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Enzyme Supplementation to Diets Containing Peas or Canola Meal Fed to Young Pigs

by

Laura C. Hargreaves 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

in

Animal Science

Department of Agricultural, Food, and Nutritional Science

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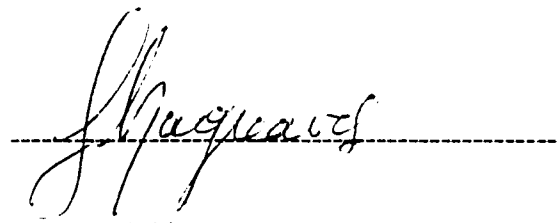

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Enzyme Supplementation to Diets Containing Peas or Canola Meal Fed to Young Pigs submitted by Laura C. Hargreaves in partial fulfillment of the requirements for the degree of Master of Science in Animal Nutrition.

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To Richard

For your trust, your understanding,
and your love

Abstract

Two experiments were carried out with young pigs to determine the effects of an exogenous enzyme mixture supplemented to diets containing either peas or canola meal, on the digestibility of amino acids and other nutrients. Pigs were weaned at 18 to 21 d of age and fitted with a simple T-cannula at the distal ileum 6 to 7 d after weaning. After 5 to 7 d of adaptation to diets, ileal digesta and feces were collected. Peas or canola meal were the main sources of dietary protein in the experimental diets. The exogenous enzyme mixture contained xylanase and protease activities.

In the first experiment, enzyme supplementation to diets containing peas plus wheat had no effect on the apparent ileal or fecal digestibility of any dietary nutrient.

In the second experiment, enzyme supplementation to diets containing canola meal plus wheat improved ($P<0.05$) the apparent ileal digestibility of starch. There was no effect on the ileal or fecal digestibility of any other dietary nutrient.

These studies have shown that the addition of a xylanase-protease mixture of enzymes to diets containing peas or canola meal fed to young pigs may improve ($P<0.05$) the ileal digestibility of starch in young pigs fed canola meal plus wheat, but has no effect on the ileal or fecal digestibility of any other dietary nutrient.

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1. Introduction

The efficiency of pork production in Alberta may be limited by the performance of piglets immediately post-weaning, due to the immaturity of their gastrointestinal tract in terms of digestive capacity. This often results in low feed intake and the development of diarrhea, symptoms of 'post-weaning growth check' which may last for up to two weeks (Li, 1992), and can increase the costs of production. Furthermore, diets for weaned pigs can be expensive, especially when imported ingredients are used. Therefore, the use of locally grown, less expensive feed ingredients may contribute to reduced feed costs for producers. In addition, the use of supplemental enzymes in diets for young pigs may contribute to improved digestibility of dietary nutrients, and possibly reduce the severity of the post-weaning growth check, which may contribute to the increased efficiency of pork production.

A. Peas

Background

Peas (*Pisum spp.*) are an annual grain legume producing large, edible seeds. The common pea (*P. sativum*) is one of only two species in the *Pisum* genus (Smartt, 1984). Peas were originally grown in the Middle East as a winter crop, but they are now grown in many climates worldwide, often during the summer in cooler temperate regions. Several distinct subspecies (varieties) of *P. sativum* have been identified based upon morphological features, including plant size, color or number of flowers or seeds, pod length or number, and other characteristics. 'Field' varieties (subspecies *arvense*, with colored flowers and dark seeds) are usually grown as livestock feed, whereas 'garden' varieties (subspecies *hortense*; white flowers, larger seeds) are used as human food or feed for swine (Castell, 1990). The edible pods, and sucrose-storing (Smartt, 1984) round seeds, of 'sugar' peas (subspecies *axiphium*) are intended for human food (Castell, 1990).

In 1998, the number of acres sown for peas in Alberta increased 33% over that sown in 1997 (Anonymous, 1998). The increased production of peas in Western Canada is partly due to the economic efficiency of pea production (Jaikaran *et al.*, 1995). As legumes, peas can use atmospheric nitrogen

through symbiosis with nitrogen-fixing soil bacteria (*Rhizobium spp.*). This supplies almost all of the plant's nitrogen requirement (Pate, 1977); peas therefore do not deplete the soil of valuable nutrients, and require less fertilizer than cereals. A short growing season relative to other legumes (Savage and Deo, 1989) makes peas suitable as a Western Canadian crop. The increased production of peas in recent years makes them readily available as an alternative protein source to imported soybean meal (Sauer *et al.*, 1990), the traditional protein source to which alternative feedstuffs are usually compared. Peas which may be unsuitable for human consumption, due to pests or physical damage, retain value as livestock feed (Savage and Deo, 1989). Furthermore, the use of locally grown feed ingredients, such as field peas translates into lower handling costs for livestock producers compared to imported protein sources.

Nutritive Value

Peas have potential as a high protein, high carbohydrate feedstuff for swine (Savage and Deo, 1989; Fan *et al.*, 1994). The crude protein content averages 25% (Sauer *et al.*, 1990). The content of amino acids is correlated with the amount of crude protein (Gatel and Grosjean, 1990); the amino acid content usually increases with increasing protein content (Fan *et al.*, 1994) and there is a tendency towards increasing amino acid digestibility with increasing protein content (Buraczewska *et al.*, 1989; Gatel and Grosjean, 1990). Therefore, peas having a higher protein content may have greater nutritional value not only for their total protein content, but also for their tendency towards higher amino acid digestibility compared to peas with lower protein content. Amino acid content is correlated with protein content, however the content of amino acids is also dependent on the relative proportions of albumins and globulins, the main storage proteins in peas (Fan *et al.*, 1994). Albumins, deposited early in pea seed development, are rich in sulphur-containing amino acids whereas globulins (vicilins), deposited later in development, are low in these amino acids (Savage and Deo, 1989), therefore the total content of sulphur-containing amino acids will decrease with pea seed maturity. While the sulphur-containing amino acid content in peas may be low, the lysine content is high (1.8%; Gatel, 1992), making peas a good complement for cereal grains, which are usually adequate in sulphur-containing amino acids but often lacking in lysine (Gatel, 1992). When fed in

combination with wheat, the protein quality of peas equals that of soybean meal in combination with wheat (Sauer *et al.*, 1990). Variations in proximate composition exist, due to differences in analytical methods, and also due to environmental conditions and genetic factors among the many varieties (Savage and Deo, 1989; Sauer *et al.*, 1990). White-flowered cultivars are often reported to have higher digestible energy contents and protein digestibility compared to varieties having colored flowers (Sauer *et al.*, 1990, Gatel 1992). Cooking or other processing treatments may slightly lower the protein content (Savage and Deo, 1989), or increase the amount of available energy through disruption of starch granules (Goodlad and Mathers, 1992).

Peas contain relatively high levels of total carbohydrates: ranging from 56.6 to 74.0% (Savage and Deo, 1989), with a high proportion of total polysaccharides (both starch and non-starch polysaccharides (NSP); Fleming, 1981; Sauer *et al.*, 1990; Goodlad and Mathers, 1991). Starch content ranges from approximately 214 to 486 g/kg (Savage and Deo, 1989), although higher contents have been reported (490 to 500 g/kg; Grosjean and Gatel, 1986). Relatively low levels of amylose (20 to 38%) contribute to the high digestibility of pea starch (over 98%; Savage and Deo, 1989). Sucrose is the main type of sugar (38% of total sugars), with the remainder being mainly alpha-galactosides (Grosjean and Gatel, 1986; Gatel and Grosjean, 1990). Crude fibre levels in peas range from 1.1 to 9.9% (Sauer *et al.*, 1990); this includes NSP, oligosaccharides, resistant starch, polyphenols, and proteins bound to cell walls (Gdala, 1998). The NSP make up the bulk of pea dietary fiber, along with the structural complexes formed between NSP and lignin (an alcohol polymer) (Gdala, 1998). The average NSP content in peas is 18.5% (DM basis; Gdala, 1998), but the composition of NSP is significantly affected by the stage of maturity of the seed: according to Gdala (1998) mature whole seeds contain approximately 16 to 20% NSP, with the proportions of specific NSP residues being very similar between white- and colored-flowered varieties.

Anti-nutritive Factors

The nutritive value of peas is affected by the digestibility of energy and protein as well as by the nutritional composition. Several compounds, known as anti-nutritive factors (ANF), exist naturally in plant feedstuffs and may adversely affect metabolic processes, including the digestibility of nutrients and energy. Many varieties of peas contain ANF which have

varying effects on nutrient digestibility and availability (Savage and Deo, 1989). Protease (trypsin) inhibitors, tannins and lectins are some of the most commonly discussed, although other dietary components such as fiber, or certain oligosaccharides have also been recognized as having anti-nutritive activity, due to their influence on the digestibility of other dietary nutrients (Leterme *et al.*, 1990; Birk and Smirnoff, 1992; Gdala 1998).

The level of trypsin inhibitor activity in peas is dependent on variety (genetic factors), and also varies with environmental conditions (Leterme *et al.*, 1990). According to Gatel and Grosjean (1990), winter varieties contain 2 to 4 times the levels of trypsin inhibitors than spring varieties. The levels are highest in the pea cotyledon: 13 times higher than in the hull (Gatel and Grosjean, 1990, Sauer *et al.*, 1990). Peas contain similar amounts of trypsin inhibitors as field beans, and approximately one-tenth the trypsin inhibitor activity of soybeans (Sauer *et al.*, 1990; Valdebouze, 1980); Gatel and Grosjean (1990) reported that peas contain 5 to 20 times less trypsin inhibitor activity than soybean meal.

Lectins (also called hemagglutinins) are glycoproteins which bind to cell surface carbohydrates of the intestinal mucosa, causing damage to the microvilli (Wareham *et al.*, 1994), and interfering with nutrient absorption (Liener, 1981; 1990). Intestinal enterocyte turnover is increased; nitrogen balance may also be affected through increased amounts of nitrogen excreted in feces. Morphological changes in other organs (atrophy of the thymus gland, or hypertrophy of the pancreas and liver) can result when lectins are absorbed (Wareham *et al.*, 1994). Levels of lectins in peas and other legumes vary widely, due to the limitations of the chemical assays used; according to Savage (1989), pea seeds are non-toxic with respect to the levels of lectins.

Tannins are high-molecular weight phenolic compounds found mainly in the hulls of seeds, having an aromatic ring with various chemical groups (Wareham *et al.*, 1994). Their effects are similar to other ANF: complex formation with proteins or carbohydrates; inhibition of digestive enzymes, and alteration of the integrity of the mucosa. Most legumes contain significant amounts of tannins; dark-flowered peas contain higher amounts of total phenols than white-flowered varieties: 2.0 and 7.5 mg/g, respectively (Gdala, 1998). In agreement with this, Griffiths (1981) found that tannins are usually concentrated in the varieties of peas and other legume

seeds having colored flowers; one white-flowered variety of peas was found to be almost completely devoid of phenolic compounds.

Certain oligosaccharides found in peas are considered to have an anti-nutritive character due to their poor digestibility and the resulting effects on gastrointestinal function, which include diarrhea, flatulence, proliferation of microorganisms in the colon, and increased acidity of intestinal contents and urine (de Groot, 1987). Flatulence-producing oligosaccharides, such as the alpha-galactosides of sucrose (raffinose, verbascose, stachyose) occur in significant amounts in beans, peas, lentils, and several oil-extracted meals, including soybean and rapeseed (canola) meal (Saini, 1989; Dierick and Decuypere, 1994; Gdala, 1998); stachyose and verbascose are the predominant types found in peas (Gdala, 1998). Monogastric digestive systems lack the necessary alpha-galactosidase enzyme for the complete intestinal hydrolysis of these oligosaccharides, which pass largely intact into the cecum and large intestine where they are rapidly fermented to produce gaseous products (Sosulski, 1982; Dierick and Decuypere 1994; Gdala, 1998). The buildup of gas produces distention, nausea and discomfort, while the presence of intact oligosaccharides in the intestine can alter lumen osmolarity, leading to the influx of water and resulting in diarrhea (de Groot, 1987; Saini, 1989; Gdala, 1998). Partial hydrolysis of alpha-galactoside sugars may be possible in the mature monogastric animal, via the action of invertase (sucrase) in the intestine (Kidder and Manners, 1978), as well as microbial action (Millard and Chesson, 1984). However, levels of invertase are negligable at birth (Kidder and Manners, 1978); in piglets these oligosaccharides have been found to depress the ileal digestibility of nutrients, including protein and starch (Dierick and Decuypere, 1994). Polysaccharides which are fermented in the large intestine do provide energy substrates in the form of volatile fatty acids (short chain fatty acids), however the amount of energy provided via fermentation of nutrients is less than that provided by nutrients digested anterior to the distal ileum (Just *et al.*, 1983; Gdala, 1998) A considerable proportion of potential energy is lost as gases and heat from the fermentation process (25 to 40%; Bergman, 1990); fermentation of carbohydrates is only approximately 70% as efficient (Gdala, 1998) as enzymatic digestion of carbohydrates in the small intestine.

Early weaned piglets (weaned prior to 4 weeks of age) may initially be susceptible to some ANF in peas (Leterme *et al.*, 1990), such as trypsin

inhibitors. Alpha-galactosides in particular may have anti-nutritive effects on early weaned piglets, due to low levels of invertase at birth (Kidder and Manners, 1978). However, many authors (eg., Ogle and Hakansson, 1988; Leterme *et al.*, 1990; Sauer *et al.*, 1990; Kehoe *et al.*, 1995) have reported that peas, especially the white-flowered, spring seeded varieties grown in Canada, contain relatively low levels of ANF which do not adversely affect the performance of pigs. Peas have been included in diets for weanling pigs at levels of up to 50% without adverse effects on growth performance, with the addition of supplemental amino acids where required (Kehoe *et al.*, 1995).

B. Canola Meal

Background

Canola meal is the byproduct of oil extraction from canola seed. Rapeseed, the parent of canola, is a member of the *Cruciferae* family and is closely related to mustard and cabbage (Bowland, 1975; Bell, 1984). Rapeseed was grown 3000 years ago in India, and 2000 years ago in China and Japan. It is adapted to temperate regions, and as a cool-season crop in subtropical countries; two species are presently grown in Canada: *Brassica campestris* L. (a Polish variety) was introduced in 1936, and *Brassica napus* L. was introduced a few years later from Argentina (Baidoo, 1984; Bell, 1984). Certain bitter substances, such as glucosinolates (found in all cruciferous seeds and plants), and erucic acid, have limited the use of rapeseed meal as an alternative to soybean meal and other imported protein sources (Bell, 1984; Thacker, 1990). However, the discovery of low-glucosinolate varieties in 1967 precluded the development of the first low-erucic acid cultivar in 1968; in 1974 the first 'double low' cultivar (low in both erucic acid and glucosinolates) was produced (Bell, 1984). The name 'canola' was adopted in 1979 to describe all 'double low' (or double-zero) cultivars, having less than 5% erucic acid in canola oil and less than 3mg/g glucosinolates in canola meal (Baidoo, 1984; Aherne and Bell, 1990). The development through selective breeding of double-low varieties has resulted in more extensive use of canola meal in swine diets (Fan *et al.*, 1996). In recent years, production in Western Canada has increased and the use of canola meal is common for all ages of pigs (Thacker, 1990).

Nutritive Value

Canola meal is an excellent source of protein, averaging 38.3% (43% on a dry matter basis) (Bell, 1984). The crude protein (CP) content of the meal varies depending upon the component cultivars: meal from *B. campestris* contains approximately 35% CP while meal from *B. napus* contain 38 to 40% CP; meal from a mixture of these can contain 37 to 38% CP (Thacker, 1990). Variation in CP and amino acid contents exists among samples of canola meal, mainly due to differences in growing conditions, location, and genetics: the amino acid composition of the main storage proteins (globulins) varies among the different cultivars (Fan *et al.*, 1996). However, the nutritive value of protein in canola meal is determined by the digestibility of protein and amino acids as well as the total content of protein and amino acids, therefore variability in CP and amino acid contents does not reflect the real differences in protein nutritive value among different canola meal samples (Fan *et al.*, 1996). The content of lysine in canola meal is usually similar, or slightly lower (Baidoo, 1984; Bell, 1984) than traditionally-used soybean meal, while canola meal contains higher levels of the sulfur-containing amino acids (Sauer *et al.*, 1982; Baidoo, 1984; Bell 1984). However, the amino acid digestibility in canola meal is lower: i.e., the digestibility of lysine is approximately 10 percentage units lower than in soybean meal (Sauer *et al.*, 1982). Since lysine is usually the first limiting amino acid in many cereals, higher levels of canola meal providing excess CP may be used in diet formulation in order to compensate for the low lysine digestibility (Sauer *et al.*, 1982; Thacker, 1990). Alternatively, synthetic lysine can be provided, if canola meal replaces enough of soybean meal on a weight/weight basis (Fenwick, 1982). However, despite lower digestible amino acid levels compared to soybean meal, the ratio of amino acids to lysine in canola meal remains relatively constant as the dietary inclusion level of canola meal is altered; therefore canola meal is considered an excellent source of complementary amino acids (Lin *et al.*, 1987; Knabe *et al.*, 1989). Processing methods to extract the oil can influence amino acid levels: solvent extraction leaves more available lysine due to less damage caused by expeller prepared meals (Clandinin, 1961). Finally, the selection of the appropriate nitrogen-to-protein conversion factor also has important nutritional implications with respect to the protein quality of canola meal (Bell, 1984) and inclusion levels in diets. The % nitrogen X 6.25 is commonly used to determine the %CP for

most feedstuffs, despite its inaccuracy for most cereals and oilseeds; the correct conversion factor for canola meal is 5.53 (Bell, 1984) which should be considered when diets are formulated to fulfill the protein requirement of the animal.

Canola meal has a lower digestible energy content than soybean meal (approximately 25%), which is a reflection of its higher crude fiber content (Bowland, 1975; Bell, 1984; Thacker, 1990). This results from the high proportion of hull relative to the size of the seed and the higher levels (30%) of hulls in the meal (Slominski and Campbell, 1990). However, canola meal contains a higher content of ether extract (4% crude fat; Bell, 1984) than soybean meal (Thacker, 1990). The seed contains approximately 40% oil (Baidoo, 1984; Bell, 1984), therefore canola meal is considered a good source of energy. Double low cultivars have a relatively higher energy content due to the high digestibility of the oil fraction (Bourdon and Aumaitre, 1990), which is another advantage of these varieties compared to the original cultivars.

Canola meal contains approximately 12% crude fiber, most of which is contained in the lignin-rich hulls (Bell 1984). Yellow-seeded varieties have thinner hulls and therefore a lower crude fiber content than dark-seeded (brown) varieties: up to 4% less (Aherne and Kennelly, 1982; Mitaru *et al.*, 1984; Thacker, 1990). Starch content is low; most of the carbohydrate is in the form of NSP (including pectins, cellulose and pentosans), which make up approximately 16 to 22% (Bell, 1984; Mitaru *et al.*, 1984; Slominski and Campbell, 1990). The non-cellulosic components account for approximately 13 to 16% of the total NSP (Slominski and Campbell 1990). Canola meal has a high proportion of water-insoluble polysaccharides, which explains the relatively low digestible energy content (Bell, 1984; Slominski and Campbell, 1990). Cellulose is also a main component of the seed hulls, and may contribute up to 24.1% of the total polysaccharides in the meal (Fan *et al.*, 1996). Slominski and Campbell (1990) found that pectic-type substances (consisting mainly of galacturonic acid residues) comprised the bulk of non-cellulosic polysaccharides. Arabinose, xylose, galactose and rhamnose were found to be the main pectic sugars, and a relatively high content of xylose (13%) indicates the presence of xylan and xyloglucans (hemicellulose components) (Slominski and Campbell, 1990).

Fan *et al.* (1996) stated that fiber sources, including pectins and cellulose, can have a negative effect on amino acid digestibility. Water-

soluble fiber sources, such as pectins, act as a 'cementing' materials, forming viscous gels and a subsequent 'unstirred' water layer which acts as a physical barrier to protein digestion and absorption (Mitaru *et al.*, 1984; Fan *et al.*, 1996). Viscous gels may decrease protein digestibility by blocking enzyme-substrate interactions, reducing proteolytic hydrolysis and reducing the mixing of intestinal contents as well as decreasing the access of hydrolysis products to absorptive sites on the intestinal epithelium (Fan *et al.*, 1996). The low digestibility of amino acids in canola meal may be due in part to the relatively large proportion of hulls in the meal: protein which is associated with the hulls is often inacessible to digestive enzymes due to the low digestibility of the cellulose and lignin in the hulls (Bell, 1984; Slominski and Campbell, 1990; Bourdon and Aumaitre, 1990). In addition, the compact structure of the storage globulins in canola meal resist solubilization and therefore digestive enzyme penetration, resulting in lower protein and energy digestibility values (Valette *et al.*, 1993). Fibrous components may also decrease amino acid digestibility by directly binding to proteins. Mitaru *et al.* (1984) found that lignin in canola meal hulls had deleterious effects on protein and amino acid digestibilities. They state that lignin, an insoluble polymer of phenyl propyl alcohols and acids, may decrease protein digestibility by hydrophobically binding to amino acids, withholding them from intestinal absorptive sites. Differences in dietary cellulose and pectin content could therefore contribute to differences in apparent ileal digestibility values of amino acids; and these differences may be related to differences in hull level and composition among varieties of canola meal. Furthermore, according to Slominski and Campbell (1990) unabsorbed amino acids may not be degraded to any great extent in the colon due to the presence of more preferable substrates for microbial action, such as poorly-digestible polysaccharides.

Insoluble fiber (such as the lignin component of the canola hulls, or resistant starch which may be included in measurements of dietary fiber) may increase the endogenous CP and amino acid content in digesta at the distal ileum, reducing apparent ileal CP and amino acid digestibility values (Slominski and Campbell, 1990; Fan *et al.*, 1996). Mosenthin and Sauer (1991) found that dietary fiber could increase the sloughing of intestinal cells, and increase the volume of pancreatic juice, leading to increased losses of endogenous nitrogen. Imbeah *et al.* (1988) found higher amino acid

digestibilities in soybean meal than in canola meal, and suggested that differences in the rate of secretion of endogenous enzymes may be partly responsible for differences in amino acid digestibilities between feedstuffs such as soybean meal and canola meal; however they found no significant differences in the rate of secretion of pancreatic juice, protein or enzymes between pigs fed soybean meal or canola meal diets. Valette *et al.* (1993) found that *in vitro* protein digestibility between casein (milk) and rapeseed proteins was not significantly different after 24 hours, although there were significant differences during the first 12 hours, which they attributed to the nature of the protein source. Canola meal protein (globulins) may initially be less susceptible to denaturation by digestive enzymes, due to their complexity and chemical stability compared to casein protein fractions (Gray and Cooper, 1971; Valette *et al.*, 1993). This could contribute to the lower CP and amino acid digestibilities of canola meal compared to other protein sources, such as soybean meal (Sauer *et al.*, 1982; Imbeah *et al.*, 1988) or skim milk (Danielsen *et al.*, 1994). According to Mitaru *et al.* (1984), NSP such as pectin may further contribute to the lower digestibility of canola meal protein by coating protein molecules, decreasing their accessibility to digestive enzymes. Components of dietary fiber, such as the NSP of canola meal, which include cellulose (24.1%) and pectin (Fan *et al.*, 1996), may reduce the overall transit time in the gastrointestinal tract (Sauer and Ozimek, 1986). This could leave less time for digestion and absorption of dietary protein (Mitaru *et al.*, 1984; Bell, 1984) and result in decreased CP and amino acid digestibilities as found by some authors (eg., Imbeah *et al.*, 1988).

Anti-nutritional Factors

Anti-nutritional substances of concern in canola meal include tannins, sinapine, and glucosinolates. Glucosinolates (also called mustard oil glucosides or thioglucosides), are the main ANF, located in the embryo (Bell, 1984). This family of compounds includes gluconapin, glucobrassiconapin, progoitrin, goitrin, epiprogoitrin plus many others which themselves are not toxic but produce various cyanates (goitrogenic compounds) when hydrolyzed by bacterial or endogenous myrosinase (Bell, 1984; Thacker, 1990) These hydrolysis byproducts can have detrimental metabolic effects, including hepatotoxicity and goitrogenic symptoms, as cyanates compete with iodine for uptake into the thyroid gland; decreased thyroxine and tri-iodothyronine

release can impact nutrient utilization by body cells (Baidoo, 1984; Bell, 1984). There can be thyroid, liver, and kidney enlargement proportional to the amount of glucosinolates consumed (Bourdon and Aumaitre, 1990). The hydrolysis products have a bitter taste which can reduce feed intake; some may cause embryonic death or abnormalities (Bell, 1984). Some heat-processing methods may destroy most of the glucosinolates present and inactivate endogenous myrosinase in the canola seed (Bell, 1984). Levels of glucosinolates in canola meal have been reduced to about 15% of the levels of earlier varieties, due to genetic selection (Thacker, 1990); effects of glucosinolates in current crops is less than the effects of fiber (Bell, 1984; Thacker, 1990). For finisher pigs, soybean meal can be completely replaced by canola meal without decreasing animal performance (Sauer *et al.*, 1982); double-zero varieties of canola meal are now used more extensively in diets for all classes of swine (Fan *et al.*, 1996).

Tannins are polyphenolic compounds found mainly in the hulls of canola seeds (Liener, 1990). Their concentration in the seed is related to the seed color (Liener, 1990), with darker seeds usually having higher levels of tannins. Canola meal contains approximately 3% tannins (Thacker, 1990). Tannins may form complexes with dietary proteins, carbohydrates, or divalent metals, inhibit digestive enzymes, alter the integrity of the intestinal mucosa (Liener, 1990), and subsequently affect the digestibility of protein and energy (Baidoo, 1984; Thacker, 1990). Mitaru *et al.* (1984) found that the ileal digestibility of certain amino acids was lower in high tannin than in a low tannin sorghum. They suggested that tannins have a greater affinity for hydrophobic amino acids (eg., glycine, tryptophan); hydrophobic amino acids were among those which showed the greatest improvement in digestibility when the high tannin sorghum was reconstituted, de-activating the tannins. Dietary proteins or enzymes with many hydrophobic amino acid residues may therefore preferentially form protein-tannin complexes (Mitaru *et al.*, 1984), characterized by hydrogen bonds and interactions of the tannin's hydroxyl groups with the carbonyl groups of the protein (Fan *et al.*, 1996). In this manner tannins may decrease the activities of pepsin, trypsin, chymotrypsin and intestinal brush border peptidases. The decrease in trypsin activity could stimulate the hypersecretion of pancreatic enzymes through interruption of the normal trypsin-cholecystokinin-pancreatin feedback mechanism (Fan *et al.*, 1995). The morphology of the intestinal mucosa may

also be altered; resulting in decreased transmembrane uptake. Tannins which are absorbed can have toxic effects (Liener, 1990), although their large size suggests absorption is unlikely (Wareham *et al.*, 1990). Decreased digestion and absorption of nutrients along with increased endogenous secretions, may explain the lower digestibility of canola meal compared to soybean meal (Fan *et al.*, 1996).

Until recent years, there has been a lack of agreement regarding optimum levels of canola meal in diets for young pigs, due to variation in protein and amino acid digestibility values, which was partially due to variation in processing methods among processing plants but also due to genetic differences among varieties (Fan *et al.*, 1996); variation in age and weight of pigs used in investigations, the duration of the test, diet composition or feeding methods (Baidoo, 1984). Prior to the development of the double-low varieties, the use of canola meal in swine diets was limited to well under 5% of the diet depending upon the age of pig (Bell, 1984.) However, the double-low varieties can be used at much higher levels without adverse effects on pig performance: levels exceeding 20% of the diet can be used although lysine must often be supplemented (Bell, 1984).

C. Digestive Capacity of the Piglet

The digestive tract of newly-weaned piglets is considered immature, due to the limited production of digestive enzymes, compared to the mature pig (Aumaitre, 1972; Aumaitre and Corring, 1978; Kidder and Manners, 1972, 1978; Corring *et al.*, 1978). The activities of pepsin and pancreatic enzymes (including trypsin, chymotrypsin, and amylase), and mucosal carbohydrases (including sucrase, maltase, and isomaltase) are low, or negligable, at birth (Aumaitre, 1972; Aumaitre and Corring, 1978; Kidder and Manners, 1972, 1978; Corring *et al.*, 1978). The secretion of hydrochloric acid is also low until approximately 5 weeks of age (Aumaitre, 1972; Kidder and Manners, 1972; Manners, 1976). However, since the neonatal pig's diet consists entirely of sow's milk for the first few weeks, it only requires the enzymes necessary to digest the protein (mainly casein), carbohydrate, and fat in milk. Rennin is produced in the stomach from birth (Kidder and Manners 1972; 1978) for milk protein hydrolysis; rennin is only specific for casein and does not hydrolyze

the maternal immunoglobulins secreted in colostrum. Activities of both lactase and lipase is high at birth (Kitts *et al.*, 1956; Aumaitre, 1972; Kidder and Manners, 1972; Manners, 1976) for digestion of lactose and fats in milk. It is usually accepted that during the first few weeks of life (prior to 4 weeks of age), the digestive capacity of young piglets is insufficient for the digestion of plant protein sources (Hays *et al.*, 1959; Wilson and Leibholz, 1981; Owsley *et al.*, 1986; Walker *et al.*, 1986; Inborr *et al.*, 1993).

Piglets are weaned onto diets based on complex carbohydrates and plant proteins from a relatively simple high fat, milk protein diet: sows' milk contains approximately 40% fat, which provides about 60% of the piglet's dietary energy intake (Kidder and Manners, 1972). In contrast, plant carbohydrates are a main energy source (providing 80% of the dietary energy intake) in creep and starter feeds (Aumaitre and Corring, 1978). Young pigs have the potential for rapid growth rates: the ability to double their initial birth weight within 1 week and quadruple this within 3 weeks. This means they also have a large requirement for nutrients and energy (Bellis, 1957; Kidder and Manners, 1972). Prior to weaning, piglets may consume limited amounts of creep feed from 2 to 3 weeks of age, possibly due to the decline in yield and nutritional composition of the sow's milk after the third week of lactation (Hughes and Varley, 1980). English *et al.* (1982) suggest that before this time, piglets may be reluctant to consume creep feed because of insufficient digestive capacity. The intake of creep feed varies widely among piglets (English *et al.*, 1982; Lindemann *et al.*, 1986); the creep diet acts as a supplement rather than the main source of nutrition prior to weaning. Consequently, at weaning the piglet experiences a sudden shift in diet composition, which confers a corresponding shift in the importance of different digestive enzymes for the digestion of these new dietary nutrients (Kitts *et al.*, 1956; Aumaitre, 1972). While digestive capacity begins to increase at about 3 to 4 weeks of age (Aumaitre and Corring, 1978; Kidder and Manners, 1978), and some (i.e., sucrase, maltase and salivary amylase) will have reached a maximum at 4 weeks of age (Hudman *et al.*, 1957; Lloyd *et al.*, 1957), many enzyme activities do not reach substantial levels until 6 to 8 weeks of age (Kitts *et al.*, 1956; Corring *et al.*, 1978). Therefore, piglets weaned before 6 to 8 weeks of age are considered immature in terms of digestive capacity (Aumaitre and Corring, 1978). Plant carbohydrates are not well digested by young piglets (Kidder and Manners, 1978); flatulence-producing

oligosaccharides, present in many legumes, have been found to depress the ileal digestibility of organic matter, protein and starch in diets fed to young pigs (Dierick and Decuypere, 1994). Digestive capabilities play a major role in the performance of the young pig (Lindemann *et al.*, 1986): an inability to digest carbohydrate in starter diets will reduce the supply of energy available to the piglet, and along with decreased protein digestibility, may limit growth. Several authors (Lewis *et al.*, 1955; Hays *et al.*, 1959; Maner *et al.*, 1961; Leibholz, 1981) have attributed the poor performance of piglets on diets of soybean meal or other plant proteins (relative to milk protein) to inadequate digestive capacity. In addition, undigested carbohydrate entering the large intestine may encourage microbial proliferation (Kidder and Manners, 1972), which may contribute to post-weaning diarrhea and a subsequent delay in growth often seen in newly-weaned pigs.

D. Supplemental Enzymes

Background

Since Lahrmann in 1882 used 'digestive ferments' for predigesting casein (Cunningham and Brisson, 1957b), enzyme additives have been used by many investigators (eg., Lewis *et al.*, 1955; Calder *et al.*, 1959; Baird *et al.*, 1976; Inborr and Ogle, 1988; Officer, 1995) in attempts to improve the utilization of dietary nutrients by young pigs. Supplemental enzymes may remove or destroy ANF, including NSP; augment the existing digestive capacity of young pigs; increase the availability of certain dietary nutrients, or target certain dietary components such as NSP, which are potential energy sources for monogastric animals (Dierick and Decuypere, 1994).

Destruction of ANF

Many NSP are considered to be anti-nutritive (Chesson, 1987; Newman, 1994), due to their effects on the efficiency of nutrient digestion and utilization. Two of the most common NSP in swine feeds commonly used in Western Canada are arabinoxylans and beta-glucans (Bedford, 1995). Monogastric animals lack many of the enzymes necessary to hydrolyze the glycosidic bonds joining the monosaccharides of arabinoxylans and beta-

glucans (Leegwater, 1986; Bedford, 1995). Consequently, these NSP remain largely intact in the small intestine, and may interfere with the utilization of other nutrients (Aman and Graham, 1990; Dierick and Decuypere, 1994). Viscous NSP may coat starch granules and other dietary nutrients, reducing their digestion (Ellis *et al.*, 1996, Bedford and Schulze, 1998). Structural NSP of cell walls may encapsulate dietary nutrients, preventing the access of digestive enzymes (the 'cage effect'; Simon, 1998). Other effects include reduced passage rate of digesta and subsequent feed intake reduction, elevation of microbial activity in the intestine (Bedford, 1995), reduced utilization of vitamins and minerals (Newman, 1994), and disturbed gastric motility (Annison and Choct, 1994; Dierick and Decuypere, 1994; Ellis *et al.*, 1996). The presence of bulky, hydrophilic NSP (Low, 1989) in the lumen may also stimulate the influx of water in order to maintain osmolarity, which may lead to diarrhea (Schutte, 1991), and stimulate endogenous secretions (Low, 1985; 1989). Ellis *et al.*, (1996) suggests that increased digesta viscosity impairs the 'sieving' function normally performed by the pyloric sphincter, and results in large particles passing into the small intestine. Since nutrient digestibility is related to particle size (Sauer *et al.*, 1977b; Bedford and Schulze, 1998), this may impair nutrient digestion. Increased viscosity may indirectly alter gastric inhibitory peptide (GIP) output, which subsequently affects gastric, pancreatic and mucosal secretion (Low, 1985; 1989; Ellis *et al.*, 1996). Increased production of digestive enzymes can increase the amounts of nitrogen excreted with the feces (Chesson, 1990). In addition, indigestible protein conjugated with NSP passes into the large intestine where microbes deaminate the proteins and nitrogen is excreted with the feces (Annison and Choct, 1994). Digesta viscosity can affect the morphology and functional development of the digestive tract. Simon (1998) found that the weight and length of the small intestine was highly correlated (R=0.97) with the viscosity of the digesta in the jejunum. Increases in viscosity may shorten the turnover rate of mucosal cells, increase the number and size of goblet cells, and mucin production. The rate of mucin formation may affect the thickness of the unstirred water layer in the lumen, which affects the diffusion of nutrients (Simon, 1998). Fat utilization in particular may be impaired, as the fat micelles which form in the small intestine are relatively large (Campbell and Bedford, 1992; Bedford, 1995). A reduced feed passage rate may encourage microbial proliferation; this along with reduced digestion rate due to

increased digesta viscosity will increase the amounts of nutrients available for small intestinal microbes (Bedford and Schulze, 1998).

Reductions in digesta viscosity may be possible with the use of single-enzyme preparations (eg., beta-glucanase or xylanase) which can act on the interior of the monosaccharide polymer backbone, reducing the size of the polymer which then reduces viscosity in the intestine and enables more rapid digestion (Chesson, 1987; Dierick and Decuypere, 1994; Bedford, 1995). Few hydrolytic events are needed to shorten polysaccharide chains and reduce their gel-forming properties (Chesson, 1987). Cereal endosperm cell walls encapsulate starch and protein; enzymes may aid in the degradation of the cell walls, releasing the encapsulated nutrients (Chesson, 1987; Bedford and Schulze, 1998). However, Chesson (1987) suggests that xylanases and other exogenous enzymes may be hindered by the presence of various side-chains protruding from the polysaccharides of the endosperm. In addition, plant cell wall polysaccharides have complex structure, and require the activities of several enzymes working in synergy to effect the degradation of the cell wall structures and the release of encapsulated starch or protein (Chesson, 1987). Single enzyme preparations are probably ineffective toward the complex cell wall polysaccharides; their main role is likely in reduction of digesta viscosity via polysaccharide chain cleavage (Chesson, 1987). Alternatively, NSP hydrolyzing enzymes may indirectly enhance cell wall breakdown by stimulating gastric motility via the release of enteroglucagon (Gee *et al.*, 1996; Bedford and Schulze, 1998).

Enzymes may affect the quantities and composition of intestinal flora. According to Simon (1998), improved nutrient digestibility and a shift in the main sites of nutrient absorption toward the uppermost sections of the intestine could reduce microbial proliferation in the distal segments due to a reduction in substrate (Inborr and Ogle, 1988). However, partial degradation of NSP to oligomers, and partial solubilisation of insoluble NSP improves the availability of these substrates, in addition to reducing the passage rate of digesta, and may encourage microbial proliferation in the upper tract (Campbell and Bedford, 1992). Addition of a xylanase to a wheat-based broiler diet was shown to reduce bacterial numbers by 60% in the ileum and also encouraged a shift in the predominant species of bacteria present (Bedford and Schulze, 1998).

Augmentation of Digestive Capacity

The immature digestive tract of young pigs has been shown to produce low amounts of most digestive enzymes until approximately 8 to 10 weeks of age (Aumaitre, 1972; Aumaitre and Corring, 1978; Kidder and Manners, 1978). Consequently, piglets' digestive capacity at weaning (commonly around 3 to 4 weeks of age in Western Canada) is inadequate for the digestion of the vegetable protein-based diets which are offered (Maner *et al.*, 1961; Leibholz, 1981; Dierick, 1989). The use of supplemental enzymes in diets for newly weaned pigs in order to augment the low levels of enzymes is therefore justified (Dierick and Decuypere, 1994). Due to the various nutritional and psychological stressors encountered at weaning, many piglets experience a post weaning 'growth check' period (characterized by low intake of feed, reduced digestibility of dietary nutrients and the potential for the development of diarrhea), which may last as long as two weeks (Leibholz, 1981; Li, 1992). However, adaptation to a change in diet and environmental stressors encountered at weaning can be rapid; Aumaitre (1972) showed that adaptation to a change in diet took 5 days in young pigs which were changed from a milk to a starch diet. Therefore, the use of supplemental enzymes may only be justified during the immediate post-weaning period, before piglets have adapted to new diets (Dierick and Decuypere, 1994).

Targeting Specific Dietary Components

Monogastric animals do not possess the enzymatic capability to digest many NSP, including pentosans, cellulose, pectins, or the flatulence-producing oligosaccharides found in many legumes and oilseed meals (Campbell and Bedford, 1992; Dierick and Decuypere, 1994; Bedford, 1995). Pentosan and beta-glucan NSP are potential energy sources if they can be completely hydrolyzed to their monomeric sugars in the small intestine (Chesson, 1987; Campbell and Bedford, 1992; Newman, 1994). Some of these monomers (i.e., xylose and arabinose) may be almost completely absorbed from the small intestine; and some (eg., xylose and glucuronic acid) may be metabolized via the pentose phosphate and glucuronic acid pathway (Dierick and Decuypere, 1994; Salway, 1994), possibly contributing to the energy requirement of the animal.

Flatulence-producing oligosaccharides such as raffinose, stachyose and verbascose, are present in significant amounts in beans, peas, lentils, and

several oil-extracted meals, including soybean and rapeseed (Dierick and Decuypere, 1994). These oligosaccharides (alpha-galactosides) have been linked to decreased ileal digestibility of protein and starch in young piglets (Dierick and Decuypere, 1994), and contribute to the development of diarrhea, due to their osmotic activity which encourages the secretion of water into the intestinal lumen (de Groot, 1987; Saini, 1989; Gdala, 1998). Partial hydrolysis of these oligosaccharides is possible via the action of invertase (sucrase), which is negligable at birth but rises rapidly for the first few weeks of life through 2 years of age (Kidder and Manners, 1978). The supplementation of diets for young pigs with alpha-galactosidase enzymes may increase the digestion of these sugars in the small intestine, and improve the use of feed by young pigs.

Use of Supplemental Enzymes in Diets for Young Pigs

Most of the reported results of trials wherein exogenous enzymes were supplemented to diets for young pigs have been inconsistent, due to factors such as enzyme type and dose variation, differences in diet composition and age of animals used (Cowan *et al.*, 1996; Bedford and Schulze, 1998). Some investigators (eg., Inborr and Ogle, 1988) have reported reductions in post-weaning diarrhea which may be due to improvements in starch digestion in the upper small intestine and subsequent reductions in microbial proliferation in the distal ileum. Others (eg., Collier and Hardy, 1986) have reported improvements in weight gain and feed conversion efficiency. Improvements in nutrient digestibility are more likely to be found when poorly-digestible diets are fed compared to highly-digestible diets (Chesson, 1987). Inconsistencies in reported results could also be due to the presence or lack of other, contaminating enzyme activities (Chesson, 1987; Dierick, 1989), which may be rate limiting enzymes in the hydrolysis process (Dierick and Decuypere, 1994). Commercial enzyme products are usually mixtures or 'cocktails' exhibiting activity towards a range of substrates (Newman, 1994); distinguishing which enzyme activity is responsible for the observed effects can be difficult (Chesson, 1987) however, according to Rexen (1981) these crude preparations may be advantageous considering the synergy of action of many enzymes (Chesson, 1987).

The use of supplemental enzymes to reduce the effects of gel-forming NSP in poultry diets is well-documented (Newman, 1994), however there is relatively little information with regard to the effects of viscosity on digestion

in pigs, due in part to the problems encountered when viscosity is measured. Greater water intake means that the digesta of pigs is not as viscous as that of chickens, and low viscosity measurements often approach the detection limits of the machinery being used therefore enzyme effects on viscosity are hard to determine (Bedford and Schulze, 1998). Growing pigs are not usually affected by dietary NSP (beta-glucans or pentosans) (Campbell and Bedford, 1992), since their digesta has a relatively high water content than that of chickens; in addition, older pigs may have an endogenous beta-glucanase of microbial origin (Campbell and Bedford, 1992). Therefore, many investigators (eg., Lindemann *et al.*, 1986; Campbell and Bedford, 1992; Dierick and Decuypere, 1994; Bedford, 1995) suggest that the augmentation of the immature digestive capacity of early- or newly-weaned pigs may be the most applicable use of enzyme preparations.

E. Factors Affecting the Digestibility of Protein

Introduction

The nutritive value of a protein is the ability to provide a pattern of amino acids in the proper concentrations similar to body proteins (Kakade, 1974). Nutritive value is affected by protein digestibility, as well as by the quantity and quality of the protein in a feedstuff. The quantity and quality of protein is influenced mainly by environmental and genetic factors; however many other factors affect the digestibility of dietary proteins for monogastric animals. These include animal factors (eg., age, levels of endogenous secretions), protein source (composition and levels of various nutrients), and experimental factors (techniques used to collect samples for chemical analyses, and methodology used to determine protein digestibility values).

Protein Quantity

Monogastric animals have protein and amino acid requirements for body maintenance and growth processes, as outlined by NRC (1998). Below this requirement, tissue proteins are broken down to provide amino acids for the synthesis of more essential proteins required for maintenance (Kakade, 1974), such as neurotransmitters or digestive enzymes. However, when certain amino acids are in excess of the levels required for maintenance or

growth, this excess puts an additional burden on the liver and kidney, which must degrade the amino acids which cannot be stored (Kakade, 1974; Wang and Fuller, 1989). Furthermore, an excess of one amino acid may affect the utilization of other, structurally similar, amino acids by reducing their absorption from the intestine (Buraczewska, 1981).

Protein Quality

The quality of dietary protein, defined by the amino acid profiles of the different protein fractions present in the feedstuff, also affects nutritive value (Kakade, 1974; Wang and Fuller, 1989). Ideally, protein and amino acids should be provided in similar proportions to those required by the body (Kakade, 1974). However, plant protein sources are usually deficient in one or more amino acids, often lysine in cereals or sulphur-containing amino acids in legumes, due to the low levels of these amino acids in the main protein fractions of cereals (prolamins) and legumes (globulins) (Kakade, 1974). Genotype and environment affect the total and relative amounts of the different seed protein fractions, each of which has a unique amino acid composition (Kakade, 1974; Sauer and Ozimek, 1986). Variation in the amino acid content of the protein fractions of the feedstuff affects protein quality (Sauer and Ozimek, 1986), as some feedstuffs provide levels of CP and amino acids which more closely resemble the daily requirement of an animal. Variation in amino acid content is related to the total amino acid content of the feed; cereals have relatively low levels of amino acids compared to protein sources such as soybean meal or canola meal. Environmental factors also contribute to variation in amino acid content: growing conditions, fertilizer application, and genetic factors all influence the amino acid variation both within and among varieties of a feedstuff. Variation in total protein or amino acid content may occur due to differences in methodology used to determine these (Sauer *et al.*, 1990). Measurements of nitrogen content are often used to determine the amount of crude protein in feedstuffs, utilizing nitrogen-to-protein conversion factors specific for the protein source. Traditionally, proteins have been assumed to contain approximately 16% nitrogen; subsequently 6.25 is the most commonly used conversion factor (Tkachuk, 1969). However, this conversion factor is not appropriate for most protein sources of plant origin (Tkachuk, 1969); Bell (1984) states that the use of the appropriate nitrogen-to-protein conversion

factor is essential for the determination of protein quality and will affect the accuracy of protein digestibility values.

Digestibility

The digestion of dietary protein is a complex process involving both mechanical and chemical actions which release amino acids from protein (Alpers, 1987). Digestibility is usually defined as a rate measurement of nutrient hydrolysis by digestive enzymes (Sauer and Ozimek, 1986). Low (1980) defines apparent digestibility as a measure of an animal's capacity to secrete sufficient enzymes of the appropriate types to effect the hydrolysis in the presence of suitable mineral, electrolyte and pH conditions; the susceptibility of the nutrient's physical and chemical structure to enzymatic hydrolysis within the intestinal lumen; and a measure of the ability of the mucosal cells to remove the digestion products from the lumen.

Proteolytic hydrolysis results from the chemical actions of proteolytic enzymes (Low, 1980), therefore the levels of these enzymes will affect the degree of protein hydrolysis which can occur. Levels of digestive enzymes (in particular, pancreatic proteases and intestinal carbohydrases) are low in many young animals (Kidder and Manners, 1978; Li, 1992), and many authors have reported that the low digestibility of dietary nutrients in young pigs may be due to insufficient digestive capacity (eg., Bailey *et al.*, 1956; Corring *et al.*, 1978; Kidder and Manners, 1978). This is due in part to physical immaturity, but is also related to the composition of the diet: after a few weeks of age, young pigs adapt to different diets, in terms of digestive capacity (levels of particular digestive enzymes). It has been suggested that the digestive immaturity of the young pig is a function of the diet itself: given the stimulus of a plant-based diet, the young piglet would develop the appropriate digestive capacity (Kitts *et al.*, 1956). However, there is no doubt that the piglet is born with limited digestive capacity, and at least for the first few weeks, young pigs are not able to adapt their digestive function to a change in diet composition (Kidder *et al.*, 1968; Low 1980). Weaning, in particular, can adversely affect enzyme levels, possibly due to psychological stressors or changes in the nature of the environment or diet (Leibholz, 1981). Young pigs have been shown to adapt over time (with age) to diets based upon plant protein and carbohydrate sources; as their levels of specific digestive enzymes increase, their digestive capacity also increase (Leibholz, 1981; Makkink *et al.*,

1994). Makkink *et al.* (1994) found that soy protein concentrate was less well digested than milk protein. Digestion of milk protein is high in early-weaned piglets and does not increase whereas the digestion of vegetable protein sources is low post-weaning but increases with the age of the piglet. These authors found that pancreatic enzyme activities increased with age after weaning. The rate and extent of protein digestion and absorption are principal factors for the increase in performance of young pigs fed different protein sources.

Amino acid sequence and the chemical nature of amino acids in protein affect the digestibility of the molecule due to the specificity of digestive enzymes for certain peptide bonds (Sauer and Ozimek, 1986; Stryer, 1987). Trypsin preferentially hydrolyzes the arginyl and lysyl peptide bonds in a protein; therefore the content of these amino acids in protein determines the ability of trypsin to hydrolyze the amino acid chain. Furthermore, since arginyl-prolyl bonds are resistant to proteolytic attack by trypsin, high amounts of proline coupled with low amounts of arginine and lysine in many cereals, may contribute to the relatively low digestibility of many cereal proteins (Kakade, 1974; Sauer and Ozimek, 1986). Similarly, pepsin and chymotrypsin preferentially cleave the peptide bonds next to aromatic amino acids: Zebrowska (1973) found relatively high proportions of phenylalanine, tyrosine, and leucine in duodenal digesta which was attributed to the specific actions of pepsin. Therefore proteins containing low amounts of phenylalanine, tyrosine, or tryptophan will be less susceptible to hydrolysis by these enzymes (Sauer and Ozimek, 1986; Stryer, 1987).

The interactions of the different amino acids within the chain confers a unique tertiary structure on the protein molecule, which affects its solubility in gastric juice, and its susceptibility to hydrolysis by digestive enzymes (Sauer and Ozimek, 1986). Native proteins exist as highly organized molecules, folded into compact, three-dimensional structures stabilized by various hydrophobic, hydrogen and disulphide bonds between the amino acid residues of the protein chain (Stryer, 1987). Hydrophobic amino acid residues are grouped together in the interior of the molecule, while polar, hydrophilic residues are present on the exterior of the molecule, and act as proton donors or acceptors, solubilizing the protein molecule depending on the pH of the luminal environment (Stryer, 1987). The solubility of a protein in gastric juice varies depending upon the nature of exposed amino acid residues and

the conditions of the gastrointestinal lumen. Protein solubility affects the digestion of protein: the primary limiting step in digestion is the rate at which dietary proteins are dissolved and hydrolyzed (Asche *et al.*, 1989). These authors report that dried skim milk has a higher digestibility value than soybean meal because it is more soluble in gastric juice and can therefore be digested more proximally in the small intestine than soybean meal protein.

Physical hindrance of proteolytic enzymes to protein molecules or their component amino acids affects the digestibility of a protein. The tertiary structure of a protein causes some amino acid residues to be located inside the compact molecule, where they are physically inaccessible to digestive enzymes (Stryer, 1987). Physical hindrance of enzymes to protein occurs when protein is located within relatively indigestible fractions of a feedstuff, such as aleurone or other cellulosic supportive structures (Kakade, 1974; Sauer and Ozimek, 1986). The low digestibility of cell wall structures in monogastric animals reduces the digestibility of the protein contained within these, as contact between the protein molecules and digestive enzymes is limited (Sauer *et al.*, 1974). Sauer *et al.* (1974) found a low digestibility value for lysine, which is often the first limiting amino acid in many feedstuffs, which they attributed to the poor digestibility of the supportive structures within which it is often deposited. The more digestible amino acids such as glutamate are usually located in the highly digestible endosperm fractions of feedstuffs (Taverner *et al.*, 1981).

Amino Acid Absorption

Amino acids are absorbed via various transport mechanisms, including carrier-mediated mechanisms (active transport and facilitated diffusion), and passive diffusion (non-carrier- mediated), depending upon their molecular structure and electrochemical properties (Buraczewska, 1981; Webb, 1990). The capacity for nutrient absorption varies with location along the length of the small intestine: it is usually accepted that the ileum is the site of greatest absorption, as the optimal pH range for most intestinal proteases is greater than that found in the duodenum (Webb, 1990). Specific carriers exist for amino acids of similar structure (Buraczewska, 1981); however structurally dissimilar amino acids may share a transport system (Webb, 1990). The relative contribution made by each of these transport

mechanisms to overall nutrient absorption depends upon substrate concentration (Webb, 1990; Hopfer, 1987). Active transport may be quantitatively the most important mechanism when intraluminal concentrations of substrates are low; passive diffusion makes a relatively larger contribution when intraluminal concentrations are higher (Webb, 1990). The binding sites of carrier proteins can be saturated (Alpers, 1987; Webb, 1990) by high concentrations of substrate; therefore a limited rate of molecule transport exists which is specific to each transport mechanism (Rhoades and Pflanzer, 1989). Consequently, the apparent digestibility of individual amino acids may be affected by their differential rates of absorption by different transport mechanisms, determined by their concentrations within the gut lumen, as well as by the rates of digestion of the intact dietary molecule. Zebrowska (1973) found that the rate of absorption of amino acids was much slower than the rate of their release from the peptide chains. Li *et al.* (1993) found that increasing the dietary CP level resulted in lower ileal CP digestibility values, which could have been due to decreased absorption of amino acids when the transport capacity of the active transport carrier proteins was exceeded at higher intraluminal concentrations. In addition, active transport of monosaccharides and amino acids requires the co-transport of Na+ ; changes in the lumen environment, including Na+ 'flux', could alter the trans-enterocyte membrane gradients and potentials, subsequently altering nutrient transport (Alpers, 1987). In addition, competition for transport may exist among nutrients: arginine and leucine were found to actively inhibit the absorption of other amino acids (Buraczewska, 1981). Competition for transport is greater among those amino acids for which a protein carrier has a greater affinity (Webb, 1990); however some amino acids stimulate the transport of others: alanine, phenylalanine, leucine, and methionine were found to increase intestinal transport of basic amino acids; arginine, lysine, ornithine and possibly methionine were found to stimulate threonine absorption (Buraczewska, 1981). Peptides are absorbed more rapidly than free amino acids, by a different transport system (Vorotyntseva *et al.*, 1984; Webb, 1990) which requires energy to transport amino acids against concentration gradients independent of Na+ . Protons may be co-transported with peptides and this electrochemical proton gradient may be the driving force for the concentration of peptides against their concentration gradient (Webb, 1990). Absorption of peptides is affected by size

(only di- and tri-peptides can be transported), and amino acid composition (glutamate plus tyrosine was absorbed at nearly twice the rate of glutamate plus methionine, from the intestine of the rat; Webb, 1990). Peptides compete with each other for transport, and may also stimulate the absorption of some other peptides (Webb, 1990).

Apparent ileal amino acid digestibilities vary with the content of CP and amino acids in the diet (Fan *et al.*, 1994). Ileal amino acid digestibilities tend to increase with increasing CP content. Since the dietary amino acid content is dependent on the dietary CP content, and apparent ileal digestibilities of amino acids depend on the CP content of the diet, amino acid digestibilities may then also depend upon the amino acid content of the diet. Fan *et al.* (1994) investigated the effects of dietary amino acid content on amino acid digestibilities, and showed that increases in apparent ileal digestibilities of amino acids with increasing dietary CP content were greatest at the lower dietary CP (amino acid) levels. These increases in apparent ileal amino acid digestibilities were greatest at the lower CP (and amino acid) levels, but became negligable at the higher CP levels, because endogenous protein accounts for a smaller proportion of CP in digesta. Relatively small changes in levels of endogenous amino acids secreted into the intestinal lumen may elicit relatively large changes in apparent digestibility values, especially for the limiting amino acids, and/or for amino acids for which the endogenous level is high. Threonine and glycine are high in mucin and bile, respectively (Sauer and Ozimek, 1986). Therefore, apparent ileal amino acid digestibility values are only meaningful under standardized conditions: i.e., under conditions of similar dietary CP content, and therefore under conditions of similar dietary amino acid content, due to variations in the increases of apparent ileal amino acid digestibility values with variations in overall CP level (Fan *et al.*,1994). Increases in amino acid digestibility values became smaller at increasingly higher dietary CP levels, (to a threshold level) after which there were no further increases, and the apparent amino acid digestibility values became independent of dietary amino acid levels. However, not all of the amino acids reached their threshold levels for ileal digestibility values at the same dietary CP content; therefore Fan *et al.* (1994) suggest that the dietary amino acid contents affect their respective apparent ileal digestibility values, possibly independent of dietary CP level.

Endogenous Secretions

The amino acid content of endogenous secretions can affect apparent ileal amino acid digestibility values. Fan *et al.* (1994; 1995) found low apparent digestibility values for certain amino acids, due to the relatively high concentrations of these amino acids in endogenous secretions. Glycine is a major component of bile salt conjugates, accounting for more than 90% of the amino acids in bile juice (Li *et al.*, 1994; Fan *et al.*, 1995). While bile salt conjugates are hydrolyzed in the distal ileum by microbes and the major proportion (90%) is reabsorbed into the enterohepatic circulation, deconjugated glycine escapes reabsorption and enters the large intestine (Li *et al.*, 1994). Mucin protein is rich in threonine, serine and proline (Lien *et al.*, 1997); low ileal digestibility values for threonine may also result from its relatively low rate of absorption (Buraczewska, 1979).

Fat Content of the Diet

Several authors (eg., Imbeah and Sauer, 1991; Li and Sauer, 1994) have shown improvements in apparent ileal digestibility values of amino acids with increasing fat levels in the diet. Increased levels of fat may delay gastric emptying, which could result in a slower rate of passage of the diet in the small intestine, and higher amino acid digestibility values based on the assumption that time required for the digestion of protein and/or absorption of amino acids is a limiting factor. According to den Hartog *et al.*, (1989), dietary fat may obstruct the access of microorganisms in the large intestine to digesta, thereby negatively affecting the processes of fermentation and microbial proliferation. Less nitrogen may then be excreted in feces, resulting in improved nitrogen balance.

Fiber Content of the Diet

The effect of dietary fiber on nutrient digestibility depends upon the chemical nature of fiber, and its ability to interact with other nutrients in the intestinal lumen. Soluble fiber sources, such as pectin form viscous gels in the small intestine, altering passage rates of digesta, pH, viscosity of digesta and enzyme activities, water-holding capacity or the cation-exchange capacity of digesta (Dierick and Decuypere, 1994), and altering the microbial population of the intestine (Luckey, 1987), all of which may interfere with the processes of digestion and absorption (Mosenthin *et al.*, 1994; den Hartog *et*

al., 1989). Increased levels of fiber in the diet usually increase the passage rate of digesta (Ravindran *et al.*, 1984; den Hartog *et al.*, 1989), which provide less opportunity for enzymatic digestion in the small intestine, assuming that the time required for digestion is a limiting factor (den Hartog *et al.*, 1989; Imbeah and Sauer, 1991). Amino acid digestibilities are usually lower in diets containing canola meal compared to diets containing soybean meal, due to the higher amount of crude fiber (Sauer *et al.*, 1982; Fan *et al.*, 1995). However, increases in the viscosity of digesta due to high levels of gel-forming NSP may resist the peristaltic movements of the intestine (Annison and Choct, 1994); movement of dry matter may be delayed. While this may allow digestive enzymes more time to degrade dietary components (MacDonald, 1979), the presence of hydrophilic groups on NSP (Annison and Choct, 1994; Low, 1989) may stimulate the influx of water in order to maintain osmolarity in the lumen (Schutte, 1991). Monogastric animals lack many of the enzymes necessary to hydrolyze the specific types of glycosidic bonds joining the constituent monosaccharides of NSP (Leegwater, 1986), which are therefore often termed 'low digestibility carbohydrates' (Schutte, 1991; Dierick and Decuypere, 1994). Increased levels of fiber in the diet may decrease dry matter and energy digestibility, as fiber replaces more digestible ingredients (Sauer *et al.*, 1991). Poorly-digestible NSP remain largely intact in the small intestine (Dierick and Decuypere 1994; Aman and Graham, 1990), affecting digestion and absorption through increases in digesta viscosity. The 'bulking' effect of NSP stimulates gastric acid and pepsin secretions; pancreatic output is affected by the amounts of acid entering the intestine, and mechanical stimulation of the epithelium by coarse fiber particles may increase mucin secretion (Low, 1989), as protection. According to Low (1989), the delay in gastric emptying due to increased digesta viscosity may inhibit the release of gastric inhibitory peptide (GIP), a hormone which is part of a negative feedback mechanism for gastric secretions. Increases in gastric acid secretion stimulate the release of both intestinal and pancreatic secretions, ultimately increasing the amounts of nitrogen excreted in feces (Chesson, 1990). Mosenthin *et al.* (1994) found that pectin depressed apparent ileal and fecal protein and amino acid digestibilities, which they attributed to both an increase in endogenous protein secretions as well as a decrease in the efficiency of digestion. Increased levels of poorly-digestible fiber provide a source of energy for large intestine microbes, which use indigestible protein

and produce ammonia and other sources of nitrogen, which are excreted in feces, negatively affecting nitrogen balance of the animal (Jorgensen *et al.*, 1984). The increased passage rate of fibrous digesta helps to flush microbes through the upper small intestine; however the indigestible components of fiber provide substrates for microbes in the ileum (Luckey, 1987); therefore fiber encourages colonization of the distal small intestine by bacteria and other microbes. Here they may attach to the villi, increasing epithelial cell turnover, and could penetrate the mucosa (Luckey, 1987), entering the circulation and possibly causing systemic or localized infections.

ANF

Anti-nutritional factors (ANF) are feed components having biological activity, which can affect the digestion and/or absorption of dietary nutrients (Kakade, 1974). These components may be proteins such as protease inhibitors and lectins (hemagglutinins), phenolic compounds such as tannins or sinapic acid, cyanic compounds from glucosinolate hydrolysis, or NSP. Many ANF bind with dietary nutrients, forming indigestible complexes (Kakade, 1974; Mitaru *et al.*, 1984). ANF may also bind to digestive enzymes, reducing the amounts of free, active enzyme in the intestinal lumen, and subsequently the rate of digestion of dietary nutrients (eg., Fan *et al.*, 1995). Undigested proteins and the reduced activity of digestive enzymes may stimulate pancreatic hypersecretion of pancreatic enzymes via a cholecystokinin (CCK)-secretin feedback pathway, further decreasing protein and amino acid digestibility due to increased losses of endogenous amino acids secreted in to the gut lumen (Kakade, 1974; Fan *et al.*, 1995). Some ANF can affect the integrity of the mucosal cells, binding to the enterocytes and altering absorptive capacity (Kakade, 1974).

Supplemental Amino Acids

Synthetic amino acids are often added to diets in order to improve the protein value, by increasing the levels of limiting amino acids. These amino acids are almost completely absorbed, usually at a faster rate than protein-bound amino acids (den Hartog *et al.*, 1989; Leibholz, 1989). The frequency of feeding affects the utilization of free amino acids; more frequent feeding allows a more balanced amino acid profile to be absorbed, which can be more efficiently used for protein synthesis (den Hartog *et al.*, 1989; Leibholz, 1989).

Methods for Measuring Amino Acid Digestibilities

The method used for determining amino acid digestibilities can affect the accuracy of the digestibility values. The fecal analysis method (Kuiken and Lyman, 1948) was used extensively to determine amino acid digestibilities, by measuring the percent difference between the amount of amino acid in feed, and in feces. Studies by Zebrowska (1973) revealed that amino acids entering the large intestine may be hydrolyzed and absorbed, however the benefit to the host animal is minimal. Proteins may be degraded in the large intestine however the products do not contribute to the protein status of the animal (Sauer and Ozimek, 1986), as the absorbed material is rapidly excreted in urine. The modifying effect of the large intestine microflora on nutrients such as amino acids entering the large intestine may alter the concentrations of the different amino acids in feces (Sauer *et al.*, 1974). In the large intestine, protein after fermentation may be absorbed in the form of ammonia, amides and amines, or excreted in feces. Absorbed nitrogen is excreted via the urea cycle. Microbial protein makes up a large proportion of fecal nitrogen: 62 to 76% (Mason, 1984). The estimation of the contribution of endogenous amino acids to amino acids derived from the experimental diet in feces is also a major problem inherent in the fecal analysis method (Sauer *et al.*, 1974), and often confounds the fecal digestibility coefficients for amino acids.

The relatively faster flow of digesta through the small intestine limits the proliferation of microorganisms (Luckey, 1987), and thus microbial activity. However, under certain conditions, microbial populations in the small intestine can reach appreciable levels in the distal ileum (Bach Knudsen *et al.*, 1991; Millard and Chesson, 1984). According to den Hartog *et al.* (1989), increased levels of fiber in the diet may result in a decreased rate of passage in the small intestine, possibly due to delayed gastric emptying. Higher levels of dietary fiber also result in an increased rate of passage of digesta in the large intestine, which may result from increases in gut fill, water-holding capacity, as well as increased fermentation. According to Gohl and Gohl (1977), certain types of fiber which have been shown to decrease the rates of passage of digesta have the potential to stabilize the intestinal flora. Decreased flow of digesta in the small intestine, along with increased microbial proliferation in the large intestine, could encourage a greater degree of small intestinal colonization, possibly due to backflow of digesta through

the ileo-cecal valve into the distal ileum. Fermentation producing organic acids and ammonia may confound apparent digestibility measurements by modifying dietary nutrients before they can be hydrolyzed by endogenous enzymes (Low, 1980). Millard and Chesson (1984) found losses of free sugars, and some alteration of cell wall material of the diet anterior to the ileo-cecal junction, which they attributed to the action of the microflora in the small intestine. While some degree of fermentation occurs in the ileum (hemicellulose components; Millard and Chesson, 1984) the degree of microbial proteolysis is questionable (den Hartog *et al.*, 1989). Therefore, measurements of nutrient content in ileal digesta (in particular the content of amino acids) are usually not confounded by the modifying effect of ileal microflora. However, amino acid digestibilities measured with the fecal analysis method are often higher than those measured with the ileal analysis method (eg., Sauer and Thacker, 1986; Imbeah and Sauer, 1991; Li and Sauer, 1994; Fan *et al.*, 1995), due to microbial activity in the large intestine. In addition, Sauer *et al.* (1981) found that the ileal analysis method was able to discriminate between samples of barley differing in lysine digestibility ($P<0.05$), whereas the fecal analysis method did not reveal these differences. The digestibility values obtained with the ileal analysis method are a more accurate indication of the digestive capacity of the animal. For this reason the ileal analysis method is superior to the fecal analysis method for determining digestibility values for protein and amino acids (eg., Jorgensen *et al.*, 1984; Lin *et al.*, 1987; Fan *et al.*, 1995).

Methods for Collecting Ileal Digesta

Collection of ileal digesta requires that animals be slaughtered, or surgically modified. The slaughter method is the simplest, and ileal digestibility values determined with this method are comparable to ileal digestibility values determined with other collection techniques involving cannulation procedures (Moughan and Smith, 1987). However, replicate samples cannot be collected from one animal, which may allow individual animal variation to affect the results; and amounts of digesta available may be small, necessitating pooling of samples which would provide no information about individual variation. The procedure can be expensive due to the number of animals required (Kohler, 1992). Shedding of epithelial cells into the lumen after the animals are euthanized may alter the content of

nitrogenous compounds (Low, 1980); in order to avoid this, digesta must be collected while the animal is under anaesthetia (Kohler, 1992).

Surgical modifications allow for repeated collections from the same animal. One of the most commonly used procedures is the use of a simple T-cannula inserted into the distal ileum, approximately 5 to 10 cm from the ileo-cecal valve (Kohler, 1992). Recovery is usually rapid, and animals appear normal thereafter (Livingstone and McWilliam, 1985). Post-valve T-cecum cannulation (PVTC), developed by van Leeuwen *et al.* (1988) is a modification of the simple-T method which involves placement of one large T-cannula in the cecum. The majority of the cecum is removed, and the ileocecal valve protrudes into the cannula barrel when the cannula is open during sample collections; when closed, the ileocecal valve is redirected into the large intestine. Apparent digestibilities determined with the PVTC method are comparable to those obtained using pigs with simple-T cannulae (Kohler, 1992). However, according to Livingstone and McWilliam (1985), surgical procedures in which rigid materials are inserted into the lumen, causing the normally motile gut to adhere to the body wall (at the location of the cannula), inevitably will interfere with intestinal contractions and obstruct digesta flow, which could reduce absorption and secretion, particularly in the region of the cannula. Detrimental effects on growth and feed conversion efficiency have been reported: 7 and 10% reduction respectively (Livingstone and McWilliam, 1985). This could be consistent with heat loss increases and decreased energy retention or abnormalities in absorption, particularly from the distal ileum due to effects of cannulation on gut function. Presence of the cannula could also increase microbial proliferation (Livingstone and McWilliam, 1985). The frequency and duration of sampling in relation to the time and frequency of feeding is also a concern (Low, 1980; Graham and Aman, 1986; Sauer and Ozimek, 1986), as both the flow and composition of digesta will vary with time after feeding. The need to restrict animal movement in order to minimize damage to the animal or the cannula may also affect normal gastrointestinal motility (Livingstone *et al.*, 1980; Low, 1980; Graham and Aman, 1986). In addition, total digesta collection is not possible, since only part of the intestinal contents can be withdrawn from the cannula; digestibility markers must therefore be used. These have certain drawbacks: some markers may separate among the solid and liquid fractions of digesta (Uden *et al.*, 1980; Graham and Aman, 1986; Asche *et al.*, 1989). Marker

separation may be aggravated by the restrictions on animal movement (Livingstone *et al.*, 1980). Marker recovery is not always complete: Mroz *et al.* (1996) found that the recovery of chromic oxide was only 82 to 85%, which could be due to day-to-day variation in gastric emptying, and fluctuations in flow velocity resulting from migrating myoelectric complex variation along the intestine. Chromic oxide may sediment in the gastrointestinal tract, to varying degrees among different animals; some may remain in the gastric gland region (fundus) of the stomach, and in the crypt regions of the intestinal epithelium (Mroz *et al.*, 1996).

In order to collect digesta quantitatively, re-entrant cannulation techniques have been developed, in which two cannulae are inserted in tandem into the distal ileum (ileo-ileo re-entrant), or into the ileum and cecum (ileo-cecal re-entrant), with transsection of the intestine between the cannulae (Kohler, 1992). When digesta is not being sampled, it flows exteriorly through the joined cannulae, bypassing the transsected area. While these techniques allow for total collection of digesta, concern exists regarding the effect of transsection of the intestine on normal neuromuscular function of the intestine (Kohler, 1992). Digesta flow may be altered as a result of the interruption of the normal migrating myo-electric complex (Low, 1980). Other problems relate to blockage of digesta in the cannulae, which is related to diet composition (crude fiber content in particular), viscosity of digesta, the amount of digesta passing through the cannulae, and the mesh size of the screen through which the diet was ground (Sauer and Ozimek, 1986; Kohler, 1992).

Both simple-T and re-entrant cannulation techniques directly disturb the small intestine, although cannulation does not seem to affect the rate of passage of digesta (Sauer and Ozimek, 1986). According to Mroz *et al.* (1996), the placement of one cannula disturbs the normal intraluminal pressure and the migrating myoelectric complex to a lesser extent compared to the insertion of two cannulae. There is no need for manipulations with collection and re-introduction of digesta, which might affect mechanisms controlling gastric emptying and intestinal peristalsis; there is less need to restrain pigs during collection. There are usually no differences between ileal digestibilities determined in pigs using simple-T or re-entrant cannulation (Sauer and Ozimek, 1986), although fecal digestibilities may be higher in cannulated than in intact pigs (Sauer and Ozimek, 1986; Kohler *et al.*, 1990).

Influence of Feed Intake

In order to minimize problems with blockage of re-entrant cannulae, pigs are restricted in terms of feed intake. This is not the normal situation, as growing pigs are normally fed *ad libitum*. However, feed intake levels (*ad libitum* or restricted) do not significantly affect ileal nutrient digestibilities when these are determined with cannulated pigs, although there may be a small increase in fecal digestibilities with decreasing feed intake (Sauer and Ozimek, 1986).

Influence of Dietary Protein Level

Apparent protein and amino acid digestibility values are influenced by the protein content of the diet (den Hartog *et al.*, 1989; Li *et al.*, 1993), increasing with increased protein content of the diet. Increases in apparent digestibility values are greatest at lower protein levels because endogenous protein excretion constitutes a higher proportion of total protein excretion at lower dietary protein levels (Sauer *et al.*, 1989). Similarly, ileal amino acid digestibilities can be affected by the amino acid content of the diet, in particular the digestibilities of the limiting amino acids (eg, sulphur-containing amino acids in legumes, or lysine in many cereals), or the amino acids which are high in endogenous secretions (eg, threonine and glycine; Li *et al.*, 1993) At low dietary CP levels (low amino acid levels) the amino acids in endogenous secretions constitute a relatively greater proportion of the amino acids in ileal digesta.

Direct vs Difference Method for Determining Digestibility Values

According to Sauer *et al.*, (1990), variations in digestibility values among different samples of the same feedstuff could be due in part to differences in methodology for determining digestibility values. Amino acid digestibility values in feedstuffs can be determined directly, wherein the assay feedstuff provides the sole source of amino acids, requiring the formulation of only one diet to be assayed; amino acid digestibility values of feedstuffs with high crude protein content are usually determined with this method. Fan *et al.* (1994) showed a quadratic relationship between dietary amino acid levels and apparent ileal amino acid digestibilities: digestibility values initially increased with increasing dietary CP (amino acid) levels, but reached a plateau after which there were no further increases and apparent amino

acid digestibility values became independent of dietary amino acid levels. However, the direct method is not suitable for feedstuffs with a low CP (and amino acid) content, because of the relatively greater contribution of endogenous amino acids at low levels of dietary amino acid intake (Fan and Sauer, 1995). The difference method is more suitable for determining nutrient digestibilities in feedstuffs of lower CP content, or in feedstuffs which contain high levels of ANF or crude fiber (Sauer *et al.*, 1989). This method involves the formulation of both an assay and a basal diet; the assay diet contains a mixture of both the basal and assay feedstuffs, and digestibilities are calculated by difference (Sauer *et al.*, 1989). This method is accurate only if there are no interactions between the digestibility values of nutrients in the basal and assay feedstuffs (Fan and Sauer, 1995).

Effect of Chemical Analysis Procedures

Procedures used to prepare samples for chemical analysis, such as freeze-drying or oven drying, may affect the digestibility values. Jorgensen *et al.*, (1984) lost more than 5% of the nitrogen content of feces due to oven- or freeze-drying, compared to analyses performed on wet samples. Piccaglia and Galletti (1987) found that oven-drying of samples prior to fiber analysis could confound results due to the condensation of lignin and carbohydrate during oven-drying which overestimates neutral detergent fiber (NDF) values.

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2. Enzyme Supplementation to Diets Containing Peas Fed to Young Pigs

A. Introduction

Peas are an uncommon ingredient in diets for young pigs, due to concerns regarding ANF, palatability, limiting amino acid content and the variability of CP, all of which have long been considered factors contributing to low feed intake and decreased protein and amino acid digestibility, compared to traditionally formulated starter diets (Grosjean and Gatel, 1986; Savage and Deo, 1989; Gatel and Grosjean, 1990). Consequently, the use of peas in diets for piglets has been limited to 15% of the diet or less, without the supplementation of limiting amino acids (Grosjean and Gatel, 1986; Sauer *et al.*, 1990). However, increased production of spring-seeded, white-flowered cultivars, which contain fewer ANF (Leterme *et al.*, 1990; Leterme *et al.*, 1992; Jaikaran *et al.*, 1995) than many European, winter-seeded, or dark-flowered varieties, and are usually more palatable (Kehoe *et al.*, 1995), may allow peas to be included in diets for weaned pigs at higher levels while avoiding the negative effects on digestibility and performance that have been seen previously.

The immaturity of the piglet's digestive tract has been thoroughly investigated by many researchers (eg., Aumaitre, 1972; Aumaitre and Corring, 1978; Kidder and Manners, 1972; 1978; Corring *et al.*, 1978). The young piglet is born with limited digestive capacity; most digestive enzymes are produced at very low, or negligible levels at birth, and do not approach adult levels until approximately 8 weeks of age (Kidder and Manners, 1972; 1978). The newly weaned piglet (at approximately 3 to 4 weeks of age in Western Canada) is therefore inadequately equipped for the digestion of proteins and carbohydrates from the feed ingredients of plant origin (such as oilseeds, legumes, and cereal grains) which are commonly used in swine diets. In addition, pigs lack certain enzymes, such as alpha-galactosidase, necessary for the digestion of some oligosaccharides (alpha-galactosides) found in legumes such as peas (Fan *et al.*, 1994). While partial degradation of these oligosaccharides is possible via the action of intestinal invertase (sucrase; Kidder and Manners, 1978), the production of this enzyme is low at birth, therefore the newly weaned piglet is incapable of significant alpha-galactoside

degradation and so remains susceptible to their anti-nutritive effects. According to Dierick and Decuypere (1994), and Chesson (1987) supplementation of swine diets with enzymes of microbial origin could augment existing digestive capacity by increasing the volume or activity of endogenous enzymes, or by providing enzyme activity which is negligible. Enzymes could also be added in order to specifically target feed components, such as legume oligosaccharides or NSP, reducing or eliminating their anti-nutritive effects (Chesson, 1987; Dierick and Decuypere, 1994).

The majority of pea carbohydrate exisits as polysaccharides (both starch and NSP) (Fleming, 1981; Sauer *et al.*, 1990). NSP make up a large proportion of the total polysaccharides, along with the structural complexes formed between NSP and lignin (an alcohol polymer) (Gdala, 1998). The average NSP content in peas is 18.5% (160 to 201 g/kg, DM basis); major NSP components are cellulose, pectic substances and hemicellulose, as indicated by the major residues: glucose, uronic acids and xylose, respectively (Gdala, 1998). Water soluble NSP are known to cause problems in poultry due to increased digesta viscosity which may lead to decreased feed intake, increased microbial proliferation, decreased intestinal pH and decreased nutrient digestibility and/or absorption, due to reduced diffusion of digestive enzymes, their substrates and the products of digestion (Burnett 1966; Bedford *et al.*, 1991; Bedford and Classen, 1992; Campbell and Bedford, 1992; Bedford, 1995; Bedford and Schulze, 1998). In addition, morphological and structural modifications of the digestive tract have been attributed to increased digesta viscosity, including increased small intestinal and cecum weights, and increased pancreatic secretion (Simon, 1998). Pigs, which tend to consume more water than poultry, have not been as severely affected by increases in digesta viscosity; however some investigators (eg., Cowan *et al.*, 1996; Bedford and Schulze 1998; Simon, 1998) have suggested that vegetable protein NSP may still adversely affect nutrient digestibility in pigs, especially at higher inclusion levels in the diet which may result in significant increases in intestinal viscosity. In addition, increased viscosity in the stomach may impair the normal 'sieving' action of the pyloric sphincter, allowing large particles to exit into the small intestine where their degradation may be less complete than that of smaller particles (Bedford and Schulze, 1998). Finally, the possible encapsulation of dietary nutrients by soluble NSP, or the 'locking in' of nutrients by insoluble NSP (cage effect), may remain an obstacle to

efficient nutrient digestion by the pig (Cowan *et al.*, 1996; Simon 1998). Enzyme supplementation to vegetable protein diets may therefore contribute to improvements in nutrient digestibility through: 1. reduced viscosity of digesta which would encourage reduced transit time and allow increased feed intake; 2. dispersion of gels formed by vegetable protein NSP, which would free up dietary nutrients and make them available for attack by the endogenous digestive enzymes; and 3. partial degradation of insoluble NSP of cell walls, releasing nutrients which may be 'locked in' (cage effect) by the cell wall NSP (Bedford and Schulze, 1998; Simon, 1998). There is a scarcity of information on the effect of supplemental enzymes on the nutrient digestibility of diets containing peas fed to young pigs. Cowan *et al.* (1996) reported an improvement in energy digestibility in peas fed to piglets, and implied that the improvement seen could have been due to a 'nutrient-freeing' effect of the enzyme preparation on encapsulated nutrients, as described by Carre *et al.* (1992). The majority of reports on enzyme addition to piglet diets have involved the use of pentosanases or beta-glucanases, meant to target cereal components such as the xylans, beta-glucans or arabinoxylans of cereal hemicellulose (Inborr and Ogle, 1988; Bedford *et al.*, 1992; Inborr *et al.*, 1993; Li *et al.*, 1996). Hydrolysis of the starch or cellulose component of cereals by supplemental enzymes has also been investigated (eg., Cunningham and Brisson 1957a; Calder *et al.*, 1959; Officer, 1995); and there are a number of reports on the efficacy of proteolytic enzymes in diets containing soybean meal or other commonly used, non-legume protein sources (eg., Lewis *et al.*, 1955; Cunningham and Brisson 1957b; Calder *et al.*, 1959; Inborr and Ogle, 1988). Unfortunately, many reports do not specifically characterize the enzyme preparations used, or refer to them by a commercial name (eg., Inborr and Graham, 1991); and fewer reports indicate the activities, or optimum conditions (i.e., pH), of the enzymes in the preparations used (Dierick and Decuypere, 1994). This could be due to limitations of the methods used to extract, or purify, the enzymes; the preparations used are usually 'crude', having various types and levels of activity although the preparation may be referred to by a single enzyme name (Chesson 1987; Dierick 1989; Campbell and Bedford, 1992). As a result, the activity responsible for any effects which may be seen can often not be confirmed. While the use of such 'crude', multi-activity preparations may be useful with respect to the synergism which is often an important factor in the degradation

of complex nutrients such as dietary fiber (Chesson 1987), these mixtures may not necessarily allow the specific targeting of particular dietary components, and may even be detrimental: Officer (1995) suggested that a certain decrease in feed intake post-weaning was normal, and even necessary to allow the piglet to maintain a certain level of efficiency in nutrient digestibility; diarrhea may result from overloading the piglet's immature absorptive capacity with products of exogenous/supplemental enzyme action. However, several authors (eg., Chesson, 1987; Dierick and Decuypere, 1994) suggest that the use of crude, commercial enzyme preparations, which may have several unidentified enzyme activities, may allow for the highest possible improvements in digestibility via degradation of the complex polysaccharides (i.e., NSP) in many feed ingredients.

The objectives of this study were to investigate the effects of supplemental enzymes on the digestibility of CP, gross energy, fiber, amino acids and starch in diets containing peas fed to young pigs.

B. Materials and Methods

Animal Management

Eight piglets (PIC), weaned at 18 to 21 d of age with an average BW of 6.5 kg, were obtained from the University of Alberta R.D. Sinclair Swine Research Unit. They were housed individually in metabolic crates (85 cm high X 70 cm long X 65 cm wide), in a temperature-controlled (25 to 28ºC) barn. A cereal-based starter diet, formulated to supply digestible energy and nutrients according to the recommendations of the National Research Council (1988) for starter pigs, was provided *ad libitum*. Water was freely available from low-pressure drinking nipples.

Each pig was fitted with a simple T-cannula at the distal ileum 6 to 7 d after weaning. One pig was euthanized during surgery due to complications. All of the pigs recovered from anaesthesia within 2 to 3 h after surgery, and recovered well from the surgical procedure. After a 6 to 7 d recuperation period, the pigs were divided according to body weight into two groups, and fed one of two dietary treatments according to a crossover experimental design. The diets were fed at a rate of 5% of the average BW per d, which was determined immediately prior to the start of each experimental period. The

daily meal allowance was offered in three meals of equal amounts: at 0800, 1600 and 2400 h. All pigs usually consumed their meal allowance within 1 to 2 h. Each experimental period lasted 8 d (6 d adaptation to diet followed by a 48 h collection period). Collection of feces was performed for a total of 24 h: from 0800h on d 6 to 0800 h on d 7 of each experimental period. Collection of ileal digesta was then performed from 0800 to 1600 h on d 7, from 0000 to 0800 h and from 1600 to 2400 h on d 8 of each experimental period, for a total of 24 h. The average BW of the pigs was 9.5 kg and 12.4 kg at the beginning of the first and second experimental period, respectively. The average BW was 15.0 kg at the end of the second experimental period. At the conclusion of the experiment, the pigs were euthanized by CO_2 inhalation and lethal injection (T61[1], 5 cc/9 kg intravenously). Dissections were performed to ensure that cannulation had not caused intestinal abnormalities.

The experimental protocol was approved as submitted by the Animal Care Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta. The piglets used in this experiment were cared for in accordance with the guidelines established by the CCAC (1980).

Preparation of Cannulae

Cannulae were prepared according to procedures adapted from Sauer (1976). A finely polished 'T' shaped stainless steel mould was heated in a muffle furnace at 200 to 250°C for 15 to 30 minutes. Thereafter, it was removed from the furnace and immersed in a solution of Plastisol[2] for 3 to 5 minutes (until the Plastisol layer on the barrel reached a thickness of approximately 3 mm). The mould covered with Plastisol was then re-heated in the furnace until the color of the Plastisol changed from white to a dark yellowish color; it was removed and cooled in a container of cold water until the stainless steel mould was cool enough to touch. The cannula was then slit along the length of the flange and peeled off of the mould. The cannulae were soaked in ethanol until they reached the desired hardness (usually several hours). Retaining collars were fashioned by heating and then immersing a glass bottle stopper of the appropriate size into the Plastisol,

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[1] Tetracaine hydrochloride. Hoechst Roussel Canada Ltd., 4045 Cote Verta, Montreal, Quebec.

[2] Auburn Plastics Engineering, Chicago, Illinios 60609.

following the same procedure as described for preparing the cannulae. Plugs for each cannula were made by pouring the Plastisol into a piece (approximately 10 cm) of steel pipe, with an internal diameter of 1 to 2 mm smaller than the internal diameter of the cannula, and a large steel nut fitted onto one end. The hardened cannulae, collars and plugs were trimmed and sanded with an electric sander.

Surgical Procedures and Pre- and Post-Operative Care

The piglets were fasted for 12 h prior to surgery. Each animal was brought under general anaesthesia using a gas mixture of Halothane[3] and oxygen, and placed on its left side. The flank area of the right side of the body (from approximately the 3rd or 4th last rib caudally) was shaved with an electric clipper, cleaned thoroughly with Betadine[4], and draped, leaving the surgical area exposed. An excision of approximately 7 cm in length was made through the skin and subcutaneous fat, parallel and approximately 3 cm caudal to the last rib. Underlying muscle layers were separated by blunt dissection; the peritoneum was carefully lifted away from the underlying intestines and cut along the original incision line. The distal ileum was identified by location of the ileocecal sphincter. Prior to making the incision in the ileum, catgut (2-0)[5] was positioned as a purse-string suture line, through the serosal layer of the intestine, approximately 5 cm cranial to the ileocecal junction. A scalpel blade was used to make the incision inside the suture outline. The flanges of the cannula were inserted into the incision and the suture was tied. Approximately 2 mm below this suture line, a second purse-string suture was positioned around the base to further secure the cannula. Extruded mucosa was carefully trimmed with a scalpel.

A fistula was created between the last two ribs by excising a circular piece of skin and penetrating the muscle layers using finger manipulation and a pair of forceps. The peritoneum was carefully pushed up through the fistula and cut, completing the fistula. The cannula barrel was then pulled through the fistula until its base and flanges (which remained inside the

[3] Fluothane; Ayerst Laboratories, Saint-Laurent, Quebec.

[4] 7.5% Povidone-iodine. Purdue Frederick Inc., Toronto, Ontario.

[5] Chromic absorbable suture. DAVIV + GECK Cynamid Canada Inc., Baie d'Urfe, Quebec.

ileum) made contact with the inside of the body wall; parts of the small intestine were prevented from becoming lodged in between the body wall and the flanges of the cannula. The retaining collar was fixed over the barrel and as close as possible to the skin using a plastic locking tie. Terramycin[6] (0.5 mL) was administered into the abdominal cavity before the incision was closed.

The pigs were returned to their metabolic crates following surgery and fasted for approximately 8 to 12 h; at the first regular mealtime (08:00, 16:00 or 00:00 h) after this fasting period limited amounts (25 to 30g) of the starter diet were provided and this was gradually increased over the next 2 to 3 d according to each pig's appetite, until the pigs consumed the starter diet at a rate of 5% of the average body weight. During the recuperation period, the crate temperature was maintained at 30 to 32°C with the use of infrared heat lamps suspended over each crate. The stitches were removed 7 to 10 d after surgery. From the second week after surgery onwards, the area around the cannula of each piglet was washed two to three times daily with warm water, and Ihle's paste[7] was applied around the retaining collar in order to prevent skin irritation.

Experimental Diets

Each of the two experimental diets were formulated to contain 18% CP, digestible energy, vitamins and minerals according to NRC (1988) recommendations, on an as-fed basis (Table 2-1). D,L-methionine was included where necessary to fulfill the respective NRC (1988) requirements. The enzyme mixture supplemented to the PWE diet was provided by Finnfeeds International[8], and contained 6,250 units/g of xylanase from *Trichoderma longibrachiatum*, and 3,000 units/g of protease from *Bacillus subtilis*. The peas used in the peas plus wheat (PW) and peas plus wheat plus supplemental enzymes (PWE) diets were 'Integra' variety (smooth, yellow), obtained from Westlock County, Alberta, from the 1996 crop. Wheat was obtained from the University of Alberta Feed Mill. Chromic oxide was

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[6] Oxytetracycline Hydrochloride. Dominion Veterinary Laboratories Ltd., Winnipeg, Manitoba.

[7] 25% Zinc Oxide. Valmo Laboratories, Trois-Rivieres, Quebec.

[8] Box 777, Marlborough, Wiltshire, United Kingdom SN8 1XN.

included as a digestibility marker. The peas were cleaned of debris and weeds prior to use; both the peas and wheat were ground through a 2 mm screen prior to diet preparation.

Experimental Design

The crossover design is used when expense and complexity demand that the experiment be as short as possible; and the changeover principle removes variation among animals (Gill and Magee, 1976). This design is therefore appropriate for studies with early- weaned pigs because of the possibility of effects resulting from gastrointestinal development. Sources of potential variation were groups of pigs, pigs within groups, dietary treatment and experimental period.

Procedures for Collection of Feces and Ileal Digesta

Collection of feces was performed for 24 h, from 0800 h on d 6 to 0800 h on d 7 of each experimental period. Feces were collected into plastic containers, and immediately frozen at -20°C until chemical analyses were performed. Feces which became contaminated with feed or urine were not collected.

Collection of ileal digesta was performed for 24 h, from 0800 to 1600 h on d 7, from 0000 to 0800 h and from 1600 to 2400 h on d 8 of each experimental period. At the beginning of each digesta collection, the cannula plugs were removed, and clear, soft plastic bags (approximately 12 cm long by 2 cm internal diameter) were attached to the cannula barrels using pieces of thin wire twisted around the barrel. Formic acid (5 mL, 10% v/v) was added to the bags prior to attachment to the barrel, to minimize bacterial activity. Bags were changed as required (when they were approximately half full); contents were immediately frozen at -20°C until chemical analyses were performed. The first bag collected from each pig was discarded as it usually contained digesta which may have been present in the barrel of the cannula for some time.

Fecal and digesta samples were pooled within pig, diet and period prior to chemical analyses.

Chemical Analyses, Calculations and Statistical Analyses

Samples were obtained from each diet, each time the pigs were fed. These samples were pooled within dietary treatment prior to chemical analyses. Digesta and fecal samples were freeze-dried, and ground as finely as possible using a countertop coffee mill (household type). Samples of each diet were ground as finely as possible using the coffee mill previously described. All samples were thoroughly mixed before subsamples were taken for analyses, and all chemical analyses were performed in duplicate.

Analyses for dry matter and ash were determined according to AOAC (1997) methods; chromic oxide was determined according to the procedure of Fenton and Fenton (1979); crude protein (% nitrogen X 6.25) analysis was performed using a LECO® model FP-428 Nitrogen Determinator (LECO® Corporation, St. Joseph, Michigan). Gross energy content was determined with a LECO bomb calorimeter. Analyses for acid-detergent fiber (ADF) and neutral detergent fiber (NDF) were carried out according to the principles outlined by Goering and van Soest (1970), and analyses for amino acids (except methionine, cysteine, tryptophan and proline) were performed according to procedures described by Sedgwick *et al.* (1991).

Apparent ileal (or fecal) digestibility values can be defined as the difference between the amount of nutrient in the diet, and in ileal digesta (or feces), divided by the amount in the diet. The apparent ileal and fecal digestibility values of dry matter, crude protein, gross energy, starch, acid- and neutral-detergent fiber, hemicellulose, cellulose and amino acids were calculated according to the following formula:

$$D_D = 100\% - [(I_D \times A_F) / (A_D \times I_F)] \times 100\%$$

where D_D is the apparent digestibility of a nutrient in the assay diet (%), I_D is the marker concentration in the assay diet (%), A_F is the nutrient concentration in ileal digesta or feces (%), A_D is the nutrient concentration in the assay diet (%), and I_F is the marker concentration in ileal digesta or feces (%).

Data were subjected to statistical analyses using the General Linear Model procedure of SAS (1988). Treatment means were compared using the Student-Newman-Keuls' multiple range test procedure.

C. Results and Discussion

All of the pigs remained healthy throughout the course of the experiment. They usually finished their meal allowances within 1 to 2 h of feeding. Postmortem dissections performed at the conclusion of the second experimental period revealed no intestinal abnormalities.

The apparent ileal and fecal digestibility coefficients of dry matter, crude protein, gross energy, starch, fiber and amino acids in the experimental diets containing peas are presented in Tables 2-3 and 2-4, respectively. There were no differences ($P>0.05$) of dietary enzyme supplementation on ileal or fecal digestibility coefficients of any dietary nutrient. Of all the amino acids, the apparent ileal digestibility values for arginine and lysine are among the highest, and the apparent ileal digestibility values for glycine and threonine are among the lowest. All of the apparent fecal CP and amino acid digestibility coefficients were higher than their corresponding apparent ileal digestibility values, illustrating the modifying effect of the microflora in the large intestine, on the digestibility values of CP and amino acids. This further substantiates the ileal analysis method as a more sensitive, superior method for determining digestibilities of CP and amino acids.

The stress associated with abrupt changes in diet and environment at weaning is thought to be responsible for many of the symptoms of the post-weaning growth check often seen in pigs (Li, 1992). These symptoms include a loss of appetite (low feed intake), diarrhea, loss of body weight and condition, and subsequent delayed growth which may lead to production losses (Morkeberg *et al.*, 1992). The physical change in environment (separation from the sow, relocation to a new pen) is stressful, however the abrupt change in diet can cause drastic morphological changes to occur in the intestinal epithelium (Leibholz, 1981), and subsequently alterations in absorption (Hampson, 1986; van Beers-Schreurs *et al.*, 1998).

However, according to Dierick and Decuypere (1994), recovery of the piglets from the initial psychological and nutritional shocks of weaning, and their subsequent adaptation to a change in diet is rapid, therefore the benefits of enzyme supplementation will be short lived (i.e., 2 weeks). The addition of supplementary enzymes to diets for early weaned pigs may only be beneficial if the enzymes supplemented can compensate for the low levels or activities of endogenous enzymes in young pigs, or during the transition period

immediately post weaning, when the diet is changed from sow's milk to a solid nursery (starter) diet, before the piglet's gastrointestinal tract has been able to adapt in terms of digestive enzyme biosynthesis and secretion, gastrointestinal motility, and other aspects of intestinal function (Corring, 1980; Dierick and Decuypere, 1994). According to Corring (1980), and Aumaitre (1972), the process of adaptation to a change in diet takes from 5 to 7 days. The pigs used in this experiment were allowed to adapt to the experimental diets for 5 to 7 days before ileal digesta was collected.

The piglets used in this experiment were 5 weeks old at the start of the first experimental period, and 6 weeks old at the beginning of the second period. Several authors (eg., Corring *et al.*, 1978) have confirmed that by approximately 4 weeks of age, the production of most digestive enzymes has begun to increase rapidly, or has already reached appreciable levels compared with neonatal production. According to measurements reported by Aumaitre (1972), by 5 weeks of age the piglet is producing significantly higher amounts of amylase, maltase and sucrase than the levels produced at birth. Hudman *et al.* (1957) showed a rapid increase in pancreatic amylase activity from birth to 4 weeks of age. Manners and Stevens (1972) found that between 3 to 4 weeks of age there is a rapid increase in sucrase production. Kidder and Manners (1972) found that gastric acid is established by the seventh week of life, and pancreatic proteolytic activity increases from 4 to 36 days of age; pancreatic lipase is high at birth, but still increases to 6 weeks of age (Kidder and Manners, 1972). Lewis *et al.* (1957) showed a linear increase in gastric pepsin activity from 3 to 6 weeks of age; Corring *et al.* (1978) showed that after 4 weeks of age, enlargement of pancreatic cells, as well as increases in the number of cells occurred, and this coincided with more rapid development of pancreatic enzyme activity. The results reported by these authors suggest that by the age of 4 weeks, the piglet's digestive capacity is fast approaching that of an adult pig. In agreement with this, Officer (1995) saw no response of 4 to 5 week old piglets to enzyme supplemented diets and suggested that this was because endogenous enzyme activity was not limiting. Similarly, in the present study, the digestion and/or absorption of dietary nutrients in the diets containing peas may not have been the limiting factor.

Alternatively, the type and/or concentrations of the enzymes used in the present study were insufficient to elicit a response (Officer, 1995). Cowan *et al.* (1996) state clearly that the improvement in the availability of energy in

peas they observed in piglets was dependent on both the dose and type of enzymes used, and also was correlated to the substrate specificity of the enzymes used. Due to the variability and complexity of the many different types of component NSP present in feed ingredients, Chesson (1987) feels that enzyme supplements should be appropriate to the target substrates, according to the particular glycosidic linkages of the constituent polysaccharides. In addition, the differences in constituent monosaccharides which exist between monocotyledonous and dicotyledonous plants also must be considered; an enzyme preparation which is appropriate for the degradation of wheat (monocotyledonous) polysaccharides may not be effective against legume (dicotyledonous) polysaccharides (Chesson, 1987).

Officer (1995) suggests that not only will multi-enzyme supplements have limited benefit in wheat based diets for piglets, they may actually be detrimental. Feed intake often decreases post weaning, but does not usually last longer than approximately 2 weeks (Hampson, 1986; Officer, 1995). Officer (1995) states that some reduction in feed intake is normal, and is a mechanism used by piglets to maintain efficiency in nutrient digestibility (Veum and Mateo, 1981). However, 3 weeks of feed intake decrease shown by piglets fed diets supplemented with a multi-enzyme preparation may be due to a gradual accumulation of digestion products which overload absorptive capacity and eventually impair intake (Officer, 1995). In addition, accumulated products of digestion would confound digestibility measurements, resulting in underestimation of the degradative effect of the supplemental enzymes used in the present experiment.

According to Dierick (1989), the variability and common lack of response by piglets to the addition of supplemental enzymes could be due to the denaturation of the enzymes in the stomach. Supplemental enzymes are proteins: polypeptide chains folded in a unique configuration which is maintained by various interactions between the constituent amino acid residues of the chain and the solvent in which the enzyme is dissolved: hydrogen and various covalent bonds, hydrophobic interactions between certain residues of the protein, or stabilizing disulfide bonds can all be affected by changes in temperature, osmolarity, or pH of the surrounding luminal environment (Webb, 1990). Changes in the ionization states of the amino acid residues, which may occur with changes in hydrogen ion concentration in the stomach, can potentially alter the chemical reactivity of the enzyme

(Webb, 1990). In addition, the stabilizing interactions between amino acid residues are susceptible to denaturation and destabilization caused by a change in pH. Most pancreatic and mucosal enzymes have optimum pH ranges from 5.2 to 9.2, and would have minimal or zero activity in the very acid conditions of the stomach (the pH in gastric contents ranges from 1 to 4 in a 5 week old piglet) (Webb, 1990; Kidder and Manners, 1978). Since levels of gastric acid increase gradually from 2 to 3 weeks of life and substantial levels are present by 7 weeks (Aumaitre, 1972; Kidder and Manners, 1972), denaturation of the enzyme proteins contained in an exogenous enzyme preparation could potentially occur in the relatively acid environment of the piglet's stomach.

According to Leterme *et al.* (1996), unreliable partitioning of dietary components in the different fiber fractions as determined by fiber analysis methods such as the van Soest procedure, may mean that NDF and ADF methods are unsuitable for determining fiber content in some feed ingredients, including legumes. The ADF fraction may include significant xylose and uronic acids components, when it should theoretically contain only cellulose and lignin; NDF residues may contain resistant starch. Some of the starch in peas exist as resistant starch, which may not be completely degraded by alpha-amylase (Gdala, 1998). Even though thermostable alpha-amylase was included as part of the van Soest procedure used in the present investigation, this enzyme treatment may be inefficient for legumes: even with the use of alpha-amylase, Leterme *et al.* (1996) found that 36 to 40% of pea starch remained in the NDF fraction. The high degree of variability seen in the results of analysis of fiber digestibility (Tables 2-3, 2-4) suggest that the van Soest procedure may not be appropriate for legumes, and the reliability of the results obtained with this procedure may therefore be questionable.

According to Jorgensen *et al.* (1984), and Piccaglia and Galletti (1987), freeze drying or oven drying of digesta or feces samples prior to chemical analysis may affect values obtained for apparent digestibilities. Jorgensen *et al.* (1984) found that more than 5% of the nitrogen in their samples was lost due to oven or freeze drying. Piccaglia and Galletti (1987) found that oven drying of samples prior to fiber analysis could confound NDF results due to the condensation of lignin and carbohydrate during oven drying. All of the digesta and fecal samples collected in the present experiment were freeze-dried prior to proximate analysis, which, according to the results of Jorgensen

et al. (1984) and Piccaglia and Galletti (1987), may have confounded the resultant digestibility values.

According to Li *et al.* (1993), decreases in ileal protein digestibility reported with increasing dietary crude protein level may be due to decreased efficiency of amino acid transport into the enterocyte, since amino acid absorption is active (i.e., Alpers, 1987), and involves many different transport systems. The absorptive capacity of these transport systems may be exceeded with an increase in dietary crude protein level. This was also suggested by Officer (1995), who claimed that a long term reduction in feed intake post-weaning observed in piglets fed a cereal-based diet supplemented with exogenous enzymes, could be due to the accumulation of the products of digestion in the intestinal lumen, thereby impairing the flow of digesta through the intestine. Since, according to Low (1980), apparent digestibility is actually a measure of the ability of the mucosal cells to absorb the products of digestion (rather than a measure of the actual digestive enzyme activity), digestibility values can be underestimated if the products of digestion are not absorbed, even though they may have been digested. Zebrowska (1973) found that the rate of absorption of amino acids was much slower than the rate of their release from the peptide chains. Therefore, the protease activity of the enzyme preparation used in the present experiment may have enhanced proteolysis of the supplemented diet, however the level of enhancement may have been limited by absorptive capacity. The trend ($P>0.05$) toward decreased digestibility of most amino acids (Table 2-3) after supplementation of enzymes to the pea diet could indicate a possible overloading of the absorptive capacity of the intestine.

Intestinal bacteria may contribute to inaccurate estimations of nutrient digestibility. According to Low (1980), a nutrient may appear to have been digested and absorbed because its presence may not be detected during chemical analysis of digesta, however it may actually have been altered in some way by intestinal bacteria to another compound which may not have been measured. For this reason, nutrient digestibilities are correctly referred to as 'apparent' digestibilities. The production of organic acids by microbes is an example of the alteration of nutrients by microbial fermentation. While this occurs predominantly in the large intestine and is the basis for the accuracy and sensitivity of the ileal analysis method for measuring digestibility coefficients over the fecal analysis method, populations of

microbes do exist in the small intestine (Friend *et al.*, 1963; Kidder and Manners, 1978; Just *et al.*, 1983; Luckey, 1987; Bergman, 1990), and may confound digestibility values.

Pea starch is not completely digested in the ileum, because some starch in legumes exists as resistant starch (Gdala, 1998); and approximately 1/3 of starch exists as amylose, which is not as well digested by monogastrics as amylopectin (Leterme *et al.*, 1990). In addition, legume starches tend to be entrapped within the parenchyma of the seed and due to physical hindrance the amylase enzyme cannot penetrate to contact and degrade the starch granules (Wursch *et al.*, 1986).

Partial hydrolysis of the alpha-galactoside sugars in peas may be possible via the action of invertase (sucrase), which is negligable at birth but rises rapidly for the first few weeks of life through 2 years of age (Bailey *et al.*, 1956; Kidder and Manners, 1978). The piglets used in this study may have been old enough to produce some invertase, and may have been able to digest a portion of these sugars in the pea diets. However, since the levels of these sugars in ileal digesta were not measured, this was not determined.

Amino acid sequence and the chemical nature of amino acids in protein affect the digestibility of the amino acids due to the specificity of digestive enzymes for certain peptide bonds (Sauer and Ozimek, 1986; Stryer, 1987). The relatively high apparent digestibilities of arginine and lysine which were observed in this study tend to support the hypothesis that enzyme specificity is an important determinant of amino acid digestion and/or absorption (Li, 1992). According to Low (1980), arginine and lysine would be expected to appear first after enzymatic hydrolysis of the protein molecule, due to the known specificity of most intestinal and pancreatic proteases. Subsequently, these amino acids are the first available for absorption.

The amino acid content of endogenous secretions may affect the apparent ileal digestibility values of these amino acids (Fan *et al.* 1994; 1995). The relatively low ileal digestibilities of threonine and glycine found in this study may result in part from their relatively high concentrations in endogenous secretions (Sauer and Ozimek, 1986; Li *et al.*, 1993). Glycine is a major component of bile salt conjugates, and accounts for greater than 90% of the total amino acids secreted in bile juice (Souffrant, 1991). While most of the bile salts are reabsorbed via the entero-hepatic circulation, de-conjugated glycine escapes reabsorption and is degraded in the large intestine (Li *et al.*,

1993). Therefore, the content of glycine in ileal digesta may account for the relatively low apparent ileal digestibility of this amino acid. Similarly, mucin protein is rich in threonine (Neutra and Forstner, 1987). Dietary fiber has been shown to increase the production of mucous and other endogenous secretions (Low, 1985); therefore, the relatively high amounts of crude fiber in canola meal may have contributed to the relatively high amounts of mucin protein in ileal digesta, as evidenced by the apparent ileal digestibility value for threonine found in this experiment. According to Neutra and Forstner (1987), small intestinal secretions, including bile juice and mucous, supply the largest proportion of endogenous nitrogen to the endogenous nitrogen secretions in the small intestine.

The net disappearance of the parameters measured in the large intestine is presented in Table 2-5, expressed quantitatively as g/kg dry matter intake (DMI). The addition of supplemental enzymes decreased ($P<0.05$) the disappearance of ADF in the large intestine of piglets fed the PWE diet. Enzyme supplementation increased ($P<0.05$) crude protein disappearance in the large intestine and there was a trend (non-significant) toward increased disappearance of amino acid with the addition of supplemental enzymes in the large intestine of pigs fed the PWE diet. Of the dispensable amino acids, aspartic acid glutamic acid and glycine disappeared to a large extent. Of the indispensable amino acids, leucine disappeared to the largest extent. These results are in agreement with several other studies (eg., Sauer *et al.*, 1991; Li *et al.*, 1994; Fan *et al.*, 1995). Endogenous protein in the form of intestinal and pancreatic secretions contains a relatively large proportion of certain amino acids, including aspartic acid, glutamic acid, and leucine (Fan *et al.*, 1995); and glycine, a major constituent of bile salt conjugates, mostly escapes reabsorption in the distal small intestine and enters the large intestine where it is fermented (Fan *et al.*, 1995). The large disappearance of these amino acids in the large intestine of pigs fed the diets containing peas could be an attempt by the body to recycle these amino acids (Sauer *et al.*, 1990). Protein secreted via pancreatic and small intestinal juice contains a relatively large proportion of aspartic acid, glutamic acid, and leucine (Corring and Jung, 1972; Buraczewska, 1979; Li, 1992). The preferential fermentation of undigested endogenous protein and/or amino acids by the microflora in the large intestine may be part of a mechanism whereby the host animal is able to recover those amino acids lost as endogenous protein, in the form of

ammonia. Under certain conditions (i.e., when dietary nitrogen *per se* is limiting for the synthesis of dispensable amino acids), absorbed ammonia may contribute to the protein status of the pig (Li, 1992).

In conclusion, enzyme supplementation of diets containing peas as the primary protein source in diets fed to young pigs had no effect ($P>0.05$) on apparent ileal or fecal digestibility coefficients of crude protein, amino acids, or other dietary nutrients. The lack of response to the inclusion of the enzymes could be due in part to the age of the piglets, in addition to the time allowed for adaptation to the experimental diets. Both of these factors may have masked the effectiveness of the enzyme preparation, and resulted in the lack of response seen in this experiment. Alternatively, the type of enzymes or dosage level used may have been inappropriate for the dietary ingredients (peas); or the digestion and/or absorption of dietary nutrients in the pea diets may not have been a limiting factor.

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Table 2-1. Formulation (% as fed) of Diets Containing Peas Fed to Young Pigs

	Diets[a]	
	PW	PWE
Ingredients		
Peas	56.5	55.5
Wheat	37.2	38.2
Canola oil	3.05	2.95
Enzyme Mixture[b]		0.1
Dicalcium Monophosphate	0.9	0.9
Calcium Carbonate	0.9	0.9
Salt	0.2	0.2
D,L-Methionine	0.05	0.05
Vitamin premix[c]	1.0	1.0
Chromic oxide[d]	0.2	0.2
Total	100.0	100.0

[a] PW = peas + wheat; PWE = PW diet + enzymes

[b] Contains 6250 units/g of xylanase from *Trichoderma longibrachiatum*, and 3000 units/g of protease from *Bacillus subtilis*. Finnfeeds International, Box 777, Marlborough, Wiltshire, United Kingdom SN8 1XN.

[c] Provided per kg premix: vitamin A, 10^6 IU; vitamin D_3, 10^5 IU; vitamin E, 8000 IU; choline, 10^5 mg; Fe, 15000 mg; Zn, 12000 mg; niacin, 4000 mg; pantothenic acid, 2500 mg; Cu, 2000 mg; riboflavin, 1200 mg; Mn, 1200 mg; menadione, 200 mg; folic acid, 160 mg; Se, 30 mg; biotin, 25 mg; iodine, 20 mg; vitamin B_{12}, 3 mg. BASF Canada Inc., Regina, SK S4N 5X9.

[d] Chromic oxide powder, Fisher Scientific, Fair Lawn, NJ 07410.

Table 2-2. Partial Chemical Chemical Analyses of the Experimental Diets Containing Peas Fed to Young Pigs

	Diets*	
	PW	PWE
Dry Matter (%)	88.02	88.59
Crude Protein (g/kg)	169.0	163.8
Ash (%)	4.20	4.59
Digestible Energy (Mcal/kg)	3430	3422
Starch (%)	41.48	38.63
Fiber (%):		
NDF	19.35	19.37
ADF	6.34	6.31
Hemicellulose	13.00	13.06
Cellulose	4.24	4.21
Amino Acids (%):		
Indispensable		
Arginine	1.215	1.256
Histidine	0.388	0.418
Isoleucine	0.665	0.668
Leucine	1.202	1.23
Lysine	1.086	1.137
Phenylalanine	0.788	0.817
Threonine	0.635	0.668
Valine	0.753	0.757
Dispensable		
Alanine	0.767	0.784
Aspartic acid	1.632	1.635
Glutamic acid	3.525	3.587
Glycine	0.804	0.831
Serine	0.791	0.816
Tyrosine	0.421	0.426

* PW=peas + wheat; PWE= PW diet + enzymes

Table 2-3. Apparent Ileal Digestibilities (%) of Dry Matter, Crude Protein, Gross Energy, Starch, Fiber and Amino Acids in Diets Containing Peas Fed to Young Pigs

	Diets[a]		
	PW	PWE	SEM[b]
Dry Matter	70.0	70.4	1.39
Crude Protein	72.9	70.5	3.44
Gross Energy	70.7	70.9	1.72
Starch	93.7	92.3	0.83
Fiber:			
NDF	33.2	39.1	8.82
ADF	20.9	26.3	3.66
Hemicellulose	39.3	45.4	12.14
Cellulose	43.1	30.3	6.63
Amino Acids: Indispensable			
Arginine	86.5	86.0	1.66
Histidine	79.2	78.8	1.97
Isoleucine	72.8	69.7	3.53
Leucine	76.8	74.1	2.84
Lysine	79.6	77.9	2.62
Phenylalanine	77.2	76.4	2.44
Threonine	66.0	66.2	3.00
Valine	60.6	52.6	6.65
Dispensable			
Alanine	68.7	67.2	3.30
Aspartic acid	75.0	73.1	2.49
Glutamic acid	86.5	85.8	1.58
Glycine	58.3	61.5	2.44
Serine	75.6	75.8	2.72
Tyrosine	80.3	77.9	2.96

[a]PW=peas + wheat; PWE=PW diet + enzymes
[b]Standard error of the least squares means; 7 observations per treatment

Table 2-4. Apparent Fecal Digestibilities (%) of Dry Matter, Crude Protein, Gross Energy, Starch, Fiber and Amino Acids in Diets Containing Peas Fed to Young Pigs

	Diets[a]		
	PW	PWE	SEM[b]
Dry Matter	90.7	91.1	0.44
Crude Protein	85.2	87.6	0.87
Gross Energy	89.6	89.9	0.48
Starch	99.8	99.7	0.03
Fiber:			
NDF	85.9	82.3	1.66
ADF	74.2	67.3	5.19
Hemicellulose	90.9	82.3	4.38
Cellulose	68.9	64.9	4.53
Amino Acids:			
Indispensable			
Arginine	94.4	95.4	0.50
Histidine	90.4	91.6	1.66
Isoleucine	84.6	85.3	1.55
Leucine	88.9	89.7	0.87
Lysine	88.9	90.9	0.91
Phenylalanine	89.4	90.1	0.91
Threonine	81.5	84.3	1.92
Valine	78.8	79.6	2.50
Dispensable			
Alanine	84.1	85.8	1.10
Aspartic acid	86.8	88.5	1.00
Glutamic acid	92.8	93.8	0.82
Glycine	83.8	85.7	1.54
Serine	88.4	90.3	0.64
Tyrosine	87.3	88.5	1.23

[a] PW=peas + wheat; PWE=PW diet + enzymes
[b] Standard error of the least squares means; 7 observations per treatment

Table 2-5. Disappearance (g kg^{-1} DMI) of Dry Matter, Crude Protein, Gross Energy (kcal kg^{-1}),Starch, Fiber and Amino Acids in the Large Intestine of Young Pigs Fed Diets Containing Peas

	Diets[a]		
	PW	PWE	SEM[b]
Dry Matter	209.69	207.68	13.74
Crude Protein	18.24[c]	27.87[d]	2.95
Gross Energy	674.14	687.68	59.35
Starch	22.29	25.47	2.81
Fiber:			
NDF	99.07	67.68	9.66
ADF	34.16	22.11	3.26
Hemicellulose	64.99	47.69	10.92
Cellulose	10.81	14.76	3.38
Amino Acids:			
Indispensable			
Arginine	0.85	1.04	0.18
Histidine	0.38	0.47	0.03
Isoleucine	0.76	0.92	0.12
Leucine	1.29	1.70	0.27
Lysine	0.89	1.30	0.27
Phenylalanine	0.85	0.99	0.15
Threonine	0.87	1.07	0.10
Valine	1.20	1.81	0.36
Dispensable			
Alanine	1.04	1.29	0.21
Aspartic acid	1.69	2.22	0.24
Glutamic acid	1.97	2.53	0.34
Glycine	1.82	1.78	0.15
Serine	0.90	1.04	0.15
Tyrosine	0.26	0.40	0.14

[a] PW=peas + wheat; PWE=PW diet + enzymes

[b] Standard error of the least squares means; 7 observations per treatment

[c,d] Means within a row having different superscripts differ ($P<0.05$)

3. Enzyme Supplementation to Diets Containing Canola Meal Fed to Young Pigs

A. Introduction

Nutritional benefit may be gained from the NSP of vegetable proteins, such as canola meal, if these NSP could be hydrolysed in the small intestine (Dierick, 1989; Dierick and Decuypere, 1994). Canola meal contains 19.6% NSP (as % DM; Chesson, 1987; Dierick, 1989); this may be hydrolyzable since Millard and Chesson (1984) found substantial modifications to cell wall material anterior to the terminal ileum of pigs, including partial solubilisation of some hemicellulose components. Partial hydrolysis of the alpha-galactosides in canola meal (such as raffinose and stachyose) is possible via intestinal invertase (sucrase), to fructose and some other sugars (Dierick and Decuypere, 1994). Levels of invertase are low at birth but increase through 2 years of age (Kidder and Manners, 1978), which may explain the relatively high digestibility of alpha-galactosides in pigs (60-90%; Dierick and Decuypere, 1994). Levels of NSP in canola meal are fairly high and therefore, according to Dierick (1989), a potentially large benefit exists from intestinal NSP hydrolysis. Slominski and Campbell (1990) incubated poultry diets with 1 and 3% enzymes, and found substantial hydrolysis of canola meal polysaccharides after incubation with 1% enzymes, but no significant improvement with 3%. They suggest that relatively rapid hydrolysis of the small water-soluble fraction of NSP occurs, followed by relatively slow release of component sugars from the larger water-insoluble (and therefore inaccessible) cell wall fraction. They also supplemented the same levels of enzymes *in vivo*, and found low nutrient digestibility with unsupplemented diets (2 to 3% NSP digestibility); the addition of 1% of their commercial enzyme preparation increased this to 36.6%. They attributed the majority of this effect to enhanced digestibility of the non-cellulosic polysaccharide components, and suggest that a low level of water-soluble NSP in canola meal may be a limiting factor to intestinal NSP digestibility, as well as the effectiveness of enzyme supplementation.

Most reports of enzyme-supplemented diets for young pigs give inconsistent results (Officer, 1995). Enzymes are usually used in growth experiments, supplemented to complex diets composed of many different

ingredients; enzyme supplementation does not consistently improve performance parameters such as average daily gain (ADG), feed intake (FI) or feed conversion ratio (FCR). Few trials have investigated the effects of enzyme supplementation of oilseed meal diets on nutrient digestibility, and those that do rarely report improvements in digestibility. Inborr *et al.* (1993) reported an increase of 21 to 27% in the digestibility of NSP from a cereal-soybean meal diet (Officer, 1995), however Baird *et al.* (1976) found no effect on nutrient digestibility or nitrogen retention with the addition of 1% enzymes (amylases and proteases) to a soybean meal diet fed to piglets.

Some authors feel that improvement in digestibility is only possible with less digestible ingredients, such as high-NSP feedstuffs. Many of the available reports on the effect of adding supplemental enzymes to diets for young pigs have used diets containing traditionally-used ingredients such as soybean meal as the main protein source (eg., Baird *et al.*, 1976; Caine *et al.*, 1997). The digestibility of soybean meal is relatively high compared to some other protein sources (eg., Fan *et al.*, 1995) without supplementation, therefore significant improvements in nutrient digestibility may not be possible with diets which contain traditionally used protein sources and highly digestible cereals. Rexen (1981) found improvements in growth and feed utilization only with relatively poor quality barley and not with barley of better quality, using cellulase, pectinase and protease enzymes in livestock diets. Feed ingredients of lower quality and thus lower cost (such as canola meal) may therefore be a more economical alternative to traditionally used ingredients, such as soybean meal (Rexen, 1981); hydrolysis of canola meal NSP in the small intestine may offer the greatest potential for nutritional improvement of relatively low quality feeds.

The objectives of this study were to investigate the effects of supplemental enzymes on the digestibility of CP, gross energy, fiber, amino acids and starch in diets containing canola meal fed to young pigs.

B. Materials and Methods

Animal Management

Eight piglets (PIC), weaned at 18 to 21 d of age with an average BW of 6.5 kg, were obtained from the University of Alberta R.D. Sinclair Swine

Research Unit. They were housed individually in metabolic crates (85 cm high X 70 cm long X 65 cm wide), in a temperature-controlled (25 to 28ºC) barn. A cereal-based starter diet, formulated to supply digestible energy and nutrients according to the recommendations of the National Research Council (1988) for starter pigs, was provided *ad libitum*. Water was freely available from low-pressure drinking nipples.

Each pig was fitted with a simple T-cannula at the distal ileum 6 to 7 d after weaning. All of the pigs recovered from anaesthesia within 2 to 3 h after surgery. After a 6 to 7 d recuperation period, the pigs were divided according to body weight into two groups, and fed one of two dietary treatments (Table 3-1) according to a crossover experimental design (Gill and Magee, 1976). The diets were fed at a rate of 5% of the average BW per d, determined immediately prior to the start of each experimental period. The daily meal allowance was offered in three meals of equal size: at 0800, 1600 and 2400 h. All pigs usually consumed their meal allowance within 1 to 2 h. Each experimental period lasted 8 d (6 d adaptation to diet followed by a 48 h collection period). Collection of feces was performed for a total of 24 h: from 0800h on d 6 to 0800 h on d 7 of each experimental period. Collection of ileal digesta was then performed from 0800 to 1600 h on d 7, from 0000 to 0800 h and from 1600 to 2400 h on d 8 of each experimental period, for a total of 24 h. The average BW of the pigs was 9.5 kg and 12.4 kg at the beginning of the first and second experimental period, respectively. The average BW was 15.0 kg at the end of the second experimental period. At the conclusion of the experiment, the pigs were euthanized by CO_2 inhalation and lethal injection (T61[1], 5cc/9kg intravenously). Dissections were performed to ensure that there were no intestinal abnormalities.

The experimental protocol was approved as submitted by the Animal Care Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta. The piglets used in this experiment were cared for in accordance with the guidelines established by the CCAC (1980).

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[1] Tetracaine hydrochloride. Hoechst Roussel Canada Ltd., 4045 Cote Ver ta, Montreal, Quebec

Preparation of Cannulae

Cannulae were prepared according to procedures adapted from Sauer (1976). A finely polished 'T' shaped stainless steel mould was heated in a muffle furnace at 200 to 250°C for 15 to 30 minutes. It was removed from the furnace and immersed in a solution of Plastisol[2] for 3 to 5 minutes (until the Plastisol layer on the barrel reached a thickness of approximately 3 mm). The mould covered in Plastisol was then re-heated in the furnace until the color of the Plastisol changed from white to a dark yellowish color; it was removed and cooled in a container of cold water until the stainless steel mould was cool enough to touch. The cannula was then slit along the length of the flange and peeled off of the mould. The cannulae were soaked in ethanol until they reached the desired hardness (usually several hours). Retaining collars were fashioned by heating and then immersing a glass bottle stopper of the appropriate size into the Plastisol, following the same procedure for preparing the cannulas. Plugs for each cannula were made by pouring the Plastisol into a piece (approximately 10 cm) of steel pipe, having an internal diameter of 1 to 2 mm smaller than the internal diameter of the cannula, and a large steel nut fitted onto one end. The hardened cannulae, collars and plugs were trimmed and sanded with an electric sander.

Surgical Procedures and Pre- and Post-Operative Care

The piglets were fasted for 12 h prior to surgery. Each animal was brought under general anaesthesia using a gas mixture of Halothane[3] and oxygen, and placed on its left side. The flank area of the right side of the body (from approximately the 3rd or 4th last rib, caudally) was shaved with an electric clipper, cleaned thoroughly with Betadine[4], and draped, leaving the surgical area exposed. An excision of approximately 7 cm in length was made through the skin and subcutaneous fat, parallel and approximately 3 cm caudal to the last rib. Underlying muscle layers were separated by blunt dissection; the peritoneum was carefully lifted away from the underlying intestines and cut along the original incision line. The distal ileum was

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[2] Auburn Plastics Engineering, Chicago, Illinios 60609.

[3] Fluothane; Ayerst Laboratories, Saint-Laurent, Quebec.

[4] 7.5% Povidone-iodine. Purdue Frederick Inc., Toronto, Ontario.

identified by location of the ileocecal sphincter. Prior to making the incision in the ileum, catgut (2-0)[5] was positioned as a purse-string suture line, through the serosal layer of the intestine, approximately 5 cm cranial to the ileocecal junction as a complete outline to the incision. A scalpel blade was used to make the incision inside the suture outline. The flanges of the cannula were inserted into the incision and the suture was tied. Approximately 2 mm below this suture line, a second purse-string suture was positioned around the base to further secure the cannula. Extruded mucosa was carefully trimmed with the scalpel.

A fistula was created between the last two ribs by excising a circular piece of skin and penetrating the muscle layers using finger manipulation and a pair of forceps. The peritoneum was carefully pushed up through the fistula and cut, completing the fistula. The cannula barrel was then pulled through the fistula until its base and flanges (which remained inside the ileum) contacted the inside of the body wall; parts of small intestine were prevented from becoming lodged in between the body wall and the cannula. The retaining collar was fixed over the barrel and as close as possible to the skin using a plastic locking tie. Terramycin[6] (0.5 mL) was administered into the abdominal cavity before the incision was closed.

The pigs were returned to their metabolic crates following surgery and fasted for approximately 8 to 12 h; at the first regular mealtime (08:00, 16:00 or 00:00 h) after this fasting period limited amounts (25 to 30g) of the starter diet were provided and this was gradually increased over the next 2 to 3 d according to each pig's appetite, until the pigs consumed the starter diet at a rate of 5% of the average body weight. During the recuperation period, the crate temperature was maintained at 30 to 32°C with the use of infrared heat lamps suspended over each crate. The stitches were removed 7 to 10 d after surgery. From the second week after surgery onwards, the cannula area of each piglet was washed two to three times daily with warm water, and Ihle's paste[7] was applied around the retaining collar in order to prevent skin irritation.

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[5] Chromic absorbable suture. DAVIV + GECK Cynamid Canada Inc., Baie d'Urfe, Quebec.

[6] Oxytetracycline Hydrochloride. Dominion Veterinary Laboratories Ltd., Winnipeg, Manitoba.

[7] 25% Zinc Oxide. Valmo Laboratories, Trois-Rivieres, Quebec.

Experimental Diets

The two experimental diets were formulated to contain 18% CP, digestible energy, vitamins and minerals according to NRC (1988) recommendations, on an as-fed basis (Table 3-1). L-lysine-HCl was included where necessary to fulfill the respective NRC (1988) requirements. The supplemental enzyme mixture was provided by Finnfeeds International[8], and contained 6,250 units/g of xylanase from *Trichoderma longibrachiatum*, and 3,000 units/g of protease from *Bacillus subtilis*. Canola meal and wheat were obtained from the University of Alberta Feed Mill. Chromic oxide was included as a digestibility marker. The canola meal and wheat were ground through a 2 mm screen prior to the mixing of the diets.

Experimental Design

The crossover design is used when expense and complexity demand that the experiment be as short as possible; this design also removes variation among animals (Gill and Magee, 1976). This design is therefore appropriate for studies with young pigs because of the possibility of effects resulting from gastrointestinal development. Sources of potential variation were groups of pigs, pigs within groups, dietary treatment and experimental period.

Procedures for Collection of Feces and Ileal Digesta

Collection of feces was performed for 24 h, from 0800 h on d 6 to 0800 h on d 7 of each experimental period. Feces were collected into plastic containers, and immediately frozen at -20°C until chemical analyses were performed. Feces which became contaminated with feed or urine were not collected.

Collection of ileal digesta was performed for 24 h, from 0800 to 1600 h on d 7, from 0000 to 0800 h and from 1600 to 2400 h on d 8 of each experimental period. At the beginning of each digesta collection, the cannula plugs were removed, and clear, soft plastic bags (approximately 12 cm long by 2 cm internal diameter) were attached to the cannula barrels using pieces of thin wire twisted around the barrel. Formic acid (5 mL, 10% v/v) was added to the bags prior to attachment to the barrel, to minimize bacterial activity. Bags were changed as required (when they were approximately half full); contents were immediately frozen at -20°C until chemical analyses were performed. The first bag collected from each pig was discarded as it usually

contained digesta which may have been present in the barrel of the cannula for some time.

Fecal and digesta samples were pooled within pig, diet and period prior to chemical analysis.

Chemical Analyses, Calculations and Statistical Analyses

Samples were obtained from each diet, each time the pigs were fed. These samples were pooled within dietary treatment prior to chemical analyses. Digesta and fecal samples were freeze-dried, and ground as finely as possible using a countertop coffee mill (Braun, household type). Samples of each diet were ground as finely as possible using the coffee mill previously described. All samples were thoroughly mixed before subsamples were taken for each analysis procedure, and all chemical analyses were performed in duplicate.

Analyses for dry matter and ash were determined according to AOAC (1997) methods; chromic oxide was determined according to the procedure of Fenton and Fenton (1979); crude protein (% nitrogen X 6.25) analysis was performed using a LECO® model FP-428 Nitrogen Determinator (LECO® Corporation, St. Joseph, Michigan). Gross energy content was determined with a LECO bomb calorimeter. Analyses for acid-detergent fiber (ADF) and neutral detergent fiber (NDF) were carried out according to the principles outlined by Goering and van Soest (1970), and analyses for amino acids (except methionine, cysteine, tryptophan and proline) were performed according to procedures described by Sedgwick *et al.* (1991).

Apparent ileal or fecal digestibility values can be defined as the difference between the amount of nutrient in the diet, and in ileal digesta (or feces respectively), divided by the amount in the diet. The apparent ileal and fecal digestibility values of dry matter, crude protein, gross energy, starch, acid- and neutral-detergent fiber, hemicellulose, cellulose and amino acids in the four experimental diets were calculated according to the following formula:

$$D_D = 100\% - [(I_D \times A_F) / (A_D \times I_F)] \times 100\%$$

where D_D is the apparent digestibility of a nutrient in the assay diet (%), I_D is the marker concentration in the assay diet (%), A_F is the nutrient concentration in ileal digesta or feces (%), A_D is the nutrient concentration in

the assay diet (%), and I_r is the marker concentration in ileal digesta or feces (%).

Data were subjected to statistical analyses using the General Linear Model procedure of SAS (1988). Treatment means were compared using the Student-Newman-Keuls' multiple range test procedure.

C. Results and Discussion

All of the pigs remained healthy throughout the course of the experiment. They usually finished their meal allowances within 1 to 2 h of feeding. Postmortem dissections performed at the conclusion of the second experimental period revealed no intestinal abnormalities.

The apparent ileal and fecal digestibility coefficients of dry matter, crude protein, gross energy, starch, fiber and amino acids in the experimental diets containing canola meal are presented in Tables 3-3 and 3-4, respectively. There were differences ($P<0.05$) between dietary treatments only for the ileal digestibility of starch. Compared to the other amino acids, the apparent ileal digestibilities of arginine and lysine were relatively high, whereas the apparent ileal digestibilities of threonine and glycine were relatively low. All of the apparent fecal CP and amino acid digestibility coefficients are higher than their corresponding apparent ileal digestibility values, illustrating the modifying effect of the microflora in the large intestine, on the digestibility values of CP and amino acids. This further substantiates the ileal analysis method as a more sensitive, superior method for determining digestibilities of CP and amino acids.

The stress associated with abrupt changes in diet and environment at weaning is thought to be responsible for many of the symptoms of the post-weaning growth check often seen in pigs (Li, 1992). These symptoms include a loss of appetite (low feed intake), diarrhea, loss of body weight and condition, and subsequent delayed growth which may lead to production losses (Morkeberg *et al.*, 1992). The physical change in environment (separation from the sow, relocation to a new pen) is stressful, however the abrupt change to a novel diet can cause drastic morphological changes to occur in the intestinal epithelium (Leibholz, 1981), and subsequently

alterations intestinal absorption (Hampson, 1986; van Beers-Schreurs *et al.*, 1998).

However, according to Dierick and Decuypere (1994), recovery of the piglets from the initial psychological and nutritional shocks of weaning, and their subsequent adaptation to a change in diets is rapid, therefore the benefits of enzyme addition will be short lived (i.e., 2 weeks; Dierick and Decuypere, 1994). The addition of supplementary enzymes to diets for newly weaned pigs may only be beneficial if the enzymes can compensate for low levels or activities of endogenous enzymes in young pigs, or during the transition period immediately post-weaning, when the diet has been changed from sow's milk to a solid nursery (starter) diet, before the piglet's gastrointestinal tract has been able to adapt in terms of digestive enzyme biosynthesis and secretion, gastrointestinal motility, and other aspects of intestinal function (Corring, 1980; Dierick and Decuypere, 1994). According to Corring (1980), and Aumaitre (1972), the process of adaptation to a change in diet takes approximately 5 to 7 days. The pigs used in this experiment were allowed to adapt to the experimental diets for 5 to 7 days before ileal digesta was collected.

The enzyme mixture used in this experiment contained protease and xylanase activities. While xylanase (a pentosanase) is not specific for the glycosidic bonds of starch (a glucose polymer), the activity of this enzyme may possibly have been involved in the release of encapsulated starch contained within structural NSP. Although plant cell walls are complex structures which require complex mixtures of specific enzymes acting in synergy (Chesson, 1987), crude mixtures of enzymes such as xylanase (an endo-enzyme) may cleave enough glycosidic links of NSP to reduce the viscosity of digesta and/or to substantially disrupt the cell wall. This may have allowed the release of starch.

The type and/or concentrations of the protease enzymes used in this study may have been insufficient to elicit a measurable response (Officer, 1995) in dietary protein or amino acids. Cowan *et al.* (1996) state clearly that the improvement in pea energy availability they saw in piglets was dependent on both the dose and type of enzymes used, and also correlated to the substrate specificity of the enzymes used. Due to the variability and complexity of the many different types of component NSP (and NSP-protein complexes) found in feed ingredients, Chesson (1987) feels that enzyme supplements should be appropriate to the target substrates, according to the

particular glycosidic linkages of the constituent polysaccharides. In addition, the differences in constituent monosaccharides which exist between monocotyledonous and dicotyledonous plants also must be considered; an enzyme preparation which is appropriate for the degradation of wheat (monocotyledonous) polysaccharides may not be effective against rapeseed (dicotyledonous) polysaccharides (Chesson, 1987).

Officer (1995) suggests that not only will multi-enzyme supplements have limited benefit in wheat based diets for piglets, they may actually be detrimental. Feed intake often decreases post weaning, but does not usually last longer than approximately 2 weeks (Hampson, 1986; Officer, 1995). Officer (1995) states that some reduction in feed intake is normal, and is a mechanism used by piglets to maintain efficiency in nutrient digestibility (Veum and Mateo, 1981). However, Officer (1995) observed a decrease in feed intake of 3 weeks by piglets fed diets supplemented with an enzyme preparation, and suggested that this may be due to a gradual accumulation of digestion products which overload absorptive capacity and eventually impair intake. Accumulated products of digestion would confound digestibility measurements, resulting in underestimation of the hydrolyzing effect of the supplemental enzymes used in the present experiment.

Alternatively, the piglets used in this experiment may have been too mature in terms of digestive capacity, which may have masked any response to the supplemental enzymes. The piglets used in this experiment were 5 weeks old at the start of the first experimental period, and 6 weeks old at the beginning of the second period. Several authors have reported that by approximately 4 weeks of age, the production of most digestive enzymes begins to increase rapidly, or has already reached appreciable levels compared with neonatal pigs. According to measurements reported by Aumaitre (1972), by 5 weeks of age the piglet is producing significantly higher amounts of amylase, maltase and sucrase than at birth. Hudman *et al.* (1957) showed a rapid increase in pancreatic amylase activity from birth to 4 weeks of age. Manners and Stevens (1972) found that between 3 to 4 weeks of age there is a rapid increase in sucrase production. Kidder and Manners (1972) found that gastric acid is established by the seventh week of life, and pancreatic proteolytic activity increases from 4 to 36 days of age. Pancreatic lipase is high at birth, but still increases to 6 weeks of age (Kidder and Manners, 1972). Lewis *et al.* (1957) showed a linear increase in gastric pepsin activity from 3 to

6 weeks of age, Corring *et al.* (1978) showed that after 4 weeks of age, enlargement of pancreatic cells, as well as increases in the number of cells occurred, which coincided with more rapid development of pancreatic enzyme activity. The results reported by these authors suggest that at the age of 4 weeks, the piglet's digestive capacity is fast approaching that of an adult pig. In agreement with this, Officer (1995) saw no response of 4 to 5 week old piglets to enzyme supplemented diets and suggested that this was because endogenous enzyme activity was not limiting. However, other factors exist, apart from endogenous enzyme levels, which may influence piglets' performance post-weaning: a lack of response to lipase supplementation may be due to impaired fat utilization (absorption) rather than impaired fat digestion, since the addition of fat emulsifiers have been shown to improve apparent digestibility of fat during the post-weaning period (Jones *et al.*, 1992).

According to Dierick (1989), the variability and common lack of response by piglets to the addition of supplemental enzymes could be due to the denaturation of the enzymes in the stomach. Supplemental enzymes are proteins: polypeptide chains folded in a unique configuration which is maintained by various interactions between the constituent amino acid residues of the chain and the solvent in which the enzyme is dissolved: hydrogen and various covalent bonds, hydrophobic interactions between certain residues of the protein, or stabilizing disulfide bonds can all be affected by changes in temperature, osmolarity, or pH of the surrounding luminal environment (Webb, 1990). Changes in the ionization states of the amino acid residues, which may occur with changes in hydrogen ion concentration in the stomach, can potentially alter the chemical reactivity of the enzyme (Webb, 1990). In addition, the stabilizing interactions between amino acid residues are susceptible to denaturation and destabilization caused by a change in pH. Most pancreatic and mucosal enzymes have optimum pH ranges from 5.2 to 9.2, and would have minimal or zero activity in the very acid conditions of the stomach (the pH in gastric contents averages from 1 to 4 in a 5 week old piglet) (Webb, 1990; Kidder and Manners, 1978). Since levels of gastric acid increase gradually from 2 to 3 weeks of life and substantial levels of both are present by 7 weeks (Aumaitre, 1972; Kidder and Manners, 1972), denaturation of the enzyme proteins contained in an exogenous enzyme preparation could potentially occur in the relatively acid environment of the piglet's stomach.

Various ANF present in canola meal (eg., fiber and tannins) may decrease the digestibility of CP and amino acids. Several investigators (eg., Mitaru *et al.*, 1984;) have attributed the lower nutritive value of canola meal compared to soybean meal, to the relatively higher crude fiber content of the canola hulls. Lignin, a polymer of phenyl propyl alcohols and acids, is insoluble and hydrophobic. Lignin may adsorb amino acids by binding hydrophobically and witholding them from absorption (Mitaru *et al.*, 1984). A reduction in transit time due to dietary fiber could leave less time for digestion and absorption of dietary protein (Low, 1985; den Hartog *et al.*, 1989), assuming that the time required for these processes is a limiting factor. Fiber may increase the sloughing of intestinal cells leading to increased losses of endogenous nitrogen. Tannins may also bind to amino acids and decrease the access of digestive enzymes. Pectins (another fraction of the hulls) may form gels in the intestine, and may decrease protein digestibility reducing access to protein molecules by digestive enzymes, and the access of the products of digestion to absorptive sites (Low, 1985).

According to Leterme *et al.* (1996), unreliable partitioning of dietary components in the different fiber fractions as determined by fiber analysis methods such as the van Soest procedure (Goering and van Soest, 1970), may mean that NDF and ADF methods are unsuitable for determining fiber content in some feed ingredients, including legumes. The ADF fraction may include significant xylose and uronic acids components, when it should theoretically contain only cellulose and lignin; NDF residues may contain resistant starch. The van Soest procedure may not be appropriate for all feedstuffs, and the reliability of the results obtained with this procedure in the present study may therefore be questionable.

According to Jorgensen *et al.* (1984), and Piccaglia and Galletti (1987), freeze-drying or oven-drying of digesta or feces samples prior to chemical analyses may affect values obtained for apparent digestibilities. Jorgensen *et al.* (1984) found that more than 5% of the nitrogen in their samples was lost due to oven- or freeze-drying. Piccaglia and Galletti (1987) found that oven-drying of samples prior to fibre analysis could confound NDF results due to the condensation of lignin and carbohydrate during oven-drying. All of the digesta and fecal samples collected in the present experiment were freeze-dried prior to proximate analyses, which, according to the results of Jorgensen

et al. (1984) and Piccaglia and Galletti (1987), may have confounded the resultant digestibility values.

According to Li *et al.* (1993), decreases in ileal protein digestibility reported with increasing dietary CP level may be due to decreased efficiency of amino acid transport into the enterocyte, since amino acid absorption is active (i.e., Alpers, 1987), and involves many different transport systems. The absorptive capacity of these transport systems may be exceeded with an increase in dietary CP level. This was also suggested by Officer (1995), who claimed that a long term reduction in feed intake post-weaning observed in piglets fed a cereal-based diet supplemented with exogenous enzymes, could be due to the accumulation of the products of digestion in the intestinal lumen, thereby impairing the flow of digesta through the intestine. Since, according to Low (1980), apparent digestibility is actually a measure of the ability of the mucosal cells to absorb the products of digestion (rather than a measure of the actual digestive enzyme activity), digestibility values can be underestimated if the products of digestion are not absorbed, even though they may have been digested. Zebrowska (1973) found that the rate of absorption of amino acids was much slower than the rate of their release from the peptide chains. Therefore, the protease activity of the enzyme preparation used in this experiment may have enhanced proteolysis of the supplemented diet, however the level of enhancement may have been limited by absorptive capacity. The trend toward decreased digestibility of most amino acids (Table 3-3) after supplementation of enzymes to the canola meal diet could indicate a possible overloading of the gut absorptive capacity.

Amino acid sequence and the chemical nature of amino acids in protein affect the digestibilities of amino acids due to the specificity of digestive enzymes for certain peptide bonds (Sauer and Ozimek, 1986; Stryer, 1987). The relatively high apparent digestibilities of arginine and lysine which were observed in this study (Table 3-3) tend to support the hypothesis that enzyme specificity is an important determinant of amino acid digestion and/or absorption (Li, 1992). According to Low (1980), arginine and lysine would be expected to appear first after enzymatic hydrolysis of the protein molecule, due to the known specificity of most intestinal and pancreatic proteases. Subsequently, these amino acids are the first available for absorption.

In this study, there was no improvement in the digestibility of CP or amino acids with the supplementation of protease enzymes. Fan*et al.* (1994) reported that amino acid digestibilities reach a plateau when the threshold levels of dietary amino acids are exceeded. The threshold levels are characteristic of the diet, but do correspond to a dietary CP level. The diet composition of the study by Fan *et al.* (1994) is different from that of the present study, however the protein quality of canola meal compared to soybean meal may be such that the threshold CP levels reported by Fan *et al.*, (1994) (past which changes in amino acid digestibility values become independent of the dietary amino acid/CP levels) may still be applicable to the present study. According to Lin *et al.* (1987), and Knabe *et al.* (1989), the dietary amino acid digestibility values of canola meal stays relatively constant over a wide range of dietary CP levels, compared to soybean meal; therefore despite the relatively lower amino acid digestibility of canola meal, it may be superior in protein quality (Lin *et al.*, 1987; Knabe *et al.*, 1989). Therefore, the threshold CP levels (Fan *et al.*, 1994) for soybean meal may also apply to canola meal, since the amino acid digestibility values for canola meal are likely to stay relatively constant over a wide range of dietary CP levels. In the present study, the dietary CP levels were higher for most amino acids than the threshold levels reported by Fan *et al.*, (1994); therefore, protease supplementation to the canola meal diet may not have improved amino acid digestibilities in young pigs possibly because the threshold levels of dietary CP (amino acids) may have been exceeded.

The amino acid content of endogenous secretions may affect the apparent ileal digestibility values of amino acids (Fan *et al.* 1994; 1995). The relatively low ileal digestibilities of threonine and glycine found in this study (Table 3-3) may result in part from their relatively high concentrations in endogenous secretions (Sauer and Ozimek, 1986; Li *et al.*, 1993). Glycine is a major component of bile salt conjugates, and accounts for more than 90% of the total amino acids secreted in bile juice (Souffrant, 1991). While most of the bile salts are reabsorbed via the entero-hepatic circulation, de-conjugated glycine escapes reabsorption and is fermented in the large intestine (Li *et al.*, 1993). Therefore, the content of glycine in ileal digesta may account for the relatively low apparent ileal digestibility of this amino acid. Similarly, mucin protein is rich in threonine (Neutra and Forstner, 1987). Dietary fiber has been shown to increase the production of mucin and other endogenous

secretions (Low, 1985); therefore, the relatively high amounts of crude fiber in canola meal may have contributed to the relatively high amounts of mucin protein in ileal digesta, as evidenced by the relatively low apparent ileal digestibility value for threonine found in this experiment. According to Neutra and Forstner (1987), small intestinal secretions, including bile juice and mucous, supply the largest proportion of endogenous nitrogen to the endogenous nitrogen secretions in the small intestine.

Intestinal bacteria may contribute to inaccurate estimations of nutrient digestibility. According to Low (1980), a nutrient may appear to have been digested and absorbed because its presence may not be detected during chemical analysis of digesta, however it may actually have been altered in some way by intestinal bacteria to another compound which may not have been measured. For this reason, nutrient digestibilities are correctly referred to as 'apparent' digestibilities. The production of organic acids by microbes is an example of the alteration of nutrients by microbial fermentation. While this occurs predominantly in the large intestine and is the basis for the accuracy and sensitivity of the ileal analysis method for measuring digestibility coefficients over the fecal analysis method, populations of microbes do exist in the small intestine (Friend *et al.*, 1963; Kidder and Manners, 1978; Just *et al.*, 1983; Millard and Chesson, 1984; Luckey, 1987; Bergman, 1990), and may confound digestibility values.

The net disappearance of the parameters measured in the large intestine is presented in Table 3-5, expressed quantitatively as g/kg dry matter intake (DMI). The disappearance of starch in the large intestine was reduced ($P<0.05$) in the CWE diet. Of the dispensable amino acids, aspartic acid, glutamic acid, glycine, threonine, and leucine were among those amino acids which showed the greatest disappearances in the large intestine. These results agree with those of Li *et al.* (1993), who found that the amino acids found in endogenous secretions (glycine, threonine, leucine) disappeared to the greatest extent in the large intestine. Protein secreted via pancreatic and small intestinal juice contains a relatively large proportion of aspartic acid, glutamic acid, and leucine (Corring and Jung, 1972; Li, 1992). The preferential fermentation of undigested endogenous protein and/or amino acids by the microflora in the large intestine may be part of a mechanism whereby the host animal is able to recover those amino acids lost as endogenous protein, in the form of ammonia. Under certain conditions (i.e., when dietary

nitrogen *per se* is limiting for the synthesis of dispensable amino acids), absorbed ammonia may contribute to the protein status of the pig (Li, 1992).

In conclusion, the addition of enzymes to diets containing canola meal fed to young pigs improved the ileal digestibility of starch to a small extent, but failed to improve the ileal digestibility of crude protein or other dietary nutrients. The types and/or dosage of the enzymes used in this study may have been inappropriate for the experimental diets; alternatively, the piglets used in this study may have been too mature in terms of digestive capacity. The NSP in canola meal tends to reduce the ileal digestibility of crude protein and amino acids in canola meal diets, in particular when these are fed to young pigs, due in part to relatively immature digestive capacity. However, the hydrolytic action of the xylanase used in this experiment may have cleaved sufficient numbers of glycosidic bonds of cell wall NSP, allowing the release of starch. or the digestion and/or absorption of dietary nutrients in the canola meal diets may not have been a limiting factor.

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Table 3-1. Formulation (% as fed) of Diets Containing Canola Meal Fed to Young Pigs

	Diets[a]	
	CW	CWE
Ingredients		
Canola meal	22.3	21.9
Wheat	71.1	71.5
Canola oil	3.2	3.2
Enzyme Mixture[b]		0.1
Dicalcium Monophosphate	0.6	0.5
Calcium Carbonate	0.8	0.8
Salt	0.3	0.3
Lysine-HCl	0.5	0.5
Vitamin premix[c]	1.0	1.0
Chromic oxide[d]	0.2	0.2
Total	100.0	100.0

[a] CW=canola meal + wheat; CWE=CW diet + enzymes

[b] Contains 6250 units/g of xylanase from *Trichoderma longibrachiatum*, and 3000 units/g of protease from *Bacillus subtilis*. Finnfeeds International, Box 777, Marlborough, Wiltshire, United Kingdom SN8 1XN.

[c] Provided per kg premix: vitamin A, 10^6 IU; vitamin D_3, 10^5 IU; vitamin E, 8000 IU; choline, 10^5 mg; Fe, 15,000 mg; Zn, 12,000 mg; niacin, 4,000 mg; pantothenic acid, 2,500 mg; Cu, 2,000 mg; riboflavin, 1,200 mg; Mn, 1200 mg; menadione, 200 mg; folic acid, 160 mg; Se, 30 mg; biotin, 25 mg; iodine, 20 mg; vitamin B_{12}, 3 mg. BASF Canada Inc., Regina, SK S4N 5X9.

[d] Chromic oxide powder, Fisher Scientific, Fair Lawn, NJ 07410.

Table 3-2. Partial Chemical Analyses of the Experimental Diets Containing Canola Meal Fed to Young Pigs

	Diets*	
	CW	CWE
Crude Protein (g/kg)	168.90	161.60
Dry Matter (%)	87.73	87.81
Ash (%)	4.58	4.46
Digestible Energy (Mcal/kg)	3.347	3.349
Starch (%)	39.30	41.22
Fiber (%):		
NDF	22.41	22.38
ADF	7.83	7.76
Hemicellulose	14.59	14.62
Cellulose	4.49	4.45
Amino Acids (%):		
Indispensable		
Arginine	0.913	0.826
Histidine	0.428	0.408
Isoleucine	0.592	0.581
Leucine	1.157	1.138
Lysine	1.178	1.095
Phenylalanine	0.712	0.699
Threonine	0.675	0.644
Valine	0.755	0.746
Dispensable		
Alanine	0.748	0.733
Aspartic acid	0.987	0.982
Glutamic acid	4.059	3.991
Glycine	0.89	0.834
Serine	0.757	0.727
Tyrosine	0.427	0.352

* CW= canola meal + wheat; CWE= CW diet + enzymes

Table 3-3. Apparent Ileal Digestibilities (%) of Dry Matter, Crude Protein, Gross Energy, Starch, Fiber and Amino Acids in Diets Containing Canola Meal Fed to Young Pigs

	Diets[a]		
	CW	CWE	SEM[b]
Dry Matter	68.7	67.7	1.01
Crude Protein	72.7	68.7	1.91
Gross Energy	69.2	68.6	1.19
Starch	95.9[c]	98.3[d]	0.29
Fiber:			
NDF	39.2	44.5	4.03
ADF	29.9	24.6	1.58
Hemicellulose	44.4	55.3	6.45
Cellulose	32.7	28.9	6.60
Amino Acids: Indispensable			
Arginine	83.4	80.2	1.96
Histidine	80.6	79.1	3.19
Isoleucine	71.0	68.4	4.14
Leucine	79.0	73.9	2.35
Lysine	80.3	75.4	2.69
Phenylalanine	78.5	75.6	2.72
Threonine	68.8	61.6	3.45
Valine	58.0	53.5	5.51
Dispensable			
Alanine	71.6	66.3	3.49
Aspartic Acid	63.8	58.8	4.34
Glutamic Acid	87.8	86.5	1.40
Glycine	62.4	57.1	2.73
Serine	78.9	72.2	2.01
Tyrosine	75.2	64.7	3.57

[a] CW=canola meal + wheat; CWE=CW diet + enzymes.
[b]Standard error of the least squares means; 8 observations per treatment.
[c,d]Means within a row having different superscripts are significantly different ($P<0.05$).

Table 3-4. Apparent Fecal Digestibilities (%) of Dry Matter, Crude Protein, Gross Energy, Starch, Fiber and Amino Acids in Diets Containing Canola Meal Fed to Young Pigs

	Diets[a]		
	CW	CWE	SEM[b]
Dry Matter	84.9[c]	86.0[d]	0.29
Crude Protein	80.3	81.4	0.84
Gross Energy	83.5	84.2	0.42
Starch	99.5[c]	99.8[d]	0.06
Fiber:			
NDF	69.1	71.4	1.69
ADF	52.3	54.2	3.54
Hemicellulose	78.6	79.9	1.30
Cellulose	43.9	55.8	4.25
Amino Acids:			
Indispensable			
Arginine	91.4	89.2	1.52
Histidine	89.0	86.9	1.96
Isoleucine	80.3	77.9	2.86
Leucine	86.7	84.8	1.96
Lysine	88.2	86.2	1.89
Phenylalanine	87.3	85.1	2.00
Threonine	79.6	77.6	2.64
Valine	74.4	70.9	3.90
Dispensable			
Alanine	81.9	79.9	2.61
Aspartic Acid	76.4	74.3	2.80
Glutamic Acid	92.7	92.1	0.90
Glycine	81.4	79.5	2.49
Serine	85.7	84.4	1.40
Tyrosine	85.7	81.0	2.81

[a] CW=canola meal + wheat; CWE=CW diet + enzymes.
[b] Standard error of the least squares means; 8 observations per treatment.
[c,d] Means within a row having different superscripts are significantly different ($P<0.05$).

Table 3-5. Disappearance (g kg^{-1} DMI) of Dry Matter, Crude Protein, Gross Energy (kcal kg^{-1}), Starch, Fiber and Amino Acids in the Large Intestine of Young Pigs Fed Diets Containing Canola Meal

	Diets[a]		
	CW	CWE	SEM[b]
Dry Matter	162.15	183.05	11.01
Crude Protein	11.23	18.03	3.49
Gross Energy	516.15	559.99	47.49
Starch	12.32[c]	5.40[d]	1.16
Fiber:			
NDF	64.06	74.45	17.16
ADF	16.83	24.85	3.57
Hemicellulose	47.23	46.79	12.10
Cellulose	9.52	16.35	2.63
Amino Acids:			
Indispensable			
Arginine	0.65	0.66	0.19
Histidine	0.32	0.29	0.12
Isoleucine	0.49	0.49	0.24
Leucine	0.78	1.09	0.32
Lysine	0.82	1.05	0.35
Phenylalanine	0.55	0.59	0.21
Threonine	0.65	0.92	0.22
Valine	1.09	1.15	0.42
Dispensable			
Alanine	0.69	0.88	0.31
Aspartic Acid	1.06	1.34	0.43
Glutamic Acid	1.75	1.96	0.55
Glycine	1.50	1.75	0.30
Serine	0.46	0.78	0.17
Tyrosine	0.40	0.51	0.16

[a] CW=canola meal + wheat; CWE=CW diet + enzymes.
[b]Standard error of the least squares means; 8 observations per treatment.
[c,d]Means within a row having different superscripts are significantly different ($P<0.05$).

4. General Discussion and Conclusions

This study investigated the effects of enzyme (xylanase plus protease) supplementation to diets for young pigs. Numerous studies (eg., Calder *et al.*, 1959; Baird *et al.*, 1976; Inborr and Ogle, 1988; Bedford *et al.*,, 1992; Cowan *et al.*, 1996; Li *et al.*, 1996) have reported the effects of supplemental enzymes on apparent digestibilities of crude protein, amino acids and other nutrients in diets for young pigs. The use of exogenous enzymes supplemented to diets for young pigs may contribute to the reduction in the severity of the post-weaning growth-check period (Li, 1992), which is characterized by reduced nutrient digestibility in young pigs, the development of diarrhea, and delays in growth which adversely impact pork production. Many authors (eg., Aumaitre and Corring, 1978; Corring *et al.*, 1978; Kidder and Manners, 1978) have concluded that reductions in nutrient digestibility and performance of piglets post-weaning is due to the immaturity of the digestive system of the young pig. Until approximately 8 to 10 weeks of age, the secretion of many intestinal and pancreatic enzymes (including proteases) is low in the young pig (Aumaitre and Corring, 1978; Corring *et al.*, 1978). Therefore, augmentation of the digestive capacity of the young pig may be possible with the use of exogenous enzymes (Dierick and Decuypere, 1994).

The use of enzymes such as beta-glucanase and xylanase in poultry diets is well documented (eg., Hastings, 1946; Burnett, 1966; Groot Wassink *et al.*, 1989; Bedford *et al.*, 1991; Bedford and Classen, 1992). These studies have usually concluded that reductions in viscosity of digesta due to supplementation of diets with enzymes results in improved nutrient digestibility and performance of poultry, compared to poultry fed unsupplemented diets. However, the digesta of pigs is not as viscous as that of poultry (eg., Bedford and Schulze, 1998; Simon, 1998); many authors (eg., Bedford and Schulze, 1998) feel that the viscosity of digesta does not limit the digestibility of protein and other nutrients in pigs.

In the present study, supplementation of a diet containing canola meal plus wheat with an enzyme mixture containing xylanase plus protease activities resulted in a small improvement ($P<0.05$) in the apparent ileal digestibility of starch. It is possible that the NSP-hydrolyzing action of xylanase may have contributed to the release of starch from encapsulation within structural NSP (Chesson, 1987). However, there was no effect on the

apparent ileal digestibility of dietary protein or any amino acids. This may be because the secretion of pancreatic proteases in young pigs of approximately 5 to 7 weeks of age (the age of the pigs used in this study at the time of collection of digesta and feces) is not limiting (Officer, 1995). Despite the relative immaturity of the young pig's digestive capacity compared to a mature animal, secretion of digestive enzymes in the 5 week old piglet may be sufficient, so that nutrient digestibility cannot be improved by augmenting the existing enzyme levels with exogenous enzymes. Alternatively, the digestion and/or absorption of dietary nutrients may not have been a limiting factor.

The present study is one of the first to supplement proteases to diets for young pigs. Other studies which have investigated the effects of protease supplementation to diets for young pigs have usually involved the use of soybean meal, a traditionally used protein source (eg., Rooke *et al.*, 1996; Caine *et al.*, 1997). The digestibility of soybean meal protein in young pigs is already higher than some other protein sources used in diets for young pigs (eg., Jorgensen *et al.*, 1984; Fan *et al.*, 1995). A lack of significant response to protease supplementation of soybean meal diets seen in young pigs could be due to the relatively high digestibility of soybean meal, which results in less room for a significant improvement in protein digestibility. The present study is one of the first to investigate the effects of protease supplementation to diets containing canola meal for young pigs. One other recent study (Zijlstra *et al.*, 1998) which reported the effects of protease supplementation to diets containing canola meal fed to young pigs, found no effects on nutrient digestibility. Since the digestibility of protein in canola meal is usually lower than the digestibility of protein in soybean meal, there may be more room for improvement of protein digestibility with the use of supplemental enzymes. However, the level of CP and most amino acids in the experimental diets was in excess of the threshold levels (past which amino acid digestibility does not change) of CP and amino acids reported by Fan *et al.* (1994). Enzyme supplementation to canola meal diets may not have improved the digestibility of dietary CP and amino acids, possibly because the threshold levels of CP and amino acids was exceeded. Furthermore, the lack of response of protein digestibility to protease supplementation to canola meal diets seen in the present study and also seen by Zijlstra *et al.* (1998) could suggest that the

secretion of endogenous proteases may not be a limiting factor in the digestion of dietary proteins by young pigs.

Several investigators (eg., Officer, 1995) have suggested that endogenous enzyme secretion may not limit nutrient digestibility in young pigs. However, endogenous enzyme secretion may be limiting immediately post-weaning, when piglets must experience a sudden change in diet, from primarily milk protein to plant protein starter diets. According to Aumaitre (1972), and Corring (1980), the adaptation of the intestine of the piglet to a change in diet composition is rapid. However, during this time the production of many intestinal and pancreatic enzymes may not be sufficient for the efficient digestion of dietary proteins. The use of supplemental enzymes during periods of intestinal adaptation to a change in diet (such as the immediate post-weaning period) in order to augment digestive capacity until adaptation has occurred, may prevent decreased performance due to reduced nutrient digestibility, and possibly lessen the severity of the post-weaning growth check.

In conclusion, this study shows that supplementation of diets containing peas or canola meal with a xylanase-protease enzyme mixture did not improve the apparent ileal digestibility of dietary nutrients, except for an improvement ($P<0.05$) in the digestibility of starch in the diet containing canola meal. Production of endogenous enzymes may not have been limiting. The use of supplemental enzyme mixtures in diets for young pigs may be more applicable to the immediate post-weaning period, before the intestine has fully adapted to the change in diet composition which occurs at weaning.

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