# UTILIZATION OF PEAS (Pisum sativum) BY EARLY-WEANED PIGS

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by

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UTILIZATION OF PEAS (Pisum sativum) BY EARLY-WRANED PIGS

BY

#### AUGUSTINE OWUSU-ASIEDU

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University

of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

AUGUSTINE OWUSU-ASIEDU @1998

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# **DEDICATION**

To Mrs Comfort Badu Boateng and Joyce Owusu-Ankomah for their endless support

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#### **ABSTRACT**

Four experiments were conducted to evaluate the utilization of peas and to determine the value of peas as a protein supplement for early-weaned pigs. Apparent and true ileal and total tract digestibility of protein and amino acids in raw peas supplemented with amylase, amylase + xylanase and amylase + xylanase + protease in early-weaned pigs fitted with a simple-T cannula at the terminal ileum were determined in experiment 1. The effect of amylase, amylase + xylanase and amylase + protease + xylanase supplementation on apparent and true ileal and faecal digestibility of protein and amino acids in extruded peas in early-weaned pigs fitted with simple-T cannula in the ileum was determined in experiment 2. The effect of amylase, amylase + xylanase, and amylase + xylanase + protease inclusion on apparent and true digestibility of protein and amino acids in micronized pea in early-weaned pigs fitted with simple-T cannula was evaluated in experiment 3. Experiment 4 was carried out based on the results of the first three experiments. Productive parameters were studied in 16-d old pigs (n=70) fed 7 dietary treatments; soybean control, raw, extruded and micronized peas supplemented with amylase and xylanase, with 5 pens per treatment in a completely randomised design. All diets were formulated using standard value of ingredients to have similar digestible energy and ideal protein. Pigs were fed 4% of their body weight and ad libitum for the digestibility and performance studies respectively. Blood samples were collected from the jugular vein at the start, end of phase 1 (4.0 - 10.0 kg liveweight) and phase 2 (10.0 - 20.0 kg liveweight) to

determine plasma urea nitrogen (PUN) level during the performance trial. With the exception of apparent ileal digestibility (AID) of threonine and threonine, cysteine and glycine in raw and extruded peas respectively, AID and true ileal digestibility (TID) of crude protein were not affected (P > 0.05) by enzyme supplementation of raw, extruded or micronized peas. AID of threonine and glycine increased significantly (p < 0.05) with amylase, amylase + xylanase but not amylase + xylanase + protease supplementation of raw peas. There were however, about 2 -6% non-significant (p > 0.05) improvements in AID and TID of raw, extruded and micronized peas with amylase, amylase + xylanase but not with the inclusion of the combination of amylase, xylanase and protease cocktail enzymes. Addition of amylase + xylanase to raw, extruded or micronized peas had no significant (p>0.05) influence on daily feed intake and weight gain. Pigs fed enzyme supplemented had better (p < 0.05) feed efficiency during starter phase 1 (4.0 -10.0 kg liveweight) but not starter phase 2 (10.0 - 20.0 kg liveweight). PUN levels were significantly (p < 0.05) reduced for the amylase and xylanase supplemented and the peas fed pigs compared to soybean fed pigs. It was concluded that amylase and xylanase are beneficial when raw peas are fed to 16-d old weaned pigs (4.0 - 10.0 kg liveweight) but not for older pigs. In addition, enzyme supplementation is not necessary when feeding extruded or micronized peas to early-weaned pigs. Furthermore, feeding a combination of pea/soybean meal to 16-d old weaned pigs also resulted in better protein utilization compared to soybean meal alone.

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#### LIST OF ABEVIATIONS

AA Amino acid

AD Apparent digestibility

ADFI Average daily feed intake

ADG Average daily gain

AFD Apparent faecal digestibility

AID Apparent ileal digestibility

ANF Anti-nutritional factors

AOAC Association of Official Analytical Chemists

CP Crude protein

DE Digestible energy

DM Dry matter

FCE Feed conversion efficiency

GLM General Linear Modelling

LMW Low molecular weight

NF Nitrogen free

NSP Non-Starch Polysaccharides

PROT Protease

PUN Plasma urea nitrogen

SAS Statistical analysis system

SEM Standard error of the mean

TFD True faecal digestibility

TID True ileal digestibility

#### 1.0 INTRODUCTION

In recent years, increasing emphasis has been placed on early weaning of piglets. This trend is compelled by factors such as improving the number of pigs per sow per year, the need to optimize swine farrowing facilities by pushing more sows through the unit, thereby reducing capital cost and eliminating disease transmission from sow to the piglets. Weaning as early as three weeks and in large production complexes two weeks of age has become common. The earlier piglets that are weaned, the greater the need for more complex diets to minimizes the post-weaning lag in growth (Okai et al., 1976). Baby pigs have relatively undeveloped gastrointestinal tracts (Cranwell, 1995), and therefore require highly digestible feed ingredients.

Over the years, feed ingredients like spray-dried porcine plasma, fish meals (herring meal and menhaden meal), milk and milk products, as well as refined soybean protein have been used with a greater degree of success (Kats et al., 1992). However, prices of these conventional protein sources are not only expensive, but have to be imported. This therefore, calls for the need to identify alternative protein sources, which can be fed alone or in combination with other protein sources. The prerequisite is that the alternative ingredient be agronomically efficient, locally available and above all, possesses nutrient profiles compatible with the animal's requirements.

Peas (*Pisum sativum L.*), for a long time has been recognized as a rich source of protein with high lysine content and energy for animal feed. With their comparatively high protein, essential amino acids and energy (Daveby et al., 1993;

Igbasan et al., 1996, 1997), coupled with a historic trend to increase production of peas in Western Canada (Slinkard, 1994; Miller, 1996), peas still remain an under-utilized feed ingredient in swine, especially in early-weaned pig diets. Considerable variation in crude protein content, low levels of sulphur-containing amino acids (Savage and Deo, 1989, NRC, 1998) and the presence of a number of anti-nutritional factors (Marquardt and Bell, 1988; Gatel and Grosjean, 1990), are among the few reasons cited for the under utilization of peas in early-weaned pigs. However, studies have shown that relatively high levels off peas are well used by growing pigs (Myer and Froseth, 1983; Kehoe et al., 1991) and poultry (Igbasan and Guenter, 1996a, 1997a), with proper processing methods (Poel et al., 1992; Igbasan and Guenter, 1996b, 1997b), amino acids and enzyme supplementation (Igbasan and Guenter, 1997b).

Numerous studies have looked at the nutritional values of peas for livestock and poultry (Fan et al., 1994a; Igbasan and Guenter, 1996a; 1997a) and stated the guidelines and levels of inclusion for different classes of swine (Gatel and Grosjean, 1990; Gatel, 1994; Castell et al., 1996). Myer and Froseth (1983), substituted all of the soybean meal in grower diets with cull peas (43.5%) without adverse effect on pigs fed from 20 - 41 kg live weight. Further study also showed that peas with low trypsin inhibitor activity could constitute up to 45% in weaned and growing pig diets (Grosjean and Gatel, 1989). Gatel et al., (1989) and Zivkovic et al., (1987), observed improved performance when raw peas were supplemented with methionine (first limiting amino acid in peas) and tryptophan respectively in starter pig diets. Subsequent studies however, showed that inclusion of raw peas

in diets of pigs weaned at three weeks of age resulted in reduced feed intake and growth depression (Bengala-Friere et al., 1989; Gatel and Grosjean, 1990). Recently, Kehoe et al., (1991) and Igbasan and Guenter (1996b; 1997b), showed that relatively high levels of peas are well utilized by growing pigs and poultry respectively, if the dietary amino acid requirements are met, and with proper processing methods. There is however, little or no literature information available on the use of peas in early-weaned pig diets supplemented with enzymes ( $\alpha$ -amylase and xylanase). Considering the lower levels of enzyme secretion in baby pigs, addition of exogenous enzymes and as well as synthetic amino acids to peabased diets fed to this group of pigs may be beneficial.

Like any other pulse and legume, peas contain a number of anti-nutritional factors (Savage and Deo 1989). These include; protease inhibitors, tannins and lectins or haemagglutinins (Bender, 1983; Griffiths, 1984). Nevertheless, these are inactivated by numerous processing methods. Most common techniques include cooking, pelleting, steam-flaking, extrusion and micronization. The latter has the advantage of being very quick and continuous. van der Poel (1990), reported reduction in trypsin inhibitor capacity of extruded peas without loss in availability of lysine or pepsin nitrogen solubility. A significant improvement in bioavailable energy, apparent protein and starch digestibilities were observed in birds fed micronized peas (Igbasan and Guenter 1996b). They also reported faster growth rate and better-feed conversion in these birds than that of birds fed untreated peas and wheat-soybean controlled diets. Supplementing diets containing 800 g/kg peas with pectinase has recently been reported to increase weight gain by 7.3%

units, above non-supplemented diets in chickens (Igbasan and Guenter, 1997b).

There is little information in early-weaned pig nutrition research on the use of processed peas with exogenous enzyme supplementation.

#### The objectives of the present study include:

- 1. Determination of true and apparent iteal and faecal digestibility of protein, amino acids and energy of raw peas supplemented with or without either  $\alpha$ -amylase,  $\alpha$ -amylase + xylanase or  $\alpha$ -amylase + protease + xylanase in 16-day old pigs.
- 2. Determination of true and apparent iteal and faecal digestibility of protein, amino acids and energy in extruded peas supplemented with or without  $\alpha$ -amylase,  $\alpha$ -amylase + xylanase or  $\alpha$ -amylase + protease + xylanase in early-weaned pigs.
- 3. Determination of true and apparent, iteal and faecal digestibility of protein, amino acids and energy in micronized peas supplemented with  $\alpha$ -amylase,  $\alpha$ -amylase + xylanase and  $\alpha$ -amylase + protease + xylanase in early-weaned pigs.
- 4. Performance of early-weaned (16-d old) pigs fed raw, extruded or micronized pea-based diets supplemented with α-amylase and xylanase.

The studies will provide useful information on the nutritional value of peas supplemented with enzymes in early-weaned pigs for both animal producers and feed manufacturers. It will also help compare the two most widely used processing

methods (extrusion and micronization) on nutrient utilization in early-weaned pigs and finally whether the inclusion of peas will reduce feed cost per gain. These will not only enable peas to compete more effectively with conventional protein sources but also, help develop a stable market for Canadian-grown peas.

#### 2.0 LITERATURE REVIEW

#### **Baby Pig Nutrition**

The digestive tract of the young pig changes considerably in the first few weeks of the pig's life. These changes include changes in salivary  $\alpha$ -amylase, gastric, pancreatic and small intestine secretions (Cranwell, 1995). Absorption capacity of the intestine is relatively low at this stage. The ability of newly weaned pigs to assimilate nutrients from non-milk based diets is limited by a delay in the adaptation of the gastrointestinal tract to secretion of sufficient enzymes to fully support digestive processes (Cranwell, 1995). Lactose in milk is the main source of energy in the young piglets' diet before weaning. Starch is the main energy source in the weaned pig diet and should be easily digested. As a result of insufficient production of enzymes such as amylase and protease the accessibility of the starch granules is reduced. Excess indigestible starch may result in scouring. Morphological studies have shown that transient hypersensitivity and atrophy of the intestinal mucosa at weaning greatly reduces the capacity of pigs to digest plant-based protein sources such as peas (Cranwell, 1995). The gastrointestinal system has to adapt to the considerable changes in the physiochemical properties of their feed as well as to changes in the pattern of intake in order to satisfactorily digest and absorb nutrients and maintain an acceptable growth rate (Cranwell, 1995; Jensen et al., 1994). Moughan et al., (1992) for example reported that digestibility of fat at weaning decline to 65 - 80% compared to 96% in suckling pigs.

The presence of trypsin inhibitors in soybean meal causes inactivation and hyper-secretion of pancreatic proteolytic enzymes and subsequent loss of endogenous protein in non-ruminant animals, especially baby pigs (Tamminga et al., 1995), and reduce protein digestibility in young pigs. Protease inhibitors and lectins are protein containing complexes that may be susceptible to proteolytic degradation and inactivation by protease enzymes. Numerous companies are currently developing enzymes that are specific for use in the animal industry. The efficacy of these enzymes are however, questionable, for example, Caine et al., (1997) reported no significant differences in the apparent amino acids digestibility in newly weaned pigs fed diets containing protease-treated and untreated soybean meal. Addition of enzymes (amylase, protease or xylanase) to either raw, extruded or micronized peas-based diets may be beneficial to newly weaned pigs. The effects of these enzymes or cocktails of these enzymes on utilization of processed and unprocessed peas will be studied in young pigs.

#### **Pea Production Status**

Feed peas belong to the family of cool season legume crops commonly referred to as pulses, which include chickpeas, faba beans, kidney beans and lentils. Peas have been valued for their nutrient composition since they were first cultivated. It is only in the last century that their actual nutrient attributes became known to add credibility to their reputation as a high quality feed source. Peas have been grown to a limited extent in Western Canada ever since farmers started

Table 1. Area, production and exports of peas in Western Canada, 1985 - 1997

Year	Area (Ha)	Production (T)	Export (T)
1985	74, 400	168,800	102,700
1986	131,400	238,900	103,400
1987	236,700	415,000	288,600
1988	271,100	319,700	198,500
1989	149,700	234,100	165,800
1990	123,400	264,000	117,100
1991	198,400	409,700	270,000
1992	263,000	498,000	296,800
1993	505,900	970,200	692,000
1994	696,100	1,409,000	908,000
1995	819,500	1,454,700	978,000
1996	619,200	1,394,700	940,000
1997	700,000	1,575,000	1,000,000

Slinkard (1994); Miller, (1996); Special Crop Report, (1997); UNIP, (1998).

cultivating the prairies over a century ago, with production concentrated in Manitoba, Saskatchewan and Alberta (Slinkard, 1994). Dry pea production has increased rapidly since 1985 (Miller, 1996). The real goal was the opening of the European pea market leading to high prices. As a result, pea harvest increased from 74,400 ha in 1985 to 131,400 ha in 1986 (Table 1). Dry pea harvest again doubled in 1987, while a 38 and 18% increase in pea harvest was observed in 1994 and 1995, respectively (Miller, 1996). The major reasons for this dramatic increase in production is that the net returns from dry pea production have been much greater than that from red spring wheat production, especially in the black soil zone. Emphasis on crop diversification, crop rotation, value added processing, new industries in the rural areas, and sustainability of agriculture in Western Canada are among other driving factors for the increased production.

#### **Composition of Peas**

The value of any feed ingredient in animal nutrition is determined by its ability to supply protein and/or energy in the proportions and at levels required by the animal. The value of peas is associated with the ability of the pulse to substitute for a portion of the supplemental protein and energy to produce results equal to those obtained with conventional protein and energy sources, but at a lower cost of production. Pea seeds, generally considered as protein supplement consist of two major components that differ in compositions and proportions. The seed coat (hull) represents 70 - 140 g/kg and consists mostly of non-starch

Table 2. Chemical composition of Feed Peas (90% DM basis)

Average (%)	
22.60	
0.56	
8.19	
16.65	
0.85	
46.08	
3.30	
1.20	
0.70	

Based on data from: Rhone-Poulenc Animal Nutrition, 1993; Saskatchewan Feed Testing Lab 1990; Igbasan and Guenter, 1997a; NRC, 1998).

protein with values of 450 and 250 g/kg respectively. Table 2, is a summary of the mean chemical composition of pea-seed. There are trace amounts of ash (3.30%), ether extract (1.38%) and crude fibre (5.5%), (Daveby et al., 1993; Igbasan et al., 1996; Savage and Deo, 1989).

#### Protein and Amino Acid

Comparative proximate analysis have shown that peas are intermediate sources of protein to feed grain and canola meal or soybean meal which are considered as conventional protein-rich ingredients for monogastrics. The crude protein content of peas varies from 19.0 to 39.7% (Table 3). The wide variation in protein content has been attributed to variety, location and year of cultivation, predominant growing conditions, time of harvest, stage of maturity as well as nitrogen fertilizer application (Savage and Deo, 1989; Igbasan et al., 1996). Kossen et al., (1994) reported a crude protein value of 19 - 30% for smooth and 27 - 28% for wrinkle cultivars. Gdala et al., (1992) on the other hand reported a range of 22 - 27% for white and 21 - 26.6% for coloured-flowered pea cultivars. Recently, Igbasan and Guenter (1996a) reported a range of 20.7 - 26.5% for twelve cultivars of peas. The average protein content of peas is reported to be 22.5% (NRC, 1998).

On the basis of their solubility, two main protein fractions of peas have been isolated. The water-soluble fraction, albumin, constitutes about 20% and the salt-soluble fraction, globulins, about 80%. Compositions of amino acids also differ

Table 3. Crude protein content and amino acids composition of whole seed protein of peas.

Amino acid	Content
Crude protein (% DM)	19.0-39.7
Amino acid (g/16g N DM)	
Essential	
Arginine	6.8-14.9
Histidine	1. <del>9-4</del> .8
Isoleucine	2.4-6.2
Leucine	4.2-10.9
Lysine	4.6-12.3
Methionine	0.8-2.8
Phenylalanine	2.9-6.9
Threonine	2.8-6.8
Tryptophan	0.8-1.9
Valine	2.8-7.0
Non-Essential	
Alanine	2.8-7.4
Aspartic acid	7.8-18.9
Cysteine	0.2-3.5
Glutamic acid	11.1-27.9
Glycine	2.9-7.3
Proline	2.5-6.0
Serine	2.9-7.8
Tyrosine	1.9-5.5

Based on data from: Savage and Deo, 1989; Leterme et al., 1990; Kossen et al., 1994; Igbasan et al., 1996, 1997; NRC, 1998.

between albumin and globulins (legumin and vicilin). Albumin includes most of the enzymic and metabolic proteins (Owusu-Ansah and McCurdy, 1991). Compared to globulins, albumin has a more favourable amino acid profile, with higher levels of the sulphur-containing amino acids and tryptophan (Leterme et al., 1990).

The nutritive value of dietary protein depends on the profile and bioavailability of essential amino acids. It is well recognized that deficiency of dietary essential amino acid results in reduced feed intake and consequently retarded growth. Table 3 shows the amino acid composition of pea seed. The amino acid composition of pea seed is characterized by relatively high levels of lysine (6.84 - 7.98 g/16g N) and low levels of methionine and cysteine (2.2 - 3.02 g/16g N), similar to other legumes (Leterme et al., 1990; Igbasan et al., 1996). In practical swine diets, lysine is generally the first limiting amino acid (NRC 1998). Reddy et al., (1979) showed that methionine is the first limiting amino acid in peabased diets. Arginine, aspartic acid, glutamic acid and the amide in peas constitute about 41% of the total amino acid composition. This has been found to increase significantly with increasing nitrogen content. Lysine and leucine only constitute 15%, while histidine, methionine, threonine, tryptophan and cysteine account for 11% (Leterme et al., 1990; Igbasan and Guenter, 1996a). Compared to cereal grains, peas contain much more lysine and less methionine and cysteine (NRC, 1998). Peas and cereal grains are therefore, nutritionally complementary in supplying amino acids to meet the requirements of monogastrics.

## **Pea Protein Quality**

The protein quality or biological value of peas resembles that of legume seed in general. Low digestibility, presence of ANF and relatively poor levels of sulphur-containing amino acids are among other factors reducing pea protein quality (Igbasan et al., 1996), Protein quality test evaluation showed that peas have smaller protein efficiency ratio (PER) and chemical scores than soybean meal. Davis (1981) using micro-biological tests showed that nutritional value of whole pea protein ranges from 20 to 50%, PER between 1.72 to 2.6%, biological value of 56.8 - 71.5%, with net protein utilization of 44.7 - 60.7%. Savage and Deo (1989) estimated the true digestibility of pea protein to be 78.7 - 84.8. Bhatly and Christison (1984), using mice reported that the PER of peas (1.45%) was higher than both faba beans (1.09%) and lentils (0.73%). They also reported that pea protein was more digestible (89.3%) than faba beans (83.5%) and lentils (77.8%). True amino acid digestibility using adult cockerels were 86.9, 88.6, 77.7 and 87.7 % for lysine, methionine, cysteine and threonine respectively (Brenes et al., 1993; Gatel, 1994). Fan et al., (1994b) also reported an improvement in protein quality of peas with methionine supplementation. True ileal digestibility and biological value of peas fed to growing pigs were 89 - 92 % and 62.3 % respectively (Leterme et al., 1990).

# Pea Carbohydrates

Peas when used in swine diets contribute a significant amount of energy due to the high content of starch. Carbohydrate concentrations range from 56.6 -

Table 4. Carbohydrate composition of whole pea seed (g/kg DM)

Fraction	Whole seed
LMW - sugars	64-76
Sucrose	25-31
Raffinose	2-6
Stachyose	15-48
Verbascose	17-29
Starch	298-505
NSP*	
Rhamnose	3-4
Arabinose	28-32
Xylose	11-12
Mannose	5
Galactose	8-9
Glucose	85-98
Uronic acids	23-24
TOTAL NSP*	171-177
Lignin	2-11

NSP\* Non starch polysaccharides

Based on data from: Aman and Graham, 1987; Savage and Deo, 1989; Leterme et al., 1989; Abrahamsson et al., 1993; Kossen et al., 1994; Igbasan et al., 1996; NRC, 1998.

74.0% and 62.8 - 78.6% DM basis in whole and dehulled peas respectively (Gatel and Grosjean, 1990; Igbasan et al., 1996). Mature dry peas are good sources of oligosaccharides and polysaccharides, the latter including starches which are relatively slowly digested by  $\alpha$ -amylase (Gee and Johnson, 1985) and the cell wall polysaccharides which are part of the dietary fibre complex. Table 4 shows a review of some recent results.

Low molecular weights (LMW) sugars make up to 64 - 76 g/kg DM. Oligosaccharides of the raffinose family (raffinose, stachyase and verbascose) and sucrose are the most prominent sugars. The main carbohydrate component of pea is starch. Review of the literature showed large variation in starch depending on cultivar. Smooth peas contain 43 - 51% and contain more starch than wrinkled peas (30 - 33%) (Kossen et al., 1994). Kossen et al., (1994) reported that pea starch is characterized by a higher proportion of amylose compared to cereal and tuber starches. The importance of peas as an excellent energy supplement in swine diets is the direct result of the fact that starch is the most abundant polysaccharide in peas. Cell wall materials are mostly non-starch polysaccharides (NSP). Other components include, glycoproteins and phenolics (lignin) (Selvendran and Robertson, 1990). Mature dry pea seeds contain 17 - 18% NSP. Pectic arabinans, xyloglucans and cellulose are abundant in the cotyledon (Selyendran and Robertson, 1990). In dehulled seed, arabinose, uronic acid and glucose residues are the dominating NSP component (Dandanell and Aman, 1993). Pea hull, is rich in cellulose, acidic xylans, pectic polysaccharides and xyloglucans and constitute 8 - 15% of the whole seed (Kossen et al., 1994). The

NSP units of pea hulls show a high content of glucose, uronic acid and xylose (Longstaff and McNab, 1989). These authors further reported that about 69% of the hull NSP are insoluble. The content of lignin in dehulled seeds is negligible and in the hulls amounts to about 3 - 13% depending on the variety.

#### Pea Lipid

The lipid content of feed peas is relatively low. Rhone-Poulence (1993), reported an average value of 1.38% of which linoleic acid constitutes 0.56% of pea material. Of this, 90% of the total lipid is found in the cotyledons of peas (Savage and Deo, 1989). About 50 to 60% of the total lipid contents are estimated to be in the neutral fraction. Compared with saturated fatty acids, the amount of polyunsaturated fatty acids in peas is high (Rhone-Poulence, 1993; NRC, 1998).

#### Minerals and Vitamins in Peas

Peas are good sources of many important elements, however, animal diets containing peas are generally supplemented with minerals and vitamins. Peas contain sodium (29.5 - 1500 g/kg), iron (22 - 490.0 g/kg) and phosphorus (2.2 - 5.2 g/kg), but are low in calcium (0.3 - 1.4 g/kg). Rhone-Poulence (1993), NRC (1998) reported values between 0.4 - 0.5%. The presence of phytic acid, which binds phosphorus, affects bioavailability of phosphorus, (Manan et al., 1987). Reddy et al., (1982), reported that feed peas contain approximately 1.2% phytic acid. Levels of trace minerals in peas are considered to be similar to levels found in many cereal grains (Rhone-Poulence, 1993; NRC, 1998).

Hazell and Johnson (1987) showed that diffusible iron (iron availability) in peas is very low compared to other plant sources of iron. The presence of phytate has been reported to be the major inhibitor of iron availability, (Manan et al., 1987; Savage and Deo, 1989). Calcium and phosphorus are concentrated in the cotyledons while the remaining elements are evenly distributed between the hull and cotyledons. The levels of cadmium, chromium, cobalt, lead, molybdenum, nickel and tin in fresh peas have been found to be below detection limit of the atomic absorption spectrophotometer (Lopez et al., 1986). Peas are rich in the B-group vitamins, vitamin E and ascorbic acid (Manan et al., 1987). Germination, stage of maturation and processing are among the factors affecting the levels of vitamins in peas (Savage and Deo, 1989).

#### Anti-Nutritional Factors in Peas

Field peas could provide an excellent source of dietary protein and energy for young pigs. However, their use in these groups of pigs is limited probably due to the presence of a number of anti-nutritional factors (ANF). These toxic factors are a group of non-related chemical compounds with varying effects on metabolic processes. ANF in plants and seed serve as natural protection by their effect on the biological systems of invasion; examples are bacteria and insects. The levels of these toxic factors depend on the cultivar and the analytical methods used. Savage and Deo (1989) summarized the reported levels of naturally occurring ANF in peas. Subsequently, Huisman and Tolman (1992) grouped the ANF in

peas into various classes based on their effect on the nutritional value of feedstuff and on the biological response in the animal.

#### Protease Inhibitors

Protease inhibitors which include trypsin and chymotrypsin inhibitors are low molecular weight proteins with particular property of forming reversible stoichiometric protein-protein complexes with various proteolytic and digestive enzymes, resulting in competitive inhibition and inactivation of their catalytic functions (Rackis et al., 1986). Trypsin is a proteolytic enzyme secreted by the pancreas. Inactivation or inhibition of this enzyme brings about partial or inadequate digestion of proteins resulting in fewer amino acids available for growth. In addition, the pancreas may function abnormally and dietary amino acids may be directed towards the synthesis of additional pancreatic enzymes, since pancreatic enzymes are rich in sulphur containing amino acids (Huisman and Jansman, 1991). Hypertrophy of the pancreas may divert methionine and cysteine from body tissue synthesis to production of additional pancreatic enzymes. This would further aggravate the deficiency of sulphur containing amino acids in peas based diet (Huisman and Jansman, 1991). Furthermore, inactivation of trypsin in the gut results in an increase in secretion of cholescystokinin (pancreozymin) in the intestinal mucosa (Huisman and Jansman 1991). This hormone stimulates the pancreas to produce more digestive enzymes such as chymotrypsin, amylase and elastrase. The increase in pancreatic enzyme production causes hypertrophy and hyperplasia of the pancreas and hence an increase in weight. The trypsin inhibitor

content of peas (150 - 10800  $\mu$ /g) is reported to be about a tenth of that of soybeans but similar to the levels in field beans (Leterme et al., 1990; 1992), but are however, enough to cause poor growth performance in non-ruminants (Gatel and Grosjean, 1990). About 90% of the trypsin inhibitor in peas have been found in the kernel and 10% in the testa. According to Jansman et al., (1994), the main nutritional effect of trypsin inhibitors is not the reduction in digestive capacity but relate more to an increase in the loss of endogenous protein, like digestive enzyme rich in essential amino acid; for example, methionine. Chymotrypsin inhibitor is a proteolytic enzyme similar to that of trypsin inhibitor. Chymotrypsin inhibitor content in peas ranges from 74 to 240  $\mu$ g/g (Griffiths, 1984). Cooking has been found to completely eliminate trypsin inhibitor activity.

#### Amylase Inhibitors

Amylase inhibitors are inhibitors of pancreatic and salivary amylase. Compared to other pulses, peas contain a significantly smaller amount of these compounds which interfere with the pancreatic and salivary enzymes involved with carbohydrate digestion (Griffiths, 1984). Amylase inhibitors are however completely inactivated at 100°C.

#### Tannins and Phenolic Acid

Tannins consist of a diverse group of water insoluble polyphenolic compounds with molecular weight between 500-3000 daltons. These polyphenolic compounds

have the ability to form complexes with carbohydrates, protein and other polymers in feed (Hagerman, 1988). Tannins are generally grouped into hydrolyzable and condensed tannins. The latter is of particular importance in monogastric nutrition, in that, they form complexes with dietary nutrients thereby rendering them more resistant to the action of digestive enzymes (Jansman and Longstaff, 1993). Condensed tannins which do not split into sugar and phenolic carboxylic acids upon treatment with either acid or alkali, may also bind salivary protein or other endogenously secreted proteins (Marquardt and Bell, 1988) and inhibits their activities. Tannins and phenolic content of field pea seed ranged from 0.2 to 0.4 g/kg and 26 mg/kg respectively. Higher concentrations are normally located in the testa (Savage and Deo, 1989).

Marquardt (1989) and Jansman (1993) reviewed the nutritional effect of consumption of tannins by non-ruminants. Reduced feed intake due to astringent taste, decreased nutrient utilization, impaired growth and direct toxicity are among the negative effects of consumption of tannins. Tannins can also interfere with protein anabolism and mucosal epithelial cells (Jansman, 1993). The phenolic group of tannins binds to enzymes and other proteins by hydrogen bonding to amide groups to form insoluble complexes. The polyphenolics and tannins react with the  $\alpha$ -amino group of lysine and polymerize into tannin-protein complexes forming large blocks of amino acids resistant to digestive enzymes (Jansman, 1993).

Phenolic compounds are generally non-toxic but their absorption by mammals necessitates detoxification. Detoxification of these polyphenolic

compounds involves methylation, which would put further stress on the limited methionine content of peas (Savage and Deo, 1989). Since most of the tannins and the phenolic compounds are contained in the testa, dehulling would reduce the toxic content of the seed.

### Phytic Acid and Oxalic Acid

Important storage for phosphorus in phytic acid is a hexa-phosphate ester of inositol which can form complexes with divalent cations ( $Ca^{2+}$ ,  $Mg^{2+}$ ) and thereby reducing their availability (Liener, 1989). The phytic acid content in peas ranges from 4.7 to 8.2 g/kg (Manan et al., 1987). In plants, phosphorus is organically bound as phytate (NRC, 1998). Phytic acid affinity to basic residues of protein have been suggested as the reason for their inhibition of some digestive enzymes such as  $\alpha$ -amylase, pepsin and pancreatin (Liener, 1989). Aside from inactivation of enzymes, phytic acid also forms poorly soluble compounds that are not readily absorbed from the intestine. In addition, they form chelates with calcium ions, which are essential for the activities of a number of digestive enzymes. Digestion of phytate depends on the enzyme phytase, which tends to be limiting in monogastric animals. Liener (1989) also demonstrated inhibition of proteolytic activities by phytic acid.

Oxalic acid like phytic acid has the capacity to form insoluble salts with divalent ions. Manan et al., (1989) reported the oxalic acid content of peas to be 6.67 g/kg. They are highly concentrated in the testa and therefore dehulling may reduce their levels.

# Lectins (Phytohaemagglutinins)

Lectins (phytohaemagglutinins) are proteins characterized by their unique ability to bind sugar or glycoproteins (Liener, 1989; Huisman and Jansman, 1991). Phytohaemagglutinins agglutinate red blood cells in vitro and in vivo, as well as interfere with digestive and absorptive processes in the digestive tract, affecting systemic metabolism (Grant and Van Driesche, 1993). Besides, lectins bind to epithelial cells lining the small intestine resulting in reduced intestinal permeability and nutrient transport. Enzymatic activity as well as hormonal regulations are also affected. Dietary lectins are required as the first step to bind gut epithelial cells (Grant and Van Driesche, 1993), after which they can damage the brush border and reduce the activities of brush border enzymes (Huisman and Jansman, 1991). Peas contain about 0.25% lectins, and are reported to have little deleterious nutritional effects (Grant and Van Driesche, 1993).

#### Peas in Swine Diet

Field peas have been fed extensively to pigs (Myer and Froseth, 1983; Kehoe et al., 1991 and Castell et al., 1996). The prime nutritional advantages of pea-meal are the relatively high lysine content, favourable amino acid balance and energy content (Igbasan and Guenter, 1996a). Henry and Bourdon (1978) reported that peas can be effectively used up to a level of 30% in the diet of finishing pigs provided that the diet is adequately balanced with essential amino acids, particularly tryptophan which seems to be the second limiting amino acid when peas are given in conbination with corn. Comprehensive review of the use of

peas in the diets of pigs and other domestic animals has been reported (Castell et al., 1996; Henry and Bourdon, 1978) as well as feeding trials with several cultivars of field peas (Igbasan and Guenter, 1996b; 1997b; Gatel and Grosjean, 1990).

Using maize-soybean meal diets adequate in lysine, methionine and tryptophan containing 0, 15 or 30% field peas and no significant differences were reported in average daily gain or efficiency of feed utilization of pigs fed the test diets from 26 to 99 kg liveweight. Similar results were obtained for finishing pigs (51-100 kg) by replacing all of the soybean meal in the diet with 30% field peas (Bourdon and Henry, 1978). Bell and Wilson (1970) fed 0, 6, 12, 18 or 24% field peas to pigs (23 to 90 kg) replacing barley, wheat, soybean meal and herring fishmeal on an iso-nitrogenous basis and found no differences in daily gain or efficiency of feed utilization. Myer and Froseth (1983), substituted all of the soybean meal in grower diets with cull pea (43 - 45%) without adverse effect on pigs fed from 20 to 41 kg live weight. Davis (1981) found that whereas the use of up to 28% peas had no effect on dietary energy intake, 53% peas caused 8% decrease in dietary energy intake. Henry and Bourdon (1977) stated that 30% of peas could be used during the finishing period, with appropriate attention to essential amino acids. Several feeding trials on weaning pigs indicate that proportion of peas used in starter diet is smaller than levels found acceptable for grower-finisher pigs. Fekete et al (1984) concluded after studies on 3380 piglets starting at an average weight of 6.7 kg that it was not advisable to use peas immediately after weaning but from twelve days after weaning, and that, 15% peas

could be used. They further stated that amounts above 15% decreased performance and the pigs never fully recovered. Taverner and Curic (1983) showed that when peas contributed 30% of the total diet fed to pigs the digestibility of protein in the peas was 82%, compared to that of soybean (91%). Also, the digestibility of total energy in peas was 85%, was comparable to that of soybean.

Pea meal is reported to be highly palatable to pigs and is recommended at levels of up to 25 to 30% in diets of growing-finishing pigs (Bell and Wilson, 1970). The prime nutritional advantages of pea meal are the relatively high lysine content and favourable essential amino acids balance. Low levels of methionine and relatively high fibre content compared with other sources of protein are however drawbacks. Bengala-Friere et al., (1989) reported that the inclusion of raw peas in diets for pigs weaned at three weeks resulted in reduced feed intake and growth. However, nutrient digestibility and performance were not adversely affected when peas were extruded (Castell et al., 1996).

Castell et al., (1996) indicated that for pigs weighing less than 20 kg, it seems prudent to limit raw peas to 10 to 15% of the diets. However, higher rates of raw peas in starter diets may be justified if amino acid supplements are included (Gatel et al., 1989). Kehoe et al., (1991) observed that while gains were lower (811 versus 846 g/d) when 505 g/kg Tipu peas replaced 155 g/kg of soybean meal, supplementing the pea diet with methionine and threonine increased the growth rate to 930 g/d in grower pigs. Confirming the fact that relatively high levels of peas are well utilized by growing pigs if the dietary amino acid requirements are met. Henry and Bourdon (1978) reported that peas can be effectively used up to a

level of 30% in diets of finishing pigs provided that the diet is adequately balanced with essential amino acids, particularly tryptophan which seems to be the second limiting amino acid when peas are given in conjunction with corn. However, lower digestibility coefficients were observed for pea cultivars containing high levels of tannins, when peas constituted 35% of the total diet for growing and finishing pigs, (Hlodversson, 1987).

The high feeding value of low trypsin inhibitor peas was confirmed in three experiments with amino acid balanced diets containing 0 or 40 - 45% peas. Pigs used diets containing peas as efficiently as the control diets (Gatel et al., 1989). Peas can be incorporated without limit, with low trypsin inhibitor activity peas (up to 40 - 45%) in amino acid balanced diets for growing-finishing pigs. Grosjean et al., (1989) showed that when one considers the digestible energy value and also increases the addition of synthetic tryptophan and methionine to the diet, it is possible to obtain with diets containing 30% high trypsin inhibitor pea, a performance practically identical to that obtained with low trypsin inhibitor peas. They further stated that animal's performance is essentially linked to the limiting amino acids content (tryptophan, sulphur amino acids) of peas.

Bell and Wilson (1970) in their studies indicated that from the nutritional point of view, up to 40% cull pea or better grade peas can be incorporated into swine grower-finisher rations without affecting animal performance. Grosjean and Gatel (1986) in their growth trials with weaned piglets or growing-finishing pigs observed that peas can be widely used in diet for these groups of pigs, and with low trypsin inhibitor activity varieties, peas can be used up to 45% without any

consequence on growth and carcass performance. Contrary reduced growth rate, feed conversion efficiency were observed when peas were fed to both piglets and growing fattening pigs, especially at growth phase below 60kg liveweight (Grosjean et al., 1989; Matre et al., 1990). Other studies revealed that with early-weaned piglets fed diets containing 15 - 45% peas, impaired growth performance was observed (Bangala Freire et al., 1989; Castell et al., 1996). The reduced performance was attributed to reduced feed intake in the peas-fed pigs and attributed to improper balancing of the content of available essential amino acids in the diets.

Lettner et al., (1986) reported that up to 30% of broilers' diets could be made up of peas without detrimental effects. Under commercial production, Brenes et al., (1989) reported that birds fed 80% peas performed better than soybean meal-fed birds. In another study with chicks, Brenes et al., (1993) showed that satisfactory growth performance could be obtained with 48% pea inclusion rate. Earlier reports on the other hand showed a significant reduction in growth rate, feed intake and feed efficiency when peas were included at 40%. In these studies, methionine supplementation, as well as heat treatment had no effect.

Review of the literature showed inconclusive results in the use of peas in laying hens. Castanon and Perez-La-Zac (1990) observed no detrimental effects on egg production, feed consumption or feed utilization with 50% inclusion rate. Ivusic et al., (1994) further reported that peas could be added at higher level (59%). Conversely, incorporation of 17 and 37% peas in laying hens resulted in 20 and 45% reduction in egg production respectively. Subsequent studies again

showed a reduction of about 47% in egg production at 37% peas in the diet.

However, heat processing coupled with methionine supplementation eliminated this observed difference (Davidson, 1977).

Castanon and Perez-Lan-Zac (1990) showed that including up to 50% peas in eight week studies with leghorn had no effect on egg production, feed intake or feed conversion efficiency except for egg weight which increased slightly with increasing levels of peas in the diet. In a longer term study, Ivusic et al., (1994) using up to 59% peas in the diet, compared to corn/soy control diet, reported paler egg yolk colour and thinner shells from layers fed the highest inclusion level of peas. They, therefore, concluded that equivalent performance could be achieved when layer diets are properly balanced for energy and amino acids.

### Pea Processing

The nutritional value of feed peas can be improved by various processing methods. These include; dehulling, heat processing, enzyme and amino acid supplementation. Of these, heat treatments are the most successful processing methods that could improve increased availability of nutrients through modification of nutrients themselves and reducing the effect of ANF. Methods of thermal processing described in the literature comprise extrusion, autoclaving, steaming, boiling in water or buffer media, dielectric, heating, micronization, toasting and pelleting (Madsen and Mortensen, 1989; and van der Poel et al., 1991,1992). Low accessibility of pea protein and starch to enzymatic attack as a result of strong cellular cohesion in pea cotyledons have been found to be accountable for the

poor digestibility as well as the overall performance of birds and swine fed unprocessed peas (Carre et al., 1991). Heating has been found to increase thermal molecular oscillation which disrupts the binding forces within and between polypeptide chain, causing an unfolding of the molecules (Michaelsen, 1992). Disulphide bonds within proteins are often disrupted, resulting in partial loss of the secondary structure and, therefore, decreased solubility. The cross-linkage within proteins are believed to delay enzymatic hydrolysis in the stomach and the small intestine, thereby reducing digestibility (van der Poel, 1989; Michaelsen, 1992). Mechanism by which nutrients are made available after heat processing results from increased accessibility of nutrients to enzymatic attack and or inactivation of proteinaceous ANF (van der Poel, 1990).

Protease inhibitors require their secondary structural integrity in order to inactivate proteolytic enzymes (Rackis et al., 1986). Partial denaturation of protein and gelatinization of starch is believed to facilitate enzymatic breakdown of the polymers. Furthermore, energy value of dietary fibre fraction may increase and the proteins associated to fibre become more available for digestion. Other beneficial effects include reduction in lectins and enzyme inhibitors, and also assayable levels of polyphenols and tannins (van der Poel, 1990). However, excessive heating may reduce total content and availability of lysine, cysteine and methionine, particularly in the presence of carbohydrate (De Wet, 1982; Michaelsen, 1992, van der Poel et al., 1992). Therefore, accurate controls of heating conditions are essential to processing of peas for maximum nutritive value. Effectiveness of heat treatment depends on; process temperature, heating time.

particle size, initial moisture content and amount of moisture added during the heating.

Focant et al., (1989), showed that trypsin inhibitor capacity of peas may be reduced to a minimum level by steam-flaking, micronizing or extrusion, without loss in lysine availability or pepsin nitrogen solubility. Autoclaving, extrusion or micronization has been found to result in complete loss of trypsin inhibitor and haemagglutinins activities and also inactivation of lipoxygenase in peas (van der Poel et al., 1992). Moist heat treatment was found to be more efficient than dry heating for the inactivation of trypsin and chymotrypsin inhibitors in peas (Griffiths, 1984). Heat treatment can neutralize the effects of tannins and thereby prevent their interaction with the protein component of the diet (Marquardt, 1989) and increase the utilization of peas by monogastrics.

Heat treatments significantly improved protein and starch digestibility. This was attributed in part to the reduction in the ANF, and breakdown of the cell walls of peas, which allows the accessibility of nutrients to digestive enzyme action (Carre et al., 1991). Gelatinized or disrupted starch has been reported to be more rapidly degraded by enzymes than raw starch, van der Poel et al., (1992) demonstrated that extrusion at 105 °C for 3 minutes slightly reduce trypsin inhibitor activity (TIA) but had no effect on haemagglutinins activity (HA) in wrinkled seed pea. However, most HA in round seeded peas was fully inactivated. Griffiths (1984) reported that trypsin and chymotrypsin inhibitors were stable at temperatures below 100 °C. On the other hand, van der Poel (1990) showed that extrusion at 100 - 135 °C resulted in total loss of TIA and between 88 - 100% loss

in HA, while micronizing peas at 124 °C for 65 seconds resulted in complete loss of both TIA and HA. Again, van der Poel et al (1992), reported extrusion at 105 - 130 °C led to 46 - 90% and 100% loss in TIA and HA respectively in wrinkled seeded peas.

#### Micronization of Peas

Micronization is the process whereby cereal or grain legume is passed beneath a series of China-Clay ceramic tiles that have been treated to a predetermined temperature by a gas/ air mixture to produce a luminous red glow known as a "bright line spectrum". This radiates the electromagnetic spectrum in the infrared band and the grain passing beneath the heated ceramics absorbs the radiation produced. Selective absorption of the infrared rays by cereal or legume causes it to become irradiated thereby causing the molecule within to vibrate in accordance with the resonance of their own wavelength and frequency. This results in internal frictional heating and a rise in water vapour pressure. The process had the advantage of being very quick (40-90 seconds) and continuous, much cleaner to operate, takes up less space, has a greater capacity and above all produces flakes which do not go mouldy (Lawrence, 1973).

Lawrence (1973) examined the effect of the micronization process on the composition of peas and digestibility as well as nitrogen retention in growing pigs. This author reported that micronization improves starch availability. Earlier report also showed that nitrogen retention was not affected by the process but apparent digestibility of dry matter, nitrogen and gross energy was improved by 4.3% in

micronized compared to untreated peas (Lawrence, 1973). Recently, Igbasan and Guenter (1996b, 1997b) reported that micronization resulted in a significant improvement in apparent metabolizable energy, protein and starch digestibility in poultry. These authors also reported that chicks fed micronized peas grew faster and had better feed conversion than birds fed untreated peas and wheat-soybean control diets.

## Extrusion of peas

Whole seeds put through the extruder making flakes under 300 psi resulted in thoroughly gelatinize starch at normal moisture content in feed and thereby enhanced starch digestibility (van der Poel et al., 1992). The extrusion system consisted of a twin-screw extruder. The barrel about 1 m long composed of four thermally controlled sections. Basically the screw configuration consisted of positive ions, with decreasing pitch from feeder to die. The dies consist of two cylindrical holes 30 mm long and 4 mm in diameter. Flour feeding is achieved by a volumetric hopper and the amount of water required delivered by a volumetric pump at the entrance section of the barrel. Camire et al., (1990), showed that extrusion-cooking processing produces favourable properties for starch digestibility while maintaining availability of other nutrients present. The degree of extrusion is an important industrial variable since incomplete 'cook' may result in unsatisfactory storage stability, palatability and digestibility of extruded products (van der Poel et al., 1992). The degree of cook is again associated with the degree

of irreversible swelling and complete rupture of the starch granule, and subsequent effect on nutrient availability.

Focant et al., (1989) reported that extrusion did not influence dry matter intake of pea-based diets nor their apparent digestibility coefficient. However, nitrogen retention was significantly increased. The growth rate was however reduced in young rats. They concluded that improvement might be due to a probable decrease in ANF levels.

The use of either micronization or extrusion processes in animal feeding necessitates an economic study to compare the costs of these processes with their nutritional benefits. There is scarce information in the literature comparing the nutritional benefits of extruding or micronizing feeds in young pigs' diets.

### Protein Conformation and Heat Damage

Conformational stability is the ability of a protein to preserve its native conformation under various influences, such as heat, denaturing agents and extreme pH. Under the influence of such factors, proteins may undergo quite significant conformational changes (Michealsen, 1992). van der Poel, (1990) demonstrated that denatured proteins are more susceptible to digestive enzyme attack than native proteins. Heat denaturation may also bring about inactivation of anti-nutritional factors, particularly protease and amylase inhibitors, as well as lectins due to their proteinaceous nature (Mecion et al., 1993). Furthermore, tannins which are phenolic in nature, are partially inactivated by heat (van der Poel et al., 1992).

Maillard reaction consists of the reaction of aldehydes, ketones and reducing sugars with amines, amino acids, peptides and proteins (Figure 1). Lysine, through its ε-amino group is the most reactive amino acid although cysteine and methionine are also sensitive (Bender, 1978; Mauron, 1981). Formation of colourless compounds which later complex to form a brown pigment is the first stage of the Maillard reaction (Bender, 1978).

Thermal treatment can have negative effects on the nutritional value of feeds depending on the process conditions and the nature of the product treated Changes observed in the product composition may not necessarily reflect changes in its digestibility, utilization or fermentability. Damage of protein may result from excessive heating, particularly in the presence of carbohydrates (van der Poel et al., 1992; Michelsen, 1992). The chemical mechanisms of protein damage and the nutritional consequences are not well understood. Many possibilities exist for the formation of intra or intermolecular bonds, as functional groups of other food constituents. Racemization, protein-protein, protein-carbohydrate and protein-lipid reactions are among mechanisms outlined for heat damage. However, the latter, which involves free radical reactions with either triacylglycerides or free fatty acid, is of less importance in peas because of the low lipid content (2 - 4%) (Rhone-Poulence, Animal Nutrition, 1993).

Protein-protein interactions involve the formation of hydrolyzable as well as non-hydrolyzable bonds, which include internal amides, esters and thioesters. These can be formed between the carboxylic acid of aspartic or glutamic acid, with the amino group of lysine, the alcohol group of serine, for example or the thio-

group of cysteine (Michelsen, 1992). Thioesters are relatively reactive and frequently participate in the formation of other products. Lysinoalanine and lanthionine are examples of non-hydrolyzable products. They are derived from reactions with dehydroalanine formed from dehydrogenation of alanine or by elimination of water from serine. Lysinoalanine, a secondary amine, is a product from the reaction between dehydroalanine and lysine. Thioester lanthione on the other hand is formed when dehydroalanine reacts with cysteine.

Of special concern for the nutritional consequences of thermal processing of peas are protein-sugar interactions, because of the high content of starch and low molecular weight carbohydrates. The latter fraction is dominated by indigestible oligosaccharides of the raffinose family, and constitute 3 - 10% of the seed dry matter while sucrose is found at a level of 1.0 - 3.5% (Igbasan et al., 1996). On the other hand, reducing sugars such as glucose, fructose and pentoses are only minor constituents. Reaction between protein carbohydrates takes place under mild heating and during storage (Michaelsen, 1992), but treatment at high temperature and low moisture contents as in micronization and extrusion are conditions that favour the browning reactions (Mauron, 1981). In this reaction, a free amino group typical of lysine reacts with the aldehyde group of reducing sugars to form a Schiff's base intermediate. Amadori re-arrangement and elimination of water results in formation of 1-amino-1-deoxy-2-ketose compound. Compounds of this type and their condensation products are referred to as Maillard reaction products (Mauron, 1981). Reducing sugar is easily formed from sucrose, as the bond between fructose and glucose is

Possible reaction of cystine and cysteine upon heating

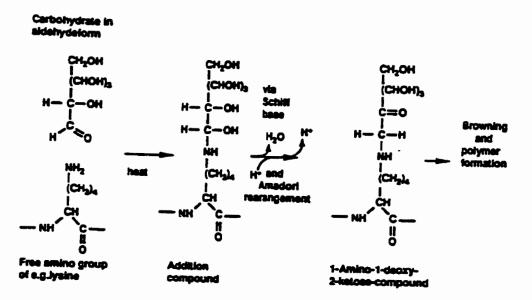


Fig. 1 Maillard reaction in a simplified scheme (Adapted from Mauron, 1981; Michealsen, 1992)

relatively unstable. Severe heating affects both essential and semi-essential amino acids. Notably, lysine and cysteine are mostly affected, while methionine, arginine, histidine, threonine and tryptophan seem to be less affected (Michaelsen, 1992).

Mauron (1981) divided the Maillard reaction in early, advanced and final stage. The early Maillard reaction stopping at the Amadori rearrangement stage, do not cause browning, though can reduce nutritional value. However, these compounds can be broken down to regenerate the initial sugar and amino acid. Advanced and final Maillard reaction results after polymerization of many reactive compounds bringing about dark brown melanoidin pigment. The consequences of this reaction include, destruction of the amino acids, reduce amino acid availability through formation of crosslinks or lead to the formation of biologically unavailable peptide residues that are absorbed but not metabolized (Mauron, 1981). High temperature and long duration increase the rate of the Maillard reaction. Water is also necessary for the initial reaction to take place.

Biological availability of lysine in micronized or extruded peas may be a concern as evidenced from the improvement in performance of chicks fed micronized peas supplemented with 0.15% L-lysine. Igbasan and Guenter (1997b) suggested that some amount of lysine might have been involved in cross-linkage reaction with either carbohydrate or other amino acids in the process of heating. The cost of heat processing must be more than counterbalanced the net improvement of the nutritional value obtained.

# **Enzymes Supplementation of Pigs Diets**

Efforts to improve digestibility on non-cellulosic dietary components are based on augmenting endogenous enzyme activity, with the assumption that digestive enzyme production is limiting as in early weaned-pigs. Supplying enzymes lacking in endogenous secretions may offer the opportunity to digest otherwise unavailable components to an absorbable form, example, α-amylase, α-galactosidase and phytase to improve starch, oligosaccharides and availability of plant phosphorus respectively. Studies in swine have shown benefit in baby and growing pigs using crude amylase. This has been demonstrated to reflect an age related pancreatic insufficiency or simple lack of substrate stimulation (Lindemann et al., 1986). Enzymes are sometimes added to feed in an attempt to improve the digestibility of complex carbohydrates and proteins. Enzymes are proteins catalyzing all metabolic processes in plants, animals and microorganisms (Sears, 1994). These biological catalysts are produced by fermenting microorganisms such as fungi and bacteria on specific substrates.

Specificity, substrate affinity, stability, pH and temperature sensitivity are among the distinctive properties of enzymes. The potential for industrial enzyme products as animal feed additive has attracted substantial interest from both feed manufacturers and animal producers as a novel means of improving performance. The use of enzymes that degrade polysaccharides of the endosperm cell wall is the most prominent (Wenk, 1992). β-glucanase and pentosanase have been used to degrade β-glucans and pentosans which interfere with digestibility of nutrients in

barley and rye (Thacker and Baas, 1996). Xylanase and glucanase have been found to expose encapsulated intracellular nutrients, particularly, starch and protein to digestive enzymes within the gut lumen (Petersson and Aman, 1989). Varied responses have been shown to the addition of  $\alpha$ -amylase and protease to diet for young pigs to aid nutrient digestibility (Wenk, 1992; van Hartingsveldt et al., 1995).  $\beta$ -glucanase inclusion significantly increased ileal digestibility of protein, crude fibre, starch or mixed linked  $\beta$ -glucans in growing pigs (Graham et al., 1988). However, Campbell and Bedford, (1992), on the other hand, observed improved weight gain and digestibility of nutrients in weaning pigs but not in growing pigs.

The cell wall of cereals, legumes and some pulses contain primarily complex carbohydrates (NSP), which can negatively affect nutrient utilization. Mechanism by which NSP-degrading enzymes work is not fully understood. Studies have shown that a major part of the effect is by altering the physical nature of the digesta. β-glucans and arabinoxylans are branched chain structures which bind water, giving rise to highly viscous aqueous solution even at low levels. Increased digesta viscosity associated with the presence of such compounds slow down the rate of diffusion of substrate and digestive enzymes, thereby hindering their effective interaction at the mucosal surface of the intestine leading to reduced absorption in the small intestine (Bedford and Classen, 1992). Failure to digest these NSP's has been reported to have serious implication to the host due to explosive microbial growth in the lower gut (Liebman, 1991). NSP's may also

interact with the microbial flora as increase in viscosity reduces mixing and rate of feed passage, bringing about increased colonization of the small intestine by microbes resulting in competition for nutrients and increased fermentation as digestion (Bedford, 1996), McClean (1993) studied supplementation of wheat middling-based diets for weaned pigs (14 or 28d) with xylanase and pectinase. This author reported a 4% increase in apparent digestibility of energy and protein. Recently, Bach-Knudsen (1995), observed that enzyme inclusion to wheat and by-product-based diets for rats only increased (0.7%) the faecal digestibility of energy. Cellulase supplementation to wheat and by-products diets for pigs have been reported to increase ileal digestibility of NSP (35.9 versus 19.2%), crude protein (70.8 versus 64.9%) and crude fat (69.6 versus 61.4%) (Dierick and Decuyperer 1995), van Lunen and Schulze (1996) reported a significant improvement in growth rate and feed conversion efficiency by 9.2 and 5.3% respectively for pigs from 10 - 18 weeks of age given a diet containing wheat and corn. Again, Liu et al (1997), observed that β-glucanase and xylanase supplementation significantly improved iteal digestibility of dry matter, energy, crude protein and most amino acids by 8% on average for three hulless barley cultivars containing 60 - 70 g/kg of β-glucans. On the other hand, Officer (1995) observed that supplementing a wheat-based weaner diet with a commercial multienzyme had no effect on weight gain and feed utilization. A similar result was obtained when early-weaned pigs were fed wheat-based diets (Inborr et al., 1993).

Although there are physiological reasons for augumenting the digestive capacity of baby pigs with supplementary enzymes, the value of supplementing enzymes to pig diets is inconclusive. Feeding trials have failed to produce definitive results in terms of improved performance. Limited nutritive value of some released carbohydrates and variable survival of enzymes during processing and within the digestive tract may account for variation in response (Chesson, 1993). Different variety and environmental factors affecting composition, the type and level of enzyme inclusion and the age of the animal studied may have effect on efficacy of enzyme addition.

### Methodological Aspect of Digestibility Studies

The potential nutritive value of feeds can be determined by chemical analysis of the constituents, however, the value of the ingested feed ingredient depends on the animal's ability to utilize them. Digestibility of ingested feed can be described as the proportion of the feed which is not excreted in the faeces and therefore, assumed to be absorbed by the animal (McDonald et al., 1995). Digestibility coefficients are important parameters in evaluating the nutritional value of feed for livestock and poultry. The classical method of estimating digestibility relies on the quantitative collection of faeces from pigs kept in metabolism cages.

In vivo methods estimate the amount of nutrients absorbed by comparing the dietary intake with the quantities of the digesta passing various sections in the intestine. Apparent digestibility measurement is defined according to the site as the large intestine have little or no nutritional value to monogastric animals. They are either excreted or incorporated into microbial protein, which account for most of the faecal nitrogen. To avoid interference of the large intestine, nutritional value of dietary proteins are estimated based on ileal apparent or true digestibility. Non-starch polysacchrides (NSP) on the other hand, are resistant to digestion by non-microbial enzyme during gut transit (Robertson, 1988). Depending on their chemical composition and physical properties, the partitioning of their digestion between small and large intestine may vary giving rise to differences in the nutrient supply. In addition, starch from various sources and certain oligosaccharides are partly resistant to digestive enzymes of the animal and therefore escape digestion in the small intestine (Abrahamson et al., 1993; Veldman et al., 1993). A suitable technique is therefore necessary to estimate these partitioning between ileal and faecal values in all cases (Laplace et al., 1994). Cannulation of animals is used as a way of obtaining such partitions.

Major concerns in using cannulation techniques include, cannulation effect on digestive processes in the animal, whether the samples obtained are representative of the whole digesta and lastly, reliability of the marker used. Various methods for determining digestibility have been developed. These are, simple T- intestinal, re-entrant (Ileo-ileal, ileo-ceacal), ileo-colic-post-valve, post valve-T and ileo-rectal anastomosis cannulation and serial slaughter or intact ileal sample (review by Batterham, 1994; Fuller et al., 1994)). Of all the mentioned techniques, only simple-T and re-entrant cannulation, preserve the whole caecum

and colon and can therefore, allow measurement of both ileal and faecal digestibility in the same animal. The others involve either total, partial removal or by-pass of the caecum or the colon. By far, simple-T cannulation is the most commonly used method. This technique has the advantage in that the surgery is relatively simple, and also collection of digesta can be made over relatively long periods. In addition, it is possible to use the same set of pigs for a number of collections, and lastly simple-T cannulation, is considered to have less effect on the physiology of the gut.

Simple-T cannulation involves insertion (implantation) of a T-shaped cannula anterior to the ileo-caecal junction (terminal ileum). Digesta is collected from the cannula after the pigs have been on the test diet for 5 - 10 days. T-cannulation rely on natural forces to divide the flow of chyme, one part continuing along the intestine and the other diverted through the cannula. The assumption made is that this provides a representative sample of the total flow and that division of the flow does not involve any fractionation of components (Fuller et al., 1994). Donkoh et al., (1994), observed similar apparent faecal nitrogen and amino acids digestibility in pigs cannulated at the end of the ileum with T-shaped cannula and intact pigs. In contrast, Jorgensen et al., (1985) reported slightly higher faecal digestibility of dry matter, protein and lysine in T-shaped cannulated pigs than in intact counterparts. The difference was ascribed to a slower passage of chyme in the former group. Yin et al., (1991) reported that T-cannulation leads to error particularly when the digesta is heterogeneous, partial separation of insoluble fibre

from other dietary components in T-shaped cannulated pigs have also been reported (Graham and Aman, 1986).

T-cannulation only permits spot sampling, calculations are therefore based on a marker. Inert markers are often used in digestibility studies. These provide means of calculating the digestibility of nutrient when complete collection of digesta from a known quantity of feed consumed is not possible (Jagger et al., 1992). Suitability of a marker depends on its recovery rate. Ideally, a marker should be totally indigestible and non-absorbable, pharmacologically inactive. progress through the tract at the rate as digesta and above all, can be readily determined. Metallic oxides, mineral salts, acid insoluble ash, silica, lignin and solid particles are among the numerous substances used as potential markers (Yin et al., 1996). Chromic oxide is one of the most widely used markers in swine trials. The assumption is that this marker flows in constant proportion to the nutrients of interest, since digestibility would be affected by separation of digesta (Graham and Aman, 1986). Chromic oxide seems to migrate in the same way as protein and amino acids, hence their digestibility would not be affected in case of separation (Yin et al., 1996). Yin et al., (1991), observed a negative, while Kohler et al., (1990), reported relatively high digestibility values for fiber.

## **Apparent and True Digestibility**

Apparent digestibility measure both the digestibility of crude protein and amino acids in the feed uncorrected for the endogenous secretions within the animal (Batterham, 1994). Faecal and iteal materials not only contain undigested

nutrients from the feed but a number of components arising from the animal system. These consist of undigested amino acids from digestive secretions and cell sloughed from the lining of the stomach and small intestine during passage of chyme. Other secretions include bile salts, enzymes, mucopolisaccharides and microorganisms arising from fermentation of undigested residues in the hindgut. A difference between nutrient intake and faecal output is termed as apparent digestibility. Correction for endogenous components is called true digestibility. The same principle applies for iteal digestibility except that microbial contribution in the lower gut is much reduced. The level of crude protein in the test diet affects apparent digestibility values. With a low protein diet, the amino acids from endogenous sources form a higher proportion of the total amino acids reaching the terminal ileum (Batterham, 1994). True digestibility however, is not affected by protein level since endogenous sources are corrected for.

Over the years, correction for endogenous nitrogen have been made using a protein-free diet or feeding graded levels of the test protein and extrapolating back to zero (Batterham, 1994). Recent methods include the use of N15 to label the amino acids in the feed or the pig so that the undigested amino acids can be distinguished from unabsorbed endogenous secretions (Huisman et al., 1992). Other method is the use of homoarginine to estimate lysine digestibility (Hagermeister and Erbesdobler, 1985). The principle behind this is that lysine in feed sources is converted to homoarginine by a guanidination process. Homoarginine is then absorbed and is immediately reconverted to lysine so that the pig does not suffer a lysine deficiency. Any homoarginine remaining in the

terminal ileum is thus, of dietary origin and is a reflection of the true digestibility of lysine (Low, 1982). The higher cost of these procedures restricts their widespread use and therefore, the use of protein-free diet though questionable, is by far the quick and easiest method to determine endogenous losses.

## **Endogenous Nitrogen**

Quantification of endogenous nitrogen and amino acid losses at the terminal ileum of the pig is of practical significance for the determination of protein and amino acids requirements and for calculation of the true ileal protein and amino acid digestibility of feed ingredients. Endogenous nitrogen is the nitrogen found in digesta or faeces when a nitrogen-free diet is fed to pigs. The nitrogen is added to the chyme as enzymes, mucin, amides, amines, bacteria and mucosal cells during passage through the digestive tract (Souffrant, 1991). A classical method for assessment of endogenous nitrogen and amino acids in digesta and faeces is to measure the ileal and faecal nitrogen and amino acids of animals fed a nitrogen-free diet (de Lange et al., 1989). This method has been criticized as altering the protein status of the animal and thereby, perhaps altering the rates at which proteins are secreted into the gut lumen (Fuller et al., 1994). In addition, the validity of using the data to calculate endogenous nitrogen secretions, when nitrogen-containing diets are fed has been questioned by de Lange et al., (1989). They attempted to overcome this problem by infusion of a mixture of amino acids intravenously while studying endogenous nitrogen secretion of pigs on a nitrogenfree diet. The results showed that endogenous protein in ileal digesta was reduced

from 18.5 to 12.7 g/kg DM intake during infusion of amino acids. Again, research showed that endogenous amino acids secretion from the small intestine of rat and growing pigs are higher under peptide alimentation than under nitrogen-free feeding (Darragh et al., 1990). Endogenous nitrogen secretion has also been found to be affected by age of the animal, protein content and source, as well as dietary fibre content (de Lange et al., 1989; Leterme, 1996).

#### General Methodology

Results of four experiments conducted in the Animal Science Research Unit (ASRU), at the University of Manitoba, Winnipeg, Manitoba are reported in this thesis. Experimental procedures, arrangement and care of animals were in accordance with Canada Council of Animal Care, (CCAC, 1980).

## **Experimental Animals**

Thirty (30) Costwold pigs (16-d old) were fasted for 24 h before surgery. Surgical procedures were performed by Drs. Nora J. Lewis and Sam K. Baidoo of University of Manitoba. Anaesthesia was induced with a mixture of halothane and oxygen by way of a facemask attached to a closed circuit breathing set up. Under local anaesthesia, the animal was placed dorsal-ventrally and the area from the last rip to the hind leg was shaved and washed with hibitane (Zeneca Pharma Inc. Montreal, Canada) and sprayed with industrial methylated spirit before surgery. An incision extending for 3 - 5 cm caudally was made about 3 cm behind the last rib through the body wall. The intestine was then exposed and exteriorized to locate the ileo-caecal junction. An incision was made approximately 5 - 15 cm anterior to the ileo-caecal junction and a "T" cannula inserted in this opening. The cannula was secured in position by means of purse-string sutures. Another incision was made between the last two ribs through the skin to hold the stalk of the cannula. The first incision was then closed from inside out.

# Housing and Care of Pigs

After surgery, the pigs were moved to an individual metabolic crate with dimensions, height, 80 cm; length 170 cm and width 65 cm with regular exercise (once a week) in floor pens, dimensions, height 91; width, 118 and length, 146 cm in ASRU. The pigs were provided with heat lamps to prevent chilling after surgery. The lamps were removed 72 hours after surgery. Room temperature was kept constant between 22 to 25 °C and pigs had free access to water from a lowpressured drinking nipple throughout the experimental period. For 3 d post surgery, excenel (Upjohn company, Orangeville, Ontario, Canada) was administered (1ml per 17kg liveweight) by intramuscular injection. The room was washed twice daily, and the cannulae and the area around the wound cleaned with Stanhexidine (Novopharm, Toronto, Canada) each day, dried with paper towel and the skin smeared with zincoderm (Rhone Merieux Canada Inc.), to avoid skin irritation due to the increased emission of digesta which were more liquid and acid than normal faeces. The pigs were weighed after each period and cared for following the guideline published by the Canada Council on Animal Care (CCAC, 1980).

## PEAS

Raw, extruded and micronized peas of same variety (Impala) and obtained from the same source were used. All the peas were tannin free and smooth. Extrusion of the whole peas was carried out in a wet extruder. The whole pea was moved through an auger and conditioner where steam was injected to raise the

temperature and moisture content to 62.8 °C and 20% respectively. The pea was then passed through the extruder screen and carried through the cylinder in 30 - 40 seconds. During the passage, the temperature of the cylinder was maintained between 137.8 - 162.8 °C. The extruded peas left the end of the cylinder through two 2.4 mm x 76.2 mm long openings in the die plate. Following extrusion, the ground pea was cooled and the moisture content reduced to 11%. Prior to commencement of the experiment all peas sample were analyzed for their chemical composition.

Micronization of the ground peas was carried out in a micronizer (Micronizing Co. UK. Ltd., Suffolk, England), made up of gas fired infrared ceramic heaters under which a conveyor carries the ground peas one layer thick. The infrared rays excite the evenly distributed molecules in the ground pea seeds which then vibrate at a frequency of 600 - 1,200 million per second resulting in rapid internal heating and a rise in water vapour pressure. The ground pea seed cooked inside out swelled and fractured. The soft, turgid ruptured product obtained was then rolled, flaked and then cooled. The micronization process takes about 45 seconds at 140 °C.

### Feed and Feeding

Raw, extruded and micronized peas used were ground through a 3-mm screen. Peas flour was then mixed with corn starch, pure crystalline cellulose, sucrose and a commercial mineral-vitamin premix to form the treatment diets

shown in Table 5. A fifth diet, protein-free, was also formulated. Chromic oxide was added to the diets 3 g/kg DM to help estimate the digestibility coefficient.

During recovery from surgery, the pigs were fed increasing amounts of standard diet until they were able to consume 4 % of their live body weight daily. Following a 10-d recuperation period, the pigs were fed 4 % of their body weight (determined 4 hours before the start of a phase), on the experimental diets (Tables 5). Their daily feed allowance was divided to three equal meals fed at 06:00, 12:00 and 18:00 h. A small quantity of water was added to the ration during feeding. Each experimental period was divided into five phases. A phase consisted of seven day adaptation to the diet, during which the pigs received the test diet at the same amount as the collection period, a one-day faecal collection from 08:00 -20:00 h and a two-day digesta collection, from 08:00 - 20:00 h each day. The ileal digesta of the cannulated pigs were collected in plastic bags attached to the stalk of the canulae containing formic acid (10% v/v) (to prevent microbial growth). After collection, the samples were immediately stored at -20 °C, until ready for analyses. The frozen ileal digesta and the faecal samples collected were separately freeze dried and milled through a 1-mm sieve. The samples from individual pig were pooled and thoroughly mixed and a representative sample collected for chemical analyses.

## Chemical Analysis

Every pea batch and each diet was analyzed for dry matter (DM), ash, fat, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), protein,

amino acids, and gross energy using standard method of analysis (AOAC, 1990). Chromic oxide was analysed using the method described by William et al., (1962).

## **Dry matter Determination**

5g of sample was weighed in a pre-weighed silica dishes and dried to a constant weight for approximately 16 - 24 hours in a forced draught oven set at 105 °C. The samples were then removed, cooled in a dessicator and re-weighed.

#### Protein

Nitrogen was determined by the Kjeldahl procedure detailed in the AOAC (1990). Approximately 0.5 - 1.0 g of each sample was weighed into a digestion tube. Samples were digested in 18-ml concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) 36% w/v using selenium as a catalyst. Nitrogen content was then measured using Kjeltec Auto 1030 Analyser, and converted to protein using a 6.25 conversion factor.

#### Amino acids

Amino acid determination was carried out by weighing a 100-mg sample and prepared for acid hydrolysis using the method of AOAC (1990). The weighed sample was carefully transferred to a side-arm hydrolysis tube. Two drops of 2-octanal were added followed by addition of 4 ml 6 N hydrochloric acid. The tube was evacuated for at least 30 seconds, after placing a stopper on. This was then placed on a pre-heating block and heated for 24 hours at 110 °C. The sample was

then removed on the next day and quickly cooled in ice. 4 ml of 25.625% w/v sodium hydroxide was added, mixed and cooled to room temperature. The cooled sample was quantitatively washed into a 50-ml volumetric flask using sample dilutor. 10-ml mixture was then filtered, frozen till ready for analysis. For methionine and the other sulphur containing amino acids, samples were prepared using performic acid oxidation followed by acid hydrolysis. Performic acid was made by mixing formic acid (88%) and hydrogen peroxide (35%) in proportion of 9:1, and allowed to stand for an hour before addition. 100 mg of sample was weighed into a stoppered hydrolysis tube and two drops of 2-octanal added. 2 ml of performic acid was then added and allowed to stand in a fridge for 20 hours. 0.5 ml concentrated HCl was then added and allowed standing in a fume hood for 6 hours. 2 ml of concentrated HCl was again added and the sample was placed on a heating block for 16 hours, after which the process was very similar to that of the neutralization procedure described previously. Chromatographic separation and quantification of amino acids was achieved by LKB 4151 Alpha plus AA analyzer (LKB Biochrom, Cambridge, UK), equipped with an LKB 4029 Programmer and a 3393A Hewlett-Packard Integrator (Hewlett-Packard Co., Avondale, USA), which uses a cation exchange column, followed by post-column reaction with ninhydrin and colorimetric detection at 570 nm. The concentration of each amino acid was calculated using the internal standard. Peak areas were recorded and calculated by Hewlett-Packard Integrator.

#### Fibre

Dietary fibre (DF) was determined by the method described by Slominski et al., (1994). In this method, DF was estimated from the sum of neutral detergent fibre (NDF) and acid detergent fibre. The NDF component was determined by using a refluxing apparatus. Tecator Equipment, (Laboratory Construction Col. Kansas City, MO), according to the procedure outlined by Van Soest and Wine (1967) and modified by Robertson and Van Soest (1977) with addition of  $\alpha$ amylase enzyme (Termamyl) (Novo Nordisk A/S, Bagsvaerd, Denmark). 0.5 to 1 g of sample was weighed into sintered glass crucible. The crucible was then placed on a hot extraction unit, making sure that the seal was good. Approximately 50-ml neutral detergent buffer was then added and boiled for 30 minutes. The heat was turned off and solution vacuumed. About 40 ml of hot distilled water and 0.05 ml of enzyme (Heat Stable Amylase Sigma A3306), was then added and allowed to stand for about 10 minutes. The solution was vacuumed off, rinsed with boiling water and transferred to the cold extraction apparatus, rinsed twice with acetone using vacuum, removed and dried 16 - 24 hours at 105 °C. The sample was then removed to a desiccator and weighed. The procedure presupposed that any and all material that was not neutral detergent fibre was removed and any material left was NDF.

The procedure for the determination of acid detergent fibre (ADF) was similar to that of NDF, except that acid detergent buffer solution was used and no enzyme was used in this case.

### **Energy**

Gross energy of samples was measured by a Parr adiabatic oxygen calorimeter (Parr Instrument Co., Moline, IL), that had originally been calibrated. Result was obtained from the pre-programmed calorimeter controller unit.

#### Fat

2 g of sample was weighed and wrapped in #1 Whatman paper and placed in extraction thimble, put in a 150 ml pre-weighed Berzelius beaker in 35 ml hexane, place on a heater 4 hours from the onset of boil, the element was lowered, extraction thimble was removed and instead replaced with collection thimble and returned to boil. Hexane was removed at approximately 5 ml toggled dried on low heat to about 0.5 ml. This was placed in oven at 100 °C for approximately 8 - 12 hours. The beaker was then weighed the next day and the weight of the extracted fat obtained.

#### Chromic Oxide

0.5 g of sample was weighed in a crucible, ashed at 500 °C for 16 hours. The ashed sample was then transferred into a beaker to which phosphoric acid and 3 ml manganese sulphate and 4 ml potassium bromate solution were gently added. The beaker was covered with watch glass, placed on a heated hot plate, until no effervescence was observed and the solution changed colour to pink. After cooling, the solution was carefully transferred to a 200-ml volumetric flask containing 25-ml calcium chloride. The flask was then made up to volume using

distilled water. The solution was analyzed, using Atomic Absorption Analysis (Perkin-Elmer, model 603A) at 267.7 nm, and measured against a working standard (0 - 30 ppm), after keeping for 8-12 hours as described by Williams et al., (1962).

### DIGESTIBILITY STUDIES USING RAW, EXTRUDED OR MICRONIZED PEAS.

## **Experimental Diets**

Four experimental diets were formulated with raw peas (A 1), A + amylase (A 2), A + amylase + xylanase (A3) and A + amylase + protease + xylanase (A 4) for trial 1. Extruded and micronized peas replaced raw peas in trial 2 and 3 respectively. The diets were formulated so that pea was the only protein source to avoid interference when looking at the digestibility of protein and amino acids. Sucrose, cellulose and cornstarch were added as diluters. Vitamin-mineral premix was also added. Chromic oxide was included as the indigestible phase marker to help estimate nutrient digestibility. A fifth diet (NF) was formulated to contain all the above ingredients except peas. This was used to evaluate endogenous nitrogen losses to help determine the true digestibility of protein and amino acids. The mixture was thoroughly mixed so as to get even distribution of components. Feed was provided in a mash form. The composition of the diets is shown in Table 5.

Table 5. Diet formulation for digestibility of raw, peas supplemented with enzyme (trial 1)

Ingredient	A 1	A 2	A 3	A 4	NF
Raw Peas	75.0	75.0	75.0	75.0	•
Com Starch	12.7	12.6	12.4	12.1	87.7
Cellulose	2.0	2.0	2.0	2.0	2.0
Sucrose	5.0	5.0	5.0	5.0	5.0
Premix*	5.0	5.0	5.0	5.0	5.0
α-amylase**	-	0.1	0.1	0.1	•
Protease**	-	•	-	0.3	-
Xylanase**	-	-	0.2	0.2	•
Chromic oxide	0.3	0.3	0.3	0.3	0.3

\*Premix provided per kg of diet: 9,000 IU vitamin A, 1,500 IU vitamin D3, 18 mg vitamin E, 1.5 mg vitamin K, 250 mg choline, 30 mg niacin, 27.5 mg calcium pentothenate, 9.4 mg B2, 1 mg B6, 25 mcg B12, 50 mcg biotin, 0.5 mg folic acid, 5.75 g calcium, 2.6 g phosphate, 3.5 g sodium chloride, 27.5 mg manganese, 105 mg iron, 125 mg copper, 0.6 mg

In Trials 2 and 3 extruded and micronized peas respectively replaced raw peas.

<sup>\*\*</sup>Provided by Finfeeds International

#### **Experimental Animals**

Thirty (ten for each trial), female Costwold piglets weaned at 16-d old, surgically fitted with a Simple T-shaped silicone cannulae with internal diameter of 18 mm at approximately 15 cm anterior to the ileo-caecal junction as described earlier, were used for this digestibility study. The pigs were weighed at the beginning of each experimental phase and housed in individual metabolic cages throughout the experimental period. The guidelines published by CCAC (1980) were followed for the care of the pigs.

## **Experimental Design**

A 5 X 5 Latin square experimental design was used as a five-phase trial. The ten cannulated pigs were randomly assigned to the five diets (A 1, A 2, A 3, A 4 and NF) each diet was allocated to two pigs at a time during each phase. After completion of a phase and collection of faeces and digesta, the combination of diet/animal was switched over to a second, third, fourth and then a fifth diet and the collection procedure repeated. The design yielded 10 determinations for each test diet.

#### **Experimental Protocol**

The pigs were fed 4% of their live body weight (determined over 4 hours before the commencement of a phase), during the experiment. Their daily feed allowance was divided into three equal meals, fed at 06:00, 12:00 and 18:00 hours. The animals had free access to water, while room temperature was kept constant

around 22 - 25 °C. The trial consisted of a 7 d adaptation period (where pigs received test diet at the same amount as during the collection period), a day of faecal collection (07:00 to 20:00 hours), followed by a two day iteal digesta collection (07:00 to 20:00 h each day at 30 minutes interval or when bag was full). Samples were collected into plastic bags and immediately frozen at -20 °C until ready for analyses. After completion of the experiment, samples collected were pooled within pig and period for the same diet, ground through a 1-mm mesh screen, mixed thoroughly and a representative sample collected for chemical analyses was determined.

# Chemical analysis and Digestibility Calculations

Diets, faeces and digesta were analyzed for dry matter (DM), crude protein (CP), gross energy (GE), amino acid (AA) and chromic oxide by individual analytical method described above.

Apparent and true Ileal and faecal digestibility of crude protein, energy and amino acids were calculated using the relative concentrations of chromic oxide in the diets, faeces and digesta. The true digestibilities were also obtained by correcting for endogenous secretion.

Apparent digestibility (AD) coefficient was given by;

 $AD = 100 - [(Id \times Ar)/(Ad \times Ir)] \times 100\%$ 

Where: Id = chromic oxide concentration in the assay diet

Ar = nutrient concentration in ileal digesta and faeces

 $A_d$  = nutrient concentration in the assay of diet

60

I/= chromic oxide concentration in ileal digesta or faeces.

The quantity of endogenous nitrogen obtained with feeding nitrogen free diet was deducted from the total quantity of nitrogen found in the digesta or faeces to obtain

the true digestibility (TD).

True digestibility (TD) = {a - (b+c)} /a

Where a and b are the ratio of the nutrient to the index substance in feed and

digesta or faeces respectively and c, the correction factor.

c = Nitrogen in digesta (faeces) when nitrogen free diet was fed.

Statistical Analysis

Crude protein, gross energy and AA digestibility values obtained were

subjected to analysis of variance using General Linear Modeling (GLM) in the

Statistical Analysis System (SAS Institute Inc., 1988). Tukey's procedure was used

to compare and separate treatment means. The  $\alpha$ -level for significance was p $\leq$ 

0.05.

Model used was,

 $B = \mu + t_i + p_j + a_k + e_{ijk}$ 

Where: B = the digestibility of the k m pig fed diet i in the j m period

 $\mu$  = The population mean

tr= the effect of the i m diet

 $p_j$  = effect of j # period

 $a_k$  = the k m animal

EXPERIMENT 4. PERFORMANCE OF EARLY-WEANED PIGS FED RAW, EXTRUDED AND MICRONIZED PEA DIETS SUPPLEMENTED WITH  $\alpha$ -AMYLASE AND XYLANASE.

#### **Materials and Methods**

## Experimental Animal and design

Seventy (70) crossbred pigs with mean initial weight of 4.0 kg, weaned at 16-day of age were randomly allotted to one of the seven dietary treatments, in a completely romdomized design. There were five pens per treatment with two pigs per pen (109.2 x 88.8 x 86.4 cm). Piglets on each treatment were balanced for litter origin, sex and weight.

#### Feed and feeding

The study was a comparison of seven experimental diets, soybean control (N 1), raw pea (N 2), raw peas + enzyme (N 3), extruded peas (N 4), extruded peas + enzyme (N 5), micronized peas (N 6) and micronized peas + enzyme (N 7) during the first phase (4.0 -10.0 kg) and later switched to L 1, L 2, L 3, L 4, L 5, L 6 and L 7 respectively, during the second phase (10.0 - 20.0 kg). Tables 6 and 7 are summaries of composition of the diets for phase 1 and 2 respectively. All diets were formulated to contain similar levels of nitrogen and energy and were balanced for lysine, methionine, threonine and tryptophan to meet NRC, (1998)

Table 6. Composition and chemical analysis of experimental diets for starter phase 1 (4.0 - 10.0 kg)

INCLUSION RATE (%)									
Ingredient	N 1	N 2	N 3	N 4	N 5	N 6	N 7		
Com	37.0	19.0	19.0	19.0	19.0	19.0	19.0		
Peas	-	30.2	30.2	30.2	30.2	30.2	30.2		
SBM	19.05	7.0	7.0	7.0	7.0	7.0	7.0		
Fish meal	6.0	6.0	6.0	6.0	6.0	6.0	6.0		
Dried whey	13.34	13.34	13.04	13.34	13.04	13.34	13.04		
Oat groats	11.0	11.0	11.0	11.0	11.0	11.0	11.0		
Canola oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0		
SDPP*	3.0	3.0	3.0	3.0	3.0	3.0	3.0		
Premix**	5.0	5.0	5.0	5.0	5.0	5.0	5.0		
L-Lysine	0.57	0.25	0.25	0.25	0.25	0.25	0.25		
DL-Methionine	0.04	0.17	0.17	0.17	0.17	0.17	0.17		
Threonine	•	0.04	0.04	0.04	0.04	0.04	0.04		
α-Amylase***	-	-	0.1	-	0.1	-	0.1		
Xylanase***	-	-	0.2	-	0.2	-	0.2		
Analyzed Composition	on (%)								
CP	21.0	20.1	20.1	20.2	20.1	20.2	20.2		
GE (MJ/kg)	3435.2	3416.2	3416.0	3426.0	3428.1	3429.2	3426.4		
Lysine	1.38	1.39	1.38	1.39	1.39	1.38	1.39		
Methionine	0.41	0.39	0.39	0.39	0.39	0.39	0.39		
Threonine	0.92	0.96	0.97	0.98	0.97	0.99	0.98		

<sup>\*</sup>SDPP (spray dried porcine plasma), CP (crude protein), DE (digestible energy), SBM (soybeam meal)

<sup>\*\*</sup>Premix provided per kg of diet: 9,000 IU vitamin A, 1,500 IU vitamin D3, 18 mg vitamin E, 1.5 mg vitamin K, 250 mg choline, 30 mg niacin, 27.5 mg calcium pentothenate, 9.4 mg B2, 1 mg B6, 25 mcg B12, 50 mcg biotin, 0.5 mg folic acid, 5.75 g calcium, 2.6 g phosphate, 3.5 g sodium chloride, 27.5 mg manganese, 105 mg iron, 125 mg copper, 0.6 mg iodine.

<sup>(\*\*\*</sup> Enzymes provided by Finfeeds International (UK)).

Table 7. Composition and chemical analysis of experimental diets for starter phase 2 (10 - 20 kg)

		1	NCLUSION R	ATE (%)		<del></del>	
Ingredient	L1	L 2	L3	L4	L 5	L 6	L 7
Com	35.0	17.0	17.0	17.0	17.0	17.0	17.0
Peas	-	35.25	35.0	35.25	35.0	35.25	35.0
SBM	24.0	7.0	7.0	7.0	7.0	7.0	7.0
Fish meal	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Dried whey	13.5	13.6	13.6	13.6	13.6	13.6	13.6
Oat groats	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Canola oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Premix*	5.0	5.0	5.0	5.0	5.0	5.0	5.0
L-Lysine	0.4	0.07	0.07	0.07	0.07	0.07	0.07
DL-Methionine	0.10	0.04	0.02	0.04	0.02	0.04	0.02
Threonine	-	0.04	0.01	0.04	0.01	0.04	0.01
α-Amylase**	-	•	0.1	•	0.1	-	0.1
Xylanase**	•	-	0.2	-	0.2	-	0.2
Analyzed Composi	ition (%)						
СР	20.8	18.5	18.5	18.5	18.5	18.5	18.5
GE (MJ/kg)	3538.9	3510.6	3510.6	3510.6	3510.6	3510.6	3510.6
Lysine	1.06	1.07	1.07	1.07	1.07	1.07	1.07
Methionine	0.40	0.39	0.39	0.39	0.39	0.39	0.39
Threonine	0.97	0.96	0.96	0.96	0.96	0.96	0.96

<sup>\*</sup>Premix provided per kg of diet: 9,000 IU vitamin A, 1,500 IU vitamin D3, 18 mg vitamin E, 1.5 mg vitamin K, 250 mg choline, 30 mg niacin, 27.5 mg calcium pentothenate, 9.4 mg B2, 1 mg B6, 25 mcg B12, 50 mcg biotin, 0.5 mg folic acid, 5.75 g calcium, 2.6 g phosphate, 3.5 g sodium chloride, 27.5 mg manganese, 105 mg iron, 125 mg copper, 0.6 mg iodi. (\*\*Provided by Finfeeds International (UK)).

requirements for early-weaned pigs. The pea-based diets were formulated such that they only differ in enzyme supplementation. The feed was steam pelleted and available ad libitum from self-feeders at all time, except during blood sampling periods and weighing.

#### Data collection

Weekly weights gain per pig and mean pen feed intake was recorded. Feed conversion efficiency (feed/gain) was obtained from the measurement of daily feed intake and weight gain per pen and per pig.

#### Blood sampling

All seventy pigs in the study were blood sampled. Pigs were bled at the start, and end of phase 1, (10.0 kg liveweight) and finally at the end of phase 2, (20.0 kg liveweight) of the experiment. Pigs were starved overnight and blood was collected via the jugular vein in the morning. Blood samples were collected in heparinized vacuum container tubes (Becton Dickinson, Rutherford, NJ), which were immediately stored in a cold room (-4 °C) for overnight. The tubes containing the samples were then centrifuged for 30 minutes and plasma pipetted into vials. These were stored frozen (-20 °C) until ready for plasma urea nitrogen analysis.

### Chemical analysis

Feed samples for each of the phases were collected, mixed and ground in a Tecator cyclotec 1093 sample mill (Hogman, Sweden) for chemical analysis.

Samples were dried in a convection oven at 105 °C for 16 - 24 hours for DM determination. Crude protein, amino acids, gross energy acid detergent fibre and neutral detergent fibre of the feed was determined according to standard procedures as described by Association Of Analytical Chemists (AOAC, 1990).

#### Plasma Urea Nitrogen

Plasma samples were analyzed for urea nitrogen concentrations using a standard kit (Procedure No. 535) from Sigma Diagnostics (Sigma Diagnostic, St. Louis, MO., USA). This is done by quantitative, calorimetric determination of blood urea nitrogen in serum or plasma at 515-540 nm, (the Sigma procedure), based on techniques described by Crocker, (1967). Prior to analyses samples were brought out of the freezer and allowed to equilibrate to room temperature. 0.02 ml of each sample was pipetted into labeled test tubes. Urea nitrogen diluted standards were prepared by pipetting the BUN reagents into test tubes and mixing thoroughly. To each tube 3.0 ml BUN acid Reagents (Catalog No. 535-3, St. Louis MO., USA) and 2.0 ml BUN Color Reagent (Catalog No. 535-5, St. Louis, MO., USA) were added and mixed thoroughly. All tubes were placed in boiling water bath simultaneously for exactly 10 minutes. The tubes were quickly removed and placed in cold tap water for 3 minutes, after which absorbance was read within 20 minutes. A urea nitrogen calibration curve was prepared using absorbance versus the corresponding urea nitrogen concentration (mg/dl), from the standard prepared. Plasma urea nitrogen values for each sample was then obtained from the calibration curve, using the corresponding absorbance. The principle behind

this is that urea concentration is directly proportional to intensity of the colour produced, which is measured spectrophotometrically between 515-540 nm.

# Diacetyl Monoxime + Urea → Pink Chromogen + Hydroxylamine

### Statistical analysis

All data collected (average daily gain, average daily feed intake, feed conversion efficiency and duration of experiment, were subjected to analysis of variance using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS Institute Inc., 1988). The  $\alpha$ -level of significance was p  $\leq$  0.05 and differences between means were separated using Duncan's multiple range tests (1955).

#### **Peas Composition**

Raw, extruded and micronized peas had very similar proximate chemical composition (Table 8). The dry matter (DM) ranges between 88 to 90.4%. Gross energy was also similar, for the raw, extruded and the micronized peas. Acid detergent fibre, neutral detergent fiber as well as ether extract was similar for the entire pea samples analyzed. The crude protein (CP) content was 20.0, 20.4 and 20.8% for raw, extruded and micronized peas respectively. All the amino acids were somewhat similar and revealed high levels of lysine (Table 8). Methionine and cysteine levels were low 0.24, 0.23 and 0.23% and 0.21, 0.20, 0.20% respectively for raw, extruded and micronized peas respectively.

Heat processing (extrusion or micronization) did not affect nutrient composition of peas and the proximal compositions were comparable to values reported in the literature (Igbasan et al., 1997, NRC, 1998). The amino acids content was similar to reported values (Fan et al., 1994a; Igbasan et al., 1997). The relatively high levels of lysine and low levels of methionine and cysteine are constant characteristics of peas and were not affected by heat treatment. In the presence of reducing sugars, amino acids can form a complex Maillard reaction when heat is applied to the protein (Mauron, 1981; Michealsen, 1992). Lysine, methionine and cysteine are the most susceptible amino acids to this reaction (Bender, 1978, Michaelsen, 1992). Under mild conditions, Maillard reaction intermediate compounds are degraded during acid hydrolysis used in

Table 8. Composition of Raw, extruded and micronized peas

		PEAS	
Nutrients (%)	Raw	Extruded	Micronized
Dry Matter	88.60	90.10	90.4
Energy (Kcal/kg)	3680.1	3688.2	3886.3
Crude Protein (N x 6.25)	19.50	20.30	20.50
Acid detergent fibre	8.40	8.26	8.23
Neutral detergent fibre	16.71	16.66	16.60
Ether extract	1.40	1.44	1.49
Indispensable amino acid			
Arginine	1.84	1.82	1.84
Histidine	0.70	0.69	0.73
Isoleucine	1.12	1.11	1.10
Leucine	1.64	1.66	1.69
Lysine	2.00	1.99	2.03
Methionine	0.22	0.20	0.21
Phenylalanine	0.96	0.94	0.94
Threonine	0.70	0.71	0.70
Valine	1.38	1.40	1.41
Dispensable amino acids			
Alanine	0.80	0.82	0.80
Aspartic acids	2.51	2.54	2.53
Glutamic acid	3.66	3.62	3.67
Glycine	0.81	0.79	0.76
Cysteine	0.21	0.20	0.20
Tyrosine	0.49	0.44	0.47
Serine	0.86	0.81	0.83

conventional amino acids analysis. On severe over heating, the resulting compounds are not recoverable after acid hydrolysis leading to detection of lower levels of reactive amino acids in the heated protein (Mauron, 1981; Moughan, 1991) and the materials turn brownish in colour. This assumption is based on the similar amino acids compositions for the raw, extruded and micronized peas analyzed. No brownish colour development associated with the advanced Maillard reaction was observed in the processed peas and the amino acid levels were similar. The heating conditions applied during processing of peas for this study were hence not severe enough to have caused such a reaction.

EXPERIMENT 1. EFFECT OF ENZYMES SUPPLEMENTATION ON DIGESTIBILITY OF RAW PEAS BY EARLY-WEANED PIGS.

Apparent ileal and faecal digestibility of crude protein and amino acids of raw pea

Results of the apparent ileal and faecal digestibility (AID and AFD) of crude protein and amino acids of raw peas supplemented with enzyme diets obtained from cannulated pigs are summarized in Tables 9 and 11 respectively. The AFD of all the diet constituents were greater than their ileal counterparts. The difference can be explained by the absorption at the large intestine of dietary origin nutrients (mineral, electrolytes) and microbial fermentation products (volatile fatty acids, ammonia). Pigs under normal circumstances do not utilize ammonia, the AFD values, therefore over estimates the effective used of dietary protein. The AID and

AFD obtained in this trial are comparable to some values stated in the literature (Leterme et al., 1990; Huisman et al., 1992; Fan et al., 1994b; Grosjean et al., 1997) but higher than that of van Barneveld et al., (1994), however, no enzyme was added in any of the studies reported.

With the exception of threonine, there was no significant difference (p > 0.05) between all the four treatments. Inclusion of  $\alpha$ -amylase to raw peas resulted in a significant (p < 0.05) improvement in AID of threonine, in non-supplemented (55.9%) compared to amylase and xylanase supplemented raw peas (73.4%). Also mean AID of indispensable amino acids tended to increase with enzyme supplementation. This observation was again true for the dispensable amino acids. The mean AID of dispensable amino acids tend to increase, though not significant (p > 0.05), with enzyme addition. Though no direct comparisons could be made to the literature as a result of scanty information on digestibility of peas using enzymes, the results are in the range reported for pea nutrients digestibility. The low digestibility of threonine has also been reported (Knabe et al., 1989). de Lange et al., (1989) attributed this to the high concentration on threonine in endogenous secretions. Furthermore, threonine has been found to be less rapidly absorbed than most amino acids (Low, 1982), and has a lower affinity for the transport mechanism across the intestinal epithelium. The low levels in pea seed could also explain the low digestibility of cysteine. Digestibility values are expressed as percentage of intake, hence, small amount of cysteine in ileal effluents could significantly decrease its apparent digestibility. The high increase in

Table 9. Apparent ileal digestibility (%) of raw peas supplemented with enzymes.

Parameter	Raw	Raw + A	Raw + A + X	Raw + A + P + X	S.E.M.
Energy	78.3	78.7	78.9	79.7	2.2
Crude Protein	75.4	82.1	82.4	83.6	2.4
Indispensable Am	ino Acids				
Arginine	80.7	89.6	85.6	84.8	2.6
Histidine	82.4	83.5	80.7	81.7	2.9
soleucine	79.6	81.2	76.0	75.8	2.4
Leucine	79.9	81.3	77.8	79.4	2.8
_ysine	80.6	86.8	81.8	82.9	2.8
Methionine	77.9	73.9	79.8	74.4	2.6
Phenylalanine	75.6	79.9	80.1	81.7	1.9
Threonine	55.9b	72.7a	73.7a	74.5a	3.1
/aline	74.5	79.4	74.7	75.0	2.6
Mean	76.2	80.9	80.8	78.9	2.6
Dispensable Amin	o Acids				
Alanine	73.2	74.2	79.8	79.8	3.3
Aspartic acid	72.1	78.8	77.8	78.6	1.7
Cysteine	55.5	63.8	62.0	60.6	2.7
Slutamic acid	80.4	84.3	84.6	84.9	3.2
Slycine	71.8	60.5	64.8	61.4	2.9
Proline	65.9	76.3	78.3	69.7	3.2
Serine	76.9	85.4	74.7	76.6	3.4
Tyrosine	79.4	83.4	79.7	81.7	2.2
lean	71.9	75.8	76.2	75.2	2.8

Means in the same row followed by different letters are significantly different (P < 0.05)

Table 10. True ileal digestibility (%) of raw peas supplemented with enzymes.

Parameter	Raw	Raw + A	Raw + A + X	Raw + A + P + X	S.E.M.
Energy	79.9	80.0	79.9	81.2	3.1
Crude Protein	84.5	86.3	86.7	87.0	2.6
Indispensable Ami	no Acids				
Arginine	86.2	91.0	92.7	90.6	2.2
Histidine	87.2	86.7	88.1	87.0	2.7
Isoleucine	87.8	85.5	87.1	83.7	3.1
Leucine	88.4	87.0	87.2	84.6	2.3
Lysine	85.4	88.3	90.6	88.4	3.4
Methionine	80.6	84.4	83.2	82.2	2.3
Phenylalanine	79.8	85.6	86.8	87.1	2.3
Threonine	80.4	82.0	83.4	83.4	2.9
Valine	83.5	84.9	84.4	87.3	3.4
Mean	84.4	86.2	87.1	86.0	2.7
Dispensable Amino	Acids				
Alanine	86.2	83.5	85.5	83.6	2.1
Aspartic acid	87.8	85.8	86.2	85.4	2.3
Cysteine	80.6	84.2	83.4	82.5	2.3
Glutamic acid	84.4	87.3	88.3	88.6	2.4
Glycine	80.6	82.8	84.2	83.3	2.5
Proline	79.9	82.9	84.4	85.3	2.2
Serine	80.9	88.1	86.4	87.7	2.7
Tyrosine	85.8	87.0	85.4	85.2	2.2
Mean	83.3	85.2	85.5	85.2	2.4

Table 11. Effect of enzyme supplementation on apparent faecal digestibility (%) of raw pea (R).

Parameter	R	R+A	R+A+X	R+A+P+X	S.E.M.
Energy	82.3	84.0	89.9	89.2	3.1
Crude Protein	83.4	84.0	83.9	83.0	3.6
Indispensable Am	ino Acids				
Arginine	86.8	90.1	87.4	87.3	3.7
Histidine	84.2	84.3	89.1	84.9	3.7
Isoleucine	82.0	84.5	77.3	79.2	5.1
Leucine	83.7	85.2	81.4	79.9	4.6
Lysine	85.9	86.9	83.9	85.3	4.1
Methionine	79.9	77.8	80.6	80.6	3.3
Phenylalanine	79.5	84.0	83.1	84.0	4.2
Threonine	80.1	79.9	80.1	77.0	4.3
Valine	79.4	82.3	80.7	79.4	4.8
Mean	82.4	83.7	82.7	81.9	4.2
Dispensable Amin	o Acids				
Alanine	85.2	84.3	80.1	77.0	4.9
Aspartic acid	83.0	81.6	82.3	80.9	3.9
Cysteine	70.4	70.9	79.4	74.7	4.7
Glutamic acid	82.3	86.7	87.0	86.0	3.6
Glycine	79.1	74.9	78.9	70.8	5.4
Proline	70.0	78.1	77.2	81.0	4.2
Serine	79.3	83.0	79.7	80.7	3.9
Tyrosine	82.1	85.7	84.3	82.6	3.7
Mean	78.9	80.6	81.1	79.2	4.3

Table 12. Effect of enzyme supplementation on true faecal digestibility (%) of raw pea (R).

Energy Crude Protein Indispensable Amin	89.6 85.6	90.1 87.2	90.5	90.4	3.7
		87.2			
Indignengable Amin	o Acide	- <del></del>	87.9	86.8	3.3
maisponsable /allin	io Acids				
Arginine	87.1	93.9	92.3	90.7	3.8
Histidine	86.3	87.6	90.4	88.0	3.3
Isoleucine	82.9	87.5	88.9	86.2	3.1
Leucine	85.0	88.3	89.0	85.1	3.6
Lysine	88.4	90.1	90.4	89.9	3.1
Methionine	79.2	86.9	86.0	84.7	3.1
Phenylalanine	80.4	87.7	87.8	87.6	3.2
Threonine	82.4	84.9	86.7	87.1	3.1
Valine	79.8	86.2	86.4	89.4	4.8
Mean	83.5	88.1	88.6	87.6	3.3
Dispensable Amino	Acids				
Alanine	88.3	85.3	86.9	84.8	3.1
Aspartic acid	84.9	87.0	88.1	87.9	3.4
Cysteine	77.8	85.9	87.6	83.4	4.7
Glutamic acid	82.0	88.4	88.7	89.0	3.3
Glycine	79.8	84.3	87.0	84.0	3.6
Proline	74.2	85.7	85.1	87.6	5.4
Serine	81.8	90.2	86.5	87.9	4.8
Tyrosine	84.7	88.9	87.9	87.9	3.3
Mean	81.7	86.9	87.2	86.4	3.3

the energy digestibility after passing through the large intestine (90.5%), compared to the ileal value of 78.9% indicates a high disappearance of nutrients in the lower gut as a result of microbial degradation. Residual amino acids are metabolized at this site.

#### True ileal and faecal digestibility of raw peas supplemented with enzymes.

The summary of the results for the true iteal and faecal digestibility (TID and TFD) of main peas nutrients is presented in Tables 10 and 12, respectively. The values of TID and TFD also showed no significant difference (p > 0.05) for all the four dietary treatments. Gdala et al., (1996), also observed no influence on iteal and faecal digestibility of dry matter, crude protein and amino acids with enzyme supplementation. Compared to the apparent digestibility, true digestibility values were slightly higher. These were expected since with the true digestibility, nitrogen from endogenous origin was corrected for. Inclusion of amylase, amylase + xylanase, or amylase + protease + xylanase in raw peas resulted in about 2.9, 5.0 and 3.0 % unit increased respectively in the TID of lysine (p=0.14). Methionine digestibility also increased marginally by 3.9, 1.6 and 0.6 % respectively (p=0.20). In addition, true digestibility of phenylalanine also increased with enzyme supplementation.

However, none of the above were statistically significant (p > 0.05). The mean TID and TFD for essential and non-essential amino acids were higher compared to that of AID and AFD. The true digestibility values observed in this experiment were comparable to numerous studies in the literature (Leterme et al.,

1990; Fan et al., 1994b; Grosjean et al., 1997). The indispensable amino acids were in all cases better digested than the dispensable amino acids. Again, this was not unexpected, since it is in line with the observation that when absorption of an individual amino acid is measured as the percentage of amino acids present, dietary essential amino acids tend to be absorbed in greater amounts than non essential amino acids, with methionine usually near or at the top of the list (Webb, 1990). The higher digestibility of the basic amino acids (arginine and lysine) may be a direct result of stimulation of intestinal transport of these amino acids by alanine, phenylalanine, leucine or methionine as reported by Webb (1990) and Knabe (1989).

EXPERIMENT 2. EFFECT OF ENZYME SUPPLEMENTATION ON DIGESTIBILITY OF EXTRUDED PEAS BY EARLY-WEANED PIGS.

Apparent ileal and faecal digestibility of crude protein, energy and amino acids of extruded peas

The apparent iteal and faecal digestibility of amino acids are shown on Tables 13 and 15 respectively. The mean apparent iteal digestibility of amino acids was almost identical to that of nitrogen, as in most feeds of plant origin (reviewed by Austic, 1983). The results in this experiment are comparable to the values obtained by Green (1988), for growing pigs fed 80% peas, but higher than those of Leterme et al., (1990) and Huisman et al., 1992). With the exception of glycine and threonine, there was no statistical difference (P > 0.05) among the animals and

Table 13. Apparent iteal digestibility (%) of extruded pea (E) supplemented with enzymes.

Parameter	E	E+A	E+A+X	E+A+P+X	S.E.M.
Energy	80.2	81.0	82.9	82.1	2.2
Crude Protein	79.8	80.3	84.6	84.0	2.7
Indispensable Am	ino Acids				
Arginine	86.0	90.3	91.8	86.1	2.1
Histidine	82.3	85.6	87.8	84.2	2.8
Isoleucine	82.2	82.5	87.2	83.3	3.9
Leucine	82.2	87.0	88.5	79.9	2.1
Lysine	82.2	88.5	87.5	87.1	2.3
Methionine	79.5	79.4	83.3	84.1	2.3
Phenylalanine	81.6	85.2	88.4	87.5	2.6
Threonine	67.4b	75.7ab	82.6a	79.3a	2.7
Valine	76.6	80.0	83.6	81.1	3.1
Mean	80.9	82.9	86.7	83.6	2.6
Dispensable Amin	no Acids				
Alanine	84.3	85.4	87.0	78.3	2.9
Aspartic acid	82.2	85.7	87.1	81.2	2.4
Cysteine	52.4b	67.9a	72.1a	62.3ab	2.4
Glutamic acid	83.2	88.9	91.1	85.3	3.4
Glycine	76.0a	71.2ab	75. <b>4a</b>	63.5b	3.5
Proline	71.3	75.5	81.1	75.5	2.7
Serine	77.6	89.2	85.8	79.3	3.1
Tyrosine	81.5	91.2	89.1	87.5	2.9
Mean	76.1	81.9	83.6	76.6	2.8

Means in the same row with different letters are significantly different (p < 0.05).

Table 14. True ileal digestibility (%) of extruded pea (E) supplemented with enzymes.

Parameter	E	E+A	E+A+X	E+A+P+X	S.E.M.
nergy	83.6	86.3	85.9	85.5	3.3
Crude Protein	85.7	88.2	90.4	89.3	3.6
ndispensable Am	ino Acids				
rginine	88.5	89.7	94.7	91.6	3.6
listidine	88.3	89.6	89.8	89.3	3.1
soleucine	89.5	89.7	89.9	90.1	3.4
eucine	90.8	90.0	91.2	90.3	4.4
ysine	88.4	89.5	90.1	88.3	4.2
lethionine	84.1	85.1	91.1	89.5	4.3
henylalanine	82.9	92.5	92.8	91.6	3.8
hreonine	83.4	85.5	90.3	90.3	2.8
aline	86.3	87.8	88.9	90.6	3.0
lean	86.9	88.8	91.0	90.2	3.6
ispensable Amin	o Acids				
lanine	87.4	89.5	89.8	92.0	3.3
spartic acid	89.6	92.1	90.3	90.8	2.8
ysteine	79.4	84.1	86.9	85.7	3.4
lutamic acid	85.7	92.0	93.1	92.6	3.6
lycine	82.8	88.1	85.8	89.6	3.2
roline	81.3	84.8	86.6	89.2	4.3
erine	81.7	92.1	88.7	89.4	3.0
yrosine	87.3	94.0	90.5	90.8	3.5
ean	84.4	89.6	90.6	88.1	3.4

Table 15. Effect of enzyme supplementation on apparent faecal digestibility (%) of extruded pea (E).

Parameter	E	E+A	E+A+X	E+A+P+X	S.E.M.
Energy	90.1	92.6	93.4	93.4	3.9
Crude Protein	81.7	84.6	87.1	84.2	3.1
ndispensable Am	ino Acids				
Arginine	86.9	90.7	92.4	87.9	3.7
listidine	83.6	87.6	88.1	88.6	3.3
soleucine	82.3	85.0	88.3	84.0	3.1
.eucine	84.2	89.0	90.3	80.4	3.6
ysine.	85.2	89.1	89.1	88.1	3.1
Methionine	80.6	80.4	85.3	82.0	3.1
henylalanine	81.4	90.7	89.9	87.8	4.4
hreonine	81.4	74.4	87.0	81.4	4.1
aline	79.1	86.7	83.9	86.4	3.8
lean	82.7	85.9	88.3	85.2	3.6
ispensable Amin	o Acids				
lanine	84.9	85.7	88.3	81.3	3.4
spartic acid	84.1	87.3	89.0	83.6	3.5
ysteine	72.8	72.0	78.9	80.1	3.7
lutamic acid	85.1	90.4	90.4	89.7	3.2
llycine	79.0	73.2	77.0	79.4	3.3
roline	72.3	77.9	82.3	79.6	3.5
erine	80.1	90.5	85.9	83.3	4.6
yrosine	83.4	92.7	89.9	89.0	3.4
ean	80.2	83.7	85.2	83.2	3.2

Table 16. Effect of enzyme supplementation on true faecal digestibility (%) of extruded pea (E).

Parameter	E	E+A	E+A+X	E+A+P+X	S.E.M.
Energy	92.1	92.4	93.6	93.6	4.3
Crude Protein	83.6	90.1	90.2	90.6	4.4
Indispensable Amin	o Acids				
Arginine	88.7	94.2	95.5	93.9	4.6
Histidine	84.6	90.8	91.3	89.7	4.5
Isoleucine	84.7	89.0	90.2	90.0	4.3
Leucine	86.1	90.1	92.0	89.9	4.7
Lysine	89.2	90.0	90.7	89.3	4.1
Methionine	79.0	87.6	92.5	90.5	4.8
Phenylalanine	84.3	92.8	93.0	89.3	4.4
Threonine	84.0	87.9	91.9	92.0	4.1
Valine	80.1	88.9	90.0	93.7	4.1
Mean	84.5	90.1	91.9	90.9	4.4
Dispensable Amino	Acids				
Alanine	89.1	91.4	90.1	89.1	4.0
Aspartic acid	84.6	92.5	91.0	89.1	4.7
Cysteine	79.0	86.7	87.6	87.8	4.8
Glutamic acid	84.9	93.0	93.9	90.4	4.3
Glycine	80.4	89.0	88.1	90.1	5.2
Proline	77.9	86.6	87.3	89.7	5.4
Serine	83.4	93.5	89.9	92.8	4.9
Tyrosine	86.0	92.7	93.0	93.0	4.6
Mean	83.2	90.7	90.1	90.2	4.7

between all the four dietary treatments. However compared to extruded, extruded + amylase, or extruded + amylase + xylanase + protease the digestibility values were systematically higher for the extruded peas + amylase + xylanase for both indispensable and dispensable amino acids. Addition of amylase to extruded peas significantly (p < 0.05) resulted in increased apparent ileal digestibility of threonine. Inclusion of enzymes to extruded peas significantly (p < 0.05) increased apparent digestibility of cysteine by 15.5, 19.7 and 9.9% units for the amylase, amylase + xylanase, and amylase + protease + xylanase supplemented peas respectively. At the faecal level, there were no differences between the four dietary treatments in the digestibility of the above nutrients. Faecal digestibility values for all nutrients were however higher than their ileal counterparts. Nitrogen digestibility in the lower gut ranges from 88.1 - 92.3%, with amylase + xylanase supplemented diet having the highest digestibility values.

True ileal and faecal digestibility of amino acids in extruded peas supplemented with enzymes.

The true iteal and faecal digestibility of amino acids were distinctively higher than the apparent values, but were however not different (Table 16 and 18). The difference between true and apparent digestibility values are the direct relation of endogenous protein secretion. The values reported here are comparable to that reported by Grosjean et al., (1997), but higher than values observed by Leterme et al., (1990), for tannin-free pea batches studied. Compared to the non-

supplemented diet, the percentage unit increases in amino acid digestibility were; 1.0, 7.0, 5.4% for methionine (p=0.72), 1.1, 1.7, and -0.1% for lysine (p=0.41), 2.1, 6.9, and 6.9% for threonine (p=0.07) and 4.7, 7.5 and 6.3% for cysteine (p=0.53), when amylase, amylase + xylanase and amylase + protease + xylanase was added to extruded peas respectively. Addition of protease in all cases resulted in reduced digestibility of lysine, methionine and arginine, however, true digestibility of threonine was not affected. True digestibility of amino acids in peas is characterized by the low digestibility of cysteine and methionine already present in limited concentrations. These results show that the true digestibility of these amino acids were improved though not statistically significant (p > 0.05) with addition of enzymes. The higher digestibility values also indicate that the protein of extruded peas was almost completely enzymically digested in the small intestine. As shown on Table 13, 14, 15 and 16, the highest digestibility was observed for the basic amino acids (arginine and lysine). Fan et al., (1994b; 1995) also reported that ileal digestibility of lysine and arginine are consistently higher. The order of amino acid digestibility (characterized by a high digestibility of lysine and methionine) was similar to that reported for sovbean meal (Green, 1988; Tanksley and Knabe, 1993). The observation in this trial partly supports the findings of Bedford et al.. (1992) that more digestion occurred in the small intestine following enzyme addition. Liu et al., (1997), observed significant increase in over all NDF and NSP digestibility following enzyme supplementation and that such modification may improve nutrients utilization in pigs. The slight improvement in amino acids digestibility observed in this study may be due to an interaction

between the supplemental enzyme and soluble NSP in the small intestine. The exogenous enzymes can selectively degrade the NSP complex and thereby open the carbohydrate matrix for the endogenous enzyme system. In this way, the nitrogen fraction closely associated with dietary fibre, which is normally not available to non-ruminants, can be utilized. Dierck and Decuypere (1996) reported significant increases in digestibility of NSP and amino acids following enzyme supplementation. The relatively lower apparent digestibilities of cysteine, threonine and glycine have been reported (Fan, et al., 1995). The relatively high content of these amino acids in digesta collected from the distal ileum of growing pigs fed protein free diet was assumed to be the reason for the low apparent digestibility coefficient. Also glycine, a major constituent of the bile salt conjugate accounts for more than 90% of the total content of the amino acids secreted in porcine bile juice (Souffrant, 1991). Bile salts are degraded in the distal ileum. Most of them are reabsorbed by way of active transport. And enters the enterohepatic circulation (Fan et al., 1994b). Deconjugated glycine may escape re-absorption and enters the large intestine (Shiau, 1987). Also small intestine secretions (mucins), supply the largest proportion of nitrogen to the endogenous nitrogen in the small intestine (Auclair, 1986). About 95% of mucin glycoprotein is very rich in threonine and serine in addition to proline. Hence the lower digestibility of glycine, threonine and serine compared to the other amino acids observed in this trial.

EXPERIMENT 3. EFFECT OF ENZYMES SUPPLEMENTATION ON DIGESTIBILITY OF MICRONIZED PEAS BY EARLY-WEANED PIGS.

# Apparent ileal and faecal digestibility of energy, crude protein and amino acids of micronized peas in early-weaned pigs

The apparent ileal and faecal digestibility of amino acids for the micronized peas supplemented with enzymes are presented in Tables 17 and 19 respectively. The apparent ileal (AID) and faecal digestibility (AFD) of amino acids were far greater than values reported in the literature (Green, 1988; Fan et al., 1995; van Barneveld et al., 1994), though no direct comparisons could be made, since no enzyme was used in their studies. The amino acid digestibility in all the four dietary treatments was not significantly different (p>0.05). Compared to the other amino acids, threonine, cysteine and glycine had lower digestibility values. There was a non-significant improvement (p > 0.05) in apparent digestibility of threonine and glycine, with enzyme supplementation. There was a reduction in threonine digestibility when amylase + protease + xylanase were supplemented. The reasons for these observations such as higher digestibility of lysine and arginine, while threonine, cysteine and glycine were consistently low, this may be due to enzyme specificity during hydrolysis where lysine and arginine are released first and threonine last or the endogenous secretion which tend to have high concentration of threonine and glycine (Fan et al., 1994b). The apparent digestibility of arginine, lysine, methionine and valine for the enzyme supplemented diets were greater than values reported by Fan et al., (1994b) and van Barneveld et al., (1994) for digestibility of raw peas in growing pigs. Again, mean apparent digestibilities of indispensable amino acids were greater in all the

Table 17. Apparent ileal digestibility (%) of micronized pea (M) supplemented with enzymes.

Parameter	M	M+A	M + A + X	M+A+P+X	S.E.M.
Energy	82.0	82.3	84.4	84.5	3.6
Crude Protein	83.7	85.2	89.8	88.6	4.1
Indispensable Am	ino Acids				
Arginine	87.2	90.9	92.3	89.3	5.5
Histidine	83.5	86.5	89.3	84.4	3.1
Isoleucine	84.4	84.6	88.4	83.7	4.4
Leucine	83.5	86.5	89.5	84.9	3.7
Lysine	84.5	89.1	91.3	87.4	4.6
Methionine	84.2	80.8	85.3	84.6	4.1
Phenylalanine	83.5	88.0	91.1	87.4	4.4
Threonine	72.3	78.4	79.3	77.3	3.8
Valine	79.2	83.6	86.7	80.9	3.9
Mean	80.2	84.7	88.0	84.4	4.2
Dispensable Amin	o Aicds				
Alanine	84.7	83.9	87.6	79.5	3.7
Aspartic acid	82.7	80.9	84.9	82.3	3.7
Cysteine	72.8	72.3	79.2	70.3	2.2
Glutamic acid	85.1	88.7	91.2	86.6	4.4
Glycine	79.1	74.1	78.5	63.7	4.1
Proline	73.6	81.4	82.4	75.9	4.7
Serine	79.7	86.5	89.7	79.9	4.2
Tyrosine	81.5	89.2	92.3	87.4	4.5
Mean	79.9	82.1	85.7	78.2	3.9

Table 18. True ileal digestibility (%) of micronized pea (M) supplemented with enzymes.

Parameter	М	M+A	M + A + X M	+A+P+X	S.E.M.
Energy	84.5	86.3	86.6	87.4	4.1
Crude Protein	85.6	88.7	90.4	90.1	4.2
Indispensable Amino	Acids				
Arginine	88.7	93.1	94.0	92.0	4.8
Histidine	89.8	92.5	93.2	90.4	4.3
Isoleucine	89.9	92.1	91.3	90.3	5.1
Leucine	90.1	93.2	93.8	91.5	3.6
Lysine	90.1	94.1	94.0	90.6	4.1
Methionine	86.3	88.0	90.7	90.7	3.1
Phenylalanine	84.3	94.2	90.5	92.6	4.2
Threonine	86.3	90.7	90.5	90.5	5.1
Valine	86.9	92.9	90.4	92.4	4.8
Mean	88.0	92.3	92.4	91.2	4.3
Dispensable Amino A	cids				
Alanine	89.7	90.7	92.3	93.5	4.8
Aspartic acid	88.6	82.6	91.6	90.7	5.4
Cysteine	81.5	90.5	91.1	89.8	4.7
Glutamic acid	85.1	92.1	93.7	92.8	4.1
Glycine	84.3	90.2	90.1	90.6	3.2
Proline	80.6	91.5	89.7	92.7	4.4
Serine	81.9	91.9	87.1	89.1	3.9
Tyrosine	86.3	94.6	84.0	91.3	4.3
Mean	84.7	90.5	89.9	91.3	4.3

Table 19. Effect of enzyme supplementation on apparent faecal digestibility (%) of micronized pea (M).

Parameter	M	M + A	M + A + X	M + A + P + X	S.E.M.			
Energy	91.2	92.9	93.5	93.6	4.0			
Crude Protein	84.7	86.3	88.4	88.0	4.1			
Indispensable Amino Acids								
Arginine	88.4	92.0	93.0	91.3	4.5			
Histidine	85.1	87.9	89.6	87.4	4.3			
Isoleucine	86.1	89.3	88.9	83.9	4.1			
Leucine	83.9	89.9	90.4	96.2	4.3			
Lysine	86.8	90.5	92.0	88.8	4.1			
Methionine	82.4	82.6	87.0	85.1	3.1			
Phenylalanine	84.2	90.3	92.3	89.1	4.6			
Threonine	80.2	79.1	80.9	80.7	4.0			
Valine	80.2	85.4	89.0	84.9	4.2			
Mean	84.1	87.4	89.2	87.5	4.1			
Dispensable Amin	o Acids							
Alanine	86.1	85.0	89.1	83.7	4.5			
Aspartic acid	84.0	86.4	90.2	84.5	4.4			
Cysteine	76.0	73.5	83.1	79.9	4.2			
Glutamic acid	85.9	90.1	92.0	86.7	4.4			
Slycine	80.7	75.3	80.3	82.0	4.7			
Proline	76.4	82.4	84.3	78.0	4.4			
Serine	80.0	89.9	89.9	82.4	4.9			
Tyrosine	84.0	90.8	92.0	89.7	4.6			
Mean	84.0	84.2	87.6	83.4	4.5			

Table 20. Effect of enzyme supplementation on true faecal digestibility (%) of micronized pea (M).

Parameter	M	M + A	M+A+X	M+A+P+X	S.E.M.
Energy	90.1	91.4	94.1	94.5	3.6
Crude Protein	85.6	90.0	90.6	90.0	3.2
ndispensable Am	ino Acids				
Arginine	89.2	94.0	95.1	93.0	5.2
listidine	87.0	93.1	93.0	89.9	4.5
soleucine	84.6	92.7	93.4	91.4	5.1
.eucine	85.3	92.8	93.4	89.0	4.3
ysine.	89.7	94.3	92.3	93.0	5.3
<b>Methionine</b>	83.5	89.7	91.8	90.0	4.8
Phenylalanine	86.3	90.9	94.5	92.0	5.5
'hreonine	84.9	93.0	92.0	92.7	5.3
'aline	83.4	92.3	93.7	93.9	5.0
<b>lean</b>	86.0	92.5	93.2	91.6	5.0
ispensable Amin	o Acids				
lanine	89.0	91.0	92.8	90.3	4.5
spartic acid	87.2	92.8	93.0	91.8	5.2
Systeine	81.4	90.8	92.5	89.9	4.6
Slutamic acid	84.1	93.6	93.4	92.7	5.1
Stycine	82.0	91.4	91.0	90.6	4.1
roline	79.0	92.5	89.9	92.4	4.1
erine	84.6	93.0	93.4	92.0	5.1
yrosine	86.4	94.9	94.7	94.3	5.4
lean	84.2	92.5	92.6	91.7	4.8

four diets than the dispensable amino acids. Webb (1990) reported that individual amino acids are not absorbed with equal efficiency and that essential amino acids are absorbed in the greater proportion than non-essential amino acids.

The apparent faecal digestibility of amino acids though not significant (p > 0.05) among the four treatments, in all cases were higher than values observed by van Barnveld, (1994) and those reported in most feed stuffs (reviewed by Austic, 1983), Compared to the other amino acids, apparent faecal digestibility of methionine, threonine, glycine and cysteine were lower. The lower faecal digestibility of methionine compared to the ileal value has also been reported and was attributed to the net synthesis of this amino acid by the microorganisms in the large intestine (Tankley and Knabe, 1993).

# True ileal and faecal digestibility of amino acids

The summary of the results of the true ilael and faecal digestibility (TID) and (TFD) of amino acids in micronized peas are presented in Tables 18 and 20 respectively. The TID and TFD values also showed no significant difference (p > 0.05) for all the four dietary treatments. Compared to the apparent values, the true digestibility values were higher in all cases. This is because the apparent digestibility values are influenced by secretion from the intestinal tract, while this is corrected for in the true digestibility values. Addition of amylase, amylase + xylanase, or amylase + xylanase + protease to micronized peas resulted in about 4.1, 4.0 or 0.5% unit increase respectively in the TID of lysine (p=0.80). While

methionine digestibility respectively increased by 1.4, 4.4 or 4.4% unit (p=0.06). Compared to the non-supplemented diet, inclusion of amylase + xylanase resulted in about 9.6% unit increased in cysteine digestibility (p=0.80). Also mean digestibility of indispensable amino acids increased about 4.0% units with the enzyme addition. A further calculation by subtracting individual enzymes effect on digestibility revealed that inclusion of protease resulted in reduced digestibility. In fact, lysine digestibility reduced by 3.4% unit. The reason for this effect is yet to be determined. It may however, be speculated in a number of ways, either due to competitive action of exogenous and endogenous protease as a result of inadequate substrate, inhibition at the cellular level as a result of excessive production of nutrients and lastly, insufficient number of villi as well as reduced villi height, in such case nutrients uptake become limited.

EXPERIMENT 4. EFFECT OF ENZYMES SUPPLEMENTATION ON PERFORMANCE OF EARLY-WEANED PIGS.

#### Average daily feed intake.

There was no influence on daily feed intake as shown in Table 21. ADFI between treatments were similar during the first week of the experiment. However, inclusion of enzymes to extruded and micronized pea based diets resulted in slight reduction in ADFI. The ADFI for the first week however, was not significantly different (p > 0.05) among all the 7 dietary treatments. Results of ADFI for week 2

was again not different from that of week 1, except for the numerical increase. Pigs fed micronized peas recorded the highest ADFI (375.8 g/day), compared to that of the soybean control diet (346.1) (p=0.14). Addition of enzymes resulted in a slight improvement, though not significant (p > 0.05) in ADFI of pigs fed the raw peas diet, whereas there was reduction in the extruded and micronized peas diets. interestingly, the ADFI during weeks 5 and 6 reduced for the enzyme treated raw pea, as well as the extruded and micronized peas fed pigs. The summary of the results of ADFI for the starter phase 1 (4.0 - 10.0 kg), starter phase 2 (10.0 - 20.0 kg) and the over all phase (4.0 - 20.0 kg) are shown in Table 21. During the starter phase of growth, pigs fed micronized peas had the highest ADFI (405.8 g/day), while the lowest ADFI (324.8 g/day) was recorded in the raw peas fed pigs (p=0.12). Comparison among the enzymes supplemented diets shows an improvement in ADFI for raw peas, while there was a drastic reduction in that of the micronized peas. However, the ADFI at this stage was not significantly different (p>0.05). Results at the starter phase 2 also show that enzyme supplementation apparently had no effect on ADFI. It can be seen that peas fed pigs compared to the soybean fed pigs recorded the higher ADFI values. Over all, ADFI for the 7 dietary treatments were somewhat similar (p>0.05). However, micronized peas fed pigs had the highest ADFI (594.9 g/day) and the lowest for the micronized peas supplemented with enzymes (513.9 g/day). However, these values are lower than the NRC (1998) expected ADFI (668 g/day) but higher than values reported by Caine et al., (1997) for protease-treated soybean for pigs at

Table 21. Effect of processing method and enzyme supplementation on average daily feed intake (ADFI) g/day of early (16-d) weaned pigs.

		\S						
Parameter	SBM-C	Raw		Extruded		Micronized		SEM
Enzyme	-	_	+	-	+	-	+	
Week 1	133.7	134.3	132.3	166.8	131.4	166.1	123.6	19.994
Week 2	346.1	352.4	368.6	334.2	335.7	375.8	324.3	26.944
Week 3	489.4	540.1	550.2	510.2	492.3	566.8	498.8	34.303
Week 4	640.2	567.9	656.5	679.7	625.8	734.3	620.9	55.028
Week 5	871.6	882.8	774.9	821.2	782.6	986.6	757.1	11.696
Week 6	1180.2	1075.8	817.5	911.7	918.9	905.2	926.4	164.80
Starter 1	341.4	364.5	346.4	393.9	343.2	405.8	317.3	28.453
Starter 2	698.5	866.5	794.5	802.1	808.6	866.6	746.5	45.573
Over all	528.6	575.5	555.6	548.7	558.9	594.9	513.9	20.001
Over all	528.6	575.5	555.6	548.7	558.9	594.9	513.9	20.0

Means in the same row were not significantly different (p > 0.05)

this stage of growth. Inborr et al., (1994) also reported no influence on feed intake in early-weaned pigs fed enzyme-supplemented diets.

# Average daily gain (ADG)

During the first week in starter phase 1, ADG was lowest for piglets' fed the raw pea diet (94.3 g/day) as presented in Table 22. Conversely, these group of pigs recorded the highest ADG (134.0 g/day) when the diet was supplemented with enzymes (p=0.5). Bengala-Friere et al., (1989), also reported a substantial reduction in weight gain when raw peas (30 and 45%) were fed to 3 week weaned piglets. However, response equivalent to that of soybean was obtained with extruded peas at both levels. Compared to the raw pea diet, there was a 29.8% increased (p=0.22) in ADG when piglets were fed the enzymes supplemented raw pea diets. The results of week 1 was however, not significantly different (p > 0.05). During the second week, piglets fed the micronized peas-based diet had the highest ADG (371.2 g/day), while those on raw peas based diet recorded the lowest ADG (279.0 g/day). Le Guen and Tolman (1994), reported values of 279 and 321 g/day in a 5 week old pigs fed either raw or ethanol treated peas. Addition of enzymes to raw peas based diet resulted in a slight (8.3 %) improvement (p=0.18) in ADG during the second week. On the other hand, enzyme inclusion to extruded or micronized peas based diets resulted in reduction in ADG during the first and second week in the starter phase 1. The results were however not significantly different (p > 0.05) and comparable to NRC (1998) expected ADG for

Table 22. Effect of processing method and enzyme supplementation on average daily gain (ADG) g/day of early (16-d) weaned pigs fed pea-based diets.

Parameter	PEAS								
	SBM-C	Raw		Extruded		Micronized		SEM	
Enzyme	_	-	+	-	+	_	+		
In. wt. (kg)	4.39	4.38	4.41	4.53	4.30	4.37	4.31	0.20	
Mid. Wt.(kg)	11.09	10.43	10.38	10.72	10.89	10.91	10.31	0.40	
Final wt.(kg)	20.49	20.15	20.19	20.34	20.34	20.40	20.28	0.14	
Week 1 (g)	121.04	94.17	134.01	109.14	127.01	125.55	122.44	17.18	
Week 2	333.09	279.04	304.28	342.61	304.28	371.19	288.09	41.37	
Week 3	447.14	417.14	424.43	395.99	408.56	463.42	402.89	31.25	
Week 4	519.64	407.85	483.37	441.66	416.25	504.07	519.46	39.38	
Week 5	519.28	494.04	479.92	448.74	501.83	574.99	534.87	49.61	
Week 6	845.91	778.99	668.75	795.51	682.13	686.19	664.28	85.51	
Starter 1	315.96	247.44	288.70	271.27	290.88	323.04	370.17	36.11	
Starter 2	574.62	532.13	519.53	502.24	539.47	580.54	553.40	32.80	
Over all	428.59	403.59	396.63	415.57	407.99	437.19	408.33	17.66	

Means in the same row were not significantly different (p > 0.05)

starter pigs. This may be due to micronization altering the form, particle size and the nutrients make up of peas and also detoxified the ANF present in raw peas, making it more digestible. It may therefore be suggested that there was no further room for enzyme improvement. Enzyme supplementation virtually had negative effect on ADG for all treatments during weeks 5 and 6 during starter phase 2. There was an average reduction of about 14.0, 14.1 and 16.0 % for the raw, extruded and micronized peas based diets respectively with enzyme inclusion. The results of the starter phase 1 show no significant difference (p>0.05) for all the treatments. However, there was a numerical improvement of 14.3, 6.7 and 12.7 % for the raw, extruded and micronized peas based diets respectively, as a result of enzyme addition. Enzyme supplementation had a negative effect, though not significant (p > 0.05), on ADG for the starter phase 2 and the overall phase. There have also been reports indicating that there is a decline of response to dietary enzymes with animal age (Chessen et al., 1993; Campbell and Bedford, 1992), and that the ANF effects of feeding raw peas appear to be less in older pigs.

## Feed Conversion Efficiency (FCE)

On weekly basis, there were no significant differences among all the treatments in FCE as shown in Table 23. However, FCE differ (p > 0.05) for the starter phase. Apparently, enzyme supplementation resulted in better FCE for all the diets as indicated in Table 23. Piglets on the raw peas based diets had poorest FCE values compared to those on heat processed and enzyme supplemented diets (Table 23).

Table 23. Effect of processing method and enzyme supplementation on average feed conversion efficiency (FCE) (feed/gain) of early (16-d) weaned pigs.

Parameter	*****			PE				
	SBM-C	Raw		Extruded		Micronized		SEM
Enzyme	-	-	+	-	+	-	+	
Week 1	1.15	1.38	1.01	1.71	1.02	1.23	1.04	0.156
Week 2	1.23	1.43	1.21	1.11	1.11	1.15	1.16	0.084
Week 3	1.19	1.26	1.32	1.30	1.19	1.22	1.24	0.046
Week 4	1.24	1.57	1.39	1.63	1.50	1.48	1.21	0.187
Week 5	1.51	1.63	1.63	1.58	1.56	1.50	1.43	0.082
Week 6	1.65	1.38	1.24	1.21	1.37	1.32	1.38	0.272
Starter 1	1.17a	1.34b	1.20ab	1.29ab	1.18a	1.18 <b>a</b>	1.02a	0.059
Starter 2	1.27	1.63	1.52	1.54	1.52	1.46	1.35	0.088
Over all	1.26	1.43	1.40	1.37	1.38	1.36	1.26	0.046

Means in the same row with different letters are significantly different (p < 0.05), SEM (standard error of the mean)

Parameter	PEAS								
	SBM-C	Ra	aw	Extr	uded	Micro	nized	SEM	
Enzyme	-	-	+	-	+	-	+		
Starter 1	21.6	22.0	20.4	21.6	22.8	20.4	21.2	0.894	
Starter 2	16.6	17.5	19.2	18.2	17.6	17.0	18.0	0.951	
Over all	38.2	39.2	40.0	39.8	39.4	37.4	39.2	1.575	

Starter 1 (4.0 - 10.0 kg), Starter 2 (10.0 - 20.0 kg), SEM (standard error of the mean)

The results of the starter phase 2 and over all phases though statistically not different (p>0.05) indicate that FCE improved slightly across treatments with enzyme supplementation. This observation also agrees with that of Pettersson et al., (1987), observed that inclusion of enzymes usually improve feed conversion. Also in studies with weanling pigs fed barley both ADG and FCE were moderately improved with enzyme treatment (Thacker et al., 1992). Study with starter pigs fed barley based diets also showed that addition of enzymes tended to increase liveweight gain and improve feed utilization (Inborr et al., 1994). It appears that through enzyme supplementation, more optimal conditions for digestion are created and endogenous losses reduced, which in turn resulted in improved performance of pigs.

Processing type as well as addition of enzyme had no significant (p>0.05) effect on the duration of the experiment (Table 24). However, enzyme supplemented fed pigs seem to have spent slightly longer period on the experiment than those fed the non-supplemented diets. This may due to the slightly reduced feed intake in these groups of pigs.

## Plasma urea nitrogen (PUN) concentrations

Plasma urea nitrogen (PUN) is often used to determine the efficiency of nitrogen utilization or protein break down. A reduction in PUN concentration is an indication of reduction in urea synthesis, hence, more efficient use of amino acids (Coma et al., 1995). The summary of the PUN levels measured at the starter phase 1 and phase 2 of the experiment is presented in Table 25. As shown in

Table 25 the PUN levels indicate that at the start of the experiment all piglets had a similar concentration with values ranging from 5.8 to 6.1 mg/dl. However at the end of phase 1 (4.0 - 10.0 kg live weight), PUN levels were significantly different (p < 0.05) with enzyme addition to the diet apparently reducing the PUN levels. Enzyme supplementation of the diets resulted in significant reduction (p < 0.05) PUN levels from 9.0 to 5.73 mg/dl for piglets fed enzyme and nonsupplemented diets respectively. In addition, with the exception of the raw peas fed pigs, the PUN concentrations were sequentially lower (p < 0.05) for the peas fed pigs than the soybean fed pigs. At the end of starter phase 2 (10.0 - 20.0 kg live weight), PUN levels were significantly lower (p < 0.05) for all the peas fed pigs compared to those on soybean meal control diet. The enzyme effect seen in starter phase 1 for the raw peas and all other treatments reduced to minimal during the second phase. This suggests that pea protein is well utilized or efficiently used with enzyme addition. This would explain the improved FCE of the enzymes supplemented pea-based diets. It must however, be noted that the PUN levels observed in this trial were within the normal range (7.0 -18.0 mg/dl).

Table 25. Effect of processing method and enzyme supplementation on plasma urea nitrogen (PUN) mg/dl

Parameter				PE	AS			
	SBM-C	R	aw	Extr	uded	Micro	onized	SEM
Enzyme	-	-	+	-	+	-	+	
Starter	5.98	5.96	5.94	5.82	5.80	6.12	5.87	0.494
Phase 1	7.95ab	9.01a	5.73c	6.36bc	6.77bc	5.36c	5.30c	0.409
Phase 2	10.60a	6.32c	6.23c	8.45b	7.82bc	8.25b	6.86bc	0.350

Means in the same row with different letters are significantly different (p < 0.05)

### **5.0 GENERAL DISCUSSION**

The summary of the comparisons of raw, extruded and micronized peas supplemented with enzymes on true ileal digestibility (TID), of some selected amino acids are presented in Table 26. Compared to raw peas, the TID of lysine, methionine and threonine are greater for the processed pea diets, with micronized peas recording the highest digestibility as shown in Table 26. The improved digestibility as a result of heat processing (extrusion or micronization), may be partially attributed to the direct result of heat altering the nature of protein thereby rendering it more susceptible to enzymatic attack as reported by van der Poel et al., (1991), with peas. The slight improvement in digestibility coefficient in both extruded and micronized peas compared to raw peas is an indication that the processed peas might have received appropriate heat treatment during processing and that there might be partial or fully denaturation of lectins and protease inhibitors present in peas. Earlier reports by Knabe et al., (1989) and Fan et al., (1995) showed that digestibility values of crude protein and most amino acids were higher in soybean meal than extruded soybean meal in growing finishing pigs. These authors attributed the observation in part to the relatively high residual TIA in the extruded soybean as a result of inappropriate heat treatment during processing. Since the digestibility of processed peas were higher in all cases than that of raw peas, it could be speculated that the processing conditions employed were optimum to destroy if not all, most of the ANF such as protease and amylase inhibitors, lectins and tannins present in raw peas. The lack of significant improvement between the processed and raw peas may also be due to the low or

Table 26. Comparisons of the true iteal digestibility of lysine, methionine, threonine and cysteine of raw, extruded and micronized peas supplemented with enzymes.

Parameter	Raw	Extruded	Micronized	
		Lysine		
No Amylase	85.4	88.4	90.1	
Amylase	88.3	89.5	94.1	
Amylase + Xylanase	90.6	90.1	94.0	
Amylase + Xylanase + Protease	88.4	88.3	90.6	
		<u>Threonine</u>		
No Amylase	80.4	83.4	86.3	
Amylase	82.0	85.5	90.7	
Amylase + Xylanase	83.4	90.3	90.5	
Amylase + Xylanase + Protease	83.2	90.0	90.1	
		<u>Methionine</u>		
No Amylase	80.6	84.1	86.3	
Amylase	84.4	85.1	88.0	
Amylase + Xylanase	83.2	91.1	90.7	
Arnylase + Xylanase + Protease	82.2	89.5	90.7	
		Cysteine		
No Amylase	80.6	79.4	81.0	
Amylase	84.2	84.1	90.5	
Amylase + Xylanase	83.4	86.9	91.1	
Amylase + Xylanase + Protease	82.5	85.7	89.8	

Based on Tables of Appendices (A summary of the results of the preliminary studies)

negligible levels of ANF in the pea cultivar used. Addition of amylase and xylanase resulted in fairly constant improvement in digestibility in all the dietary treatments in lysine, threonine, methionine and cysteine digestibility. The higher digestibility of heat-treated peas supplemented with enzymes compared to raw peas supplemented with enzyme may presumably be due to the fact that heat disrupted the cell wall complex and allowed greater accessibility of nutrients to enzyme attack. Teiter et al., (1991) demonstrated that heat treatment of rye increased the response to dietary pentosanase in chick diets. Bach-Knudsen et al., (1995), using rats reported that the main effect of enzyme treatment was on the pericarp and testa rich fraction. Caine et al., (1997) reported that xylans and cellulose from peas hull have a more resistant nature to digestion, requiring longer transit times. The improvement in the digestibility of lysine, threonine, methionine and cysteine with enzymes inclusion may be a direct response of exogenous enzyme (xylanase), breaking down xylans to release more nutrients for endogenous enzyme attack. However, digestibility was relatively reduced with the supplementation of the enzymes cocktail (Table 26), especially TID of methionine, lysine and cysteine were actually reduced. This may be due to the high digestibility coefficient after addition of amylase and xylanase to both raw and processed pea diets. Poultry studies have also shown that the effect of enzyme is smaller for high quality wheat which contains less NSP (Preston, 1997). Multi-enzyme preparations containing high levels of enzymes have been found to require maximizing the release of protein from the aleurone layer (Mulder et al., 1991). The results in these trials with the addition of the three cocktail enzymes do not confirm this

observation. The digestibilities of threonine in all cases seem to be improved to that of methionine with enzyme supplementation. This observation must be the direct effect of the improved methionine digestibility as a result of enzyme supplementation. Philip et al., (1981), noted that methionine stimulated the absorption of threonine when the concentrations of the latter were low. It is also apparent from the comparative results (Table 26), those amino acid digestibilities of heat-treated peas were higher than that of raw peas. Increased digestibilities as a result of heat treatment have also been reported (van der Poel et al., 1992). van der Poel, (1990) and Owusu-Ansah and McCurdy, (1991) suggested that conformational changes of storage proteins caused by heat could be the reason for the improved protein digestibilities and that heat treatment may also improve the digestibility of proteins by destruction of ANF and by altering the protein structure through denaturation, resulting in increased accessibility of protein to enzymatic attack.

The effect of enzyme supplementation on digestibility coefficients in general are in line with other studies (Campbell and Bedford, 1992; Gdala et al., 1996; Liu et al., 1997). The extend of differences may depend on environmental factors affecting composition, levels of enzymes inclusion, the age of the animal studied and level of fibre and ANF present (especially amylase and protease inhibitors). The slight improvement and in certain cases reduced nutrients digestibility have been explained in numerous ways. The stomach of the pig is a more hostile environment with the pH considerably lower. Activity of most fungal enzymes has been found to be sub-optimal and activity of many bacterial enzymes questionable

(Kidder and Mauner, 1980). In addition, the presence of proteolytic enzymes and the lengthy residence time in the pigs' stomach would appear to challenge the survivability of the most robust enzymes. Supplementation of barley-based pig starter diet with β-glucanase improved utilization of dietary protein by way of increasing the secretion of proteolytic enzymes and reducing the loss of endogenous protein (Jensen et al., 1994). The reduced effect of the enzyme cocktail may be due to excessive secretion of endogenous proteolytic enzymes that might have digested the exogenous enzyme. It could also be due in part to competition between exogenous and endogeous for the limited dietary protein present.

The mechanism by which enzymes operate is not fully understood. Studies in poultry have indicated that a major part of the effect is by altering the physical nature of the digesta. Non-starch polysaccharides, such as arabinoxylans, are branched chain structures, which binds water resulting in highly viscous aqueous solutions even at low levels. It presupposes that increased digesta viscosity, associated with the presence of such NSP compounds, slow the rate of diffusion of substrate and digestive enzymes thereby hindering their effective interaction at the mucosal surface of the intestine leading to reduced digestion and absorption in the small intestine (Bedford and Classen, 1992). In broiler chicken, supplementary enzymes reduced the viscosity of the small intestine chyme (Bedford et al., 1992), thereby improving nutrient digestibility and absorption (Almirall et al., 1993). A similar but weaker relationship appears to exist also in baby pigs (Bedford et al., 1992). Although there was no significant improvement of enzymes in most of the

studies, taken the slight numerical improvement in nutrients digestibility and in most cases better daily gain and feed efficiency, would indicate that the enzymes increased cell wall solubilization and making locked-up nutrients available to the animal enzyme increasing the opportunity for both endogenous and exogenous enzyme action. Cell wall of pea hulls has been found to be less digestible than the whole seed (Goodlad and Mathers, 1990).

Three studies were carried out in early weaned pigs to determine apparent and true iteal and faecal digestibility of amino acids, protein and energy of raw, extruded and micronized peas supplemented with either amylase, amylase + xylanase or amylase + xylanase + protease. A fourth study was also conducted to determine the performance of 16-d old weaned pigs' fed a combination of peas/soybean complete meal supplemented with amylase and xylanase.

Digestibility values were higher, though not significant, in most cases for the micronized, followed by the extruded and then raw pea-based diets. The true digestibilities for lysine were 85.4, 88.4 and 90.1% for raw, extruded and micronized peas respectively. True lysine digestibility increased to 88.3, 89.5 and 94.1% respectively with amylase supplementation. There was a further increase in true lysine digestibility to 90.6% for raw peas but not extruded or micronized peas with amylase and xylanase supplementation. Addition of the 3 cocktail enzymes (amylase, protease and xylanase) resulted in a reduction in true lysine digestibility 88.4, 88.3 and 90.6% for raw, extruded and micronized peas, respectively. The trend of true digestibility of threonine, methionine and cysteine is similar to that of lysine. True threonine digestibility was 80.4, 83.4 and 86.3%, that of methionine was 84.0, 84.1 and 86.3% for raw, extruded and micronized pea-based diets respectively. With amylase inclusion, threonine digestibility respectively increased to 82.0, 85.5 and 90.7%, while that of methionine was 84.4, 85.1 and 88.0 for raw, extruded and micronized peas respectively. There was a further increased true digestibility of threonine and methionine with amylase and xylanase

supplementation. Threonine and methionine digestibility were respectively, 83.4, 90.3 and 90.5% and 83.2, 91.1 and 90.7% for raw, extruded and micronized peas respectively. Addition of the 3 enzyme cocktail resulted in reduced, though not significant digestibility of threonine and methionine. A comparison of digestibility of raw, extruded and micronized peas indicated that digestibility of micronized peas was highest in all cases followed by extrusion and raw peas respectively. Also true digestibility for iieal and total tract values were greater than the apparent digestibility counterparts. Further comparison of ileal and total tract digestibility of amino acids, energy and crude protein also showed a higher digestibility values for the latter. Indicating that faecal digestibility over estimated the digestibility of amino acids. Neither heat processing (extrusion or micronization) nor enzymes supplementation significantly increased the apparent and true digestibility of crude protein and amino acids in the early-weaned pigs. However, micronization seems to improve pea utilization compared to extrusion. A greater proportion of soluble and negligible content of anti-nutritional factors in the cultivar of pea used may be the principal cause of the similar digestibility values in both processed and raw peas. It is also questionable whether the processed conditions were optimum to have resulted in significant improvement in the digestibility coefficients. Furthermore, the lack of significant improvement and the inconsistent results in the both apparent and true digestibility with exogenous enzymes supplementation. The survivability of the exogenous enzyme in the gastrointestinal tract is also questionable. There may be a possible interaction between the exogenous, endogenous enzyme as well as substrate.

In the performance study, average daily feed intake was 341.4, 364.5, 393.9 and 405.8 g/day for soybean control, raw, extruded and micronized peas diets, during starter phase I (4.0 - 10.0 kg liveweight). Inclusion of amylase and xylanase did not influence the daily feed intake values. Feed intakes were 698.5, 866.5. 802.1 and 866.6 g/day during starter phase II (10.0 - 20.0 kg liveweight), for the soybean, raw, extruded and micronized peas based diets respectively. Enzymes supplementation did not significantly influence feed intake. It must, however, be noted that feed intake in all cases tended to reduce slightly in the enzyme-supplemented fed pigs. Average daily weight gains were 315.9, 247.4, 271.3 and 323.0 g/day for the soybean control, raw, extruded and micronized peafed pigs, during starter phase 1 (4.0 - 10.0 kg liveweight). The weight gain at this period increased slightly though not significant with enzyme supplementation. Daily weight gain was 288.7, 290.9 and 370.2 g/day for raw, extruded and micronized pea-fed pigs respectively. However, this effect diminished during the starter phase 2 and the over all phase. Thus, it is apparent that enzyme effect was seen only during starter phase 1. This effect was reduced as the pigs get older (week 4, 5 and 6). Pigs fed raw peas actually performed similarly as those fed extruded and micronized pea-based diets. There was a significant effect in the feed conversion efficiency during starter phase 1 (4.0 - 10.0 kg liveweight), with raw pea-fed pigs having the poorest feed conversion efficiency (1.34) compared to 1.20 in the enzyme supplemented raw pea-based diet. Enzyme supplementation resulted in improved feed utilization for both processed and non-processed diets. In addition, plasma urea nitrogen concentrations were also lowered with enzyme

supplementation. The plasma urea nitrogen content was 7.9, 9.0, 6.4 and 5.4 d/l for soybean-control, raw, extruded and micronized pea-based diets respectively. Addition of enzyme to raw peas resulted in significant reduction in plasma urea nitrogen, during phase 1. Compared to soybean-based control diet, pigs fed on pea/soybean based diets had reduced levels of plasma urea nitrogen. It is recommended that with amylase and xylanase supplementation and diets formulated on ideal protein basis raw peas can constitute up to 30 - 35% in a 16-d old weaned pigs diet without any detrimental effect during the first 3 weeks after weaning. Although enzyme supplementation did not significantly influence the performance of extruded and micronized pea-fed pigs, it is however beneficial in reducing environmental pollution.

#### **Future Research:**

A further study is required to investigate why there was an improvement in digestibility of extruded and micronized when enzymes were added but did not translate into improved performance. A second study should also be carried out to find out reasons why inclusion of the enzyme cocktail, especially with protease resulted in reduction in digestibility of protein and amino acids.

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## **APPENDICES**

Table 27. Apparent ileal digestibility (%) of amino acids of raw extruded and micronized peas in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable		<del></del>	· · · · · · · · · · · · · · · · · · ·	
Arginine	85.7	86.0	87.2	1.156
Histidine	82.4	82.3	83.5	1.125
Isoleucine	79.6b	82.0ab	84.4a	1.481
Leucine	79.9	82.2	83.5	1.485
Lysine	83.6	82.8	84.5	1.273
Methionine	77.9b	79.5ab	84.2a	1.724
Phenylalanine	75.6b	80.3ab	83.5a	1.496
Threonine	73.4	78.1	79.3	2.248
Valine	74.0	76.6	79.2	1.393
Mean	79.1	81.1	83.2	1.264
Dispensable				
Alanine	83.2	84.3	84.7	1.512
Aspartic acid	82.1	82.2	82.7	1.414
Cysteine	55.5ab	52.4b	72.8a	3.346
Glutamic acid	80.4	83.6	85.1	1.437
Glycine	71.8	76.0	79.1	1.108
Proline	65.9b	71.3ab	73.6a	3.172
Serine	76.9	77.9	79.7	0.857
<b>Tyrosine</b>	79.4	80.3	81.5	1.324
Mean	74.4	76.0	79.9	1.771

S.E.M. (standard error of the mean). Means in the same row followed by different letters are significantly different (p < 0.05).

Table 28. True ileal digestibility (%) of amino acids of raw, extruded and micronized peas in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	86.2	88.5	88.7	1.856
Histidine	87.2	89.3	89.8	1.135
Isoleucine	89.8	90.0	89.9	1.085
Leucine	88.4	90.8	90.1	1.095
Lysine	86.9	88.4	90.1	1.146
Methionine	80.6	84.1	86.3	2.110
Phenylalanine	79.8	82.9	84.3	1.133
Threonine	80.4	83.4	86.3	2.184
Valine	83.5	86.3	86.9	1.563
Mean	84.6	87.5	88.9	1.303
Dispensable				
Alanine	86.2	87.4	89.7	1.912
Aspartic acid	87.8	89.6	88.7	1.414
Cysteine	78.1	79.4	81.5	2.241
Glutamic acid	84.4	85.7	85.1	1.137
Glycine	80.6	82.8	84.3	1.181
Proline	79.9	81.3	80.6	3.562
Serine	80.9	81.7	81.9	1.232
Tyrosine	85.8	87.3	86.3	1.780
Mean	83.4	84.5	86.3	1.780

Table 29. Apparent faecal digestibility (%) of amino acids of raw, extruded and micronized peas in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	86.8	86.9	88.4	1.783
Histidine	84.2	83.6	85.1	1.312
Isoleucine	82.0	82.3	86.1	2.114
Leucine	83.7	84.2	83.9	1.667
Lysine	85.9	85.2	86.8	1.815
Methionine	79.9	80.6	82.4	1.261
Phenylalanine	79.5	81.4	84.2	1.932
Threonine	80.1	81.4	80.2	1.442
Valine	79.4	79.1	80.2	1.577
Mean	82.4	82.7	84.1	1.667
Dispensable				
Alanine	85.2	84.9	86.1	1.514
Aspartic acid	83.0	84.1	84.0	1.639
Cysteine	70.4	72.8	76.0	2.699
Glutamic acid	82.3	85.1	85.9	2.013
Glycine	79.1	79.0	80.7	2.007
Proline	70.0	72.3	76.4	3.145
Serine	79.3	80.1	80.0	1.450
Tyrosine	82.1	83.4	84.0	1.279
Mean	78.9	80.2	81.6	1.964

Table 30. True faecal digestibility (%) of amino acids of raw, extruded and micronized peas in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	87.1	88.7	89.2	1.721
Histidine	86.3	84.6	87.0	1.663
Isoleucine	82.9	84.7	84.6	1.448
Leucine	85.0	86.1	85.3	1.817
Lysine	88.4	89.2	89.7	1.135
Methionine	79.2	79.0	83.5	1.278
Phenylalanine	80.4	84.3	86.3	2.143
Threonine	82.4	84.0	84.9	1.455
Valine	79.8	80.1	83.4	1.722
Mean	83.5	84.5	86.0	1.598
Dispensable				
Alanine	88.3	89.1	89.0	1.522
Aspartic acid	84.9	84.6	87.2	1.498
Cysteine	77.8	79.0	81.4	2.339
Glutamic acid	82.0	84.9	84.1	1.199
Glycine	79.8	80.4	82.0	2.311
Proline	74.2	77.9	79.0	3.305
Serine	81.8	83.4	84.6	0.997
Tyrosine	84.7	86.0	86.4	1.158
Mean	81.7	83.2	84.2	1.798

Table 31. Apparent iteal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with  $\alpha$ -amylase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	89.6	90.3	90.9	2.385
Histidine	83.5	86.1	86.5	1.645
Isoleucine	81.2	82.5	84.6	2.293
Leucine	80.3	87.0	86.5	2.087
Lysine	86.8	88.4	89.1	1.703
Methionine	73.9	79.4	8.08	3.453
Phenylalanine	81.9	85.2	88.0	2.849
Threonine	58.7	67.8	72.4	4.404
Valine	79.4	80.0	83.6	2.405
Mean	79.1	83.5	84.9	2.469
Dispensable				
Alanine	74.2	85.4	83.9	2.628
Aspartic acid	78.8	85.7	80.9	2.853
Cysteine	63.8	67.9	72.3	3.237
Glutamic acid	84.3	89.3	88.7	1.758
Glycine	60.5	71.2	74.1	7.373
Proline	76.3	75.5	81.4	6.124
Serine	85.4	89.6	86.5	1.719
Tyrosine	83.4	91.7	89.2	2.004
Mean	75.8	82.0	82.1	3.462

Table 32. True ileal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with  $\alpha$ -amylase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	92.0	91.1	93.1	1.663
Histidine	86.7b	90.1ab	92.5a	1.277
Isoleucine	85.5b	88.2ab	92.1a	1.469
Leucine	87.0b	90.0ab	93.2a	0.792
Lysine	88.1b	89.5ab	94.1a	1.374
Methionine	84.4	85.1	88.0	2.167
Phenylalanine	85.6b	92.5a	94.2a	1.088
Threonine	82.0b	85.5ab	90.7a	1.687
Valine	84.9	87.8	92.9	2.003
Mean	86.2	88.9	92.3	1.690
Dispensable				
Alanine	83.5b	89.9a	90.7a	1.337
Aspartic acid	85.8b	92.1a	82.6a	1.245
Cysteine	82.2b	84.1b	90.5a	1.244
Glutamic acid	87.3	92.0	92.1	1.256
Glycine	82.8b	88.1ab	90.2a	0.648
Proline	82.9b	84.8ab	91.5a	1.678
Serine	88.1	92.1	91.9	1.243
Tyrosine	87.0b	94.0a	94.6a	0.821
Mean	84.9	89.6	91.8	1.184

Table 33. Apparent faecal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	90.1	90.7	92.0	2.853
Histidine	84.3	87.6	87.9	2.625
Isoleucine	84.5	85.0	89.3	2.438
Leucine	85.2	89.0	89.9	2.703
Lysine	86.9	89.1	90.5	2.662
Methionine	77.8	80.4	82.6	4.087
Phenylalanine	84.0	90.7	90.3	2.470
Threonine	67.9	74.4	79.1	3.356
Valine	82.3	86.7	85.4	2.608
Mean	82.5	85.9	87.4	2.866
Dispensable				
Alanine	77.3	85.7	85.0	2.970
Aspartic acid	81.6	87.3	86.4	2.609
Cysteine	70.9	72.0	73.5	3.664
Glutamic acid	86.7	90.4	90.1	2.715
Glycine	67.9	73.2	75.3	8.432
Proline	78.1	77.9	82.4	7.361
Serine	87.0	90.5	89.9	2.643
Tyrosine	85.7	92.7	90.8	2.419
Mean	79.4	83.7	84.2	4.102

Table 34. True faecal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	93.9	94.2	94.0	2.636
Histidine	87.6	90.8	93.1	2.798
Isoleucine	87.5	89.0	92.7	2.325
Leucine	88.3	90.1	92.8	2.709
Lysine	90.1	90.0	94.3	2.435
Methionine	86.9	87.6	89.7	1.897
Phenylalanine	87.7	92.8	90.9	3.440
Threonine	84.9	87.9	93.0	5.009
Valine	86.2	88.9	92.3	2.436
Mean	88.1	90.1	92.5	2.854
Dispensable				
Alanine	85.3	91.4	91.0	3.158
Aspartic acid	87.0	92.5	92.8	2.347
Cysteine	85.9	86.7	90.8	2.509
Glutamic acid	88.4	93.0	93.6	2.238
Glycine	84.3	89.0	91.4	3.443
Proline	85.7	86.6	92.5	2.477
Serine	90.2	93.5	93.0	1.094
Tyrosine	88.9	92.7	94.9	3.581
Mean	86.9	90.7	92.5	2.606

Table 35. Apparent iteal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase and xylanase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	85.6b	91.8a	92.3a	1.334
Histidine	80.7b	87.8a	89.3a	0.837
Isoleucine	76.0b	87.1a	88.4a	1.780
Leucine	77.8b	88.5a	89.5a	1.121
Lysine	81.8b	87.1ab	91.3a	1.846
Methionine	79.8b	83.3ab	85.3a	1.231
Phenylalanine	80.1b	88.4a	91.1a	1.710
Threonine	72.3b	82.6a	78.4ab	1.165
Valine	74.4b	83.6a	86.7a	1.627
Mean	78.7	86.7	88.0	1.405
Dispensable				
Alanine	71.8b	87.0a	87.6a	1.286
Aspartic acid	77.8b	87.1a	84.9a	0.938
Cysteine	62.0b	71.1ab	79.2a	2.849
Glutamic acid	84.6	91.0	91.2	1.083
Glycine	64.8	75.4	78.5	3.435
Proline	78.3	80.1	82.4	2.312
Serine	74c	85.1b	89.7a	0.986
Tyrosine	79.7b	89.1ab	92.3a	2.413
Mean	74.1	83.2	85.7	1.913

Table 36. True iteal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase and xylanase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	90.7b	94.4a	94.0a	0.725
Histidine	88.1b	89.8b	93.2a	0.465
Isoleucine	87.0	89.8	91.3	1.264
Leucine	87.2b	91.2ab	93.8a	0.834
Lysine	89.3b	90.1b	94.0a	0.880
Methionine	83.2b	91.1a	90.7a	1.374
Phenylalanine	86.8	92.8	90.5	2.110
Threonine	83.4b	90.3a	90.5	0.911
Valine	84.4	88.9	90.4	1.172
Mean	86.7	90.9	91.4	1.018
Dispensable				
Alanine	85.5	89.8	92.3	1.306
Aspartic acid	86.2	90.3	91.6	1.054
Cysteine	83.0b	86.9ab	91.1a	1.235
Glutamic acid	88.3b	93.1a	93.7a	0.895
Glycine	84.2a	85.8ab	90.1a	1.347
Proline	84.4	86.6	89.7	2.346
Serine	86.4	88.7	87.1	0.913
Tyrosine	82.4	83.5	84.0	1.032
Mean .	85.0	88.4	89.9	1.266

Table 37. Apparent faecal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase and xylanase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	87.3	92.4	93.0	2.433
Histidine	84.9	88.1	89.6	2.057
Isoleucine	79.2	88.3	88.9	3.014
Leucine	79.9	90.3	90.4	6.246
Lysine	85.3	89.1	92.0	3.601
Methionine	80.6	85.3	87.0	2.390
Phenylalanine	84.0	89.9	92.3	2.465
Threonine	77.0	87.0	80.9	6.032
Valine	79.4	83.9	89.0	12.02
Mean	81.9	88.2	89.2	4.472
Dispensable				
Alanine	77.0	88.3	89.1	2.348
Aspartic acid	80.9	89.0	90.2	3.907
Cysteine	74.7	78.9	83.1	4.068
Glutamic acid	86.0	90.4	92.0	2.179
Glycine	70.8	77.0	80.3	2.331
Proline	81.0	82.3	84.3	1.562
Serine	80.7	85.9	89.9	2.887
Tyrosine	82.6	89.9	92.0	3.472
Mean	79.2	85.2	87.6	2.844

Table 38. True faecal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase and xylanase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				······································
Arginine	92.3	95.5	95.1	2.639
Histidine	90.4	91.3	93.0	2.147
Isoleucine	88.9	90.2	93.4	2.058
Leucine	89.0	92.0	93.4	2.155
Lysine	90.4	90.7	92.3	2.483
Methionine	86.0	92.5	91.8	2.449
Phenylalanine	87.8	93.0	94.5	2.708
Threonine	86.7	91.9	92.0	2.490
<b>Valine</b>	86.4	90.0	93.7	3.046
Mean	88.6	91.9	93.2	2.464
Dispensable				
Alanine	86.9	90.1	92.8	2.304
Aspartic acid	88.1	91.0	93.0	2.259
Cysteine	87.6	87.6	92.5	2.417
Slutamic acid	88.7	93.9	93.4	2.608
Slycine	87.0	88.1	91.0	2.550
Proline	85.1	87.3	89.9	2.633
Serine	86.5	89.9	93.4	2.098
Tyrosine	87.9	93.0	94.7	2.712
Mean	87.2	90.1	92.6	2.447

Table 39. Apparent iteal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase, protease and xylanase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				
Arginine	84.8	86.7	89.3	2.439
Histidine	81.7	85.5	84.4	1.789
Isoleucine	74.8	83.0	83.7	2.517
Leucine	79.4	79.9	84.9	3.842
Lysine	82.9	87.1	87.4	1.282
Methionine	74.4b	84.1a	84.6a	1.814
Phenylalanine	81.7	87.5	87.4	1.375
Threonine	73.8	79.3	77.3	1.912
Valine	73.0	81.1	80.9	2.512
Mean	78.5	83.8	84.4	2.165
Dispensable				
Alanine	71.8	78.3	79.5	3.374
Aspartic acid	78.6	81.2	82.3	1.163
Cysteine	60.6	62.3	70.3	3.142
Glutamic acid	84.9	85.3	86.6	1.217
Glycine	60.4	63.5	63.7	6.786
Proline	69.7	74.5	75.9	3.675
Serine	76.6	79.3	79.9	1.912
Tyrosine	81.7	87.5	87.4	1.375
Mean	73.0	76.5	78.2	2.830

Table 40. True iteal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase, protease and xylanase in early-weaned pigs.

Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				<del></del>
Arginine	90.9	91.6	92.0	0.796
Histidine	87.0	89.3	90.4	1.464
isoleucine	83.7	90.1	90.3	0.794
Leucine	84.6	90.3	91.5	1.261
Lysine	89.4	88.3	90.6	0.560
Methionine	82.2	89.5	90.7	1.681
Phenylalanine	87.1b	91.6a	92.6a	0.816
Threonine	83.1b	90.3a	90.5a	0.919
Valine	87.3b	90.6a	92.4a	0.411
Mean	86.1	90.4	91.2	0.856
Dispensable				
Alanine	83.6b	92.0a	93.5a	0.574
Aspartic acid	85.3	90.8	90.7	1.879
Cysteine	81.0	85.7	89.8	1.880
Glutamic acid	88.1	92.6	92.8	1.522
Glycine	83.3	89.6	90.6	2.763
Proline	85.3b	89.2ab	92.7a	1.247
Serine	87.7	89.4	89.1	0.721
Tyrosine	85.7	90.8	91.3	1.885
Mean	85.0	90.0	91.3	1.559

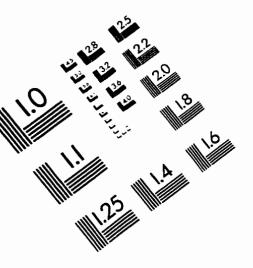
Table 41. Apparent faecal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase, protease and xylanase in early-weaned pigs.

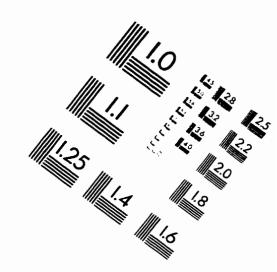
Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable		<del> </del>		
Arginine	87.4	87.9	91.3	2.651
Histidine	89.1	88.6	87.4	2.184
Isoleucine	77.3	84.0	83.9	2.217
Leucine	81.4	80.4	96.2	3.238
Lysine	83.9	88.1	88.8	1.780
Methionine	80.6	82.0	85.1	3.402
Phenylalanine	83.6	87.8	89.1	2.358
Threonine	80.1	81.4	80.7	1.297
Valine	80.7	86.4	84.9	3.117
Mean	82.7	85.2	87.5	2.471
Dispensable				
Alanine	80.1	81.3	83.7	2.755
Aspartic acid	82.3	83.6	84.5	2.338
Cysteine	79.4	80.1	79.9	1.637
Glutamic acid	87.0	89.7	86.7	1.702
Glycine	78.9	79.4	82.0	2.135
Proline	77.2	79.6	78.0	3.491
Serine	79.7	83.3	82.4	1.426
Tyrosine	84.3	89.0	89.7	2.089
Mean	81.1	83.2	83.4	2.196

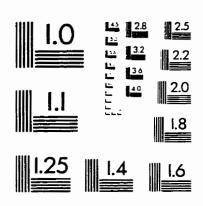
Table 42. True faecal digestibility (%) of amino acids of raw, extruded and micronized peas supplemented with α-amylase, protease and xylanase in early-weaned pigs.

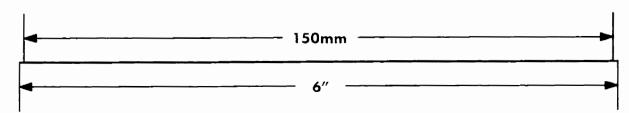
Amino acids	Raw	Extruded	Micronized	S.E.M.
Indispensable				<del></del>
Arginine	90.7	93.9	93.0	3.116
Histidine	88.0	89.7	89.9	2.312
Isoleucine	86.2	90.0	91.4	1.456
Leucine	85.1	89.9	89.0	2.058
Lysine	89.9	89.3	93.0	2.077
Methionine	84.7	90.5	90.0	4.008
Phenylalanine	87.6	89.3	92.0	1.791
Threonine	87.1	92.0	92.7	2.309
Valine	89.4	93.7	93.9	1.580
Mean	87.6	90.9	91.6	2.301
Dispensable				
Alanine	84.8	89.1	90.3	5.104
Aspartic acid	87.9	89.1	91.8	2.124
Cysteine	83.4	87.8	89.9	2.568
Glutamic acid	89.0	90.4	92.7	1.240
Glycine	84.0	90.1	90.6	2.319
Proline	87.6	89.7	92.4	1.440
Serine	87.9	92.8	92.0	2.338
Tyrosine	86.9	93.0	94.3	4.056
Mean	86.4	90.2	91.7	2.646

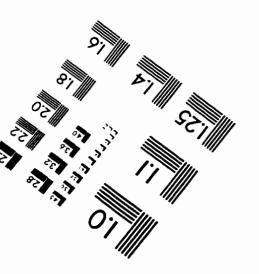
## IMAGE EVALUATION TEST TARGET (QA-3)













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