EFFECT OF A NEW MULTI-ENZYME PREPARATION ON PERFORMANCE AND NUTRIENT DIGESTIBILITY OF EARLY-WEANED PIGS

A Thesis

Submitted to the Faculty

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by Yong Li

In Partial Fulfillment of the

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FACULTY OF GRADUATE STUDIES

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Effect of new Multi-Enzyme Preparation on Performance and Nutrient Digestibility of Early-Weaned Pigs

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ABSTRACT

Two experiments were conducted to evaluate the effect of enzyme supplementation on growth performance and nutrient digestibility of pigs weaned at 18 days of age. Enzyme supplements included a conventional xylanase and β-glucanase preparation (Enzyme A) and a preparation of xylanase and β-glucanase fortified with other enzyme activities including amylase, protease and cellulase (Enzyme B). Four dietary treatments were used: positive control (wheat/corn/soybean meal/fish meal/spray dried plasma), negative control (wheat/hulless barley/soybean meal/canola meal/fish meal/spray dried plasma), negative control + Enzyme A and negative control + Enzyme B. In Experiment 1 (16 pigs per treatment), daily gain and feed conversion ratio averaged 396, 388, 405 and 419 g, and 1.39, 1.36, 1.35 and 1.32, respectively. In phase I of the experiment (4.8-10.4 kg), fecal digestibility of dry matter (82.6, 79.8, 82.7, 85.6%), protein (79.5, 75.5, 78.1, 83.3%) and energy (82.7, 78.5, 82.0, 85.2%) was significantly ($P<0.05$) improved by enzyme supplementation. Similar improvements in energy (82.2, 78.3, 82.3, 84.9%) and protein (78.9, 77.3, 77.7, 80.1%) digestibilities were observed for phase II (10.4-20.4 kg) of the experiment. Although not statistically significant, non-starch polysaccharide digestibility (64.8%) and phytate digestibility (55.3 %) tended to be greater for the enzyme supplemented groups. Complete fecal digestibility of starch was noted for all treatments (98.9%). In Experiment 2, 6 pigs per treatment were fed the same experimental diets as in Experiment 1 for a 25 d period (5.0-10.0 kg) after which time the digesta samples were collected from different segments of the gastrointestinal tract. There was a significant effect ($P<0.05$) of enzyme supplementation on intestinal starch digestibility which averaged 48.4, 27.1, 59.4, 65.9% for the medium segment and 87.9, 83.5, 90.4, 94.2% for the lower segment of the small intestine for

four treatments, respectively. There was no treatment effect on digesta viscosity, pH, plasma urea nitrogen and digestive organ weight and length. In conclusion, enzyme supplementation improved nutrient digestibility and tended to increase growth performance of early weaned pigs.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
AME	Apparent metabolizable energy
ANF	Antinutritional factor
CM	Canola meal
CP	Crude protein
DF	Dietary fibre
DM	Dry matter
ENL	Endogenous nitrogen loss
FCE	Feed conversion efficiency (feed to gain ratio)
GE	Gross energy
GI	Gastrointestinal
ME	Metabolizable energy
NCP	Non cellulosic polysaccharide
NDF	Neutral detergent fibre
NRC	National Research Council
NSP	Non-starch polysaccharide
OM	Organic matter

RPM	Rapeseed meal
SBM	Soybean meal
SEW	Segregated early weaning
SI	Small intestine
TI	Trypsin inhibitor
VFA	Volatile fatty acid

Introduction

To reduce the transfer of certain diseases from sow to piglets, segregated early weaning has become common practice in swine production. Piglets are weaned as early as two or three weeks of age. This strategy has increased efficiency of pig production and improved the number of pigs marketed per sow per year. However, early weaning imposes stress to piglets. There is evidence that early weaning causes insufficient secretion of digestive enzymes and acids; decreased feed intake and absorptive capacity (Thacker, 1998) which may depress piglets growth and affect their lifetime performance (Whitemore, 1985). Early-weaned piglets are usually fed highly digestible nutrients, such as milk products, fish meal and spray dried porcine plasma. All these highly digestible products are expensive ingredients and identification of less-expensive but readily digestible feed ingredients for early-weaned pigs would therefore be of value to the industry.

Maize is the most common cereal grain used in the pig diets. When the price of maize is high, barley can be substituted for maize. However, inclusion of barley in swine diets reduces the performance of young pigs because of the high β-glucan content (Bell and Keith, 1993; Baidoo and Liu, 1998).

Canola meal (CM) is a high quality protein supplement for growing, finishing and reproducing swine, but with the content of antinutritive factors such as glucosinalates and fibre, its dietary inclusion is limited to growing-finishing pigs. When replacing soybean meal (SBM) with CM, a linear decrease in performance was found in early-weaned piglets (Baidoo et al., 1987).

Dietary fibre (DF) in feedstuffs such as barley and canola meal may increase intestinal viscosity or encapsulate nutrients reducing their digestion by young pigs.

Studies have been undertaken to seek methods of DF removal or degradation. Interest in enzyme use has increased over the past twenty years due to economics of enzyme application. The digestive capability of young pigs may be augmented by addition of enzymes such as lipase, protease and amylase. With carbohydrase supplementation, the feeding value of less expensive feedstuffs such as barley, wheat and canola meal may be enhanced by decreasing digesta viscosity or releasing nutrients encapsulated by the cell wall structure. Therefore, the inclusion rates of these feedstuffs could be increased with less soybean meal, fish meal, synthetic amino acids (AA), and fat used in young pig diets without compromising performance. In addition, the competitiveness of barley, wheat and canola meal could be enhanced. Increased digestibility of nutrients (ie., nitrogen, phosphorus) would also reduce their excretion to the environment which is extremely important for intensive animal agriculture.

In poultry, studies have confirmed the positive effect of enzyme supplementation on animal performance. However in pigs, the results are not as consistent as in poultry. The differences in digestive physiology of pigs relative to poultry may be attributed to such different responses.

The objectives of this study were to:

1. Investigate the effect of replacing corn and soybean meal (SBM) with hulless barley and canola meal (CM) in early-weaned pig nutrition;

2. Examine the effect of a new multi-enzyme preparation on nutrient digestibility and performance of early-weaned pigs fed diets containing barley and canola meal.

Literature Review

Early-Weaned Pig Nutrition

Segregated early weaning has been used in North American for many years. It is known to improve sow productivity (increasing litters/sow/year) and piglet health condition (reduce disease transfer from dam). However, early-weaning poses environmental, immunological and nutritional challenges for piglets, which limits the post-weaning growth potential.

Effect of Weaning on Digestive Enzyme Secretions

The digestive capability of the gastrointestinal (GI) tract plays a major role in the performance of young pigs. The pigs are born with ability to digest milk. At weaning, the piglets are often fed a dry, plant protein-based diet that is not readily utilized. Weaning, change of diet and environment all have an adverse effect on digestive enzyme secretions (Efird et al.,1982; Szabo et al.,1976). Studies have shown a depression in the pancreatic enzyme levels in the piglets at the time of weaning (Lindemann et al.,1986; Efird et al., 1982; Makkink et al.,1994). The levels of pancreatic enzymes, lipase, amylase, chymotrypsin and trypsin increase with age. However, with weaning at four weeks of age, the levels of these enzymes have been shown to decrease dramatically, then recover in three to four weeks (Figure 1; Lindemann et al., 1986; Chesson, 1990).

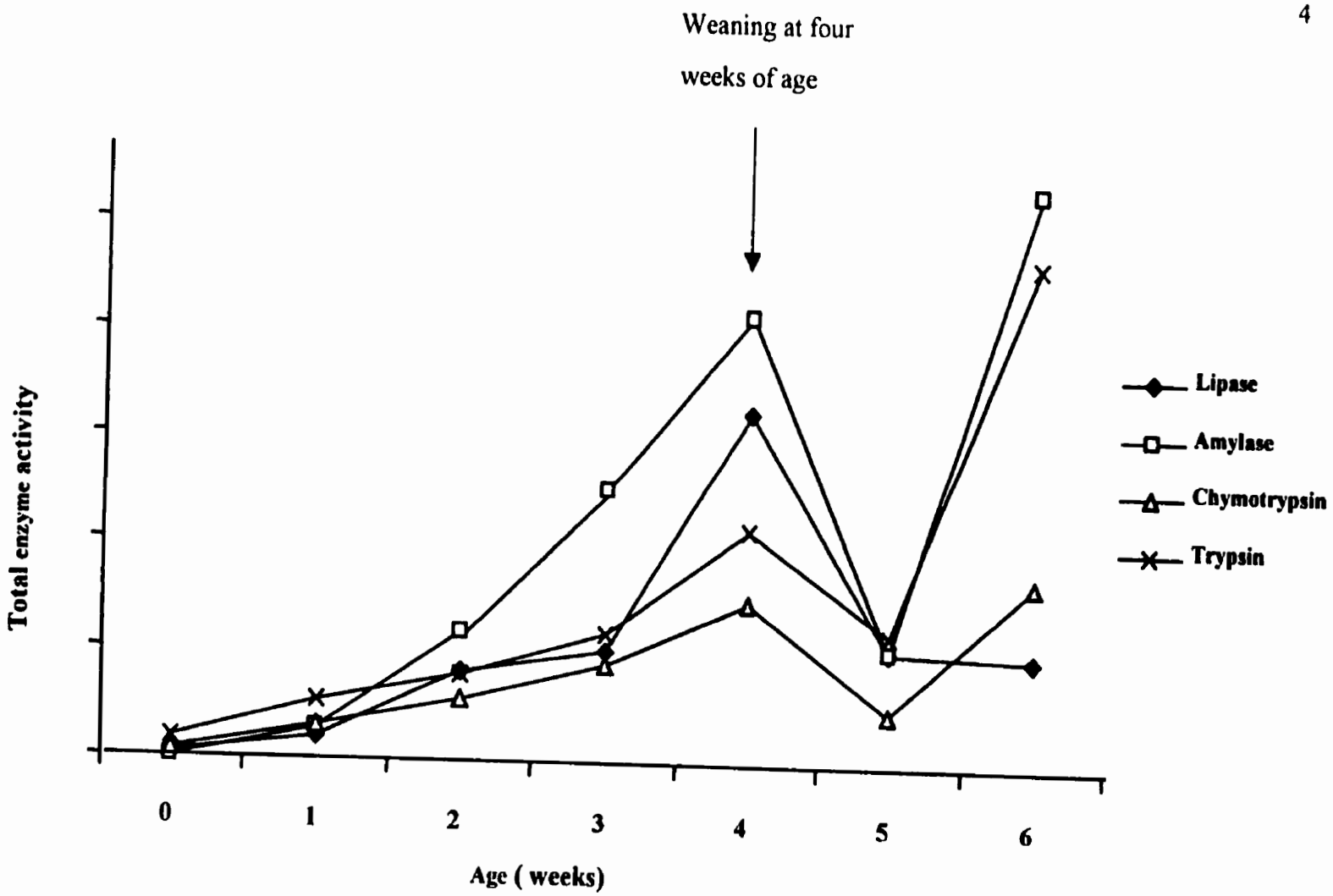

Figure 1. Effect of Age and Weaning on Pancreatic Enzyme Secretions of piglet

Modified from Lindemann et al., 1986

The reduction in enzyme activity may impair diet digestibility, cause growth depression (ie., known as postweaning lag; Okai, 1976) and lead to digestive disorder due to malabsorption (Hampson and 1986). The depression of piglet growth has deleterious effect on their lifetime performance (Whitemore, 1985). The indigestible fibre components may impede the digestion of protein and energy (Low, 1985). Incomplete digestion of nutrients in small intestine may enhance their fermentation by the bacterial population of the hind gut and cause proliferation of the microflora. Addiing enzymes to the postweaning diet may compliment the piglet's own digestive enzymes and therefore increase the digestibility of dietary nutrients.

Antinutritional Factors and their Effects on Pigs

Antinutritional factors refer to the substances intrinsic to a feed, that hampering digestion, absorption, or utilization of nutrients. This section will focus on non-starch polysaccharides (NSP), phytate and trypsin inhibitors.

A widely accepted definition of dietary fiber refers to the sum of polysaccharides and lignin that are not digested by the endogenous secretions of the digestive tract (Trowell et al.,1976). From the practical analysis standpoint, the dietary fibre is often defined as NSPs and lignin (Low,1987) . In addition, wall-inserted protein, arabinogalactan protein, polyphenols other than lignin, minerals associated with the cell walls, galactooligosaccharides, fructosans, resistant starch and Maillard reaction products could be included under this term since they are not digested by the endogenous enzymes of the small intestine (Slominski, 1997). Different feedstuffs contain different NSPs. β-

glucans in barley and arabinoxylans in wheat are partially water-soluble NSPs while the majority of NSPs in soybean meal or canola meal are water-insoluble. The NSPs are part of the cell wall structure and are closely associated with other polymeric material such as lignin and glycoprotein (Fincher and Stone, 1986; Selvendran et al.,1987). Using arabinoxylan of wheat as an example, the association of NSP with lignin and protein will significantly decrease their solubility in water (Smits and Annison, 1996).

β-Glucans of Barley

Barley is a major feed grain in Western Canada. Millions of pigs are raised annually on barley-based diets. The performance of swine fed a barley-based diet is generally inferior to those fed diets based on wheat or maize (Hollis and Palmer, 1971) due to barley's relatively high content of cell wall polysaccharides and low energy level (Larson and Oldfield, 1961). Perez et al. (1980) compared seven barley cultivars (including two hulless types) and documented that the digestible energy content decreased by 110 kcal/kg for each 1% increase in crude fibre content. Bell et al. (1983) found energy digestibility of barley declining by 19.9% as the DF content increase from 2.5 to 11.0%. Higher feeding value was found in two-row barley than in six-row barley due to the higher fibre content in the latter samples (Castell and Bowren, 1980). Replacing hulled barley by wheat in a growing pig diet has been shown to cause a linear increase ($p<0.01$) in average daily gain (ADG) and feed conversion efficiency (FCE) (Bell and Keith, 1993). A large proportion of dietary fiber is present in the hull fraction of barley (Bhatty,1986). The hull consists of two glues, the lemma and the palea, which constitute 10-13% of the dry weight of barley grain. In the hulled barley, these two glues are fused together, enclosing the seed tightly. But in hulless barley, the fusion does not occur,

so the hull can be easily removed during the threshing process. Therefore, hulless barley contains a lower concentration of crude fiber (about 2%) and higher level of protein (13.5%)(Mitchall et al, 1976; Bhatty, 1986) and would have a higher nutritive value when compared to hulled barley. However, studies have shown that hulless barley contains high NSP content, especially high β-glucan content in the endosperm and the aleurone layer of the grain (Table 1).

β-glucans are the major non-starch polysaccharides of hulless barley (ie., 75% of total NSP). Other NSP of the cell walls include arabinoxylans and mannose-containing polymers. β-glucans of the aleurone layer are for the most part water-soluble (Bacic and Stone, 1991) and consist of glucose units with a β-1,4- linked backbone and β-1,3 side linkages (Classen and Bedford, 1991). The presence of β-1,3 linkage and a branched 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-glucan and arabinoxylan fractions of the cell walls are linked to other components forming molecules of very high molecular weight ($4x10^7$ Da) (Classen and Bedford, 1991). The larger the molecule, the greater impact it has on viscosity (White et al., 1983). It is possible that viscosity is the result of macromolecular complex comprised of many different components, rather than one component.

The content of β-glucans varies from 1.5 to 8% (Thacker et al., 1996) and is a function of soil and climate conditions, agronomic practices, degree of maturity at harvest, storage conditions and barley variety (Henry, 1986). The content of β-glucans and total NSPs in three varieties, Condor, Falcon and CDC Buck were found to be 49 and 110 g/kg, 45 and 88 g/kg and 59 and 107 g/kg DM, respectively (Baidoo and Liu, 1998).

The performance of pigs fed hulless barley-based diet has not always been superior to that

Table 1. Dietary fibre content of common feedstuffs (gkg^{-1} DM)

	Maize	Wheat	Barley		Soybean meal	Canola meal
			Hulled	Hulless		
Cellulose	22	20	43	10	62	48.9
NCP[a]	75 (9)[b]	82 (8)	144 (56)	114 (50)	155 (62)	137.9
Rhamose	0	0	0	0	3 (1)	2
Arabinose	22 (3)	29 (7)	28 (6)	20 (3)	26 (9)	45.1
Xylose	30 (2)	47 (9)	56 (6)	24 (4)	19 (2)	16.1
Mannose	3 (2)	3 (2)	4 (2)	4 (1)	13 (5)	3.9
Galactose	4 (1)	4 (2)	3 (1)	3 (1)	41 (16)	16.6
Glucose	10 (1)	11 (4)	47 (39)	58 (41)	7 (6)	49.8
Uronic acid	7 (1)	5 (1)	6 (2)	2 (1)	48 (25)	43.3
β-Glucan	-	6.5 (5.2)	43.6 (28.9)	51	-	-
Pentosans	-	66.3 (11.8)	56.9 (4.8)	nd[c]	-	-
Total NSP	97(9)	119 (25)	186 (56)	124 (50)	217 (63)	179 (15)
Klason lignin	11	19	35	9	16	84[d]
Total fibre	108	138	221	133	233	

a: Non-cellulosic polysaccharides;

b: Values in parentheses are soluble NSP;

c: Not determined.

d: Includes lignin and polyphenols;

Data from: Bach Knudsen, 1997; Chesson, 1993; Slominski and Campbell, 1990; Slominski, 1997; Baidoo et al., 1998.

of pigs fed hulled barley. Regardless of a slightly lower acid detergent fiber (ADF) content of hulless barley, Thacker et al. (1988) found no significant difference in the digestibility of DM, energy or protein, average daily gain (ADG) or average daily feed intake (ADFI) in growing pigs fed hulled or hulless barley-based diets, although lower feed conversion efficiency (FCE) was noted for a hulless barley diet. Jensen et al.(1998) found hulless barley (var. Condor) to contain lower insoluble NSPs (88 *vs* 160 g/kg DM), but similar level of soluble NSPs (48 *vs* 48 g/kg) relative to hulled barley (var. Arra). The authors also reported a high digestibility of total NSPs, fat and energy in hulless barley when fed to young pigs. Baidoo and Liu (1998) found that digestibility of hulless barley for growing pigs was comparable to that of wheat.

Arabinoxylans of Wheat

In many countries, wheat is one of the most commonly used cereal grain in swine and poultry feeds. In general, wheat has a high nutritive value, superior to barley or rye. However, the feeding value of some varieties of wheat could be low due to low apparent metabolizable energy (AME) content and low DM and starch digestibility (Annison and Choct, 1991) which, in turn, may lead to significant economic losses for the producers (Bedford, 1997).

The availability of energy from wheat depends upon location, growing conditions and variety, and different values for available energy content were reported in several 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; Belgium wheat, 11.2-12.1 MJ/kg (Cwan, 1997). Many efforts have been directed to explain the relationship between chemical composition and low and high AME content of wheats. A negative correlation between water-soluble NSPs content and feeding value of wheat was noted (Annison, 1991; Classen

et al., 1995).

Arabinoxylans are the main NSP components of wheat (Table 1.) and consist of β-linked xylose backbone with arabinose side chains. In many instances, they also contain hexoses, hexuronic acids and phenolic acids (Marquardt, 1996). Arabinoxylans are the principle components of the endosperm and the aleurone layer cell walls of wheat (50-80 g/kg; Annison and Choct, 1991) and have similar antinutritional effects as β-glucans of barley. One third of the total arabinoxylan content of wheat is water-soluble (Mares and Stone, 1973). Both arabinoxylans and β-glucans are known to depress nutrient utilization in broiler chickens (Choct and Annison, 1992).

Climate and storage condition are considered the two major factors affecting the NSP content of cereals (Cowan, 1997). Addition of exogenous enzymes can reduce the variation among wheats and lead to more consistent results in animal performance (Cowan, 1997).

Dietary Fiber of Canola Meal

The worldwide demand for canola has increased from 1,022,000 tonnes in 1989 to 2,159,000 tonnes in 1997 (Statistics Canada, 1999). Canola meal (CM) is a valuable protein supplement for growing, finishing and reproducing swine. In starter pigs, dietary inclusion rate of CM should be limited due to the presence of antinutritional factors including glucosinalates, fibre, tannin, phytic acid and sinapine. This section will focus on dietary fibre and phytic acid of CM.

The content of fiber in CM is shown in Table 1. Fiber comprises about 33% of the meal (Bell and Shires, 1982) and is for the most part insoluble in water. Since the glucosinolate content of CM is fairly low, there are indications that the low metabolizable energy (ME) content of CM is associated with the high fibre content. Various approaches have been undertaken to reduce the fiber

content of CM. Among them are dehulling of canola seed (most of fibre is contained in the hull fraction; Bell and Shires,1982), development of the yellow-seeded canola with lower lignin and polyphenol content (Slominski, 1997) or the use of exogenous enzymes to depolymerize certain fiber components (Slominski and Campbell, 1990).

The performance of starter pigs tends to be reduced with high dietary inclusion rates of CM. Replacing SBM with CM in early-weaned pigs (3 wks old) resulted in a linear decrease in average daily feed intake (ADFI) and ADG by 4g and 2g, respectively for each 1% increase in the inclusion of CM (Baidoo et al., 1987). Shaw et al. (1990) observed that relative to SBM-based diet, the inclusion of 15% (equivalent to 8% CM and 5% canola oil) and 30% canola seed decreased the crude protein (CP), lysine, energy and DM digestibility in pigs weaned at 3-weeks of age. In addition, it is believed that the often seen reduction in feed intake may be related to the products of glucosinolate degradation (Mckinnon and Bowland, 1977; Ochetim et al.,1980), reduced palatability (Baidoo te al, 1986) and high fibre content of CM (Bjergegaard et al., 1991). Danielsen et al. (1994) observed, that when replacing skim milk powder with the low glucosinolate (1.1 μmol/g) dehulled rapeseed meal (RSM) containing no active myrosinase in an early-weaned pig diet, the decrease in digestibility of protein (r^2=0.90), energy (r^2=0.91), ADFI (r^2=0.86) and ADG (r^2=0.82) was negatively correlated with the dietary fiber content. In addition, an increase of 1% in the soluble fiber content resulted in 1.6% and 1% decrease in true nitrogen and energy digestibility, respectively. Bell and Kieth (1993) also observed that the digestibility of energy and protein of yellow hulls for pigs was 30 and 20%, respectively, and was much higher than that of brown rapeseed hulls (ie., 2 and 0%). The author concluded that these difference may be due to the low fibre, lignin and high CP levels in yellow hulls. The levels of CM recommended by different authors for starter pig diets are as follows: 12% (Bell

and Aherne, 1981), 6-8% (Baidoo and Aherne, 1985) and 5-10% (Thacker and Aherne, 1984).

Phytate of Canola Meal

Phytate, myo-inositol 1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate) is a polyanionic compound that is abundant in all seeds and vegetative storage tissues serving as the major reserve form of phosphorus. Under normal dietary condition, phytate phosphate is poorly utilized by monogastric animals as they lack sufficient activity of phytase enzyme for an effective phytate hydrolysis and phosphorus release. The phytate phosphorus accounts for 50-80% of the total phosphorus content of feedstuffs. Phytate is a strong chelating agent and can chelate Ca, Co, Cu, Fe, Mn, Ni, Se, and Zn, causing mineral deficiency in monogastric animals (Harland and Oberleas, 1996). Phytate can also form complexes with protein, free amino acids and starch, decreasing their availability (Kies et al., 1997).

Numerous studies have been carried out to improve phytate phosporous utilization by means of heat treatment, fermentation, malting, sprouting, irradiation or the use of phytase enzyme (Harland and Oberleas, 1996).

Trypsin Inhibitors and Dietary Fibre of Soybean Meal

The world production of soybean in 1996/1997 was about 147 million tonnes(Møller, 1998). Due to high protein content (44 - 48%) SBM is the most valuable protein supplement in animal feeds.

A transient hypersensitivity response to SBM was reported (Stokes et al., 1984), which may contribute to poor utilization of soy products by early-weaned pigs. Li et al. (1990) observed the decreased villus height and increased crypt depth due to the cell-mediated immune response in the

intestine. Such effects are attributed to the antinutritional factors (ANFs) present in the meal (ie., trypsin inhibitors, antigens glycinin and β-conglycinin, lectins, phytate).

Trypsin inhibitors (TI) of soybean include Kunitz TI family (STI or SBTI) and Bowman-Birk TI family (BBI). The STI primarily inhibits trypsin and to lesser extent chymotrypsin activity while BBI inhibits both trypsin and chymotrypsin. In rats, these inhibitors have a negative feedback mechanism which regulates pancreatic secretions. Trypsin inhibitors can form stable complexes with trypsin and chymotrypsin and decrease their activation. The inhibition triggers endocrine cell release of CCK-PZ, which stimulates the pancreas to produce more digestive enzymes (trypsin, chymotrypsin, amylase and elastase). Due to the hypertrophia and hyperplasia, the relative pancreas weight increases (Green and Lyman, 1972). Hypersecretion of enzyme also enhances the endogenous nitrogen losses (ENL). However, studies with pigs did not confirm the pancreatic hypertrophy (Yen et al.,1977) and hypersecretion of pancreatic enzymes (Li et al.,1997). Only increased volume of pancreatic juice (Li et al., 1997, 1998) and reduced performance (Yen et al.,1974; Cook et al., 1988) and nutrient digestibility (Combs et al., 1967; Li et al., 1998) were observed. Inactivation of trypsin and chymotrypsin might cause insufficient level of digestive enzymes in the pig, thus interfering with the digestive processes and resulting in endogenous and exogenous nitrogen losses (Li et al., 1998). Protease addition has been showed to effectively inactivate the protease inhibitors (Classen et al., 1993).

Today, almost all of the soybean seed is processed using solvent extraction and heating of the meal at temperatures which effectively inactivate TI, lectins (heat-sensitive) and antigen. Soybean protein concentrates and isolates with high protein content (70-96%) and low ANFs content have been developed specifically for young animals and have been reported to have no negative effect on

animal performance (MΦller, 1998).

When compared to CM, SBM contains less fiber (Table 1.) and phytate and has been reported to have less adverse effects on animal performance.

Mode of Action of Non-Starch Polysaccharides

The exact mechanism by which the non-starch polysaccharides exert their antinutritional effects is not fully understood. The prevailing explanations include the viscosity and encapsulation theories.

The Viscosity Theory

It has been proposed that the viscous NSPs (ie., β-glucan and arabinoxylan) are responsible for the poor feeding value of rye, oats, barley and some varieties of wheat for poultry since high digesta viscosity may be responsible for impaired digestion and absorption of nutrients and feed intake reduction (White et al., 1982; Fengler and Marquardt, 1988; Bedford et al., 1992; Annison, 1991). The viscosity and gelling properties tend to hinder intestinal motility (Holt et al., 1979), thereby reducing the rates of diffusion of endogenous enzymes, the mixing of digesta, digestive enzymes and other components required for digestion and absorption (Vahouny and Cassidy, 1985; Fengler and Marquardt, 1988; Wang et al., 1992). The viscous NSPs may also decrease the rate of feed passage which, in turn, may inhibit feed intake (Sudendey and Kamphue, 1995). Changes in digesta flow rate may also result in the undigested materials being fermented by the microflora of the small intestine. The increased fermentation may cause proliferation of microbial population and enhance the capability

of microflora to compete with host for nutrients (Berford et al., 1992; Choct et al., 1996). An interaction was found between the enzyme preparation and the antibiotic supplementation in barley-based diet. Supplemental enzyme(s) improved the ADG and FCE in broiler chickens, while a diet containing antibiotics and enzyme had no effect on animal performance (Elwinger and Tëglof, 1991). Choct et al. (1992) also found that the antinutritional effect of wheat pentosan was less pronounced in caecectomized than in intact chickens. Proliferation of microbial population may result in the production of toxins and bile acid degrading enzymes, therefore reducing lipid digestion (Campbell, 1983; Feighner and Dashkevicz, 1988). Since bile acids stabilize pancreatic proteases in the intestinal lumen, the digestion of protein could also be affected (Campbell et al, 1983). Since it is important for the animal to consume enough feed to meet nutrient requirements, especially under a stress condition, the reduction in feed intake may have a negative effect on animal performance (Classen, 1996).

The effect of viscosity on nutrient absorption is also reflected in the reduced transport rate. An unstirred water layer exists at the mucosal surface. The nutrients in the lumen gets into epithelial cell through the water layer by diffusion. The rate of nutrient diffusion is a function of the unstirred water layer. When the rate of digesta flow decreases, the thickness of unstirred water increases, then the rate of diffusion decreases and the disappearance rate of nutrients up to the end of the small intestine decreases (White et al, 1983). With the reduced diffusion rate, high levels of nutrients accumulate in the gut and serve as nitrogen and energy source for the gut microflora. In the isolated loops of pig jejunum, guar gum reduced the rate of glucose diffusion by 50% (Low, 1989). Addition of guar gum also delayed the appearance of glucose and α-NH_2 in the blood stream of pigs (Sambrook et al, 1982).

The Encapsulation Theory

Non-starch polysaccharides, as part of the endospermal and the aleurone layer cell walls act as a physical barrier which restricts the access of endogenous enzyme to nutrients (starch and protein) encapsulated within the cells (Chesson, 1993). For example, β-gucan accounts for 75% of the barley endosperm cell walls (Fincher and Stone, 1986). This effect could reduce or delay nutrient digestion in the small intestine. In pigs, the intestinal digesta are more watery than in the chicken (Bedford et al., 1993) and the contents of pig intestine contain less dry matter (10%) than in the chicken (20%). Consequently, the average intestinal viscosity measured in pigs is approximately 100-fold lower than that in chicken digesta (Classen and Bedford, 1991) and the negative effect of viscosity will be less pronounced in pigs. Therefore, the major antinitritive effect of NSPs in pigs will be more related to nutrient encapsulation than viscosity.

Studies have shown that increasing the content of DF (e. g., NSPs) in pig diet led to greater fecal outputs of water and some solid matter (e. g., nitrogen). These extra outputs come from the hydrophilic nature of many NSPs and the increased endogenous secretions. Various NSPs (pectin, guar gum, cellulose) and NSPs-rich feedstuff (barley) have been used to investigate their effect on the gastric, biliary, pancreatic secretions and ileal or fecal nitrogen outputs. The results are summarized in Table 2.

In addition, different NSP increase the daily gastric, biliary, and pancreatic juice outputs and electrolytes in such fluids, although enzyme and electrolyte concentration is not significantly changed.

Endogenous nitrogen losses (ENL) in the ileum or feces also increase in the presence of NSPs. How the fibrous components of the diet enhance ENL is not clear, although an increased nitrogen secretion in the pancreatic juice (Partidge et al.,1982), bile (Portman et al., 1985) and

Table 2. Effect of dietary fibre (DF) on the digestive secretions in pigs

DF or DF origin	Juice volume	Enzyme activity	Source
		Gastric Secretion	
NDF in barley	+	+	Zebrewska et al., (1983)
Barley/SBM	+	+	Low (1986)
Barley	+	+	Lawerence (1972)
Guar gum	+	ND	Rainbird and Low (1986)
Barley	+	▼	Kvasnitskii, et al.,1951
Barley	+	+	Zebrewska and Low, 1983
		Biliary Secretion	
Barley	+	ND	Sambrook, 1981
Barley/wheat	+	ND	Smbrook, 1981
NDF in wheat bran	+	ND	Payne, 1986
		Salivary secretion	
Barley	+	+	Arkhipovets, 1956
Barley	+	-	Zebrewska, et al.,1983

DF or DF origin	Juice volume	Electrolytes	Enzyme activity	Nitrogen output	Source
			Pancreatic Secretion		
NDF in barley	+	ND	-	ND	Zebrewska et al., 1983
NCP in barley	+	+	+	+	Partridge,1982
NCP in barley	+	+	-	-	Zebrewska, 1983
NCP in wheat	+	+	-	+	Zebrewska et al., 1987
Wheat bran	+	ND	+	+	Langlois et al., 1986
Pectin	-	ND	-	-	Mosenthin et al., 1994
			Jejunum		
Guar gum	ND	ND	ND	+	Rainbird et al., 1984
Guar gum	ND	ND	ND	+	Low and Rainbird,1984
			Ileum		
Pectin	ND	ND	ND	+	de Lange et al., 1989
Pectin	+	-	-	+	Mosenthin et al., 1994
Pectin	ND	ND	ND	+	de Lange et al., 1989
Guar gum	ND	ND	ND	+	Low and Rainbird,1984
Guar gum	ND	ND	ND	+	Sauer et al., 1977
Guar gum	ND	ND	ND	+	Taverner et al.,1981

Note: +, DF increased secretion; -, DF has no effect on secretion; ▼, DF decreased secretion; ND, not determined. Data from Low, 1987,1989; de Lange *et al.*, 1989; Zebrewska and Low, 1987; Nyachoti, *et al.*,1996; Mosenthin *etal.*, 1994.

mucous (Satchithanandam et al., 1990; Mosenthin et al., 1994)) with increased dietary fibre content may partially explain these results. NSPs may hinder interaction between enzyme and substrates, so animals respond by increasing endogenous secretion (mucin and saliva production to reduce viscosity) and hence enhanced ENL.

Approximately 30% of AA at the distal ileum of pigs is of bacterial origin (Dugan et al., 1994). Since the high fibre diet cause the proliferation of bacteria in the small and large intestine, the increased bacteria nitrogen may be the major source of the high ENL in the ileum and feces. It was reported that, as opposed to the low viscosity fibre the feeding of high viscosity fibre resulted in increased ENL (Ikegemi et al., 1990; Larsen et al., 1993 and de Lange et al., 1989).

The results on the effect of NSPs on the secretion of endogenous digestive enzyme are controversial. Under certain conditions, NSPs did enhanced pepsin, amylase and lipase secretion. It is thought that fibre may influence the secretory activity by affecting cholecystokinin secretion or via a negative feedback mechanism (Forman and Schneeman, 1980). NSPs may interfere with enzyme substrate interaction (Schneeman, 1978).

Different methods have been used for the digestive secretions collection, measurement of lyophilized pancreatic tissue (Forman and Schneeman, 1980), intestinal contents (Shah et al., 1982) and biliary-pancreatic duct cannulation (Ikegemi et al., 1990). High variation has been observed, either in the same experiment or among different experiments.

From the feed utilization standpoint, the metabolic cost associated with the enhanced secretion of water, protein and electrolytes into the lumen by NSP components could be substantial.

The Binding Effect of NSPs

NSPs may entrap amino acids, peptides or form complexes with digestive enzymes to reduce their activities (Ikeda and Kusano, 1983; Schneeman, 1978). At certain pH, some NSPs have high charge density, so the ions can associate or chelate with the negatively charged groups. Cations may also form ionic bridges between NSPs molecules to increase their viscosity and gel-forming properties (Smits and Annison, 1996).

Studies have also shown that insoluble NSPs impede digestion. Choct and Annison (1992) observed that inclusion of alkali-extractable wheat pentosan decreased the digestibility of nutrients and bird performance. Studies with pigs demonstrated that cellulose addition can increase fecal nitrogen output (Low, 1987) and ileal nitrogen and AA concentration (Sauer et al., 1977). Danielsen et al. (1994) found nitrogen and energy digestibility to correlate with insoluble DF fraction in early-weaned pigs fed rapeseed meal.

Enzyme Supplementation

Over the last 20 years, attempts have been made to improve the nutrient value of feedstuffs such as rye, barley, wheat, canola, peas by means of genetic selection, gamma irradiation, water extraction, enzyme or antibiotic supplementation, diet acidification and processing methods involving heat treatment (ie., pelleting, extrusion, toasting, etc.).

A successful story in the area of genetic selection was the development of canola with markedly reduced glucosinalate content when compared to its parent rapeseed. In Australia, a low β- glucan variety of barley is under development (Annison and Choct, 1993). Gamma irradiation of

barley has been reported to improve its feeding value for broiler chickens, since the irradiation can degrade the β-glucans by cleaving the β-1,3 and β-1,4 bounds and can dramatically decrease its viscous properties (Classen, 1985). Similar results were reported for rye (Campbell et al., 1983; Patel et al.,1980). Water treatment is thought to remove the water-soluble NSPs and activate the endogenous enzymes necessary for effective NSPs hydrolysis (Antoniou and Marquardt, 1982). Diet acidification is based on the assumption that young pigs do not produce enough HCl to maintain a proper gastric pH, which is important to prevent the colonization of bacteria in the upper small intestine, activate pepsinogen and solublize the complex carbohydrates (Easter. 1988). Lactic acid addition has been reported to improve performance and to reduce the proliferation of E. *coli*. and piglet scour (Reviewed by Easter, 1988). Antibiotic supplementation has been reported to improve nutrient absorption and performance in chickens fed hulless barley-based diet (Classen et al., 1985) and rye-based diet (Misir and Marquardt, 1978). The improvement with antibiotic supplementation was mainly due to suppression of microflora proliferation induced by the NSP-rich cereal grains, barley and rye (Misir and Marquardt, 1978).

In recent years, the use of exogenous enzymes has been among the most popular and cost effective approaches to improve the nutritive value of feedstuffs.

Mode of Action of Enzyme

The utilization of amylase, protease and lipase in animal feeds is based on augmentation of the piglet's own digestive system which is often depressed by early-weaning or diet change. Improvements in protein (Corring et al., 1978), starch (Officer et al., 1993; Collier and Hardy, 1986; Inborr and Ogle, 1988) and fat (Cera et al., 1988) digestibilities after weaning with protease, amylase

and lipase supplementation is well documented.

Supplementation of poultry and swine diets with phytase enzyme has consistently improved phytate phosphorus availability and decreased phosphorus excretion (Lei et al., 1993; Sebastian et al, 1998).

The use of carbohydrase enzymes is to target the NSP components, primarily β-glucans, pentosans, oligosaccharides and cellulose. Exogenous enzymes can break down the water-soluble NSP, reducing or eliminating the negative effect of increased intestinal viscosity. Improvement in performance due to viscosity reduction is typical for poultry (Bedford, 1993). However, in pigs, the improvement in performance by carbohydrase supplementation may not be related to viscosity reduction (Bedford et al., 1992; Dierick and Decuypere, 1994). In some studies, enzyme addition had no effect on digesta viscosity (Bedford et al., 1992) or even increased their viscosity (Graham et al., 1988; Bedford et al., 1992; Petterson and Aman, 1989). Another hypothesis related to mode of action of carbohydrase enzymes is that the intact cells enclose the nutrients (starch, protein), making them unavailable for digestion and absorption in the small intestine (Hesseman, 1983). Addition of enzyme can disrupt the integrity of the cell, so the host digestive enzymes have free access to the cell contents (Hesselman and Aman,1986). The greater the accessibility of the cell wall contents to the digestive enzyme, the greater the rate of nutrient utilization.

The linkages between different NSPs within the cell wall structure have a synergistic effect on digesta viscosity and influence nutrient encapsulation effect. When isolated wheat arabinoxylans were added back to a diet, higher concentration was required to elicit a response comparing to using wheat (Annison and Choct, 1993). A synergistic effect of cellulase and mannanase in degradation of the cell wall material from palm-kernel meal was reported (Düsterhöft et al.. 1993). Although β-

glucanase can effectively degrade β-glucans, high levels of cellulase and xylanase are required to maximize the release of protein from the aleurone layer of wheat and barley (Murison et al., 1989; Muder et al. 1991). In general legumes and canola contain more complex polysaccharides than cereals and require more diversified enzyme combination for an effective cell wall disruption. In addition, other ANFs (amylase inhibitors, trypsin inhibitors, lectins, glucosinolates, tannins) would require specific enzymes for their inactivation. These would indicate that a multi-enzyme supplement containing amylase, protease ,lipase, cellulase, xylanase and other enzyme activities would be required to improve the nutritive value of feeds.

Effect of Enzyme Supplementation in Pigs

Studies have been undertaken to investigate the effect of enzyme supplementation on performance of all classes of pigs, especially starter pigs. A comprehensive summary of the results of such studies is given in Table 3. The enzymes employed included fungal or bacterial β-glucanase, xylanase, cellulase, pentosanase, pectinase, amylase, protease and galactosidase. The magnitude of change observed with enzyme supplementation were for daily weight gain from -8 to 45%; feed consumption from 0 to 11.5% and feed to gain ratio, -40 to 15%.

As shown in Table 3, enzyme addition has been reported to improve the performance of weaners, growers, and even finishers. However, in many studies the improvement was related to the age of pigs. Old pigs have an increased ability to digest cereal components through the increased enzyme secretion by the bacterial population in the gut (Graham et al., 1986,1988; Newman et al., 1983). Therefore, the response to β-glucanase supplementation is usually more pronounced in growing than in finishing pigs (Newman et al., 1983). As a consequence, there may be less potential

Table 3. The effect of enzyme supplementation on pig Performance

Enzyme	Diet	Response (%)[a]	Source
	Weaner		
Cellulase	Wheat/maize/soy/rice bran	ADG, 45; FCE, 9	1
β-glucanase	Enzyme-pretreated barley	ADG, 5; FCE, 5	2
Amylase/sucrase	Maize/soy	Nil	3
Amylase/β-glucanase /glucoamylase	Cooked barley/oat/soy/fish	Nil for ADG, FCE, Less diarrhea	4
Amylase/ cellulose/protease	Cooked barley/oats/soy/fish	Nil for ADG, FCE	4
Amylase/β-glucanase /glucoamylase/ protease/cellulase	Cooked barley/oats/soy/fish	Nil for ADG, FCE, Less diarrhea	4
Amylase/cellulase /protease	Cooked barley/steamed oats/ soy/fish	ADG, -8	5
β-glucanase/ amylase/protease	Cooked barley/steamed oats/ soy/fish	Nil	5
Cellulase or Cellulose/amylase	Maize/soy/ensiled rice bran	Nil for ADG, FCE,-32–40	6
Cellulase/β-glucanase	Enzyme pretreated barley	Nil for ADG, FCE	7
Xylanase/amylase	Barley/wheat	FCR, 10-15;Less diarrhea	7
Pentosanase	Rye/soy/SBM	Nil	8
β-glucanase	Barley/soy/SBM	ADG,17; Nil for FCE	8
Xylanase/amylase/pectinase	Wheat/soy/fish	FCE, 4;	9
β-glucanase/xylanase /pectinase	Barley/soy/fish	FCE, 4;	9
β-glucanase/amylase /pectinase	Barley/sugar beet/soy/fish	Nil	9
Xylanase	Rye/soy	Nil for ADG, FCE;	10
β-glucanase	Barley/soy	ADG, 17;	10
β-glucanase	Barley/soy	Nil for ADG, FCE;	11
β-glucanase/xylanase	Barley/wheat/soy	Nil for ADG, FCE;	12
β-glucanase	Barley/soy	ADG, 11.3; FI, 11.5 Nil for FCE	13

Table 3. The effect of enzyme on pig Performance

Enzyme	Diet	Response (%)[a]	Source[b]
Xylanase	Wheat/soy	ADG, 6.9; FCR, 6.3; Nil for FCE	13
Protease/amylase/lipase/ β-glucanase	Wheat/fish/meat/tallow/ Soy/blood meal	Nil for ADG, FCE	14
Cellulase/ β-glucanase/xylanase/ amylase/pectinase	Barley/CM	ADG, 11;	15
β-glucanase	Barley/SBM/fish	Nil	16
	Grower		
Cellulase/ β-glucanase/xylanase/ amylase/pectinase	Barley/CM	ADG, 9;	15
Xylanase	Wheat	Nil	16
Xylanase/protease	Wheat/barley/SBM/fish	ADG, 30; ADFI, 5.3; FCE, 14	16
Xylanase	Wheat/barley/SBM/fish	ADG, 36; ADFI, 9.2; FCE, 14	16
	Grower to Finisher		
β-glucanase	Barley	Nil	17
β-glucanase	Barley	Nil	18
Pentosanase	Rye (mash)	FCE, 10	19
Pentosanase	Rye (pellet)	FCE, 4	19
β-glucanase	Barley	Nil	11
β-glucanase	Barley	Nil	11
β-glucanase	Barley	Nil	20

a Value represents percentage response to enzyme supplementation.

b, References: 1, Sugar et, al, 1978; 2, Thomke et,al., 1980; 3, Hogberg et al., 1983; 4, Inborr and Ogle, 1988; 5, Inborr et al., 1988; 6, Tangendjaja et al., 1988: 7, Bohom, 1990; 8, Inborr and Graham, 1991; 9, Mellange et al., 1992; 10, Bedford et al., 1992; 11, Thacker et al., 1992; 12, Inborr et al., 1993; 13, Cos et al., 1993; 14, Officer, 1995; 15, Baidoo et al., 1996; 16, Dusel et al., unpublished; 17, Thacker et al., 1988; 18, Thacker et al., 1989; 19, Thacker et al., 1991; 20, Bass, 1994.

for improved performance with enzyme supplementation in older pigs.

Improved feed intake with enzyme supplementation (amylase, xylanase, β-glucanase) may be explained by the accelerated gastric emptying in piglets (Sudendey and Kamphues, 1995). Studies by Inborr and Ogle (1988) pointed out that a multi-enzyme preparation (cellulase, amylase, protease and carbohydrase) markedly decreased the incidence and severity of diarrhea in young pigs (4 kg; 2 wks of age). The positive effect could be the result of changing the site of digestion from the large intestine to the small intestine and then reducing availability of substrates for the pathogenic bacteria in the lower gut. Studies by Böhme (1990) indicated that enzyme supplementation (β-glucanase and amylase) reduced need for antibiotic treatment due to lower incidence of digestive disorders.

Addition of xylanase and β-glucanase to wheat/barley/corn-based diet decreased the variation in the final weight of pigs (Wyatt, 1995). In this context, the improved herd uniformity with enzyme supplementation would be of benefit to the pig producers. These results are consistent with those reported for poultry (Classen et al, 1988).

Research data on the effect of enzyme addition on nutrient digestibility are summarized in Table 4. Feed ingredients investigated include barley (hulled, hulless), rye, wheat, maize, oats and legumes. The range of improvement was as follows: for ileal digestibility, DM,0-6.6%; GE, 0-9%; CP, 0-9%; fat, 0-8.2; AA, 0-15%; starch, 0-2.8%; ash, 0-10%; for fecal digestibility, DM, 0-18%; GE, 0-6.8%; CP, 0-6%; fat, 0-9.1%; AA, 0-5.3%; starch, 0-3%; ash, 0-25%.

Taverner and Campbell (1998) found increased availability of energy (13%) and protein (21%) in pigs fed barley-based diet supplemented with β-glucanase due to shifted digestion site from the large intestine to the small intestine. It is believed that the anterior digestion results in improved nutrient utilizations. In the large intestine, the major products of fermentation are volatile fatty acids

Table 4. The effect of enzyme on nutrient digestibility of pigs

Enzyme	Diet	Response (%)[a]	Source[b]
	Weaner		
Cellulase	Wheat/maize/fish/soy/ rice bran	Fecal: CP, 2; CF, 6; fat, 2	1
β-glucanase/xylanase	Barley/pollard/soy	Ileal: β-glucan, 19; CP, 6; Ash,10; fat,5.5	2
β-glucanase	Barley/soy	Ileal: GE, 2.7; St[d], 2.7; DF, 2.9 Fecal: GE, 1.4; ash, 25; DF, 1.4	3
β-glucanase/cellulase/ xylanase/amylase	Wheat/barley/soy/whey	Fecal: CP, ash and OM,4	4
Xylanase/amylase/ /pectinase	Wheat/soy/fish	DNF, -3-9; fat, -6-7	5
β-glucanase/xylanase pectinsae	Barley/fish/soy	DNF, -3-9; fat, -6-7	5
β-glucanase/amylase/ pectinase	Barley/sugar beet/soy/fish	Nil	5
Xylanase	Rye/soy	Nil	6
β-glucanase	Barley/soy	Nil	6
Cellulase	Wheat	Nil	7
Cellulase/pectinase/ xylanase	Wheat	Nil	7
β-glucanase	Barley/soy	Nil	8
Amylase/xlanase/ pectinase	Wheat/hypro soy/ concentrate	Ileal: DM, GE, OM, 4-5; Fecal: DM, GE, OM, 2	9
β-glucanase/pectinase/ cellulase/hemicellulase	Wheat/hypro soy/ concentrate	Nil	9
Carbohydrase	Whole maize plant	Fecal:NDF, 10; ADF, 11; CP, 5; GE, 3.6	10
Exo-β-glucanase/ endo-β-glucanase and glucosidase/protease/ pectinase	Barley/SBM	Ileal: GE, 8; CP, 7.9; β-glucan, 12 Fecal: GE, 2.6; CP, 6	11
Cellulase	Wheat by-product	Ileal:CP,5.9; NSP, 16.7; Fat, 8.2; P[e], 6.9; DF.13.7 AA, 4.4-9.4	12
β-glucanase	Barley/SBM	Ileal: DM,6.6; CP, 8.3: GE, 6.2 β-glucan, 12; AA, 3.5-9.8; Fecal: DM,1.8; OM,1.9; GE, 5.4; AA, 2.6-5.3	13

Table 4.The effect of enzyme supplementation on nutrient digestibility of pigs

Enzyme	Diet	Response (%)[a]	Source[b]
β-glucanase	Barley/SBM	Fecal: DM, 3.7;CP, 6.9 GE, 4.3	14
β-glucanase	Wheat/SBM	Nil	14
β-glucanase	Corn/SBM	Nil	14
β-glucanase	Rye/SBM	Nil	14
α-galactosidase	Barley/wheat/SBM/pea/RPM	Nil	15
Protease	Barley/wheat/SBM/pea/RPM	Nil	15
Xylanase	Barley/wheat/SBM/pea/RPM	Nil	15
β-glucanase	Barley/wheat/SBM/pea/RPM	Nil	15
α-galactosidase/ xylanase/protease	Barley/wheat/SBM/pea/RPM	Ileal: DM,7.6; Ara,12.3; Xyl, 20.8; Man, 16.9	15
α-amylase	Barley/wheat/SBM/pea/RPM	Ileal: DM, 0.7	15
β-glucanase	Barley/SBM/fish	SI_4[c]: β-glucan, 16.3-17.4	
		Soluble β-glucan, 15.7-19.4 AA, 2.2-12.7	16
	Grower - finisher		
β-glucanase	Barley/SBM/oats/wheat	Nil	17
β-glucanase	Barley	Fecal: DM, 2; CP, 5.5	18
β-glucanase	Barley/SBM	Fceal: DM, 4.4	19
β-glucanase	Barley/SBM	Ileal: St, 1.7; β-glucan, 1.4	20
Cellulose/β-glucanase/ xylanase/amylase /pectinase	Barley/CM	Ileal: DM, 4.5; GE, 6.2; CP, 4.2; AA, 3.5-9.8; NDF, 17.8; NSP, 27.4;	21
Xylanase	Wheat/barley/rye/SBM/fish	Ileal: fat, 7.3; Feces, fat,7.2, NDP, 2.4; GE, 6.8	22
Xylanase/protease	Wheat/barley/rye/SBM/fish	Ieal: fat, 1.9; Fecal: fat,9.1; NDP, 2.2; GE, 5.8	23

a Value represents percentage response to enzyme supplementation.

b: Reference. 1, Sugar et al., 1978; 2, 3, Graham et al., 1988,1989; 4, Inborr and Graham, 1991; 5, Mellange et al., 1992; 6, Bedford et al., 1992; 7, McClean and McCcraker, 1992; 8, Thacker et al., 1992; 9,McClean et al., 1993; 10, Wenk et al., 1993; 11, Li et al., 1993; 12, Dierick and Decuypere, 1996: 13, Li et al., 1996; 14, Li et al., 1996; 15, Gdala et al., 1997; 16, Jensen et al., 1998; 17, Graham et al., 1986; 18, Thacker et al., 1988; 19, Thacker, 1992; 20, Graham et al., 1988; 21, Baidoo et al.,1998; 22,23, Dusel et al.,unpublished.

c: Last forth section of small intestine; d,starch; e, Phosphorus.

(VFA) and lactic acid which are utilized with a lower efficiency than the carbohydrates digested and absorbed directly from the small intestine (55 *vs* 75%; NRC, 1998).

Many studies have been carried out on the digestibility of β-glucans in barley-based diets (Li et al., 1993, 1996;Graham et al.,1986,1988,1989). In pigs, considerable degradation of β-glucans was observed prior to ileum. The ileal digestibility of total NSPs and β-glucans in 20, 40, 90 kg pigs was found to average 40 and 12%, 68 and 35%, and 96 and 56%, respectively (Graham et al., 1988). Such an effective degradation of β-glucans by bacteria of the small intestine may reduce the benefits from glucanase supplementation (Newman et al., 1980; Aman and Graham, 1987; Cromell et al., 1988). This may explain why the response to β-glucanase supplementation in barley-based diets for growing pigs (20-60 kg) is more pronounced than that observed in finishing pigs (60-90 kg)(Newman et al., 1983).

Inboor et al. (1993) examined the disappearance of β-glucan in various sections of the piglet GI tract and found 32.1% and 71.9% β-glucan being decomposed in the last fourth section of the small intestine without and with enzyme supplementation (β-glucanase, xylanase, amylase), respectively. With cellulase supplementation, Dierick and Decuypere (1996) detected a significant increase in NSPs (16.7%) and AA (4.4-9.4%) digestibility and commented that the improvement in AA digestibility largely resulted from the release of fibre-bound nitrogen. Similar results were reported by Li et al. (1996) and Baidoo et al. (1998).

Some studies have shown a positive effect of enzyme addition on chick performance when fed diets containing SBM (Charlton, 1996), field bean (Castanon and Marquardt, 1989) and CM (Slominski and Campbell, 1990). Supplemental amylase or amylase/xylanase preparations were also reported to improve apparent ileal digestibility of threonine and glycine, and plasma urea nitrogen in

early-weaned pigs fed peas (Owusu-Asiedu, 1998). FCE but not ADG, was also improved during the 4 to 10 kg period. The author concluded that amylase and xylanase supplementation could be beneficial for young pigs. Other studies have shown protease to break down protease inhibitors (Classen et al., 1993). Cellulase and hemicellulase enzymes increased the NSPs hydrolysis and *in vitro* dry matter digestibility of SBM, rapeseed meal (RSM) and peas (Dierick and Deceupre, 1984). Pre-treatment of SBM with a combination of cellulase, protease and β-glucanase improved protein digestibility in pigs (Näsi, 1991).

Least cost ration formulation and pollution control are two main advantages of enzyme use in pig nutrition. Application of exogenous enzymes in feeds would allow for the use of less expensive feed ingredients at higher inclusion rates without any losses in performance. Such feedstuffs include low quality cereal grains (barley, rye, and some varieties of wheat) or oilseed by-products (linseed meal, canola meal). Their use would reduce the cost of feed and will allow for greater flexibility in ration formulation. Increased AA availability with enzyme supplementation would also reduce the demand for high quality protein supplements (ie., fish meal, meat meal) or synthetic AAs (Johnson et al., 1993).

The improvement in efficiency of feed utilization with enzyme supplementation is important from an ecological standpoint. In recent years, pollution from extensive livestock production has been of concern due to increased nitrogen and phosphorus excretion into the environment and pollution of the water supplies. In the US, 100 million tonnes of animal manure is produced annually and represent the liberation of one million tonnes of phosphorus into the environment each year (Cromell, 1991). Approximately two-thirds of total phosphorus of plant origin is present in a form of phytate which is poorly utilized by monogastric animals and necessitates the addition of inorganic

phosphorus to feeds to meet the animal requirements for available phosphorus. The enzyme can also release protein and other minerals chelated by phytate and increase their utilization. Studies by Pallauf and Rimbach (1993) demonstrated that addition of 1000 U/kg phytase decreased phosphorus excretion by 56% (2.2 g/d to 0.96 g/d) in 15 kg pigs.

Factors Affecting Enzyme Effectiveness use in Pigs

In general, the effect of enzyme supplementation on pig performance is not as consistent as that seen in poultry. Although some studies demonstrated marked improvement in performance (see Table3)(Inborr et al., 1991; Jensen et al., 1998; Baidoo et al., unpublished; Dusel et al., unpublished), many studies showed no effect of enzyme supplementation. In a systematic study, Thacker et al. (1988, 1989, 1992) demonstrated a minimal improvement in pig performance with enzyme supplementation. Other researchers indicated that enzyme supplementation increased digestibility of nutrients, but the increase was not reflected in an improvement in animal performance. Studies by Officer (1995) even suggested that enzyme supplementation was detrimental to piglets fed wheat-based diet. Bedford et al. (1992) also found supplemental xylanase to increase the digesta viscosity of piglets fed rye-based diet.

The difference in digestive physiology between swine and chickens may influence the effectiveness of enzyme addition. With longer GI tract and greater mean transit time, pigs can degrade considerable amounts of β-glucans (Graham et al., 1988; Weitzen and Aherene, 1986; Hesselman and Aman,1986) and arabinoxylans (Thacker and Baas, 1996) prior to the end of ileum due to microbial action in the small intestine and some endogenous enzyme activity present in the feed (Graham et al., 1986).

Dietary factors including feed quality, NSP concentration, feed processing, antibiotic and fat addition may all influence the effectivenes of enzyme addition. If enzymes are added to diets which contain readily digestible ingredients, the magnitude of enzyme effect would be low (Johnson et al.,1993). On the other hand, if there is enough 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 enzyme addition would be difficult to detect (McNab, 1993). For example, McNab (1993) found that the beneficial effect of enzyme supplementation in piglets fed vegetable protein was more pronounced than when the same enzyme was added to a diet containing a high quality animal protein. Similar results were reported by Officer (1992) for early-weaned pigs fed high quality diets.

Studies by Choct and Annison (1992) and Fengler and Marquardt (1988) clearly demonstrated that the monogastric animal is capable of tolerating certain dietary concentrations of NSPs without performance losses. Studies by Bhatty et al. (1979) indicated that although a high viscosity barley depressed the performance of young chicks it had no negative effect when fed to pigs. With a high tolerance of pigs to lower quality feeds, the effect of enzyme supplementation may be masked by some positive characteristics associated with the feed quality. This may also explain the results of many experiments with barley (Thacker et al.,1988, 1989, 1992) in which β-glucanase addition was beneficial for poultry but little effect on growing-finishing pig performance was noted.

The effectiveness of enzyme use depends not only on the type and level of activity, but the enzyme stability under various conditions (ie., temperature, pH, protease). Stability is inherent to each enzyme. In the study by McNab (1993), ten commercial enzyme preparations were evaluated and were found to have different tolerance to high temperature, pH and protease treatment in vitro.

During feed processing (pelleting, expansion, extrusion), enzyme preparations encounter hostile conditions (moist and heat), which may reduce their activity. Studies by Inborr and Bedford (1994) have demonstrated that during pelleting conditioning for 30 seconds or 15 minutes, the recovery of β-glucanase activity at 75°C was 66 and 49%, at 85°C - 50 and 31% and at 95°C - 10 and 11%, respectively. There is a negative quadratic effect of pelleting temperature and positive effect of enzyme level on ADG and FCE. Many other studies have also demonstrated significant losses of activity of phytase (Jongbloed and Kemme, 1990), and β-glucanase and xylanase (Pickford, 1992) on pelleting.

A number of methods have been developed to stabilize the enzyme preparation, including encapsulation, adsorption, micro-granulation, coating or post-pelleting application. Studies by Inborr and Graham (1991b) and Inborr and Bedford (1994) suggested that with reduced activity, enzyme addition can still improve the performance of animals, indicting that there is sufficient level of activity remaining in the pelleted feed.

Thacker and Baas (1996) evaluated ten commercial enzyme products (Biofeed, Prozyme SF, Endofeed, Amylofeed, Roxzyme, Allzyme PT, Allzyme BG, Biofeedplus, Porzyme TP, Avizyme TX) and found pH to have a similar effect on all enzyme activities. Little activity was found at pH 2.5 which then slightly increased at pH 3.5. The highest activity was observed at pH 4.5-5.5 which then declined at pH 6.5. In this context, the pH values in the GI tract of the pig were reported to be as follows: 2-4.5 for stomach (Thacker and Baas, 1996), 4-6.2 for duodenum, 5.5-6.9 for jejunum, 7.0 -7.4 for ileum (Chesson, 1987). It has also been documented that enzyme activity will decrease in the GI tract (Thacker and Bass, 1996; Inborr and van der Meulen, 1993). However, studies by Thacker and Bass (1996) indicated that at the beginning of duodenum, 47% and 28% of the total β-glucanase

activity added to the diet was still present after two and four hours after feeding, respectively.

Materials and Methods

Materials

Commercial soybean meal, canola meal, wheat, spray-dried plasma, whey powder and fish meal were obtained from a local feed company (Feed-Rite, Winnipeg, Manitoba). Hulless barley (var. CDC Buck) was obtained from the Glenlea Research Station, University of Manitoba. Enzyme supplements included xylanase, β-glucanase, α-amylase, amyloglucosidase, invertase, protease and cellulase were provided by Canadian Bio-Systems, Calgary, Canada.

Animal Experiments

Two experiments were conducted to determine the effect of substitution of soybean meal with canola meal (3% of a diet) and corn with hulless barley (26% of a diet) on performance and nutrient utilization of early-weaned pigs fed soybean/canola meal/wheat/hulless barley/fish meal/sprayed-dried plasma-based diet with and without enzyme supplementation.

In Experiment 1, a total of 64 Cotswold pigs (32 females, 32 castrated males) weaned at 18 d of age were selected. The average weight was 4.8 ± 0.65kg. The pigs were randomly assigned to one of the four treatments (16 pigs per treatment) by litter origin, weight and sex. The pigs were housed in flatted-deck pens, two in each pen. The initial room temperature was 28°C and decreased gradually to 22°C at the end of experiment. Throughout the experiment the pigs had free access to water from the low-pressured nipple drinkers. The experiment consisted of two phases, phase I from 4.8 ± 0.65 to 10.4 ± 1.46 kg and phase II from 10.4 ± 1.46 to 20.4 ± 2.34 kg body weight (average body weight for per pen). At the beginning and the end of each phase, blood samples were taken

from the jugular vein in the morning and collected in heparinized vacutainer tubes (Becton Dickinson, Rutherford, NJ). The tubes were stored overnight in the cold room at 4°C, then centrifuged and plasma samples were pipetted into vials and stored at -20°C for plasma urea nitrogen analysis.

Feed was provided ad libitum. Individual weights and pen feed consumption were recorded at weekly intervals. At the end of each phase, fecal samples were collected. Prior to collection, the pen decks were washed, and the fresh samples of feces were collected over three hour period During morning. The samples were then frozen and freeze-dried.

Experiment 2 consisted of one 25 d phase with 24 piglets weaned at 16d of age and randomly assigned to four dietary treatments (6 pigs per treatment). The initial weight was 5.0 ± 0.57 kg and the pigs were housed individually. Animal care, assignment, blood and fecal sample collection were the same as in Experiment 1. On Day 23 and 24 of the experiment (first day is defined as day zero), three pigs from each treatment were randomly selected and euthanized with carbon dioxide four hour after morning feeding. The abdominal cavity was exposed and the gastrointestinal (GI) tract was ligated into the following sections: stomach, three equal segments of the small intestine (upper, middle and lower), cecum and colon. Each ligate of the GI tract was weighed and the length of the small intestine, cecum and colon was measured. The contents of the stomach and the three intestinal sections were transferred into plastic sample bags, frozen and freeze-dried. The kidney, pancreas and liver were removed and weighed. The digesta pH and viscosity in each ligate was determined immediately.

Experimental procedures, arrangement and care of animals were approved by the University of Manitoba Animal Protocol Management and Review Committee and followed the guide of the

Canada Council on Animal Care (CCAC, 1980).

Experimental Diets

Two basal diets were used in each experiment. Diet 1 was based on wheat, corn, soybean meal, fish meal, spray-dried plasma and whey and served as a positive control (Table 5 and 6). Diet 2 differed from Diet 1 in that 25 % of the soybean meal was substituted with canola meal and 100% corn was substituted with hulless barley (var. CDC Buck). Diet 2 was mixed as a single unit and then divided into three equal batches, a control diet and two test diets which were supplemented with either Enzyme A or B. Enzyme A was composed of the conventional xylanase and glucanase preparations while Enzyme B consisted of xylanase, β-glucanase, α-amylase, protease, invertase and cellulase. Composition of the enzyme blends and enzyme activities and/or inclusion rates are shown in Table 7. For the first phase of Experiment 1 (4.8 - 10.4 kg) and Experiment 2 (5-10 kg) the diets were formulated to contain 21% crude protein, 1.4% lysine, 1.03% methionine and cystine, 0.86% threonine and 16.35 MJ/kg digestible energy. In the second phase of Experiment 1 (10.4-20.4 kg), the diets contained 19% crude protein, 1.2% lysine, 0.99% methionine and cystine, 0.74% threonine and 15.7 MJ/kg digestible energy.

In Experiment 1, acid insoluble ash (McCarthy et al., 1974) was used to determine the digestibility of dietary components. Using selected samples, its usefulness was compared to that of chromic oxide which was used as an external marker in Experiment 2. In this study, the chromic oxide was added to two randomly selected diets and both chromic oxide and acid insoluble ash were then used to determine the apparent digestibility of dry matter. There was no significant difference in digestibility of dry matter as determined using acid insoluble ash or chromic oxide (Table 8).

Table 5. Composition and calculated analyses of basal diets used in phase I of the pig experiments 1 and 2

	Diet 1	Diet 2
Ingredient (%)		
Wheat	46.0	46.0
Corn	23.0	0
Hulless barley	0.0	23.0
Soybean meal	12.5	9.3
Canola meal	0.0	3.0
Fish meal	4.0	4.0
Spried dried plasma	3.0	3.0
Whey	4.0	4.0
Vitamin-mineral premix[a]	5.0	5.0
Canola oil	2.0	2.0
Lysine-HCl	0.4	0.5
Threonine	0.14	0.25
Chromic oxide	0.2	0.2
Calculated conmposition		
Protein (%)	20.5	21
Digestible energy (MJ/kg)	16.35	16.19
Lysine (%)	1.4	1.4
Methionine+Cystine (%)	1.00	1.03
Threonine (%)	0.86	0.86
Calcium (%)	0.9	0.9
Available phosphorus (%)	0.43	0.43
Determined composition		
Protein (%)	20.5	21.3
Energy (MJ/kg)	16.74	16.68
NSP (%)	9.6	9.8
-- Glucose (%)	3.2	3.6
Phytate (%)	0.27	0.27

[a]The premix provided per kilogram diet: Ca, 9.0g; P, 4.25g; Salt, 3.0g; Na, 1.2g; Mg, 125mg; Mn, 35mg; Fe, 152.5mg; Zn, 137.5mg; Cu, 125mg; I, 0.75mg; Vitamin A, 11750IU; Vitamin D_3, 1500IU; Vitamin E, 50IU; Vitamin K, 1.75mg; Choline chloride, 750mg; Niacin, 38mg; Calcium pantothenate, 35.75mg; Riboflavin, 10mg; Thiamine, 1mg; Pyridoxine, 1mg; Vitamin B_{12}, 27.5mg; Biotin, 100mg; Folic acid, 0.5mg.

Table 6. Composition and calculated analyses of basal diets used in phase II of the pig experiment 1

	Diet 1	Diet 2
Ingredient (%)		
Wheat	46.0	46.0
Corn	26.0	0
Hulless barley	0.0	26.0
Soybean meal	14.5	9.3
Canola meal	0.0	3.0
Fish meal	4.0	4.0
Whey	4.0	4.0
Vitamin-mineral premix[a]	5.0	5.0
Canola oil	2.0	2.0
Lysine-HCl	0.4	0.5
Threonine	0.14	0.25
Chromic oxide	0.2	0.2
Calculated conmposition		
Protein (%)	19.0	19.1
Digestible energy (MJ/kg)	15.72	15.71
Lysine (%)	1.2	1.2
Methionine+Cystine (%)	0.95	0.99
Threonine (%)	0.74	0.74
Calcium (%)	0.9	0.9
Available phosphorus (%)	0.43	0.43
Determined composition		
Protein (%)	19.5	19.2
Energy (MJ/kg)	16.64	16.50
NSP (%)	9.1	10.6
-- Glucose (%)	2.8	3.6
Phytate (%)	0.27	0.27

[a]See table 5 for premix composition.

Table 7. Composition of enzyme blends and enzyme activities and/or inclusion rates of experimental diets

Enzyme		Enzyme A	Enzyme B
Xylanase	(units kg^{-1} diet)	412	552
β-Glucanase	(units kg^{-1} diet)	307	528
α-Amylase[1]	(FAA units kg^{-1} diet)		8094
Invertase	(%)		0.002
Protease	(HUT units kg^{-1} diet)		1410
Cellulase	(%)		0.0025

[1] Includes α-amylase and amyloglucosidase

Table 8. Digestibility of dry matter as determined using two different markers

Marker	Diet 2	Diet 2+ Enzyme A
Chromic oxide	80.5 ±2.9[1]	85.3 ±2.5
Acid insoluble ash	79.8 ±2.7	84.2 ±2.6

[1] Mean ±SD (n=6), no significant difference between methods was observed

Chromic oxide is frequently used as an external marker; however, problems determining digestibility using chromic oxide, because of interference from other minerals in the rations, has been reported (Saha & Gilbreath, 1991). Moreover, mineral concentrations are much higher in undigested materials. Acid-insoluble ash has been suggested an alternative marker to chromic oxide (McCarthy et al., 1974; Wunsche et al., 1991; Moughan et al., 1991), and as compared to the total collection method, has been proven to be an accurate indicator for digestible dry matter, protein and energy determination (McCarthy et al. 1977; Yen et al, 1983; Moughan et al, 1990). Some research data have also indicated higher recovery rate of acid-insoluble ash than that of chromic oxide (Wunsche et al., 1984; Moughan et al., 1991). However, Yen et al (1983) indicated that acid insoluble ash was only a satisfactory marker for nutrient digestibility determination in 12-week old pigs as opposed to 19-week old pigs.

Digestibility coefficients of the analyzed components were calculated using the following formula:

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

N_f = Nutrient concentration in digesta or feces (DM)
N_d = Nutrient concentration in feed (DM)
C_f = Cr_2O_3 concentration in digesta or feces
C_d = Cr_2O_3 concentration in feed

Chemical Analyses

Prior to analysis, all feed, digesta and fecal samples were finely ground in a coffee grinder.

Samples were analyzed for dry matter using AOAC method(1990). 5g sample was weighted in a pre-weighted silica dishes and dried to a constant weight for approximately 16-24 hours in a forced drought oven set at 105°C. The sample were then removed, cooled in a desiccator and weighted. Crude protein (Kjeldahl N x 6.25) was determined using the AOAC (1990) method. Approximately 0.5-1.0 g of the sample was weighted into a digestion tube and then digested in 18-ml concentrated sulphuric acid (36% w/v H_2SO_4) using selenium as a catalyst. The nitrogen content was then measured using Kejltec Auto 1030 Analyzer, and the protein was calculated using a 6.25 conversion factor. Chromic oxide was analyzed according to the procedure described by Williams et al. (1962). 0.5 g sample was weighted in a crucible and ashed at 500°C for 16 hours. The ashed sample was then transferred into a beaker to which phosphoric acid and 3 ml manganese sulphate and 4 ml potassium bromate solution were gently added. The beaker was covered with the watch glass, placed on a hot plate and digested until no effervescence was observed and the solution changed into the pink color. After cooling, the solution was carefully transferred into 200-ml volumetric flask containing 25-ml calcium chloride. The flask was then made up to volume using distilled water. Chromic oxide was then determined using Atomic Absorption Spectrophotometer (Perkin-Elmer, model 603A) at 267.5 nm against a working standard (0-30 ppm). Gross energy was determined using a Parr adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL.), results were calculated by the calorimeter controller unit which was pre-programmed. Acid insoluble ash was analyzed according to the method of McCarthy et al. (1974). 10g sample was weighted in a flask containing 100 ml 4N HCL for 30 min, then filtered through ashless filter paper, washed with boiling water until free of acid and finally ashed at 650°C for a minimum of 6h. Non-starch polysaccharides (NSP) were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure

described by Englyst and Cummings (1984), with some modifications (Slominski and Campbell, 1990). In brief, a 100 mg sample was treated with dimethylsulfoxide and incubated overnight with a solution of starch degrading enzymes amylase and pullulanase(Sigma, St. Louis, MO) at 45°C. Ethanol was then added, the mixture left for 1 hour, centrifuged and the supernatant was discarded. The dry residue was dissolved in 1 ml of 12 M H_2SO_4 and incubated for 1 hour at 35°C . Six ml of water and 5 ml of myo-inositol(internal standard) solution were then added and the mixture was boiled for 2 hours. One milliliter of the hydrolysate was then taken and neutralized with 12M 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 determined using the NSP procedure in which starch gelatinization with dimethylsulfoxide was substituted by boiling the samples with water for 30 min. Starch hydrolyzing enzymes (ie., amylase, pullunase and amyloglucosidase) were excluded from the procedure and the starch content was calculated as total sample glucose (no enzyme added) minus NSP glucose. The content of phytate was determined according to the method of Haug and Lantzsch (1983). Briefly, a 40 mg sample was accurately weighted and shaken with 10 ml 0.2 N HCL for 3 hours at room temperature. After filtration, 1 ml filtrate and 2 ml ferric solution in triplicate were placed in hydrolysis tubes and boiled for 30 min. After cooling in ice water for 15 min the tubes were allowed to adjust to room temperature, after which the samples were shaken and centrifuged at 3000 rpm for 30 min. One ml of supernatant was then transferred into another tube and 1.5 ml biphyride solution (Aldrich, Canada) was added. After10 min the absorptance was measured (Ultrospec 2000, Pharmacia Biotech, Cambridge, England) at 519 nm against distilled water. Phytate was quantified from a standard curve developed using solutions of known amounts of phytic acid

(Sigma, Canada). Plasma samples were analyzed for nitrogen concentrations using a standard kit (Procedure No. 535) from Sigma Diagnostics (Sigma Diagnostic, St. Louis, MO., USA). This was done by quantitative, colorimetric determination of plasma urea nitrogen at 540 nm, based on the technique described by Crocker (1967). 0.02 ml sample was pipetted into the test tubes. Urea nitrogen diluted standards were prepared by pipetting the kit reagents into test tubes and were mixed thoroughly. To each tube 3.0 ml of blood urea nitrogen acid Reagent (Catalog No. 535-5, St. Louis MO., USA) were added which were then mixed thoroughly. All tubes were placed in a boiled water bath and boiled for exactly 10 min. The tubes were then quickly removed and placed in the cold tap water for 3 min, after which time the absorbance was read within 20 min. The urea nitrogen standard calibration curve was prepared, and plasma urea nitrogen values for each sample were calculated. Viscosity was determined in the supernatants of digesta using a Brookfield digital viscometer (LVTDVCP-11, Brookfield Engineering Laboratories INC., Stoughton, MA). Fresh digesta were used for pH determination with the aid of pH/ISE meter (Orion Research INC. Boston, MA).

Statistical Analysis

Data from two experiments was subjected to analysis of variance (ANOVA) according to Steel and Torrie (1980) using the General Lineal Models of statistical analysis system (SAS Institute, 1988). Student-Newman-Keuls test was used to compare and separate treatment means.

Model used was as follows:

$X_{ijk} = \mu + \alpha_i + \beta_j + \xi_{ijk}$

μ = the population mean;

α_i = the effect of i_{th} diet;

β_j = the k_{th} animal;

ξ_{ijk} = error term.

Results and Discussion

Animal Performance

The growth performance data of early-weaned pigs fed the experimental Diet 1 (positive control), Diet 2 (negative control) and Diet 2 supplemented with Enzyme A and B are shown in Table 9. The average daily gain (ADG) in phase I tended to decrease when soybean meal and corn were substituted with canola meal and barley, respectively (P= 0.20). These results are in agreement with earlier studies by Bell and Keith (1993), who reported a linear increase in average daily gain (ADG) and feed conversion ratio (FCR) in growing pigs fed diets in which barley was substituted with wheat. In another study, replacing soybean meal (SBM) with canola meal (CM) in diets for early-weaned pig (3 weeks old) resulted in a linear decrease in average daily feed intake (ADFI) and ADG by 4 g and 2 g, respectively, for each 1% increase in canola meal inclusion rate (Baidoo et al., 1987). A significant decrease in voluntary feed intake and growth rate (P<0.001) of early-weaned pigs with increased level of dehulled, high protein rapeseed was also found by Danielsen et al. (1994).

As compared to the negative control (Diet 2), the ADG tended to increase numerically (244 vs 263 and 274 g) in phase I for Diet 2 supplemented with Enzyme A and B (P=0.19). In addition, the performance characteristics for both enzyme supplemented diets were equal to that of positive control. As expressed by standard deviation of the mean, enzyme supplementation also decreased the variation in final weight of pigs from 61.7 to 47.8 for Enzyme A and to 48.6 for Enzyme B.

Table 9. Effect of enzyme supplementation on performance of early-weaned pigs (Phase I and Expeiment 1)

	Week	Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B	P value
Daily feed intake (g)[1,2]	1	128.6 ± 33.2[2]	123.2 ±25.0	128.6 ± 34.0	141.1 ±23.1	
	2	268.8 ± 35.0	254.5 ± 75.3	283.9 ± 69.9	291.1 ± 62.5	
	3	529.2 ± 76.8	475.4 ±63.5	514.5 ± 100.0	530.6 ± 104.3	
	Mean (phase I)	313.8 ± 31.9	295.2 ± 33.0	315.7 ± 43.0	323.7 ± 52.3	0.56
	4	727.2 ± 87.2	713.0 ± 71.4	781.0 ± 62.3	741.0 ± 112.3	
	5	936.2 ± 123.3	864.4 ± 98.9	863.1 ± 106.4	894.4 ± 71.4	
	6	1007.3 ± 60.4	1000.2 ± 135.0	1010.2 ± 117.2	1005.1 ± 54.0	
	Mean (phase II)	832.5 ± 59.9	831.9 ± 93.3	861.7 ± 63.4	852.1 ± 67.2	0.94
	Overall	550.1 ± 44.8	529.9 ± 48.2	551.8 ± 48.7	552.8 ±37.1	0.71
Daily weight gain (g)[1,3]	1	126.8 ± 46.4	131.3 ± 31.9	142.9 ± 40.8	136.6 ± 40.3	
	2	192.9 ± 70.4	200.9 ± 75.7	223.2 ± 63.5	233.9 ±75.1	
	3	410.7 ± 75.7	378.6 ± 72.6	405.0 ± 96.3	442.3 ± 98.3	
	Mean (phase I)	247.6 ± 37.3	244.4 ±42.2	262.7 ± 40.5	273.5 ± 54.9	0.19
	4	505.4 ± 58.1	474.2 ± 91.0	512.1 ± 95.4	518.4 ± 95.2	
	5	615.2 ± 94.2	606.3 ± 79.1	614.2 ± 79.2	657.9 ± 37.2	
	6	666.9 ± 76.4	668.1 ± 110.2	675.2 ± 100.1	687.2 ± 32.4	
	Mean (phase II)	573.6 ± 71.9	576.5 ± 116.7	581.7 ± 73.0	609.1 ± 63.2	0.60
	Overall	395.6 ± 54.6	388.3 ± 61.7	405.5 ± 47.8	418.9 ±48.6	0.41
Feed/Gain[1,2]	1	1.03 ± 0.22	0.97 ± 0.27	0.95 ± 0.17	1.06 ± 0.25	
	2	1.44 ± 0.20	1.28 ± 0.18	1.30 ± 0.19	1.24 ± 0.15	
	3	1.29 ± 0.28	1.27 ± 0.12	1.28 ± 0.21	1.21 ± 0.18	
	Mean (phase I)	1.27 ± 0.19	1.21 ± 0.08	1.21 ± 0.13	1.18 ± 0.12	0.58
	4	1.43 ± 0.09	1.44 ± 0.14	1.48 ± 0.20	1.46 ± 0.27	
	5	1.53 ± 0.19	1.43 ± 0.08	1.41 ± 0.07	1.36 ± 0.09	
	6	1.50 ± 0.16	1.51 ± 0.19	1.51 ± 0.12	1.47 ± 0.12	
	Mean (phase II)	1.45 ± 0.09	1.44 ± 0.07	1.46 ± 0.07	1.40 ± 0.12	0.65
	Overall	1.39 ± 0.07	1.36 ± 0.03	1.35 ± 0.07	1.32 ± 0.06	0.16

[1]No significant treatment effects were observed ($p>0.05$).

[2]Mean ± SD (n = 8).

[3]Mean ± SD (n = 16).

According to numerous studies, the improvement in pig performance by enzyme supplementation has been highly inconsistent. Some studies documented a marked improvement in pig performance with enzyme supplementation (Sugar et al., 1978; Baidoo et al, 1998), while others showed no benefit of enzyme addition (Bedford et al., 1992; Inborr and Graham, 1991a). In a series of experiments, Thacker et al. (1988, 1989, 1992) showed only 1-3% improvement in growing to finishing pig performance with only few cases in which statistically significant difference between the control and enzyme supplemented diets were observed. Although in some studies enzyme supplementation increased digestibility of various nutrients, the increase was not reflected in the improvement in animal performance (Inborr et al., 1993; Thacker et al., 1992). Some studies even documented a detrimental effect of enzyme addition on piglet performance (Officer, 1995; Bedford et al., 1992).

There are several factors which may influence the effectiveness of enzyme use. Among them are age of animal, type of diet, variability in nutritive content of dietary ingredients or the type of enzyme preparation used in the study (ie., single enzyme activity vs multiple enzyme preparation). Traditionally, enzyme preparations have been proven effective in improving the performance of poultry, especially young chickens known to be susceptible to viscous polysaccharides (ie., β-glucan, arabinoxylan) of barley, rye or wheat. However, the difference between swine and chicken digestive physiology may influence the effectiveness of enzyme use. As compared to chicks, pigs can degrade considerable amounts of β-glucans (Graham et al., 1988; Weitzen and Aherne, 1986; Hesselman and Åman, 1986) and arabinoxylans (Thacker and Baas, 1996) prior to the ileum due to a much longer gastrointestinal tract and a greater mean transit time. Polysaccharide degradation may result from both microbial action in the pig small and large intestine and the activity of endogenous β-glucanase

and xylanase present in the feed (Graham et al., 1986).

It is a well known fact that the increase in starch digestibility with enzyme supplementation provides the birds with a significant net energy gain. However, in the pig, this net energy gain may not be as pronounced. Any undigested starch will be fermented in the lower gut with the production of volatile fatty acids and their absorption. Therefore, any exogenous enzyme addition would only shift the site of starch digestion from the large intestine to the small intestine increasing the efficiency of energy utilization but reducing the potential for improved performance with enzyme supplementation (Chesson, 1990). In addition, the viscosity in the pig small intestine is smaller than that in the chicken and enzyme supplementation may have little effect on improved pig performance due to digesta viscosity reduction. All these differences indicate that more complex enzyme preparations are needed for swine diets and the use of conventional "poultry" enzymes in swine rations may explain why pigs respond to enzyme supplementation to a lesser extent than chickens.

Exogenous enzymes are expected to augment the animal's own digestive system and digest substrates that animal's own digestive enzyme can not act on. In cases when enzymes are added to the diets which contain readily digestible ingredients, the magnitude of enzyme effect would be less pronounced (Johnson et al., 1993). In addition, if the amount of nutrients in the pig diet is sufficient for optimal performance, any increase in nutrient utilization due to enzyme supplementation will be in excess of animal requirement and consequently the effect of enzyme addition on animal performance may be difficult to detect (McNab, 1993). In addition, Campbell (cited from McNab, 1993) found that the effect of enzyme addition on pig performance was more pronounced when the animals were fed diet containing vegetable protein rather than the animal protein, both diets containing the same digestible energy and amino acids contents. Officer (1995) also found no effect

of enzyme supplementation on performance of weaned pigs fed high quality animal protein (spray dried plasma, fish meal, whey) based diet.

Studies by Fengler and Marquardt (1988) and Choct and Annison (1992) clearly demonstrated that animals are capable of tolerating certain concentrations of non-starch polysaccharides (NSP) present in their diets, without losses in performance. Studies by Bhatty et al. (1979) indicated that a high viscosity barley depressed the performance of young chick but was not detrimental to growing pigs. With a high tolerance of pigs to visrous polysaccharides in diet, the effect of enzyme addition on pig performance could be easily masked by other beneficial characteristics such as feed processing (ie., pelleting), feed particle size, antibiotic addition or type of fat.

Digestibility of Dietary Components

A total tract digestibility of dry matter, energy, starch, protein and phytate determined in Experiment 1 is shown in Table 10. In phase I of the experiment, replacing corn and soybean meal (Diet 1) with barley and canola meal (Diet 2) significantly decreased dry matter digestibility ($P<0.05$). As compared to the negative control (Diet 2), increased digestibility of dry matter, energy and protein with enzyme supplementation was observed. Dry matter digestibility increased following addition of either enzyme blends ($P<0.05$) while improvement in digestibility of energy ($P<0.01$) and protein ($P<0.05$) was only observed for Enzyme B. In addition, the digestibility of dry matter, energy and protein was greater ($P<0.05$) for Enzyme B than Enzyme A. A decrease in digestible energy content following substitution of soybean meal and corn with canola meal and hulless barley was also observed in phase 2 of the experiment ($P<0.05$). Improvement in digestibility of dry matter by Enzyme B ($P<0.05$) and energy by both Enzymes A and B ($P<0.01$) was also noted. There was

Table 10. Fecal digestibility of dry matter, energy, protein, starch and phytate in early-weaned pigs (Phase I and II; Experiment 1)(%).

Phase	Component	Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B	Sample (n)
	Dry matter	82.6 ± 2.4[1b]	79.8 ± 2.5[c]	82.7 ± 2.1[b]	85.6 ± 1.2[a]	8
	Energy	82.7 ± 2.6[ab]	78.5 ± 2.5[b]	82.0 ± 2.0[b]	85.2 ± 1.7[a]	8
I	Protein	79.5 ± 5.1[ab]	75.5 ± 3.5[b]	78.1 ± 3.4[b]	83.3 ± 3.0[a]	8
	Starch	98.7 ± 2.3	97.0 ± 2.5	99.7 ± 1.3	99.2 ± 0.7	4 (pooled)
	Phytate	51.2 ± 10.9	53.1 ± 12.7	55.2 ± 5.5	61.6 ± 5.7	4 (pooled)
	Dry matter	82.6 ± 1.4[ab]	81.7 ± 2.1[b]	82.1 ± 1.2[ab]	83.7 ± 0.7[a]	8
	Energy	82.2 ± 2.6[a]	78.3 ± 2.5[b]	82.3 ± 2.2[a]	84.9 ± 1.3[a]	8
II	Protein	78.9 ± 3.0	77.3 ± 3.1	77.7 ± 3.5	80.1 ± 1.9	8
	Starch	98.7 ± 1.7	99.1 ± 0.4	99.1 ± 1.9	99.6 ± 1.1	4 (pooled)
	Phytate	47.5 ± 8.1	48.6 ± 7.5	52.3 ± 10.4	49.5 ± 7.3	8

[1]Mean ± SD .

[abc]Means within a row with no common superscripts are significantly different (P<0.05).

no effect of enzyme addition on starch digestibility which, as determined at the fecal level, was found to be completely digested irrespective of diet composition or enzyme addition.

Digestibility of dry matter and selected dietary components in different compartments of the gastrointestinal (GI) tract as determined in Experiment 2 is shown in Table 11. Replacing corn and soybean meal with barley and canola meal markedly ($P<0.05$) decreased starch digestibility in the middle section of the small intestine (from 48 to 27%). A significant improvement in starch digestibility in the middle (by enzyme A and B) and lower (by enzyme B) segments of the small intestine was observed. Enzyme addition also tended to increase dry matter digestibility in the lower segment ($P=0.091$) and energy digestibility in the middle section of the small intestine ($P=0.069$).

As documented in Experiment 1, starch was almost completely digested (98 to 100%) when determined at the fecal level. This results are in agreement with some earlier studies which showed that even at the ileal level or prior to the end of small intestine, starch digestibility was nearly complete (Graham et al., 1986; Gdala et al., 1997). In the current study, however, enzyme addition significantly increased starch digestibility in the middle and lower segment of the small intestine. According to Jensen et al. (1998), Thacker et al.(1992) and Graham et al. (1986, 1988a, 1989) a consistent increase in ileal starch digestibility by β-glucanase addition was observed. It appears evident that the increase in ileal starch digestibility with enzyme supplementation may shift the digestion site from the large intestine to the small intestine. It is a well known fact that the direct absorption of starch as glucose from the small intestine would be considered more effective from the energy utilization standpoint than its conversion to volatile fatty acids and their absorption from the large intestine. In this regard, the efficiency of energy utilization from glucose has been reported to be significantly higher than that of volatile fatty acids (ie., 75 vs 55%; NRC, 1998). In general, an

Table 11. Dry matter, energy, protein, starch, non-starch polysaccharides (NSP) and phytate digestibilities in the middle and lower segments of small intestine and in the total tract of early-weaned pigs (Experiment 2)(%)

		Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B
	Dry matter	39.5 ± 7.2[1]	37.7 ± 4.2	40.4 ± 2.4	42.6 ± 7.0
	Energy	40.2 ± 6.2	36.9 ± 6.0	37.7 ± 3.2	45.8 ± 1.4
Middle	Protein	43.6 ± 4.2	41.3 ± 1.3	44.5 ± 11.5	46.0 ± 10.1
segment	Starch	48.4 ± 15.7[a]	27.1 ± 10.0[b]	59.4 ± 22.9[a]	65.9 ± 9.4[a]
	NSP	21.0 ± 10.2	14.4 ± 18.8	20.7 ± 15.1	22.6 ± 7.2
	Phytate	2.3 ± 15.0	3.8 ± 8.8	3.9 ± 7.4	6.0 ± 4.3
	Dry matter	68.6 ± 2.2	65.1 ± 1.4	68.7 ± 2.8	70.4 ± 2.7
	Energy	68.8 ± 2.9	65.0 ± 2.0	68.6 ± 4.6	70.4 ± 3.8
Lower	Protein	71.1 ± 2.4	68.4 ± 4.0	71.7 ± 2.0	72.2 ± 5.6
segment	Starch	87.9 ± 3.7[ab]	83.5 ± 5.9[b]	90.4 ± 4.4[ab]	94.2 ± 2.1[a]
	NSP	38.5 ± 8.9	32.6 ± 5.2	43.7 ± 8.8	42.4 ± 7.5
	Phytate	19.1 ± 9.3	18.9 ± 10.0	27.8 ± 3.2	20.2 ± 9.8
	Dry matter	81.1 ± 1.4	78.8 ± 2.8	81.1 ± 2.7	81.8 ± 1.8
	Energy	79.5 ± 1.9	77.1 ± 3.9	79.4 ± 3.6	80.9 ± 2.6
Total tract	Protein	79.5 ± 4.4	77.9 ± 3.2	78.3 ± 4.4	81.0 ± 3.2
	NSP	56.5 ± 3.9	56.6 ± 6.6	60.3 ± 3.4	64.3 ± 5.1
	Phytate	62.8 ± 10.2	55.2 ± 22.8	52.9 ± 6.6	64.8 ± 26.3

[1]Mean ± SD (n= 4).

[ab]Means within a row with no common superscripts are significantly different (P<0.05).

anterior digestion of nutrients would also decrease proliferation of the microbial population in the lower gut, therefore decreasing the digestive upsets. This may explain why there is less digestive disorders observed with enzyme supplementation (Inborr and Ogle, 1988). In addition, Böhme (1990) suggested that improved starch digestibility in the small intestine by xylanase and β-glucanase supplementation may be attributed to the release of starch encapsulated by the cell wall structure. In this context, both aranbinoxylans and β-glucans are the major components of the cell walls of barley and wheat. They are linked to other cell wall components such as cellulose, lignin, polyphenols or glycoproteins to form an effective physical barrier which would restrict the access of endogenous enzyme to nutrients enclosed within the cells (Classen and Bedford, 1991). This, in turn, could reduce or delay the digestion of nutrients in the small intestine. When comparing seven barley cultivars (including two hulless types), Perez et al. (1983) determined the digestible energy content to decrease by 110 kcal kg^{-1} for each 1% increase in crude fibre content. Moreover, in early-weaned pigs fed different levels of canola meal, the decrease in digestibility of protein and energy was negatively correlated with the fibre content. Since the canola meal-based diets used in this particular study contained very low level of glucosinolates (1.1 μmol g^{-1}), the major negative effect was thought to originate from the high fibre content of a diet (Danielsen et al, 1994). It is believed, that carbohydrase enzymes can disrupt the cell wall integrity through degradation of aranbinoxylans, β-glucans, cellulose and other cell wall components. This, in turn, would allow the host digestive enzymes to access the cell contents (Hesselman and Åman, 1985, 1986) and could result in increased nutrient digestibility.

Since starch is the main energy source in animal diets, more pronounced improvement in energy digestibility observed in the current study by Enzyme B supplementation would indicate that

additional starch-hydrolyzing enzymes are necessary to augment the insufficient secretion of pancreatic amylases in early-weaned pigs. The results of the current study are in agreement with the reports by Inborr et al. (1991a, 1993) which indicated that the use of single β-glucanase preparation did not influence the starch digestibility while a combination of amylase, xylanase and β-glucanase increased starch digestibility in the small intestine. The results of the current study confirmed the hypothesis that a multi-enzyme preparation would be necessary for an effective breakdown of the cell wall structure and more complete nutrient digestion by young monogastric animals.

Although in the phase I of Experiment 1 the inclusion of Enzyme B increased the total tract digestibility of protein by 8% ($P<0.05$), only a tendency and no significant increase in protein digestibility was observed in two segments of the small intestine in Experiment 2. In general, the literature data on the effect of enzyme supplementation on protein utilization are contradictory. Some reports showed an increased protein digestibility with enzyme supplementation (Graham et al., 1988; Thacker et al., 1988, 1992a; Bedford et al., 1992; Li et al, 1996) while others reported no effect of either single enzyme source (Jensen et al., 1998; Graham et al., 1988a; Gdala et al., 1997; Thacker et al., 1992b) or multi-enzyme preparation (Gdala et al., 1997; Inborr et al., 1993). Bedford et al. (1992) speculated that supplemental enzyme may increase the digestion of soybean meal protein. Baidoo et al. (1998) reported the increase in protein digestibility in an enzyme supplemented canola meal-based diet. It is believed that the combination of various enzyme activities may release the protein from the aleurone layer of barley and wheat or improve protein digestibility in soybean and canola meal. In addition, protease can strengthen the ability of piglets to digest protein. Increased protein digestibility in pea-based diet by xylanase/amylase addition (Owusu-Asiedu, 1998) and soybean meal diet by cellulase/β-glucanase/protease addition (Nasi, 1991) in pigs were observed.

Protease has also been shown to break down the protease inhibitors, androgenic protein and lectins of soybean meal (Classen et al., 1993). Decreased endogenous nitrogen losses by enzyme supplementation may also contribute to the increase in the overall protein utilization (Inborr and Bedford, 1994; Jensen et al, 1994).

There was no significant difference in phytate digestibility between Diet 1 and Diet 2 in both experiments and no significant improvement in phytate digestibility was observed for enzyme supplemented diets (Tables 10 and 11). However, some tendency towards increased phytate digestibility with enzyme supplementation could be attributed to the trace amounts of phytase activity present in the Enzyme B. Under normal dietary condition, phytate phosphorus is poorly utilized by the monogastric animals as they lack a significant amount of endogenous phytase. In the current study, the digestibility of phytate for Diet 1 and 2 averaged 2.3 and 3.8% for the middle segment and 18.9 and 19.1% for the lower segment of the small intestine, respectively.

In general, phytate can chelate certain minerals (ie., Ca, Co, Cu, Fe, Zn) and causes mineral deficiency in animals (Harland and Oberleas, 1996). Phytate can also form complexes with protein, free amino acids and starch therefore decreasing their availability (Kies et al., 1997). It has been documented that exogenous phytase addition to poultry and pig diets can significantly improve phytate phosphorus availability and decrease phosphorus excretion (Sebastian et al, 1998).

There was no treatment effect on total NSP digestibility although there was a trend ($P>0.051$) towards their improved utilization with Enzyme B supplementation in phase I of Experiment 1. With regard to the NSP component sugars, the digestibility of mannose and galactose significantly decreased in phase 1 of Experiment 1 when corn and soybean meal were substituted with barley and canola meal. This may be attributed to the higher β-glucan content in Diet 2 as it contained a type

of hulless barley (var. CDC Buck) known for its high β-glucan content, which may has negative effect on NSP component sugar digestibility. However, as compared to the negative control (Diet 2), the digestibility of xylose, mannose and galactose increased significantly ($P<0.05$) with Enzyme A and B supplementation and was equal to (mannose, galactose) or greater (xylose) than that of positive control (Diet 1)(Table 12). Total NSP digestibility determined in the current study (30-40% in the small intestine and 60-70% in feces) was higher than that reported earlier for barley-based diets (Jensen, et al., 1998; Baidoo et al; 1998; Gdala, et al., 1997) but in agreement with the results on wheat-based diet fed to pigs (Baidoo et al., 1998). Lack of significant increase in the total NSP digestibility observed in the current study is in agreement with the results by Graham et al. (1986, 1988a) and Gdala et al. (1997). However, both enzyme supplements A and B significantly increased the digestibility of mannose (by 4.5 and 5.8%), galactose (by 5.0 and 6.8%), xylose (by 11 and 15%) and arabinose (by 9 and 11%), respectively in phase I.

In general, the degradation of NSP depends upon their source, structure and type of enzymes used. Inborr et al. (1993) suggested that fibre-degrading enzymes would be more effective on water-soluble rather than water-insoluble substrates. In the current study, however, the diets contained a substantial amounts of wheat, soybean meal and canola meal, all known to contain a relatively high proportion of water-insoluble NSP. This may partially explain why the effect of enzyme supplementation on total NSPs digestibility was not significant.

In contrast to the total NSP, a more pronounced effect of enzyme addition on β-glucans digestion was observed (Inborr et al., 1993; Jensen et al., 1998). Numerous studies have shown that the inclusion of β-glucanase improved the digestibility of β-glucans in barley-based diet and resulted in improved performance and nutrient digestibility in pigs (Graham et al.,1988; Inborr et al., 1993)

Table 12. Fecal digestibility of non-starch polysaccharides (NSP) in early-weaned pigs (Phase I and II; Experiment 1)

Phase	Component sugar	Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B
I	Rhamnose	54.9 ± 16.6[1]	51.2 ± 20.1	39.7 ± 7.9	44.3 ± 7.2
	Arabinose	53.4 ± 2.5[ab]	48.3 ± 8.5[b]	57.1 ± 4.7[ab]	59.5 ±4.5[a]
	Xylose	50.8 ±2.9[b]	50.7 ± 9.5[b]	61.8 ± 4.4[a]	65.4 ± 2.9[a]
	Mannose	88.9 ± 1.6[a]	82.5 ± 3.5[b]	87.0 ± 1.7[a]	88.3 ±1.9[a]
	Galactose	81.3 ± 0.8[a]	75.4 ± 4.0[b]	80.5 ± 2.1[a]	82.1 ±2.4[a]
	Glucose	54.5 ±18.6	52.0 ±16.2	66.1 ± 6.9	70.3 ± 9.0
	Uronic acids	64.0 ± 5.9	58.4 ± 9.3	60.6 ± 5.7	67.6 ± 6.2
	Total NSP	64.9 ± 4.0	64.1 ± 5.0	68.4 ± 3.8	71.5 ± 4.2
II	Rhamnose	43.1 ± 13.0	41.6 ± 26.9	40.4 ± 15.0	50.9 ±17.6
	Arabinose	51.3 ± 2.3	50.9 ± 6.5	52.3 ± 1.7	55.4 ± 2.4
	Xylose	54.2 ± 2.7[a]	57.6 ± 5.6[a]	61.2 ± 1.4[ab]	62.9 ± 3.2[b]
	Mannose	84.3 ± 2.0	84.6 ± 2.0	87.8 ±2.6	86.8 ± 0.9
	Galactose	77.8 ± 1.6	75.7 ± 1.3	77.7 ± 2.1	78.7 ± 1.1
	Glucose	59.2 ± 8.0	64.1 ± 6.4	69.0 ± 8.9	67.8 ± 7.2
	Uronic acids	64.2 ± 7.0	52.5 ± 5.0	57.7 ±4.7	59.7 ±9.6
	Total NSP	59.2 ± 3.8	60.7 ± 4.6	64.3 ± 2.3	65.2 ± 4.6

[1]Mean ± SD (n = 5 in phase I and n = 6 in phase II).

[ab]Means within a row with no common superscripts are significantly different (P<0.05).

Several studies indicated that pigs can degrade considerable amounts of β-glucan and arabinoxylan (Thacker and Bass, 1996; Graham et al., 1988). Growing pigs (30 to 50kg) have $10^6 g^{-1}$ lactobacilli in the duodenal and ileal digesta with β-glucan degrading ability. In some cases, no or little β-glucans were detected in the feces (Graham et al., 1986, 1989; Li et al., 1994, 1996). This may explain why enzyme supplements are often less effective in pigs than in the chickens. In addition, it has been indicated that endogenous enzymes present in barley or wheat may also play a significant role in degradation of β-glucan in the small intestine of the pig (Bach Knudsen et al., 1993).

In the current study, no significant effect of diet or enzyme addition on digesta viscosity was observed (Table 13). This is in agreement with the results by Inborr et al. (1991a) and Bedford et al. (1992) but in contrast to the study by Jensen et al. (1998) who found a decrease in intestinal viscosity of piglets fed a β-glucanase supplemented diet.

It is believed that in poultry and more specifically in young chicks the decreased viscosity following enzyme addition is responsible for the improvement in performance and nutrient digestibility. The viscosity and gelling properties of NSP tend to hinder intestinal motility, therefore decreasing the mixing of digesta, digestive enzymes and other components required for digestion and absorption (Fengler and Marquardt, 1988). Carbohydrase enzymes can break down the soluble NSPs therefore reducing or eliminating the viscosity effect. In the pig, however, the small intestine digesta contain less dry matter (10%) than in chicks (20%)(Classen and Bedford, 1991). Therefore, the average digesta viscosity in pigs is 100 fold lower than that determined in the chicken. In some cases, enzyme supplementation of the pig diets even increased the intestinal viscosity (Bedford et al., 1992; Graham et al., 1988). Therefore, it would appear that any improvement in pig performance and nutrient digestibility with carbohydrase supplementation may be attributed to the release of nutrients

Table 13. Effect of enzyme supplementation on digesta viscosity (cPs) and pH of different compartments of the gastrointestinal tract of early-weaned pigs (Experiment 2)[1]

		Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B
Stomach	Viscosity	1.69 ± 0.25[2]	1.82 ± 0.17	1.77 ± 0.20	1.80 ± 0.16
	pH	3.52±0.44	3.98±0.69	4.14±0.30	4.01±0.58
Small intestine					
Upper segment	Viscosity	1.54 ± 0.22	1.70 ± 0.14	1.74 ± 0.16	1.80 ± 0.22
	pH	5.55 ± 0.19	5.55 ± 0.19	5.49 ± 0.16	5.55 ± 0.21
Middle segment	Viscosity	2.13 ± 0.59	2.32 ± 0.39	2.05 ± 0.14	2.31 ± 0.23
	pH	6.25 ± 0.18	6.12 ± 0.39	6.26 ± 0.36	6.21 ± 0.80
Lower segment	Viscosity	3.36 ± 0.95	3.85 ± 1.0	2.93 ± 0.72	3.52 ± 1.5
	pH	6.76 ± 0.25	6.82 ± 0.14	6.41 ± 0.33	6.40 ± 0.7
Cecum	pH	6.00 ± 0.41	5.59 ± 0.36	5.68 ± 0.21	5.72 ± 0.17
Colon	pH	6.00 ± 0.45	5.69 ± 0.37	6.17 ± 0.49	5.90 ± 0.18

[1]No significant treatment effects were observed ($p>0.05$).

[2] Mean± SD (n= 6).

encapsulated by the cell wall structure, decrease of endogenous losses (nitrogen and electrolytes) or changes in gut microflora rather than viscosity reduction.

In the current study, greater digestibility of dry matter, energy and protein was observed for the Enzyme B supplemented diet than that of Enzyme A. The difference between Enzyme A and B was such that the latter contained the amylase, protease, carbohydrase and other enzyme activities (Table 7) in addition to the conventional xylanase and β-glucanase present in the Enzyme A (Table 7). This would indicate that a multi-enzyme preparation may be necessary for the young pigs as they may be deficient in certain digestive enzymes. Owusu-Asiedu (1998) also found that a combination of xylanase and amylase was more effective in increasing feed conversion ratio in young pigs (4 to 10 kg) than in older pigs (10 to 20 kg).

The digestive capability of the GI tract would appear to play a major role in the performance of young pigs since the weaning may depress the secretion of digestive enzymes (Efrid et al., 1982; Lindemman et al., 1986; Markkink et al., 1994). Although the level of pancreatic enzymes, including lipase, amylase, chymotrypsin and trypsin increases with age, weaning at four weeks of age decreased the levels of these enzymes dramatically (Lindemman et al., 1986; Chesson, 1990). The reduction in enzyme activity may cause impaired nutrient utilization, growth depression (known as post-weaning lag; Okai, 1976) and digestive disorder due to malabsorption (Hampson and Kidder, 1986). It is also believed that the depression in piglet growth at weaning has a deleterious effect on their lifetime performance (Whitemore, 1985). Incomplete digestion of nutrients in the small intestine may lead to their enhanced fermentation by the hint gut microflora and consequently may cause excessive proliferation of microbes. Addition of enzymes to augment the animal's own digestive capacity may prevent such negative effects. Since the development of microbial population increases with age, it

is reasonable to assume that the enzyme supplements containing a broad spectrum of enzyme activities including amylase, protease and lipase activities would be more beneficial for younger (5 to 10 kg) than older pigs (10 to 20 kg).

In general, replacing corn (100%) and soybean meal (25%) with barley and canola meal decreased nutrient digestibility in early-weaned pigs, but this situation was alleviated by enzyme supplementation. With enzyme supplementation, the digestibility of diet containing barley and canola meal was equal to or greater than that observed for the corn and soybean meal diet.

Plasma Urea Nitrogen

As shown in Table 14, plasma urea nitrogen level was not influenced by enzyme supplementation in Experiment 1, regardless of the significant increase in protein digestibility and a tendency towards increased ADG and FCR. This is in contrast to research by Coma et al. (1995), who demonstrated that a reduction in plasma urea nitrogen level is an indication of more efficient utilization of amino acids. Owusu-Asiedu (1998) also found a reduced plasma urea nitrogen level in piglets fed enzyme supplemented pea-based diet. However Richert et al. (1994) found that early-weaned pigs fed a high quality diet supporting the greatest performance (ADG, FCR) and digestibility of nutrients (DM, nitrogen) had the highest plasma urea nitrogen level. Therefore, it has been suggested that plasma urea nitrogen is not a good indicator of the nutritional value of the feed. It is speculated from the current research that the relationship between nitrogen utilization and plasma urea nitrogen level may by related to the composition of a diet.

Table 14. Effect of enzyme supplementation on plasma urea nitrogen level of early-weaned pigs (Phase I and II; Experiment1) (mg/dl)[1]

	Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B
Initial level	5.09 ± 1.96[2]	4.25 ± 1.03	4.77 ± 1.74	5.17 ± 2.11
End of phase I	8.40 ± 2.20	7.68 ± 2.23	8.45 ± 2.56	8.11 ± 2.08
End of phase II	10.26 ± 2.81	9.36 ± 2.21	10.17 ± 3.09	9.80 ± 2.64

[1]No significant treatment effects were observed ($p>0.05$).

[2]Mean ± SD (n = 6).

Digesta pH

The pH value determined in current research is in line with results reported by other researchers (Thacker and Bass, 1996; Bedford et al., 1992). Neither diet nor enzyme supplementation affected the pH in the stomach, small intestine and large intestine (Table 13). This is in agreement with research by Bedford et al (1992) who indicated that supplemental β-glucanase in barley-based diet had no effect on digesta pH in the small intestine. It is believed that a low pH in the GI tract is necessary to activate pepsinogens and to prevent movement of a viable bacteria into the upper small intestine. Early-weaning may reduce the capacity of piglets to produce gastric acid and may have a negative effect on digestion (Easter, 1988).

Organ Size Evaluation

The weight of pancreas, liver, spleen, kidney, stomach, small intestine, cecum, colon and the length of the small intestine were not affected ($P>0.05$) by diet or enzyme supplementation (Tables 15 and 16). Jensen et al. (1998) found that glucanase supplementation did not affect the weight of pancreas but increased the amount of protein per gram of tissue. Boychuk (1997) found no significant increase in stomach weight of finisher pigs fed a diet in which corn was substituted with micronized barley. It is assumed that the high level of dietary fibre in diet may enhance the size of organs. Kuan et al. (1983) found the length and weight of various sections of the gut to increase as the level of lucerne leaf meal increased. The concentration of NSP and other fiber components in the diets used in the current experiments was probably not high enough to affect the organ size.

Table 15. Effect of enzyme supplementation on the weight of pancreas, liver, spleen and kidney of early-weaned pigs (Experiment 2)(g/kg body weight)[1]

	Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B
Pancreas	3.1 ± 0.2[2]	3.4 ± 0.6	3.1 ± 0.6	3.4 ± 0.6
Liver	36.5 ± 2.2	37.1 ± 5.8	38.2 ± 3.8	40.3 ± 7.1
Spleen	2.7 ± 0.3	3.3 ± 0.5	2.9 ± 0.7	3.0 ± 0.7
Kidney	7.3 ± 0.7	6.6 ± 0.4	7.3 ± 1.0	7.2 ± 1.5

[1]No significant treatment effects were observed ($p>0.05$).

[2]Mean ± SD (n = 6).

Table 16. Effect of enzyme supplementation on the size of the gastrointestinal tract of early-weaned pigs (Experiment 2)[1]

	Diet 1	Diet 2	Diet 2 + Enzyme A	Diet 2 + Enzyme B
Stomach (g/kg body weight)	12.7±1.9[2]	11.9±1.5	12.8±1.5	13.5±2.1
Small intestine (g/kg body weight)	53.0±3.3	53.6±6.1	60.1±6.0	58.2±9.0
(cm/kg body weight)	113.1±24.3	109.8±27.7	105.7±10.9	112.1±16.7
Cecum (g/kg body weight)	3.2±0.3	3.7±0.8	3.2±0.4	3.5±0.9
Colon (g/kg body weight)	22.7±1.3	22.8±3.6	23.3±4.7	22.2±8.4

[1]No significant treatment effects were observed ($p>0.05$).

[2]Mean ± SD ($n = 6$).

Cost Benefit of Enzyme Supplementation

The price of diets and the cost per kg of body weight gain is shown in Tables 17,18 and 19. As compared to the positive control, in phase I the cost per kg of body gain was lower by $0.019 and $ 0.031 in pigs fed diets supplemented with Enzyme A and B, respectively; and in phase II is $0.006 lower by enzyme B Supplementation. The results of the current study demonstrate the economic advantage to the hog producers from using an enzyme supplemented diet and indicate a higher benefit from the use of a more diversified enzyme preparation (ie., Enzyme B).

The choice of grain is related to the geographical location of feed ingredient production. In situations when the price of corn and soybean increases, barley and canola meal could be valuable alternatives for more flexible ration formulation.

Increased nutrient digestibility with enzyme supplementation would also result in less nutrients being excreted into environment which is extremely important from the environmental pollution standpoint and the economics of animal production.

Table 17. Price of diets used in phase I of the pig experiment 1and 2

Ingredient	Diet (kg)					Price ($)			
	Diet 1	Diet 2	Diet 2+ Enzyme A	Diet 2 + Enzyme B	Ingredient (Price/tonne)	Diet 1	Diet 2	Diet 2+ Enzyme A	Diet 2 + Enzyme B
Wheat	46.0	46.0	46.0	46.0	121	5.57	5.57	5.57	5.57
Corn	23.0	0	0	0	112	2.58	0	0	0
Hulless barley	0.0	23.0	23.0	23.0	108	0	2.48	2.48	2.48
Soybean meal	12.5	9.25	9.25	9.25	237	2.95	2.19	2.19	2.19
Canola meal	0.0	3.0	3.0	3.0	143	0	0.43	0.43	0.43
Fish meal	4.0	4.0	4.0	4.0	985	3.94	3.94	3.94	3.94
Spried dried plasma	3.0	3.0	3.0	3.0	4900	14.7	14.7	14.7	14.7
Whey	4.0	4.0	4.0	4.0	914	3.66	3.66	3.66	3.66
Vitamin-mineral premix	5.0	5.0	5.0	5.0	854	4.27	4.27	4.27	4.27
Canola oil	2.0	2.0	2.0	2.0	690	1.38	1.38	1.38	1.38
Lysine-HCl	0.4	0.5	0.5	0.5	2850	1.14	1.43	1.43	1.43
Threonine	0.14	0.25	0.25	0.25	5000	0.7	1.25	1.25	1.25
Enzyme	0.0	0.0	Trace	Trace	.....	0	0	0.03	0.05
Total	100.0	100.0	100.0	100.0	.....	40.881	41.292	41.322	41.342
Price ($/kg)	.....	.....	.....	.....	.....	0.4088	0.4129	0.4132	0.4134

Table 18. Price of basal diets used in phase II of the pig experiment 1

	Diet (kg)					Price ($)			
Ingredient	Diet 1	Diet 2	Diet 2+ Enzyme A	Diet 2 + Enzyme B	Ingredient (Price/tonne)	Diet 1	Diet 2	Diet 2+ Enzyme A	Diet 2 + Enzyme B
Wheat	46.0	46.0	46.0	46.0	121	5.57	5.57	5.57	5.57
Corn	26.0	0	0	0	112	2.91	0	0	0
Hulless barley	0.0	26.0	26.0	26.0	108	0	2.81	2.81	2.81
Soybean meal	14.46	9.25	9.25	9.25	237	3.43	2.19	2.19	2.19
Canola meal	0.0	3.0	3.0	3.0	143	0.00	0.43	0.43	0.43
Fish meal	4.0	4.0	4.0	4.0	985	3.94	3.94	3.94	3.94
Whey	4.0	4.0	4.0	4.0	914	3.66	3.66	3.66	3.66
Vitamin-mineral premix	5.0	5.0	5.0	5.0	854	4.27	4.27	4.27	4.27
Canola oil	2.0	2.0	2.0	2.0	690	1.38	1.38	1.38	1.38
Lysine-HCl	0.4	0.5	0.5	0.5	2850	1.14	1.43	1.43	1.43
Threonine	0.14	0.25	0.25	0.25	5000	0.70	1.25	1.25	1.25
Enzyme	0	0	Trace	Trace	.....	0	0	0.03	0.05
Total	102.0	100.0	100.0	100.0	.....	26.991	26.916	26.946	26.966
Price ($/kg)	.....	.....	.....	.....	.....	0.2646	0.2692	0.2695	0.2697

Table 19. Cost per kg of gain for early-weaned pigs (Phase I and II; Experiment 1)

	Phase	Diet 1	Diet 2	Diet 2+ Enzyme A	Diet 2+ Enzyme B
Feed/Gain		1.27	1.21	1.21	1.18
Price of feed ($/kg)	Phase I	0.4088	0.4129	0.4169	0.4169
Cost/gain ($/kg)		0.519	0.500	0.504	0.492
Feed/Gain		1.45	1.44	1.46	1.40
Price of feed ($/kg)	Phase II	0.2646	0.2692	0.2732	0.2732
Cost/gain ($/kg)		0.384	0.388	0.399	0.382

Summary and Conclusions

A performance/digestibility trial and a slaughter trial were conducted to determine the effect of dietary substitution of corn and soybean meal with hulless barley and canola meal on the performance and nutrient digestibility of early-weaned pigs. Supplementation of the early-weaned pig diet with a new enzyme preparation containing xylanase, β-glucanase, amylase, protease and a broad spectrum of other enzyme activities was also investigated.

Replacing corn (100%) and soybean meal (25%) with barley and canola meal decreased digestibility of some nutrients but this situation was alleviated by enzyme supplementation. Significantly increased nutrient digestibility with enzyme supplementation did not translate to the statistically significant improvement in pig performance probably due to too few pigs in the trial. The results suggest that with enzyme supplementation, barley and canola meal could be included in the early-weaned piglet's diet without comprising performance.

With enzyme supplementation, a shift in nutrient digestion site from the large intestine to the small intestine was noted and was considered beneficial from the efficacy of nutrient utilization standpoint. Viscosity was not influenced by either enzyme inclusion suggesting, as opposed to chickens, its minor relevance to the utilization of nutrients by pigs. More pronounced increase in digestibility of nutrients with Enzyme B supplementation suggests that a multi-enzyme preparation is needed to overcome the problems associated with early weaning.

The results of the current study clearly demonstrate that the research directed towards improvements of the conventional xylanase and β-glucanase preparation has a great likehood of success and should result in further improvement in early-weaned pig performance.

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