THE EFFECT OF EXOGENOUS ENZYME SUPPLEMENTATION ON NUTRIENT DIGESTIBILITY AND PERFORMANCE OF EARLY-WEANED PIGS.

By

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

Three experiments were conducted to study nutrient digestion and utilization and growth performance of early-weaned pigs fed starter diets with or without enzyme supplementation.

In experiment 1, the effect of microbial phytase and organic acid supplementation in diets for early weaned pigs was investigated in an *in vitro assay*, a growth performance study and a digestibility trial involving 96 piglets weaned at 18 ± 1 d. Pigs were randomly assigned to 4 dietary treatments: a corn-soybean meal positive control formulated according to NRC (1998; Diet 1), a negative control (same as Diet 1 but with no added inorganic P; diet 2), diet 2 + phytase (500 units /kg; diet 3), and diet 3 + organic acids (0.35%; diet 4). Addition of microbial phytase plus organic acids improved ($P < 0.05$) phytate phosphorus utilization in pigs fed the supplemented diets and supported similar ($P > 0.05$) growth performance and bone ash content as the positive control. Crude protein and amino acid digestibilities were similar among dietary treatments. However, fecal phosphorus excretion was reduced ($P < 0.05$) by approximately 20% when diets were supplemented with microbial phytase plus organic acids.

In experiment 2, twenty-four early-weaned pigs were randomly assigned to 4 diets formulated to meet 95% of NRC (1998) requirements. The 4 diets consisted of a basal diet plus 3 diets derived by supplementing the basal diet with 3 different enzyme cocktails. Each enzyme cocktail contained a basal xylanase and glucanase preparation fortified with amylase, invertase, protease, and phytase activities plus

organic acids. Enzyme cocktail A further contained cellulase, galactanase and mannanase, while enzyme cocktail B contained pectinase. Enzyme cocktail C contained all four enzyme activities. Pigs fed multi-enzyme supplemented diets had higher ($P < 0.05$) average daily gain (ADG), feed efficiency (FE) and total tract nutrient digestibilities than those fed the non-supplemented basal diet. Phytate phosphorus utilization in pigs fed enzyme-supplemented diets was higher ($P < 0.05$) than in control pigs and this was accompanied by a reduction ($P < 0.05$) in fecal phosphorus levels.

Based on the results of Experiment 2, experiment 3 was conducted to evaluate the effect of supplementing a pig starter basal diet with multi-enzyme preparation C (similar as in Experiment 2) on growth performance of early-weaned pigs. The basal diet was formulated at 95% NRC (1998) recommendation using ingredients that are poorly digested by young pigs. The experimental period was divided into 3 phases. In all the three phases, enzyme supplementation improved ($P < 0.05$) ADG and FE.

Results of all 3 experiments indicate a benefit of including exogenous enzymes in diets for early-weaned pigs. The results further suggest that with appropriate multi-enzyme preparation, opportunities exist for improving the digestibility and utilization of dietary nutrients and to reduce the cost of feeding weaner pigs.

DEDICATION

This thesis is totally dedicated to the Holy Trinity, Lord the Father, the Son, and the Holy Ghost, who made it possible for me to overcome all impossibilities and to leave my foot prints positively, on the sands of time. All Glory, Honor and Adorations to my Dear God.

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Last but most greatest Thanks goes to my Almighty Father, Alpha and Omega, The Beginning and the End, Who in His Infinite Mercy raised me from the dead and counted me worthy of living to serve and to proclaim His goodness.

FOREWORD

This thesis is written in manuscript style. The 1st manuscript was partly published in the Manitoba Swine Update, January 2002, Vol. 14, No. 1, pp. 3 - 4. It was also presented at the ASAS-ADSA-CSAS 2002 joint meeting (July 21 - 25) and has been submitted to the Journal of Animal Science. The other two manuscripts will be submitted soon. The authors of these manuscripts are F.O. Omogbenigun, C.M. Nyachoti and B.A. Slominski, Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2.

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LIST OF ABBREVIATIONS

AA - Amino acid(s)

ADFI - Average daily feed intake

ADG - Average daily gain

AME - Apparent metabolizable energy

BW - Body weight

CP - Crude protein

DM - Dry matter

FCE - Feed conversion efficiency

FE - Feed efficiency

GE - Gross energy

GIT - Gastrointestinal tract

NSP's - Non-starch polysaccharides

PA - Phytic acid

Phytate P - Phytate phosphorus

SBM - Soybean meal

CHAPTER 1

INTRODUCTION

Over the last 20 years, the potential for commercial enzyme products as a means to enhance dietary nutrient utilization has attracted substantial interest from feed manufacturers. The use of enzymes to degrade phytate in cereal-based diets and polysaccharides of the endosperm cell wall has become most prominent.

Although, phosphorus is an essential nutrient required by swine, it is also now recognized as a major pollutant if large quantities are allowed to accumulate in the environment. Consequently, the need to reduce phosphorus levels in livestock manure is currently identified as one of the top priorities for agricultural research and development in Canada (CARD 2000). About 60 to 70% of the total phosphorus in plant-based ingredients commonly used in swine diets occur as an organic complex called phytate. Phytate is a complex salt of divalent cations with myoinositol (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate). Phosphorus tied up in the phytate complex is only partly available to pigs which lack the phytase enzyme required for hydrolyzing the phytic acid molecule (Cromwell, 1992; Ravindran et al., 1995). The inability of pigs to efficiently utilize phytate phosphorus causes many problems. First, since only a small portion of the phosphorus from plant derived ingredients is utilized, inorganic phosphorus must be supplemented to the diet in order to meet the animal's requirement. Among the mineral supplements, inorganic phosphorus sources are the most expensive, thus increasing feed cost. Second, a large amount of phosphorus excreted in the manure is a threat to the environment,

especially in areas of intensive livestock production. Excess phosphorus in manure can leach through the soil into fresh water sources where it stimulates the growth of algae, i.e., algal blooms and other aquatic plants (Sharpley and Menzel, 1987). This process, known as "eutrophication" causes a marked deterioration in the quality of fresh water by decreasing the palatability of drinking water and by the formation of carcinogens during the chlorination of drinking water (Sharpley and Sheffield 2000, as cited by Radcliffe et al., 2001). Phosphorus and nitrogen contribute to eutrophication however, phosphorus is the primary agent in fresh water eutrophication. Excess nitrogen from manure application may also leach through the soil and reduce the quality of drinking water. Therefore, phosphorus and nitrogen are currently the two elements in manure that are of greatest concern and limit the rate of manure use as organic fertilizer (Kornegay, 1996).

Another nutritional concern associated with phytate is its ability to complex with minerals and protein, thereby reducing the absorption and availability of several trace elements and amino acids (Torre et al., 1991).

The addition of phytase enzyme to swine diets may be an effective strategy to overcome the problems associated with poor phytate phosphorus availability. Research has shown that the bio-availability of phosphorus in swine diets can be improved by the use of extrinsic phytases (Lei et al.1993a, b; Mroz et al., 1994).

However, in early weaned pig nutrition, the use of phytase has not been fully optimized. *In vitro* studies by Anand and Seshadri (1995), and Maenz et al.

(1999) suggested that acidification of diets may improve phytase activity. However, limited *in vivo* research has been done to verify the result of these *in vitro* studies. Furthermore, limited research concerning the ability of phytase to improve the utilization of other dietary nutrients has been published. In such cases, the studies were conducted with growing pigs and the results have been inconsistent and contradictory. Peter and Baker (2001) failed to show any effect of supplemental phytase on nitrogen and amino acid utilization in young pigs. Similarly, Traylor et al. (2001) did not find any positive effect of supplemental phytase on amino acid digestibility in growing pigs. In contrast, Kemme et al. (1995) and Kornegay et al. (1998) observed improved amino acid digestibility as a measure of nitrogen utilization in pigs fed diets supplemented with phytase.

In a manner similar to phytase enzyme, carbohydrases are known to effectively degrade anti-nutritional factors such as the non-starch polysaccharides (NSP's) in the endosperm of wheat, barley, rye and oats (Li et al., 1996). The presence of NSP's in these ingredients limit their use as they are considered as poorly digestible ingredients in baby pig nutrition. Hesselman and Åman (1986) reported that supplementation of such ingredients with exogenous carbohydrases improved nutrient digestibility. On the contrary, Gdala et al. (1997) reported improvement in ileal digestibility of DM only, and not that of crude protein, starch and NSP. These inconsistencies have been attributed to the presence of complex substrates in feedstuffs, and the use of enzyme activities that are not suitable for degrading these complex substrates (Slominski, 2000). Previous research with

poultry (Cleophas et al., 1995) and young pigs (Graham et al., 1988) suggest that a combination of different enzyme activities is required for complete degradation of complex NSP to achieve better dietary nutrient digestibility.

With the increasing need to explore alternative and less expensive feedstuffs, research into the use of the so called poorly digestible/ low quality feedstuffs and appropriate multi-enzyme combination is currently of great interest in early weaned pig nutrition.

The broad objectives of these studies were:

1) To determine the impact of microbial phytase, organic acids and their combination on phytate phosphorus availability, protein and amino acid digestibilities and phosphorus excretion in early-weaned pigs.
2) To examine the effect of supplementing a pig starter diet based on a wide selection of lower quality ingredients with multi-enzyme preparations on nutrient digestibility and growth performance of early weaned pigs.

CHAPTER 2

LITERATURE REVIEW

2.1. General Introdution

During the last few years, hog production has become a very important part of livestock production, especially in North America where pig production represents a large source of revenue. This has therefore, led to increasing interest in looking at ways to maximize production of good quality pork products at a lower cost to producers. Some of the ways to maximize pork production are to increase sow productivity by reducing the weaning age and being able to provide and maintain a good plane of nutrition for the early weaned piglets. Nutrition is an important aspect of pig production, representing about 65 - 70% of the cost of production (Prairie Swine Centre Inc., 2000). Therefore, there is a need to look at ways to reduce this cost, by formulating feeds to provide adequate and necessary nutrients for young pigs, while simultaneously reducing the environmental problems caused by supplying excessive nutrients, especially nitrogen and phosphorus in swine diets. All the above will be discussed in detail in the following review.

2.2. Early-Weaned Pig Nutrition

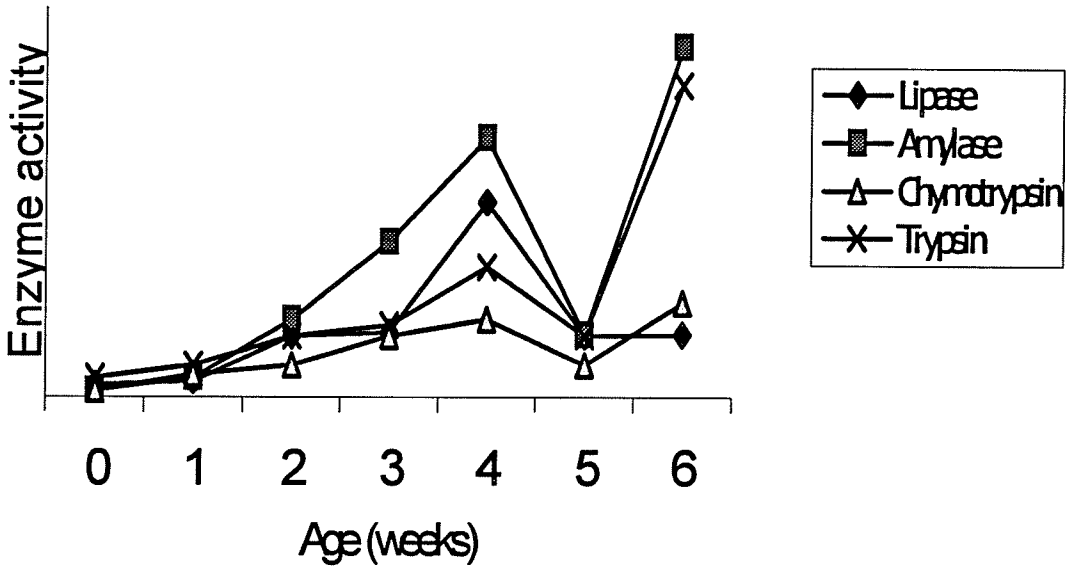
Early weaning has been used for many years, and it is widely used in North America because of the associated advantages. It improves sow productivity (increasing litters/sow/year) and also leads to maximum use of farm facilities. Moreover, it reduces the incidence of disease transfer from the sow to the piglets. However, early weaning is known to cause immediate reduction in post-weaning growth (Okai et al., 1976), a condition commonly referred to as post-weaning lag.

This is due to the change in diet, environment, and a poorly developed immune system of young pigs.

2.2.1. Effect of Early-Weaning on Nutrient Digestion in Piglets

The digestive capability of the gastrointestinal tract (GIT) plays a major role in the growth and development of young pigs. Pigs are born with the ability to digest milk, however, at weaning they are fed solid diets containing plant ingredients that are not readily utilized because of the immature GIT and a lower secretion of digestive enzymes (Effird et al., 1982). Lindemann et al. (1986) reported that weaning, change of diet and environment all have adverse effect on digestive enzyme secretions in pigs. In studies conducted by Effird et al. (1982) and Makkink et al. (1994) it was also shown that levels of pancreatic enzymes declined drastically in piglets at the time of weaning, and gradually increased after three to four weeks post-weaning as illustrated in Figure 2.1 modified from Lindemann et al. (1986). This initial reduction in enzyme activity impairs diet digestibility, and causes digestive disorders due to mal-absorption and leads to growth depression (Hampson and Kidder, 1986). The depression of piglet growth has deleterious effect on their lifetime performance (Whittemore, 1985). The indigestible fibre and phytate components may impede the digestion of protein, minerals, starch and use of dietary energy (Low, 1985; Maenz et al., 1999). Incomplete digestion of nutrients in the small intestine may enhance their fermentation by the bacterial population of the gut and cause proliferation of the microflora. Adding exogenous enzymes to

Figure 2.1 Effect of weaning on pancreatic enzyme secretions of piglets weaned at 18 days.



Modified from Lindemann et al. (1986).

the post-weaning diet may complement the piglet's endogenous digestive enzymes and therefore increase dietary nutrient utilization. This is particularly important for phosphorus and nitrogen as it will minimize their amounts excreted into the environment thus reducing the impact of swine production on the environment.

2.3. Environmental Impact of Pork Production

Over the years, swine production has increased tremendously in various parts of the world, including Western Canada. In Manitoba alone, hog production has more than doubled over the past decade. In the past five years, Manitoba's hog supply increased by approximately 58 percent (MAF, 2001). This level of growth has been associated with increasing concerns on its possible effects on the environment. Major complaints have included water pollution and odors which are due to the amount of nutrients, especially nitrogen and phosphorus excreted in pig manure. This limits the application of manure to agricultural soils so as to prevent nutrient toxicity in soil. Kornegay (1996) reported that phosphorus and nitrogen are the two major elements in manure that are of greatest concern and limit the rate of manure use as organic fertilizer. Miner (1999), also reported that excessive or continuous application of effluent to crop land created a situation in which more nitrogen is being applied than used by the crop, in which case excess nitrogen will be transported beyond the root zone and will eventually appear in ground water as an increase in nitrate concentration. Excess nitrogen and phosphorus in manure can also leach through the soil into fresh water sources where they stimulate the growth

of algae (Sharpley and Menzel, 1987). These algae and other aquatic plants compete with fishes and other marine animals for oxygen (University of North Carolina, 1995). This process, known as "eutrophication", also causes a marked deterioration in the quality of fresh water by decreasing the palatability of drinking water and by formation of carcinogens during chlorination of drinking water (Sharpley and Sheffield, 2000., cited by Radcliffe et al., 2001). Excessive levels of nitrate nitrogen in streams and lakes that supply drinking water can also pose a human health hazard, because increased consumption of nitrate nitrogen can alter the body's ability to transport oxygen, causing a condition called methemoglobinemia, also known as Blue Baby Syndrome which can be fatal in infants (University of North Carolina, 1995). In order to reduce these environmental problems, nutrient management strategies are being proposed as solutions. This involves the optimal use of dietary nutrients by the animals. Dietary manipulations have been suggested as the most effective approaches to reducing manure nutrient excretion (Miner, 1999). Among these approaches, supplementing pig feeds with exogenous enzymes offers an opportunity to improve nutrient utilization by pigs. This will not only reduce nutrient excretion, but will also reduce the problems associated with anti-nutritional factors found in some feed ingredients.

2.4. Anti-nutritional Factors in Feed Ingredients

These are a group of toxic non-related chemical compounds found in plants and may be present in the leaves, seeds/grains and roots. Anti-nutritional factors

serve as biological means by which plants are protected from invasion from bacteria and insects. However, the levels of these toxic factors in plants depend on the cultivar and the methods of processing the grains. Examples of anti-nutritional factors include the legume-based protease, trypsin and chymotrypsin inhibitors, tannins, saponins and gossypol. A recent addition to this group is soyatoxin found in soybeans grown in Brazil (Vasconcelos et al., 2001). Other group of anti-nutritional factors include oxalates and hydrocyanides found in tubers, and the cereal based anti-nutritional factors such as the non-starch polysaccharides (NSP's), α -amylase inhibitors and phytates. This review will focus on phytate and the NSP's.

2.4.1 Phytate Phosphorus

2.4.1.1 Occurrence

Phytate phosphorus also known as phytic acid (PA) occurs in a wide variety of plant materials, with the greatest concentration being found in mature seeds. The range of phytate phosphorus contents of some common feed ingredients are presented in Table 2.1. Phytate phosphorus constitutes about 60 to 85% of the total phosphorus in cereals and oilseed meals and varies from 0.21% in oats and corn to 0.97% in wheat bran. The concentration of phytate phosphorus depends on the part of the plant and type of plant from which feedstuffs are derived. Oilseed meals and cereal by-products contain larger amounts than whole cereal grains (Graf et al., 1987). The phytate content of seeds may also vary depending on climate conditions,

Table 2.1. Phytate phosphorus content of some feed ingredients

Ingredient	Phytate Phosphorus (g/100g DM)	Phytate phosphorus as % of total P	*References
Corn	0.17 - 0.29	66 - 85	1, 2, 3, 4
Corn gluten meal	0.31 - 0.36	46 - 65	3, 4, 5
Barley	0.19 - 0.33	57 - 70	4, 6, 7
Wheat	0.17 - 0.38	60 - 80	4, 5, 6, 7
Wheat bran	0.88 - 0.96	70 - 72	3
Rice bran	0.91 - 1.03	76.9	2
Oats	0.22 - 0.35	59 - 78	5, 6, 7
Sorghum	0.21 - 0.28	64 - 69	2, 5
Foxtail millet	0.19	70	2
Finger millet	0.14	58	2
Pigeon Peas	0.24	75	2
Soybean meal	0.37 - 0.42	57 - 61	2, 3, 4, 6
Cotton seed meal	0.75 - 0.90	70	5
Rapeseed meal	0.54 - 0.78	43 - 70	5, 8

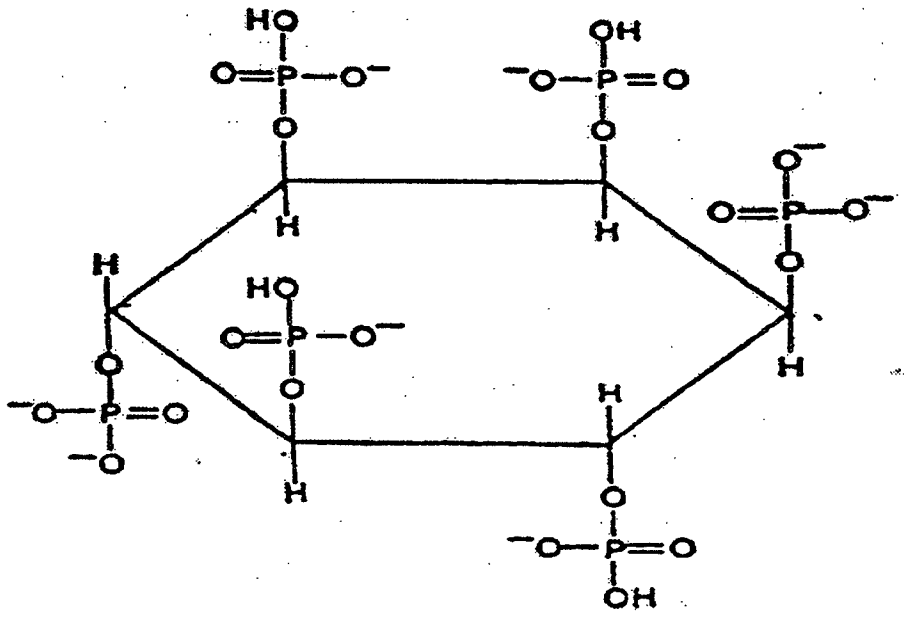
*References: 1. Cossa et al. (1997); 2. Ravindran et al. (1994); 3. Kirby and Nelson (1988); 4. Jongbloed and Kemme (1990a); 5. Nelson et al. (1968); 6. Lolas et al. (1976); 7. Ewing (1963); 8. Nwokola and Bragg (1977).

location, irrigation, type of soil, fertilizer application, and year during which plant variety is grown (Reddy et al., 1989). Nahapetian and Bassiri (1976) reported variations in the phytate content of wheat grown in different years and attributed this variation to environmental factors such as rainfall and temperature. Nitrogen and phosphorus fertilization was found to affect the phytic acid content of oats (Saastamoinen, 1987). In contrast, Barrier-Guillot et al. (1996) showed that nitrogen and phosphorus fertilization, date of harvest, and variety did not significantly affect the phytate phosphorus content of wheat. Seed variation in phytate content can also be attributed to differences in seed morphology. O'Dell et al. (1972) found that 85% of phytate is located in the aleurone layer, 13% in the germ and 2% in the starchy endosperm of wheat, whereas, in corn it is primarily located in the germ portion of the seed in a water soluble form (Harland and Oberleas, 1996). In oilseeds such as canola, phytate is found primarily in the cotyledon and in the crystalline globoids in the cell of the radicle (Yiu et al., 1982). However, earlier studies by deBoland et al. (1975) and Prattley and Stanley (1982) showed that in soybean, phytate is complexed with proteins and has no specific area of localization.

2.4.1.2 Structure of phytic acid

When the phytate molecule is chelated, as it normally is in seed-based ingredients and feeds, it is called phytin. Phytic acid is the acid form of the anion, phytate. As shown in Figure 2.2, phytin is a myo-inositol phosphate that contains

Figure 2.2. Structure of myoinositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate (phytic acid)



Source: Cheryan (1980).

6 phosphate units with 12 replaceable protons in each molecule of phytic acid in mature seeds (Angel and Applegate, 2000). Costello et al. (1976), using ^{31}P nuclear magnetic resonance-pH titration methods, found that six of these protons are in the strong acid range (pKa 1.5), one in the weak acid range (pKa 5.7), two with pKa 6.8 to 7.6, and three with pKa above 10. They concluded that at all pH values normally encountered in feedstuffs, phytic acid will be negatively charged and this makes it a strong chelating compound which has the ability to bind cations and/or protein.

2.4.1.3 Physiological Function of Phytate Phosphorus

Phytin is the chief storage form of phosphorus in seeds. In mature seeds, phytic acid occurs primarily as a complex salt of mono- and divalent cations and/ or proteins (Lott, 1984). It accumulates in seeds during the ripening period and serves several important physiological functions during dormancy and germination, including initiation of dormancy, antioxidant protection during dormancy (Williams, 1970) and storage of phosphorus, high energy phosphoryl groups, and cations for use during germination (Graf et al., 1987).

2.4.1.4. Phytic acid-mineral interactions

Phytate phosphorus has been known to complex with other feed nutrients, such as minerals, proteins and starch, which reduces the availability of these nutrients for animal utilization (Thompson, 1986). As mentioned previously, phytic acid has strong chelating potential due to the presence of many negatively charged

groups. Consequently, it may act as an anti-nutritional factor by binding di- and trivalent cations such as Zn, Ca, Mg, Fe, and Cu to form insoluble salts potentially rendering these minerals unavailable for intestinal absorption (Angel and Applegate, 2000). Phytic acid can complex with a cation and other phosphate groups, or complex between two phosphate groups of a molecule, or between groups of different phytic acid molecules (Cheryan, 1980). Diets are composed of a complex mixture of minerals which likely results in interactive effects in forming complexes with phytic acid (Maenz et al., 1999). In a simple solution at high Ca:Zn ratios, Ca^{2+} enhanced Zn incorporation into phytate-mineral complexes (Byrd and Matrone, 1965). These authors also suggested that the mixed mineral Ca-Zn-phytate complex is even more stable than Zn-phytate complexes at low Zn concentration. These complexes were found to cause a 65% and 50% inhibition of phytate phosphorus hydrolysis, respectively, by microbial phytase (Sandberg et al. 1993; Maenz et al., 1999). Phytate will form stable complexes with minerals of varying affinities depending upon the metal ions involved. Maddaiah et al. (1964) found that zinc has the highest affinity for phytic acid at pH 7.4, followed by Cu^{2+}, Co^{2+}, Mn^{2+}, and Ca^{2+}. Vohra et al. (1965) reported that phytate form complexes with minerals in the following descending order: Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, and Ca^{2+}.

Formation of insoluble phytate complexes also depends on other factors, including pH, concentration of minerals and source of phytic acid. Magnesium and calcium salts of phytic acid tend to be soluble at lower pH and insoluble at higher pH (Grynspan and Cheryan, 1983). The solubility of canola phytate was investigated

by Alli and Houde (1987) and found to be insoluble in water but highly soluble under acidic conditions and relatively insoluble under neutral and alkaline conditions. The molar ratio of mineral to phytic acid also affects the formation of phytate complexes. Grynspan and Cheryan (1983) studied the effects of pH and molar ratio on the formation of calcium phytate complexes by using calcium and phytic acid (Ca:Phytic acid) molar ratios that ranged from 0.5 to 12.7 and the pH ranged from 2 to 11. They reported that Ca and phytate phosphorus were highly soluble below pH 4 at all molar ratios, above pH 4 the extent of solubility drop depending upon the Ca:Phytic acid ratio, above pH 6 the greatest Ca precipitation occurred at molar ratios between 4 and 6.5. They also found that phytate phosphorus solubility decreased with increasing Ca:Phytic acid ratio with complete precipitation occurring above a molar ratio of 5. They emphasized that pentacalcium phytate salt predominates when calcium is not limiting. However, Graf (1983) demonstrated that phytic acid exhibits a high affinity for Ca^{2+} over a wide pH range. The study revealed the chelation of Ca^{2+} by phytate at an acidic pH of 2.0 and that certain Ca-phytate complexes were soluble. Molecules of phytate which have one or two calcium ions are termed mono- and di- calcium complexes respectively, and are found to be more soluble than the tri-, tetra-, penta- and hexa- calcium complexes.

2.4.1.5. Phytic acid-protein interactions

Phytic acid is naturally associated with proteins in the aleurone layers of cereal grains and the protein bodies of oil seeds (deBoland et al., 1975). The

formation of phytate-protein complexes has been shown to be dependent on pH (Cheryan, 1980). At low pH (2 - 3.5), proteins have a net positive charge, phytic acid binds proteins as a result of strong electrostatic attraction between the cationic residues on the protein and the anionic phosphate ester of phytate. At high pH, both phytic acid and protein are negatively charged creating a situation whereby phytate complexes with protein in the presence of divalent cations, e.g., Ca^{2+}, Mg^{2+} and Zn^{2+}. These divalent cations could also bind to the protein and phytic acid molecule and act as a linkage to form protein-mineral-phytate complex (Cheryan, 1980; Grynspan and Cheryan, 1983). These complex interactions at high and low pH are illustrated with Figures 2.3 and 2.4. The binding of phytic acid with proteins may affect the digestibility of proteins and amino acid availability. However, Thompson and Serraino (1986) studied the effect of phytic acid on the *in vivo* digestibility of proteins and absorption of amino acids in rapeseed flour and observed no significant differences in protein digestibility of rats fed high (5.7%) or low (2.4%) phytic acid diets. This is similar to the findings of McDonald et al. (1978) who found that nutritive value of rapeseed protein is not affected by phytic acid levels. Recently, however, studies conducted on phytic acid molecules and phytase supplementation in monogastric animals suggested that phytate-protein interactions interfere with protein and amino acid digestibility (Kornegay et al., 1998).

Kemme et al. (1995) found that amino acid utilization in chicks fed soybean

Figure 2.3. Interaction between phytic acid and protein at low pH.

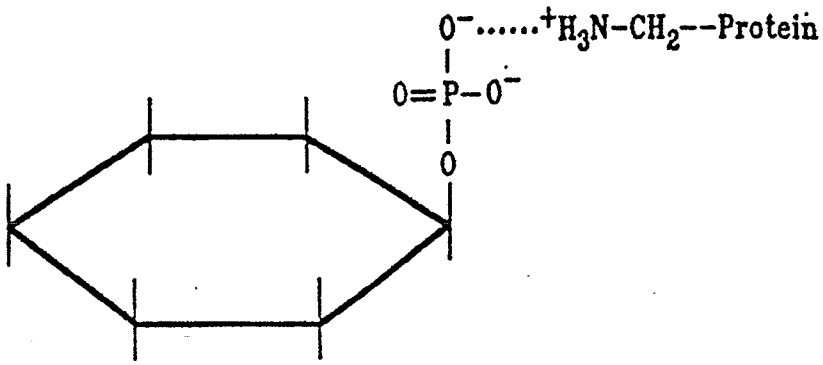
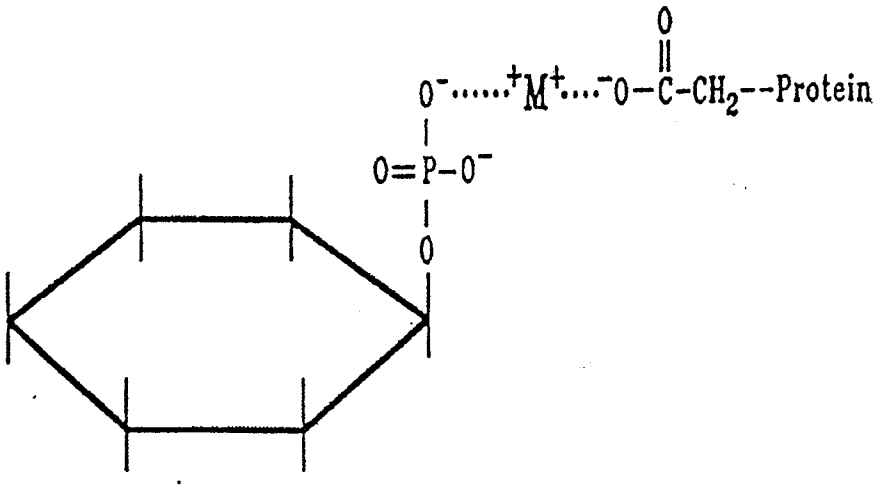


Figure 2.4. Interaction between phytic acid, minerals and protein at high pH.



M = multivalent cation

Source: Cheryan (1980).

meal can be improved with exogenous phytase, indicating that phytase releases amino acids that are bound to phytate. Similarly, Kornegay et al. (1998) reported an improvement in amino acid digestibility in growing pigs fed corn-soybean diets that were supplemented with microbial phytase. On the contrary, O'Quinn et al. (1997), Harper et al. (1997) and Valaja et al. (1998), found no improvement in nitrogen and amino acid digestibilities in corn-soybean diets supplemented with microbial phytase and fed to growing and finishing pigs. Overall, most of the studies on the effect of phytase on nitrogen and amino acid digestibilities have been conducted with growing and finishing pigs with highly inconsistent results. Therefore, there is the need to conduct studies to further elucidate the effect of supplementing corn-soybean diets with microbial phytase on nitrogen and amino acid digestibilities, especially in young pigs where there is currently a dearth of information.

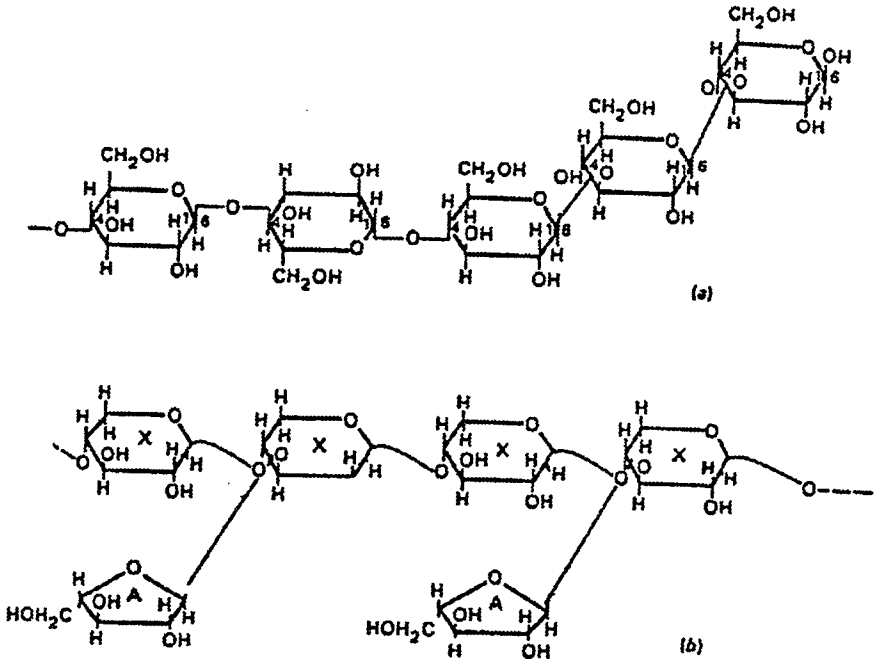
Phytic acid has also been known to reduce starch hydrolysis. Thompson and Yoon (1984) suggested that phytic acid may impede digestion of starch. An *In vitro* experiment conducted by these researchers on the rate of wheat starch hydrolysis in the presence of phytic acid showed that phytic acid reduced starch hydrolysis by 60%. Similarly, Knuckles and Betschart (1987) reported that phytate decreased *in vitro* starch digestion by amylase with the greatest inhibition occurring at pH 4.15, and reduced inhibition at lower pH. However, at high pH (6.0) the inhibitory effect of phytate on starch hydrolysis was not as pronounced as expected inspite of the fact that protein would have a net negative charge at this high pH, and further

increase the inhibition. This may be attributed to lack of divalent cations to form protein-cation-phytate complexes.

2.4.2. Non-Starch Polysaccharides

Non starch polysaccharides (NSP's) and cell walls are the major components of fiber and are composed of cellulose and non-cellulosic polysaccharides. Some NSP's, e.g. β-glucans and arabinoxylans are called 'viscous polysaccharides' because of their ability to absorb water and swell thereby increasing the viscosity of digesta and reducing digestion and absorption of nutrients. In cereal grains the non-cellulosic polysaccharides consist of β-glucans and arabinoxylans while in soybean and canola, arabinans, arabinogalactans, galactans, galactomannans, mannans and pectic polysaccharides predominate (Slominski, 2000). As shown in Figures 2.5 a & b (β-glucans and pentosans, respectively), the primary components of these complex sugars, i.e., pentoses, hexoses, 6-deoxyhexoses, and hexauronic acids, are joined by glycosidic bonds between the hemi-acetal group of one sugar and the hydroxyl group of another. This complexity in structure is further increased by covalent bonding to non-carbohydrate compounds, such as methyl and acetyl groups, phenolic acids, proteins and lignin, and non-covalent bonding within and between polysaccharides (Marquardt, 1996). This makes them unavailable to animals unless diets are supplemented with commercial enzymes to facilitate the breakdown of these bonds.

Figures 2.5. Structure of (a) β-glucan and (b) Pentosan



Source: Thacker and Bass (1996).

2.4.2.1. β-Glucans

β-glucan is a partially soluble, cell wall polysaccharide originating from the aleurone layer and starchy endosperm of barley and oats. β-glucans are the major NSP's of barley (i.e., 75% of total NSP). It consists of glucose units linked together by β-1,4 and β-1,3 linkages (Classen and Bedford, 1991). The β-1,3 linkages confer upon the molecule a step-like structure as shown in Figure 2.5a, which interferes with hydrogen bonding between adjacent chains resulting in increased water solubility (Thacker and Bass, 1996). Basic and Stone (1991) also found that the β-glucans of the aleurone layer are highly water soluble. The presence of the β-1,3 linkage and a branch structure prevents compact folding of the molecules and increases water holding capability, which results in high viscosity and gel forming properties (Fadel et al. 1987; Wang et al., 1992). β-glucans and arabinoxylan fractions of the cell walls are linked to other components thus forming molecules of very high molecular weight ($4X10^7$ Da) (Classen and Bedford, 1991). The larger the molecule the greater the impact it has on viscosity (White et al., 1983). Concentration of β-glucans vary widely in barley due to many factors. Henry (1986) and Thacker et al. (1992) reported that the amount of β-glucans in barley can vary from 1.5 to 8% depending on genotype and environmental conditions such as soil and climate conditions, agronomic practices, degree of maturity at harvest and storage condition. Thacker et al. (1992), reported that barley grown in areas with low rainfall will have higher levels of β-glucans than barley grown under conditions of adequate moisture. Hulless barley may contain higher β-glucans than hulled

varieties because of differences in genotype and environmental conditions, and may therefore cause differences in the digestibility of nutrients. Thacker et al. (1988) found no significant difference in the digestibility of dry matter, energy or protein, average daily gain or average daily feed intake in growing pigs fed hulled or hulless barley-based diets, although a lower FCE was noted for the hulless barley diet. Jensen et al. (1998) reported that hulless barley contain lower insoluble NSP (88 *vs* 160 g/kg DM), but similar level of soluble NSP (48 *vs* 48 g/kg) relative to hulled barley.

2.4.2.2 Pentosans/ Arabinoxylans

Pentosans comprise the majority of the cell wall carbohydrate not only in rye, but also in wheat and triticale (Campbell and Bedford, 1992). Like β-glucans, pentosans are viscous and prone to solubilization within the intestinal lumen. Petersson and Åman (1987) reported that the absolute viscosity observed for wheat, triticale and rye is correlated to the level of solubilized pentosans present as NSP. Structurally, pentosans consist of a β-linked xylose backbone with arabinose side chains as shown in Figure 2.5b. In some instances they also contain hexoses, hexuronic acids and phenolic acids (Classen and Bedford, 1991; Marquardt, 1996).

In swine nutrition, the most important pentosan containing ingredient is wheat because of its high nutritive value compared to rye, barley, and triticale. However, the availability of energy and nutrients from wheat depends upon factors such as location, growing conditions and variety, because these factors dictate the

concentration of pentosans in wheat, and are therefore responsible for different available energy content values reported for different countries: UK wheat, 13.01-15.24 MJ/kg; Australian wheat, 9.18-14.98 MJ/kg; Canadian wheat, 12.93-15.18 MJ/kg; Belgian wheat, 11.2-12.1 MJ/kg (Cowan, 1997). Many studies have been conducted to explain the relationship between chemical composition and low and high apparent metabolizable energy (AME) contents of wheats. A negative correlation between water-soluble NSP content and feeding value of wheat for poultry was noted by Annison (1991) and Classen et al. (1995).

2.4.2.3. Arabinans, arabinogalactans, galactans, mannans, galactomannans and pectic polysaccharides.

Oil seed meals such as canola and soybean meal contain complex polysaccharides which may reduce their digestibility in early-weaned pigs. The main component sugar found in canola meal is glucose (Slominski and Campbell, 1990), with the majority of the glucose existing as cellulose. The main non-cellulosic sugar components are arabinose, galactose and uronic acids accompanied by smaller amounts of xylose and mannose (Slominski and Campbell, 1990; Bach Knudsen, 1997). A study of the carbohydrates of canola meal by Siddiqui and Wood (1977) revealed that rhamnogalacturonans, acidic arabinogalactans and arabinans are the major non-cellulosic polysaccharides. Similar to other legume seeds, polysaccharides in soybean comprise largely cellulose, associated with arabinoxylan and glucuronoxylans (Ravindran, 1988). In addition, soybean hulls contain a

considerable amount of galactomannans (Aspinall and Whyte, 1964).

In many oil seed meals, the rhamnogalacturonans contain a high proportion of neutral sugars linked to the galacturonic backbone. These side chains include arabinans and arabinogalactans. Siddiqui and Wood (1977) indicated that galacturonic acid residues were predominant in the uronic acid residues of canola pectins and that these polysaccharides contained a relatively high proportion of the neutral sugars arabinose, xylose, galactose, and rhamnose which are joined together by complex β-1-4, β-1-3 and α-1-5 bonds. This prevents the hydrolysis of these sugars by dietary enzymes, hence they may not be utilized by young pigs unless the diets are supplemented with exogenous enzyme preparations.

2.5. Enzyme supplementation in swine diets.

In order to overcome the problem caused by the anti-nutritional factors in swine feeds, diets must be supplemented with exogenous enzymes that are capable of breaking the bonds within these factors, so as to increase the digestibility of such diets. The use of commercial enzymes that degrade phytate and polysaccharides of the endosperm cell wall in cereal-based diets has become most prominent. As mentioned previously, the major cell wall polysaccharides are the β-glucans in barley and oats and the arabinoxylans (pentosans) in rye, wheat, and triticale. These can be degraded by preparations of carbohydrases such as glucanase and pentosanase. Exogenous enzyme application may be considered in 2 ways. First, the use of preparations to supply enzymes that augment the digestive capabilities of the

animal. This is particularly so for newly hatched birds and baby pigs whose digestive systems are still immature and some enzymes (e.g., α-amylase and protease) are not produced in sufficient quantities. Second, the supply of certain enzymes in diets to break down cell wall polysaccharides (NSP) to improve their digestibility. As documented in scientific literature, the use of single enzyme preparations of xylanase, glucanase and phytase in the feed industry have been reasonably successful. This is due to the straightforward relationship between target substrate and enzyme i.e., arabinoxylans- xylanase, β-glucans- β-glucanase, and phytic acid- phytase, however, this success is lower compared to when enzymes are applied in concert.

It is well documented that microbial enzyme preparations effectively degrade the viscous polysaccharides (NSP's) in feed ingredients. In chickens, it has been shown that the anti-nutritional activity of viscous polysaccharides is not a function of their polymers but of the intestinal viscosity that they create (Peterson and Åman, 1989). Based on reviews by Campbell and Bedford (1992) and Classen (1996), it may be concluded that the nutritional and economic value of barley, oats, rye and wheat can be improved by the addition of appropriate preparations of β-glucanase and β-xylanase enzymes. Peterson and Åman (1989) and Bedford et al. (1991) concluded that inclusion of endo-β-xylanase in rye-based diets led to marked reduction in digesta viscosity and sticky droppings, with an improved litter quality. In addition to viscosity reduction, other advantages that might be derived from supplementing diets with enzymes are listed as follows: i) release of available

phosphorus from phytate hydrolysis, ii) elimination of nutrient encapsulating effect of the cell walls and therefore improved amino acid and energy availability, iii) solubilization of cell wall polysaccharides for more effective hind gut fermentation and improved overall energy utilization, iv) hydrolysis of certain types of carbohydrate-protein linkages and therefore improved availability of amino acids, v) release of readily available energy from oligosaccharide hydrolysis, vi) elimination of the anti-nutritive properties of certain dietary components including oligosaccharides, NSP's, glucosinolates, lectins or trypsin inhibitors (Slominski, 2000).

However, the use of single enzyme activities may not always give a positive response. This is particularly so in young pigs where use of single enzymes have been found to have little or no effect (Officer, 1995). Consequently, there has been considerable interest in the use of a combination of enzyme activities so as to target the different polysaccharides and anti-nutritive factors normally present in diets. Indeed, Cleophas et al. (1995) have reported that a cocktail of enzyme activities produced more beneficial effects than a single enzyme activity. Similarly, Zyla et al. (1996) reported that growing turkeys fed a corn-soybean meal diet supplemented with an enzyme cocktail containing phytase, acid phosphatase, protease and pectinase retained more phosphorus and gained more weight than those fed the same diet with only phytase supplementation.

2.5.1. Enzyme "Cocktail"

As mentioned above, supplementation of diets with a single enzyme activity may not always yield appreciable results. Instead, current evidence indicates that multi-enzyme preparations might produce significant results. For example, five different types of xylanolytic enzymes and five different types of pectinases are necessary to completely hydrolyze xylans and pectins (Cleophas et al., 1995). Research conducted by Slominski (2000) on Canadian wheat and hulless barley showed that supplementation of barley-based diets with ß-glucanase and wheat-based diets with xylanase resulted in less pronounced response than when both enzyme activities were used in concert. In another study, phosphorus retention in 7-12 d old turkeys fed corn-soybean meal-based diets was found to be 53.6% and 60% when supplemented with phytase or phytase plus pectinase, acid phosphatase and protease, respectively (Zyla et al., 1996). Cell walls, which have a structural function, are intrinsically more resistant to degradation. Murison et al. (1989) and Mulder et al. (1991) found that ß-glucanase alone is sufficient to disrupt barley endosperm walls. They further reported that multi-enzyme preparation containing high levels of enzymes active against cellulose and arabinoxylan are required to maximize the release of protein from the aleurone layer. An in-depth study of the published literature, Collier and Hardy (1986) and Inborr and Ogle (1988) support the view that addition of enzyme cocktails (amylase and protease) to the diets of weaned animals produces a significant increase in ADG and FCE. This

apparently, is not shown when protease or amylase alone is added to the diet (Cromwell et al., 1988).

2.6. Mode of Action of Enzyme

The use of enzymes in animal nutrition focused on two main reasons. The utilization of amylase, protease and lipase in early-weaned pig nutrition is to augment the piglet's own digestive system which is depressed by weaning or diet change, and also the use of enzymes to break down target nutrients such as non-starch polysaccharide cell walls and phytates. Improvement in digestibilities of protein (Corring et al., 1978), starch (Officer et al., 1993; Inborr and Ogle, 1988) and fat (Cera et al., 1988) due to supplementation of diet with protease, amylase and lipase, respectively is well documented. The use of phytase enzyme in poultry and swine diets has consistently improved phytate phosphorus availability and decreased phosphorus excretion (Mroz et al. 1994; Sebastian et al., 1998). Similarly, considerable research has been done on the use of cabohydrases in monogastric diets with good results, especially in poultry (Hesselman and Åman, 1986).

2.6.1. Phytase Enzyme and Phytate Hydrolysis

Phytases play an important role in the breakdown of plant phytate, which is the major storage form of plant phosphorus and occurs in high concentrations in many ingredients. There are two main types of phytase, 3-phytase and 6-phytase. 3-phytase is mainly produced by microorganisms and it initiates phytate dephosphorylation at the 3rd position of myo-inositol. The 6-phytase is found in

plants and it starts dephosphorylation at position 6 (Nayini and Markakis, 1986). Wheat phytase activity was compared to the microbial phytase enzyme preparation, Natuphos®, in a study by Eeckhout and De Paepe (1996) and shown to exhibit similar activities at pH 5.5 *in vitro*. When pH was lowered to 3.0, microbial phytase was very active (80% of its activity at pH 5.5) while wheat phytase was no longer active. For phytate phosphorus to be utilized by pigs, the orthophosphate molecules must be cleaved from the inositol ring of phytic acid and subsequently absorbed. This dephosphorylation is catalyzed by phytase (myo-inositol hexaphosphate phosphohydrolase), which is a specific phosphatase that hydrolyzes the stepwise removal of a phosphate ester from a phytic acid molecule. This hydrolysis takes place within the GIT. The dephosphorylation of phytate molecules occurs in a well defined manner. Kies (1996) illustrated that the phytase enzyme does not degrade a phytate molecule (inositol-6-phosphate) as far as possible, followed by the next phytate molecule, but that first, one phosphate-group is cleaved off from many phytate molecules, resulting in many inositol-5-phosphate molecules. Then the next phosphate group is cleaved off resulting in many inositol-4-phosphate molecules, and so on.

2.6.1.1. Sources of Phytase Enzyme

There are three possible sources of phytase: the bacterial microflora of the gut (intestinal phytase), the endogenous plant phytase in feed ingredients and the exogenous microbial phytase. A significant level of phytase activity was identified

in the small intestine brush border membrane of chickens (Bitar and Reinhold, 1972; Maenz et al., 1997). This may suggest its presence in pigs as well, since they have closely similar intestinal microbes as chickens but this has not been established yet. Dietary phytase is that which is inherently localized in feed ingredients with amounts varying between different ingredients. The highest activity of phytase was found in rye (5130 units/kg), wheat bran (2957 units/kg), wheat (1193 units/kg) and barley (582 units/kg) (Ravindran et al., 1995). Corn and sorghum contain very little activity (i.e. ~20 units/kg), which may partly explain why phytate digestibility and phytate phosphorus utilization is low in diets based on these cereals. However, despite the presence of endogenous phytase in animal feeds and in the GIT of monogastric animals, the overall phytate digestibility has been reported to average 40-50% for swine, leaving about 50-60% unutilized. Therefore, the use of microbial phytase preparation has long been shown to improve phytate phosphorus digestibility and utilization. Its use is also encouraged especially in countries with dense livestock and human populations (Jongbloed and Kemme, 1990b) so as to minimize total phosphorus load on the land. Microbial phytase is an activity derived from *Aspergillus ficuum* and it is able to release phosphate from phytic acid. Addition of microbial phytase to diets for growing pigs was found to increase the apparent bioavailability of phosphorus by 24% and reduce the amount excreted by 35%. Fecal phosphorus was reduced by 50% when broiler feed was similarly treated (Simons et al. 1990; Sebastian et al., 1998). Similarly, Lei et al. (1993a) reported that supplementation of swine diets with exogenous phytase improved phytate

phosphorus availability and decreased phosphorus excretion. Because the effectiveness of microbial phytase depends on its activity in feed, several researchers have tried to determine the level of microbial phytase (ranging from 0 to 1500 Units/kg feed) that will produce appreciable improvement in phosphorus retention in poultry and swine. Lei et al. (1993b) reported an optimal level of 400-600 Units/kg feed. It is well documented in the literature that levels below 400 Units/kg do not have an significant effect on phytate phosphorus hydrolysis while levels above 600 Units/kg do not yield improvements that are more significant than 400-600 Units/kg. Harper et al. (1997) showed that supplementation of low-phosphorus growing-finishing pig diets with phytase at 500 Units/kg improved performance, phosphorus digestibility, bone mineralization and reduced phosphorus excretion by 30% than diet with 250 Units/kg.

2.6.2. Carbohydrases

The use of carbohydrase enzymes targets the NSP components, primarily the β-glucans, pentosans, oligosaccharides and cellulose. In poultry, carbohydrase enzymes can break down the water-soluble NSP, therefore reducing or eliminating the negative effect of increased intestinal viscosity which is more pronounced in birds than pigs. Bedford (1993) found an improvement in performance of chickens due to viscosity reduction. However, in pigs improvement in feed utilization due to carbohydrase supplementation may not be related to viscosity because pigs are not affected by highly viscous dietary gums such as β-glucan or pentosan (Honeyfield

et al. 1983; Bedford et al., 1992; Dierick and Decuypere 1994). Bhatty et al. (1979) found that a high-viscosity hulless barley that has consistently given substantial growth depression in young chicks gave comparatively good results when fed to growing pigs, probably because of differences in gut physiology. Pigs differ physiologically from young chicks in that the digesta has a higher water content, and since β-glucan-induced viscosity is logarithmically related to concentration, simple dilution can essentially eliminate the viscosity problem, and the associated constraints on lumenal diffusion (Hesselman and Åman, 1986). In some studies with early-weaned pigs, enzyme addition had no effect on digesta viscosity (Graham et al. 1988; Bedford et al., 1992). Another hypothesis related to the mode of action of carbohydrases in swine diets is that they disrupt the cell wall that encapsulates nutrients (particularly starch and protein) thus exposing them to digestive enzymes within the gut lumen, thereby increasing their rate of digestion and absorption in the small intestine (De Silva et al. 1983; Hesselman and Åman, 1985, 1986). The greater the accessibility of the cell contents to the digestive enzymes, the greater the rate of nutrient utilization.

2.7. Factors Affecting Enzyme Use in Pigs

Generally, the effect of enzyme supplementation on pig performance has not been as consistent as that seen in poultry. Many studies have demonstrated marked improvement in performance while others have shown no improvement with enzyme supplementation. In systematic studies by Thacker et al. (1989, 1992), a

minimal improvement in pig performance was demonstrated with addition of enzymes to swine diets. Other researchers indicated that enzyme supplementation increased digestibility of nutrients, but the increase was not reflected in improved animal performance. Studies by Officer (1995) even suggested that enzyme supplementation was detrimental to piglets fed a wheat-based diet. All these inconsistencies could be attributed to factors that may influence enzyme activities. Such factors are mainly diet-related and they include feed quality, feed processing methods and gut pH. If enzymes are added to diets containing readily digestible ingredients, the magnitude of enzyme effect will be low (Johnson et al., 1993). Furthermore, McNab (1993) suggested that if there are enough dietary nutrients for the animal to achieve optimal performance, any increase in nutrient availability due to enzyme supplementation would exceed the animal requirement and the effect of the enzyme addition will be difficult to detect. Beneficial effect of enzyme supplementation in piglets fed vegetable protein was more pronounced than when the same enzyme was added to a diet containing high quality animal protein (McNab, 1993). Likewise, Johnson et al. (1993) reported that excess nutrients will be excreted as a waste if enzymes are added to diets that contain readily digestible ingredients. Therefore, when the amount of readily available phosphorus in the diet is enough to meet the animal's requirement for maintenance and growth development, increased phytate hydrolysis will avail excess phosphorus, which will subsequently be excreted and enzyme application will tend to be ineffective. Levels of other nutrients such as calcium and vitamin D in the feed also affect the

effectiveness of enzyme supplementation in animals. The general function of vitamin D is to elevate plasma calcium and phosphorus to levels that support normal bone mineralization and development (De Luca, 1979). Early researchers reported that phytate phosphorus utilization is low in rats fed diets deficient in vitamin D (Pileggi et al., 1955). More recently, Edwards (1993) determined that the supplementation of a corn-soybean meal diet with vitamin D3 (1, 25-OH_2 D_3) and phytase resulted in increased weight gain and bone ash, lowered incidence of rickets, and improved phytate phosphorus retention in chickens.

During feed processing (pelleting, expansion and extrusion), enzyme preparations encounter hostile conditions (moisture and heat), which may reduce their activity. Studies by Inborr and Bedford (1994) demonstrated that in feeds that were pelleted after conditioning for 30 seconds or 15 minutes, the recovery of β-glucanase activity at 75°C was 66 and 49%; 50 and 31% at 85°C and 10 and 11% at 95°C, respectively. There seems to be a negative quadratic effect of pelleting temperature and positive effect of enzyme level on ADG and FCE. Jongbloed and Kemme (1990b), also reported a loss in phytase activity due to pelleting of diets. Many methods have been developed to stabilize enzyme preparation, and these include encapsulation, adsorption, micro-granulation, coating and post-pelleting application (Jongbloed and Kemme, 1990b).

Enzyme activity could also be affected by gut pH. For example, at low pH, phytase is known to reduce the chelating ability of phytate to dietary minerals, starch and proteins. However, this chelating ability increases as the pH of the

medium increases thus reducing phytase efficacy (Maenz et al., 1999). Inborr and van der Meulen (1993) and Thacker and Bass, (1996) also reported a reduction in enzyme activity due to increased gut pH. This may further strengthen the hypothesis that reducing the acidity of the gut may be necessary to achieve maximum activity of microbial phytase.

2.8. Acidification of Diets for Pigs

Growth lag immediately post-weaning is a well established phenomenon in early-weaned pigs (Okai et al., 1976). Undoubtedly, this is a manifestation of an array of interacting environmental, social and physiological factors, not the least of which is digestive immaturity (Etheridge et al., 1984). It has been adequately demonstrated that the weanling pig is ill-prepared enzymatically to digest complex carbohydrates found in most cereal-based weaner diets. This may be due to their lower capacity to produce adequate amount of gastric acid which is required for optimal nutrient digestion (Corring et al., 1978). Easter (1988) reported that incomplete digestion in the upper gut results in passage of fermentable substrates into the large intestine and may lead to diarrhoea. This problem can be reduced by the addition of organic acids to weaner diets to lower gut pH to acidic levels. The need for dietary acids presumes that there is a deficiency in the pig's ability to maintain proper gastric pH and that inclusion of acids will reduce gut pH to the level required for optimum enzyme activity for maximum utilization of dietary components. Mature pigs can adjust stomach pH by secretion of hydrochloric acid

from the parietal cells and their stomach can reach very acid levels, i.e., pH 2.0-3.5 (Kidder and Manners, 1978 as cited by Easter, 1988). However, the situation is very different in young pigs. Initially they produce a very low amount of hydrochloric acid which increases with age (Corring et al., 1978). This is apparently the consequence of limited secretory capacity and not lack of stimulation. Easter (1988) reported that maximal acid output in response to intravenous betazole hydrochloride infusion averaged 3.4 mmol H^+/h for pigs at 9-12 days and increased to 7.6 mmol H^+/h when pigs reach an age of 27-38 days.

Organic acids can exert their effects by acting on dietary enzyme supplements, intestinal enzymes, dietary minerals, intestinal microbes and by preventing gluconeogenesis and lipolysis in early-weaned pigs (Vogt et al. 1981; Giesting and Easter, 1985). As reported earlier, phytase action is known to improve with increasing acidity of the gut. Simons et al. (1990) reported that acidic conditions are more favorable for phytase action. Apart from increasing the activity of phytase, organic acids have also been reported to improve protein digestion by improving the activity of the enzyme pepsinogen. Easter (1988) reported that hydrochloric acid is involved in the activation of pepsinogens, and that pepsin has two pH optima, 2.0 and 3.5, thus it is likely that pigs having an elevated gastric pH also experience a net reduction in efficiency of protein digestion (Giesting and Easter, 1985). The mineral content of the diet is also known to influence phytate hydrolysis and utilization at low and high pH by reducing the effectiveness of exogenous and supplemental phytase. Indeed, it has been suggested that phytic

acid binds minerals thus forming mineral-phytate complexes that may be resistant to phytase hydrolysis (Kim and Atallah 1993). Ravindran and Kornegay (1993) reported that acidification of diets with organic acids can improve enzyme activity by forming complexes with various cations and acting as chelating agents resulting in increased intestinal absorption of minerals. In their studies, the addition of 2% fumaric acid to a weaner diet promoted the apparent absorption of Ca, P, Mg and Zn by 13, 11, 3, and 33%, respectively. Similarly, Höehler and Pallauf (1993) observed an improved availability of Ca, P and Mg by adding citric acid (1.5%) to a corn-soybean meal diet fed to piglets. Moreover, a review of *in vitro* studies by Anand and Seshadri (1995) and Maenz et al. (1999) showed that diet acidification with citric and phthalic acid improved phytase activity by acting as competitive chelators of minerals that may compete for binding sites on the phytate molecule. It is important to note, however, that others have failed to show an effect of phytic acid and organic acids on mineral balance (Radecki et al., 1988).

Other possible actions of organic acids include an antimicrobial effect in which potentially detrimental bacteria are destroyed by reduced gastric pH. Vogt et al. (1981) reported reduced microbial count in broiler chicks fed various levels of organic acids. Organic acids may also serve as intermediate metabolites of the TCA cycle. Giesting and Easter (1985), suggested that pigs under stress following weaning may benefit from administration of glucogenic, TCA cycle intermediates such as citric or fumaric acid, which may prevent some tissue wastage resulting from high rates of gluconeogenesis and lipolysis.

Most of the research done with organic acids showed an improved utilization of dietary nutrients by young pigs. Therefore, this implies that supplementing pig starter diets with organic acids might not only enhance dietary nutrient utilization, but will also offer a means to reduce feed cost and the problems associated with excretion of minerals like phosphorus and nitrogen in pig manure.

2.9. Conclusion

There is considerable data available on the use of microbial phytase to improve phytate phosphorus digestibility in young and growing pigs. Although several studies have shown that phytase is highly active in improving phytate digestibility thereby reducing phosphorus excretion in manure, there are many inconsistencies on the effect of phytase on digestibility of other nutrients, especially protein and amino acids. Furthermore, addition of organic acid to pig starter diet is known to reduce gut acidity and is generally assumed to improve the action of phytase on phytate phosphorus digestibility. However, there is lack of information on their combined effect on digestibility of nutrients such as protein and amino acids in pigs weaned as early as 18 days of age. Similarly, there is lack of information on digestibility of these nutrients, including phytate phosphorus when diets for early-weaned pigs are supplemented with a cocktail of enzyme plus organic acids. A series of experiments to be conducted in this research will therefore focus on the effects of microbial phytase plus organic acids on digestibility of phytate phosphorus, protein and amino acids in young pigs weaned at 18 days of age.

Furthermore, experiments will be conducted to clearly assess the efficacy of an enzyme synergy (combination of various enzymes plus organic acids) in young pigs by using diets that are limiting in nutrient content. This will ensure that nutrients availed due to enzyme supplementation are utilized for performance. Therefore the experiments to be conducted will be based on digestibility and pig performance.

CHAPTER 3

MANUSCRIPT 1

THE EFFECT OF SUPPLEMENTING MICROBIAL PHYTASE AND ORGANIC ACIDS TO A CORN-SOYBEAN BASED DIET FED TO EARLY-WEANED PIGS.

3.1. ABSTRACT.

The effect of microbial phytase and organic acid supplementation in diets for early weaned pigs was investigated in an *in vitro* assay and a growth performance/ digestibility trial involving 96 piglets weaned at 18 ± 1 d of age and assigned to four dietary treatments in a completely randomized design. The dietary treatments were, a positive control (corn-soybean meal formulated according to NRC, 1998; Diet 1), negative control (same as positive control but with no added inorganic P; Diet 2), Diet 2 + phytase (500 units /kg; Diet 3), and Diet 3 + organic acids (Nutri-acid® at 0.35%; Diet 4). In the *in vitro* assay, the 4 diets were incubated under simulated gastrointestinal tract conditions. Addition of microbial phytase increased ($P = 0.003$) phytate hydrolysis from 34.0% (Diet 2) to 87.5%. This was further increased to 90.1%, due to the addition of organic acids. In the growth trial, each dietary treatment was randomly assigned to six replicate pens with four pigs per pen, balanced for initial BW and sex. Feed intake and BW were determined weekly. At the end of the 3rd wk., a mobility test was conducted on one pig randomly selected from each pen as a measure of bone development. Pigs fed the diet containing phytase plus organic acids had similar ($P = 0.06$) score for mobility compared to the positive control fed pigs. At the end of wk. 4, six pigs per treatment were killed and samples of digesta from sections of the gut, and the 3rd metatarsal bone were collected for nutrient digestibility and bone ash measurements, respectively. There were no differences in ADFI, ADG and FE among treatments ($P > 0.05$). However, ADG was 6.5 % higher in piglets fed the diet containing phytase plus organic acids

compared to the positive control. Piglets fed microbial phytase and organic acid supplemented diets had a higher ($P = 0.003$) bone ash content than those fed the control diets. Apparent ileal DM and CP digestibilities were similar ($P > 0.10$) among dietary treatments and averaged 80.7 and 79.4%, respectively. Of all amino acids, only apparent ileal digestibility of isoleucine, histidine and aspartic acid were increased ($P < 0.05$) by microbial phytase and organic acid supplementation. Overall, there were slight numerical improvements in amino acid digestibilities due to microbial phytase and organic acid supplementation, with digestibility of essential amino acids averaging 79.4, 77.7, 80.1 and 81.6 % for Diets 1, 2, 3, and 4, respectively. Apparent ileal phosphorus digestibility was increased ($P = 0.0001$) by 21 percentage units and the amount of phosphorus excreted reduced ($P = 0.03$) by 19.4% as a result of microbial phytase plus organic acid supplementation when compared to the control diets. In conclusion, addition of microbial phytase and organic acid to pig starter diets improved phosphorus digestion and utilization. Phosphorus excretion was also reduced, and there was numerical increase in dietary amino acid utilization due to the addition of microbial phytase and organic acids to corn-soybean meal diets fed to young pigs.

3.2. INTRODUCTION

About 60 to 70% of phosphorus in plant-based ingredients commonly used in swine rations occurs as phytate phosphorus; a form that is only partly available to pigs due to their inability to produce enough phytase, the enzyme required for

hydrolysing the phytate molecule (Cromwell, 1992; Ravindran et al., 1994, 1995). Consequently, the addition of phytase from exogenous sources (e.g., microbial phytase) to swine diets to hydrolyze the phytate molecule, and therefore release the bound phosphorus has been an area of intensive research (Jongbloed et al., 1992; Lei et al., 1993a,b; Kornegay and Qian, 1996). Results of many of these studies also suggest potential environmental benefits through reduction in fecal phosphorus excretion in the range of 30 to 40%. It has been shown that gut pH has a significant influence on microbial phytase activity. For instance, Shieh et al. (1969) observed the highest rate of phytate hydrolysis at pH 2.5. This was later confirmed in *in vitro* studies by Eeckhout and De Paepe (1996), who showed that although microbial phytase was active at pH 5.5, full activity was realized at pH 3.0 (80% more than at pH 5.5), presumably due to dissociation of phytate molecule from insoluble phytate-mineral complexes and consequently providing better substrate availability. A review of *in vitro* studies by Anand and Seshadri (1995) and Maenz et al. (1999) showed that diet acidification with citric and phthalic acid increased phytate hydrolysis by microbial phytase since organic acids may act as competitive chelators of minerals (i.e., divalent cations) therefore leaving the phytate molecule more susceptible to phytase action. Furthermore, the addition of organic acids to diets for weanling pigs is believed to lower gut pH to acidic levels, and this might increase the effectiveness of microbial phytase addition to diets. However, result from different studies concerning the effects of these two additives are highly inconsistent, especially when digestibilities of nutrients other than phosphorus are

of interest. Furthermore, there is limited information on protein and amino acid digestibilities in early-weaned pigs fed diets supplemented with microbial phytase and organic acids. Studies conducted by Peter and Baker (2001), in nursery pigs fed soybean meal-based diets did not indicate any significant effect of microbial phytase on utilization of soybean meal protein. Similarly, O'Quinn et al. (1997) and Valaja et al. (1998) reported that microbial phytase supplementation had no effect on apparent ileal and total tract crude protein and amino acid digestibilities in finishing pigs. On the contrary, other researchers have found significant improvements in amino acid utilization due to addition of microbial phytase to swine diets. For instance, Kemme et al. (1995) and Kornegay et al. (1998) reported improvements ($P < 0.05$) in amino acid digestibilities in growing pigs fed a corn-soybean meal based diet supplemented with microbial phytase. Therefore, the objective of the present study was to determine the impact of microbial phytase, organic acids and their combination on *in vitro* phytate hydrolysis, *in vivo* phytate phosphorus availability, protein and amino acid digestibilities and phosphorus excretion in early-weaned pigs fed a corn-soybean meal-based diet.

3.3. MATERIALS AND METHODS

3.3.1. Experimental Diets

The composition of the four corn-soybean meal-based diets used in the *in vitro* assay and growth performance trial is shown in Tables 3.1. The growth experiment was conducted in two phases each lasting 2 wk. Dietary treatment in

Table 3.1. Ingredient and nutrient composition of experimental diets

	DIETS			
	PHASE 1		PHASE 2	
Item	1	2 - 4[a]	1	2 - 4[a]
Ingredient (%)				
Corn	35.00	35.65	49.60	50.20
Lactose	14.00	14.00	-	-
Soybean meal	22.50	22.50	26.60	26.60
Oat groats	5.00	5.00	-	-
Spray dried porcine plasma	8.00	8.00	-	-
Canola meal	3.00	3.00	3.00	3.00
Vegetable oil	2.50	2.50	2.75	2.75
Peas	4.00	4.00	4.00	4.00
Biophos	1.55	0.65	0.90	-
Limestone	1.25	1.53	1.10	1.40
Vitamin premix[b]	1.00	1.00	1.00	1.00
Mineral premix[c]	0.50	0.50	0.50	0.50
Chromic oxide	0.30	0.30	0.30	0.30
DL-Methionine	0.09	0.08	0.03	0.03
Lysine HCl	0.31	0.31	0.22	0.22
Calculated nutrient composition[d]				
DE, Kcal/kg	3482	3505	3523	3544
Crude protein, %	23.19	23.24	20.19 (21.55)	20.24 (19.99)
Methionine, %	0.39	0.39	0.35	0.35
Lysine, %	1.60	1.60	1.30 (1.41)	1.30 (1.38)
Phytate P, %	0.20	0.19	0.23 (0.27)	0.24 (0.33)
Calcium, %	0.85	0.80	0.75	0.70
Available P, %	0.45	0.26	0.35	0.16

[a]Diets 3, and 4 contained Phytase (500 U/kg); and phytase (500 U/kg) plus Nutri-acid® (0.35g kg^{-1}), respectively.

[b]Supplied per kg diet: vitamin A, 8255 IU; vitamin D3, 1000 IU; vitamin E, 10.9 IU; vitamin B12, 0.115 mg; vitamin K, 1.1 mg; Niacin, 36.8 mg; Choline chloride, 781.2 mg; Biotin, 0.25 mg, and Folic acid, 0.75 mg;

[c]Supplied per kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.28 mg.

[d]Based on NRC (1998) feed composition data; Values in parenthesis were derived from chemical analysis of the phase 2 diets.

each phase, consisted of 1) a positive control formulated according to NRC (1998); 2) a negative control which was the same as diet 1 except that available phosphorus was reduced, 3) diet 2 plus microbial phytase (500 U/kg), and 4) diet 3 plus Nutri-acid®. Microbial phytase (3-phytase) and Nutri-acid® were provided by Canadian Bio-Systems Inc., Calgary, Alberta. The unit of phytase activity was defined as the amount of enzyme that liberates 1 μmol phosphate per minute at 37^{0}C and pH 5.5. Nutri-acid® was a broad spectrum gut acidifier composed of citric, malic, phosphoric, sorbic, tartaric, lactic acids and aluminate. Chromic oxide was used as an internal marker. All diets were pelleted at 54°C.

3.3.2. *In Vitro* Experiment

An *in vitro* procedure similar to that used in earlier studies (Slominski, 1999; Figure 3.1) was used to study the degree of phytate hydrolysis under simulated gut conditions. The phase 2 diets were used for this assay, and they were finely ground to pass through a 1 mm sieve. Four samples of each diet were incubated at 40°C in an incubator shaker set at 20 rpm for the appropriate time length as shown in Figure 3.1. To 5 g diet, 500 mg of pepsin (Sigma Chemical Co., St. Louis, MO 63178, USA) was added and incubated with 50 mL of a mixture of 0.07 M HCl and 54 mM NaCl in the incubator shaker for 2 h. 1.6 mL of 2 M NaOH and 25 mg pancreatin (P7545, 8 x U.S.P., Sigma) were then added and the slurry was incubated for an additional 5 h, during which time the pH was checked on an hourly basis. At the end of the incubation period, samples were frozen, freeze-dried and

Figure 3.1. *In vitro* procedure used in determining the degree of phytate hydrolysis.

STOMACH

5 g diet + 500 mg pepsin (P 7000, Sigma)
+ 50 mL of 0.07 M HCL/54 mM NaCl

↓

Incubate[1] for 2 h at 40°C (pH = 3.42)

↓

SMALL INTESTINE

Add 1.6 mL of 2.0 M NaOH
and 25 mg pancreatin (P7545, Sigma)

↓

Incubate[1] for 5 h at 40°C
(mean pH = 5.95)

↓

Freeze and freeze dry[2] the samples

↓

Analyze for phytate phosphorus content.

---

[1]New Brunswick Scientific, Edison, NJ.

[2]Virtis, 815 Route 208, Gardiner, NY 12525-9989.

then analyzed for phytate content (Haug and Lantzsch, 1983; Figure 3.2). Phytate hydrolysis was calculated as the difference in phytate content of the diet and the hydrolyzed material.

3.3.3. Animal Experiment

Ninety-six Cotswold piglets averaging 6.41 ± 0.71 (mean ± SD) kg BW and weaned at 18 ± 1 d (mean ± SD) were blocked on the basis of sex and BW and then assigned randomly from within the block to the four dietary treatments. Each dietary treatment was assigned to six replicate pens each with 4 pigs per pen. Pigs had unlimited access to feed and water. Individual BW and pen feed disappearance were monitored weekly. Room temperature was initially set at 29.5°C and gradually reduced by 1.5°C per wk. The trial lasted 28 d and was divided into two phases with phase 1 and 2 lasting from d 0 - 14 and d15 - 28, respectively. At the end of wk 3, a mobility test (Han et al. 1998) was carried out on 6 pigs per treatment by 2 swine specialists who were unaware of the dietary treatments. The following scale was used for scoring the mobility:

1 = unable to walk or stand,

2 = walks with great difficulty,

3 = walks with slight difficulty,

4 = good movement,

5 = very mobile.

Figure 3.2. Procedure for phytate phosphorus determination (Haug and Lantzsch, 1983).

Sample

(100 mg of diet or 30 mg of excreta)

↓

Add 10 mL 0.2 N HCl

↓

Shake for 3 h at room temperature

↓

Filter

↓

Transfer 0.5 mL filtrate into hydrolysis tube

↓

Add 0.5 mL distilled water and 2 mL ferric solution

Boil for 30 min

↓

After cooling in ice water for 15 min

allow to adjust to room temperature for 15 min

↓

Centrifuge[3] for 30 min at 3000 rpm

↓

To 1 mL supernatant add 1.5 mL

bipyridine solution

↓

After 10 min read the absorbance[4] at

519 nm against distilled water

---

[3]Centra GP8, International Equipment Co., Needham Heights, MA[4]Ultrospec 2000, Pharmacia Biotech, Cambridge, England.

At the end of the trial, one pig selected at random from each pen, was held under halothane general anesthesia and then euthanized via cardiac puncture with sodium pentobarbital to facilitate collection of digesta from the stomach, the terminal ileum and caecum. The pH of digesta collected from these gut segments was measured and used as a basis to simulate the GIT conditions in the *in vitro* study. Also, the left hind leg was recovered from each pig and placed in a labeled sample bag and frozen. Digesta samples were then kept frozen at -20°C until required for chemical analysis. All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

3.3.4. Sample Preparation and Chemical Analyses

Digesta samples were freeze-dried, and along with diet samples, finely ground to pass through a 1 mm mesh prior to chemical analyses. All analyses were performed in duplicate. Chromic oxide was analyzed according to the procedure described by Williams et al. (1962). Briefly, a 0.4 g or 1 g sample of excreta or feed, respectively, was weighed into a crucible and ashed at 600°C for 12 h. After cooling, 3 mL of 7.6% w/v manganese sulphate monohydrate and 4 mL of 4.5% w/v potassium bromate solution were gently added, and the crucible was covered with a watch glass, placed on a hot plate and digested until no effervescence was observed and the solution changed to a purple color. After cooling, the solution was

carefully transferred into a 200-mL volumetric flask containing 100 mg calcium as CaCl. The flask was made up to marked volume using de-ionized water. Chromic oxide was then determined using atomic absorption spectrophotometery (Perkin-Elmer, model 603 A) at 357.9 nm against 5 standards (0, 2, 4, 6, 8, 10 μg/mL) that were prepared as the standard curve.

In order to recover the 3rd left metartarsal bone, the frozen feet were thawed for 20 minutes in warm water, and then defleshed. The bones were then cleaned to remove all adhering tissue and then broken into smaller pieces. Broken bones were wrapped in Whatman's No. 42 filter paper, stapled at the tip and then defatted in hexane for 48 h. The bones were then air dried and ashed in the furnace at 600°C for 12 h. Bone ash was expressed as a percentage of dry fat-free bone weight (modified from Spencer et al., 2000).

Diet and digesta samples were analyzed for DM according to AOAC (1990). 5 g sample was weighed into a pre-weighed silica dish and dried to a constant weight for 16-24 h in a forced air oven set at 105°C. The sample was then removed, cooled in a desiccator and weighed.

Phytate phosphorus content in the diet and digesta samples was determined by using the method of Haug and Lantzsch (1983), shown in Figure 3.2. Total phosphorus (%) was determined according to the AOAC (1990) procedure. 1g of sample was placed in 50 mL borosilicate tube, in which it was ashed for 12 h at 550°C. After ashing, tubes were removed from furnace for acid extraction, by adding 10 mL of a solution made of 5N HCl and $HN0_3$ (1% v/v), and heated in a

sonicator bath at 65°C for 1 h, after which the caps were removed and the tubes were allowed to settle overnight. Standards were prepared between a range of 0 - 15 μg/mL phosphorus. The absorbance of samples was read against that of the standard solutions at 400 nm using a Pharmacia Ultrospec 2000 spectrophotometer.

Crude protein (N x 6.25) content of the diets and ileal digesta samples was determined using Leco NS 2000 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI., USA).

Ileal digesta and diets were analyzed for amino acid content on an LKB Biochrom 4151 Alphaplus amino acid analyzer, in an ion exchange column (Biochrom Science Park, Cambridge, UK), using the standard hydrolysis procedure (Andrews and Balder, 1985). Ileal digesta and diets (100 mg dry matter basis) were weighed into hydrolysis tubes. To each sample, two drops of 2-octanol and 4mL of 6 M HCl were added. The hydrolysis tube were capped and evacuated for 60 seconds. After tightly securing the lids, the hydrolysis tubes were placed in an electrically heated block at 110°C for 24 h. After samples were cooled to room temperature, 4 mL of 25% w/v NaOH was added. The samples were allowed to return to room temperature before diluting with sodium citrate buffer (pH 2.2) to a total volume of 50 mL. After vigorously mixing the samples, 10 mL was filtered through Whatman # 40 filter paper and through a 0.22 µm nylon syringe filter. Samples (50 μL) were then loaded onto the ion exchange column.

3.3.5. Calculations and Statistical Analysis

Digestibility coefficients of the chemically analyzed components were calculated using the following formula (Gabert and Nyachoti, 2000)

Digestibility (%) = $[1- (N_f \times C_d) / (N_d \times C_f)] \times 100$

N_f = Nutrient concentration in digesta or feces (DM)

N_d = Nutrient concentration in diet (DM)

C_f = Cr_2O_3 concentration in digesta or feces

C_d = Cr_2O_3 concentration in diet

Phosphorus excretion (kg /tonne of feed consumed) was calculated as: % phosphorus in feces x DM of feces.

Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC). When a significant *F* value for treatment ($P < 0.05$) was observed in the analysis of variance, treatment means were compared using Duncan's multiple range test (Duncan, 1955). The design used was completely randomized and the model was:

$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

Y_{ij} = Individual observation of the treatment;

μ = Population mean;

α_i = Effect of the i^{th} treatment;

ε_{ij} = The error term.

3.4. RESULTS AND DISCUSSION

3.4.1. Animal Performance

Average daily feed intake, ADG and FE determined during phase I and II are presented in Table 3.2. There were no differences in ADFI among treatments during both phases. In phase 1, ADG tended ($P = 0.07$) to decrease from diet 1 (positive control) to diet 2 (negative control). With the inclusion of microbial phytase (diet 3) and further addition of nutri-acid to the treatment (diet 4), there was a 17.2% and 18.2% increase ($P = 0.07$) in ADG, respectively, when compared to the positive control diet. ADG for phase 2 also followed the same trend as in phase 1, without any significant difference. This unit increase observed in ADG from diet 3 and 4 are in agreement with results of earlier studies by Radcliffe et al. (1998) who found no effect of adding microbial phytase and citric acid on growth of young pigs fed corn-soybean meal diets. Similarly, Han et al. (1998) observed no effect of adding plant phytase, microbial phytase and citric acid to diets for young pigs, on ADG and ADFI. However, as in the current study, FE was improved ($P = 0.03$) during phase I (d 0 - 14) due to the addition of microbial phytase and nutri-acid compared to the negative control diet. However, this improvement was not observed in the 2nd phase or over the entire experimental period ($P = 0.38$).

According to results reviewed from many studies, especially with young pigs, improvement in pig performance by phytase and organic acid supplementation has

Table 3.2. Effect of supplemental microbial phytase and organic acids on performance of early-weaned pigs.

	DIETS[1]					
Item	1	2	3	4	SEM[2]	*P*[3]
ADFI, g/d						
d 0 - 14	285.1	304.8	300.5	306.1	8.4	0.76
d 14 - 28	558.1	570.5	590.7	584.2	12.6	0.83
Overall	421.6	437.6	445.6	445.1	9.7	0.79
ADG, g/d						
d 0 - 14	218.6^{ab}	209.1^{b}	256.1^{ab}	258.3^{a}	21.9	0.07
d 14 - 28	351.2	325.1	337.5	351.2	10.9	0.71
Overall	284.9	267.1	296.8	304.7	14.2	0.21
Gain/Feed						
d 0 - 14	0.76^{ab}	0.69^{b}	0.86^{a}	0.85^{a}	0.07	0.03
d 14 -28	0.63^{a}	0.57^{b}	0.57^{ab}	0.60^{ab}	0.03	0.06
Overall	0.67	0.61	0.67	0.68	0.03	0.38

[1]Diet 1 = Positive control formulated to NRC (1998) standard

Diet 2 = Diet 1 without inorganic phosphorus (negative control)

Diet 3 = Diet 2 plus microbial phytase (500 U/kg)

Diet 4 = Diet 3 plus organic acid.

[2]SEM = standard error of mean.

[3]*P*= observed significance due to effect of treatment.

[ab]Means with same superscripts are not significantly different (P > 0.05)

been poor. In most studies, phytase and organic acid supplementation increased digestibility of phytate phosphorus and some other nutrients, but the increase was not reflected in improved animal performance (Thacker et al., 1992; Inborr et al., 1993; Radcliffe et al., 1998). This variability in the effectiveness of phytase might be due to the various factors such as age of pig, diet, and type of enzyme preparation (i.e., single *vs* multi-enzyme activity), all of which are known to influence phytate hydrolysis. Phytase supplementation was found to improve performance in growing and finishing pigs more than in early-weaned pigs (Harper et al., 1997); an observation that could be explained by the low gastric acidity and increased microbial fermentation in the gut of older pigs. In the present study, the lack of improvement in piglet performance as a result of enzyme and organic acid supplementation could be explained by the fact that diet 2 was formulated to contain adequate levels of all other nutrients except for phosphorus to meet the piglets' requirements for growth. In that case then, phytase and organic acid addition to diets 3, and 4 might have only improved phytate phosphorus utilization but not other nutrients and consequently growth. It is possible that because only a single enzyme activity was used in the current experiment, improvement in the utilization of dietary nutrients other than phytate phosphorus was not high enough to impact piglet performance.

3.4.2. Hydrolysis of Phytate Phosphorus

The extent of phytate phosphorus hydrolysis in the *in vitro* and *in vivo* studies is shown in Table 3.3. In the *in vitro* study, phytate phosphorus hydrolysis

Table 3.3. Effects of supplemental microbial phytase and organic acids on phytate hydrolysis *in vitro* and along the digestive tract.

	DIETS[1]					
Item	1	2	3	4	SEM[2]	*P*[3]
In vitro, %	39.8[b]	34.0[b]	87.5[a]	90.1[a]	25.1	0.003
In vivo, %						
Stomach	22.5[b]	11.0[b]	42.6[a]	56.1[a]	17.5	0.0001
Ileum	71.9[b]	71.8[b]	84.0[a]	86.0[a]	6.6	0.0001
Caecum	78.7[c]	78.9[c]	92.6[b]	97.6[a]	8.4	0.0001

[1]Diets are the same as in Table 3.2

[2]SEM = standard error of mean

[3]*P* = observed significance due to effect of treatment.

[abc] Means with same superscripts are not significantly different (P > 0.05)

increased ($P = 0.003$) with the supplementation of diets with phytase and organic acids. There was a linear increase due to the action of phytase and organic acids showing 90.1% hydrolysis of phytate. The present results are in close agreement with that of Nernberg (1998) who observed a 90% *in vitro* phytate hydrolysis in a wheat canola-based diet supplemented with phytase at 1100 U/kg. This is also similar to results reported by Zyla and Koreleski (1993) where they reported a complete breakdown of rapeseed meal phytate at 40°C following addition of phytase and acid phosphatase. However, in the current study, full hydrolysis was not attained when phytase, and phytase plus organic acids, were added to the corn soybean meal-based diet. Similarly, Zyla et al. (1995) reported that phytase enzyme was not able to completely dephosphorylate phytates in corn-soybean meal diets under simulated conditions of the gastrointestinal tract of turkey. This was attributed to the lack of intestinal microorganisms. Another reason adduced for the incomplete *in vitro* phytate phosphorus hydrolysis is the mineral content of diet especially calcium and phosphorus. Ballam et al. (1985), reported that phytate hydrolysis is greatly reduced as calcium content of the diet increases.

In the *in vivo* trial, phytate hydrolysis followed a similar trend as in the *in vitro* study, with a higher ($P = 0.0001$) amount of phytate being hydrolyzed with the inclusion of phytase and organic acids in the diets compared to the control diets. However, phytate digestibility at the ileal and caecal levels for diets 1, and 2 were higher than the levels seen *in vitro* (Table 3.3). This observation could be attributed to the presence of microorganisms in the gut, which are known to influence nutrient

digestion (Vogt et al., 1981). It was also observed in both the *in vitro* and *in vivo* studies that there was phytate hydrolysis in diet 2, despite the fact that it did not contain supplemental phytase or phytase plus organic acids. This may be due to the action of the endogenous phytase present in feed ingredients (Ravindran et al., 1995). It is also possible that endogenous phytase may be present in the gut of pigs considering that they have closely similar digestive physiology as chickens. In this regard, Bitar and Reinhold (1972) and Maenz et al. (1997) identified a significant level of phytase activity in the small intestinal brush border membrane of the chicken. In the current *in vivo* study, it was also observed that the addition of organic acids to phytase further improved phytate hydrolysis in the cecum.

3.4.3. Utilization of Phytate Phosphorus

A mobility scoring system and bone ash percentage were used as indicators of bone development in the current study. Supplementing the negative control diet with phytase or phytase plus organic acids improved ($P = 0.003$) both the mobility score ($P = 0.06$) and bone ash content to the same level observed in pigs fed the positive control diet (Table 3.4). Because no inorganic phosphorus was added to diets 2, 3, and 4, the observed improvement in mobility score and bone ash content for pigs fed diets 3 and 4 relative to those fed the negative control diet, indicate that the phosphate groups liberated due to improved phytate hydrolysis were

Table 3.4. Mobility score and bone ash values of young pigs fed corn-soybean meal diets supplemented with microbial phytase and organic acids.

	DIETS[1]					
Item	1	2	3	4	SEM[2]	*P*[3]
Mobility score	4.7[a]	3.8[b]	4.7[a]	4.8[a]	0.5	0.06
Bone Ash (%)	44.9[a]	39.6[b]	45.0[a]	45.7[a]	2.8	0.003

[1]Diets are the same as in Table 3.2

[2]SEM = standard error of mean.

[3]*P*= observed significance due to effect of treatment.

[ab]Means with same superscripts are not significantly different (P > 0.05)

utilized for bone development. Supplementing microbial phytase with or without organic acids in the negative control diet improved ($P = 0.003$) bone ash content to the same level as in the positive control diet. Bone ash is often used as an indicator of bone mineralization, with high levels indicating increased incorporation of minerals into the bone structure (Radcliffe et al., 1998). Because, mobility and bone ash content values for diets 3, and 4 were not different from the positive control, the present result suggests that addition of phytase alone or phytase plus organic acids may completely replace the use of inorganic phosphorus in diets of young pigs. This is in close agreement with the findings of Han et al. (1998) indicating that supplementing a corn soybean meal-based diet with microbial phytase (1200 U/kg) and endogenous wheat phytase (15% of diet) improved ($P = 0.002$) mobility of early-weaned pigs relative to diets without any of the phytase sources.

3.4.4. Digestibility of DM and Phosphorus Excretion

Digestibility of DM and phosphorus, phosphorus excretion and the percentage reduction in phosphorus excretion are shown in Table 3.5. There were no differences ($P = 0.23$) in DM digestibility among treatments. Harper et al. (1997) and Jongbloed et al. (1996) were also unable to demonstrate an effect on DM digestibility as a result of supplementing weanling and growing pig diets with phytase or phytase plus lactic acid. In the current study, diets supplemented with

Table 3.5. Effect of supplementing corn SBM-based diet with phytase and organic acids on ileal DM and P digestibility (%) and P excretion (kg/ tonne of feed consumed).

	DIETS[1]					
Item	1	2	3	4	SEM[2]	*P*[3]
DM digestibility(%)	80.8	80.2	81.0	80.9	0.32	0.23
P digestibility (%)	29.8[b]	28.8[b]	48.3[a]	53.4[a]	11.0	0.0001
P excretion (kg/ tonne of feed)	3.1[a]	2.9[a]	2.7[ba]	2.5[b]	0.23	0.03
Reduced P excretion (%)	-	-	12.9	19.4	-	-

[1]Diets are the same as in Table 3.2

[2]SEM = standard error of mean

[3]*P*= observed significance due to effect of treatment.

[ab]Means with same superscripts are not significantly different (P > 0.05)

phytase or phytase plus organic acids had similar phosphorus digestibility, which were higher (P = 0.0001) than those of the positive and negative control diets. Addition of phytase in diet 3 only led to numerical reduction (P > 0.05) in the amount of phosphorus excreted when compared to diets 1, and 2. However, with the inclusion of organic acids (diet 4) there was significant reduction (P = 0.03) in the amount of phosphorus excreted per tonne of feed consumed compared to the positive control.

The result of the current study indicate a 22 percentage unit increase in phosphorus digestibility with the addition of 500 U phytase /kg diet. Harper et al. (1997) and O'Quinn et al. (1997) reported a range of 23 to 44% improvement in total tract phosphorus digestibility when 500 U/kg dietary phytase was added to a sorghum-soybean meal-based diet fed to growing pigs. Results of short term digestibility trials using weanling or very young pigs have indicated increases in phosphorus digestibility from 30% and above depending on levels of phytase in the diet (Simons et al., 1990; Lei et al.,1993a,b; Mroz et al., 1994; Kornegay and Qian, 1996).

Estimates of the reduction in phosphorus excretion with phytase and organic acid use have relevance because regular application of swine manure to agricultural soils can result in excessive accumulation of soil phosphorus (Mueller et al., 1994). In the future, it is likely that application of swine manure may be limited based on phosphorus content in an effort to reduce the potential for water pollution. In the present study, estimates of the reduction in phosphorus excretion

with 500 U phytase /kg diet, and 500 U phytase /kg diet plus organic acids was 12.9% and 19.4%, respectively, relative to pigs fed the positive control diet. This level of reduction in fecal phosphorus excretion is in close agreement with the values reported by Harper et al. (1997) who found a 21.5% reduction in phosphorus excretion with 500 U phytase /kg of diet. However, these values are somewhat lower compared to the levels (35 to 42%) reported by Lei et al. (1993a) and Kornegay and Qian (1996) in younger pigs fed diets supplemented with higher levels of inorganic P and phytase than that used in the present study.

3.4.5. Apparent Crude Protein and Amino Acid Digestibility.

Apparent ileal crude protein and amino acid digestibilities are shown in Table 3.6. There were no differences in apparent ileal crude protein digestibility among dietary treatments ($P = 0.26$). Supplementing the negative control diet with phytase alone or in combination with organic acid generally had no effect ($P > 0.10$) on apparent ileal amino acid digestibilities, except for isoleucine ($P = 0.0004$) and histidine ($P = 0.08$) whose digestibilities were improved (Table 3.6). Of all the dispensable amino acids, only the digestibility of aspartic acid was increased ($P = 0.02$) due to phytase plus organic acid supplementation compared to the positive and negative control diets. Nonetheless, the current observation is in agreement with that of Peter and Baker (2001), and Traylor et al. (2001) indicating no improvement in amino acid utilization in growing pigs fed soybean meal based diets

Table 3.6. Effect of phytase and organic acids supplementation on apparent ileal digestibilities (%) of protein and amino acids in corn-soybean meal-based diets.

	DIETS[1]					
Item	1	2	3	4	SEM[2]	*P*[3]
Crude Protein	78.9	78.7	79.5	80.9	0.8	0.3
Amino acids						
Indispensable						
Valine	77.1	77.4	76.0	78.1	1.3	0.2
Isoleucine	77.1[b]	76.9[b]	77.8[b]	86.2[a]	0.8	0.01
Threonine	73.2	66.8	71.4	74.1	2.2	0.3
Leucine	81.2	78.6	78.3	80.6	1.5	0.3
Phenylalanine	81.2	77.5	77.7	81.7	1.8	0.7
Histidine	77.5[b]	75.8[b]	81.8[a]	80.1[a]	0.7	0.08
Lysine	81.2	81.1	83.2	83.7	1.4	0.7
Arginine	86.7	87.2	84.3	88.1	1.2	0.3
Dispensable						
Aspartic acid	73.2[b]	74.0[b]	76.6[ab]	79.6[a]	1.4	0.02
Serine	78.7	72.7	75.8	78.4	1.7	0.3
Glutamic acid	78.5	77.6	79.8	81.1	1.5	0.3
Proline	77.6	72.8	76.1	76.1	1.5	0.4
Glycine	69.2	65.1	66.8	71.8	1.9	0.5
Alanine	75.2	71.2	72.4	76.1	2.0	0.4
Tyrosine	77.6	77.9	78.3	83.1	1.5	0.5

[1]Diets are the same as in Table 3.2

[2]SEM = standard error of mean

[3]*P*= observed significance due to effect of treatment.

[ab]Means with same superscripts are not significantly different (P > 0.05)

supplemented with microbial phytase. Similarly, O'Quinn et al. (1997) and Valaja et al. (1998) reported that microbial phytase supplementation had no effect on apparent ileal and total tract crude protein and amino acid digestibilities in finishing pigs. However, others have found significant improvements in amino acid utilization due to addition of microbial phytase to swine diets. For instance, Kemme et al. (1995) and Kornegay et al. (1998) reported improvements ($P < 0.05$) in amino acid digestibilities in growing pigs fed a corn-soybean meal based diet supplemented with microbial phytase.

The primary objective of supplementing swine diets with microbial phytase is to facilitate the breakdown of the phytate molecule, so as to avail the phytate bound phosphorus for pig utilization. Because phytic acid is naturally associated with proteins in the aleurone layers of cereal grains and the protein bodies of oil seeds (deBoland et al., 1975) and is able to bind proteins and divalent cations such as Ca^{2+}, Mg^{2+} and Zn^{2+} to form protein-mineral-phytate complexes (Cheryan, 1980; Grynspan and Cheryan, 1983), it is believed that facilitating its breakdown might also improve the availability of proteins and amino acids and minerals to the pig. In a study with rats, however, Thompson and Serraino (1986) could not demonstrate an effect on digestibility of proteins and absorption of amino acids in rapeseed flour containing 3 - 6% phytic acid. On the contrary, a recent study by Kornegay et al. (1998) showed improvements ($P < 0.05$) in amino acid digestibilities in growing pigs fed a corn-soybean meal based diet supplemented with microbial phytase Although in the current study amino acid digestibilities were generally unaffected

by microbial phytase supplementation, numerical improvements ($P > 0.10$) ranging from 1.6 to 7.8% were observed due to phytase plus organic acid addition (Table 3.6). This might be due to the fact that the diets were formulated to be sufficient in protein and amino acid contents, in that case the effect of phytase addition on protein and amino acid digestibilities will not be easily detected.

The current study indicates a potential for microbial phytase and organic acids to eliminate the addition of inorganic phosphorus in the diets of early-weaned pigs. However, further research is needed to determine if a higher level of organic acid plus microbial phytase may further increase phytate phosphorus utilization at the ileal level, and other dietary nutrients to enhance piglet performance.

3.5. IMPLICATIONS

The results of the present study clearly demonstrate that the addition of microbial phytase plus organic acids significantly improves phytate phosphorus utilization in young pigs fed diets formulated without inorganic phosphorus. Estimates from these data indicate that 500 U/kg of supplemental phytase plus 0.35% of Nutri-acid® can completely replace phosphorus from inorganic sources while reducing fecal phosphorus excretion by approximately 20%. This is of significant importance to the swine industry as it not only offers a means to reduce feed cost but it also provides a strategy to reduce the risk of environmental pollution that currently limits manure application to agricultural land.

CHAPTER 4

MANUSCRIPT 2

THE EFFECT OF MULTI-ENZYME SUPPLEMENTATION OF LOWER QUALITY DIETS ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN EARLY-WEANED PIGS.

4.1. ABSTRACT.

A 28-d trial was conducted to evaluate the effect of a multi-enzyme preparation on growth performance and nutrient digestibilities in early-weaned pigs fed diets based on a variety of non conventional feed ingredients. The trial involved 24 piglets weaned at 18 ± 1 d of age and assigned to four dietary treatments in a completely randomized design. The dietary treatments were, a basal diet (corn/wheat/wheat screenings/millrun/hulless barley/soybean meal/peas/canola meal, formulated at 95% of NRC (1998) requirement except for available P which was reduced by 44% (Diet 1); Diet 1 + multi-enzyme A (Diet 2), Diet 1 + multi-enzyme B (Diet 3), and Diet 1 + multi-enzyme C (Diet 4). All three multi-enzyme supplements contained the same level of xylanase (250 units/kg complete feed), glucanase (150 units/kg), amylase (0.001%), protease (0.0003%), invertase (0.002%) and phytase (400 units/kg) activity and differed in the type of carbohydrase preparation used. In this regard, multi-enzyme A contained cellulase, galactanase and mannanase, multi-enzyme B the pectinase preparation, while multi-enzyme C was a combination of cellulase, galactanase, mannanase and pectinase. A source of organic acid, Nutri-acid® was also added to the enzyme supplemented diets. Each dietary treatment was randomly assigned to six replicate pens with one pig per pen, balanced for initial BW and sex. Feed intake and BW were determined weekly. During the 4th wk of the trial, fresh fecal grab samples were collected from each pen. At the end of the trial, all pigs were euthanized and digesta samples were collected from jejunum, ileum and cecum for nutrient digestibility

determination. The 3rd metatarsal bone was recovered and used to determine bone ash content. Pigs fed enzyme-supplemented diets had a higher ADG ($P = 0.02$) and FE ($P = 0.01$) than those fed the non-enzyme control diet. Average daily feed intake was similar among treatments ($P > 0.10$). Enzyme supplementation improved ($P = 0.002$) jejunal phytate digestibility, but had no effect on digestibilities of DM, starch and non-starch polysaccharides (NSP) ($P > 0.10$). Ileal DM, starch, NSP, GE, CP and phytate digestibilities were higher ($P < 0.05$) in enzyme-supplemented diets than in the non-enzyme control diet. Ileal digestibilities of all nutrients were similar among Diets 2, 3 and 4, except for NSP whose digestibility was further improved ($P = 0.007$) when Diet 4 was fed relative to Diets 2 and 3. Cecal digestibilities of DM ($P = 0.02$), phytate ($P = 0.002$) and starch ($P = 0.006$) were improved in pigs fed Diets 2, 3 and 4 compared to Diet 1, but there were no differences among the enzyme-supplemented diets. Total tract digestibilities of all nutrients were increased ($P < 0.01$) when Diets 2, 3, and 4 were fed, and followed a similar trend as in the ileum. There were no differences ($P > 0.10$) in total tract digestibilities of all nutrient among Diets 2, 3, and 4 except for NSP digestibility which was increased ($P = 0.0001$) by an average of 10% when Diet 4 was fed, relative to Diets 2 and 3. Bone ash content was higher ($P = 0.006$) in pigs fed Diets 2, 3 and 4 compared to control. In conclusion, addition of a multi-enzyme preparation to pig starter diets based on non conventional ingredients, improved both the ileal and total tract DM, CP, starch, GE, NSP and phytate digestibilities and dietary phosphorus utilization.

4.2. INTRODUCTION

Swine production is an industry that is growing tremendously in the World and North America inclusive. In Western Canada it represents a large percentage of farm income. However, to optimize production, it is imperative that cost of production is lowered. This may be achieved by using locally available feed ingredients as opposed to depending on supply from external sources, which are often quite expensive. An example is Western Canada where wheat and barley are more cultivated than corn. However, these ingredients are used to a limited extent in young pig diets because of the presence of anti-nutritional factors in their endosperm. Hesselman and Åman (1986) and Li et al. (1996) found that the presence of anti-nutritional factors such as the non-starch polysaccharides in the endosperm of wheat, barley, rye and oat reduced their nutritional value. In recent years, efforts to improve the nutritive value of these feed ingredients has focused on means to eliminate the effects of the anti-nutritional factors. One such method is the use of exogenous enzymes. It is well documented that bacterial and fungal preparations effectively degrade most of the viscous polysaccharides e.g., ß-glucans and arabinoxylans in barley, oats, rye and wheat (Li et al., 1996). Likewise, microbial phytase preparations are known to improve the utilization of phytate phosphorus (Cromwell, 1992; Simons et al., 1990). However, the use of exogenous enzymes to degrade a variety of indigestible dietary components other than those listed above, has met with inconsistent results. This has been attributed to the presence of complex substrates in feedstuffs and the use of enzyme activities not

suitable for degrading such complex substrates (Slominski, 2000). Previous research with poultry (Cleophas et al., 1995) and young pigs (Graham et al., 1988) suggests that a combination of different enzyme activities is required for complete degradation of complex NSP and higher dietary nutrient digestion. Similarly, *in vitro* studies by Meng et al. (2002) showed that a combination of carbohydrases led to significant improvements in NSP depolymerization in soybean meal, canola meal and peas than when the carbohydrases were used individually; this multi-enzyme preparation was further used to improve starch and NSP digestibilities, body weight gain and feed conversion ratio in broiler chickens and adult roosters.

Although the use of multi-enzyme preparations to enhance nutrient digestion and utilization has been extensively evaluated in studies with chickens, only a few studies have looked at the use of multi-enzyme preparations in pig diets. Furthermore, the few studies conducted with pigs have mainly involved growing pigs and provided inconsistent results (Officer, 1995; Li et al.,1996). The objective of the current study was to examine the effect of supplementing a pig starter diet based on a wide selection of poorly digested ingredients with multi-enzyme preparations on nutrient digestion and growth performance of early weaned pigs.

4.3. MATERIALS AND METHODS

4.3.1. Pigs and Housing

Twenty-four Cotswold pigs averaging 6.52 ± 0.83 (mean ± SD) kg BW and weaned at 18 ± 1 d (mean ± SD) were blocked on the basis of sex and BW and

then fed a commercial diet for a 7-d adaptation period. The average weight at the end of the adaptation period was 7.0 ± 0.42 (mean ± SD) kg. Pigs were then assigned randomly from within block to dietary treatments. Each dietary treatment was assigned to six replicate pens each with 1 pig per pen and pigs had unlimited access to feed and water. Individual BW and pen feed disappearance were monitored weekly. Room temperature was initially set at 29.5°C and gradually reduced by 1.5°C per wk. Fecal grab samples were collected from each pen during the last wk of the experiment. At the end of the 28 d trial, all the pigs were killed as described in manuscript 1 and digesta collected from the jejunum, ileum and cecum. The samples were kept frozen at -20°C until required for chemical analysis. The left hind leg was also recovered from each pig and placed in a labeled sample bag and stored frozen. All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

4.3.2. Experimental Diets

All pigs were fed a commercial starter diet for an adaptation period of 1 wk and then randomly assigned to the experimental diets. The ingredient and nutrient composition of the basal diet is shown in Table 4.1 and 4.2, respectively. It was formulated at 95% of NRC (1998) recommended levels, available P was reduced by 44%. This basal diet contained corn/wheat/wheat screenings/millrun/hulless barley/soybean meal/peas and canola meal, (Diet 1); Diet 1 + multi-enzyme A

Table 4.1. Ingredient composition of basal diet[a].

Ingredient	%
Corn	30.00
Wheat	5.00
Wheat screenings	6.00
Hulless Barley	7.00
Millrun	4.00
Soybean meal	20.00
Canola meal	6.11
Vegetable oil	1.03
Peas	8.00
Blood meal	1.00
Dried whey	8.00
Biophos	0.37
Limestone	1.62
Vitamin premix[b]	1.00
Mineral premix[c]	0.50
Chromic oxide	0.30
DL-Methionine	0.02
Lysine HCl	0.05

[a]Diet was formulated and then divided into 4 portions for the addition of enzymes (1g kg^{-1}) and organic acid (0.35 g kg^{-1}).

[b]Supplied per kg diet: vitamin A, 8255 IU; vitamin D3, 1000 IU; vitamin E, 10.9 IU; vitamin B12, 0.115 mg; vitamin K, 1.1 mg; Niacin, 36.8 mg; Choline chloride, 781.2 mg; Biotin, 0.25 mg, and Folic acid, 0.75 mg;

[c]Supplied per kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I,0.28mg

Table 4.2. Calculated nutrient composition[1] of the basal diet.

Item	
DE, kcal/kg	3405
Crude protein, %	20.23
Threonine, %	0.73
Cystine, %	0.34
Methionine, %	0.33
Lysine, %	1.12
Phytate phosphorus, %	0.25
Calcium, %	0.80
Available phosphorus, %	0.25

[1]Based on NRC (1998) feed composition data.

(Diet 2); Diet 1 + multi-enzyme B (Diet 3); and Diet 1 + multi-enzyme C (Diet 4). All three multi-enzyme supplements contained the same level of xylanase (250 units/kg complete feed), glucanase (150 units/kg), amylase (0.001%), protease (0.0003%), invertase (0.002%) and phytase (400 units/kg) activity and differed in the type of carbohydrase preparation used. In this regard, multi-enzyme A contained cellulase, galactanase and mannanase, multi-enzyme B the pectinase preparation while multi-enzyme C was a combination of cellulase, galactanase, mannanase and pectinase. A source of organic acid, Nutri-acid® was also added to the enzyme supplemented diets. Enzyme supplements and organic acids were provided by Canadian Bio-Systems Inc., Calgary, AB. Millrun and wheat screenings were provided by Ritchie Smith Feeds, Abbotsford, BC. Chromic oxide was added as an indigestible marker and all diets were pelleted at 54°C.

4.3.3. Sample Preparation and Chemical Analyses

Samples collected from the jejunum were analyzed for DM, starch, NSP and phytate content. Ileal and fecal samples were analyzed for DM, starch, NSP, phytate, gross energy and CP, whereas cecal samples were analyzed only for DM, starch and phytate. All samples were prepared and chemically analyzed as described in manuscript 1, except for energy, NSP and starch analyses. Gross energy was measured using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL) that had originally been calibrated. Non-starch polysaccharides were

determined by gas-liquid chromatography (component neutral sugars) and by calorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984), with some modifications by Slominski and Campbell (1990). Briefly, 100 mg sample was treated with dimethylsulfoxide and incubated overnight with a solution of starch degrading enzymes (amylase and pullulanase; Sigma Chemical Co., St.Louis, MO) at 45°C. 43 mL of ethanol was then added and the mixture left to stand for 1 h and then centrifuged at 2400 x *g* for 30 min and the supernatant was discarded. The precipitate was dissolved in 1 ml of 12 M H_2SO_4 and incubated for 1 h at 35°C. Six mL of de-ionized water and 5 mL of myo-inositol (internal standard) solution were then added and the mixture was boiled for 2 h. One mL of the hydrolysate was then taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetate anhydride in the presence of 1-methylimidazole. Component sugars were separated using SP-2340 column and Varian Vista 6000 Gas Chromatograph. Starch was analyzed according to the procedure described by Macrae and Armstrong (1968). Briefly, 100 mg of sample and 50 mg of corn starch (standard; Sigma Chemical Co., St. Louis, MO) were respectively weighed into hydrolysing tubes. Twenty mL of 80% ethanol was added to the tubes, which were then incubated in a water bath at 75°C for 60 min. After cooling at room temperature, the tubes were centrifuged at 2400 x *g* for 15 min, after which the supernatant was carefully removed with suction, and 25 mL of 0.15 N sodium acetate buffer (pH 5) and 100µl of α-amylase (Termanyl) added to the precipitate. The mixture was then incubated at 100°C for 30 min, after which

time the samples were cooled down to 50°C and then 100µl of amyloglucosidase (10 mg/ 1 mL sodium acetate buffer) was added. The tubes were then capped and incubated at 45°C overnight in a water bath. In the following morning, tubes were cooled to room temperature and then centrifuged at 2400 x *g* for 10 min. 0.01 mL of samples from each tube was then pipetted into cuvets. One mL of glucose reagent (Sigma Chemical Co., St. Louis, MO) was also added in to the cuvets. The absorbance was read at 340 nm against de-ionized water.

4.3.4. Calculations and Statistical Analysis

Analysis of growth performance data, calculations of digestibility values determined in the jejunum, ileum, cecum and feces and their respective statistical analyses were performed according to the methodology described in manuscript 1 of this thesis.

4.4. RESULTS AND DISCUSSION

4.4.1. Animal Performance

Average daily feed intake, ADG and FE are shown in Table 4.3. There were no differences in ADFI among treatments ($P > 0.10$). Pigs fed enzyme-supplemented diets had a higher ADG ($P = 0.02$) and FE ($P = 0.01$) than those fed the basal diet. However, there were no differences in ADG and FE among pigs fed diets supplemented with different carbohydrase preparations. The present results are in agreement with that of Bedford et al. (1992) who observed an improvement

Table 4.3. Effect of multi-enzyme supplementation on performance of early-weaned pigs.

	DIETS					
Item	1	2	3	4	SEM[1]	P^2
ADFI, g/d	432.3	435.4	456.4	413.6	15.2	0.42
ADG, g/d	223.6[b]	252.1[a]	262.8[a]	249.1[a]	14.4	0.02
Gain/Feed	0.52[b]	0.58[a]	0.58[a]	0.61[a]	0.03	0.01

[1]SEM = standard error of mean.

[2]*P* = observed significance due to effect of treatment.

[ab]Means with different superscripts are significantly different ($P < 0.05$)

in weight gain and feed efficiency when a combination of glucanases was added to a rye- and barley-based diets and fed to 5 wk old pigs. Similarly, Baidoo et al. (1997) reported an improvement in feed efficiency of growing pigs fed hulless barley diets supplemented with a blend of ß-glucanase, xylanase, amylase and pectinase. Most of the studies with pigs on the use of multi-enzyme have been done with pigs of initial weight of 19 kg and above, thus there is little or no information on multi-enzyme supplementation in pigs weaned as early as 18 d. Furthermore, in the growing and finishing pigs, results on the effect of multi-enzyme preparation have been contradictory. Feeding a wheat-soybean-based diet supplemented with a multi-enzyme preparation (ß-glucanase, hemi-cellulase, pentosanase and cellulase), Officer (1995) found no improvement in ADFI, ADG and FCE of pigs weaned at 27- 29 d. However, Grandhi (2001) found improvement in FCE but not in ADFI and ADG when barley diets supplemented with commercial carbohydrases were fed to pigs of 20 kg BW.

The apparent contradictions in the effectiveness of enzyme supplementation among studies is mainly attributable to differences in age of the pigs and the composition of diets used. In general, the impact of enzyme supplementation on nutrient digestion declines with pig age, particularly because digestive capacity in pigs improves with age as the enzyme system matures and gut microbial population increases (Lindemann et al., 1986). Also, the extent to which enzyme supplementation improves nutrient digestibility tends to be low when using diets containing highly digestible ingredients (Johnson et al., 1993). From a practical

swine nutrition perspective, it would appear that supplementing enzymes to pig diets will be more beneficial when using diets based on ingredients that are of lower quality and poorly digested similar to those used in the current study.

4.4.2. Digestibility of Nutrients in the Jejunum

Dry matter, starch, NSP, and phytate digestibilities at the jejunum level are shown in Table 4.4. Enzyme supplementation increased (P = 0.002) phytate digestibility and there were no differences in phytate digestion among pigs fed enzyme supplemented diets (P > 0.10). Dry matter, starch and NSP digestibilities at the jejunal level were not influenced by enzyme supplementation (P > 0.10; Table 4.4), although there were numerical increases in DM and starch digestibilities following enzyme supplementation. Similarly, NSP digestibilities were not different among dietary treatments (P > 0.10; Table 4.4), indicating that little or no NSP is digested in the upper gut. This may be due to the slow action of exogenous enzymes and a low number of intestinal microorganisms present in the stomach and jejunum of the young pig when compared to the lower sections of the gastrointestinal tract (Gdala et al., 1997).

In manuscript 1 of this thesis, it was observed that feeding microbial phytase or phytase and organic acids improved phytate digestibility in the stomach. Based on this fact, it was expected that observed improved phytate digestibility would be in post-stomach sections of the gut of pigs fed diets that were supplemented with these additives. This might be the case in the current study where phytate

Table 4.4. Digestibility of DM, starch, NSP and phytate in the jejunum of early-weaned pigs fed diets supplemented with multi-enzyme preparations (%).

	DIET					
Component	1	2	3	4	SEM[1]	*P*[2]
DM	58.2	60.1	59.5	60.0	0.9	0.7
Starch	75.2	80.2	78.6	81.7	2.8	0.8
NSP	-17.0	0.8	-11.3	0.9	9.0	0.4
Phytate	21.7[b]	42.6[a]	36.0[a]	38.9[a]	9.2	0.002

[1]SEM = standard error of mean.

[2]*P* = observed significance due to effect of treatment.

[ab]Means with different superscripts are significantly different ($P < 0.05$)

digestibility was improved in the jejunum of piglets fed enzyme supplemented diets. However, jejunal phytate digestibility values observed due to enzyme supplementation in the present study are lower than values obtained in the stomach of pigs in manuscript 1, despite that piglets of same age were used in both studies. This may be due to differences in feed composition. Diets used in manuscript 1 were made of ingredients with readily available nutrients rather than the poorly digestible feedstuffs used in the present trial. A study by Han and Wilfred (1988) revealed that soybean phytate was more readily and extensively hydrolyzed than cottonseed phytate and the difference was attributed to variations in cellular location of phytate in different feedstuffs.

4.4.3. Ileal Digestibility of Nutrients.

Ileal digestibilities of DM, starch, phytate, gross energy and CP were similar among enzyme supplemented diets ($P > 0.10$) and all were higher ($P < 0.05$) than the control treatment (Table 4.5). However, NSP digestibility was further improved ($P < 0.05$) in pigs fed Diet 4 compared to Diets 2, and 3. Gdala et al. (1997) reported improved ($P < 0.05$) ileal DM digestibilities in soybean / rapeseed meal-based diets fed to 8 - 12 wk old piglets when supplemented with α-amylase alone or a multi-enzyme preparation containing α-galactosidase, xylanase and protease relative to non-enzyme supplemented diets. Although, unlike in the current study, enzyme supplementation had no effect on ileal digestibilities of starch, total NSP and crude protein in the study by Gdala et al. (1997), DM digestibility was similar to that

Table 4.5. Digestibility of selected components in the ileum of early-weaned pigs fed diets supplemented with multi-enzyme preparations (%).

	DIET					
Component	1	2	3	4	SEM[1]	P^2
DM	60.1[b]	65.8[a]	66.1[a]	66.7[a]	3.1	0.01
Starch	86.7[b]	92.6[a]	94.6[a]	95.3[a]	3.9	0.02
NSP	10.1[c]	14.9[b]	16.4[b]	21.4[a]	4.7	0.007
Gross Energy	62.8[b]	70.0[a]	69.7[a]	71.4[a]	3.8	0.0008
CP	62.1[b]	71.5[a]	71.4[a]	73.2[a]	5.0	0.0002
Phytate	59.2[b]	71.7[a]	69.1[a]	69.7[a]	5.6	0.04

[1]SEM = standard error of mean.

[2]P = observed significance due to effect of treatment.

[abc] Means with same superscripts are not significantly different ($P > 0.05$)

observed in the current study (65% *vs.* 66.2%). Li et al. (1996), using 28-d old piglets fed hulless barley or wheat/ soybean meal diets supplemented with 0.2% ß-glucanase (ß-glucanase refers to a mixture of enzymes with endo- and exo-ß-glucanase and ß-glucosidase activities), found improved ileal digestibilities of DM, crude protein and gross energy. Similarly, in a review by Slominski (2000), it was reported that broiler chickens fed a wheat/hulless barley/canola meal-based diet supplemented with either enzyme A (xylanase and ß-glucanase) or enzyme B (xylanase + ß-glucanase fortified with a broad spectrum of other enzyme activities) had increased ($P \leq 0.05$) DM, starch, NSP and phytate digestibilities compared to the non-supplemented basal diet. DM and starch digestibility values obtained in the present study (66.2 and 94.2%) are close to those (67.8 and 98.1%) reported by Slominski (2000). Similarly, ileal NSP digestibility values obtained in the current study are close to those reported by Slominski (2000) in a broiler trial where birds were fed diets supplemented with multi-enzyme preparations. Using similar type of ingredients and the same group of carbohydrases in a growing pig trial, it was further reported in the review by Slominski (2000) that the digestibility results followed the same trend as in the broiler trial, and the values were slightly closer to those obtained in the current trial.

In the present study, ileal phytate digestibility was improved ($P = 0.04$) when Diets 2, 3, and 4 were fed relative to Diet 1. This is in close agreement with ileal phytate digestibility values reported in manuscript 1. However, these values were higher than those reported by Li (2000) in young pigs fed wheat-hulless

barley-based diets supplemented with microbial phytase and carbohydrases.

Differences observed in the literature for the ileal digestibility of nutrients (especially, starch, energy, NSP and crude protein) may be attributed to the age of pigs used in the different experiments. Graham et al. (1988) suggested that in pigs the fiber degrading capacity in the small intestine increases with age. Multi-enzyme supplementation may therefore be more beneficial in diets for early-weaned rather than for growing or finishing pigs. Differences in response to multi-enzyme supplementation between different studies may also depend on dietary NSP content, for example, diets containing barley tend to have lower digestibilities of nutrients like starch and crude protein depending on the amount of barley included in the diet. The mixed linked ß-glucans of barley are mainly localized in the cell walls of the endosperm. They represents about 75% of the polysaccharides in barley, the remainder being mainly cellulose, arabinoxylans, mannose containing polymers, protein and phenolic compounds (Fincher, 1975; de Silva et al., 1983). Similarly, in wheat the non-cellulosic polysaccharides consist mainly of arabinoxylans and β-glucans while in soybean, canola or peas arabinogalactans, arabinans, galactans, galactomannans, mannans and pectic polysaccharides predominate (Slominski, 2000). In cereal grains, arabinoxylans and β-glucans are found in the cell walls of the protein rich aleurone layer and starchy endosperm (Basic and Stone, 1991) and can act as a physical hindrance for nutrient hydrolysis and absorption. Similarly, the cell wall polysaccharides of soybean, canola seed or peas may also be responsible for the nutrient encapsulating effect. Grinding and pelleting of feed ingredients

during preparation of the feed significantly reduces this physical hindrance, although some part of the grain/seed could be unaffected, leaving starch and intracellular protein surrounded by cell walls and unavailable for digestion in the ileum as was seen in diet 1 of the present study. However, with the addition of carbohydrase enzymes that contain xylanase, glucanase, cellulase, pectinase, galactanase and mannanase activities, the cell wall polysaccharides are broken down and the cell walls, at least partly, depolymerized (Fleming and Kawakami, 1977; Hesselman and Åman, 1985; Meng et al., 2002) which could lead to improved utilization of nutrients as was observed in the current study.

Furthermore, the inclusion of microbial phytase in the enzyme preparation used in the present study, assisted in the break down of complex linkages that potentially existed between phytate molecules, starch and protein. This action of microbial phytase was reported in manuscript 1 of this thesis, and was shown to improve digestibility of phytate along with a slight improvement ($P > 0.05$) of other dietary components. In the current study, ileal phytate digestibility increased ($P = 0.04$) by an average of 18% over the control diet. This is in close agreement with ileal phytate digestibility values reported in manuscript 1.

Energy digestibility values determined in the current study were significantly higher ($P = 0.0008$) in enzyme supplemented diets relative to the control, and the results were similar to values reported by Li et al. (1996) in a trial where a mixture of carbohydrases was supplemented to a hulless barley-wheat-based diet fed to 28 d old piglets. Similarly, Graham et al. (1986) and Jensen et al. (1998) also observed

a consistent increase in starch and energy digestibility following ß-glucanase supplementation. The increase in energy digestibility observed in the present study might have been due to the improved ileal starch digestibility. It is well known that the absorption of starch as glucose in the small intestine is more effective in terms of energy utilization than its conversion to short chain fatty acids in the large intestine (NRC, 1998). Therefore, the increase in starch digestibility in the ileum will represent an increase in ileal glucose uptake and a subsequent increase in ileal energy utilization.

4.4.4. Digestibility of Nutrients in the Cecum.

Cecal digestibilities of DM and phytate were similar in pigs fed Diets 2, 3, and 4 ($P > 0.10$) and all were higher ($P < 0.05$) than the control (Table 4.6). Starch digestibility was also higher ($P = 0.006$) in pigs fed enzyme supplemented diets. Furthermore, starch digestibility improved ($P < 0.05$) in pigs fed Diet 4 compared to Diets 2. The observed values of cecal nutrient digestibilities in the present study are similar to those reported by Bedford et al. (1992) who found numerical increases in the digestibility of DM and starch in the cecum of growing pigs fed a rye and barley-based diet supplemented with enzyme relative to the same diet without enzyme. However, Gdala et al. (1997) failed to show any increase in digestibility of DM and starch in the cecum of 14 - 21 wk old pigs fed a corn-soybean-rapeseed meal diet supplemented with carbohydrases.

As stated earlier, the discrepancies in digestibility values between the

Table 4.6. Digestibility of DM, starch and phytate in the cecum of early-weaned pigs fed diets supplemented with multi-enzyme preparations (%).

	DIET					
Component	1	2	3	4	SEM[1]	*P*[2]
DM	66.2[b]	69.1[a]	69.0[a]	69.9[a]	1.6	0.02
Starch	89.2[c]	93.2[b]	96.1[ab]	96.5[a]	3.2	0.006
Phytate	66.3[b]	84.7[a]	85.1[a]	86.0[a]	11.5	0.002

[1]SEM = standard error of mean.

[2]*P* = observed significance due to effect of treatment.

[abc] Means with same superscripts are not significantly different ($P > 0.05$)

current study and the literature data might be due to the age of animals used or differences in feed composition. Li et al. (1996) reported that the effect of enzyme supplementation on nutrient digestibility was negligible in pigs above 20 kg BW. In the current study, very young pigs (18 ± 1 d) with an average BW of 13 kg (at end of trial) were used and this might be the reason for the observed significant effect of enzyme supplementation on nutrient digestibility in the ileum and cecum.

4.4.5. Total Tract Digestibility of Nutrients.

Total tract nutrient digestibilities are shown in Table 4.7. There was an increase ($P < 0.05$) in DM, starch, NSP, gross energy, crude protein, and phytate digestibilities as a result of feeding enzyme supplemented diets when compared to non-enzyme supplemented control diet. Although, starch utilization was almost complete for the control diet, enzyme supplementation further increased ($P = 0.006$) its fecal digestibility. This is similar to findings of Graham et al. (1989) and Slominski (2000) indicating that starch was almost completely digested at the fecal level irrespective of dietary treatments. This is probably because all the diets were pelleted, as Graham et al. (1989) found no difference in starch digestibility in the gut, when a barley-based diet fed to growing pigs was either pelleted or supplemented with ß-glucanase.

Complete starch digestibility at the fecal level may also be due to the action of the microbes in the large intestine, which to a great extent can ferment complex polysaccharides. This was clearly the case in the present study, where NSP

Table 4.7. Total tract digestibility of selected components in early-weaned pigs fed diets supplemented with multi-enzyme preparations (%).

	DIET					
Component	1	2	3	4	SEM[1]	*P*[2]
DM	75.6[b]	78.1[a]	77.2[a]	80.0[a]	1.8	0.01
Starch	94.4[b]	98.6[a]	97.6[a]	98.6[a]	2.0	0.006
NSP	48.9[c]	61.2[b]	59.6[b]	66.8[a]	7.5	<0.001
Gross energy	77.8[b]	79.8[a]	79.8[a]	81.1[a]	1.4	0.01
CP	67.1[b]	71.2[a]	71.6[a]	74.2[a]	2.9	0.014
Phytate	69.4[b]	96.8[a]	96.3[a]	96.0[a]	13.5	0.001

[1]SEM = standard error of mean.

[2]*P* = observed significance due to effect of treatment.

[abc] Means with same superscripts are not significantly different (*P* > 0.05)

digestibility was relatively higher at the fecal than ileal and jejunal levels irrespective of dietary treatment. However, piglets fed enzyme supplemented diets had higher ($P < 0.0001$) fecal NSP digestibilities than those fed the control diet. Furthermore, fecal NSP digestibility increased by 6.4 percentage units when feeding Diet 4 compared to Diets 2 and 3. These values are in close agreement with values reported by Slominski (2000), and showing an increase of 5.9 percentage units when NSP digestibility values of enzyme supplemented diets was compared to a non-enzyme supplemented control diet.

Similarly, pigs fed Diets 2, 3, and 4 had improved fecal digestibilities of gross energy ($P = 0.012$), and crude protein ($P = 0.014$) compared to those of Diet 1. However, there were no differences in digestibilities of gross energy and crude protein among pigs fed Diets 2, 3, and 4. On average, gross energy and crude protein digestibilities of the enzyme-containing diets increased by 2.4 and 5.2 percentage units respectively over the control (Diet 1). This is similar to the findings of Li et al. (1996) who found an increase of 2.6 and 5.4 percentage units in gross energy and crude protein digestibilities, when a hulless barley/ soybean meal diet fed to young pigs was supplemented with 0.2% ß-glucanase (endo- and exo- ß-glucanase, and ß-glucosidase activities). Conversely, Thacker et al. (1992) in studies with 7 - 8 wk old pigs, reported no effect of ß-glucanase supplementation on the fecal digestibilities of crude protein and gross energy.

However, as previously noted, improvements in fecal nutrient digestibilities as a result of supplementation of growing pig diets with the conventional enzyme

preparations have not been significant (Thacker et al., 1992; Officer, 1995; Li et al., 1996).

The improvements in fecal crude protein and phytate digestibilities observed in the current study may have significant environmental implications as reduced amount of nitrogen and phosphorus would be excreted into the environment. In a study with chickens, Jacob et al. (2000) observed increased fecal nitrogen digestibility in 5 wk old broilers fed diets supplemented with a wide range of enzyme activities, which was associated with a 12.2 % reduction in daily nitrogen output.

4.4.6 Utilization of Phytate Phosphorus

Total tract phosphorus digestibility, bone ash percentage, and fecal phosphorus excretion are illustrated in Figures 4.1, 4.2, and 4.3, respectively. Pigs fed enzyme supplemented diets had higher total tract phosphorus digestibilities (P = 0.001; Figure 4.1) and bone ash content (P = 0.06; Figure 4.2) and lower amounts of phosphorus excreted (P = 0.04; Figure 4.3) than those fed the control diet. There were no differences in any of these parameters among the enzyme-supplemented diets (P > 0.10).

The result of the current study indicating a 56% increase in phosphorus digestibility with the addition of enzyme complex is higher than values reported in literature. O'Quinn et al. (1997) reported a 44% improvement, while Harper et al. (1997) found 33% increase in total tract phosphorus digestibilities when 500 U/kg dietary phytase was added to a sorghum-soybean meal-based diet fed to growing

Figure 4.1. Total tract phosphorus digestibility of early-weaned pigs fed multi-enzyme supplemented diets (%). [ab] Means with same superscripts are not significantly different ($P > 0.05$).

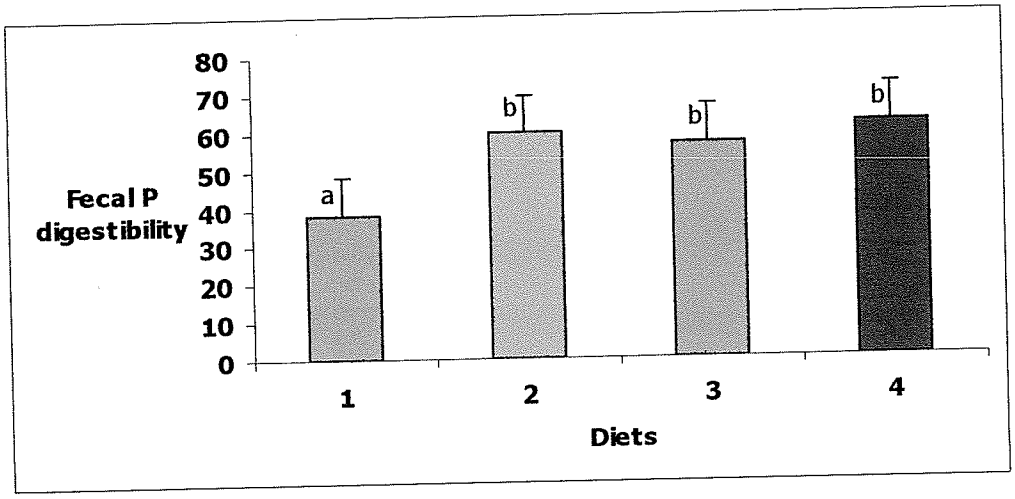


Figure 4.2. Bone ash content of early-weaned pigs fed multi-enzyme supplemented diets (%). [a] Means with same superscripts are not significantly different ($P > 0.05$).

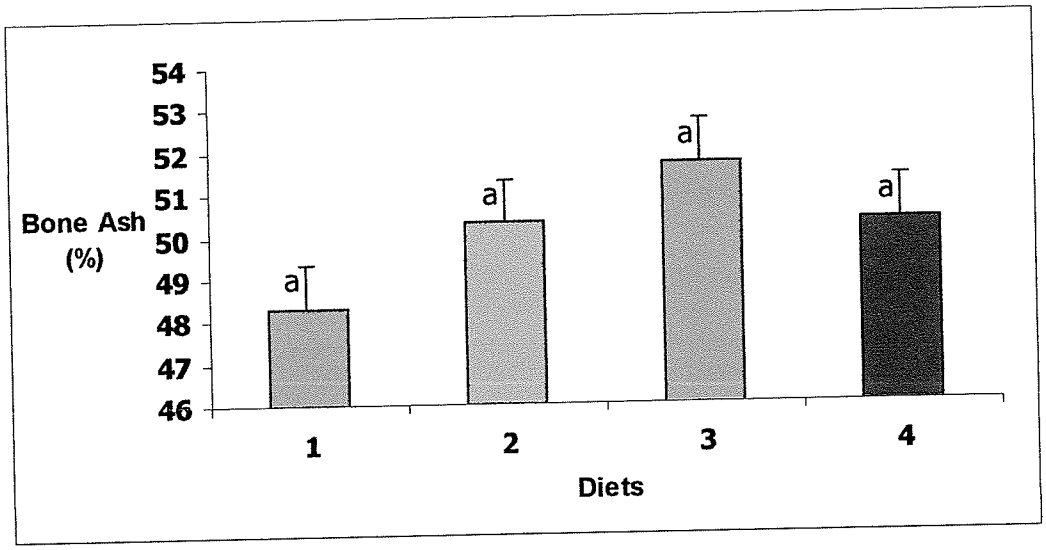
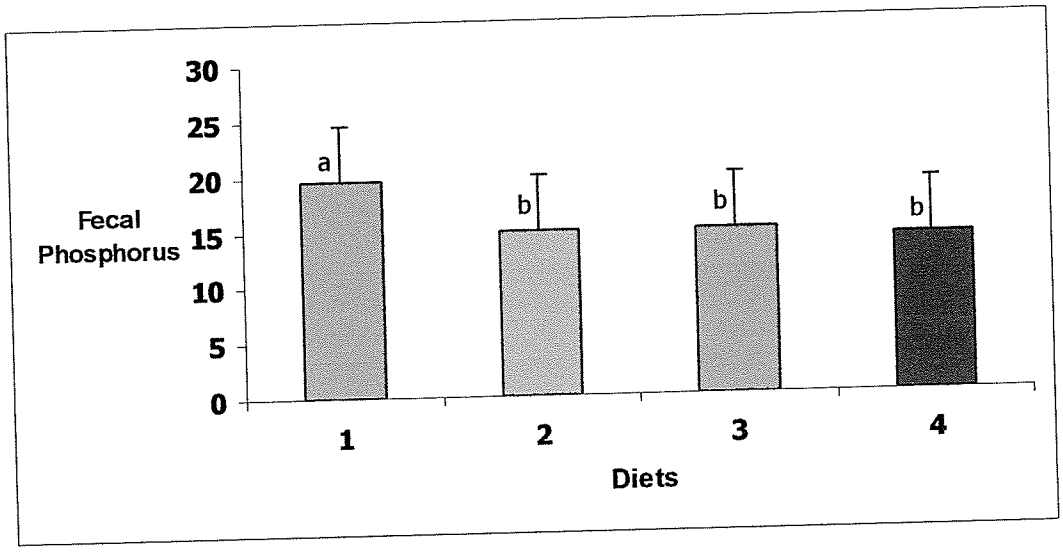


Figure 4.3. Fecal phosphorus excretion of early-weaned pigs fed multi-enzyme supplemented diets (Kg/ tonne of manure). [ab] Means with same superscripts are not significantly different ($P > 0.05$).



pigs. The improvement observed in the current study over literature values might be due to the fact that multi-enzyme preparation was used. In addition, there was an improvement of about 5.1% in the bone ash content of pigs fed Diets 2, 3, and 4 compared to those fed Diet 1. It can be argued that the additional phosphate liberated due to improved phytate hydrolysis in Diets 2, 3 and 4 were utilized for bone development. When the average amount (kg) of phosphorus excreted per tonne of feces was calculated for pigs fed Diets 2, 3, and 4, compared to that of the control, there was a 25.3% reduction ($P = 0.04$) in fecal phosphorus output. This was slightly higher than the reduced phosphorus value (20%) observed due to microbial phytase and organic acids supplementation in manuscript 1 and could be due to the fact that a broad range of enzyme activities was used in the present study. However, this reduction in fecal phosphorus excretion observed in the current study is in close agreement with the values reported by Lei et al. (1993a) and Harper et al. (1997) who reported 21.5 to 35% reduction in phosphorus excretion with 500 phytase U/kg of diet fed to young pigs.

The basal diet used in the current study was formulated with a range of feedstuffs that are known to be poorly digested by young pigs. Supplementation of this diet with different enzyme cocktails and organic acids improved the digestibility of all dietary nutrients examined thus indicating a potential for the simultaneous use of poorly digestible feed ingredients and appropriate enzyme combinations in diets for early-weaned pigs. However, further research is needed to determine the optimal inclusion levels of these lower quality ingredients in diets for early-weaned

pigs and to conduct an in-depth growth trial to determine if the improvements in nutrient digestibilities could be translated into improved growth performance of young pigs.

4.5. IMPLICATIONS

The results of the current study clearly demonstrate that, with an appropriate enzyme combination, young pigs can effectively utilize diets containing ingredients that are normally poorly utilized. This will increase the flexibility in feed formulation allowing for the use of locally grown alternative feedstuffs, with the potential to reduce feed costs. Application of such enzyme preparations also has tremendous potential as a tool for minimizing nitrogen and phosphorus excretion into the environment.

CHAPTER 5

MANUSCRIPT 3

GROWTH PERFORMANCE OF EARLY-WEANED PIGS FED A MULTI-ENZYME-SUPPLEMENTED DIET.

5.1. ABSTRACT

The effect of multi-enzyme supplementation of poorly digestible diets for early weaned pigs was investigated in a 38-d growth performance study involving 48 piglets weaned at 18 ± 1 d of age. A basal diet consisting of wheat, wheat screenings, millrun, peas, canola meal, and soybean meal and formulated to supply 95% of NRC (1998) nutrients requirements except for available P which was reduced by 44%, was fed as control (Diet 1) or plus multi-enzyme (Diet 2). The multi-enzyme preparation was the same as multi-enzyme C used in manuscript 2, and it contained xylanase, glucanase, amylase, invertase, protease, cellulase, galactanase, mannanase, pectinase and phytase activities. Each dietary treatment was assigned in a completely randomized design to six replicate pens with four pigs per pen, balanced for initial BW and sex. The experiment was divided into three phases, during which feed intake and BW were determined. At the end of phase I, there was no difference ($P = 0.24$) in ADFI between the pigs fed the control and enzyme-supplemented diets. Similarly, there was no difference ($P = 0.40$) in ADG between the pigs fed the 2 different diets. However, pigs fed the enzyme-supplemented diet had a higher ($P < 0.0001$) FE than those fed the control diet. In phase II, ADFI followed the same trend as in phase I with no significant difference ($P = 0.99$) between the pigs fed the enzyme-supplemented diet and those fed the control diet. However, pigs fed the enzyme-supplemented diet had a higher ADG ($P = 0.001$) and FE ($P = 0.04$) than those fed the control diet. Similarly, values observed in phase III and overall followed the same trend observed in phase II.

Pigs fed the enzyme-supplemented diet had a better ADG ($P < 0.001$) and FE ($P < 0.0001$) than those fed the non-supplemented diet, and there was no difference ($P > 0.10$) in ADFI between the two groups of pigs in phase III and overall. Overall and in all the 3 phases, ADG and FE of piglets fed the enzyme-supplemented diet were improved ($P < 0.05$) compared to those fed the non-supplemented diet.

5.2. INTRODUCTION

Hog production is a fast-growing industry in the World and represents a major source of income in North America. One of the ways to optimize production is to lower the cost of production. Feed is the single most expensive input in pork production representing about 60 - 70% of the total production cost in intensive swine production (Prairie Swine Centre Inc., 2000). Furthermore, starter diets are often very expensive because of the speciality ingredients used. This is because baby pigs have a relatively undeveloped gastrointestinal tract (Cranwell, 1995) and therefore require highly digestible feed ingredients. Over the years, feed ingredients like spray dried porcine plasma, fish meal (herring and menhaden), milk and milk products as well as refined soy bean meal have been used with a greater degree of success (Kats et al., 1992). However, these conventional ingredients are expensive. The current goal in swine production is to reduce the cost of production. This in turn, calls for the need to identify and increase the use of alternative and locally grown feed ingredients such as wheat and wheat products, barley, peas, canola meal, etc. However, the use of these ingredients in young pig feeds is limited due

to the presence of anti-nutritive factors, such as non-starch polyssacharides (NSP's), phytate and oligossacharides which depress the efficiency of nutrient utilization and pig performance. One way of reducing the anti-nutritive effects posed by feeding these alternative ingredients to pigs is through exogenous enzyme supplementation. Several published data have reported improvement in ileal and total tract digestibility of nutrients following enzyme supplementation of such diets (Graham et al., 1986; Peterson and Åman, 1989; Li et al. 1996; Jensen et al., 1998).

It has been suggested that the use of exogenous enzymes is most effective in young pigs than growing and finishing pigs, due to the fact that young pigs have a less developed digestive tract and thus less digestive enzymes (Li et al. 1996). In manuscript 2, it was observed that the use of these non conventional ingredients and an appropriate broad range of enzyme activities led to improved nutrient digestibility and reduced nutrient excretion in young pigs. However, it is important that the nutrients that were availed by supplementing diets with enzymes are used for piglets' performance such as growth. This is because an increase in nutrient digestibility does not necessarily translate to increased utilization, as the diet might already contain nutrients in sufficient amounts to meet the pigs' requirement. Therefore, any availed nutrients due to enzyme supplementation will represent a waste of the nutrient and its subsequent appearance in the feces (Johnson et al., 1993). The objective of the current study was to determine if the improved nutrient digestibility observed by supplementing lower quality diets with multi-enzyme

preparation in manuscript 2 could translate to improved nutrient utilization in terms of growth performance of young pigs.

5.3. MATERIALS AND METHODS

5.3.1. Pigs and Housing

Forty-eight Cotswold pigs averaging 6.21 ± 0.74 (mean ± SD) kg BW and weaned at 18 ± 1 d (mean ± SD) were blocked on the basis of sex and BW. Pigs were then assigned randomly from within blocks to dietary treatments. Each dietary treatment was assigned to six replicate pens each with 4 pigs per pen and pigs had unlimited access to feed and water. Individual BW and pen feed disappearance were monitored weekly. Room temperature was initially set at 29.5°C and gradually reduced by 1.5°C per wk. All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

5.3.2. Experimental Diets

The dietary treatments were formulated at 95% of NRC (1998) recommended levels except for P that was reduced by 44%. These diets contained wheat, wheat screenings, millrun, peas, canola meal, and soybean meal and fed as is (Diet 1) or with multi-enzyme supplementation (Diet 2). Ingredient and nutrient composition of the diets are shown in Table 5.1 and 5.2, respectively. Both diets were prepared

Table 5 .1. Ingredient composition of basal diets (%)

Ingredient	Phase I[a]	Phase II[a]	Phase III[a]
Wheat	28.00	51.00	60.10
Wheat screenings	7.00	7.00	7.00
Oat groats	7.00	-	-
Lactose	4.90	-	-
Soybean meal	13.30	15.38	8.24
Peas	8.82	12.00	12.00
Canola meal	11.00	10.07	8.45
Vegetable oil	1.10	0.93	0.97
Spray dried porcine plasma	3.30	-	-
Blood meal	1.00	-	-
Dried whey	10.27	-	-
Biophos	0.31	0.32	0.25
Limestone	1.70	1.43	1.19
Vitamin premix[b]	1.00	1.00	1.00
Mineral premix[c]	0.50	0.50	0.50
DL-Methionine	0.05	0.02	-
Lysine HCl	0.32	0.05	-
Threonine	0.13	-	-
Chromic oxide	0.30	0.30	0.30

[a]Diet was formulated and then divided into 2 parts for the addition of enzymes (1g kg^{-1}).

[b]Supplied per kg diet: vitamin A, 8255 IU; vitamin D3, 1000 IU; vitamin E, 10.9 IU; vitamin B12, 0.115 mg; vitamin K, 1.1 mg; Niacin, 36.8 mg; Choline chloride, 781.2 mg; Biotin, 0.25 mg, and Folic acid, 0.75 mg;

[c]Supplied per kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.28mg

Table 5.2. Calculated nutrient composition[1] of the basal diets.

Item	Phase I	Phase II	Phase III
DE, Kcal/kg	3298	3298	3298
Crude protein, %	22.02	21.19	18.48
Threonine, %	0.97	0.84	0.84
Cystine, %	0.42	0.42	0.42
Methionine, %	0.37	0.32	0.30
Lysine, %	1.52	1.19	1.15
Phytate phosphorus, %	0.25	0.31	0.29
Calcium, %	0.85	0.75	0.62
Available phosphorus, %	0.28	0.24	0.22

[1]Based on NRC (1998) feed composition data.

for 3 different growth phases. Phase 1 was a 7-d period while phases 2 and 3 lasted 10 and 21 days, respectively. The multi-enzyme preparation contained the same enzyme "cocktail" (xylanase, glucanase, amylase, invertase, protease, cellulase, galactanase, mannanase, pectinase and phytase activities) as multi-enzyme C used in an earlier study (manuscript 2), but it did not contain organic acids. Enzymes were provided by Canadian Bio-Systems Inc., Calgary, Alberta. Millrun and wheat screenings were provided by Ritchie Smith Feeds, Abbotsford, BC. Both diets were pelleted at 54°C.

5.3.3. General Conduct of Study

At the beginning of each phase, feeds were weighed into the feeder in each pen and were monitored to reduce wastage of feed. More feeds were weighed into the feeder depending on the rate of feed disappearance from the feeders. At the end of each phase, residual feeds in the feeder were removed and weighed. Average daily feed intake was then calculated as the difference between the amount of feed given and the remnant, divided by the number of pigs in the pen and the number of days in the phase. Average daily gain for each phase was determined by calculating the weight gain of each pig from the end of the previous phase to the beginning of a new phase and dividing it by the number of days in the phase. Feed efficiency for each phase was calculated by dividing the ADG by the average daily feed intake. All these 3 parameters were also calculated overall, i.e., by calculating the parameters from d 0 - 38.

5.3.4. Calculations and Statistical Analysis

Data were statistically analyzed according to the methodology described in manuscript 1 of this thesis.

5.4. RESULTS AND DISCUSSIONS

Average daily feed intake, ADG and FE determined during phase I, II, and III are presented in Table 5.3. In phase I, there were no differences in ADFI (P = 0.24) and ADG (P = 0.40) between the pigs fed the control and enzyme-supplemented diets. Values for ADFI were higher, and values for ADG were lower, and this led to a statistically significant FE. Pigs fed the enzyme-supplemented diet had a higher ($P < 0.0001$) FE than those fed the control diet. In phase II, ADFI followed the same trend as in phase I with no significant difference (P = 0.99) between the pigs fed the enzyme-supplemented diet and those fed the control diet. However, pigs fed the enzyme-supplemented diet had a higher ADG (P = 0.001) and FE (P = 0.04) than those fed the control diet. Similarly, values observed in phase III and overall followed the trend observed in phase II (Table 5.3). Pigs fed the enzyme-supplemented diet had a significantly better ADG ($P < 0.001$) and FE ($P < 0.0001$) than those fed the non-supplemented diet.

The present results are in agreement with those reported in manuscript 2 of this thesis in which, ADG (P = 0.02) and FE (P = 0.01) were improved when pigs were fed enzyme-supplemented diets relative to the control. Similarly, there was no difference in ADFI irrespective of dietary treatments ($P > 0.10$) in manuscript 2.

Table 5.3. Effect of multi-enzyme supplementation on performance of early-weaned pigs.

	DIETS			
Item	Control	Enzyme[1]	SEM[2]	*P*[3]
ADFI, g/d				
d 0 - 7	158.6	145.7	8.5	0.24
d 7 - 21	500.2	509.0	13.6	0.99
d 21 - 38	598.5	593.8	18.7	0.20
Overall (d 0 - 38)	409.3	406.1	24.5	0.25
ADG, g/d				
d 0 - 7	80.8	86.5	22.9	0.40
d 7 - 21	277.0[a]	303.4[b]	19.5	0.001
d 21 - 38	356.4[a]	412.1[b]	16.1	<0.0001
Overall (d 0 - 38)	231.1[a]	257.4[b]	17.4	0.001
Gain/Feed				
d 0 - 7	0.51[a]	0.58[b]	0.06	<0.0001
d 7 - 21	0.55[a]	0.60[b]	0.08	0.04
d 21 - 38	0.60[a]	0.69[b]	0.09	<0.0001
Overall (d 0 - 38)	0.56[a]	0.63[b]	0.07	<0.0001

[1]Enzyme supplement was a cocktail of xylanase, glucanase, amylase, invertase, protease, cellulase, galactanase, mannanase, pectinase and phytase activities.

[2]SEM = standard error of mean.

[3]*P* = observed significance due to effect of treatment.

[ab]Means with same superscripts are not significantly different (P > 0.05)

Both the results of the present study and that of manuscript 2 are in close agreement with the findings of Bedford et al. (1992), who observed an improvement in weight gain and feed efficiency when a mixture of glucanases were added to rye- and barley-based diets fed to 5 wk old pigs. Baidoo et al. (1997) also reported an improvement in feed efficiency in growing pigs fed hulless barley diets supplemented with a mixture of ß-glucanase, xylanase, amylase and pectinase. Similarly in an early-weaned pig trial, Slominski (2000) reported improvements in growth performance of pigs fed wheat, hulless barley-based diets supplemented with a broad spectrum of carbohydrase activities compared to those fed the non-supplemented diets. On the contrary, Thacker et al. (1992) found no improvement ($P > 0.05$) in growth rate, feed intake and feed efficiency when growing and finishing pigs were fed barley-based diets supplemented with a combination of ß-glucanase, *Aspergillus niger,* and propionic acid. Officer (1995) also found no improvement in ADFI, ADG and FCE of 4 wk old pigs fed wheat-soybean-based diets supplemented with multi-enzyme preparations (ß-glucanase, hemi-cellulase, pentosanase and cellulase). However, Grandhi (2001) found improvement in FCE but not in ADFI and ADG when barley-based diets supplemented with commercial carbohydrases were fed to pigs of 20 kg BW.

As mentioned in manuscript 2, the contradictions in the effectiveness of enzyme supplementation among different studies is mainly attributable to differences in age of the pigs, composition of diets used and more importantly the enzyme supplements used. In general, the impact of enzyme supplementation on

nutrient digestion reduces with increasing age of pigs. Graham et al. (1988) and Li et al. (1996) reported that the fibre-degrading capacity increases with age in the small intestine of the pig, suggesting that enzyme supplementation may be more beneficial in diets for early-weaned rather than for growing or finishing pigs. The present data indicating improved growth performance of early weaned pigs as a result of enzyme supplementation are in agreement with this suggestion. In addition, enzymes that contain xylanase, glucanase, cellulase, pectinase, galactanase, mannanase and phytase activities are known to break down many dietary fiber constituents (Meng et al. 2002), which in the current study represented the major anti-nutritional factors present in the diets. Consequently, the association between NSP's, phytate, starch and protein were disrupted thus leading to improved availability of nutrients for digestion and utilization.

The effect of enzyme supplementation in the present study was evaluated on a diet formulated to supply 95% of recommended nutrient requirements for early weaned pigs (NRC, 1998). Another approach would have been to include a commercial diet formulated to meet NRC (1998) recommendations to serve as a positive control. However, due to a limitation in pig supply the former approach was chosen. Furthermore, by using a slightly nutrient-limiting diet any effect due to enzyme supplementation could be easily detected. Indeed, it has been reported that the extent to which enzyme supplementation improves nutrient digestibility tends to be low when using diets containing highly digestible ingredients (Johnson et al., 1993). Availability of nutrients in the basal diet used in the current study was further

constrained by formulating the diet with ingredients that are known to be poorly digested by early-weaned pigs. With this design, any additional nutrients that could become available as a result of enzyme supplementation will be used for growth with little, if any, increase in the proportions excreted. In conclusion, the data obtained in the current study along with that reported in manuscript 2, suggest that the multi-enzyme preparation used was able to boost pig performance by enhancing nutrient utilization in a pig starter diet based on lower quality ingredients. This is important from practical pork production standpoint and suggests that ingredients known to be poorly digested by early-weaned pigs can be utilized in starter diets with enzyme supplementation.

5.5. IMPLICATIONS

With an appropriate multi-enzyme preparation, the ability of early-weaned pigs to utilize starter diets containing lower quality ingredients can be improved considerably. As these ingredients are normally cheaper than the specialty ingredients commonly used in pig starter diets, it suggests that enzyme preparation provide an opportunity to reduce the cost of feeding weaner pigs.

CHAPTER 6.

GENERAL DISCUSSION

Exogenous enzymes are widely used in feed formulation as dietary supplements to help eliminate or reduce the effect of anti-nutritional factors in diets. In swine nutrition, several benefits of supplementing microbial phytase to diets for young pigs have been reported in the literature. These include improvements in digestibility and utilization of phytate phosphorus (Kornegay and Qian 1996) and reduced phosphorus excretion (Harper et al., 1997). It is also reported that the addition of organic acids to corn-soybean meal based-diets may improve microbial phytase activity by acting as a competitive chelator of minerals that may bind to the phytate molecule and render it resistant to phytase action (Maenz et al. 1999), and by lowering the pH of the gut to a desirable level required for optimum phytase action (Ravindran and Kornegay, 1993).

Similarly, supplementation of diets with carbohydrases has been shown to improve utilization of nutrients by reducing the effect of anti-nutritional factors present in diets (Graham et al., 1988). The use of a concert of carbohydrase activities seems to be more effective than a single enzyme activity (Cleophas et al., 1995).

However, many of the studies showing beneficial effects of enzyme supplementation have been performed in poultry and only a few in pigs. Furthermore, most of the studies with pigs have involved growing and finishing pigs and generated inconsistent results. While some published data report no

improvements (Radecki et al. 1988; Officer, 1995) others (Dierick and Decuypere 1994; Li et al., 1996) have reported improvements when cereal based-diets supplemented with exogenous enzymes were fed to growing pigs. Li et al. (1996) attributed the source of contradiction in the literature to the fact that most of these experiments were conducted with growing and finishing pigs that could perform well without supplementing their diets with exogenous enzymes. This is because of the more developed GIT in growing/ finishing pigs and therefore, a higher fiber degrading ability (Graham et al., 1988). The type of ingredients used in formulating the diets could also contribute to this contradiction. Johnson et al. (1993) reported that the effect of exogenous enzymes was not obvious in pigs fed diets containing readily digestible ingredients. Experiments in this thesis were therefore conducted to study the effect of supplementing pig starter diets with exogenous enzymes on nutrient digestibility and pig performance. The diets were formulated by using poorly digestible ingredients in order to limit the nutrient levels, and were fed to pigs weaned at 18 ± 1 d.

Experiment 1 was conducted to determine the impact of microbial phytase, organic acids and their combination on phytate hydrolysis and phytate phosphorus utilization, protein and amino acid digestibilities and phosphorus excretion in early-weaned pigs fed a corn-soybean meal-based diet. Results showed that supplemental phytase plus organic acids can completely replace phosphorus from inorganic sources without any adverse effect on growth performance of early-weaned pigs and reduce phosphorus excretion by 20%. This observation is in close agreement

with that of Harper et al. (1997) who found a 21.5% reduction in phosphorus excretion when diets were supplemented with 500 phytase U/kg of diet. The present data showing no benefit of microbial phytase and organic acids supplementation on protein and amino acid digestibility are in support of the findings of Traylor et al. (2001). This suggests that microbial phytase and organic acids may be effective in dissociating the complex bond that exists between phytate molecules and protein, however other enzymes are still needed to facilitate the digestibility of protein and amino acids. There was improvement in bone ash content and a reduction in the amount of fecal phosphorus of pigs fed the supplemented diets. These results are in close agreement with the findings of Han et al. (1998) who found an improvement in bone ash content of pigs fed microbial phytase-supplemented diets without any effect on growth parameters. It can be concluded that the phosphate groups liberated from phytate molecules, due to microbial phytase and organic acid supplementation, were metabolized by the pig.

However, the use of a single enzyme activity as done in experiment 1 may not always give a positive response especially with regard to utilization of nutrients that are not directly targeted by the enzyme. It was observed in experiment 1 that supplementation of corn-soybean meal diets with microbial phytase and organic acids only improved the utilization of phytate phosphorus and not that of protein and amino acids, and did not improve growth performance. This is because of the direct relationship between target substrate and enzyme, i.e., phytic acid- phytase (Eeckhout and De Paepe, 1996). Therefore, in order to improve the utilization of

other dietary nutrients, the use of different enzyme activities together may be a reasonable approach. Cleophas et al. (1995), Zyla et al. (1996) have shown that the addition of 2 or more enzymes together led to improved hydrolysis of anti-nutritional factors and improved utilization of dietary nutrients. The use of such multi-enzyme preparations may be needed particularly when using diets based on poorly digestible/ lower quality ingredients which may be attractive so as to lower feed costs. Lower quality ingredients are known to contain varieties of anti-nutritional factors which can only be successfully degraded by the use of a combination of enzyme activities used in concert.

Therefore, experiment 2 was conducted to determine the effect of multi-enzyme supplementation on nutrient digestibility and utilization in early-weaned pigs fed diets that were formulated with poorly digestible/ lower quality ingredients. Furthermore, the diets were formulated to be limiting in nutrient contents so that the nutrients availed due to multi-enzyme supplementation will be utilized by pigs and the effects will be easily noticed. The population of pigs used in experiment 2 was small (n = 24) therefore, experiment 3 (n = 48) was conducted in order to verify the results of the growth parameters observed in experiment 2. Digestibility results obtained in experiment 2, showed that supplementation of the lower quality diets with multi-enzyme preparations, improved the digestibility of all nutrients measured especially in the ileum, cecum and feces. These digestibility results were in agreement with results of a study by Li et al. (1996) and those reported by Slominski (2000). This is an indication that with the use of an appropriate enzyme

combination, young pigs can effectively utilize diets containing ingredients that are normally poorly digested. Results obtained in both experiment 2 and 3 indicated improvement in ADG and FE of piglets fed the multi-enzyme-supplemented diets compared to the non-supplemented diets. These are closely in agreement with the findings of Baidoo et al. (1997) who reported an improvement in FE of growing pigs fed hulless barley diets that were supplemented with a multi-enzyme preparation. This observation is also in close agreement with that of Grandhi (2001) who found an improvement in FCE when barley-based diets supplemented with commercial carbohydrases were fed to pigs of 20 kg BW. The improvement observed in growth performance of young pigs indicates that the dietary nutrients that were availed due to multi-enzyme supplementation were utilized by pigs.

In conclusion, microbial phytase and organic acid supplementation was effective in improving phytate utilization in young pigs. Furthermore, the addition of microbial phytase plus carbohydrases improved the digestibility and utilization of phytate and other dietary nutrients. This suggests that the use of multi-enzyme preparations is better than that of a single enzyme activity. The studies further implied that feed cost may be reduced by the use of appropriate multi-enzyme preparations, because this will enhance the use of inexpensive, locally grown feed stuffs and may replace the use of inorganic phosphorus in swine diets.

CHAPTER 7.

CONCLUSIONS

1. Supplementing a corn-soybean meal diet with microbial phytase with or without organic acids significantly improved phytate phosphorus utilization in young pigs and may completely replace the need for inorganic phosphorus in swine diets without compromising growth performance.

2. Addition of microbial phytase alone or together with organic acids significantly reduced fecal phosphorus excretion by 20%.

3. Replacement of phosphorus from inorganic sources with microbial phytase plus organic acids may offer a means to reduce feed cost because inorganic-phosphorus is the 3rd most expensive ingredient in swine diets.

4. Young pigs can effectively utilize diets containing ingredients that are normally poorly digested, if such diets are supplemented with appropriate multi-enzyme preparations.

5. On the average, supplementation of diets with the multi-enzyme preparations significantly improved the ileal digestibilities of DM, starch, NSP, GE, CP and phytate by 6.1, 7.5, 7.5, 7.6, 9.9, and 11 percentage units, respectively compared to the non- supplemented diet.

6. Inclusion of microbial phytase and organic acids in the multi-enzyme preparation showed significant improvement in phytate phosphorus utilization, even better than when they were added alone as in manuscript 1.

7. Total tract digestibility of phosphorus was increased by 56% due to supplementation of diets with a multi-enzyme preparation. Similarly, bone ash content increased by 5% and fecal phosphorus level was reduced by 25% in pigs fed enzyme-supplemented diets.

8. Enzyme supplementation improved nutrient digestibilities and these improvements were reflected in better average daily gain and feed efficiency.

9. Therefore, use of appropriate enzyme combination may allow for the use of locally grown alternative feedstuffs. This will increase flexibility in feed formulation and may further serve as a tool to reduce the cost of weaner pig diets.

CHAPTER 8.

REFERENCES

Alli, I. and R. Houde. 1987. Characterization of phytate in canola meal. 8th Progress Report. Research on canola seed, oil, meal, and meal fractions. Winnipeg: Canola Council of Canada, pp. 159-165.

Anand, A.N. and S. Seshadri. 1995. A quantitative model for prediction of iron bioavailability from Indian meals: an experimental study. Inter. J. Food Sci. Nutr. 46:335-342.

Andrews, R. and N. Baldar. 1985. Amino acid analysis of feed constituents. Science Tools 32:44-48.

Angel, R. and T.J. Applegate. 2000. Phytase use - What do we know: A review. Department of Animal Sciences, Purdue University, West Lafayette, IN 47907. pp., 1-14.

Annison, G. 1991. Relationship between the levels of soluble non-starch polysaccharide and the apparent metabolizable energy of wheats assayed in broiler chickens. J. Agric. Food Chem. 39: 1252-1259.

AOAC. 1990. Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists. Washington DC.

Aspinall, G.O. and J.N.C. Whyte. 1964. Polysaccharides of soybeans. I. Galactomannans from the hull. Journal of the Chemical Society. 5: 5058-5063.

Bach Knudsen, K.E. 1997. Carbohydrate and lignin contents of plant materials used

in animal feeding. Animal Feed Science and Technology. 67: 319-338.

Baidoo, S.K., Y.G. Liu and Grandhi, R.R. 1997. Exogenous microbial enzymes and hulless barley utilization by young pigs. Proceedings of Manitoba Swine seminar 11:135-140.

Ballam, G.C., T.S. Nelson and L.K. Kirby. 1985. Effect of different dietary levels of calcium and phosphorus on phytate hydrolysis by chicks. Nutr. Rep. Int. 32:909-913.

Barrier-Guillot, B., P. Casado, P. Maupetit, C. Jondreville, F. Gatel and M. Larbier. 1996. Wheat phosphorus availability: 1. In vitro study; Factors affecting endogenous phytase activity and phytic phosphorus content. J. Sci. Food Agric. 70:62-68.

Basic, A. and B.A. Stone. 1991. Chemistry and composition of aleurone cell wall components from wheat and barley. Aust. J. Plant Physiol. 8:475-495.

Bedford, M.R. 1993. Mode of action of feed enzymes. J. Appl. Poult. Nutr. 2:85-92.

Bedford, M.R., Classen, H.L. and Campbell, G.L. 1991. The effect of pelleting, salt, and pentosanase on the viscosity of intestinal contents and performance of broilers fed rye. Poult. Sci. 70:1571-1577.

Bedford, M.R., J.F. Patience, H.L. Classen and J. Inborr. 1992. The effect of dietary enzyme supplementation of rye- and barley-based diets on digestion and subsequent performance in weanling pigs. Can. J. Anim. Sci. 72:97-105.

Bhatty, R.S., G.I. Christion and B.G. Rossnagel. 1979. Energy and protein digestibilities of hulled and hulless barley determined by swine feeding. Can.

J. Anim. Sci. 59:585-588.

Bitar, K. and J.G. Reinhold. 1972. Phytase and alkaline phosphatase activities in intestinal mucosa of rat, chicken, calf, and Man. Biochim. Biophys. Acta. 268 :442-452.

Byrd, C.A. and Matrone, G. 1965. Investigation of chemical basis of zinc-calcium -phytate interaction in biological systems. Proc. Soc. Exp. Biol. Med.119: 347-354.

Campbell G.L. and M.R. Bedford. 1992. Enzyme application for monogastric feeds: A review. Can. J. Anim. Sci. 72:449-466.

Canadian Adaptation and Rural Development (CARD). 2000. National Research and Development Priorities, Winnipeg MB., July 15, 2000.

CCAC. 1993. Guide to the Care and Use of Experimental Animal (vol 1). Canadian Council of Animal Care. Ottawa, ON, Canada.

Cera, K.R., D.C. Mahan and G.A. Reinhart. 1988. Weekly digestibilities of diets supplemented with corn oil, lard or tallow by weanling swine. J. Anim. Sci. 66:1430-1437.

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13:297-335.

Classen, H.L. 1996. Enzymes in action. Feed Mix, 4 (2):22-28.

Classen, H.L. and M.R. Bedford. 1991. The use of enzymes to improve the nutritive value of poultry feeds. In:Recent Advances in Animal Nutrition (W. Haresign and D.J.A. Cole, eds.). Butterworths, Oxford, pp. 95-116.

Classen, H.L., T.A. Scott, G.G. Irish, P. Hucl, M. Swift and M.R. Bedford. 1995. Proceedings of the WPSA 10th European Symposium on Poultry Nutrition, Antalya-Turkey, 169-175.

Cleophas G.M.L., W. van Hartngsveldt, W.A.C Somers and J.P.K. van der Lugt. 1995. Enzymes can play an important role in poultry nutrition. World Poultry-Misset 4:12-15.

Collier, B. and B. Hardy. 1986. The use of enzymes in pig and poultry feeds. Part 2. Results of animal trials. Feed Compounder. 6:28-30.

Corring, T., A. Aumaitre and G. Durang. 1978. Development of digestive enzymes in piglet from birth to 8 weeks. I. Pancreas and pancreatic enzymes Nutr. Metab. 22:231-243.

Cossa, J., K. Oloffs, H. Kluge and H. Jeroch. 1997. Investigation into the total P and PP content in different varieties of grain maize. 11th European Symposium on Poultry Nutrition, Proceedings of the World's Poultry Science Association, Faaberg, Denmark. pp 444-446.

Costello, A.J.R., T. Glonek and T.C. Myers. 1976. Phosphorus-31 nuclear magnetic resonance-pH titrations of myoinositol hexaphosphate. Carbohydr. Res. 46:159-164.

Cowan, W.D. 1997. Feed enzymes, mode of action, stability and application systems for wheat-based diets. Proceedings of 8th Western Nutrition Conference. 15-22.

Cranwell, P.D. 1995. Development of the neonatal gut and enzyme system. In: The

Noenatal Pig Development and Survival. Edited by M.A. Varley. Cab International, UK.

Cromwell, G.L. 1992. The biological availability of phosphorus from feed stuffs. Pig News Info. 75N-78N.

Cromwell, G.L., A.H. Cantor, T.S. Stahly and J.H. Randolph. 1988. Efficiency of β-glucanase addition to barley-based diets on performance of weanling and growing-finishing pigs and broiler chicks. J. Anim. Sci. 66 (suppl. 1): 461 (abstr.).

deBoland, A.R., G.B. Garner and B.L. O'Dell, 1975. Identification and properties of phytate in cereal grains and oilseed products. J. Agric. Food Chem. 23:1186-1192.

De Luca, H.F. 1979. The vitamin D system in the regulation of calcium and phosphorus metabolism. Nutr. Rev. 37:161-193.

De Silva, S., K. Hesselman and P. Åman. 1983. Effects of water and beta-glucanase treatment on non-starch polysaccharides in endosperm of low and high viscous barley. Swed. J. Agri. Res. 13:211-219.

Dierick, N.A. and J.A. Decuypere. 1994. Supplementary enzymes to improve utilization of pig diets. Proceedings 45th Annual Meeting of EAAP, Edinburgh.

Duncan, D.B. 1955. Multiple range and multiple *F* tests. Biometrics 11:1-42.

Easter, R.A. 1988. Acidification of diets for pigs. A review. Department of Animal Sciences, University of Illinois, Urbana, Illinois, USA. pp., 61-71

Edwards, H.M., Jr. 1993. Dietary 1,25-dihydroxycholecalciferol supplementation

increases natural phytate phosphorus utilization in chickens. J. Nutr. 123:567-577.

Eeckout, W. and M. De Paepe. 1996. *In vitro* and *in vivo* comparisons of microbial and plant phytase. In: Phytase in animal nutrition and waste management (Coelho, M.B. and E.T. Kornegay, eds.). A BASF Corporation manual, Mount Olive, NJ., pp. 237-240.

Effird, C., W.D. Armstrong and D.L. Herman. 1982. The development of digestive capacity in young pigs: effects of weaning regimen and dietary treatment. J. Anim. Sci. 55:1370-1379.

Englyst, H.N. and J.H. Cummings. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst, 109:937-942.

Etheridge, R.D., R.W. Seerley and T.L. Huber. 1984. The effects of diet on fecal moisture, osmolarity of fecal extracts, products of bacterial fermentation and loss of minerals in feces of weaned pigs. J. Anim. Sci. 58:1403-1410.

Ewing, W.R. 1963. Poultry Nutrition, Fifth Edition (Revised), The Ray Ewing Company, Pasadena, California.

Fadel, J., R.K. Newman, C.W. Newman and A.E. Barnes. 1987. Hypocholesterolemic effects of β-glucans in different barley diets fed to broiler chicks. Nutr. Rep. Int. 57-257.

Fincher, G.B. 1975. Morphology and chemical composition of barley endosperm cell walls. J. Inst. Brew. 81:116-122.

Fleming, M. and K. Kawakami. 1977. Studies of the fine structure of ß-glucans of barley extracted at different temperatures. Carbohydr. Res. 57:15.

Gabert, V.M. and C.M. Nyachoti. 2000. Aspects of true ileal amino acid digestibilities and endogenous amino acid losses in swine. *Presented at the Symposium on the Occassion of the Retirement of Dr. R.R. Marquardt. University of Manitoba, Winnipeg, MB. September 27, 2000.*

Gdala, J., H.N. Johansen, K.E. Bach Knudsen, I.H. Knap, P. Wagner and O.B. Jϕrgensen. 1997. The digestibility of carbohydrates, proteins and fat in the small and large intestine of piglets fed non-supplemented and enzyme supplemented diets. Anim. Feed Sci. Technol. 65:15-33.

Giesting D.W. and R.A. Easter. 1985. Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. J. Anim. Sci. 60:1288-1294.

Graf, E. 1983. Calcium binding to phytic acid. J. Agric. Food Chem. 31:851-855.

Graf, E., K.L. Empson and J.W. Eaton. 1987. Phytic acid: a natural antioxidant. J. Biol. Chem. 262:11647-11650.

Graham, H., J.G. Fadel, C.W. Newman and R.K. Newman. 1989. Effect of pelleting and ß-glucanase supplementation on the ileal and fecal digestibility of a barley-based diet in the pig. J. Anim. Sci. 67:1293-1298.

Graham, H., K. Hesselman, E. Jonsson and P. Åman. 1986. Influences of ß-glucanase supplementation of a barley-based diet in the pig gastrointestinal tract. Nutr. Rep. Int. 34:1089-1096.

Graham, H., W. Löwgren, D. Petterson and P. Åman. 1988. Effect of enzyme

supplementation on digestion of a barley/pollard-based pig diet. Nutr. Rep. Int. 38:1073-1079.

Grandhi, R.R. 2001. Effect of dietary ileal amino acid ratios, and supplemental carbohydrase in hulless-barley-based diets on pig performance and nitrogen excretion in manure. Can. J. Anim. Sci. 81:125-132.

Grynspan, F. and M. Cheryan. 1983. Calcium phytate: effect of pH and molar ratio on in vitro solubility. J. Am. Oil Chem. Soc. 60:1761-1764.

Hampson, D.J. and D.E. Kidder. 1986. Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. Res. Vet. Sci. 40:24-31.

Han, Y.W. and A.G. Wilfred. 1988. Phytate hydrolysis in soybean and cottonseed meals by *Aspergillus ficuum* phytase. J. Agric. Food Chem. 36:259-262.

Han Y.M., K.R Roneker, W.G. Pond and X.G. Lei. 1998. Adding wheat middlings, microbial phytase, and citric acid to corn-soybean meal diets for growing pigs may replace inorganic phosphorus supplementation. J. Anim. Sci. 76: 2649-2656.

Haug, W. and H.J. Lantzsch. 1983. Sensitive method for the rapid determination of phytate in cereal and cereal products. J. Sci. Food Agric. 34:1423-1427.

Harland, B.F. and D. Oberleas, 1996. Phytic acid complex in feed ingredients. In: Phytase in animal nutrition and waste management (Coehlo M.B. and E.T. Kornegay, eds.). A BASF Corporation manual, Mount Olive, NJ., pp. 70-76.

Harper, A.F., E.T. Kornegay and T.C. Schell. 1997. Phytase supplementation of low-

phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75:3174-3186.

Henry, R.J. 1985. A comparison of non-starch carbohydrates in cereal grains. J. Agric. Food Chem. 36:1243-1253.

Henry, R.J. 1986. Genetic and environmental variation in pentosan and β-glucan contents of barley and their relationship to malting quality. J. Cereal Sci. 4:269-277.

Hesselman, K. and P. Åman. 1985. A note on microscopic studies on water- and beta-glucanase treated barley. Swed. J. Agric. Res. 15:139-143.

Hesselman, K. and P. Åman. 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chicks fed on barley of low- or high-viscosity. Anim. Feed Sci. Technol. 14:83-93.

Höehler, D. and J. Pallauf. 1993. Untersuchungen zum Einfluss von Zitronensäure auf die Mineralstoffverwertung beim Ferkel anhand einer Mais-Soja-Diät mit und ohne Zn - ergänzung. J. Anim. Physiol. a. Anim. Nutr. 69:133.

Honeyfield, D.C., J.A. Proseto and J. McGinnis. 1983. Comparative feeding value of rye for poultry and swine. Nutr. Rep. Int. 28:1253-1260.

Inborr, J. and M.R. Bedford. 1994. Stability of feed enzymes to steam pelleting during feed processing. Anim. Feed Sci. Technol. 46:179-196.

Inborr, J. and R.B. Ogle. 1988. Effect of enzyme treatment of piglet feeds on performance and post-weaning diarrhoea. Swed. J. Agric. Res. 18:129-133.

Inborr, J. and J. van der Muelen. 1993. Residual activity of added enzymes in relation to fibre digestibility in the terminal ileum of growing pigs. In:Enzymes In Animal Nutrition (Wenk, C. and M.E. Boessinger, eds.). Kartause Ittingen, Switzerland. pp. 34-37.

Inborr, J., M. Schmitz and F. Ahrens. 1993. Effect of adding fibre and starch degrading enzymes to barley/wheat based diet on performance and nutrient digestibility in different segments of the small intestine of early weaned pigs. Anim. Feed Sci. Technol. 44:113-127.

Jacob, J.P., S. Ibrahim, R. Blair, H. Namkung and I.K. Paik. 2000. Using enzyme supplemented, reduced protein diets to decrease nitrogen and phosphorus excretion of broilers. Asian-Aus. J. Anim. Sci. 13:1561-1567.

Jensen, M.S., K.E. Bach Knudsen, J. Inborr and K. Jakobsen. 1998. Effect of ß-glucanase supplementation on pancreatic enzyme activity and nutrient digestibility in piglets fed with diets based on hulled and hulless barley varieties. Anim. Feed Sci. Technol. 72:329-345.

Johnson, R., P. Williams and R. Campbell. 1993. Use of enzymes in pig production. In: Enzymes In Animal Nutrition (Wenk, C. and M.E. Boessinger, eds.). Kartause Ittingen, Switzerland. pp. 49-60.

Jongbloed, A.W., P.A. Kemme, Z. Mroz and R. Jongbloed 1996. The effect of organic acids in diets for growing pigs on the efficacy of microbial phytase. In: Phytase in Animal Nutrition and Waste Management (Coelho M.B. and E.T. Kornegay, eds.). A BASF Corporation manual, Mount Olive, NJ., pp 515.

Jongbloed, A.W., Z. Mroz, and P.A. Kemme. 1992. The effect of supplementary *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. J. Anim. Sci. 70:1159-1168.

Jongbloed, A.W. and P.A. Kemme. 1990a. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. Netherlands J. Agric. Sci. 38:567.

Jongbloed, A.W. and P.A. Kemme. 1990b. Effect of pelleting mixed feeds on phytase activity and the apparent absorpability of phosphorus and calcium in pigs. Anim. Feed Sci. Technol. 28:233-242.

Kats, L.J., M.D. Tokach, R.D. Goodband and J.L. Nelssen. 1992. Influence of protein source fed to the early-weaned pig during phase I (d 0 - 9) on the response to various protein sources fed during phase II (d 9 - 28). J. Anim. Sci. 70 (Suppl. 1): 60 (abstr.).

Kemme, P., A.W. Jongbloed, Z. Mroz and M. Mäkinen. 1995. Apparent ileal amino acid digestibility as affected by phytate, microbial phytase, and lactic acid. J. Anim. Sci. 73 (Suppl. 1): 173 (Abstr.).

Kies, K. 1996. Phytase - Mode of action. In: Phytase in Animal Nutrition and Waste Management (Coelho M.B. and E.T. Kornegay, eds.). A BASF Corporation manual, Mount Olive, NJ., pp. 205-212.

Kim, M. and M.T. Atallah. 1993. Intestinal solubility and absorption of ferrous iron

in growing rats are affected by different dietary pectins. J. Nutr. 123:117 -124.

Kirby, L.K. and T.S. Nelson. 1988. Total phytate phosphorus content of some feed ingredients derived from grains. Nutr. Rep. Int. 37:277-280.

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

Kornegay, E.T. 1996. Nutritional, environmental, and economic considerations for using phytase in pig and poultry diets. In:Nutrient management of food animals to enhance and protect the environment (Kornegay, E.T., ed.). CRC Press, Boca Raton, Florida, pp. 277-302.

Kornegay, E.T. and H. Qian. 1996. Replacement of inorganic phosphorus by microbial phytase for young pigs fed a maize-soyabean-meal diet. Br. J. Nutr. 76:563-578.

Kornegay, E.T., J.S. Radcliffe and Z. Zhang. 1998. Influence of phytase and diet composition on phosphorus and amino acid digestibilities, and phosphorus and nitrogen excretion in swine. In: BASF Tech. Symp., Carolina Swine nutr. Conf., Durham, NC. pp 125-155.

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

Lei, X.G., P.K. Ku, E.R. Miller, M.T. Yokohama and D.E. Ullrey. 1993b.

Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weanling pigs. J. Anim. Sci. 71:3368-3375.

Li, S., W.C. Sauer, S.X. Huang and V.M. Gabert. 1996. Effect of ß-glucanase supplementation to hulless barley or wheat-soybean meal diets on the digestibilities of energy, protein, ß-glucans, and amino acids in young pigs. J. Anim. Sci. 74:1649-1656.

Li, Y. 2000. Effect of a new multi-enzyme preparation on performance and nutrient digestibility of early-weaned pigs. M.Sc. Thesis, Department of Animal Science, University of Manitoba, Winnipeg, MB. Canada.

Lindemann, M.D., S.G. Cornelius, S.M. El Kandelgy, R.L. Moser and J.E. Pettigrew. 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J. Anim. Sci. 62:1298-1307.

Lolas, G.M., N. Palamidis and P. Markakis. 1976. The phytic acid-total phosphorus relationship in barley, oats, soybeans and wheat. Cereal Chem. 53:867.

Lott, J.N.A. 1984. Accumulation of seed reserves of phosphorus and other minerals. In: Seed Physiology and Development (Murray, D.R., ed.). Academic Press, New York, pp 139-166.

Low, A.G. 1985. The role of dietary fibre in digestion, absorption and metabolism. In: 3rd International Seminar on Digestive Physiology in the Pig. National Institute of Animal Science, Copenhagen, Denmark. Landhusholdningsselskabets Forlag, Copenhagen, Beretning Fra Statens Husdyrbrugsforsog, No. 580:157-179.

Macrae, J.C. and D.G. Armstrong. 1968. Enzyme method for determination of α - linked glucose polymers in biological materials. J. Sci. Food. Agric. 19:578-581.

Maddaiah, V.T., A.A. Kurnick and B.L. Reid. 1964. Phytic acid studies. Proc. Soc. Exp. Biol. Med. 115:391-393.

Maenz, D.D., C.M. Engele-Schaan and H.L. Classen. 1997. Endogenous phytase activity in the small intestinal brush border membrane of broiler chicks and laying hens. Poult. Sci. 76 (Suppl. 1):71 (Abstr.).

Maenz, D.D., Engele-Schaan, C.M., Newkirk, R.W. and Classen, H.L. 1999. The effect of mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in slurry of canola meal. Anim. Feed Sci. Technol. 81:177-192.

MAF. 2001. Manitoba Agriculture and Food. www.gov.mb.ca/agriculture/livestock /pork. Accessed on 3/4/02.

Makkink, M., J.M. Berntsen, B.M.L. op den Kamp, B. Kemp and M.W. Verstegen. 1994. Gastic protein breakdown and pancreatic enzyme activities in response to two different dietary protein sources in newly weaned pigs. J. Anim. Sci. 72:2843-2850.

Marquardt, R.R. 1996. Enzyme enhancement of the nutritional value of cereals: role of viscous, water-soluble, non starch polysaccharides in chick performance. In: Proceedings of the 1st Chinese Symposium of Feed Enzymes (Marquardt, R.R. and Z.H. Han, eds.). Nanjin, China. pp. 5-17.

McDonald, B.E., S.A. Lieden and L. Hambraeus. 1978. Evaluation of the protein quality of rapeseed meals, flours, and isolate. Nutr. Rep. Int. 17:49-56.

McNab, J.M. 1993. Optimal use of enzymes for special ingredients. In: Enzymes In Animal Nutrition (Wenk, C. and M.E. Boessinger, eds.). Kartause Ittingen, Switzerland, pp. 97-124.

Meng, X.F., F.O. Omogbenigun, C.M. Nyachoti and B.A. Slominski. 2002. Degradation of cell wall polysaccharides by a combination of carbohydrase enzymes: *In vivo* and *in vitro* studies. J. Anim. Sci. 80 (Suppl. 1): 253 (Abstr.).

Miner, J.R. 1999. Alternatives to minimize the environmental impact of large swine production units. J. Anim. Sci. 77:440-444.

Mroz, Z., A.W. Jongbloed and P.A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. J. Anim. Sci. 72:126-132.

Mueller, J.P., J.P. Zublena, M.H. Poore, J.C. Barker and J.T. Green. 1994. Managing pasture and hay fields receiving nutrients from anaerobic swine waste lagoons. NC Coop. Ext. Serv. AG-506.

Mulder, M.M., J.A. Lomax, P.M. Hotten, E. Cowie and A. Chesson. 1991. Digestion of wheat aleurone by commercial polysaccharides. Anim. Feed Sci. Technol. 32:185-192.

Murison, S.D., M.M. Mulder and P.M. Hotten. 1989. Enzymatic solubilization of aleurone cell walls and release of protein. In: Proc. 5th Cell Wall Meeting,

(S.C. Fry, C.T. Brett and J.S.G. Reid, eds.). University of Edinburgh, 30th August - 2nd September 1989. pp.197.

Nahapetian, A. and A. Bassiri. 1976. Variations in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, zinc, and iron in wheat varieties during two years. J. Nutr. 110:1458-1472.

Nayini, N.R. and P. Markakis. 1986. Phytases. In: Phytic acid: chemistry and applications (E. Graf, ed.). Pilatus Press, Minneapolis, pp. 101-118.

Nelson, T.S., L.W. Ferrara and N.L. Storer. 1968. Phytate phosphorus content of feed ingredients derived from plants. Poult Sci. 47:1342-1346.

Nernberg, L.W.J. 1998. Improved phosphorus availability in poultry fed wheat/ canola meal-based diets supplemented with phytase enzyme. M.Sc. Thesis, Department of Animal Science, University of Manitoba, Winnipeg, MB. Canada.

NRC. 1998. National Research Council. Nutrient Requirements of Swine. (10th Ed.). National Academy Press, Washington, DC.

Nwokola, E.N. and D.B. Bragg. 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. Can. J. Anim. Sci. 57:475-480.

O'Dell, B.L., A.R. deBoland and S.R. Koirtyohann. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food Chem. 20:718-721.

Officer, D.I. 1995. Effect of multi-enzyme supplements on the growth performance

of piglets during the pre- and post-weaning periods. Anim. Feed Sci. Technol. 56:55-65.

Officer, D.J., E.S. Batterham and D.J. Farrell. 1993. Wheat starch reduces piglet (5-20 kg) performance. In: Manipulating pig production IV (E.S. Batterham, ed.). Australian Pig Science Association (Abstr.) pp. 232.

Okai, D.B., F.X. Aherne and R.T. Hardin. 1976. Effect of creep and starter composition on feed intake and performance of young pigs. Can. J. Anim. Sci. 56: 573-586.

O'Quinn, P.R., D.A. Knabe and E.J. Gregg. 1997. Efficacy of Natuphos® in sorghum-based diets of finishing swine. J. Anim. Sci. 75:1299-1307.

Peter, C.M. and D.H. Baker. 2001. Microbial phytase may not affect protein utilization. Feedstuffs, January 22, 2001 edition, pp 10 - 20.

Petersson, D. and P. Åman. 1987. The variation in chemical composition of triticale grown in Sweden. Acta. Agric. Scand. 37:20-26.

Petersson, D. and P. Åman. 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Br. J. Nutr. 63:139-149.

Pileggi, V.J., H.F. De Luca and H. Steenbock. 1955. The role of vitamin D and intestinal phytase in the prevention of rickets in rats on cereal diets. Arch. Biophys. 58:194-204.

Prairie Swine Centre Inc. 2000. Pork production reference guide. pp 19.

Prattley, C.A. and D.W. Stanley. 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem.

6:243-253.

Radcliffe, D., M. Cabrera, W. Hanna, N. Dale and G. Gascho. 2001. Manure Phosphorus: Problems and solutions.WATT Poultry USA, July 2001 edition, pp. 44-52.

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

Radecki, S.V., M.R. Juhl and E.R. Miller. 1988. Fumaric acid and citric acid as feed additives in starter pig diets: Effect on performance and nutrient balance. J. Anim. Sci. 66:402-408.

Ravindran, G. 1988. Non starch polysaccharides of seeds of soybean (*Glycine max.* L.). Journal of the National Science Council of Sri Lanka. 16: 223-228.

Ravindran V. and E.T. Kornegay. 1993. Acidification of weaner pig diet: A review. J. Sci. Food Agric. 62:313- 333.

Ravindran V., W.L. Bryden and E.T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Poultry and Avian Biology Reviews. 6:125-143.

Ravindran V., G. Ravindran, and S. Sivalogan. 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chem. 50:133-136.

Reddy, N.R., M.D. Pierson, S.K. Sathe and O.K. Salunkhe. 1989. Phytates in cereals and legumes. CRC Press, Boca Raton, Florida, pp. 152.

Saastamoinen, M. 1987. Effect of nitrogen and phosphorus fertilization on the phytic acid content of oats. Cereal Res. 15:57-60.

Sandberg, A.F., T. Larsen, and Sandstrom, B. 1993. High dietary calcium level decreases colonic phytate degradation in pigs fed rapeseed diet. J. Nutr. 123: 559-566.

SAS Statistical Analysis System, 1988. SAS Institute Inc. Cary NC. USA.

Sebastian, S., S.P. Touchburn and E.R. Chavez. 1998. Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a review. World Poult. Sci. J. 54:27-47.

Sharpley, A.N. and R.G. Menzel. 1987. The impact of soil and fertilizer phosphorus on the environment. Adv. Agron. 16:297-301.

Shieh, T., R.J. Wodzinshki, and J.W. Ware. 1969. Regulation of the formation of acid phosphatases by inorganic phosphate and *Aspergillus ficuum*. J. Bacteriol. 100:1161-1165.

Siddiqui, I.R. and P.J. Wood. 1977. Carbohydrates of rapeseed: a review. Journal of the Science of Food and Agriculture. 28: 530-538.

Simons, P.C.M., H.A.J. Versteegh, A.W. Jongbloed, P.A. Kemme, P. Slump, K.D. Bos, M.G.E. Wolters, R.F. Beudeker, and G.J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64: 525-540.

Slominski, B.A. 2000. A new generation of enzymes for animal feeds: Proceedings of the 21st Western Nutrition Conference, September 28 & 29, 2000.

Winnipeg, MB., pp. 1-29.

Slominski, B.A. and L.D.Campbell. 1990. Non-starch polysaccharides of canola meal: Quantification, Digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53:175-184.

Smits, C.H.M. and G. Annison. 1996. Non-starch polysaccharides in broiler nutrition - towards a physiologically valid approach to their determination. World's Poult. Sci. J. 52:203-221.

Spencer, J.D., G.L. Allee, and T.E. Sauber. 2000. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 78:675-681.

Thacker, P.A. and T. Baas. 1996. Use of enzyme in swine rations: a promise unfulfilled? Proceedings of the 16th Western Nutrition Conference. Alberta, Canada. pp. 177-195.

Thacker, P.A., G.L. Campbell and J.W.D. GrootWassink. 1988. The effect of β-glucanase supplementation on the performance of pigs fed hulless barley. Nutr. Rept. Int. 38:91- 99.

Thacker, P.A., G.L. Campbell and J.W.D. GrootWassink. 1989. The effect of sodium bentonite on the performance of pigs fed hulless barley-based diets supplemented with β-glucanase. Nutr. Rep. Int. 40:613-620.

Thacker, P.A., G.L. Campbell and J.W.D. GrootWassink. 1992. The effect of organic acids and enzyme supplementation on the performance of pigs fed barley-based diets. Can. J. Anim. Sci. 72:395-402.

Thompson, L.U. 1986. Phytic acid: a factor influencing starch digestibility and blood glucose response. In: Phytic acid: chemistry and applications (E. Graf, ed.). Pilatus Press, Minneapolis, pp. 173-174.

Thompson, L.U. and M.R. Serraino. 1986. Effect of phytic acid reduction on rapeseed protein digestibility and amino acid absorption. J. Agric. Food Chem. 34:468-469.

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

Torre, M., A.R. Rodriguez and F. Saura-Calixto. 1991. Effects of dietary fibre and phytic acid on mineral availability. Crit. Rev. Food Sci. Nutr. 1:1-22.

Traylor, S.L., G.L. Cromwell, M.D. Lindemann and D.A. Knabe. 2001. Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. J. Anim. Sci. 79: 2634-2642.

University of North Carolina. 1995 Water quality and the North Carolina Swine Industry. http://www.ces.ncsu.edu/whpaper/WQswine.html. Accessed on 2/22/02.

Valaja, J., S. Plaami and H. Siljander-Rasi. 1998. Effect of microbial phytase on digestibility and utilization of phosphorus and protein in pigs fed wet barley protein with fibre. Anim. Feed Sci. and Technol. 72:221-233.

Vasconcelos, I.M., A.A.B. Baia, E.A. Siebra, J.T.A. Oliviera, A. -de-F.F.U. Carvalho, V.M.M. Melo, C.R. Carlini and L.I.-de-M. Castelar. 2001. Nutritional study of two

Brazilian soybean (Glycine max) cultivars differing in the contents of antinutritional and toxic proteins. J. Nutr. Biochem. 12: 55-62.

Vogt, H., S. Matthes and S. Harnisch. 1981. Preservatives/organic acids in broiler and laying rations. Conference on Feed Additives, Budapest, Hungary.

Vohra, P., G.A. Gray and F.H. Kratzer. 1965. Phytic acid-metal complexes. Proc. Soc. Exp. Biol. Med. 120:447-450.

Wang, L., R.K. Newman and P.J. Hofer. 1992. Barley ß-Glucans alter intestinal viscosity and reduce plasma cholesterol concentration in chicks. J. Nutr. 122:2292-1297.

White, W.B., H.R. Bird, H.L. Sunde and J.A. Marlett. 1983. Viscosity of ß-D-glucan as a factor in the enzymatic improvement of barley for chicks. Poult. Sci. 62:853-862.

Whittemore, C.T. 1985. Nutrition of sow and weaner. The Feed Compounder. January, 1985. pp.42-49.

Williams, C.H., D.J. David and O. Lismoa. 1962. The determination of chromic oxide in fecal samples by atomic absorption spectrophotometry. J. Agric. Sci. 59:381-389.

Williams, S.G. 1970. The role of phytic acid in the wheat grain. Plant Physiol. 45: 376-381.

Yiu, S.H., H. Poon, R.G. Fulcher and I. Altosar. 1982. The microscopic structure and chemistry of rapeseed and its products. Food Microstruct. 1:135-143.

Zyla, K. and J. Koreleski. 1993. In vitro and in vivo dephosphorylation of rapeseed

meal by means of phytate-degrading enzymes derived from *Aspergillus niger*. J. Sci. Food Agric. 61:1-6.

Zyla, K., D.R. Ledoux, M. Kujawski and T.L. Veum. 1996. The efficacy of an enzymic cocktail and fungal mycelium in dephosphorylating corn-soybean meal-based feeds fed to growing turkeys. Poult. Sci. 75:381-387.

Zyla, K., D.R. Ledoux and T.L. Veum. 1995. Complete enzymic dephosphorylation of corn-soybean meal feed under simulated intestinal conditions of turkey. J. Agric. Food Chem. 43:288-294.