NUTRIENT DIGESTIBILITY AND PERFORMANCE RESPONSES OF GROWING PIGS AND BROILERS FED PHYTASE AND XYLANASE SUPPLEMENTED WHEAT-BASED DIETS

By

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ABSTRACT

The effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on nutrient utilisation and performance was determined in growing pigs (from 20 to 60 kg body weight) and broilers (from hatch to three weeks of age). No synergistic interactions ($P > 0.05$) were detected between phytase and xylanase on any of the response criteria measured in both pigs and broilers. There were no enzyme effects ($P > 0.05$) on growth performance in in both pigs and broilers. Phytase increased ($P < 0.05$) the apparent ileal and total tract digestibility of P in pigs, and in broilers by 21 and 51%, and by 17.7 and 8.7%, respectively. In conclusion, phytase improved P digestibility in both pigs and broilers, but showed no synergistic effect with xylanase on any response criteria measured, probably due to the low non-starch polysaccharides concentration in the basal diets.

DEDICATION

This thesis is dedicated to my parents, Andrew and Agnes.

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FOREWORD

This thesis was written in a manuscript format and it is composed of two manuscripts. Manuscript I has been submitted to the Journal of Animal Science for publication, and manuscript II will be submitted to Poultry Science. Part of the work in manuscript I was presented at the ASAS-ADSA joint meeting in July, 2006. Part of the work in the same manuscript will be presented at ASAS-ADSA Midwestern Section meeting in March, 2007. Part of the work in Manuscript II was presented at the PSA meeting in July, 2006 and the Western Nutrition Conference in September, 2006. Authors to manuscript I are T. A. Woyengo, J. S. Sands, W. Guenter, and C. M. Nyachoti, whereas those to manuscript II are all the above-mentioned authors plus M. A. Mirza. In the thesis, the two manuscripts were written according to the guidelines for the American Society of Animal Science manuscript preparation.

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LIST OF ABBREVIATIONS

AA	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibility
AME	Apparent metabolisable energy
ATTD	Apparent total tract digestibility
BW	Body weight
BWG	Body weight gain
CP	Crude protein
d	Day
DE	Digestible energy
DM	Dry matter
Exp	Experiment
FCR	Feed conversion ratio
FTU	*Fytase units*
G:F	Gain to feed ratio
GE	Gross energy
GIT	Gastro-intestinal tract
NC	Negative control
NSP	Non-starch polysaccharides
PA	Phytic acid

PC	Positive control
SBM	Soybean meal based
wk	Week
XU	Xylanase units

1.0 GENERAL INTRODUCTION

Approximately 60 to 70% of P in vegetable feedstuffs is phytic acid (**PA**)-bound (Maenz, 2001). Phytic acid-bound P is poorly digested by pigs and poultry because they do not produce a sufficient amount of phytase, an enzyme that liberates P from PA (Bedford, 2000). Furthermore, PA is negatively charged at stomach and small intestinal pH conditions and thus it can react with positively charged molecules such as dietary divalent cations and amino acids (**AA**) and endogenous enzymes, thereby reducing nutrient digestibility (Lenis and Jongbloed, 1999).

In addition to PA, wheat, which is one of the major feed ingredients used in formulating pig and poultry feeds in Canada (AAFC, 2005), also contains anti-nutritional components, such as non-starch polysaccharides (**NSP**) in its cell wall, which are indigestible and capable of reducing utilisation of nutrients entrapped within the cells (Bedford and Schuzle, 1998; Kim et al., 2005a). The major component of NSP in wheat, soluble arabinoxylans (Kim et al., 2005a), can increase digesta viscosity, which in turn, can decrease nutrient intake and digestion by reducing digesta passage rate, accessibility of enzymes to their substrates and absorption of nutrients (Bedford, 2000).

The presence of PA and NSP in wheat-based pig and poultry diets can thus result in reduced efficiency of nutrient utilisation and hence increased cost of feeding. Their presence in wheat-based diets also poses a major environmental risk due to excessive excretion of unabsorbed nutrients, especially N and P (Lenis and Jongbloed, 1999). Phytase and xylanase supplementation to wheat-based diets may alleviate the anti-nutritional effects that are associated with PA and NSP, respectively. There is, however,

limited information available on the effect of combining phytase and xylanase on nutrient utilisation in pigs and broilers fed wheat-based diets. In wheat, both PA and arabinoxylans are highly concentrated in the aleurone cells (Joyce et al., 2005). Phytase and xylanase could thus act synergistically in improving the nutritive value of wheat-based diets for pigs and poultry because xylanase can hydrolyse arabinoxylans in the cell wall to release PA for the action of phytase. Reports on the effect of combining phytase and xylanase in wheat-based diets are, however, inconsistent. For instance, in broilers, Selle et al. (2003b) reported synergism between the 2 enzymes, but Wu et al. (2004a) could not show similar effects. In pigs, Codagan and Selle (2000) and Kim et al. (2005b) did not show synergism between the 2 enzymes. The objective of this study was to determine the effects of supplementing wheat-based diets with phytase and xylanase alone or in combination on nutrient utilisation and performance of growing pigs and broilers.

2.0 LITERATURE REVIEW

2.1 Phytic Acid

Phytic acid is a constituent of plant seeds in which it serves as the major storage form of P (Maenz, 2001). Chemically, it consists of an inositol ring with 6 phosphate groups and 12 protons, i.e, 2 protons per phosphate group (Figure 2.1; Maenz, 2001; Cowieson et al., 2004). And of the 12 protons, 6 dissociate at pKa value of approximately 1.5, 3 at pKa values between 5.7 and 7.6, and the remaining 3 at pKa values greater than 10 to leave a negatively charged PA at these acidic, neutral and basic pH conditions (Maenz, 2001, Adeola and Sands, 2003). In cereal grains, grain legumes and oilseeds, from which pig and poultry feeds are commonly derived, PA occurs as phytate (i.e., a mixed salt of cations, mainly K and Mg, and to a lesser extend Ca, Fe and Zn) in spherical inclusions called globoids, which are located within protein bodies (Ockenden et al., 2004; Joyce et al., 2005; Lin et al., 2005). In soybean, however, most of the phytate (85 to 90 %) in protein bodies does not occur as spherical inclusions; it interacts directly with the protein (Prattley and Stanely, 1982).

Phytic acid is not uniformly distributed in plant seeds, but localized within certain seed components. For instance, in wheat (Joyce et al., 2005) and barley (Ockenden et al., 2004) it is concentrated in the aleurone cells, whereas in corn (Lin et al., 2005) and oilseeds like soybean (Prattley and Stanely, 1982) and canola (Yiu et al., 1982) it is concentrated in the embryonic cells. Its concentration in various feed ingredients that are commonly used in formulation of pig and poultry feeds also varies depending on several

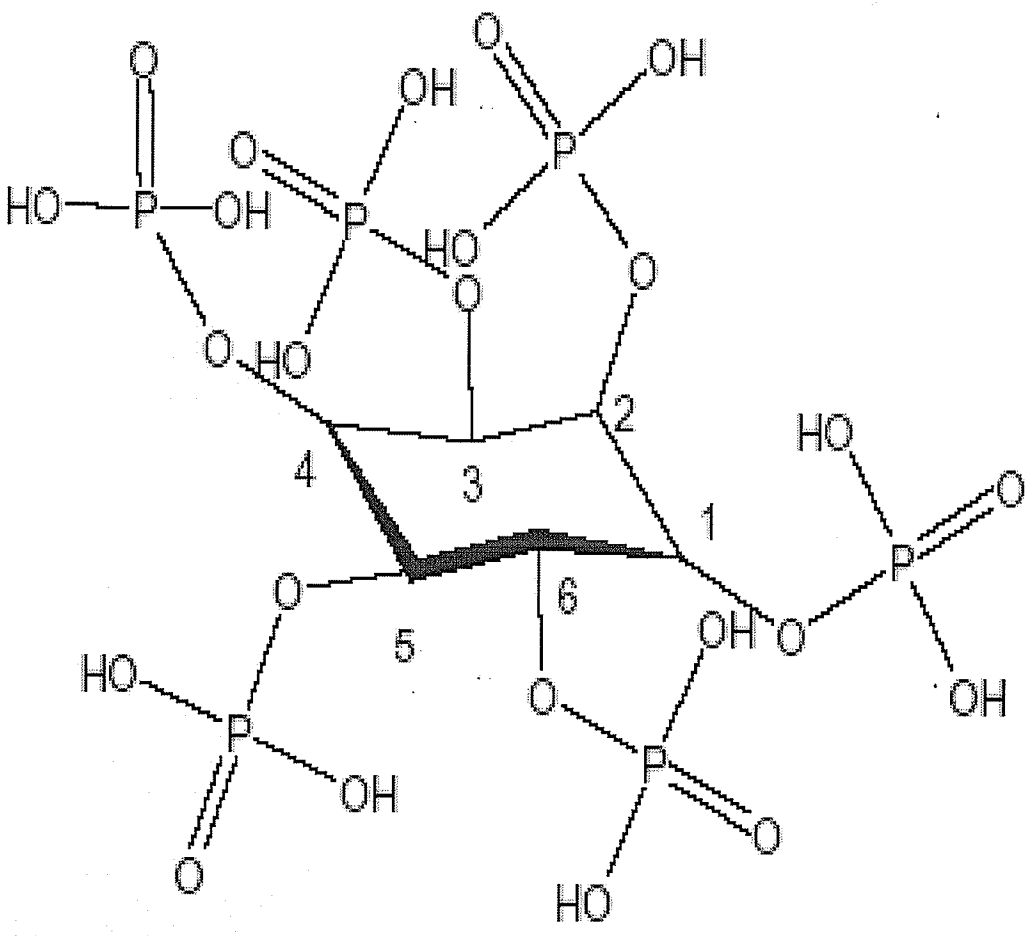

Figure 2.1:Phytic acid (Cowieson et al., 2004)

factors including type and variety of ingredient, and year of harvesting (Steiner et al., 2006). It is, however, generally higher in cereal milling by-products and oil seed meals than in grain legumes and cereal grains as indicated by phytate-P concentration in these feed ingredients (Table 2.1). Its high concentration in cereal by- products is due to its high concentration in the aleurone cells of cereal grains, which are part of the by-products (Steiner et al., 2006).

2.2 Effect of Phytic Acid on Mineral Digestibility

Phytic acid contains approximately 60 to 70% of the total P in vegetable feedstuffs that are commonly used to formulate pig and poultry feeds (Kornegay, 2001; Steiner et al., 2006). The digestibility of this PA-bound P by non-ruminants such as pigs and poultry is low because they lack capacity to hydrolyse PA (Kornegay, 2001). For instance, Cowieson et al. (2006a) found the digestibility of PA-bound P in 42-day (**d**) old broilers to be only 10%.

Phytic acid can also reduce the digestibility of other minerals because in its natural state in plants, it is complexed with minerals in globoids within protein bodies (Ockenden et al., 2004; Joyce et al., 2005; Lin et al., 2005). Furthermore, because of its negative charges at acidic, neutral and basic pH conditions, PA can complex cations at all pH conditions in the gastro-intestinal tract (**GIT**), thereby reducing the bioavailability of the cations, especially multivalent cations, which form insoluble complexes with PA at small intestinal pH conditions (Maenz, 2001; Adeola and Sands, 2003).

Table 1.1. Concentration of phytate-P and phytase activity of common feed ingredients

Ingredient	Phytate P (g/kg)	Phytase activity (FTU/kg)	Reference
Wheat	2.9±0.37	2886±645	Steiner et al., 2006
Barley	2.6±0.31	2323±645	Steiner et al., 2006
Triticale	2.8±0.30	2799±501	Steiner et al., 2006
Rye	2.4±0.23	6016±1578	Steiner et al., 2006
Corn	2.05	<50	Selle et al., 2003a
Sorghum	2.4±0.54	<50	Selle et al., 2003a
Wheat bran	7.9±0.05	9945±427	Steiner et al., 2006
Rye bran	4.9±0.23	9241±1452	Steiner et al., 2006
SBM	4.5±0.40	<50	Selle et al., 2003a
Canola meal	6.7±1.06	<50	Selle et al., 2003a
Peas	1.67	58	Selle et al., 2003a
Lupins	1.60	<50	Selle et al., 2003a

Studies on the effect of PA on mineral digestibility have shown a negative relationsip between dietary PA concentration and mineral digestibility. Kemme et al. (1999) reported reduced ash digestibility in growing pigs from 56.9 to 54.7% due to an increase in dietary PA concentration from 9.4 to 14.3 g/kg. An increase in the level of PA concentration in diets for broilers from 10.4 to 13.6 g/kg was also shown to result in reduced ileal digestibility of Ca and Fe from 37.7 to 36.0% and 21.8 to 20.3%, respectively (Ravindran et al., 2006). Urbano et al. (1999) similarly reported decreased Ca digestibility from 82.1 to 59.8% in rats fed lentil-based diets due to an increase in dietary PA concentration from 4.77 to 6.46 mg/g.

2.3 Effect of Phytic Acid on Digestibility of Amino Acids and Energy

The digestibility of AA and energy, like that of minerals, has also been found to be negatively affected by PA. Ravindran et al. (2000), feeding broilers on wheat-sorghum-soybean meal based (**SBM**) diets reported a decrease in ileal digestibility of N from 83.0 to 80.7% due to an increase in dietary PA concentration from 10.4 to 15.7 g/kg. In their study, the ileal digestibility of all the essential AA was also reduced by the increase in dietary PA concentration. More recently, Cowieson et al. (2006a) precision fed growing broilers on 5 g of casein for 48 h and observed a decrease in total AA digestibility by 8.9 and 11.9% due to addition of 0.5 and 1 g of PA to 5 g of casein, respectively. An increase in PA concentration in corn-SBM-based diets for broilers from 10.0 to 13.6 g/kg by addition of rice bran has also been reported to result in a decrease in

apparent metabolisable energy (**AME**) value of the diet by 2.1% (3353 vs 3281 kcal/kg; Ravindran et al., 2006).

Four mechanisms have been proposed to explain the detrimental effect of PA on AA and energy digestibility. First, PA in its natural state is complexed with AA in protein bodies (Ockenden et al., 2004; Joyce et al., 2005; Lin et al., 2005) and thus it can minimize the availability of these nutrients for digestion and absorption (Lenis and Jongbloed, 1999).

Second, the terminal amino groups on proteins and free amino groups on basic AA possess positive charges at pH below the iso-electric point, i.e, at pH less than 5 (Prattley et al., 1982; Maenz, 2001). Consequently, at acidic pH in the stomach, PA can interact with protein directly to form electrostatic bonds (Prattley and Stanely, 1982), thereby reducing the availability of protein for digestion in the stomach (Cowieson et al., 2004). This hypothesis was recently confirmed by an *in vitro* study by Kies et al. (2006a) who incubated 10 g of casein with or without 1 mg of PA and observed its (casein) complete dissolution at pH 2 in absence of PA, and almost complete precipitation (99%) in presence of PA.

Third, protein possesses a net negative charge at a pH above the isoelectric point but below pH 10 (Maenz, 2001). Hence in the small intestine, where the pH is within the range of 5 to 10, PA can bind protein through multivalent cations to form a PA-mineral-protein complex that is resistant to enzymatic hydrolysis (Maenz, 2001; Adeola and Sands, 2003). This ability of PA to bind protein via cations was clearly demonstrated by Prattley et al. (1982), who incubated serum albumin with PA either in presence or absence of Ca and observed formation of PA- protein complexes only in presence of Ca.

Fourth, endogenous proteolytic enzymes, like any other protein, can be bound by PA in both the stomach and small intestines, thereby reducing their ability to digest dietary proteins (Cowieson et al., 2006a; Selle et al., 2006). For instance, Singh and Krikorian (1982) measured the activity of trypsin *in vitro* at pH 7.5 using casein as substrate and reported an increase in inhibition of enzyme activity from 2.7 to 42.5% (after incubation for 20 min) due to an increase in level of phytate concentration from 10 to 90 mM.

For energy digestibility, PA can cause a reduction directly by binding energy generating molecules such as carbohydrates, lipids and protein (Thompson et al., 1987; Selle and Ravindran, 2006) and indirectly by binding endogenous enzymes and metal cofactors of enzymes involved in hydrolysis of energy generating molecules (Thompson et al., 1987), and by binding Na, which is involved in AA and glucose absorption from the GIT (Selle et al., 2006).

2.4 Effect of Phytic Acid on Endogenous Nutrient Losses

The effect of PA on endogenous losses has been investigated by Cowieson et al. (2004, 2006a). Cowieson et al. (2004) precision fed 6 wk-old broilers on a glucose solution and observed an increase in endogenous excretion of essential AA, non-essential AA, Ca, Fe, Na and S by, 29, 26, 68, 32, 300, and 47 %, respectively, over a 48-h period due to addition of 1 g of PA to the glucose solution. Cowieson et al. (2006a), also precision feeding broilers, found that addition of 1 g of PA to a solution containing 5 g of casein resulted in increased excretion of Ca, Mg, Mn, and Na by 187, 39, 87, and 174,

respectively, over a 48-h period. The increased endogenous nutrient losses due to PA ingestion is believed to be due to its ability to bind endogenous enzymes and their cofactors; by binding the enzymes and cofactors, it may increase their secretion through negative feed back mechanism and hence their losses via feces (Cowieson et al., 2004, 2006a). Phytic acid may also promote endogenous nutrient losses by binding mucin, thereby increasing its secretion through negative feedback mechanisms, and by binding and reducing the re-absorption of endogenous minerals that are secreted into the GIT via the bile duct (Selle et al., 2006).

The increased endogenous losses of N and minerals due to PA is likely to have adverse effects on the efficiency of nutrient utilisation in the body. This is because increased endogenous losses of nutrients can result in increased dietary demand of the lost nutrients by the animal, and of energy required to synthesise enzymes and mucin (Nyachoti et al., 1997).

2.5 Phytases

Phytases are enzymes that catalyse step-wise cleavage of phosphate groups from PA (Selle and Ravindran, 2006). The site of initial removal for some phytases is the phosphate group at the C_6 position on the inositol ring whereas for others it is the phosphate group at position C_3 (Kornegay, 2001). Phytases whose site of initial hydrolysis is the phosphate group at the C_6 position are known as 6-phytases whereas those whose initial site of initial hydrolysis is the phosphate group at the C_3 position are known as 3-phytases (Selle and Ravindran, 2006). As mentioned above the removal of

the phosphate groups from PA by phytase occurs in a step-wise manner. Their preferred molecule for removal of the phosphate group is, however, a fully phosphorylated PA followed by penta-, tetra-, tri-, di and mono-esters of inositol in descending order of preference (Wyss et al., 1999; Lassen et al., 2001; Vats and Banerjee, 2004). They thus normally hydrolyse all the available fully phosphorylated PA molecules to penta-esters of inositol before they hydrolyse the latter to tetra-esters of inositol and so on (Wyss et al., 1999). Phytases are normally inhibited by their product (inorganic P; Greiner et al., 1993; Hu et al., 1996; Greiner et al., 2002; Lopez et al., 2004), and like other enzymes, they have pH and temperature ranges at which they function optimally (Greiner and Konietzny, 2006). Their activity is measured in *fytase* units; where 1 *fytase* unit is the amount of phytase that liberates 1 μmol inorganic phosphate per minute from 0.0051 mol/L sodium phytate at pH 5.5 and at temperature of 37°C (Engelen et al., 1994). *Fytase* units are commonly abbreviated as FTU, though other abbreviations including FYT, U, and PU have also been used (Selle and Ravindran, 2006). In the current study, FTU will be used to denote *fytase* units.

2.6 Sources of Phytases

Phytases are produced to a greater extent by micro-organisms and plants, and to a lesser extent by animals (Maenz, 2001; Pandey et al., 2001). Among the micro-organisms, the major phytase producers are fungi, yeast and bacteria (Table 2.2; Pandey et al., 2001). Most of phytases produced by these micro-organisms are 3-phytases

Table 2.2. Sources and properties of phytases

Origin	Expression host	Temperature optimum (°C)	pH optimum	Reference
Fungi				
Aspergillus. niger	-	-	2.5-4.5	Tomschy et al., 2002
A. niger PhyA	*A. oryzae*	50	2.5–5.5	Lassen et al., 2001
A. fumigatus	*A. fumigatus*	-	4.0, 6.0–6.5	Pasamontes et al., 1997
A. ficuum	*A. niger*	58	5.5, 2.5	Ullah et al., 2003
A. pediades PhyA	*A. oryzae*	50	5.0–6.0	Lassen et al., 2001
Peniophora lycii	-	-	4.0–4.5	Tomschy et al., 2002
P. lycii	*A. oryzae*	58	3.0	Ullah et al., 2003
P. lycii PhyA	*A. oryzae*	50-55	4.0–4.5	Lassen et al., 2001
Ceriporia sp. PhyA1	*A. oryzae*	55-60	5.5–6.0	Lassen et al., 2001
Ceriporia sp. PhyA2	*A. oryzae*	40-45	5.0–6.0	Lassen et al., 2001
Rhizopus oligosporus	-	65	5.0	Casey and Walsh, 2004
Bacteria				
Escherichia coli	-	55	4.5	Greiner et al., 1993
E. coli	*Bacillus subtilis*	-	2.0-4.5	Adeola et al., 2004
E. coli	*Schizosaccharomyces pombe*	55	3.5	Seonho et al., 2005
Klebsiella sp. strain ASR1	*E. coli*	45	5.0	Sajidan et al., 2004
Obesumbacterium proteus	*E. coli*	40–50	4.9	Zinin et al., 2004
Plants				
Barley (PI)	-	55	6.0	Sung et al., 2005
Barley (PII)	-	50	5.0	Sung et al., 2005
wheat	-	50	5.0–6.0	Paik, 2003
Lupinus albus Var. Amiga	-	50	5.0	Greiner et al., 2002

(Maenz, 2001) except for a few like Basidiomycete fungi- and (Lassen et al., 2001) and *Escherichia coli* bacteria- (Greiner et al., 1993) derived phytases, which are 6-phytases.

In plants, phytases occur in seeds, where their major role appears to be the release of P from PA during germination for utilisation by the developing plant (Centeno et al., 2001). The plant phytases are 6-phytases (Maenz, 2001; Schlemmer et al., 2001), and among feed ingredients that are commonly used in formulation of pig and poultry diets, they are less concentrated in grain legumes, oil seed meals and non-viscous grains (corn and sorghum) than in viscous cereal grains and their milling by-products (Table 2.1).

Plant phytases have been shown to significantly hydrolyse PA in poultry (Paik, 2003) and pigs (Rapp et al., 2001b). They are, however, not as effective as microbial phytase. For instance, PA hydrolysis in the stomach of minipigs fed a diet supplemented with the microbial (*Aspergillus niger*) phytase at 818 FTU/kg was found to be 17% higher than in those fed a diet with supplemented plant (wheat) phytase at 1192 FTU/kg (Rapp et al., 2001b). The recovery of the wheat phytase in the duodenum of the same animals was also lower than that of *Aspergillus niger* phytase (45 vs 70%; Rapp et al., 2001a). Phillippy (1999) has similarly reported a higher recovery of *A. niger* phytase than wheat phytase (95 vs 70%) after 1 h of preincubation with 5 mg of pepsin/mL.

The poorer performance of plant phytases compared with microbial phytases is due to their narrower optimum pH range (Greiner and Konietzny, 2006). The optimum pH for plant phytases ranges from 5.0 to 6.0 whereas that for *A. niger*-, *Peniophora lycii*- and *Escherichia coli*-derived phytases, the most commonly utilized microbial phytses (Lei and Stahl, 2001; Greiner and Konietzny, 2006; Selle and Ravindran, 2006), ranges from 2.0 to 5.5 (Table 2.2), which is closer to the physiological pH (2 to 5) of the

stomach of pigs and poultry (Simon and Igbasan, 2002). The poorer performance of plant phytases compared with microbial phytases is also attributed to greater resistance of the latter to protease enzymes in the GIT due to a higher degree of glycosylation (Phillippy, 1999).

Animal phytases are produced by intestinal mucosa (Yang, 1991; Applegate et al., 2003). Their activity has been found to be higher in the duodenum than in other intestinal segments of rats (Lopez et al., 2000) and poultry (Maenz and Classen, 1998) and their production appears to be induced by the presence of its substrate (PA) in the intestines (Lopez et al. 2000) and reduced by increased concentration of its product (inorganic P) in the same GIT compartment (Hu et al., 1996).

Intestinal phytase has been reported to increase PA hydrolysis by 24% in rats fed a purified diet after intestinal perfusion with pure PA for 20 d (Lopez et al., 2000). Its effect on hydrolysis of PA in conventional (unpurified) diets has, however, been reported to be negligible (Jongbloed et al., 1992; Rapp et al., 2001a), and it is attributed to the failure of PA to induce mucosal phytase production since in conventional feeds, it occurs as phytate in protein bodies and thus it rarely comes in contact with the intestinal mucosa (Lopez et al., 2000).

2.7 Phytases for the Pig and Poultry Feed Industry

An ideal phytase for the pig and poultry feed industries would be the one that is resistant to acidic pH and protease enzymes in the stomach and small intestines, where P absorption takes place; be cheap to produce; and be resistant to high temperatures (65 to

80°C) that are encountered during feed pelleting (Lei and Stahl, 2001). Of the phytases, plant phytases appears to be the cheap because they are already present in feed ingredients. However, because microbial phytases compared with other phytases have greater ability to hydrolyse PA in the GIT, a lot of research has been focused on the identification and testing of efficacy of the former for use in the animal feed industry (Pandey et al., 2001). It has, however, been difficult to obtain native microbial phytases with all the above-mentioned attributes (Lei and Stahl, 2001).

Thus, several microbial phytases have been genetically modified by processes such as genetic transformation in an effort to achieve these desired characteristics (Lei and Stahl, 2001). Currently, there are a few phytase products mainly derived from *Aspergillus niger, Peniophora lycii* and *Escherichia coli* that function optimally at acidic pH (Table 2.2) and are commercially available (Selle and Ravindran, 2006). Of these, *E coli*-derived phytase is relatively more resistant to proteolytic acitivity in the stomach (Onyango et al., 2005a, b).

2.8 Sites of Supplemental Microbial Phytase Activity in the Digestive Tract

Supplemental microbial phytases hydrolyse PA in some but not all GIT compartments in poultry and pigs. In nursery pigs, the activity of a fungal (*A. niger*) phytase has been found to be highest in the stomach (50% of the dietary activity) followed by the upper 2 m of the small intestine (30% of the dietary activity) and negligible in the lower 2 m of the small intestine (5% of the dietary activity; Yi and

Kornegay, 1996). In growing pigs, the activity of the same fungal (*A. niger*) phytase has similarly been found to be highest in the stomach and duodenum combined (89% of the dietary activity) and negligible in the ileum (< 0.03% of dietary activity; Jongbloed et al., 1992). In broilers, Yu et al. (2004) observed highest activity of another fungal (*P. lycii*) phytase in crop and gizzard followed by duodenum and jejunum, and negligible in ileum. Onyango et al. (2005a), feeding broilers on diets supplemented with either bacterial (*E. coli*) or *P. lycii* phytase at 1000 FTU/kg observed highest activity of *P. lycii* phytase in the crop (404 FTU/kg) followed by the gizzard (63 FTU/kg), and negligible in the jejunum (25 FTU/kg) and ileum (6 FTU/kg). For *E. coli* phytase, however, although the activity was highest in the crop (649 FTU/kg), it remained relatively high in the proventriculus and gizzard combined (406 FTU/kg) and jejunum (554 FTU/kg), and was only low in the ileum (91 FTU/kg). The reason why the major sites of activity of supplemental fungal phytases are the crop and gizzard in poultry, and stomach in pigs, is because they have maximal activity at the acidic pH that is within the pH range present in these GIT segments and are susceptible to proteolysis that occurs in the small intestine (Simon and Igbasan, 2002). The reason why *E. coli* phytase compared with the fungal phytases remains active up to jejunal segment is because it is more resistant to proteolysis that occurs in the small intestine (Onyango et al., 2005a).

2.9 Effect of Supplemental Microbial Phytase on Nutrient Digestibility and Performance of Pigs and Poultry

By hydrolysing PA in the GIT, the supplemental phytases are expected to increase

nutrient digestibility and hence performance of pigs and broilers.

2.9.1 Effect on P Availability and Performance. Phytase supplementation has been shown to improve availability of P in piglets and growing-finishing pigs (Brana et al. 2006; Kim et al., 2005b). It has also been shown to increase the availability of P in broilers and laying hens (Selle and Ravindran, 2006). In piglets, Lei et al. (1993) reported increased P retention by 45% due to supplementation of corn-soybean meal diets with phytase at 750 FTU/kg, whereas Kim et al. (2005b) observed increased total tract P digestibility by 14 percentage units due to supplemenation of wheat-based diets with phytase at 500 FTU/kg. In growing pigs, Jongbloed et al. (1992) observed an increased ileal and total tract digestibility of P by 18.5 and 27 percentage units, respectively, due to supplementation of corn-SBM-based diets with phytase at 1500 FTU/kg. Similarly, supplementation of corn-based diets for growing pigs with 500 FTU/kg was reported to increase AID of P by 12 percentage units (Radcliffe et al., 2006) and total tract P digestibility and retention by 9.9 and 10.2 percentage units, respectively (Adeola et al., 2004).

Because of the ability of phytase to increase the availability of P, several studies have been conducted to investigate the effect of reducing the dietary concentration of available P and supplementation of the resulting low available P diet with phytase. Results from these studies generally indicate that supplemental phytase has a positive effect on performance of pigs fed low P diets. Stahl et al. (2000) reduced the available P in P-adequate corn-SBM-based diets for piglets from adequate to inadequate by 0.22 percentage units to investigate the effect of phytase on performance. They found that reduction in dietary available P resulted in reduction of average daily gain (**ADG**) and

gain to feed ratio (**G:F**) from 572 to 488 g and 0.559 to 0.542, respectively. But phytase supplementation at 1,200 FTU/kg increased ADG to 567 g and G:F ratio to 0.589, which were similar to values obtained with the P adequate diet. Matsui et al (2000) similarly reported reduced ADG (from 610 to 432 g) of piglets fed corn-SBM-based diets due to reduction of dietary available P from 0.34 to 0.20%, and an increase in the same parameter due to phytase supplementation at 1,000 FTU/kg. The ADG value for phytase supplemented diet (569 g) was similar to 610 g obtained with the diet that was adequate in P. The effect of phytase on performance of growing-finishing pigs fed a low P diet was investigated by Harper et al. (1997). The pigs were fed a P adequate diet (containing 0.50 and 0.40% P, and 0.58 and 0.48% Ca during the grower and finisher phases, respectively), and a low available P diet (containing 0.40 and 0.35% P, and 0.53 and 0.43% Ca for grower and finisher phases, respectively) either unsupplemented or supplemented with phytase at 500 FTU/kg. They found decreased average daily feed intake (**ADFI**) and ADG of the pigs to be decreased by 8.8 and 18.4%, and 13.5 and 20.5% in grower and finisher phases, respectively, due to reduction of P and Ca in the diet. But phytase supplementation to the low P and Ca diet increased ADFI and ADG of the pigs fed the low P diets by approximately 13 and 9% and by 23 and 19% in grower and finisher phases, respectively, and reached those of pigs fed P and Ca adequate diets.

The effect of phytase supplementation on P availability and performance of poultry has also been investigated. The results have generally been similar to those of pig studies. In broilers, Rutherfurd et al. (2004) observed increased true ileal P digestibility in 4-week (**wk**) old broilers by 14 percentage units after supplementing corn-SBM-based diets with phytase at 750 FTU/kg. Supplementation of corn-, wheat- and barley-based

diets with phytase at 500 FTU/kg was also shown to increase P retention by 14, 4 and 6 percentage units, respectively, for broilers at 3 wk of age (Juanpere et al., 2005). Wu et al. (2003) fed one-day-old broiler chicks on wheat- and corn-based diets supplemented with 500 FTU/kg for a period of 21 d and reported increased average weight gain, feed intake, and reduced feed/gain (irrespective of diet type) from 832 to 869 g/bird, 1156 to 1194 g/bird, and from 1.404 to 1.389 g/g, respectively. The AID and retention of P (g/kg DM intake) were also increased from, 0.478 to 0.540 and 3.46 to 3.61, respectively, irrespective of diet type. Dilger et al. (2004) reported that phytase supplementation at 1000 FTU/kg to a low Ca (0.51%) and available P (0.12%) corn-based diet increased retention of P in 3 wk old broilers by 14.1 percentage units. The BW gain and feed intake were lower for the low Ca and available P diet (445 and 713 g, respectively) compared with Ca (1.0%) and P (0.50% available) adequate diet (514 and 791 g, respectively), but were, respectively, increased by 19 and 10% due to the supplementation to reach those of the Ca and P adequate diet. Silversides et al. (2004) similarly observed improved body weigh gain (**BWG**) of broiler chicks (from hatch to 21 d of age) fed a low available P diet (0.23% available P) from 671 to 793 g due to phytase supplementation at 1250 FTU/kg. In their study, the BWG for the phytase-supplemented diet was similar to 799 g observed for the P adequate diet (0.40 available P). In laying hens, Um and Paik (1999) observed increased P retention (by 13 percentage units) and egg production per hen per d (2.2%) between 21 and 40 wk of age due to supplementation of corn-SBM-based diets with phytase at 500 FTU/kg. Boling et al. (2000) reported that reduction of available P in diets for laying hens from 0.45 to 0.1% resulted in reduction in egg production from 89 to 81%, but supplementation of the low available P diet with phytase at 300 FTU/kg

increased the egg production to 88%, which was similar to the production obtained with hens fed the P adequate diet.

Tibia ash is the most sensitive indicator of P adequacy in growing broilers (Onyango et al., 2005b) because most of the absorbed P is deposited in bones (Leeson and Summers, 2001) and the tibia grows faster than all other bones in the body (McLean et. al., 1961 cited by Yan et al., 2005). Viveros et al. (2002) reported increased tibia ash by 9.7% in broilers fed corn-SBM-based diets supplemented with phytase at 500 FTU/kg for 6 wk. Similarly, Onyango et al. (2004) observed that reduction of Ca and available P in diets for broilers from 1.0 to 0.50% and 0.51 to 0.24%, respectively, resulted in a reduction in tibia ash at 22 d of age from 51.2 to 45.3%, but supplementation of the low Ca and available P diet with phytase at 1000 FTU/kg increased the tibia ash to 51.1%, which was similar to the tibia ash of broilers fed the P adequate diet.

It is apparent from these studies that supplementation of phytase to diets for pigs and poultry can result in reduced requirement of added available P in the diets without a significant effect on performance due to liberation of PA-bound P by phytase. The amount of P that can be liberated from PA is, however, variable and depends on several factors including the dietary level of Ca and available P, source and level of phytase, age and size of the animals, and size and location of PA in feed, which in turn, depends on ingredient type (see Section 2.10).

2.9.2 Effect on Availability of Non-phosphorus Minerals. As mentioned previously, PA reduces the availability of minerals. Thus, by hydrolysing PA, phytase can increase the availability of these nutrients. Murry et al. (1997) reported increased absorption of Ca (from 8.16 to 8.50 g/d) in piglets fed pearl-SBM based diets due to

phytase supplementation at 700 FTU/kg. Li et al. (1998) and Adeola et al. (2004) reported that total tract Ca availability in growing pigs increased by at least 13%, after supplementation of corn-based diets with phytase at 750 FTU/kg whereas Kim et al. (2005b) observed that total tract Ca availability increased by 14%, after supplementing wheat-based diets for piglets with phytase at 500 FTU/kg. Radcliffe et al. (2006) supplemented corn-SBM-based diets for growing pigs with phytase at 500 FTU/kg and reported increased ileal Ca, availability by 8.4%. Shelton et al. (2005) observed reduced ADG (from 342 to 277g) of piglets due to removal of trace mineral premix in diets and improved ADG of the pigs fed diets lacking the trace mineral premix to levels similar to that of pigs fed the trace mineral premix containing diet (350 vs 342 g) due to phytase supplementation at 500 FTU/kg. In poultry, Silversides et al. (2004) reported that supplementation of wheat-SBM-based diets for broilers with phytase at 1250 FTU/kg improved Ca retention by 47%. Cowieson et al. (2006b) also reported that supplementation of corn-SBM-based diets for broilers with phytase at 150 FTU/kg increased the retention of K, Mg, CU, Fe and Mn by 30, 31, 43, 33 and 23%, respectively.

The effect of phytase on the availability of cations has, however, not been consistent as that on P. For instance, Harper et al. (1997) did not observe an increase in Ca availability in growing pigs and finishing pigs due to phytase supplementation, whereas Dilger et al. (2004) and Cowieson and Adeola (2005) did not report increased digestibility of the same in broilers due to phytase supplementation. Since in these studies P digestibility was improved by phytase supplementation, the lack of effect of phytase on Ca digestibility indicates that the effect of phytase on availability of non-P minerals is not

only dependent on factors that affect PA hydrolysis but on other factors. One of these factors may be the capacity of PA to bind the minerals (see Section 2.10.4).

2.9.3 Effect on Availability of Amino Acids and Energy. The effect of phytase on protein/amino acids, and energy availability has also been studied. Murry et al. (1997) reported increased absorption of N (from 33.82 to 34.83 g/d) in piglets fed pearl-SBM based diets due to phytase supplementation at 700 FTU/kg. Mroz et al. (1994) reported increased apparent ileal digestibility of methionine and arginine, and apparent total tract crude protein (**CP**) digestibility by 2.8, 5.1 and 3.0% after supplementation of a corn-tapioca-SBM-based diet for growing pigs with phytase at 800 FTU/kg. Radcliffe et al. (2006) supplemented corn-SBM-based diets for growing pigs with phytase at 500 FTU/kg and observed increased ileal CP, total AA, lysine, arginine, phenylalanine, threonine and methionine-cysteine digestibilities by 5.1, 3.4, 4.5, 2.3, 2.5, 6.1 and 3.6%. In broilers, phytase supplementation to a wheat-soybean meal-sorghum-based diet (Ravindran et al., 2001) and a corn-SBM-based diet at 1000 FTU/kg (Ravindran et al., 2006) improved AA digestibility by a mean of 4.4 and 5.3%, respectively. With regard to energy availability, addition of phytase to corn-SBM-based diets for broilers at 500 FTU/kg (Ravindran et al., 2001) and at 1000 FTU/kg (Onyango et al., 2004) resulted in improved AME value of the diets by 2.3 and 2.1%, respectively. And like Ca, the effect of phytase on AA and energy availability is also variable because in other pig (Johnston et al., 2004; Kim et al., 2005b) and broiler (Onyango et al., 2005b) studies improvements have not been noted due to phytase supplementation. Also, because in these studies P digestibility was improved by phytase supplementation, the effect of phytase on AA and energy digestibilities, like that on Ca availability, appears not only dependent on PA

hydrolysis but on other factors such as the degree of association beetween PA and non-mineral nutrients (see Section 2.10.4).

2.10 Factors Affecting Efficacy of supplemental Microbial Phytase

2.10.1 Effect of Dietary Ca and Available P Concentrations. As mentioned previously, PA can react with multivalent cations to form insoluble PA-mineral complexes at intestinal pH. The susceptibility of such PA-mineral complexes to hydrolysis by phytase, however, reduces with an increase in molar ratios of multivalent cations to PA, indicating that an increase in dietary concentration of multivalent cations can result in reduced efficacy of phytase on PA hydrolysis (Maenz, 2001; Adeola et al., 2004). Among the multivalent cations that are commonly included in pig and poultry diets, Zn^{2+} has been found to be the most potent mineral as an inhibitor of PA hydrolysis followed by Fe^{2+}, Mn^{2+}, Fe^{3+}, Ca^{2+} and Mg^{2+} in decreasing order of potency (Maenz and Classen, 1998). Also, inorganic P, the end product of PA hydrolysis, inhibits the catalytic activity of phytase (Greiner et al., 1993), indicating that an increase in dietary concentration of inorganic (available) P can result in decreased hydrolysis of PA by phytase. In general, therefore, an increase in dietary concentration of multivalent cations and available P can potentially reduce the efficacy of supplemental phytase. Calcium and P compared with other minerals have, however, high potential of reducing the efficacy of phytase because they are included in pig and poultry diets at a higher rate (Lie et al., 1994). Hence most studies have concentrated on determining the effect these two minerals have on the efficacy of phytase.

Li et al. (1999) found that reduction of dietary Ca level from adequate (0.8%) to inadequate (0.4%) in the presence of microbial phytase at 750 FTU/kg increased the digestibility of phytate P in piglets by 10.9 and 5.7 percentage units at the duodenal and ileal levels, respectively. Zyla et al. (2000) reported greater improvement in BWG of broilers from hatch to 21 d of age (by 13% vs 8.3%) due to phytase supplementation at 750 FTU/kg when dietary Ca was low (0.59%) than when it was high (0.79%). Tamin et al. (2004) reported that an increase in dietary Ca from 0.1 to 0.2, 0.4, 0.7 and 0.9% resulted in a decrease in PA-bound P hydrolysis in broilers by 52, 55, 58, 72, and 72%, respectively, due to supplementation with phytase at 500 FTU/kg.

With regard to dietary available P, Fan et al. (2005) found significant improvement in ileal P digestibility (by 8.3%) in growing pigs fed corn-rice-rapeseed-cottonseed meal-based diets after phytase supplementation when the basal diets were low in available P (0.19%), but not when the basal diet was adequate in available P (0.21%; by 3.3%). Phytase supplementation at 500 FTU/kg to wheat-based diets for broilers was also shown to result in greater improvement in ileal P digestibility (by 23.7 vs 7.4%) when the basal diet was low in inorganic P (0.30%) than when it was adequate in the same mineral (0.45%; Wu et al., 2003).

It is thus apparent from these studies that reducing the dietary concentration of both Ca and available P from adequate to inadequate level can increase the efficacy of phytase. Logically, the efficacy of phytase on PA hydrolysis would be highest in diets with zero levels non-PA-bound Ca and P. It is, however, not practical to feed such kind of diets to pigs and poultry because PA-bound Ca and P (that are released by phytase) alone cannot meet the animals' requirements. Studies have shown that Ca and available P

can be reduced by 0.1 and 0.1 percentage units, respectively, in pigs (Harper et al., 1997) and by approximately 0.50 and 0.25 percentage units, respectively, in broilers (Onyango et al., 2004) without significant effects on performance when phytase is supplemented. Zyla et al. (1999a), however, reported that reduction of dietary Ca and available P by 0.14 and 0.30 percentage units, respectively, resulted in reduced performance of broilers, which was not fully restored by a fungal (*Aspergillus oryzae*) phytase supplementaion, indicating that phytase may not fully restore performance if dietary available P is reduced by 0.30 percentage units or more. However, the actual magnitude by which Ca and available P should be reduced to optimize phytase activity is likely to vary depending on other factors that affect the hydrolysis of PA by phytase.

2.10.2 Level of Phytase. The general industry practice has been to supplement pig and poultry diets with phytase at about 500 FTU/kg (Kies et al., 2006b). However, in some recent studies phytase has shown to be effective when it is supplemented at higher doses than at current recommended dosage. Shirley and Edwards (2003) fed broiler chicks a P adequate diet (0.7% total P), and a low P diet (0.46% total P) supplemented with phytase at 0, 93.75, 187.5, 350, 750, 1,500, 3,000, 6,000, and 12,000 FTU/kg and found the BWG of chicks fed a low P diet with phytase at 750 FTU/kg to be inferior to that of the positive control. However, a further increase in level of phytase to 12,000 FTU/kg increased the BWG to that of the positive control diet. Brana et al. (2006) supplemented low P diets (lower than normal by 0.1 percentage units) for piglets and growing pigs with graded levels of phytase at 250, 500, 750, 1,000 or 10,000 FTU/kg and similarly observed higher performance for diets with the highest level of phytase. In piglets, the G:F ratio for the low P diet (526 g/kg) was lower than 577 g/kg observed for

P the adequate diet, but was significantly increased by phytase when the enzyme was supplemented at 10,000 FTU/kg (588 g/kg), and not at 500 FTU/kg (551 g/kg). At 10,000 FTU/kg, the G:F ratio was similar to that for the P adequate diet. In growing pigs, the G:F ratio for the low P diet was also lower than that for the P adequate diet (436 vs 453 g/kg), but was increased by phytase supplementation at 500 FTU/kg or greater. The G:F for the diet supplemented with phytase at 500 FTU/kg (445 g/kg) was similar to that observed for the P adequate diet and an increase in level of phytase from 500 to 10,000 FTU/kg further improved G:F to a value (474 g/kg) that was superior to that for the P adequate diet.

Two reasons why performance of pigs and broiler could be higher at high levels of phytase than at current recomendation have been proposed. First, at low level of supplementation, phytase hydrolyses PA to partially phosphorylated PA products, whose further hydrolysis is known to require high amount of phytase (Wyss et al., 1999; Cowieson et al., 2006b). Second, if phytase is supplemented at a high level, a larger amount of it may escape proteolysis by pepsin in the stomach and be available to act on PA in the upper part of the small intestine where the pH conditions favours both phytase activity and P absorption (Kies et al., 2006b).

2.10.3 Source of Phytase. Extensive proteolysis and nutrient absorption occurs in the small intestine. Consequently, for phytase to be effective, it must either extensively hydrolyse PA before it reaches the small intestine or be able to continue hydrolysing PA in the small intestine (meaning that it is able to resist the proteolysis) or both. Matsui et al. (2000) found fungal (*A. niger*) phytase at 1000 FTU/kg to be as effective as yeast (*Schwanniomyces occidentalis*) phytase at 4000 FTU/kg due to a lower susceptibility of

the former than the latter to inactivation by pepsin. However, *A. niger* phytase, although superior to yeast phytase, was found to be inferior to *E. coli*-derived phytase with regard to total tract P digestibility (51.3 vs 61.5%) in broiler chicks (Adedokun et al., 2004). Augspurger and Baker (2004) also found other fungal (*A. ficuum* and *P. lycii*) phytases to be inferior to *E. coli* phytase on PA hydrolysis in the GIT of broilers. In their study, *E-coli* phytase at 1000 FTU/kg was more effective than the 2 fungal phytases at 10,000 FTU/kg. The superior performance of *E-coli* phytase compared with these fungal phytases has been attributed to the fact that it is more resistant to hydrolysis by endogenous proteolytic enzymes in the GIT (Onyango et al., 2005a).

2.10.4 Effect of Type of Ingredient. The availability of P and other PA-bound nutrients in response to phytase supplementation appears to vary with dietary ingredient composition. Nernberg (1998) found the hydrolysis of PA in canola meal by phytase (*in vitro*) to be higher than that of wheat, whereas Adeola et al. (2004) found the hydrolysis of PA in SBM by the same enzyme *in vitro* to be higher than that of corn. Biehl and Baker (1997) observed improved G:F ratio of young chicks (8 to 20 d of age) from 459 to 468 g/kg after supplementation of a corn-SBM-based diet with phytase at 1,200 FTU/kg, but not after supplementation of a corn-peanut meal-based diet with the same amount of phytase. Ravindran et al. (1999b), working with broilers, observed greater improvement in mean ileal digestibility of AA for wheat (by 6.9 percentage units) followed by SBM (3.3 percentage units), corn (2.4 percentage units) and then canola meal (2.0 percentage units) due to phytase supplementation at 1,200 FTU/kg. Rutherfurd et al. (2002) also observed greatest improvement in mean ileal digestibility of AA in broilers for wheat- (13%) followed by SBM- (12%), rapeseed meal- (10%) and then a corn-based diet (6%).

The differences in efficacy of phytase on PA hdrolysis due to variation in ingredient composition has been attributed to differences in location of PA and composition of cations that are associated with PA in the ingredients (Adeola et al., 2004). The availability of PA for hydrolysis by phytase is higher in oilseed meals than in wheat probably because in the former, it is uniformly distributed throughout the embryonic cells (Prattley and Stanely, 1982; Yiu et al., 1982) whereas in the latter, it is highly concentrated in aleurone cells (Joyce et al., 2005), where the accessibility by phytase may be limited by presence of NSP in the cell walls (Kim et al., 2005a,b). Among the oil seed meals, the availability of PA for phytase hydrolysis is higher in SBM than in other oilseed meals probably because in the former it interacts directly with protein (and thus it is likely to be more available to phytase hydrolysis) whereas in the latter, PA occurs as phytate in globoids (Adeola et al., 2004). The hydrolysis of PA may also be lower in ingredients where the ratio of Ca to other cations (K, Mg, Zn and Fe) that associates with PA to form phytate is higher because phytates with a higher concentration of Ca form larger globloids and are more insoluble than those with low Ca concentration (Adeola et al., 2004). For instance, phytate globoids found in corn are larger than those found in SBM (Onyango et al., 2005a), indicating that the former has more insoluble phytate than the latter. This could be one of the reasons why the hydrolysis of PA in corn was found by Adeaola et al. (2004) to be lower than in SBM.

The efficacy of phytase with regard to improving the availability of PA-bound protein and other non-phosphorus nutrients may additionally vary with type of ingredient due to variation in capacity of PA to bind the nutrients (Ravindran et al., 2006). For instance, the capacity of PA to bind protein in the stomach may depend on a number of

terminal amino groups and free amino groups on basic AA that the preotein has, which in turn, may depend on AA composition and structure of protein (Adeola and Sands, 2003). It has been suggested that the higher AA digestibility for wheat compared with corn is due to AA composition and structure of protein in wheat that makes it (wheat protein) to be more susceptible to PA binding (Ravindran et al., 2006; Selle and Ravindran, 2006).

The effectiveness of supplemental phytase also depends on endogenous phytase concentration in the basal diets. This is because endogenous phytase can hydrolyse PA to partially dephosphorylated PA products, thereby releasing some of the PA-bound P. Furthermore, the partially dephosphorylated products resulting from the hydrolysis of PA by endogenous phytase have low capacity to bind nutrients such as Ca and AA (Wyss et al., 1999; Cowieson et al., 2006b). Juanpere et al. (2004) fed broilers on diets based on either untreated or autoclaved barley and observed improved AME (12.6 vs 13.5 MJ/kg) and P retention (58 vs 69%) and Ca retention (23 vs 35%) after phytase supplementation to a diet based on autoclaved but not untreated barley due to higher concentration of endogenous phytase in the latter (196 FTU/kg) than in the former (12 FTU/kg). Later on, Juanpere et al. (2005) observed a greater improvement in retention of P in 3 wk old broilers fed a corn-based diet (by 12 percentage units), than in those fed wheat- and barley-based diets (by 4, and 6 percentage units, respectively) after supplementation with phytase at 500 FTU/kg due to high endogenous phytase activity in wheat- (459 FTU/kg) and barley- (276 FTU/kg) based diets compared to the corn-based one (6 FTU/kg). In the same study, AME and Ca availability were improved by phytase supplementation only for the corn-based diet. Scott et al. (2001) similarly reported increased P absorption (by more than 50%) in laying hens after supplementing corn-based diets with phytase at 1000

FTU/kg diet, but not after supplementing wheat-based diets with the same amount of phytase due to the presence of endogenous phytase in wheat.

Thus, it appears that the efficacy of phytase with regard to PA hydrolysis and hence P digestibility is higher for SBM than for other ingredients due to both the location and size of the phytate globoids. Among the cereal grains, the response to phytase supplementation with regard to P and Ca digestibilities appears to be lower for ingredients with high endogenous phytase activity like wheat and barley and higher for those with low endogenous phytase activity like corn and heat-treated barley and wheat. For AA availability and energy, the response to phytase supplementation appears to be higher for wheat and SBM than for corn and other ingredients probably due to higher susceptibility of proteins found in these ingeredients to be bound by PA. In wheat, however, the capacity of PA to bind AA may depend on its level of endogenous phytase. For example, the endogenous phytase activity in a wheat-based diet was higher (459 vs 340 FTU/kg) in the study of Juanpere et al. (2005), where phytase did not improve AME than in the study of Ravindran et al. (1999b), where phytase improved AA digestibility.

2.10.5 Age of Animal. The effect of age on response to phytase supplementation has been investigated, and in pigs, it appears to increase with age. Kemme et al. (1997) fed growing-finishing pigs on low available P diets with identical feedstuff composition either without or with added microbial phytase and observed greater improvement in P digestibility at 60 kg BW than at 30 kg BW (16.7 vs 14.7 percentage units). Similarly, Adeola et al. (2004) reported that supplementation of phytase at 750 FTU/kg to the low P corn-based diets of piglets (13 kg) and growing pigs (19 kg) resulted in greater improvement in P digestibility at 19 kg BW than at 13 kg BW (31 vs 15 percentage

units). In terms of performance, Harper et al. (1997) reported improved ADG by 0.086 kg (13%) and 0.172 kg (23%) during the growing phase (19 to 52 kg) and finishing phase (52 to 109 kg), respectively due to phytase supplementation to low available P corn-SBM-based diets. Brana et al. (2006) also fed low available P corn-based diets to pigs either unsupplemented or supplemented with phytase to investigate its effect on performance of piglets and growing-finishing pigs. They found that phytase improved ADG in growing-finishing pigs, but not in piglets.

In broilers, however, the effect of age on efficacy of phytase has not been clearly established. For instance, Lan et al. (2002) observed greater improvement in feed conversion ratio (**FCR)** in broilers between d 22 and 42 than between d 1 and 21 (8.5 vs 4.6%), whereas Dilger et al. (2004) observed greater improvement in FCR at a younger age for broilers (between d 1 and 22; 4.3%) than at an older age (between d 23 and 43; 0%) due to phytase supplementation to low available P diets.

It is not clear why the efficacy of phytase would increase with increase in age of pigs. It could probably be due to an increase in acid (HCl) secretion in the stomach (which increases the solubility of PA and increases the activity of phytase) and a decrease in gastric emptying (which increases time the PA is acted upon by phytase in the stomach) with increase in age as has been suggested by Adeola et al. (2004).

2.11 Arabinoxylans

Arabinoxylans are components of cell wall polysaccharides of cereals (Izydorczyk and Biliaderi, 1995). They consist of a linear backbone of (1→4)-linked β-D

xylose units to which arabinose units are attached as side branches (Izydorczyk and Biliaderi, 1995). The arabinoxylans can be categorized as either water soluble or insoluble (Izydorczyk and Biliaderi, 1995; Maes and Delcour, 2002). Generally, the backbone xylan chains of water-soluble arabinoxylans are less branched than that of the water insoluble arabinoxylans (Maes and Delcour, 2002). Among the cereals that are commonly used in formulation of pig and broiler diets, arabinoxylans are highly concentrated in wheat, triticale and rye (Chesson, 1993). In wheat, they are highly concentrated in the walls of the aleurone and endosperm cells (Guillon et al., 2004) in which they constitute 60 to 70% of the cell walls (Izydorczyk and Biliaderi, 1995; Zijlstra et al., 1999; Kim et al., 2005a).

2.12 Anti-nutritive Effects of Arabinoxylans

Arabinoxylans are indigestible by non-ruminants like pigs and poultry because these animals do not have the enzymic capacity (Bedford and Schulze, 1998; Kim et al., 2005a). And by being indigestible, they can reduce the availability of nutrients within the cells by encapsulation (Bedford and Schuzle, 1998; Kim et al., 2005a). Furthermore, the soluble arabinoxylans can increase digesta viscosity, which in turn, can reduce feed intake by decreasing digesta passage rate, and reduce nutrient digestibility by reducing the interaction of enzymes with their substrates and absorption of digested nutrients in the small intestine (Bedford and Schuzle, 1998; Kim et al., 2005a). They can also further reduce lipid digestibility by binding bile salts and the lipids (Carre et al., 2002). In addition to reducing nutrient digestibility, arabinoxylans can adsorb endogenous enzymes in the GIT, thereby increasing their secretion through a negative feedback mechanism

and hence endogenous N losses (Silva and Smithhard, 2002; Wang et al., 2005). The arabinoxylans can also increase the endogenous N losses by promoting microbial growth and multiplication in the small intestine, which utilizes endogenous AA, resulting in their reduced (endogenous AA) reabsorption (Bartelt et al., 2002).

Several studies have been conducted to evaluate the anti-nutritive effects of arabinoxylans. Zyla et al. (1999b) reported a positive correlation between arabinoxylans concentration and viscosity of wheat digested *in vitro*, whereas Carre et al. (2002) found negative correlation between *in vitro* viscosity and AME of wheat in broilers. Boros et al. (2002) observed a reduction in FCR (from 2.647 to 3.360) and fat digestion (from 76.0 to 69.7%) of 3-wk old broilers due to an increase in dietary concentration of soluble arabinoxylans from 3.36 to 7.74%. Zijlstra et al. (1999) found a negative correlation between digestible energy (**DE**) in growing pigs and total NSP, insoluble NSP and xylose concentrations in wheat-based diets. Soluble NSP concentration in the diets was, however, not related to the DE, indicating that viscosity might not be a problem in pigs.

2.13 Xylanases

Xylanases are enzymes that hydrolyse xylans to xylo-oligosacchrides and then to xylose (Sapre et al., 2005), and are produced mainly by fungi and bacteria with a purpose of providing them with sugars (Bedford and Schuzle, 1998). Xylanases can be classified into two categories; β-1, 4 endoxylanases and β-D-xylosidases (Bedford and Schuzle, 1998). β-1, 4 endoxylanases randomly breaks linkages of xylan chains to xylo-oligosacchrides, whereas β-D-xylosidases sequentially cleaves xylose units from xylan

chains starting from the non-reducing end (Chesson, 1993; Bedford and Schuzle, 1998). Because of their ability to hydrolyse xylans, xylanases can be added in wheat-based diets for pigs and poultry to alleviate the ant-nutritional effects of arabinoxylans.

To reduce the anti-nutritional effects of arabinoxylans, what is required is disruption of the structure of the arabinoxylan to increase the accessibility of digestive enzymes to their substrates within the cells and reduce viscosity (Chesson, 1993). Thus, β-1, 4 endoxylanases compared with β-D-xylosidases are more effective in reducing the anti-nutritional effect of arabinoxylans because they randomly cleave xylan chains to disrupt the structure of the former whereas β-D-xylosidases have low ability to hydrolyse arabinoxylans with high concentration of arabinose units (Bedford and Schuzle, 1998). In general, the optimal temperature and pH of xylanases, respectively, ranges from 50 to 60°C and 3.0 to 10.0 (Table 2.3), indicating that in pigs they are active in the small intestine whereas in poultry they are active in both the crop and small intestine since their pH optima is within the range of pH found in these GIT segments.

2.14 Effect of Xylanase on Nutrient Digestibility and Performance

By hydrolysing arabinoxylans, xylanase is expected to increase the feed intake, availability of nutrients and hence performance of pigs and broilers. Diebold et al. (2004) reported increased ileal digestibility of energy (80.1 vs 81.4%), CP (83.6 vs 85.2%), and of most AA due to supplementation of wheat-based diets for nursery pigs with xylanase at 5,600 XU/kg. Barrera et al. (2004) reported increased ileal digestibility of CP (from 78.5 to 84.1%) and that of most AA, and improved ADG (from 82 to 331 g) of

Table 2.3. Temperature and pH optima for xylanases

Origin	Temperature optimum (°C)	pH optimum	Reference
Trichoderma reesei	50	3.0–8.5	Janis et al., 2001
Trichoderma reesei AF35, AF53	60	4.0	Nogawa et al., 1999
Trichoderma reesei (Xyln III)	55	6.0	Xu et al., 1998
Aspergillus niger	50	5.0	Deng et al., 2006
Bacillus spp.	50	6.5, 8.5, 10.5	Sapre et al., 2005

growing pigs fed wheat-based diets due to xylanase supplementation at 11,000 XU/kg. Wu et al. (2004b) observed increased AME, BWG and FCR of 3 wk old broilers fed wheat–based diets by 1.1, 2.6 and 1.5%, respectively, due to supplementation of the diets with xylanase at 1000 XU/kg. The duodenal, jejunal and ileal digesta viscosities were also reduced by 11.3, 20.9 and 18.1%, respectively, due to the supplementation. Lazaro et al. (2003) supplemented rye-based diets for broilers (4 to 25 d) with an enzyme preparation containing 858 units of β-glucanase and 864 units of xylanase/g) at a rate of 500 ppm to determine the effect of enzyme on digesta flow rate in the GIT and performance of broilers. Enzyme supplementation reduced the time required to recover 1% (0.78 vs. 0.98 h) and 50% (4.2 vs. 6.5 h) of the marker in feces. Enzyme supplementation also improved weight gain (31.7 vs 38.3 g/d), feed intake (63.6 vs 66.7 g/d) and FCR (1.96 vs 1.71 g/g) and reduced intestinal viscosity from 321 to 86 cps. Xylanase supplementation has also been found to improve the pancreatic lipase and chymotrypsin activities in broilers fed wheat-based diets (Engberg et al., 2004).

Some studies have, however, not shown beneficial effect of xylanase. For example, Diebold et al. (2005) did not observe an improvement in ileal energy and AA digestibilities, and total tract energy and CP digestibilities in nursery pigs due to supplementation of wheat-based diets with xylanase at 5600 XU/kg. Mavromichalis et al. (2000) and Kim et al. (2005b) also did not observe improved ADG, feed efficiency, and apparent nutrient digestibility of nursery pigs fed on wheat-based diets after supplementation of the diets with xylanase at 4000 XU/kg, whereas Preston et al. (2000) observed a non-significant effect of xylanase on performance of broilers fed wheat-based

diets supplemented with xylanase at 2500 XU/kg. This variable response to xylanase supplementation is due to several factors, which are discussed below.

2.15 Factors Affecting the Efficacy of Xylanase

2.15.1 Dietary Arabinoxylans Concentration. The anti-nutritional effect of arabinoxylans and hence the efficacy of xylanase appears to be partly dependent on dietary concentration as suggested by studies of Adeola and Bedford (2004) and Jozefiak et al. (2006). Adeola and Bedford (2004) observed improved performance of White Pekin ducks after xylanase supplementation to wheat-based diets when the wheat was high in NSP (15.3%), but not when it was low in NSP (9.4%). Jozefiak et al. (2006) reported increased growth performance and reduced ileal digesta viscosity of broilers fed rye-based diets (which was high in total NSP and soluble arabinoxylans; 13.78 and 2.73%, respectively), but not wheat- and triticale-based diets, which were lower in total NSP and soluble arabinoxylans (11.46 and 1.06%, and 13.06 and 1.23%, respectively).

2.15.2 Structure of Arabinoxylans. The anti-nutritional effect of arabinoxylans and efficacy of xylanase appears also to be partly dependent on the structure of arabinoxylans. Austin et al. (1999) found the viscosity of wheat to decrease with an increase in the number of side branches and with an increase in the spread of the branches along the linear backbone of xylose units. They attributed this to the fact that side branches reduce the interactions between adjacent linear xylans (and hence the formation of tight junctions), and that widely distributed side branches reduces the chances of the adjacent xylan chains to interact than side branches that are concentrated in one place.

2.15.3 Level of Xylanase. The general commercial practice has been to supplement pig and broiler diets with xylanase at 4000 and 2500 UX/kg, respectively, (Cadogan et al. 2002; Silva and Smithard, 2002). Recently, however, Barrera et al. (2004) supplemented wheat-based diets for growing pigs with xylanase at 5,500, 11,000 and 16,500 XU/kg and reported increased AID of CP and AA after increasing the level of xylanase in these diets from 5,500 to 11,000 U/kg but not beyond 11,000 U/kg. This suggests xylanase at higher than currently recommended doses might be more effective in hydrolysing arabinoxylans, but this would most likely depend on cost of the xylanase and others factors that affect the efficacy of the enzyme such as dietary level and composition of arabinoxylans.

2.15.4 Age of Animal. The response to xylanase generally reduces with age and it is attributed to increased fermentation capacity of microbes in the GIT and the ability of the host animal to secrete digestive enzymes (Bedford, 1995). Palander et al. (2005) investigated the effect of supplementing wheat-based diets for turkeys with an enzyme preparation containing activity of xylanase activity and of β-glucanase (an enzyme that hydrolyses β-glucans, major NSP found in some cereal grains like barley) on digesta viscosity at different ages. They found greater reduction in small intestinal digesta at 4 wk of age (by 29 percentage units) than at 8 wk of age (15 percentage units) and 12 wk of age (11 percentage units). Boros et al. (2002) similarly reported greater improvement in fat digestibility in broiler chicks at 7 d of age than at 21 d of age (14 vs 9 percentage units). In pigs, Simmins and Wiseman (2003) found the improvement in ADG of pigs after supplementation of an enzyme preparation contining xylanase and β-glucanase to be twice as much in the growing phase than in the finisher phase (7.6 vs 3.7%).

2.16 Effect of Combining Phytase and Xylanase

In wheat, both PA and arabinoxylans are concentrated in aleurone cells (Guillon et al., 2004; Joyce et al., 2005). Thus, supplemental phytase and xylanase are expected to act synergistically in improving nutrient digestibility and performance of pigs and broilers because: (i) xylanase can hydrolyse arabinoxylans, which are located in the cell walls to increase the accessibility of phytase to PA, which is located within the cells, and (ii) by reducing the digesta viscosity, xylanase can increase the absorption of nutrients released by the action of phytase (Selle et al., 2003b; Kim et al., 2005a, b). There is, however, limited and inconsistent information on the effect of combining phytase and xylanase on nutrient digestibility and performance of pigs and broilers. Selle et al. (2003b) supplemented wheat-based diets for broilers with phytase and xylanase alone and in combination and observed a synergistic interaction between phytase and xylanase on BWG and AA digestibility. A combination of phytase and xylanase improved BWG and overall ileal digestibility of AA in broilers at 28 d of age by 7.3 and 4.5%, respectively, which were greater than improvements observed for phytase (5.6 and 3.6%) and xylanase (1.7 and 0.4%) individually. Ravindran et al. (1999a) similarly reported greater improvement in AME of wheat-based diets for broilers (by 19%) when phytase and xylanase were supplemented in combination than when the two enzymes were supplemented alone (5.3% for phytase and 9.7% for xlanase). Wu et al. (2004a), however, did not observe any synergistic interaction between phytase and xylanase on nutrient utilisation and performance of broilers fed wheat-based diets. Cadogan and Selle

(2000) and Kim et al. (2005b) also did not observe any synergism between the two enzymes on nutrient utilisation and performance of nursery pigs fed wheat-based diets.

Synergism between phytase and xylanase on nutrient utilisation and performance occurs when xylanase hydrolyses arabinoxylans to increase the accessibility of phytase to PA (Kim et al., 2005b). This implies that it can only occur when arabinoxylans and digesta viscosity are limiting the accessibility of phytase to PA. Thus, the variable response to supplementation of a combination of phytase and xylanase could be due to differences in the concentration and composition of arabinoxylans present.

2.17 Conclusion

It can be concluded that PA and arabinoxylans in wheat-based diets for pigs and poultry can reduce nutrient utilisation and performance, and that phytase and xylanase supplementation can alleviate the antinutritional effects of PA and arabinoxylans, respectively. However, the effectiveness of phytase depends on dietary levels of Ca and available P, source and level of phytase, age of the animals, and type of ingredient, whereas that of xylanase depends on concentration and composition of arabinoxylans in the diet (wheat), source and level of xylanase, and age of the animal.

Most PA in wheat is located in the aleurone cells and thus the presence of arabinoxylans in walls of the aleurone cells can reduce the efficacy of phytase because they (arabinoxylans) can limit the accessibility to PA by phytase. Thus, addition of xylanase to phytase-supplemented wheat-based-diets could increase the efficacy of phytase because xylanase can hydrolyse arabinoxylans to increase the accessibility of

phytase to PA. There is, however, limited and inconsistent information on the effect of combining phytase and xylanase on nutrient utilisation and performance of pigs and poultry. There is thus need for more information on the effect of combining phytase and xylanase on nutrient utilsation and performance of pigs and poultry fed wheat-based diets.

It was hypothesised that phytase and xylanase individually will improve nutrient utilisation and performance of pigs and broilers, and that a combination of the two will result in a synergistic improvement in nutrient utilisation and performance.

The objectives of the study were:

1. To determine the effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on ileal and total tract nutrient digestibility, and on performance of growing pigs

2. To determine the effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on digesta viscosity, ileal and total tract nutrient digestibility, tibia ash, and performance of broiler chicks.

3.0 MANUSCRIPT I

NUTRIENT DIGESTIBILITY AND PERFORMANCE RESPONSES OF GROWING PIGS FED PHYTASE AND XYLANASE SUPPLEMENTED WHEAT-BASED DIETS[1]

3.1 ABSTRACT Three experiments were conducted to evaluate the effect of supplementing phytase and xylanase on nutrient digestibility and performance of growing pigs fed wheat-based diets. In experiment (**Exp**). 1, 10 diets were fed to 60 pigs balanced for sex from 20 to 60 kg body weight (**BW**) to determine the effect of combining phytase and xylanase on apparent total tract digestibility (**ATTD**) of nutrients at about 20 and 60 kg BW, and performance. The 10 diets included a positive control (**PC**) diet (0.23% available P; 0.60% Ca), and a negative control (**NC**) diet (0.16% available P; 0.50% Ca) supplemented with phytase at 0, 250 and 500 FTU/kg and xylanase at 0, 2000 and 4000 XU/kg in a 3 x 3 factorial arrangement. In Exp. 2, 6 barrows (initial BW = 35.1 kg) cannulated at the distal ileum were fed 4 wheat-based diets in a 4 x 4 Latin square design with 2 added columns to determine the effect of combining phytase and xylanase on apparent ileal digestibility (**AID**) of nutrients. The 4 diets were NC used in Exp. 1 either without or with phytase at 500 FTU/kg and xylanase at 4000 XU/kg alone or in combination. In Exp. 3, 36 barrows (initial BW = 55.5 kg) were fed 4 diets based on pre-pelleted (at 80°C) and crumbled wheat for 2 wk to determine the effect of phytase

[1]I actively participated in statistical analysis and interpretation, but not collection of data for Exp. 3

supplementation on ATTD of nutrients. The 4 diets fed were a PC (0.22% available P; 0.54% Ca), and a NC diet (0.13% available P; 0.43% Ca) alone or with phytase at 500 or 1000 FTU/kg. All diets in the three experiments contained chromic oxide (0.5% in Exp. 1 and 2, and 0.2% in Exp. 3) as an indigestible marker. No synergistic interactions ($P > 0.05$) were detected between phytase and xylanase for any of the response criteria measured in Exp. 1 and 2. There were no dietary effects ($P > 0.05$) on performance in Exp. 1. In Exp. 1, phytase at 250 FTU/kg increased ATTD of P and Ca at 20, and at 60 kg BW by 51 and 11%, 54 and 10%, respectively, but increasing the level of phytase to 500 FTU/kg only increased ($P < 0.05$) ATTD of P at 20 kg BW. In Exp. 2, phytase at 500 FTU/kg increased ($P < 0.05$) AID of P and Ca by 21 and 12%. In Exp. 3, phytase at 500 FTU/kg improved $P < 0.05$) ATTD of P by 36%, but with no further effect at higher level of phytase. Xylanase at 4000 XU/kg improved ($P < 0.05$) AID of lysine, leucine, phenylalanine, threonine, aspartic acid, glycine and serine in Exp 2. In conclusion, phytase and xylanase respectively improved P and AA digestibilities, but did not interact synergistically probably due to a low arabinoxylans content in the NC diet.

3.2 INTRODUCTION

Wheat is a major pig feed ingredient in Canada (AAFC, 2005). However, wheat, like most other vegetable feed ingredients, is not a good source of P because most of its P is bound to phytic acid (**PA**), which is poorly digested by pigs (Bedford, 2000). Phytic acid can also reduce the digestibility of other nutrients by binding the nutrients, and digestive enzymes in the gut (Lenis and Jongbloed, 1999). In addition to PA, wheat contains non-starch polysacchrides (**NSP**) in its cell wall, which are indigestible and

capable of reducing nutrient digestibility by encapsulation and by increasing digesta viscosity (Kim et al., 2005a). Phytic acid and NSP in wheat can thus reduce efficiency of nutrient utilisation and increase environmental pollution due to excessive excretion of unabsorbed nutrients, especially N and P (Lenis and Jongbloed, 1999). Supplementation of wheat-based diets with phytase and xylanase may alleviate the negative effects of PA and NSP because phytase and xylanase can hydrolyse PA and arabinoxylans, respectively (Bedford, 2000). There is, however, limited information on the effect of combining phytase and xylanase on nutrient utilisation in pigs fed wheat-based diets. In wheat, both PA and arabinoxylans are highly concentrated in the aleurone cells (Joyce et al., 2005). Phytase and xylanase could thus act synergistically in improving the nutritive value of wheat-based diets for pigs because xylanase can hydrolyse arabinoxylans in cell wall to release PA for the action of phytase. Reports on the effect of combining phytase and xylanase in wheat-based diets are, however, inconsistent. For instance, Selle et al. (2003b) have reported synergism between the 2 enzymes in broilers, but Kim et al. (2005b) could not show similar effects in pigs. The objective of this study was to determine the effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on nutrient digestibility and performance of growing pigs.

3.3 MATERIALS AND METHODS

Three experiments were conducted at the T. K. Cheung Center for Animal Science Research, University of Manitoba. The phytase and xylanase used in the experiments were Phyzyme® XP (5,000 FTU/g) and Porzyme® 9300 (4,000 XU/g),

respectively, from Danisco Animal Nutrition (Marlbourough, UK). The cultivar of the wheat used in making diets was AC-Barrie, and was grown at Glenlea Research Farm, University of Manitoba and harvested during Fall, 2004. All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and pigs were handled in accordance with guidelines described by the Canadian Council on Animal Care (CCAC, 1993).

3.3.1 Experiment 1

The experiment was conducted to determine the effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on ATTD of nutrients and on performance of growing pigs from 20 to 60 kg BW. Sixty Cotswold growing pigs, obtained from the Glenlea Swine Research Unit, University of Manitoba, and weighing approximately 20 kg, were used in the experiment. Pigs were divided on the basis of BW and sex into 3 groups of 20 pigs each (10 barrows and 10 gilts), housed individually in pens (1.5 x 1.2 m) with smooth sides and plastic covered expanded metal floors and fed the experimental diets until they reached approximately 60 kg. The actual initial and final BW of all pigs were 19.9 ± 1.2 and 60.2 ± 2.4 (mean ± SD) kg, respectively. The length of time each pig stayed on the experimental diets varied depending on its growth rate.

The 10 diets used in the experiment included a PC diet and a NC diet supplemented with phytase at 0, 250 and 500 FTU/kg and xylanase at 0, 2000 and 4000 XU/kg in a 3 x 3 factorial arrangement to give nine treatment combinations (Tables 3.1 & 3.2). The PC was formulated to meet or exceed the NRC (1998) nutrient requirements for growing pigs weighing between 20 and 50 kg. The NC was similar to the PC except that

Table 3.1. Ingredient and chemical composition of the basal diets, as fed basis

Item	Diets used in Exp. 1 and 2		Diets used in Exp. 3[1]	
	Positive control (PC)	Negative control (NC)	Positive control (PC)	Negative control (NC)
Ingredient (%)				
Wheat	60.22	62.69	56.00	56.00
Wheat middling	8.95	8.95	-	-
Corn	-	-	20.00	20.00
Soybean meal	6.00	5.50	13.79	14.31
Canola meal	10.00	10.00	-	-
Peas	10.00	10.00	-	-
Canola oil	2.32	0.73	3.50	3.50
Biophos	0.23	-	-	-
Dicalcium phosphate	-	-	0.50	-
Limestone	1.09	0.94	0.90	0.90
Iodized salt	0.30	0.30	0.50	0.50
L-Lys	0.27	0.27	0.11	0.09
DL-Met	0.02	0.02	-	-
L-Thr	0.10	0.10	-	-
Premix[2]	0.50	0.50	4.50	4.50
Calculated nutrient content				
DE, kcal/kg	3358	3283	3404	3423
CP, %	18.24	18.34	15.48	15.73
Ca, %	0.60	0.50	0.54	0.43
Total P, %	0.53	0.49	0.45	0.36
Available P, %	0.23	0.16	0.22	0.13
Digestible amino acids, %				
Lysine	0.86	0.85	0.75	0.75
Methionine	0.25	0.25	0.26	0.26
Threonine	0.55	0.55	0.54	0.55
Analysed composition				
DE, kcal/kg[3]	3495	3571	3633	3630
CP, %	18.64	18.30	19.04	19.11
Ca, %	0.77	0.60	0.74	0.57
Total P, %	0.53	0.47	0.58	0.44
NSP, %	9.91	9.56	-	-

[1]Wheat was pelleted at 80°C and crumpled prior to diet formulation to inactivate endogenous phytase. [2]Supplied per kilogram of diet: vitamin A, 8250 IU; vitamin D_3, 825, IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B1, 1 mg; vitamin B2, 5 mg; niacin, 35 mg; pantothenic acid, 15; vitamin B12, 25 µg; biotin 200 µg; folic acid, 2 mg; Cu, 15 mg, iodine, 0.21; Fe, 100; Mn, 20 mg; Se, 0.15 mg and Zn, 100 mg. [3]The DE for basal diets for Experiment 1 was determined at 20 kg BW.

Table 3.2. Enzyme dosages and analysed activities for the diets

Item	Phytase, FTU/kg		Xylanase, XU/kg	
Diets used in Exp. 1 and 2				
1 (PC)	0	(717)[1]	0	(122)
2 (NC)	0	(690)	0	(116)
3	250	(943)	0	(161)
4	500	(1039)	0	(102)
5	0	(618)	2000	(1578)
6	250	(708)	2000	(3866)
7	500	(1043)	2000	(1901)
8	0	(702)	4000	(4152)
9	250	(802)	4000	(5160)
10	500	(912)	4000	(5559)
Diets used in Exp. 3				
1 (PC)	0	(198)	0	ND[2]
2 (NC)	0	(227)	0	ND
3	500	(526)	0	ND
4	1000	(1114)	0	ND

[1]Values in parentheses represent results from enzyme analysis
[2]ND = not determined

Ca and available P contents were reduced by 16 and 30%, respectively, to maximize response to enzyme supplementation. The diets were fed as mash.

The experiment was conducted as a completely randomized block design with the three groups as blocks. The 10 diets were randomly allocated to pigs in such a manner that each diet was assigned to 1 barrow and 1 gilt from each group to give 2 replicates per diet in each group and hence 6 replicates per diet in overall. Body weight and feed consumption were recorded weekly for calculation of average daily feed intake (**ADFI**), average daily gain (**ADG**) and gain to feed ratio (**G:F**). Chromic oxide (Cr_2O_3) was added to all diets at a rate of 0.5% as an indigestible marker and fed during the first and last 10 days of the experiment to determine ATTD of nutrients at approximately 20 and 60 kg BW. Samples of each diet and wheat were collected at the start of the experiment, and representative fecal samples collected from each pen over the last 3 days (**d**) of Cr_2O_3 feeding were stored at –20°C until required for analysis.

3.3.2 Experiment 2

This experiment was conducted to determine the effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on AID of nutrients in growing pigs. Six crossbred barrows (Yorkshire-Landrace♀ and Duroc♂), obtained from a local commercial swine herd (Genesus Genetics, MB, Canada), and with an average body weight of 35.1 ± 1.6 (mean ± SD) kg were used in this experiment. Pigs were housed in adjustable metabolic crates with smooth, transparent plastic sides and plastic

covered expanded metal floor. After a 7-d adaptation period, pigs were surgically fitted with a simple T-cannula at the distal ileum as described by Nyachoti et al. (2002). After surgery, pigs were returned to the metabolic crates and allowed a 14-d recovery period. During this period they were fed twice daily increasing amounts of a commercial grower diet and had unlimited access to water.

After the recovery period, pigs were fed 4 experimental diets, which consisted of a NC same as that used in Exp. 1 or NC supplemented with phytase at 500 FTU/kg, xylanase at 4000 XU/kg, or phytase at 500 FTU/kg plus xylanase at 4000 XU/kg (Tables 3.1 and 3.2). All diets contained Cr_2O_3 (0.5%) as an indigestible marker and were fed as mash. The experiment was conducted according to a 4 x 4 Latin square design with 2 added columns. Each period consisted of 9 d; the first 7 d were for adaptation and the last 2 d for ileal digesta collection. Pigs were fed the diets at 2.6 times maintenance energy requirement (ARC, 1981) based on their BW at the beginning of each period. Daily feed allowance was offered in 2 equal portions at 0800 and 1530. Ileal digesta were collected continuously for 12 h from 0800 to 2000 on d 8 and 9 as described by Nyachoti et al. (2002) and stored at –20°C until required for analysis.

3.*3.3 Experiment 3*

This experiment was conducted to determine the effect of supplementing phytase to wheat-based diets on ATTD of nutrients in growing-finishing pigs. Thirty-six Yorkshire growing-finishing barrows, obtained from a commercial swineherd in Manitoba (Iceman Genetics, MB, Canada), and with an average BW of 55.5 kg were

utilized in this trial. Pigs were grouped based on BW into 3 groups, and housed individually in pens similar to those that were used in Exp. 1. After a 4 d acclimatization period to the new surroundings, during which period they were fed a common commercial grower diet, pigs were randomly assigned to 4 experimental diets, which included a PC, and a NC diet either unsupplemented or supplemented with phytase at 500 or 1000 FTU/kg (Tables 3.1 and 3.2). The PC diet was formulated to meet or exceed the NRC (1998) nutrient requirements of growing pigs weighing between 50 and 80 kg. The NC was the same as the PC except that Ca and available P contents were reduced by 20 and 35%, respectively, to maximize response to enzyme supplementation. The wheat used in the diets was pre-pelleted at 80°C in order to inactivate any endogenous phytase and then crumbled prior to diet mixing. The enzyme was added to the diets at a higher rate (500 and 1000 FTU/kg) than in Exp. 1 (250 and 500 FTU/kg) and 2 (500 FTU/kg) because pelleting of wheat used in this experiment (Exp. 3) was expected to reduce the endogenous phytase activity in the basal diets. All diets contained Cr_2O_3 (0.2%) as an indigestible marker.

The experiment was conducted as a randomized complete block design with the groups as blocks. The pigs were assigned to the experimental diets in such a manner that each diet was assigned to 3 pigs within each group to give nine replicates per diet. The experiment lasted for 2 wk. Representative fecal samples were collected from each pig during the last 3 d and stored frozen at –20°C until required for analysis.

3.3.4 Sample Preparation and Chemical Analyses

Fecal samples for Exp. 1 were dried in an oven at 60°C for 4 days and pooled for each pig and period of collection (i.e., first and last ten days of the experiment), finely ground in a grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT), and thoroughly mixed for analysis. Ileal digesta samples for Exp. 2 were pooled for each pig and each period, homogenized in a blender (Waring Commercial, Torrington, CT), sub-sampled, freeze-dried, and ground as described previously for Exp. 1 for analysis. Fecal samples for Exp. 3 were dried as those for Exp. 1, pooled for each pig, and further prepared for analysis as described for Exp. 1. Diet samples for all the three experiments were similarly ground for analysis. All samples (diet, ileal digesta and feces) were analysed for dry matter (**DM**), gross energy (**GE**), crude protein (**CP**), Ca, P and Cr. Diet samples were additionally analysed for NSP (Exp. 1) and enzymes (phytase; Exp. 1, 2 & 3; and xylanase; Exp. 1 & 2) whereas diets and ileal digesta for Exp.2 were additionally analysed for amino acids (**AA**).

Dry matter was determined according to the method of AOAC (1990) and GE was determined using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Crude protein (N x 6.25) was determined using a N analyser (Model NS-2000; LECO Corporation, St. Joseph, MI). Samples for Ca and P analyses were ashed and digested according to procedures described by AOAC (1990) and read on a Varian Inductively Coupled Plasma Mass Spectrometer (Varian Inc, Palo Alto, CA, USA). Diets and ileal digesta samples (Exp. 2) for AA analysis were prepared by acid hydrolysis according to the method of AOAC (1984), and as modified by Mills et al. (1989). Briefly, about 100 mg of each sample was digested in 4 mL of 6 *N* HCl for 24 h at 110°C,

followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then made up to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an LKB 4151 Alpha plus AA analyzer (LKB Biochrom, Cambridge, UK). Samples for analysis of sulphur containing AA (methionine and cysteine) were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not determined. Samples for Cr analysis were ashed and digested according to procedures described by William et al. (1962) and read on Varian Inductively Coupled Plasma Mass Spectrometer (Varian Inc, Palo Alto, CA). Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The neutral sugars were analysed as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990), whereas uronic acids were determined using the procedure described by Scott (1979). Analysis of phytase and xylanase in diets was carried out at Danisco Animal Nutrition (Marlbourough, UK).

3.3.5 Calculations and Statistical Analysis

Apparent ileal and total tract digestibility coefficients were calculated using the following equation:

$$\% \text{ apparent nutrient digestibility} = 100-[(C_d/C_f * N_f/N_d)*100]$$

where: C_d = Cr_2O_3 concentration in diet (% DM); C_f = Cr_2O_3 concentration in feces or ileal digesta (% DM); N_f = nutrient concentration feces or ileal digesta (% DM); N_d = nutrient concentration in the diet (% DM). Digestible energy (**DE**) content of the positive and negative control diets was calculated using the following equation:

DE (Kcal/kg) = [(energy digestibility of diet, %) * (gross energy value of diet, Kcal/kg)]/100

Data from the 3 experiments were subjected to ANOVA using Mixed procedure (SAS, 2002). The differences between PC and NC diets, and main effects of enzymes and their interactions in Exp. 1 were determined using the following contrasts:

PC vs NC 'diet -1 1 0 0 0 0 0 0 0 0 '

Main effect of phytase 'diet 0 1 0 -1 1 0 -1 1 0 -1
diet 0 0 1 -1 0 1 -1 0 1 -1'

Phytase level (250 vs 500 FTU/kg) 'diet 0 0 -1 1 0 -1 1 0 -1 1'

Main effect of xylanase 'diet 0 1 1 1 0 0 0 -1 -1 -1
diet 0 0 0 0 1 1 1 -1 -1 -1'

Xylanase level (2000 vs 4000 XU/kg) 'diet 0 0 0 0 -1 -1 -1 1 1 1'

Phytase*xylanase interaction 'diet 0 1 0 -1 0 0 0 -1 0 1
diet 0 0 0 0 1 0 -1 -1 0 1
diet 0 0 1 -1 0 0 0 0 -1 1
diet 0 0 0 0 0 1 -1 0 -1 1'

Main effects of enzymes and their interactions in Exp. 2 were determined using the following contrasts:

Main effect of phytase 'diet 2 -1 0 -1'

Main effect of xylanase 'diet 2 0 -1 -1'

Phytase*xylanase interaction 'diet 1 -1 -1 1'

The differences between PC and NC diets, and effects of phytase in Exp. 3 were determined using the following contrasts:

PC vs NC 'diet -1 1 0 0'

Main effect of phytase 'diet 0 2 -1 -1'

Phytase level (500 vs 1000 FTU/kg) 'diet 0 0 1 -1'

In all the three experiments, differences were considered significant at $P < 0.05$.

3.4 RESULTS

3.4.1 Experiment 1

The analysed chemical composition of the PC and NC diets and enzyme activities of the 10 dietary treatments are presented in Tables 3.1 and 3.2, respectively. The analysed values of CP and total P were similar to calculated values whereas those of DE and Ca were higher than calculated values in Table 3.1. The PC and NC diets were similar in NSP and endogenous phytase and xylanase activities. The addition of phytase and xylanase enzymes in diets generally resulted in increased activities of the respective enzymes by margins similar to those that were anticipated except for diets 6, 9 and 10 whose xylanase activity was increased by a much higher margin than anticipated after adding the xylanase enzyme (Table 3.2). The ADFI, ADG and G:F were not affected ($P > 0.05$) by dietary treatment (Table 3.3).

Table 3.4 shows the ATTD values. No interactions were detected ($P > 0.05$) between phytase and xylanase on the ATTD of the components measured in this study. The PC and NC had similar ($P > 0.05$) ATTD values for all nutrients measured in this study except for DM at 20 kg BW and Ca at both 20 and 60 kg BW whose digestibilities were lower ($P < 0.05$) for PC than for NC. Phytase supplementation did not influence ($P > 0.05$) DE and ATTD of DM and CP and xylanase supplementation had no effect ($P >$

Table 3.3. Feed intake and performance of growing pigs fed wheat-based diets in Experiment 1

Diet		Parameters		
		ADFI (kg/d)	ADG (kg/d)	G:F (kg/kg)
PC		1.444	0.776	0.538
NC		1.479	0.784	0.531
Phytase	0 FTU/kg	1.492	0.790	0.530
	250 FTU/kg	1.448	0.787	0.544
	500 FTU/kg	1.478	0.811	0.549
Xylanase	0 XU/kg	1.459	0.787	0.539
	2000 XU/kg	1.474	0.808	0.549
	4000 XU/kg	1.486	0.793	0.535
SEM		0.050	0.030	0.014
Contrasts[1]				
PC vs NC		NS	NS	NS
Phytase		NS	NS	NS
Phytase level		NS	NS	NS
Xylanase		NS	NS	NS
Xylanase level		NS	NS	NS
Phytase*xylanase		NS	NS	NS

[1]PC = positive control; NC = negative control; NS = non-significant

Table 3.4. Apparent DE and total tract digestibility of DM, CP, Ca and P in growing pigs fed wheat-based diets in Experiment 1

		Digestibility of nutrient, % DM[1]									
		DE, kcal/kg		DM		CP		Ca		P	
Diet		20 kg	60 kg	20 kg	60 kg	20 kg	60 kg	20 kg	60 kg	20 kg	60 kg
PC		3495	3617	78.2	81.9	76.3	82.7	43.6	44.3	27.1	24.0
NC		3571	3594	81.0	81.9	78.7	81.4	52.8	57.0	24.2	22.8
Phytase	0	3574	3639	81.3	83.0	78.0	82.2	55.9	59.6	22.4	23.2
	200	3593	3671	81.7	83.8	79.1	84.0	61.8	65.4	33.8	35.7
	500	3581	3661	81.6	83.5	79.1	83.3	64.1	64.2	38.1	36.2
Xylanase	0	3568	3638	81.1	82.9	78.7	83.3	57.4	60.7	31.1	32.3
	2000	3576	3642	81.3	83.1	78.8	82.6	60.2	63.8	31.2	31.0
	4000	3605	3689	82.2	84.2	78.8	83.6	64.2	64.8	32.0	31.8
SEM		22	17	0.50	0.37	0.72	0.62	1.52	1.49	1.39	1.56
Contrast[2]											
PC vs NC		NS	NS	*	NS	NS	NS	*	**	NS	NS
Phytase		NS	NS	NS	NS	NS	NS	**	**	**	**
Phytase level		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Xylanase		NS	NS	NS	*	NS	NS	**	NS	NS	NS
Xylanase level		NS	*	NS	*	NS	NS	NS	NS	NS	NS
Phytase*xylanase		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]20 kg = digestibility at 20 kg BW; 60 kg = digestibility at 60 kg BW.
[2]PC = positive control; NC = negative control; NS-non=significant; * = $P < 0.05$; ** = $P < 0.0.1$

0.05) on these response criteria except for DE and ATTD of DM at 60 kg BW, which were slightly but significantly increased ($P < 0.05$) by xylanase supplementation at 4000 XU/kg.

Phytase supplementation at 250 FTU/kg increased ($P < 0.05$) ATTD of Ca and P, but increasing the level of phytase to 500 FTU/kg only further increased ($P < 0.05$) the ATTD of P at 20 kg BW. Xylanase supplementation had no influence ($P > 0.05$) on ATTD of P, but at 4000 XU/kg it increased ($P < 0.05$) the ATTD of Ca at 20 kg BW.

3.4.2 Experiment. 2

Data for AID measurements are presented in Table 3.5. No interactions were detected ($P > 0.05$) between phytase and xylanase on AID of all nutrients measured in this experiment except for a antagonistic interaction ($P < 0.05$) on AID of lysine, alanine, aspartic acid and glycine. The effect of either of the enzymes separately was similar ($P > 0.05$) to the combination. Phytase supplementation did not affect ($P > 0.05$) DE, and the AID of DM, CP and AA, but increased ($P < 0.05$) the AID of Ca and P. Xylanase supplementation also did not affect ($P > 0.05$) ileal DE, and the AID of DM, Ca and P. Xylanase, however, increased ($P < 0.05$) the AID of amino acids, lysine, leucine, phenylalanine, threonine, glycine and serine.

Table 3.5. Apparent ileal DE and nutrient digestibility of growing pigs fed wheat-base diets in Experiment 2

	Diets[1]					Contrasts[2]		
Nutrient	Control	Control + + phy	Control + xyl.	Control + phy + xyl	SEM	Phytase	Xylanase	Phy*xyl
DE, kcal/kg	2824	2864	2860	2872	34.0	NS	NS	NS
Digestibility, %								
DM	70.9	72.2	72.6	72.2	0.90	NS	NS	NS
CP	80.9	82.6	83.4	82.8	0.77	NS	NS	NS
P	42.1	53.2	44.0	51.3	2.06	**	NS	NS
Ca	47.2	57.1	55.5	57.5	2.38	*	NS	NS
AA								
Arginine	86.7	89.3	89.0	89.6	0.91	NS	NS	NS
Histidine	85.1	87.0	88.9	86.0	1.17	NS	NS	NS
Isoleucine	79.0	82.2	82.6	81.6	1.75	NS	NS	NS
Leucine	82.9	85.5	86.1	85.4	0.86	NS	*	NS
Lysine	83.4	85.6	87.1	85.1	0.92	NS	*	*
Methionine	85.6	85.7	85.5	81.3	1.40	NS	NS	NS
Phenylalanine	86.0	89.0	89.4	88.6	0.98	NS	*	NS
Threonine	76.6	81.2	81.7	81.4	1.23	NS	*	NS
Valine	77.0	81.5	81.8	79.6	1.60	NS	NS	NS
Alanine	77.8	81.5	82.8	80.4	1.01	NS	NS	*
Aspartic acid	77.6	81.5	82.5	80.7	1.09	NS	NS	*
Cysteine	78.1	79.1	76.9	78.7	2.03	NS	NS	NS
Glutamic acid	90.8	92.4	93.4	92.7	0.63	NS	NS	NS
Glycine	75.0	80.1	81.5	78.7	1.70	NS	*	*
Proline	87.4	89.7	90.1	90.2	0.91	NS	NS	NS
Serine	80.3	84.5	85.2	84.5	1.17	NS	*	NS
Tyrosine	82.2	87.1	86.2	86.2	1.22	NS	NS	NS

[1]Phy = control + phytase at 500 FTU/kg; Xyl = control + xylanase at 4000 XU/kg; Phy+xyl = control + phytase at 500 FTU/kg and xylanase at 4000 XU/kg.
[2]Phy*xyl = interaction between phytase and xylanase; NS-non=significant; * = $P < 0.05$; ** = $P < 0.01$

3.4.3 Experiment. 3

Analysed chemical composition of the PC and NC diets and of phytase activity in the four diets are presented in Tables 3.1 and 3.2, respectively. The analysed values of DE, CP, Ca and total P were higher than the calculated values in Table 3.1. The addition of phytase at 500 and 1000 FTU/kg in the NC diet generally resulted in increased activities by margins similar to those that were anticipated. Data for DE and ATTD measurements are presented in Table 3.6. The PC diet had a higher value of ATTD for P was higher than the NC diet ($P < 0.05$). The 2 diets were, however, similar ($P > 0.05$) in ATTD of all other components measured in this experiment. The DE and ATTD of CP and Ca were not influenced ($P > 0.05$) by phytase supplementation. Phytase supplementation increased ATTD of DM and P ($P < 0.05$) without further improvements with 1000 FTU/kg.

3.5 DISCUSSION

The growth performance of pigs fed the NC diet was similar to those fed the PC diet despite the lower Ca and P concentration in the former. Furthermore, enzyme supplementation to NC had no effect on pig performance in Exp. 1 despite the significant increase in Ca and P digestibilities. These results are contrary to the findings of Harper et al. (1997) who reported reduced performance of growing (19 to 52 kg BW) and finishing (52 to 109 kg BW) pigs fed corn-based diets due to reduction of dietary P and Ca from adequate (0.50 and 0.40% P, and 0.58 and 0.48% Ca during the grower and finisher phases, respectively) to inadequate (0.40 and 0.35% P, and 0.53 and 0.43% Ca for grower

Table 3.6. Digestible energy and total tract nutrient digestibility of growing-finishing pigs fed diets based on pre-pelleted and crumbled wheat in Experiment 3

	Diets[1]					Contrasts[2]		
Parameter	PC	NC	NC + 500 FTU/kg	NC + 1000 FTU/kg	SEM	PC vs NC	Phytase	Phytase level
DE, kcal/kg	3633	3630	3661	3659	13.7	NS	NS	NS
Digestibility, %								
DM	87.0	86.4	88.9	87.5	0.77	NS	*	NS
CP	87.1	86.0	87.1	87.2	0.47	NS	NS	NS
Ca	58.0	53.4	56.1	57.6	1.73	NS	NS	NS
P	47.6	40.0	54.5	53.8	1.69	**	**	NS

[1]Diets were based on pre-pelleted and crumbled wheat; PC = positive control, NC = negative control; NC + 500 FTU/kg = negative control plus phytase at 500 FTU/kg; NC + 1000 FTU/kg = negative control plus phytase at 1000 FTU/kg

and finisher phases, respectively) levels, and improved performance of the pigs due to phytase supplementation to the low Ca and available P diets. It should, however, be noted that in the current study, the basal diets used in Exp.1 were high in endogenous phytase, which can increase the digestibility of Ca and P. Thus, the lack of treatment effect on performance in Exp. 1 could be attributed to the fact that Ca and P were not limiting performance due to the presence of endogenous phytase in the basal diets. It appears that phytase supplementation would have increased performance if the endogenous phytase in the basal diets was inactivated, for example, by pelleting. It also appears that the performance of pigs would have been increased by phytase supplementation if the Ca and available P in the diets were reduced to lower levels than in the current study.

The ATTD of components measured in the current study were generally higher for the basal diets in Exp. 3 than Exp. 1. This could be attributed to increased availability of nutrients for digestion after pelleting, which results in cell and starch granule rupture, thereby releasing the nutrients (O'Doherty et al., 2001). It could also be attributed to the older age of the pigs that were used in Exp. 3 compared to Exp. 1. This is because the digestive capacity of animals increases with age due to an increase in mucosal surface area (Iji et al., 2001). The ATTD of P for the PC diet in Exp. 3 was higher ($P < 0.05$) than that for the NC whereas in Exp 1 it was numerically ($P > 0.05$) higher than that for the NC. Adeola et al. (2004) have also reported higher ATTD of P for a P-adequate diet compared to a P inadequate diet (0.93 vs 0.38%), and they attributed it to the higher concentration of inorganic (available) P in the PC diet than in the NC. In contrast, the ATTD of DM (20 kg BW) and Ca (20 and 60 kg BW) digestibilities for the PC diet in Exp. 1 were lower than the NC. The higher Ca digestibility in the NC diet could be

explained by lower Ca content in NC than in the PC diet, which was adequate in Ca. Low dietary Ca level, is known to result in increased efficiency of Ca absorption from the gastro-intestinal tract (**GIT**) in an effort to maintain normal plasma Ca level (Weaver et al., 1996). Traylor et al. (2001) have also reported increased Ca digestibility in growing pigs due to a reduction in dietary Ca concentration. The increased DM digestibility in the NC diet could have been due to increased digestibility of Ca and other nutrients (not measured in this study) due to its increased efficiency of absorption from the GIT.

Approximately 60 to 70% of P in plant feedstuffs is PA-bound (Maenz, 2001). The bioavailability of this PA-bound P for non-ruminants such as pigs is low since these animals do not produce a sufficient amount of phytase enzyme required to hydrolyze PA to release P for absorption and utilization (Bedford, 2000). Supplementation with exogenous phytase can, however, increase the availability of P (Maenz, 2001). Several studies have shown that phytase supplementation results in increased digestibility of P. Adeola et al. (2004) reported that phytase supplementation at 250 FTU/kg to corn-based diets increased ATTD of P in growing pigs by at least 20%, whereas Kim et al. (2005b) observed that ATTD of P increased by 35% after supplementation of wheat-based diets for weaner pigs with phytase at 500 FTU/kg. Similarly, Radcliffe et al. (2006) reported that AID of P in growing pigs increased by 17% after supplementing a corn-based diet with phytase at 500 FTU/kg. Results of the present study are consistent with findings of these studies. In the current study, however, an increase in phytase supplementation from 250 to 500 FTU/kg did not result in a significant increase in ATTD of P at 60 kg in Exp. 1. Harper et al. (1997) similarly reported a non-significant increase in ATTD of P in pigs heavier than 50 kg due to increasing the phytase supplementation level from 250 to 500

FTU/kg. This lack of effect of increasing the level of supplemental phytase on P digestibility in pigs heavier than 50 kg compared to those lighter than 50 kg could be attributed to the fact the NC diet was not defcient enough in available P to realize the positive effect of phytase because it (NC diet) was formulated to contain 0.16% available P whereas the requirement of available P by pigs heavier than 50 kg according to NRC (1998) is 0.19%.

Supplementation of phytase at 500 FTU/kg to the NC diet resulted in a similar improvement in ATTD of P in Exp. 1 (at 60 kg BW) and 3 (15.3 vs 14.5 percentage units), which was surprising and unexpected. It had been assumed that phytase supplementation to the NC diet in Exp. 3 compared to Exp. 1 would result in greater improvement in P digestibility because wheat used in Exp. 3 had been pelleted at 80°C, which is most likely to inactivate most of the endogenous phytase. This lack of difference in improvement of ATTD of P in the 2 experiments due to phytase supplementation could be attributed to higher P digestibility (40.0 vs 22.8%) for the NC diet in Exp. 3 than Exp. 1. The response to phytase supplementation with regard to P digestibility has been shown to be high when the P digestibility in the basal diet is low (Johnston et al., 2004).

Phytic acid in its natural state in feedstuffs is complexed with minerals and protein, and in the stomach and small intestine has potential to complex positively charged nutrients and endogenous enzymes that are involved in nutrient digestion, thereby reducing nutrient digestibility, because it is negatively charged at all pH conditions in the GIT (Lenis and Jongbloed, 1999; Maenz, 2001). Thus, by hydrolysing PA, phytase is not only expected to increase the digestibility of P, but of other nutrients as well. In the current study, phytase supplementation increased both AID (Exp. 2) and

ATTD (Exp. 1) of Ca, which is in agreement with results of Johnston et al. (2004) who reported improved AID and ATTD of Ca in growing-finishing pigs due to phytase supplementation. The ATTD of Ca in Exp. 3 was, however, unaffected by phytase supplementation. Also, increasing the level of phytase from 250 to 500 FTU/kg in Exp. 1 did not result in significant increase in ATTD of Ca. The lack of effect of phytase on ATTD of Ca in Exp. 3 could be due to high digestibility of Ca in the large intestine of pigs used in Exp. 3 due to increased hindgut fermentation capacity. An increase in digesta fermentation in the hind gut can result in increased production of volatile fatty acids, which in turn, can acidify the digesta and enhance solubility and hence absorption of Ca (Kruger et al., 2003). The increased absorption in the hindgut can mask the actual effect of phytase on Ca digestibility as demonstrated by the results of Radcliffe et al. (2006) who reported increased Ca digestibility at the ileal but not fecal level of pigs heavier than 48 kg due to phytase supplementation. The lack of effect of increasing the level of phytase supplementation from 250 to 500 FTU/kg on ATTD of Ca in Exp. 1 could be due the high level of endogenous phytase in wheat that was used in this experiment. In the GIT, PA normally exerts its negative effect on mineral digestibility by binding them in the small intestine (Lenis and Jongbloed, 1999). Wheat phytase is capable of hydrolysing PA into partially dephosphorylated PA products (Schlemmer et al., 2001) that have low capacity to bind nutrients in the small intestine (Cowieson et al., 2006b). Thus, it is possible that the endogenous phytase in the basal diet plus supplemental phytase at 250 FTU/kg were adequate for hydrolysis of PA to partially dephosphorylated PA products that have low capacity to bind Ca.

In the present study, phytase supplementation did not affect the AID of AA and CP, and ATTD of CP, which is contrary to results of Mroz et al. (1994), but in agreement with those of Johnston et al. (2004). The effectiveness of phytase with regard to increasing protein/AA digestibility depends on the capacity of PA to bind the protein and the susceptibility of the PA-protein complex to phytase hydrolysis (Selle et al., 2006). The lack of effect of phytase on CP in Exp. 1 and 2, and on AA digestibilities in Exp. 2 could be due to the presence of the endogenous phytase in the basal diets used in these 2 experiments. Phytic acid mainly reduces protein digestibility by reacting with dietary protein and digestive enzyme (pepsin) at acidic conditions in the stomach to form binary PA-protein complexes (Lenis and Jongbloed, 1999). It can also reduce protein digestibility by reacting with divalent cations, dietary proteins and endogenous enzymes at less acidic pH in the small intestine to form ternary PA-mineral-protein complexes (Lenis and Jongbloed, 1999). It is, thus, possible that the endogenous phytase in the basal diet hydrolysed PA in the stomach, resulting in partially dephosphorylated PA that lacked capacity to bind the proteins in the stomach and in the small intestine.

The lack of effect of phytase supplementation on ATTD of CP in Exp. 3 in which the most of the endogenous phytase in the basal diets is likely to have been inactivated could be related to the age of pigs. The fermentative capacity of the pigs increases with age (Kim et al., 2005a). The hind gut hydrolysis of CP is thus most likely to have been high in pigs fed phytase unsupplemented diet due to increased substrate availability, thereby resulting in a lack of difference between pigs fed unsupplemented and phytase supplemented diets. Phytase supplementation has been reported to improve CP digestibility at ileal, but not at the fecal level (Radcliffe et al., 2006). Phytase

supplementation did not affect the digestibility of energy at both the ileal (Exp. 2) and fecal (Exp. 1 and 2) levels. Phytic acid negatively affects energy digestibility by binding dietary protein and lipids, and endogenous enzymes that are involved in digestion of energy sources (protein, carbohydrates, and lipids; Selle and Ravindran, 2006). The lack of effect of phytase supplementation on energy digestibility in Exp. 1 and 2 could thus be explained by the low capacity of PA to bind dietary protein and endogenous enzymes in the GIT due to the presence of endogenous phytase in the basal diets. In Exp. 3, however, it could be attributed to increased organic matter fermentation in the hindgut.

Nutrient utilisation in wheat-based diets is also limited by the presence of NSP in the wheat cell wall, which reduces the availability of nutrients in wheat for use by the animal by encapsulation (Kim et al., 2005a). Apart from limiting nutrient availability for digestion, NSP can adsorb endogenous enzymes in the GIT (Silva and Smithhard, 2002), thereby resulting in their increased secretion through a negative feedback mechanism (Wang et al., 2005). The arabinoxylans can also increase the endogenous N losses by promoting the growth of microorganisms in small intestine, which utilizes endogenous AA, resulting in in their (endogenous AA) reduced reabsorption (Bartelt et al., 2002). In the current study, xylanase supplementation at 4000 XU increased the AID of some AA (Exp. 2) and ATTD of Ca at 20 kg BW (Exp. 1), and marginally increased ATTD of energy at 60 kg BW (Exp. 1). The improved ATTD of Ca at 20 kg BW could have been due to increased absorption in hindgut. Supplementation of wheat-based diets with an enzyme preparation that is rich in xylanase has been found to increase total volatile fatty acid concentration in the cecum due to increased fermentation of enzyme degradation products in the same GIT segment (Wang et al., 2005). The volatile fatty acids can

acidify the digesta and enhance solubility and hence absorption of Ca (Kruger et al., 2003). The lack of effect of xylanase on ATTD of Ca at 60 kg BW could be due to both increased hindgut fermentation capacity and lower dietary requirement of these mineral at this BW. The improved AID of AA could be due to their increased availability of amino acids that are highly associated with arabinoxylans. The improved AID of AA could also be attributed to reduced secretion of endogenous AA due to hydrolysis of arabinoxylans by xylanase.

The influence of xylanase on nutrient digestibility depends on the amount of arabinoxylans in the diet and their association with nutrients (Kim et al., 2005a). Adeola and Bedford (2004) reported increased nutrient utilisation due to xylanase supplementation to wheat– based diets for White Pekin ducks when the diets had high (15.3 %), but not low (9.4 %) concentration of NSP. The NSP concentration in the negative control diet used in the current study was 9.56 %. The low response to xylanase supplementation with regard to digestibility of nutrients other than AA and Ca in the current study could thus be explained by the low dietary NSP (i.e., arabinoxylans) concentration.

In wheat, both PA and arabinoxylans are concentrated in aleurone cells (Joyce et al., 2005) and thus the presence of arabinoxylans in the walls of these cells can limit the accessibility of phytase to PA. It was, thus, hypothesised that phytase and xylanase could act synergistically in improving nutrient digestibility because xylanase can hydrolyse arabinoxylans to release PA for phytase action. In the current study, however, phytase and xylanase did not synergistically interact on any of the response criteria measured. Kim et al. (2005b) also did not observe synergistic interaction between phytase and

xylanase on nutrient utilisation and performance of weanling pigs fed wheat-based diets. Selle et al. (2003b), however, observed a synergistic effect of the two enzymes in broilers fed wheat-based diets. Synergism between phytase and xylanase on nutrient utilisation and performance occurs when xylanase hydrolyses arabinoxylans to increase the accessibility of phytase to PA (Kim et al. 2005b). This implies that synergism can only occur when arabinoxylans are limiting the accessibility of phytase to PA. Adeola and Bedford (2004) found xylanase to be effective with regard to improving nutrient digestibility and utilisation in wheat-based diets when diets were high (15.3% and 45.68 cps), but not low (9.4% and 5.86 cps) in NSP concentration and digesta viscosity, respectively. The basal diets used in the current study and that reported by Kim et al. (2005b) were low in NSP concentration (9.6 and 10.6 %, respectively). Although Selle et al. (2003b) did not report NSP concentration in the basal diet used in their study, it is most likely to have been high because the digesta viscosity (10.21 cps) for the same diet was higher than the 5.86 cps reported by Adeola and Bedford (2004) for the low NSP wheat-based diet. The lack of synergism between phytase and xylanase on nutrient utilisation and performance of pigs in the current study and that reported by Kim et al. (2005b) could thus be due to low NSP concentration of the basal diets.

In conclusion, phytase supplementation to wheat-based diets for growing pigs improved P and Ca digestibilities whereas xylanase supplementation increased Ca digestibility and apparent AA digestibility, but neither of them affected performance. Furthermore, there was no synergistic interaction between phytase and xylanse, which could probably be due to the high endogenous phytase activity and low NSP concentration in the basal diets.

4.0 MANUSCRIPT II

NUTRIENT UTILIZATION AND PERFORMANCE RESPONSES IN BROILERS FED WHEAT-BASED DIET

4.1 ABSTRACT An experiment was conducted to determine the effect of supplementing phytase and xylanase individually or in combination on nutrient utilisation and broiler performance. Three hundred and twenty male Ross broilers were divided into 80 groups of 4 birds balanced for body weight (**BW**) and fed 10 wheat-based diets (8 groups/diet) from 1 to 23 days (**d**) of age. The 10 diets were a positive control (**PC**) diet (0.90% Ca and 0.43% available P) and a negative control (**NC**) diet (0.82% Ca and 0.28% available P) supplemented with phytase at 3 levels, 0, 250 and 500 FTU/kg and xylanase at 3 levels, 0, 1250 and 2500 XU/kg in a 3 x 3 factorial arrangement to give 9 treatment combinations. Chromic oxide (0.3%) was added to the diets as a marker to determine nutrient digestibility. No interactions ($P > 0.05$) between phytase and xylanase were detected on any of the response criteria measured. Birds fed the NC diet had lower ($P < 0.05$) feed intake, BW gain and tibia ash than the PC diet. The 2 diets were, however, similar ($P > 0.05$) in feed conversion ratio. Phytase (250 FTU/kg) supplementation did not affect ($P > 0.05$) feed intake and BW gain. It, however, improved ($P < 0.05$) feed conversion ratio, tibia ash, apparent ileal digestibility (**AID**) of P, and apparent total tract digestibility (**ATTD**) of P and Ca by 2.0, 3.5, 17.7, 8.7 and 8.4%, respectively, but with no further effect ($P > 0.05$) on these responses at a higher level of phytase supplementation. Also, phytase at 500 FTU/kg did not improve ($P > 0.05$) tibia ash to that of the PC diet. Xylanase supplementation did not affect ($P > 0.05$) performance and mineral utilization responses except for ATTD of Ca, which was

increased ($P < 0.05$). Enzyme supplementation did influence ($P > 0.05$) the apparent ileal and total tract digestible energy, and AID of AA, and ATTD of N. In conclusion phytase supplementation improved P and Ca digestibilities and utilisation, but showed no synergistic effect with xylanase on any response criteria measured, probably due to the low concentration of NSP in the basal diet.

4.2 INTRODUCTION

Most P in vegetable feedstuffs is phytic acid (**PA**)-bound (Kornegay, 2001). Phytic acid-bound P is poorly hydrolysed by non-ruminants such as broilers because they have low capacity to hydrolyse PA (Bedford, 2000). Phytic acid can also bind other nutrients and digestive enzymes in the gut, thereby reducing nutrient digestibility and availability to the body (Lenis and Jongbloed, 1999). Wheat, which is one of the major feed ingredients used in formulating broiler diets in Canada (AAFC, 2005), also contains anti-nutritional components, such as non-starch polysacchrides (**NSP**) in its cell wall (Kim et al., 2005a), which are indigestible by non-ruminants and can reduce utilisation of nutrients entrapped within the cells (Bedford and Schuzle, 1998; Kim et al., 2005a). The major component of NSP in wheat, soluble arabinoxylans (Kim et al., 2005a) increases digesta viscosity, which in turn, decreases nutrient digestion by reducing gastro-intestinal tract (**GIT**) passage rate of digesta, accessibility of enzymes to their substrates and absorption of nutrients (Bedford, 2000).

The presence of PA and NSP in wheat can reduce the efficiency of nutrient utilisation thus increasing cost of feeding wheat-based diets to poultry. Reduced nutrient

utilisation can result in environmental pollution due to excessive excretion of unabsorbed nutrients, especially N and P (Lenis and Jongbloed, 1999). Phytase and xylanase supplementation to wheat-based diets may, however, alleviate the anti-nutritional effects that are associated with PA and NSP, respectively. This is because phytase can hydrolyse PA to release the bound nutrients and digestive enzymes, whereas xylanase can hydrolyse NSP (arabinoxylans) to release encapsulated nutrients and to reduce digesta viscosity (Bedford, 2000). There is, however, limited information on the effect of combining phytase and xylanase on nutrient utilisation in broilers fed wheat-based diets. Furthermore, results of a few studies on the effect of combining phytase and xylanase in broilers diets are inconsistent. For instance, Selle et al. (2003b) have reported synergism between the 2 enzymes, but Wu et al. (2004a) could not show similar effects. In wheat, phytic acid is highly concentrated in aleurone cells (Joyce et al., 2005), whose cell walls are composed mainly of arabinoxylans (Guillon et al., 2004). It was thus hypothesized that phytase and xylanase could act synergistically in improving the nutritive value of wheat-based diets because xylanase can hydrolyse arabinoxylans to release PA for the action of phytase whereas phytase can hydrolyse PA to release bound nutrients and enzymes. The objective of this study was thus to determine the effect of supplementing wheat-based diets with phytase and xylanase alone or in combination on nutrient digestibility and performance of broilers from hatch to three weeks of age.

4.3 MATERIALS AND METHODS

4.3.1 Birds and Housing

Three hundred and twenty one-day-old male broiler chicks of the Ross strain were obtained from a commercial hatchery (Carleton Hatcheries LTD, Grunthal, MB) and used in this experiment, which lasted for 23 days (**d**). The chicks were individually weighed upon arrival and divided into 80 groups of 4 birds balanced for body weight (**BW**). They were then group-weighed and each group housed in a cage in an electrically heated Petersime battery brooders (Incubator Company, Gettysburg, OH). The brooder and room temperatures were set at 32 and 29°C, respectively during the first week. Thereafter, heat supply in the brooder was switched off and room temperature was maintained at 29°C throughout the experiment. Light was provided for 24 hours throughout the experiment. All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee (Protocol No. F03-029/1), and birds were handled in accordance with guidelines described by the Canadian Council on Animal Care (CCAC, 1993).

4.3.2 Experimental Diets

The 10 experimental diets included a positive control (**PC**) diet, and a negative control (**NC**) diet supplemented with phytase at three levels, 0, 250 and 500 FTU/kg and xylanase at three levels, 0, 1250 and 2500 XU/kg in a 3 x 3 factorial arrangement to give nine treatment combinations (Tables 4.1 & 4.2). The PC diet was formulated to meet or

Table 4.1. Ingredient and chemical composition of the positive and nutrient reduced wheat-based diets

Item	Positive control (PC)	Negative control (NC)
Ingredient (%)		
Wheat (13.8 %)	55.89	58.00
Soybean meal (45.8 %)	18.40	17.30
Canola meal (34.7 %)	3.00	3.00
Peas	12.00	12.00
Triticale	3.00	3.00
Canola oil	2.70	2.25
Biophos	1.15	0.45
Limestone	1.51	1.65
Iodized salt	0.20	0.20
Sodium Bicarbonate	0.20	0.20
L-Lys (79 %)	0.34	0.36
DL-Met (99 %)	0.27	0.25
L-Thr	0.10	0.10
Premix[1]	1.24	1.24
Calculated nutrient content		
ME, Kcal/kg	2939.00	2940.00
CP, %	21.23	21.02
Ca, %	0.90	0.82
Total P, %	0.66	0.51
Available P, %	0.43	0.28
Digestible amino acids, %		
Lys	1.21	1.20
Met	0.57	0.55
Analysed nutrient and NSP contents		
AME, Kcal/kg	2850.05	3014.44
CP, %	19.66	19.74
Ca, %	1.01	0.77
Total P, %	0.64	0.49
NSP, %	9.09	8.95
Lys, %	1.11	1.23
Met, %	0.51	0.54

[1]Supplied per kilogram of diet: vitamin A, 8255 IU; vitamin D_3, 3000, IU; vitamin E, 30 IU; vitamin K, 2 mg; Thiamine (vitamin B_1), 4 mg; riboflavin (vitamin B_2), 6 mg; niacin, 41.2 mg; folic acid, 1 mg; biotin, 0.25 mg; pyridoxine, 4 mg; choline, 1300.5; Pantothenic acid, 11 mg; vitamin B_{12}, 0.013 mg Mn, 70 mg; Zn, 80 mg; Fe, 80 mg; Cu, 10 mg; and Na, 1.7 g

Table 4.2. Enzyme dosages and analysed activities of the experimental diets for broilers

Diet	Phytase, FTU/kg	Xylanase, XU/kg
1 (PC)	0 (544)[1]	0 (157)
2 (NC)	0 (524)	0 (118)
3	250 (671)	0 (114)
4	500 (836)	0 (157)
5	0 (519)	1250 (1792)
6	250 (606)	1250 (1829)
7	500 (1200)	1250 (1880)
8	0 (497)	2500 (5469)
9	250 (738)	2500 (4557)
10	500 (913)	2500 (6377)

[1]Values in parentheses represent results from enzyme analysis

exceed the NRC (1994) nutrient requirements for broiler chicks. The NC diet was the same as the PC except that the Ca and available P levels were reduced by approximately 10 and 35%, respectively. All the diets contained chromic oxide (0.3%) as an indigestible marker and were fed as mash.

The phytase and xylanase enzymes used in the current experiment were Phyzyme® XP (5,000 FTU/g) and Porzyme® 9300 (4,000 XU/g), respectively, from Danisco Animal Nutrition (Marlbourough, UK). The cultivar of the wheat used in making diets was AC-Barrie, and was grown at Glenlea Research Farm, University of Manitoba and harvested during Fall, 2004.

4.3.3 Experimental Procedures

The 10 diets were randomly allocated to 80 pens to give 8 replicates per diet. The BW and feed consumption for each pen were determined weekly on d 7, 14 and 20 after withdrawing feed for 2 hours. On d 21, 22 and 23, excreta samples were collected from each pen and stored frozen at –20°C for the determination of total tract nutrient digestibility. Care was taken during the collection of excreta samples to avoid contamination from feathers and other foreign materials. On the last day of the experiment (d 23), 2 birds were randomly selected from each pen and euthanised by cervical dislocation. Left tibiae and contents of jejunum (from the end of duodenum to Meckel's diverticulum) and ileum (from Meckel's diverticulum to approximately 1 cm above the ileal-cecal junction) were obtained for determination of tibia ash, digesta viscosity and ileal nutrient digestibilities, respectively. Jejunal digesta was immediately

prepared and analysed for digesta viscosity as described below whereas ileal digesta samples and the tibiae were stored frozen at –20°C until the analyses could be carried out.

4.3.4 Sample Preparation and Chemical Analyses

The tibiae were defleshed by autoclaving at 121°C for 1 minute and dried in an oven at 45°C for 2 d. They were then fat extracted using hexane for 2 d, dried in a fume hood for 2 d to allow the hexane to evaporate and ashed at 550°C in a muffle furnace for 4 hours for the determination of tibia ash. Jejunal digesta was mixed to obtain a homogenous mixture, which was then centrifuged at 9000 rpm in duplicate tubes for five minutes in order to separate feed particles from the liquid phase. The supernatant (0.5 ml) from each tube was analyzed for viscosity, which was measured in Centipoise (**cps**) units at 30 rpm and 40°C using the Brookfield digital viscometer (model LVDVII+CP, Brookfield Engineering Laboratories, Stoughton, MA).

Ileal and excreta samples were freeze-dried and finely ground in a grinder (CBG5 Smart Grind; Applica Consumer Products, Inc., Shelton, CT) to pass through 1 mm screen and thoroughly mixed before analysis. The excreta samples were analysed for dry matter (**DM**), gross energy (**GE**), N, Ca, P and Cr. Ileal samples were also analysed for the same chemical components as the excreta samples except that they were analysed for amino acids (**AA**) instead of N. Diet samples were similarly ground and analysed for all chemical components that were determined in excreta and ileal samples, and for NSP and enzyme activities (phytase and xylanase).

Dry matter was determined according to the method of AOAC (1990), and GE was determined using the Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co.,

Moline, IL). Nitrogen was determined using a N analyser (Model NS-2000; LECO Corporation, St. Joseph, MI). Samples for Ca and P analyses were ashed and digested according to AOAC (1990) procedures and read on a Varian Inductively Coupled Plasma Mass Spectrometer (Varian Inc, Palo Alto, CA, USA). Samples for amino acids (**AA**) analysis were prepared by acid hydrolysis according to the method of AOAC (1984), and as modified by Mills et al. (1989). Briefly, about 100 mg of each sample was digested in 4 mL of 6 *N* HCl for 24 h at 110°C, followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then made up to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an LKB 4151 Alpha plus amino acids analyzer (LKB Biochrom, Cambridge, UK). Samples for analysis of sulphur containing AA (methionine and cysteine) were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not determined. Samples for Cr analysis were ashed and digested according to procedures described by William et al. (1962) and read on a Varian Inductively Coupled Plasma Mass Spectrometer (Varian Inc, Palo Alto, CA). Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The neutral sugars were analysed as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990), whereas uronic acids were determined using the procedure described by Scott (1979). Analysis of phytase and xylanase in diets was carried out at Danisco Animal Nutrition (Marlbourough, UK).

4.3.5 Calculations and Statistical Analysis

Apparent ileal and total tract nutrient digestibilities were calculated using chromic oxide as the indigestible marker by the following equation:

$$\% \text{ apparent ileal or total tract nutrient digestibility} = 100-[(C_d/C_f * N_f/N_d)*100]$$

where: $C_d = Cr_2O_3$ concentration in diet (% DM); $C_f = Cr_2O_3$ concentration in either ileal digesta or excreta (% DM); N_f = nutrient concentration in ileal digesta or excreta (% DM); N_d = nutrient concentration in the diet (% DM).

Tibia ash was calculated as follows: Tibia ash (%) = 100*(weight of tibia ash/weight of fat free tibia)

Data were analysed using GLM procedure (SAS, 2002) in a completely randomized design. The differences between PC and NC diets, and main effects of enzymes and their interactions were determined using the following contrasts:

PC vs NC 'diet -1 1 0 0 0 0 0 0 0 0 '

Main effect of phytase 'diet 0 1 0 -1 1 0 -1 1 0 -1
diet 0 0 1 -1 0 1 -1 0 1 -1'

Phytase level (250 vs 500 FTU/kg) 'diet 0 0 -1 1 0 -1 1 0 -1 1'

Main effect of xylanase 'diet 0 1 1 1 0 0 0 -1 -1 -1
diet 0 0 0 0 1 1 1 -1 -1 -1'

Xylanase level (1250 vs 2500 XU/kg) 'diet 0 0 0 0 -1 -1 -1 1 1 1'

Phytase*xylanase interaction 'diet 0 1 0 -1 0 0 0 -1 0 1
diet 0 0 0 0 1 0 -1 -1 0 1
diet 0 0 1 -1 0 0 0 0 -1 1

diet 0 0 0 0 0 1 -1 0 -1 1'

Differences were considered significant at $P < 0.05$.

4.4 RESULTS

Analysed chemical composition of the 2 basal (positive and nutrient reduced) diets and enzyme activities of the ten experimental diets are shown in Tables 4.1 and 4.2, respectively. The analysed chemical composition of the basal diets was fairly close to the calculated values. The NSP content of wheat used in the current study was 8.24%. Both PC and NC diets were similar in NSP. The PC diet had slightly higher endogenous phytase (544 vs 524 FTU/kg) and xylanase (157 vs 118 XU/kg) activities than the NC diet. The addition of phytase and xylanase enzymes in diets generally resulted in increased activities of the respective enzymes by margins similar to those that were anticipated except for xylanase at a higher dose (2500 UX/kg), whose activity was increased by a much higher margin than anticipated.

Data for feed intake, body weight gain **(BWG)**, feed conversion ratio (**FCR**) and tibia ash are presented in Table 4.3. There were no interactions ($P > 0.05$) between phytase and xylanase on any of the response criteria measured. Birds fed the NC diet had lower ($P < 0.05$) feed intake, BWG and tibia ash than those fed the PC diet. The 2 diets were, however, similar ($P > 0.05$) in FCR. Phytase (250 FTU/kg) supplementation did not affect ($P > 0.05$) feed intake and BWG. But it improved ($P < 0.05$) FCR and tibia ash, though there was no further effect ($P > 0.05$) at higher level of phytase on these

Table 4.3. Feed intake, body weight gain (BWG), feed conversion ratio (FCR) and tibia ash of broilers

Diet		Feed intake (g)	BWG (g)	FCR (g/g	Tibia ash (%)
PC		906.9	691.8	1.312	47.6
NC		744.1	578.7	1.285	41.7
Phytase	0 FTU/kg	772.4	602.9	1.281	42.6
	250 FTU/kg	777.4	619.3	1.255	44.1
	500 FTU/kg	779.7	618.6	1.261	45.5
Xylanase	0 XU/kg	774.9	612.0	1.267	44.3
	1250 XU/kg	771.9	609.0	1.268	44.3
	2500 XU/kg	782.7	619.9	1.262	43.6
SEM		10.0	7.75	0.0077	0.54
Contrasts[1]					
PC vs NC		**	**	NS	**
Phytase		NS	NS	*	**
Phytase level		NS	NS	NS	NS
Xylanase		NS	NS	NS	NS
Xylanase level		NS	NS	NS	NS
Phytase*xylanase		NS	NS	NS	NS

[1]PC = positive control; NC = negative control; effects of phytase and xylanase were determined using

responses. Also, the tibia ash values for phytase-supplemented diets did not reach ($P > 0.05$) that of the PC diet. Xylanase supplementation did not influence ($P > 0.05$) feed intake, BWG, FCR and tibia ash, though the FCR for diets with the highest dosage was better ($P < 0.05$) than the PC diet.

Data for digesta viscosity; apparent ileal and total tract DE, AID and ATTD of Ca and P, and ATTD of N are shown in Table 4.4. The digesta viscosity for the PC diet was lower ($P < 0.05$) than that for the NC diet. The digesta viscosity was reduced ($P < 0.05$) by phytase and xylanase supplementation, but only when the enzymes were supplemented at higher levels (i.e., 500 FTU/kg and 2500 XU/kg, respectively). The AID of Ca and ATTD of P for NC diet were higher ($P < 0.05$) than those for the PC diet. The PC and the NC diets were, however, similar ($P > 0.05$) in AID of P and ATTD of Ca. Xylanase supplementation did not influence ($P > 0.05$) AID of Ca and P, and ATTD of P, but increased ($P < 0.05$) ATTD of Ca. There was, however, no further effect ($P > 0.05$) at higher level of xylanase on ATTD of Ca. Phytase supplementation also did not influence ($P > 0.05$) AID of Ca, but increased ($P < 0.05$) AID of P and ATTD of P and Ca. And similar to xylanase, there was no further effect ($P > 0.05$) at higher level of phytase on AID of P and ATTD of P and Ca. Phytase and xylanase did not affect ($P > 0.05$) apparent ileal and total tract DE, and AID of AA and ATTD of N. The mean apparent ileal and total tract DE, and AID of AA and ATTD of N for the enzyme supplemented diets were 3273.1 and 3303.8 kcal/kg, and 75.6 and 63.5%, respectively (data not shown).

Table 4.4. Digesta viscosity, and apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of Ca and P in broilers

Diet		Viscosity (cps)	AID, %		ATTD, %	
			Ca	P	Ca	P
PC		2.79	50.9	44.9	44.2	42.5
NC		3.65	64.8	40.4	47.5	50.2
Phytase	0 FTU/kg	3.22	61.7	35.1	52.3	47.3
	250 FTU/kg	3.38	63.3	41.3	56.7	51.4
	500 FTU/kg	2.73	63.5	42.3	55.0	41.0
Xylanase	0 XU/kg	3.32	62.5	39.9	50.6	50.9
	1250 XU/kg	3.19	61.2	39.2	57.6	49.0
	2500 XU/kg	2.83	64.8	39.6	55.8	49.8
SEM		0.13	2.21	1.99	1.20	0.95
Contrasts[1]						
PC vs NC		**	*	NS	NS	**
Phytase		**	NS	*	*	**
Phytase level		**	NS	NS	NS	NS
Xylanase		*	NS	NS	*	NS
Xylanase level		NS	NS	NS	NS	NS
Xylanase level		NS	NS	NS	NS	NS

[1]PC = positive control; NC = negative control; NS = non-significant; * = $P < 0.05$; ** = $P < 0.01$

4.5 DISCUSSION

The objective of the current study was to determine the influence of phytase and xylanase individually or in combination on nutrient utilisation and performance of broilers. Phytase was expected to increase nutrient utilisation and performance because: (i) its substrate (PA) contains approximately two-thirds of the total P in vegetable feedstuffs (Kornegay, 2001), which is poorly digested by broilers (Bedford, 2000); (ii) PA in its natural state is complexed with minerals and proteins, and thus it can reduce the availability of these nutrients for utilisation (Ockenden et al., 2004; Lin et al., 2005); and (iii) it (PA) is negatively charged at all pH conditions in the GIT and thus can bind positively charged nutrients and endogenous enzymes in the GIT, thereby reducing nutrient utilisation (Lenis and Jongbloed, 1999; Adeola and Sands, 2003). Xylanase was expected to increase the nutrient utilisation and performance because: (i) its substrate (arabinoxylans) reduces nutrient intake and digestibility by increasing digesta viscosity and decreasing passage rate; and (ii) in wheat, arabinoxylans are the major component of NSP, which reduces nutrient availability by encapsulation (Bedford and Schuzle, 1998; Kim et al., 2005a). A combination of phytase and xylanase was expected to further increase nutrient utilisation and performance because in wheat, PA is highly concentrated in aleurone cells (Joyce et al., 2005), whose cell walls are composed mainly of arabinoxylans (Guillon et al., 2004), and thus xylanase can hydrolyse arabinoxylans to rupture the cell walls and increase the accessibility of phytase to PA (Juanpere et al., 2005).

In the current study, the analysed xylanase activity in diets with high dose (2500 FTU/kg) of the same enzyme was two times higher than expected, and it is not clear why the analysed activity was high. The basal (NC) diet to which enzymes were supplemented in the current study was formulated to be deficient in Ca and available P so as to maximize the response to phytase supplementation. The BWG of broilers fed this NC diet was poorer than that of birds fed the PC (nutrient adequate) diet, confirming that Ca and available P were indeed limiting nutrients in this diet. Phytase supplementation in the present study did not improve BWG, which is in contrast with results of previous studies (Dilger et al., 2004; Onyango et al., 2005b; Cowieson et al., 2006b) showing significant improvement in performance of growing broilers due to phytase supplementation to low Ca and P diets. In the current study, phytase supplementation only increased P digestibility whereas the studies where improvement in BWG has been reported, phytase also improved the digestibility of Ca, AA or both. The failure of phytase to improve BWG in the current study could thus be explained by its failure to increase the ileal digestibility of other nutrients. It could also have been due to inadequate release of P from PA by phytase because in the current study, phytase improved ATTD of P by 4.1 percentage units whereas in the studies where improvement in BWG has been reported, phytase improved ATTD of P by at least 9.4 percentage units (Dilger et al., 2004; Onyango et al., 2005b; Cowieson et al., 2006b). The poor response to phytase supplementation could be due to high endogenous phytase activity in the basal diets used in the current study. Endogenous and supplemental phytases have been reported to act additively on hydrolysis of PA (Zimmermann et al., 2003).

Tibia ash is the most sensitive indicator of P adequacy in growing broilers (Onyango et al., 2005b) because most of the absorbed P is deposited in bones (Leeson and Summers, 2001) and the tibia grows faster than all other bones in the body (McLean et. al., 1961 cited by Yan et al., 2005). In the present study, phytase supplementation increased tibia ash, which agrees well with results of others (Dilger et al., 2004; Onyango et al., 2005b). Phytase, however, did not improve tibia ash to that of the PC diet, which could probably be due to inadequate release of P from PA by phytase. In the present study, the NC diet compared with the PC diet, was formulated to be 35% lower in available P yet phytase improved P digestibility by only 17%. Phytase supplementation in the present study improved FCR in broilers only when it was supplemented at 250 FTU/kg. This could have been due to greater numerical increase in BWG than in feed intake due to phytase supplementation at this level. It was, however, not clear why this happened when phytase was supplemented at 250 FTU/kg, but not at 500 FTU/kg.

Xylanase supplementation did not influence broiler performance in the current study. Similarly Ravindran et al. (1999a) have reported lack of effect of xylanase supplementation on performance of broilers fed wheat-based diets. Wu et al. (2004b), however, reported improved performance of broilers due to xylanase supplementation. Xylanase supplementation to wheat-based diets increases performance by increasing feed intake and nutrient digestibility (Kim et al., 2005a). The improvement in nutrient intake and digestibility (and hence performance) by xylanase supplementation depends on the concentration and composition of arabinoxylans in wheat and hence its nutrient encapsulating and viscosity-causing effects (Cowieson et al., 2005). For instance, Adeola and Bedford (2004) observed improved performance of White Pekin ducks after xylanase

supplementation to wheat-based diets when the wheat was highly viscous (45.68 cps), but not when it was less viscous (5.86 cps). The digesta viscosity for the basal diet that was used in the current study was 3.65 cps. The lack of effect of xylanase on performance could thus be due to the low viscosity of wheat used in the formulation of these diets.

The AID of Ca and ATTD of P for the PC group were lower than those fed the NC diet. Cowieson and Adeola (2005) and Onyango et al. (2005b) have also reported similar results, and it is attributed to its (Ca) increased efficiency of absorption from GIT in an effort to maintain normal plasma Ca level (Weaver et al., 1996). The higher ATTD of P for the NC diet than for the PC diet could similarly be explained by increased efficiency of utilisation of the absorbed P (i.e., its reduced excretion via urine) due to its lower concentration in the NC diet.

It is well documented that phytase supplementation to poultry diets increases ileal and total tract P digestibility (Kornegay, 2001; Maenz, 2001; Silverside et al., 2004; Cowieson and Adeola, 2005; Watson et al., 2006). The increased P digestibility implies its improved utilisation, and reduced excretion and hence reduced environmental pollution. Similarly in the current broiler study, phytase supplementation improved AID and ATTD of P, however, increasing the level of phytase supplementation from 250 to 500 FTU/kg did not influence the AID of P, which is contrary to the results of Silversides et al. (2004). These authors reported a significant increase in P digestibility after increasing the level of phytase supplementation from 150 to 450 FTU/kg in a wheat-based diet that was low in available P. It should, however, be noted that diets used in the present experiment had high levels of endogenous phytase, and were supplemented at 250 FTU/kg and fed as mash (untreated), whereas those used in the study reported by

Silversides et al. (2004) were pelleted (heat treated) at a temperature above 80°C after enzyme addition, which may have inactivated of most the endogenous phytase and significantly reduced the effective dose of the supplemental phytase.

The liberation of P from PA in response to increasing levels of supplemental phytase is quadratic (Zyla et al., 1999b). This is because the cleavage of the first phosphate group from fully phosphorylated PA by phytase occurs at a faster rate and requires lower amount of phytase whereas the cleavage of the phosphate groups from partially dephosphorylated PA occurs at a slower rate and requires higher amounts of phytase (Wyss et al., 1999; Cowieson et al., 2006b). The endogenous phytase activity in the NC diet that was used in the current study was high (524 FTU/kg). The lack of effect of increasing the level of phytase supplementation from 250 to 500 FTU/kg in the current study, compared with that of Silversides et al. (2004), could thus be attributed to the fact that endogenous phytase plus supplemental phytase at 250 FTU/kg had already reached its maximum dose required to partially desphophorylated PA, further addition of phytase enzyme to 500 FTU/kg was inadequate to further hydrolyse the partially dephosphorylated PA. Cowieson et al. (2006b) supplemented a low Ca and P diet for broilers with increasing levels of phytase and observed a greater improvement in P digestibility (59 vs 66%) when the supplemental phytase was increased from 0 to 150 FTU/kg, and a further increase in the digestibility of the same mineral (66 vs 76%) when the level of phytase supplementation was increased from 150 to 24,000 FTU/kg, but not when to it was increased from 150 to 1,200 FTU/kg (66 vs 67%).

Phytase supplementation did not influence the AID of Ca, but improved its ATTD. The lack of effect of phytase on AID of Ca is contrary to the results of Silversides

et al. (2004) but is in agreement with those of Dilger et al. (2004). The efficacy of phytase with regard to liberation of Ca from phytate depends on various factors. These include dietary levels of PA, Ca and other divalent cations, and pH in the small intestine (Maenz, 2001; Adeola and Sands, 2003); and the degree to which dietary PA is dephosphorylated (Cowieson et al., 2006a). High molar ratios of divalent cations to PA and intestinal pH, that ranges from 4 to 7 encourages the formation of mineral-PA complexes that are resistant to phytase hydrolysis (Maenz, 2001), whereas partially dephosphorylated PA compared to the fully phosphorylated one has a lower capacity to bind nutrients (Cowieson et al., 2006a). Since in the current study PA hydrolysis was increased by phytase supplementation (as evidenced by the increased AID of P), the lack of effect of phytase on Ca digestibility could be explained by low capacity of PA to bind Ca due to the presence of endogenous phytase in the basal diet. In the GIT, PA normally binds to Ca and other divalent cations in the small intestine because the less acidic pH here favours the formation of PA-mineral complexes (Lenis and Jongbloed, 1999). The optimum pH for wheat phytase is pH 5.5 (Paik, 2003), which is within the range of pH in the crop of broilers (pH 5.2-5.8; Yu et al., 2004). Endogenous phytase could thus have hydrolysed PA in the crop, resulting in partially desphosphorylated PA that had low capacity to bind Ca in the small intestine. The improved ATTD of Ca observed in the present study due to phytase supplementation might have been due to its improved utilisation as a result of increased P absorption, leading to reduced excretion via urine.

Although protein, AA and energy digestibilities are expected to be increased by phytase supplementation, this has not been consistently observed among studies. For instance, Dilger et al. (2004) and Ravindran et al. (2001, 2006) reported improvements,

but Zhang et al. (1999) and Onyango et al. (2005b) could not show similar effects. In the current study, AID of AA and energy, and ATTD of N and energy were not influenced by phytase supplementation despite the improved AID of P. Factors other than those that affect PA hydrolysis have been suggested to influence the effect of phytase on AA/protein digestibility. They include: (i) the location of PA and protein in the feed material (Adeola and Sands, 2003); (ii) chemical and structural properties of proteins in the feed material (Selle et al., 2006); (iii) degree to which PA is dephosphorylated in the GIT before it interacts with proteins (Cowieson et al., 2006a); and (iv) dietary level of Na (Selle et al., 2006). The first 3 factors are known to influence the capacity of PA to bind protein and the ability of phytase to liberate the bound proteins (Ravindran et al., 2006; Selle et al., 2006), whereas the last one (dietary Na) influences AA absorption from the gut (Selle et al., 2006). The lack of effect of phytase on AA digestibility in the current study might also have been due to low capacity of PA to bind proteins due to the presence of endogenous phytase in the basal diet. Phytic acid mainly reduces protein digestibility by reacting with dietary protein and digestive enzyme (pepsin) at acidic conditions in the stomach (proventriculus) to form binary PA-protein complexes (Cowieson et al., 2004). It can also reduce protein digestibility by reacting with divalent cations, dietary proteins and endogenous enzymes at less acidic pH in the small intestine to form ternary PA-mineral-protein complexes (Lenis and Jongbloed, 1999). The endogenous phytase in the basal diets used in the present study was high (524 FTU/kg). Thus it is possible that the endogenous phytase hydrolysed PA in the crop, resulting in partially dephosphorylated PA that lacked capacity to bind the proteins in the proventriculus and the small intestine. It has recently been found that phytase at low

levels (150 to 300 FTU/kg) is adequate with regard to improving the digestibilities of AA and energy, but not of P (Cowieson et al., 2006b). The lack of effect of phytase on AID and ATTD of energy could be attributed to its lack of effect on AA digestibility and probably on binding endogenous enzymes that are responsible for energy digestion.

Phytase supplementation at 500 FTU/kg reduced digesta viscosity in the present study. This was surprising and unexpected because phytase does not hydrolyse arabinoxylans, the compounds that are responsible for the high digesta viscosity in wheat-based diets (Kim et al., 2005a). The reduced digesta viscosity could probably have been due to alteration/disruption of arabinoxylan structures in wheat during PA hydrolysis because in wheat, both arabinoxylans and PA are highly concentrated in the aleurone cells (Joyce et al., 2005).

As expected, xylanase supplementation in the current study reduced digesta viscosity. Its supplementation, however, did not increase the AID and ATTD of all nutrients measured except ATTD of Ca. This is contrary to the findings of Selle et al. (2003b) who observed improved nutrient digestibility after xylanase supplementation of wheat-based diets for broilers. It is known that the effectiveness of xylanase on improving nutrient utilisation in poultry depends on the digesta viscosity (Adeola and Bedford, 2004) and the degree of association between arabinoxylans and nutrients (Kim et al., 2005a). The response to xylanase supplementation is poor when the intestinal digesta viscosity is less than 10 cps (Bedford and Schuzle, 1998). The digesta viscosity (3.65 cps) for the basal diet used in the current study was lower than 10 cps. The lack of increase in AID and ATTD of nutrients measured in the current study after xylanase

supplementation might thus have been due to low digesta viscosity or a weak association between NSP and the nutrients.

The reason why xylanase increased ATTD of Ca in the current study is not clear. The AID of P for the enzyme-supplemented diets was generally lower than the ATTD of the same mineral, suggesting that some of it was absorbed in the hindgut. On the other hand the AID of Ca for the enzyme-supplemented diets was generally higher than the ATTD of the same mineral, indicating that some of the absorbed Ca was lost via urine. It is thus most likely that xylanase exerted its positive effect ATTD of Ca by reducing the excretion of Ca via urine. It is, however, difficult to explain how it (xylanase) reduced Ca excretion since it neither influenced Ca nor P digestibilities, which are known to affect Ca utilisation/retention.

In the current study, phytase and xylanase did not synergistically interact on any of the response criteria measured. Similarly Wu et al. (2004a) did not observe synergistic interaction between phytase and xylanase on nutrient utilisation and performance of broilers fed wheat-based diets. Selle et al. (2003b), however, observed a synergistic effect of the two enzymes. Synergism between phytase and xylanase on nutrient utilisation and performance occurs when xylanase hydrolyses arabinoxylans to increase the accessibility of phytase to PA (Kim et al., 2005b). This implies that it can only occur when arabinoxylans and digesta viscosity are limiting the accessibility of phytase to PA. The basal diets used in the current study and that reported by Wu et al. (2004a) were lower in digesta viscosities (3.65 and 2.8 cps, respectively) compared to the 10.21 cps basal diet used in the study reported by Selle et al. (2003b). The lack of synergism between phytase and xylanase on nutrient utilisation and performance of broilers in the current study and

that reported by Wu et al. (2004a) could thus be explained by low digesta viscosity of the basal diets.

In conclusion, phytase supplementation improved FCR, tibia ash, AID of P, and ATTD of P and Ca whereas xylanase only improved ATTD of Ca. Phytase did not improve any of the other parameters measured in the present study probably due to the presence of high endogenous phytase activity in the basal diet. Phytase and xylanase did not synergistically improve nutrient utilisation and performance of broilers probably due to a low arabinoxylan concentration and hence digesta viscosity in the basal diets. It thus appears that exogenous phytase and xylanase may not interact significantly in wheat-based diets with high endogenous phytase activity and low NSP concentration.

5.0 GENERAL DISCUSSION

The objective of the current study was to determine the effect of supplementing a wheat-based diet with phytase and xylanase alone or in combination on nutrient utilisation and performance of growing pigs and broilers. Phytase is more effective with regard to PA hdrolysis and hence should improve performance when diets are limiting in Ca and available P (Zyla et al. 2000; Wu et al., 2003; Fan et al., 2005). The levels of these two minerals in the NC diets to which the ezymes were supplemented were thus reduced so as to maximise response to to phytase supplementation on PA hdrolysis. The NC diet was, however, limiting in Ca and available P in the broiler study but not in the pig study since the performance for the NC diet compared with that of the PC diet was lower only in the broiler study. The similar performance of pigs fed the PC and NC diets could be attributed to the age of the pigs and endogenous phyase level in the basal diets, which increases the availability of P. Growing pigs used in the current study have lower dietary requirement of available P (0.23%; NRC, 1998) than broilers in the starter phase (0.45%; NRC, 1994). The dietary level of available P can limit performance only when it is sufficiently reduced (Brana et al., 2006). In most studies with pigs where reduction in dietary available P resulted in reduced performance, the dietary available P in the basal diet was lower than the NRC (1998) recommended levels by at least 0.1 percentage unit (Harper et al., 1997; Matsui et al., 2000; Brana et al., 2006), and this reduction was achieved because the diets were based on corn, which is low in available P (0.08; NRC, 1994). In the current study, the NC diet fed to pigs was lower than the recommended level by 0.07 percentage units, and could not be reduced beyond this level due to the

higher content of available P in wheat (0.13%; NRC 1994) compared to corn. On the other hand, the available P in the NC diet that was fed to broilers was lower than that in the PC diet by a bigger margin (0.15 percentage units). The endogenous phytase activities in the PC (717 FTU/kg) and NC (690 FTU/kg) diets for pigs were higher than the activities (313 and 419 FTU/kg) reported by Liao et al. (2005) in piglet diets with similar amounts of wheat. Thus, the lower reduction in dietary available P coupled with high endogenous phytase activity in the basal diets could be attributing to the lack of effect of reducing dietary available P on performance in the present studies.

Phytase supplementation did not improve growth performance of pigs despite the fact that it (phytase) is more effective in growing pigs (with regard to improving performance) and it significantly increased Ca and P digestibilities. Also, phytase supplementation did not improve growth performance of broilers despite the significant increase in Ca and P digestibilities. This response to phytase supplementation in both the pig and broiler studies is in contrast to results of Harper et al. (1997) and Dilger et al. (2004) who, respectively, reported improved growth performance of growing pigs and broilers due to phytase supplementation at 500 and 1000 FTU/kg to corn- and pelleted wheat-based diets. In the pig study, the lack of a phytase effect on performance could be attributed to the small difference in dietary available P between the PC and NC diets and the high endogenous phytase activity in the basal diets, whereas in broilers, it could have been due to the failure of phytase to sufficiently increase the digestibility of Ca and P to impact performance, the low dosage of phytase and the high endogenous phytase activity in the basal diets.

Scott et al. (2001) reduced P concentration in corn- and wheat-based diets for layers by approximately 0.12 percentage units and supplemented the resulting low P diets with Phytase to investigate the effect of phytase on nutrient utilisation. They reported increased egg production of layers fed corn-based diets but not wheat-based diets due to the presence of endogenous phytase in wheat. The egg production by hens fed the phytase suplemented corn-based diets increased to that of hens fed the P adequate diet. The amount of phytase required to release a given amount of P from PA *in vitro* was found to be higher for untreated wheat than for autoclaved (phytase inactivated) wheat (Zyla et al., 1999b), which may have been due to higher dosage of phytase that is required to further hydrolyse the partially dephosphoylated PA products that results from endogenous phytase hydrolysis (Wyss et al., 1999; Cowieson et al., 2006b). Wu et al. (2003) observed increased BWG of broiler chickens fed wheat-based diets due to phytase supplementation when the dietary available P had been reduced from adequate (0.45%) to inadequate by 0.15 percentage units. In their study the diets had been pelleted at 65°C, which is most likely to inactivate most of the endogenous phytase. In the current study, the endogenous phytase activities in basal diets for broilers, like those for pigs were high (544 and 524 FTU/kg for PC and NC diets, respectively); they were similar to a value (459 FTU/kg) reported by Juanpere et al. (2005) for wheat-based diet for broilers to phytase supplementation did not affect performance.

It thus appears that in the current study, the growth performance of pigs would have been increased by phytase supplementation if the diets were lower in both the available P and endogenous phytase than in the present study. It also appears that the growth performance of pigs would have been increased by phytase supplementation if

pigs used in the study were lighter than 20 kg because at that BW (< 20 kg), they have a high dietary available P requirement. Concerning the performance of broilers, it appears that their performance could have been increased if the diets were low in endogenous phytase and a higher dose of phytase was added to the diets.

Xylanase supplementation did not affect performance of either pigs or broilers. Xylanase generally increases performance of pigs and poultry fed wheat-based diets by hydrolysing arabinoxylans, which reduce feed intake and nutrient digestibility by nutrient encapsulation and by increasing digesta viscosity (Kim et al., 2005a). The effectiveness of xylanase with regard to alleviating the antinutritional effects of arabinoxylans depends on both the concentration and composition of the latter (Bedford, 1995; Austin et al., 1997). The NSP concentrations in the NC diets for both pig and broiler studies to which xylanase was supplemented were 9.56 and 8.95%, respectively. Since xylanase has been shown to be effective when concentration of NSP in the wheat-based diets is higher than 13% (Adeola and Bedford, 2004; Jozefiak et al., 2006), the lack of effect of xylanase on the performance of both pigs and broilers could be attributed to the fact that arabinoxylans were not limiting performance.

Phytase supplementation improved P and Ca digestibilities in both pigs and broilers, which is in agreement with several previous studies (Silversides et al., 2004; Kim et al., 2005b; Radcliffe et al., 2006). The improved P digestibility indicates that the phytase evaluated here can be used to formulate diets for growing pigs and broilers with lower amount of added inorganic sources of P. Since P is the third most expensive nutrient in pig and broiler diets, the result also represents potential cost savings with the use of phytase as eveinced by results of Ahmed et al (2004), who after feeding broilers a

phytase supplemented corn-based diet for 42 d reported increased profits per kilogram broiler by 15% due to reduction in cost of feeding by phytase.

The magnitude of improvement of P digestibility at both ileal and total tract levels was, however, greater in pigs than in broilers. Also, improvement in Ca digestibility in pigs was significant at both ileal and fecal levels whereas in broilers it was only increased at the total tract level. This lower efficacy of phytase in broilers compared with pigs could be due to greater hydrolysis of PA to partially dephosphorylated PA products by endogenous phytase. The major sites of phytase activity are the crop and gizzard in poultry (Yu et al., 2004), and stomach in pigs (Jongbloed et al., 1992; Yi and Kornegay, 1996). The pH ranges in the crop of broilers, and in the proventriculus and gizzard of broilers and stomach of pigs are pH 5.2-5.8, and 2-5, respectively (Simon and Igbasan, 2002; Yu et al., 2004), whereas the optimum pH for wheat phytase is pH 5.5 (Paik, 2003). Endogenous phytase could thus have hydrolysed PA more efficiently in the crop of broilers than in the stomach of pigs due to more favourable pH conditions in the crop, which can result in increased availability of partially desphosphorylated PA, whose further hydrolysis require a higher dosage of phytase and have low capacity to bind Ca in the small intestine. Furthermore, plant phytases like wheat phytase are susceptible to proteolysis that occurs in the stomach (Phillippy, 1999). For instance, Rapp et al. (2001b) reported a 45% recovery of the wheat phytase in the duodenum of mini pigs. Therefore, the relatively lower impact of wheat phytase on PA hydrolysis in pigs compared with broilers could also have been due to the fact that its (endogenous phytase) activity was reduced to some extent by pepsin that is found in the stomach.

Xylanase supplementation did not affect digestibility of all the nutrients measured in both pig and broiler studies except for Ca digestibility in both experiments and AA in the pig experiment. This limited response to xylanase could be due to the low concentration of NSP in the basal diets used in the two experiments. It is, however, not clear why xylanase supplementation improved AA digestibility in pigs but not in broilers. It had been assumed that xylanase would be more effective in broilers than in pigs because its optimum pH (3.0 to 10.0; Table 2.3) is close to the range of pH (5.2-5.8) found in the crop and thus it could hydrolyse most of the arabinoxylans in crop before it is inactivated by proteolytic enzymes found in the proventriculus and small intestine. There is thus need to determine the activity of xylanase used in the current studies in all segments of GIT in both pigs and broilers.

Phytase and xylanase did not interact synergistically on all response criteria measured in the two studies. For phytase to interact with xylanase, xylanase must disrupt the structure of arabinoxylans in cell walls to increase the accessibility of phytase to PA (Kim et al., 2005a, b). Since the NSP concentration in the basal diets used in both studies was low, the lack of synergism between phytase and xylanse could be attributed to the fact that arabinoxylans were not limiting the accessibility of phytase to PA. It thus, appears that exogenous xylanase and phytase may not interact significantly wheat-based diets with low concentration of arabinoxylans.

6.0 SUMMARY AND CONCLUSIONS

Phyatse supplementation to wheat-based diets for growing pigs and broilers improved Ca and P digestibilities, but not performance probably due to a high level of endogenous phytase in the basal diets used in the studies where performance was measured. Xylanase supplementation did not affect the digestibility of all components measured in the two studies (except AA digestibility in the pig study and Ca digestibility in both studies) and the performance of pigs and broilers probably due to a low concentration of arabinoxylans in the wheat used in both studies. Phytase and xylanase did not interact synergistically on all response criteria measured in both studies probably due to the low concentration of arabinoxylans in the wheat used in the studies.

Further research is suggested to:

1. Determine the effect of supplementing phytase to diets based on pre-pelleted wheat on nutrient utilization and performance of broilers and growing pigs especially those that are lighter than 50 kg.
2. Determine the effect of supplementing high doses of phytase (above 500 FTU/kg) to diets based on untreated wheat on nutrient utilization and performance of broilers and growing pigs especially those that are lighter than 50 kg.
3. Investigate the effect of further reducing dietary levels of Ca and available P (by including feed ingredients that have lower concentration of available

P than wheat in the diet) and supplementation of the resulting low Ca and P diet with phytase on nutrient utilization and performance of growing pigs.

4. Determine the effect of supplementing a combination of phytase and xylanase to diets based on wheat with high concentration of arabinoxylans on nutrient utilization and performance of both pigs and broilers.

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