentucky, USA) was used. One unit of xylanase is defined as that amount of enzyme that liberates 1 μmol of xylose in one minute at pH 5.3 and 50 °C.

Experimental Design and Dietary Treatments

The experimental design involved a 2 x 2 factorial arrangement of treatments with two diet forms (ground wheat [GW] and whole wheat [WW] inclusion after pelleting) and two levels of xylanase (0 and 1000 XU/kg diet). For comparative purposes, a corn-soy diet was also included. Details of dietary treatments are shown in Table 1.

Two types of diets were used: corn-soy and wheat-soy (Table 2). The diets were formulated to meet or exceed the NRC (1994) recommendations for all nutrients. In wheat-soy diets, whole wheat replaced ground wheat (10 and 20% whole wheat replacing GW during 1-21 d and 22-35 d, respectively). The birds were fed the starter diets from day 1-21 and the finisher diets from day 22-35. All diets were cold-pelleted (65-70 °C). Diets were offered *ad libitum* and water was available at all times.

Table 1. Details of the dietary treatments.

Diet	Treatments	
	Diet Form[1]	Xylanase[2]
1	Corn-soy	-
2	GW-soy	-
3	GW-soy	+
4	WW-soy	-
5	WW-soy	+

[1] GW, ground wheat; WW, whole wheat (10 and 20% replacing GW during 1-21 d and 22-35 d, respectively).

[2] 1000 XU/kg diet.

General Experimental Procedures

Day-old male broiler (Ross) chicks were obtained from a commercial hatchery and randomly assigned to 20 floor pens (46 birds/ pen) on wood shavings in an environmentally controlled room with 24-hour fluorescent lighting. Each of the five dietary treatments was randomly assigned to four pens of 46 birds per pen. Room temperature was maintained at 32 ± 1 ^{0}C during the first week and gradually decreased to 21 ^{0}C by the end of the five week.

Body weights and feed intake were recorded on a pen basis at weekly intervals. Mortality was observed and recorded daily. Feed/gain was corrected for mortality.

Digestive Tract Measurements

On days 21 and 35, two birds (closest to the mean pen weight) were selected from each replicate pen, and fasted for six hours. After fasting, body weights were recorded and the birds were killed by cervical dislocation. The gastrointestinal tract and organs were carefully excised. Empty weight and length of the duodenum (pancreatic loop),

jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to 1 cm above ileocaecal junction), and caeca (left and right) were recorded. The empty weight of the crop, proventriculus, gizzard and the weight of pancreas, liver and heart were recorded for each bird.

Table 2. Ingredient composition and calculated analysis of the basal diets.

Ingredient	Starters		Finishers	
	Corn-soy	Wheat-soy	Corn-soy	Wheat-soy
			%	
Corn	58.50	0.00	63.10	0.00
Wheat[1]	0.00	64.80	0.00	70.80
Soybean meal	35.99	29.50	31.39	23.14
Vegetable oil	1.25	1.31	2.21	2.56
Dicalcium phosphate	1.75	1.62	1.25	1.10
Limestone	1.57	1.65	1.39	1.50
Lysine·HCL	0.02	0.19	0.03	0.25
DL-Methionine	0.37	0.38	0.23	0.25
Salt	0.25	0.25	0.20	0.20
Vitamin-mineral premix[2]	0.30	0.30	0.20	0.20
Calculated analysis				
AME (MJ/kg)	12.27	12.37	12.91	13.00
Crude protein, %	22.1	22.60	20.3	20.30
Lysine, %	1.15	1.15	1.05	1.05
Methionine + Cysteine, %	0.94	0.94	0.76	0.76
Calcium, %	1.09	1.09	0.90	0.90
Total P, %	0.71	0.72	0.60	0.61
Phytate P, %	0.26	0.27	0.25	0.26
Non-phytate P, %	0.45	0.45	0.35	0.35

[1] Ground wheat; in experimental diets, 10 and 20% whole wheat replaced ground wheat post pelleting during 1-21 and 22-35 d, respectively.

[2] Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

Carcass Measurement

On day 35, two more birds (close to mean body weight of the pen) were fasted, weighed and then killed by cervical dislocation, followed by exsanguination. After the removal of feathers, viscera, shanks and neck, the weights of the eviscerated hot carcass, abdominal fat pad and breast muscle were measured for each bird.

Data Analysis

For bird performance data, pen means served as the experimental unit for statistical analysis. For digestive tract measurements and carcass characteristics, individual birds were considered as the experimental unit. All data were analysed using one-way ANOVA of the General Linear Models (GLM) procedure according to a completely randomised design (SAS, 1997). Effects of whole form (Diets 2, 3 vs 4, 5), xylanase (Diets 1, 3 vs 2, 4) and diet type (diet 1 vs 2, 3, 4, 5) were made using orthogonal contrasts. Multiple comparisons between dietary treatments were made using the Least Significant Difference (LSD) test. Significant differences were considered at $P<0.05$, although probability values up to $P<0.10$ are shown in the text if the data suggested a trend.

RESULTS

During the starter phase (1-21 d), birds fed diets containing 10% whole wheat reduced ($P<0.05$) feed intake, but had similar ($P>0.05$) weight gains and feed/gain compared to those fed diets containing ground wheat (Table 3). During the finisher phase (22-35 d), however, 20% whole wheat inclusion reduced ($P<0.05$ to 0.001) weight gains and feed intake, but improved ($P<0.01$) feed/gain compared to those fed diets containing ground wheat. In general, the trends observed for weight gains ($P=0.07$), feed intake ($P<0.001$) and feed/gain ($P<0.05$) over the 35-day experimental period were similar to those observed during the finisher phase.

During the starter phase (1-21 d), xylanase supplementation improved ($P<0.05$ to 0.001) weight gains and feed intake. These improvements were due largely to improvements in ground wheat diets supplemented with xylanase (Table 3). Xylanase supplementation lowered ($P<0.05$) feed/gain in both ground wheat and whole wheat

diets, but the differences were not significant ($P>0.05$) in ground wheat diets. Similar trends were observed for weight gains ($P<0.05$ to 0.01), feed intake ($P<0.05$) and feed/gain ($P<0.05$) during 22-35 d and over the 35-day experimental period. The only exception was that xylanase supplementation had no effect ($P>0.05$) on feed intake during 22-35 d.

Birds fed the corn-based diet grew faster and were more efficient in utilising feed than those fed wheat-based diets (Table 3). Feed/gain values of birds fed the corn-based diet were lower ($P<0.05$) than those fed wheat-based diets throughout the experiment. Addition of xylanase to ground wheat-based diets improved weight gains and feed intake to levels comparable ($P>0.05$) to those fed the corn-based diets. Birds ground wheat-based diets with added xylanase had comparable ($P>0.05$) feed/gain during the starter phase (1-21 d), but had higher ($P<0.05$) feed/gain during the finisher phase (22-35 d) as well as over the entire experimental period (1-35 d) compared to those fed the corn-based diet.

Compared to those fed the corn-based diet, birds fed diets containing whole wheat with supplemental xylanase had reduced ($P<0.05$) weight gains and feed intake throughout the experimental period (Table 3), but the feed/gain values were comparable ($P>0.05$).

Relative weights (g/100g body weight) of organs of broilers as influenced by dietary treatments are shown in Table 4. Whole wheat inclusion had no effect ($P>0.05$) on the relative weights of crop and proventriculus at 21 or 35 days of age, and that of pancreas and liver at 21 days of age, but increased ($P<0.05$ to 0.001) the relative weights of gizzard (12.5%) and heart at 21 days of age, and of gizzard (43.0%) and pancreas (9.1%) at 35 days of age compared to those fed diets containing ground wheat. Interestingly, whole wheat inclusion reduced ($P<0.05$ to 0.01) the relative weights of liver and heart at 35 days of age compared to those fed diets containing ground wheat.

Overall xylanase supplementation had no effect ($P>0.05$) on the relative weights of crop, proventriculus, gizzard, pancreas, liver and heart at 21 or 35 days of age. Xylanase supplementation reduced ($P<0.05$) the relative weights of proventriculus and pancreas in ground wheat diets at 21 days of age.

Table 3. Weight gain, feed intake and feed/gain of male broilers[1] as influenced by whole wheat inclusion and xylanase supplementation.

Diet	Treatments		Weight gain (g/bird)			Feed intake (g/bird)			Feed/gain (g/g)[4]		
	Diet Form[2]	Enzyme[3]	1-21 d	22-35 d	1-35 d	1-21 d	22-35 d	1-35 d	1-21 d	22-35 d	1-35 d
1	Corn-soy	-	806^{a}	1229^{a}	2035^{a}	1053^{a}	2080^{ab}	3133^{a}	1.331^{b}	1.693^{c}	1.600^{b}
2	GW-soy	-	697^{c}	1117^{b}	1814^{b}	966^{b}	2031^{b}	2998^{b}	1.393^{a}	1.821^{a}	1.686^{a}
3	GW-soy	+	786^{a}	1205^{a}	1991^{a}	1057^{a}	2132^{a}	3190^{a}	1.368^{ab}	1.770^{ab}	1.661^{a}
4	WW-soy	-	717^{bc}	1130^{bc}	1847^{b}	979^{b}	1964^{c}	2943^{b}	1.393^{a}	1.739^{bc}	1.648^{a}
5	WW-soy	+	738^{b}	1118^{bc}	1856^{b}	976^{b}	1876^{d}	2853^{c}	1.341^{b}	1.678^{c}	1.590^{b}
	Pooled SEM		13.3	15.5	24.5	14.0	19.2	19.5	0.0155	0.0217	0.0149
	LSD		40.0	46.8	73.8	42.1	57.8	58.7	0.0466	0.0653	0.0449
	Probabilities[5]										
	Contrast Diet 2, 3 vs 4, 5		NS	*	†	*	***	***	NS	**	**
	Contrast Diet 2, 4 vs 3, 5		***	*	**	**	NS	*	*	*	*
	Contrast Diet 1 vs others		***	***	***	**	**	***	*	*	*

[a,b] Means in a column with different superscripts differ ($P<0.05$).

[1] Each mean represents four pens of 46 birds each.

[2] GW, ground wheat; WW, whole wheat (10 and 20% replacing GW during day 1-21 and 22-35, respectively).

[3] 1000 XU/kg diet.

[4] Corrected for mortality

[5] NS, not significant; † $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 4. Relative weight (g/100g body weight) of organs (crop, proventriculus, gizzard, pancreas, liver and heart) of male broilers[1] at 21 and 35 days of age as influenced by whole wheat inclusion and xylanase supplementation.

Diet	Treatments		Crop		Proventriculus		Gizzard		Pancreas		Liver		Heart	
	Diet Form[2]	Enzyme[3]	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d
1	Corn-soy	-	0.35^{a}	0.33^{a}	0.47^{b}	0.30^{a}	1.84^{bc}	1.07^{c}	0.31^{c}	0.23^{a}	2.98^{b}	2.47^{b}	0.70^{a}	0.51^{ab}
2	GW-soy	-	0.37^{a}	0.38^{a}	0.50^{ab}	0.29^{a}	2.11^{ab}	0.99^{c}	0.40^{a}	0.22^{a}	3.25^{ab}	2.79^{a}	0.60^{b}	0.53^{a}
3	GW-soy	+	0.36^{a}	0.31^{a}	0.48^{ab}	0.29^{a}	1.57^{c}	1.01^{c}	0.33^{bc}	0.21^{a}	3.35^{ab}	2.62^{ab}	0.65^{ab}	0.51^{ab}
4	WW-soy	-	0.38^{a}	0.31^{a}	0.54^{a}	0.29^{a}	1.92^{ab}	1.35^{b}	0.38^{ab}	0.25^{a}	3.38^{a}	2.48^{b}	0.73^{a}	0.48^{b}
5	WW-soy	+	0.36^{a}	0.30^{a}	0.48^{b}	0.29^{a}	2.23^{a}	1.52^{a}	0.36^{abc}	0.24^{a}	3.28^{ab}	2.46^{b}	0.68^{ab}	0.49^{ab}
	Pooled SEM		0.021	0.030	0.022	0.012	0.109	0.058	0.022	0.012	0.141	0.080	0.030	0.016
	LSD		0.061	0.086	0.062	0.035	0.314	0.166	0.063	0.035	0.404	0.231	0.087	0.047
	Probabilities[4]													
	Contrast Diet 2, 3 vs 4, 5		NS	NS	NS	NS	*	***	NS	*	NS	**	*	*
	Contrast Diet 2, 4 vs 3, 5		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Contrast Diet 1 vs others		NS	NS	NS	NS	NS	*	*	NS	*	NS	NS	NS

[a,b] Means in a column with different superscripts differ ($P<0.05$).

[1] Each mean represents four pens of 46 birds each.

[2] GW, ground wheat; WW, whole wheat (10 and 20% replacing GW during day 1-21 and 22-35, respectively).

[3] 1000 XU/kg diet.

[4] NS, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Birds fed diets containing ground wheat or whole wheat with supplemental xylanase had relative weights of crop, proventriculus, gizzard pancreas, liver and heart, at 21 or 35 days of age comparable ($P>0.05$) to those fed the corn-soy diet (Table 4). The only exception was that the relative gizzard weights of birds fed the whole wheat diet with added xylanase were 21.1- 42.1% greater ($P<0.05$) than those fed the corn-based diet.

Relative weight (g/100g body weight) and length (cm/100g body weight) of the intestinal tract of male broilers by dietary treatments are shown in Tables 5 and 6, respectively. Whole wheat had no effect ($P>0.05$) on the relative weight and length of duodenum, jejunum, ileum, caeca and small intestine at 21 days or 35 days of age. The only exception was that whole wheat inclusion increased ($P<0.05$) the relative weight of jejunum at 35 days of age.

Xylanase supplementation had no effects ($P>0.05$) on the relative weight and length of the duodenum, jejunum, ileum, caeca and small intestine at 21 or 35 days of age. The exception was that xylanase supplementation reduced ($P<0.05$ to 0.01) the relative weights of duodenum and caeca, and the relative length of the duodenum, jejunum, ileum, caeca and small intestine at 21 days of age (Tables 5 and 6). Birds fed diets containing ground wheat or whole wheat with supplementation of xylanase had relative weight and length of the duodenum, jejunum, ileum, caeca and small intestine at 21 or 35 days of age comparable ($P>0.05$) to those fed the corn-soy diet, except that the relative weight and length of the jejunum and small intestine of birds fed a diet with combination of whole wheat and xylanase were greater ($P<0.05$) than those fed the corn-based diet.

Dietary treatments had no effects ($P>0.05$) on carcass recovery and breast muscle yield (Table 7). Neither whole wheat inclusion nor xylanase supplementation influenced ($P>0.05$) the weight of abdominal fat pad. Abdominal fat pad weights of birds fed ground wheat-based diets were 15% lower compared to those fed the corn-based diet, but the difference was not significant ($P>0.05$). Compared to those fed the corn-based diets, birds fed enzyme supplemented diets containing ground wheat or whole wheat had lower ($P<0.05$) relative weights of the abdominal fat pad.

Table 5. Relative weight (g/100g body weight) of the intestinal tract of male broilers[1] at 21 and 35 days of age as influenced by whole wheat inclusion and xylanase supplementation.

Diet	Treatments		Duodenum		Jejunum		Ileum		Caeca		Small intestine[4]	
	Diet Form[2]	Enzyme[3]	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d
1	Corn-soy	-	0.59^{b}	0.41^{a}	1.34^{b}	0.87^{b}	1.06^{b}	0.66^{b}	0.36^{b}	0.28^{ab}	2.98^{b}	1.93^{c}
2	GW-soy	-	0.72^{a}	0.40^{a}	1.58^{a}	0.93^{ab}	1.15^{ab}	0.68^{ab}	0.42^{ab}	0.27^{b}	3.44^{a}	2.01^{bc}
3	GW-soy	+	0.61^{b}	0.43^{a}	1.49^{ab}	0.92^{ab}	1.17^{ab}	0.79^{a}	0.37^{b}	0.30^{ab}	3.27^{ab}	2.13^{abc}
4	WW-soy	-	0.68^{ab}	0.45^{a}	1.55^{ab}	1.04^{a}	1.25^{a}	0.76^{ab}	0.47^{a}	0.32^{a}	3.48^{a}	2.25^{a}
5	WW-soy	+	0.59^{b}	0.46^{a}	1.36^{ab}	1.01^{a}	1.11^{ab}	0.73^{ab}	0.37^{b}	0.31^{ab}	3.06^{ab}	2.21^{ab}
	Pooled SEM		0.035	0.023	0.083	0.042	0.054	0.040	0.023	0.016	0.153	0.084
	LSD		0.101	0.066	0.239	0.120	0.156	0.116	0.067	0.045	0.438	0.242
	Probabilities[5]											
	Contrast Diet 2, 3 vs 4, 5		NS	NS	NS	*	NS	NS	NS	NS	NS	NS
	Contrast Diet 2, 4 vs 3, 5		**	NS	NS	NS	NS	NS	**	NS	NS	NS
	Contrast Diet 1 vs others		NS	NS	NS	*	NS	NS	NS	NS	NS	*

[a,b] Means in a column with different superscripts differ ($P<0.05$).

[1] Each mean represents 8 observations .

[2] GW, ground wheat; WW, whole wheat (10 and 20% replacing GW during 1-21 d and 22-35 d, respectively).

[3] 1000 XU/kg diet.

[4] Small intestine = duodenum + jejunum + ileum.

[5] NS, not significant; * $P<0.05$; ** $P<0.01$.

Table 6. Relative length (cm/100g body weight) of the intestinal tract of male broilers[1] at 21 and 35 days of age as influenced by whole wheat inclusion and xylanase supplementation

Diet	Treatments		Duodenum		Jejunum		Ileum		Caeca		Small intestine[4]	
	Diet Form[2]	Enzyme[3]	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d
1	Corn-soy	-	2.85^{c}	1.46^{a}	7.37^{b}	3.55^{b}	7.00^{c}	3.46^{b}	1.49^{c}	0.85^{a}	17.22^{c}	8.47^{b}
2	GW-soy	-	3.41^{a}	1.50^{a}	8.42^{a}	3.82^{ab}	7.93^{ab}	3.68^{ab}	1.75^{ab}	0.90^{a}	19.75^{ab}	9.00^{ab}
3	GW-soy	+	3.00^{bc}	1.50^{a}	7.79^{ab}	3.62^{ab}	7.52^{abc}	3.60^{ab}	1.56^{bc}	0.91^{a}	18.31^{abc}	8.72^{ab}
4	WW-soy	-	3.28^{ab}	1.58^{a}	8.39^{a}	3.91^{ab}	8.39^{a}	3.89^{a}	1.78^{a}	0.89^{a}	20.06^{a}	9.39^{a}
5	WW-soy	+	3.03^{bc}	1.55^{a}	7.59^{ab}	3.99^{a}	7.44^{bc}	3.83^{ab}	1.51^{c}	0.87^{a}	18.05^{bc}	9.36^{a}
	Pooled SEM		0.123	0.050	0.332	0.129	0.305	0.146	0.073	0.029	0.691	0.283
	LSD		0.353	0.142	0.953	0.370	0.877	0.418	0.211	0.083	1.984	0.813
	Probabilities[5]											
	Contrast Diet 2, 3 vs 4, 5		NS	NS	NS	NS	NS	NS	NS	NS	*	NS
	Contrast Diet 2, 4 vs 3, 5		*	NS	*	NS	*	NS	**	NS	NS	NS
	Contrast Diet 1 vs others		*	NS	NS	0.06	*	0.08	NS	NS	*	0.05

[a,b] Means in a column with different superscripts differ ($P<0.05$).

[1] Each mean represents 8 observations for each treatment.

[2] GW, ground wheat; WW, whole wheat (10 and 20% replacing GW during 1-21 d and 22-35 d, respectively).

[3] 1000 XU/kg diet.

[4] Small intestine = duodenum +jejunum + ileum.

[5] NS, not significant; * $P<0.05$; ** $P<0.01$.

Table 7. Carcass characteristics (g/100g body weight) of male broilers at 35 days of age[1] as influenced by whole wheat inclusion and xylanase supplementation.

Diet	Treatments		Carcass recovery	Breast muscle	Abdominal fat pad
	Diet Form[2]	Enzyme[3]			
1	Corn-soy	-	70.0[a]	19.2[a]	1.20[a]
2	GW-soy	-	69.8[a]	19.8[a]	1.02[ab]
3	GW-soy	+	69.8[a]	20.0[a]	0.97[b]
4	WW-soy	-	69.7[a]	19.8[a]	0.83[b]
5	WW-soy	+	69.2[a]	19.4[a]	0.93[b]
	Pooled SEM		0.41	0.44	0.081
	LSD		1.78	1.26	0.233
	Probabilities[4]				
	Contrast Diet 2, 3 vs 4, 5		NS	NS	NS
	Contrast Diet 2, 4 vs 3, 5		NS	NS	NS
	Contrast Diet 1 vs others		NS	NS	**

[a,b] Means in a column with different superscripts differ ($P<0.05$) and multiple comparisons were made using LSD's test.

[1] Each mean represents 8 observations.

[2] GW, ground wheat; WW, whole wheat (10 and 20% replacing GW during 1-21 d and 22-35 d, respectively).

[3] 1000 XU/kg diet.

[4] NS, not significant; ** $P<0.01$.

DISCUSSION

The primary aim of the present study was to examine the effects of whole wheat inclusion and xylanase supplementation in broilers fed wheat-based diets. During the starter phase (1-21 d), post-pelleting inclusion of 10% whole wheat reduced feed intake by 3.4%, but had no effect on weight gains and feed/gain compared to those fed diets containing ground wheat. These results are in disagreement with those reported in Chapter 9, which demonstrated the beneficial effects of inclusion of 20% whole wheat in broilers housed in cages to 21 days of age. The study reported in Chapter 9 showed that post-pelleting inclusion of 20% whole wheat improved weight gains and feed efficiency by 2.1 and 5.8%, respectively. Two reasons may be responsible for the observed discrepancy. One may be the reduction in inclusion levels of whole wheat from 20 to 10%. The other relates to the possible consumption of wood shavings in floor-reared birds, which may have stimulated the development of gizzard of all birds.

Examination of gizzard contents at 21 d of age revealed the presence of wood shavings in most birds.

During the finisher phase (22-35 d) and over the whole experimental period (1-35 d), whole wheat inclusion reduced weight gains, but improved feed efficiency, compared to those fed diets containing ground wheat. Improved feed efficiency with whole wheat inclusion was due largely to a reduction in feed intake. These results are in general agreement with several reports (Uddin *et al.*, 1996; Hetland *et al.*, 2002), but in disagreement with others (Taylor and Jones, 2001; Bennett *et al.*, 2002). Similar to the present results, Uddin *et al.* (1996) reported that weight gains of birds fed diets containing 15-30% of whole wheat (15 and 30% during 24-33 d and 33-42 d, respectively) was reduced by 2.4%, but feed efficiency was improved by 2.1% compared to those fed diets containing ground wheat. Hetland *et al.* (2002) reported that weight gains of birds fed diets containing moderate to high levels of whole wheat (12.5-33 and 30-44% during 10-24 and 25-38 d, respectively) were numerically reduced, but feed efficiency was significantly improved by 5.1-6.7% over the experimental period (10-38 d) compared to those fed diets containing ground wheat. Relative gizzard weights of birds fed diets containing whole wheat at 24 and 38 d of age were increased by 56-86% and 36-100%, respectively. Taylor and Jones (2001), however, reported that weight gains and feed efficiency of birds were not influenced by inclusion of 20% whole wheat in wheat-based diets. Relative gizzard weights of birds fed diets containing 20% whole wheat were 7.8-10.7% higher compared to those fed diets containing ground wheat. Bennett *et al.* (2002) found that the feed efficiency of broilers fed diets containing whole wheat during the starter phase (1-26 d), was reduced by the inclusion of 20% whole wheat compared to those fed diets containing ground wheat. Feed efficiency was not affected during the finisher (27-48 d) phase even with the inclusion levels of 35-50% whole wheat.

In the present study, xylanase supplementation improved feed efficiency of birds fed ground wheat and whole wheat diets, although differences were not significant in ground wheat diets over the 35-day experimental period. These results are in general consistent with the study reported in Chapters 6, 8 and 9 that the use of supplemental xylanase in wheat-based diets for broilers is beneficial. The effects of xylanase supplementation in improving the performance of birds fed wheat-based diets are well documented (Annison and Choct, 1991; Bedford and Schulz, 1998) and have been previously discussed.

As expected, birds fed unsupplemented ground wheat-based diets had the lowest weight gains and highest feed per gain value. Birds fed corn-based diets grew faster and were more efficient in utilising feed compared to those fed ground wheat-based diets. Addition of xylanase to ground wheat-based diets improved weight gains and feed intake to levels comparable to corn-based diets, but feed efficiency remained poorer, although the feed efficiency of birds fed ground wheat-based diets with added xylanase during the starter phase (1-21 d) was not significant from those fed corn-based diets. These results, in general, show that bird performance could be improved to levels comparable to those fed corn-based diets when exogenous xylanase are added to wheat-based diets. Similarly, Marquardt *et al.* (1994) found that the addition of an enzyme preparation with predominant xylanase activity to wheat-based diets improved broiler performance to levels comparable to those fed corn-based diets.

Published data on the effects of combining whole wheat and xylanase in broiler diets are limited. Xylanase supplementation was found to be more effective in improving feed efficiency in the whole wheat diet compared to the ground wheat diet. The apparent additivity of the effects of whole wheat and xylanase suggests that the mechanisms involved are different and could be further exploited to improve feed efficiency. Birds fed diets with whole wheat and supplemental xylanase had feed/gain comparable to those fed corn-based diets throughout the experimental period (1.341 vs 1.331, 1.678 vs 1.693, and 1.590 vs 1.600 during 1-21, 22-35 and 1-35 d, respectively).

In the present study, improvements in feed efficiency in birds given whole wheat paralleled improvements in relative gizzard weights. Feed efficiency of birds fed diets containing whole wheat were improved, compared to those fed diets containing ground wheat. Relative weight of the gizzard at 21 and 35 days of age were increased by 12.8 and 43.6%, respectively. These results are consistent with those reported in Chapter 9 and previous published data (Preston *et al.*, 2000; Plavnik *et al.*, 2002). Preston *et al.* (2000) reported that there were numerical improvements (3%) in feed efficiency and 50% greater relative gizzard weights in birds fed 33% whole wheat compared to those birds fed diets containing ground wheat. Similarly, Plavnik *et al.* (2002) found that the inclusion of whole wheat (10-20% replacing ground wheat) in corn-wheat-soy diets lowered feed intake and improved feed efficiency by 5.7-5.8%. Whole wheat inclusion caused numerical improvements (10%) in gizzard weights. Bennett *et al.* (2002) found that the feed efficiency of broilers fed diets containing the whole grain, during the starter phase (1-26 d), was reduced by the inclusion of 20% of whole wheat compared to

those fed diets containing ground wheat. Feed efficiency, however, was not affected during the finisher (27-48 d) phase even with the inclusion of 35-50% of whole wheat. Inclusion of whole wheat increased gizzard weights by 18-37%.

In general, whole wheat inclusion had no effects on the relative weights and length of duodenum, jejunum, ileum, caeca and small intestine. Whole wheat inclusion also had no effect on the relative weight of the crop and proventriculus, but decreased the relative weight of the liver and heart at 35 days of age. These results were in general agreement with the data reported in Chapter 9 and other published reports (Preston *et al.*, 2000; Taylor and Jones, 2001; Banfield *et al.*, 2002). The significance of the observed reductions in the weight of the heart and liver with whole wheat inclusion is unclear.

Whole wheat inclusion increased relative pancreas weights at 35 days of age. The results from the study reported in Chapter 9 also showed that 20% whole wheat inclusion increased relative pancreas weights by 20%. These results would suggest increased pancreatic activity and since the increases in pancreatic weights are associated with improved feed efficiency, and it is tempting to speculate that this may reflect increased secretion of digestive enzymes leading to improved digestibility of nutrients.

Xylanase supplementation reduced the relative weight of the proventriculus and pancreas in ground wheat-based diets. These results are consistent with the 21-day study reported in Chapter 9 and the reports of Brenes *et al.* (1993). The study reported in Chapter 9 showed that xylanase supplementation reduced the relative pancreas weight by 10.3%. Brenes *et al.* (1993) also reported that the relative pancreas weight was reduced by 16% with addition of xylanase. Xylanase supplementation had no effect on the relative weight and length of the small intestine at 35 days of age. These results are consistent with data reported in Chapter 9, but in disagreement with the data reported in Chapter 8. The results reported in Chapter 8 showed that the addition of xylanase to ground wheat-based diets reduced the relative weight and length of small intestine by 15.5 and 16.5%, respectively.

Whole wheat inclusion had no effects on carcass recovery, and the relative weight of breast muscle and abdominal fat pad. Published data on the effects of whole wheat on carcass measurements is contradictory. Bennett *et al.* (2002) reported that whole wheat inclusion (5, 20 and 35-65% whole wheat during 0-6, 6-13, 27-48 d) in wheat-barley-based diets had no effect on carcass yield and abdominal fat pad weights of broilers. Plavnik *et al.* (2002) similarly reported that whole wheat inclusion (20%

whole wheat replacing ground wheat during 1-28 and 29-49 d) in corn-wheat-based diets had no effect on the relative weight of abdominal fat pad compared to those fed diets containing ground wheat. In a subsequent trial, however, inclusion of whole wheat (25% whole wheat during 1-21 and 22-45 d) in broilers fed corn-wheat-based diets significantly increased the relative weight of the abdominal fat pad (from 1.57 to 1.77 g/100g body weight) and decreased breast meat (from 1.51 to 1.42 g/100g body weight), compared to those fed diets containing ground wheat. Preston *et al.* (2000) also reported that the abdominal fat pad of birds fed diets containing 33% whole wheat was increased by 5.3% (from 1.89 to 1.99 g/100g body weight), compared to those fed diets containing ground wheat. Nahas and Lefrancois (2001) reported that whole wheat inclusion (10 and 20% whole wheat replacing ground wheat during 7-21 and 21-38 d, respectively) increased the relative weight of the abdominal fat pad (from 2.21 to 2.64 g/100g body weight) in birds fed corn-wheat-based diets.

Xylanase supplementation had no effect on carcass recovery, breast meat, and abdominal fat pad, irrespective of the wheat form used. Brenes *et al.* (1993) also reported that the addition of xylanase to wheat-based dies had no effect on abdominal fat pad in broilers.

CONCLUSIONS

In this floor pen trial, post-pelleting inclusion of whole wheat inclusion reduced weight gains, but improved feed efficiency in broilers fed wheat-based diets. Improved feed efficiency by whole wheat inclusion was associated with the development of gizzard and a reduced feed intake. Xylanase supplementation improved the performance of broilers in both ground wheat and whole wheat diets. Feed efficiency of birds fed diets with whole wheat and supplemental xylanase were comparable to those fed the corn-based diet. Neither whole wheat inclusion nor xylanase supplementation influenced carcass recovery, breast muscle or the relative weight of abdominal fat pad.

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Chapter 11

GENERAL DISCUSSION

Microbial phytase has become popular in recent years due to its ability to release phytate-bound phosphorus (P) and to reduce P output in the manure, which is a major problem in many parts of the world. Of the two types of phytase products available in the market, the microbial phytases produced by submerged liquid fermentation have been the subject of numerous investigations over the past decade. The effectiveness of these phytases in releasing phytate-bound nutrients (P, protein/amino acids and minerals) in diets based on a range of plant-derived feedstuffs for poultry is well documented (Chapter 2). Another type of microbial phytase, produced by solid state fermentation, has recently become available. Because of the fermentation technology employed, this phytase contains side activities of several other enzymes (including protease, β-glucanase, xylanase, fungal cellulase, amylase and gluco-amylase). Limited data, however, are available on the efficacy of microbial phytase produced by solid state fermentation in poultry diets. The presence of multiple enzyme activities may enhance the efficacy of this type of phytase and this hypothesis formed the major focus of this thesis.

Five studies were conducted to examine the effects of microbial phytase produced by the solid state fermentation on the performance, apparent ileal digestibility and utilisation of nutrients in broilers. Bird performance and toe ash data from the first two studies (Chapters 3 and 5) showed that the optimum dose of microbial phytase produced by solid state fermentation is 500 PU/kg diet. This finding compares closely with the recommendation for microbial phytase produced by submerged liquid fermentation (Coelho and Kornegay, 1999). However, it should be noted that the lowest dose tested in our studies was 500 PU/kg diet and it is possible that the optimum dose may have been lower than the 500 PU/kg diet. Influence of dose levels lower than 500 PU/kg diet need to be evaluated in future studies.

Toe ash and ileal phytate P degradation data from the current work (Chapters 3 and 5) provided direct evidence for the effectiveness of microbial phytase in releasing phytate-bound P and improving P bioavailability in wheat- or corn-based diets for broilers. The data reported in Chapter 6 showed that the addition of microbial phytase

improved P availability in sorghum or barley. The apparent ileal P digestibility coefficient of sorghum was increased by 42.8% (from 0.362 to 0.517) and that of barley by 22% (from 0.610 to 0.745) with supplemental phytase. The data from the *in vitro* study (Chapter 7) demonstrated that the phytase produced by the solid state fermentation released more phytate-bound P (11.0% and 7.8% in wheat- and corn-based diets, respectively) than a phytase preparation produced by submerged liquid fermentation. These data provide evidence that microbial phytase produced by solid state fermentation is as effective or better than microbial phytase produced by submerged liquid fermentation in releasing phytate-bound P in broiler diets.

Phosphorus is an essential mineral to support adequate skeletal development of rapidly growing birds. For this reason, it has become a common practice to provide an adequate margin of safety for this nutrient in broiler diets. The toe ash data from the work reported in Chapters 3 and 5 suggest that P requirements for broiler starters and finishers may be lower than those recommended (0.45 and 0.35% non-phytate P, respectively) by the NRC (1994). Lowering the safety margins for P in practical formulations may, therefore, provide an alternative approach to lower P pollution by minimising P contents in the manure. Toe ash content in males and females was maximised by the addition of 0.06% inorganic phosphate to diets containing 0.30 and 0.20% non-phytate P during the starter and finisher phase, respectively. Toe ash content of males fed diets containing four dietary levels of non-phytate P (0.30, 0.36, 0.42 and 0.48%, and 0.20, 0.26, 0.32 and 0.38%, during the starter and finisher phase, respectively) was 9.68, 10.57, 10.67 and 10.69%. Corresponding values in the females were 9.71, 10.35, 11.23 and 11.16%, respectively. These data suggest that P required for bone mineralisation was around 0.36 and 0.26% for broiler starters and finishers, respectively. These findings are consistent with those of Waldroup *et al.* (2000) who, based on weight gain, feed conversion ratio, and tibia ash response criteria, estimated non-phytate P requirements of broilers starters to be 0.32-0.34, 0.18 and 0.39%, respectively. In contrast, using tibia ash response criteria, Yan *et al.* (2001) reported the non-phytate P requirements for broiler finishers (22-42 days) to be 0.33%, which was in close agreement with the NRC (1994) recommendations for broiler finishers.

The addition of microbial phytase produced by solid state fermentation was found to be effective in lowering P output in manure of broilers fed diets based on wheat or corn (Chapters 3 and 5). Compared to adequate-P diets, excreta P levels in

birds fed low-P wheat- and corn-based diets containing 500 PU/kg phytase produced by solid state fermentation were lowered by 35%. Similar reductions with the addition of phytase produced by submerged liquid fermentation have been previously reported in corn-based diets (Kornegay, 1999; Waldroup *et al.*, 2000; Yan *et al.*, 2000). Kornegay (1999) summarised the data from 23 experiments and generated equations for the calculation of percentage reduction in P excretion with the addition of phytase. Based on these equations, it was demonstrated that phosphorus excretion can be reduced by 32%, when 500 FTU phytase/kg was added to a low P diet compared to an adequate-P diet. Waldroup *et al.* (2000) recorded 28% reductions in excreta P contents (from 1.21 to 0.87%), compared to those in the adequate-P diets (0.45% non-phytate P), when 800 FTU/kg phytase was added to corn-based starter diets. Yan *et al.* (2000) similarly reported that the addition of 1000 FTU/kg phytase to low-P (0.30% non-phytate P) corn-based diets reduced the excreta P contents at 21 days of age by 36% compared to adequate-P (0.45% non-phytate P) diets. Our work also showed that excreta nitrogen contents of birds fed 500 PU/kg phytase-supplemented low-P diets were reduced by 1.6 and 0.9-6.4% for corn- and wheat-based diets, respectively, compared to adequate-P diets (Chapters 3 and 5).

Inorganic phosphates are the most expensive mineral sources used in poultry diets. The phosphorus replacement value for microbial phytase is therefore commercially relevant and of interest. Widely ranging P replacement values have been reported for phytase produced by submerged liquid fermentation depending on the response criteria used, and dietary Ca and P levels (See Chapter 2). Depending on the response criteria used, around 550-1000 and 250-300 FTU phytase/kg diet are required to replace 1 g of phosphorus in form of defluorinated phosphate or mono-calcium phosphate for broilers and laying hens, respectively. Interestingly, all values have been generated with corn-soy diets and no estimates are available for poultry fed wheat-based diets.

Phosphorus replacement values for microbial phytase produced by solid state fermentation in broilers fed wheat-based diets have been reported in Chapter 3. Equations based on performance responses, obtained with supplemental non-phytate P and phytase produced by submerged liquid fermentation, have been previously used to calculate the equivalency value of phytase for inorganic P (Kornegay *et al.*, 1996; Yi *et al.*, 1996a). Application of these assumptions and methodology to phytase produced by solid state fermentation, however, produced apparently spurious results. Non-phytate P

equivalency values of the 0.30% non-phytate P diet with 500 PU/kg diet during the starter phase for male and female broilers were calculated to be 0.54 and 0.53%, respectively. Corresponding non-phytate P equivalency values during the finisher phase were 0.42 and 0.39%, respectively. These estimates translate into P release values of 72 to 131% from phytate. Possible reasons for the spurious P replacement values observed with phytase product have been discussed in Chapter 3. Overall, the data suggest that the use of weight gain response may not be appropriate to estimate P replacement values for microbial phytase with multiple enzyme activities, as part of the weight gain responses may have been due to the action of other enzymes. On the other hand, bone mineralisation criteria such as tibia ash and toe ash are more appropriate to estimate P replacement values, since any improvement in these parameters can only occur as a result of P released. These criteria have been reported to be sensitive response criteria for assessing the influence of phytase on P utilisation in broilers (Ravindran *et al.*, 1995; Kornegay *et al.*, 1996; Yi *et al.*, 1996a). However, for these criteria to be accurate and sensitive, a low-P basal diet must be fed. Also the birds need to have limited P stores at the start of the evaluation or the birds to need to be growing rapidly so that the P requirements would be high (Kornegay and Yi, 1999).

Phytic acid is considered primarily as a factor limiting P availability from plant-derived feedstuffs, but current evidence show that the deleterious effects of phytic acid in the nutrition of poultry go much beyond just limiting P availability (Ravindran, 2001). The effects of microbial phytase on nutrients other than P have attracted interest in recent years due to the associated economic benefits. In its native state, phytate is also complexed with various cations, protein, lipids (Cosgrove, 1966) and starch (Thompson and Yoon, 1984). Supplementation of diets with phytase produced by submerged liquid fermentation have been shown to cause protein responses in poultry diets (Yi *et al.*, 1996b, Sebastian *et al.*, 1997; Camden *et al.*, 2001; Ravindran *et al.*, 1999b, 2000, 2001) by releasing the phytate-bound protein/amino acids and improving their utilisation. The effects of microbial phytase on protein utilisation have been reviewed in Chapter 2 of this thesis and in two recent reports (Selle *et al.*, 2000; Kies *et al.*, 2001). In general, improvements in apparent ileal nitrogen digestibility with addition of microbial phytase in poultry diets are variable. Review of literature showed that the addition of 400-1200 FTU/kg microbial phytase produced by submerged liquid fermentation increased the apparent ileal nitrogen digestibility by 2.0-4.0% of broilers depending on dietary level of non-phytate P and diet type (Chapter 2).

The protein effects of microbial phytase produced by solid state fermentation were examined in this thesis (Chapters 3 and 5-8). The results showed that the responses on the apparent ileal nitrogen digestibility of broilers fed diets based on wheat or corn were inconsistent. The addition of 500 PU/kg phytase to low-P (0.30% non-phytate P) diets increased the ileal nitrogen digestibility of broilers fed wheat-based diets by 3.1-5.3% (Chapter 3). In a subsequent study, however, addition of 1000 PU/kg diet phytase caused only numerical improvements (2.2%; from 0.757 to 0.772) in apparent ileal nitrogen digestibility of wheat (Chapter 6). The data reported in Chapter 3 showed that the addition of 500 PU/kg diet phytase to low-P corn-based diets improved the apparent ileal nitrogen digestibility by 2.1% (from 0.809 to 0.826). In the studies reported in Chapter 6, phytase supplementation had no effect on the apparent ileal nitrogen digestibility of corn. Inconsistent protein responses have also been reported in studies conducted with microbial phytase produced by submerged liquid fermentation in broilers. While most reports have shown positive responses (Yi *et al.*, 1996b; Namkung and Leeson, 1999; Ravindran *et al.*, 1999b; Camden *et al.*, 2001), some have reported no effects (Sebastian *et al.*, 1997; Zhang *et al.*, 1999). Inconsistent responses with the addition of microbial phytase observed with both types of phytase may be related to the source and concentration of phytate, dietary protein levels, the inherent digestibility of the protein component, Ca and P levels and phytase inclusion rate (Selle *et al.*, 2000). Clearly, studies were warranted to identify factors causing these variable responses. The basis for the protein digestibility responses with phytase addition are not well understood, but appear to be related to the capacity of phytic acid to bind protein/amino acids and to the ability of the enzyme to release these bound nutrients by hydrolysing phytic acid (Selle *et al.*, 2000). In the case of the phytase product evaluated in this thesis, it is also likely that part of the improvements in protein digestion may have been caused by the presence of side enzyme activities, especially proteases.

The energy effects of microbial phytase, especially in wheat-based diets, are also being increasingly recognised. Supplemental microbial phytase produced by submerged liquid fermentation has been shown to improve the AME in poultry diets based on wheat (Ravindran *et al.*, 2000), corn (Ledoux *et al.*, 1999; Namkung and Leeson, 1999), sorghum (Farrell *et al.*, 1992; Selle *et al.*, 1999), and wheat-sorghum (Ravindran *et al*, 2001). Overall, the addition of microbial phytase produced by submerged liquid fermentation to poultry diets increases the AME by 1.1-6.3% depending on dietary level

of non-phytate P and diet type (Chapter 2). In the studies reported in this thesis, however, inconsistent energy responses by the addition of microbial phytase were observed. In the studies reported in Chapters 3 and 5, the addition of 500 PU/kg phytase produced by solid state fermentation to low-P diets improved the AME of wheat or wheat-based diets by 1.4-6.7%, but had no effect in the studies reported in Chapters 6 and 8. Similarly, the addition of phytase improved the AME of corn by 2.6% (Chapter 8) and had no effect on the AME of broilers fed diets based on corn (Chapter 5). In general, the magnitude of responses with improvements in the AME of wheat-based diets with addition of phytase produced by solid state fermentation, appear to be higher than those reported with microbial phytase produced by submerged liquid fermentation. These were confirmed by the results of the *in vitro* study reported in Chapter 7, wherein the phytase produced by solid state fermentation was found to release 2.9% more reducing sugars in wheat-based diets compared to those produced by submerged liquid fermentation. Data from this thesis has shown that inclusion of microbial phytase would permit formulation of commercial diets with reduced levels of not only P, but also of energy and protein. Energy and protein replacement values, however, need to be evaluated in various types of practical diets for poultry.

Possible mechanisms contributing to improvements in AME with supplemental phytase have been discussed (Chapter 2). The work reported in Chapter 3 and 5 and data from studies conducted with microbial phytase produced by submerged liquid fermentation (Coelho and Kornegay, 1999) support the thesis that improvements in AME are associated with improved protein digestibility. The work reported in Chapter 6, however, showed that improvements in AME with supplemental phytase are not always associated with enhanced digestibility of protein and starch. Other factors are clearly involved and these need to be elucidated in future studies.

A piece of significant work reported in this thesis was the comparison of AME, apparent ileal nitrogen digestibility and nutrient utilisation in male and female broilers fed diets containing low and adequate dietary levels of P (Chapter 4). The data showed that AME values were similar between sexes at 21 days of age, but favoured the males at 42 days of age. Hughes *et al.* (2000), however, reported that the AME of wheat-based diets in male broilers were lower compared to the females (15.15 vs 15.32 MJ/kg dry matter). Hughes (2001) examined the effect of sex on the variability of responses in energy utilisation in broilers given a wheat-based diet containing high concentrations of soluble non-starch polysaccharide and found that the AME in males were lower than the

females (14.2 vs 14.6 MJ/kg dry matter). The lower energy utilisation by the males was due largely to a higher degree of variability in the males, with a relatively large proportion of males showing a poor capacity for energy uptake. Further examination of these data, using covariance analysis on the relationship between endogenous energy losses and gross energy intake, revealed that there are fundamental differences between individual males and females in their digestive physiology with males having higher endogenous energy losses than the females (Hughes, 2003). The data reported in Chapter 4 also showed that the ileal nitrogen digestibility tended to favour the females. These results are consistent with those of Ten Doeschate *et al.* (1993) who found that female broilers showed nitrogen digestibility coefficients that were, in general, 3% higher than those of male birds. Wallis and Balnave (1984), however, reported that amino acid digestibility was not influenced by the gender of broilers. The present data, when considered along with previous published data (See Chapter 4), suggest that the effect of gender on energy utilisation and the apparent ileal nitrogen digestibility in broiler chickens is inconsistent and inconclusive.

The presence of 'side enzyme activities' in phytase preparations may impact on bird responses to supplemental phytase. Evidence for the potential beneficial effects of the side activities present in microbial phytase produced by solid state fermentation were first demonstrated by the feed efficiency data reported in Chapter 3. Over the 42-d trial period, feed efficiency of broilers fed phytase-supplemented low-P diets were consistently superior to those fed diets with adequate level of P. Data reported in Chapters 6 and 8 further confirm the potential benefits of side activities of enzymes, especially of glycanases, present in this phytase product. The finding that the addition of phytase improved apparent ileal digestibility of nitrogen and starch digestibility in barley by 10.4 and 5.8%, respectively, may be indicative of the activity of β-glucanase (Chapter 6). The findings in Chapter 8 that the phytase produced by solid state fermentation was as effective as exogenous xylanase in improving performance of broilers fed wheat-based diets containing adequate P levels, was noteworthy. The toe ash data from this study suggests that the observed performance responses with supplemental phytase are not related to P effects, but likely to reflect the effects on nutrients other than P.

Potential beneficial effects of other enzymes present in microbial phytase produced by solid state fermentation on protein and energy was also confirmed by the results from the *in vitro* study wherein the release of reducing sugars and α-amino

nitrogen by the phytase evaluated were compared with a phytase produced submerged liquid fermentation with no detectable side activity in wheat- and corn-based diets (Chapter 7). Phytase produced by solid state fermentation released more α-amino nitrogen (1.7 and 6.2% for wheat- and corn-based diets, respectively) than the phytase produced by submerged liquid fermentation. Phytase produced by solid state fermentation also released 2.9% more reducing sugars in the wheat-based diet. These *in vitro* results, however, need to be confirmed in *in vivo* studies. In a recent study (Murai *et al.*, 2002), it was observed that the addition of a microbial phytase with side enzyme activities improved final body weight, feed efficiency and metabolisable energy (from 15.3 to 15.6 MJ/kg diet) in broilers given a barley-based diet, compared to those fed diets with a phytase product with no detectable side activities.

To the author's knowledge, the effect of microbial phytase on gut morphology of broilers fed wheat-based diets has not previously investigated. Interestingly, the addition of phytase increased villus height in the duodenum and decreased the number of goblet cells in the jejunum compared to those in the unsupplemented basal diet. Phytase supplementation had no effect on crypt depth, epithelial thickness, and the ratio of crypt depth to villus height in the duodenum, jejunum and ileum. The observed effects may be due, partly, the presence of glycanases in this phytase product.

Whole grain feeding for broilers has attracted attention in recent years due to the associated economic benefits. Published data on the effects of whole wheat feeding in broilers are, however, contradictory (Chapter 2). Conflicting results were also found in the two studies reported in the present work (Chapters 9 and 10). The first study reported in Chapter 9 demonstrated that there are beneficial effects of 20% whole wheat inclusion in broilers housed in cages and fed wheat-based diets to 21 days of age. However, the second study (Chapter 10), conducted with broilers reared on floor pen with wood shavings litter, failed to reproduce the results. The study reported in Chapter 10 showed that during the starter phase (1-21 d), birds fed diets containing 10% whole wheat had comparable weight gains and feed efficiency, compared to those fed diets containing ground wheat. During the finisher phase (22-35 d) and over the entire experimental period (1-35 d), whole wheat inclusion significantly reduced weight gains by 3.2 and 2.7%, respectively, but improved feed efficiency by 4.8 and 3.3%, respectively, compared to those fed diets containing ground wheat. Two explanations may be given for the observed discrepancy between the two studies. First, the reduction in inclusion levels of whole wheat from 20 to 10% during the starter phase. The other

may be the consumption of litter (wood shavings) by the floor-reared birds, which may have stimulated the development of the gizzard in all birds. Presence of wood shavings was consistently observed in the gizzard contents of most birds.

In most published reports, whole wheat has been included after the pelleting of diets. Only one report (Taylor and Jones, 2001) has examined the effects of whole wheat inclusion prior to pelleting on the performance of broilers fed wheat-based diets. No studies have compared the influence of the method of whole wheat inclusion (pre- or post-pelleting) on the performance of broilers fed wheat-based diets. A finding from the work reported in Chapter 9 was that post-pelleting inclusion of whole wheat resulted in greater improvements in feed efficiency, AME and relative weights of gizzard of broilers compared to pre-pelleting inclusion of whole wheat. The two whole wheat studies consistently demonstrated that post-pelleting inclusion of whole wheat improved feed efficiency. These improvements were associated with reductions in feed intake and improvements in relative gizzard weights resulting in greater improvements in AME. The study reported in Chapter 9 showed that post-pelleting inclusion of whole wheat improved weight gains (3.9%), feed efficiency (5.8%) and AME (6.0%) compared to those birds fed diets containing ground wheat. The relative gizzard weights of birds fed diets with 20% post-pelleting inclusion of whole wheat were 73% higher compared to those fed diets containing ground wheat. In the second study, post-pelleting inclusion of whole wheat improved feed efficiency and relative gizzard weights over the 35-day experimental period by 3.8 and 44%, respectively. Two recent reports (Svihus and Hetland, 2001; Hetland *et al.*, 2002) showed that ileal starch digestibility of birds fed diet containing whole wheat was significantly improved compared to those fed diets containing ground wheat. The relative gizzard weight and AME data from the present work, along with the published data on ileal starch digestibility, may explain the beneficial effects of whole wheat feeding. The improvements observed in performance with pre-pelleting inclusion were, however, not associated with gizzard development.

The effects of xylanase supplementation in improving the performance and the AME of broilers fed wheat-based diets are well documented (Annison and Choct, 1991; Bedford and Schulz, 1998). Overall, the responses to dietary supplementation with xylanase are variable and factors affecting enzyme response in wheat-based diets include type of enzyme, wheat quality and, breed and age of birds (Bedford, 1997). The major mode of action appears to involve degradation of non-starch polysaccharides in

the cell wall matrix (Bedford and Schulze, 1998), thereby releasing the nutrients encapsulated within the cell and lowering digesta viscosity. The principal factor controlling the magnitude of response to enzymes is intestinal viscosity especially when the viscosity is high (Bedford, 1997; Bedford and Schulze, 1998). The work reported in this thesis (Chapter 6, 8-10) demonstrated that xylanase supplementation improved the performance of broilers fed diets based on wheat. Improved bird performance by xylanase supplementation was associated with reduced digesta viscosity and improved AME. The observed effects of xylanase supplementation in reducing the relative weight and length of the small intestine (See Chapter 8) may be a reflection of reduced digesta viscosity.

Published data on the effects of combining whole wheat and xylanase in broiler diets are limited. The data reported in Chapter 10 showed that dietary xylanase supplementation was more effective in improving feed efficiency in whole wheat diets compared to ground wheat diets. The 'apparent additivity' of the combination of whole wheat and xylanase suggests that the mechanisms involved are different and could be further exploited. Birds fed diets with whole wheat and supplemental xylanase had feed/gain comparable to those fed corn-based diets throughout the experimental period (1.341 vs 1.331, 1.678 vs 1.693, and 1.590 vs 1.600 during 1-21, 22-35 and 1-35 d, respectively). These results demonstrated that the combination of whole wheat and xylanase in broilers fed wheat-based diets are beneficial in terms of feed efficiency.

The work reported in this thesis showed inconsistent effects of xylanase supplementation on digestive tract measurements of broilers fed wheat-based diets. In the study reported in Chapter 8, xylanase supplementation reduced the relative weight and length of the small intestine by 11.4 and 14.1%, respectively, but this response was not seen in the studies reported in Chapters 9 and 10. The variable responses of xylanase supplementation on digestive tract measurements may be related, partly, to differences in the quality of the wheat used and the resultant interactions with gut microflora (Choct *et al.*, 1996; Bedford and Apajalahti, 2001). The heavier relative size of the intestine in birds fed the unsupplemented wheat-based diet was probably due to the increased digesta viscosity caused by the presence of water-soluble non-starch polysaccharide in the wheat, and consequently increased microbial activity that stimulated intestinal growth.

The major contribution of this thesis was to provide comprehensive data on the effects of microbial phytase, produced by solid state fermentation, on bird performance,

digestibility and utilisation of nutrients in broilers fed diets based on wheat or corn (Chapters 3, 5-8). Influence of gender on the performance, AME and nutrient utilisation in broilers was also compared (Chapter 4). To the author's knowledge, the study reported in Chapter 5 was the first study which examined the effects of microbial phytase on broiler performance and nutrient utilisation in wheat-based and corn-based diets in the same study. The study reported in Chapter 8 was also the first study wherein the effects of microbial phytase on gut microstructure of broilers fed wheat-based was examined. Presented data demonstrated that a microbial phytase produced by solid state fermentation is as effective as phytases produced by submerged liquid fermentation in improving P availability in broiler diets. It is noteworthy that the microbial phytase produced by solid state fermentation was as effective as xylanase in improving the performance of broilers fed wheat-based diets containing adequate P levels (Chapter 8). Potential benefits of side enzyme activities present in the phytase preparation was confirmed by the results of the *in vitro* study (Chapter 7). The work reported in this thesis also demonstrated the beneficial effects of whole wheat inclusion (Chapters 9 and 10) and xylanase supplementation (Chapters 6, 8-10) in broilers fed wheat-based diets. Presented data indicated that the energy and protein effects of supplemental phytase are variable, but in general wheat-based diets appear to be more responsive to phytase addition than corn-based diets (Chapters 5 and 7). Clearly, studies are warranted to identify the factors causing these variable responses by supplemental phytase.

The widespread use of microbial phytases in broiler diets is currently limited by high temperature pelleting of diets required, which results in significant losses of phytase activity. Spraying of liquid form of phytase onto broiler diets after pelleting is a more realistic approach, as post-pelleting fat spraying systems are available in feed mills and no additional cost is required. Recently, a successful case of post-pelleting application of dry phytase produced by solid state fermentation onto broilers diets has been demonstrated (Edens *et al.*, 2002). The latter approach has advantages in terms of a relatively lower cost associated with purchasing and shipping of dry phytase and also less warehouse space for storage, compared to the liquid form of phytase. Clearly future research efforts will focus on identifying phytase-producing fungi, which will produce more heat-resistant phytases to eventually allow increased use of microbial phytase in the poultry industry.

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