

**CHARACTERIZATION OF THE NUTRITIONAL VALUE OF MANITOBA-
GROWN PEAS (*Pisum sativum*) FOR PIGS.**

By

Melanie Joy Friesen

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Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirements

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ABSTRACT

Four studies were conducted to investigate the chemical composition, AA digestibilities, feeding value, and economic value of field peas for pigs.

In Study 1, nine pea cultivars (yellow-, and green-seeded) were analyzed for dry matter, crude protein, fat, NDF, ADF, starch and NSPs. Crude protein, starch and NSPs averaged 22.1% (range 20.0 to 24.2%), 385 mg g⁻¹ (range 331 to 428), and 87.7 mg g⁻¹ (range 75 to 104) respectively among the cultivars.

In Study 2, apparent, standardized and true ileal AA digestibilities of four pea-based diets (Profi, Swing, Croma and a mixture of AC Melfort, CDC Mozart, and Eclipse) were determined using ileal cannulated barrows (average initial body weight = 24.4 ± 1.8 kg) according to a 4 × 4 Latin square design. True ileal digestibilities were determined with the homoarginine or reactive lysine techniques. Apparent and standardized ileal AA digestibilities of the essential AA were different ($P < 0.05$) only for phenylalanine and leucine among diets, and averaged 74.3% and 80.8%, respectively. True ileal AA digestibilities determined with the homoarginine method were similar among diets except for lysine, phenylalanine and valine. Overall true digestibilities for all AA were 97.0% and 99.8%, for the homoarginine and reactive lysine methods, respectively.

In Study 3, the performance of nursery pigs fed increasing dietary levels of peas was evaluated. Ninety-six 17-day old weaned Cotswold (Platinum) pigs (6.05 ± 1.08 kg initial body weight) were randomly assigned to a corn-soybean-meal-based control (4 pens of 4 pigs each) and four pea-based diets (5 pens of 4 pigs each) containing 15, 20,

25 or 30% peas (cultivar Swing) during a five week experiment. There were no differences ($P > 0.05$) in average daily feed intake, average daily gain, and gain:feed among the diets and the overall values were 500 g d^{-1} , 346 g d^{-1} and 0.70, respectively.

Based on the results of Study 3, Study 4 was conducted to evaluate the economic value of peas by determining shadow prices, inclusion levels, and savings from the use of peas in commercial swine diets. Prices for corn, wheat, barley, peas, soybean meal 47%, canola meal 35%, and animal fat in February and August, from 2000-2003 were used for analysis in a linear feed formulation program. The starter, grower, and finisher diet shadow prices for peas ranged from \$146.40 to \$220.30, \$150.50 to \$227.40, and \$150.50 to \$212.90/metric tonne, respectively. The actual price of peas ranged from \$128.67 to \$201.67/metric tonne, which is generally lower than the shadow prices indicating that the inclusion of peas in most diets will reduce the total cost of the complete feed. The optimum inclusion level (i.e. most economical, lowest complete feed cost) for peas in the starter, grower, and finisher diets ranged from 36 to 44%, 56 to 69%, and 32 to 35% peas, respectively.

Results from the present studies indicate that peas are highly variable in their composition and that correcting apparent ileal AA digestibilities for minimum endogenous losses diminishes the differences of ileal AA digestibilities among pea cultivars. Furthermore, peas can be fed up to 30% to weaned pigs without affecting performance and generally have a good economic value and where appropriate they can be used to reduce the cost of complete swine feed.

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To God be the glory.

FOREWORD

This thesis was prepared in a manuscript format. There are four manuscripts corresponding to four chapters. Manuscript III was partially presented at the Pulse Conference in Edmonton, Alberta, December 2002. The findings from Manuscripts I, II, and III will be presented at the Canadian Society of Animal Science Annual Meeting in Saskatoon, Saskatchewan, June 2003. All manuscripts were formatted to meet the Guidelines for the Canadian Journal of Animal Science manuscript preparation. The titles of the manuscripts are: Chemical composition of Manitoba grown peas and suitable conditions for guanidination of lysine in peas (*Pisum sativum*); Apparent, standardized, and true ileal amino acid digestibilities of peas (*Pisum sativum*) fed to growing pigs determined with the homoarginine and the reactive lysine methods; Effect of increasing dietary pea (*Pisum sativum*) levels on nursery pig performance; and Economic value of peas (*Pisum sativum*): shadow prices, inclusion levels and savings from the use of peas in commercial swine diets. The authors for all four of the manuscripts are: M. J. Friesen, G. Crow, S. Arntfield, W. Guenter, and C. M. Nyachoti.

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LIST OF ABBREVIATIONS

AA	Amino acid
AC	Agriculture & Agri-Food Canada
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibilities
ANF	Anti-nutritive factor
AOAC	Association of Official Analytical Chemists
BW	Body weight
Ca	Calcium
CaCl	Calcium chloride
CCAC	Canadian Council on Animal Care
CDC	Crop Development Center
CP	Crude protein
Cu	Copper
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
EHC	Enzymatically hydrolyzed casein
ENL	Endogenous gut nitrogen losses
FFLP	Feed Formulation Linear Program
GA	Glucuronic acid

HCl	Hydrochloric acid
K	Potassium
ME	Metabolizable energy
Mg	Magnesium
Min	Mineral
Mn	Manganese
Mt	Million tonnes
MV	Missing value
N	Nitrogen
N/A	Not available
NaOH	Sodium hydroxide
NDF	Neutral detergent fibre
NE	Net energy
NRC	National Research Council
NSP	Non-starch polysaccharide
OMIU	O-methyl-iso-urea
P	Phosphorus
SAS	Statistical Analysis System
Se	Selenium
SEM	Pooled standard error of the mean
SID	Standardized ileal digestibilities
TIA	Trypsin inhibitor activity
TIU	Trypsin inhibitor units

Vit

Vitamin

Zn

Zinc

1.0 GENERAL INTRODUCTION

The production of peas in Western Canada has increased over recent years. The production of peas peaked in 2000, however in 2003 total world supply is expected to increase by 13% to 10.8 Mt due to the higher production in Canada, the European Union, and Australia (Skrypetz and Oleson, 2003). The plans for the future are to increase feed pea supplies to 4 million tonnes by the year 2010 (Gowans et al. 2000). With this growth there has also been increased interest in utilizing peas as a feedstuff for swine in place of imported and often expensive soybean meal. Two of the most important nutrients sought after in feed ingredients are energy and protein, of which peas are an excellent source. On top of that, the most limiting amino acid in swine diets is lysine, which peas are relatively high in compared to other sources of protein for pigs. However, peas remain under-utilized as a feed ingredient for swine. Possible reasons for this are the variability of protein content in peas, as well as the low levels of sulphur containing amino acids (NRC, 1998; Fan and Sauer, 1999) and the presence of some anti-nutritional factors (Stefanyshyn-Cote, 1998).

To optimize the use of peas in the swine industry, a means to ascertain the content and availability of nutrients (especially energy and amino acids) in individual batches prior to feeding is needed. Little work has been done with cultivars grown in Canada, and in particular Manitoba. Determining the true ileal amino acid digestibility coefficients in peas offer an excellent opportunity to enhance utilization of locally grown peas as source of protein in swine diets.

True ileal amino acid digestibilities rather than apparent or standardized ileal amino acid digestibilities need to be determined for accurate feed formulation. True ileal digestibility coefficients are derived by correcting the apparent ileal amino acid digestibilities for the amount of endogenous amino acid losses (ENL; Rademacher et al. 2000). There are a number of methods that have been used for the determination of ENL. The traditional methods of feeding nitrogen free diets and mathematical regression have been criticized for underestimating the amount of ENL, and overestimating availability of the amino acids (Tamminga et al. 1995; Boisen and Moughan, 1996; Nyachoti et al. 1997a). Different studies have shown that the endogenous losses of amino acids from the small intestine of monogastrics is higher following dietary intake of protein or peptides (Darragh et al. 1990; de Lange et al. 1990; Moughan and Rutherford, 1990; Butts et al. 1991; Butts et al. 1993). Alternative methods that have been developed and shown to provide more reliable results include the isotope dilution technique, the peptide alimentation ultrafiltration method (a.k.a. enzymatically hydrolyzed casein method (EHC)), and the homoarginine technique. Recently another method has been developed, the reactive lysine method, potentially providing a quick means of assessing the available amino acid content based on feed analysis.

The ^{15}N -isotope dilution method is too complex and expensive for regular use as a method of measuring true ileal amino acid digestibility and due to the fact that peas contain complex plant proteins the enzymatically hydrolyzed casein method would not be the best choice for analysis either. The homoarginine method is relatively quick and simple, and has been shown repeatedly to give similar results to the other two aforementioned methods. Reactive lysine is the amount of lysine in the feed that is

nutritionally available to the animal. The reactive lysine technique has the potential to reduce costs of production and improve production consistency by providing a simple means to measure the available lysine content of heat processed feed ingredients (van Barneveld, 2001). Reactive lysine can further be used to assess the extent of heat damage and storage on lysine availability. Before that can be done in practical situations, however, digestibility trials using the reactive lysine method need to be completed to determine the digestibility of the reactive lysine.

The overall objectives of these studies were:

1. To promote the use of locally-grown peas in the commercial swine feed industry through an improved understanding of their nutritive value.
2. To examine the true ileal amino acid digestibilities of peas in growing pigs using the homoarginine and the reactive lysine methods.
3. To determine what level of peas can be fed to weaned pigs without negatively affecting their performance.
4. To evaluate the economic value of peas in swine diets.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Peas (*Pisum sativum*) have considerable potential as a feedstuff for pigs.

Increasing production of peas in Canada in recent years has sparked great interest within the livestock feed industry and among swine producers to find economical ways to use peas in pork production, instead of exporting the majority of peas produced (Table 1). The production of peas peaked in 2000, however in 2003 total world supply is expected to increase by 13% to 10.8 Mt due to the higher production in Canada, the European Union, and Australia (Skrypetz and Oleson, 2003). This increase in production, however, is expected to be offset by increased use in livestock feeds. Not only is the production of peas increasing, but also a lot of the top 20 pea cultivars grown in 2002 were relatively new cultivars (Table 2).

Another reason why pea production has increased is that seed growers are also being pressured to grow more leguminous crops in their crop rotations to improve soil fertility and therefore increase the longevity of producing high crop yields (Jansman and Verstegen, 2002). Peas are a rich source of protein with a high lysine content for animal feed, however, they remain under-utilized as a feed ingredient for swine. Possible reasons for this are the variability of protein content in peas, as well as the low levels of sulphur containing amino acids (NRC, 1998; Fan and Sauer, 1999) and the presence of some anti-nutritional factors (Stefanyshyn-Cote, 1998). However, the level of anti-

Table 1. Feed pea supply and disposition (T) in Western Canada.

Year	Acres	Production	Imports	Total Supply*	Export	Domestic Use	Seed	Feed, Waste, and Dockage
1991	490 300	409 700	8 800	470 100	274 100	6 100	51 600	78 900
1992	675 000	504 800	7 500	571 700	339 500	6 200	94 500	96 700
1993	1 250 000	970 200	7 200	1 012 200	736 098	9 000	116 100	149 000
1994	1 720 000	1 441 000	8 400	1 451 402	928 808	15 000	135 800	222 000
1995	2 025 000	1 454 700	8 400	1 612 894	992 744	15 000	90 900	221 000
1996	1 345 000	1 173 000	8 100	1 474 350	865 696	15 000	142 200	201 000
1997	2 097 000	1 762 300	12 000	2 024 754	1 113 841	20 600	181 700	336 000
1998	2 680 000	2 336 800	10 000	2 719 413	1 705 034	20 900	142 600	431 000
1999	2 104 000	2 251 900	11 700	2 683 479	1 416 511	20 000	190 000	650 000
2000	3 065 000	2 864 300	11 600	3 282 868	2 190 000	21 000	275 600	600 000
2001	3 588 000	2 196 400	11 000	2 403 668	1 600 000	22 000	288 000	400 000

*Total supply includes carryover from previous year.

Source: Saskatchewan Agriculture and Food, 2001.

Table 2. Acreages of the top 20 field pea cultivars grown commercially in Saskatchewan from 1998 to 2002.

Cultivar	1998	1999	2000	2001	2002
Delta	MV	2 978	36 827	156 215	152 610
Swing	MV	1 270	10 231	63 099	69 309
Carneval	32 018	36 966	61 207	102 265	57 525
Alfetta	10 700	25 273	51 213	93 395	54 781
Grande	25 598	31 320	50 012	56 107	29 663
CDC Mozart	MV	MV	MV	10 083	28 125
Majoret	20 951	24 431	27 609	55 494	25 692
Nitouche	MV	MV	1 892	16 111	25 630
Scuba	MV	2 085	6 479	22 036	21 096
Keoma	16 051	17 432	21 133	22 621	18 197
Espace	3 461	12 172	11 456	30 286	16 973
Croma	MV	MV	MV	18 756	15 340
CDC Handel	MV	MV	MV	4 888	11 254
Toledo	MV	MV	MV	5 327	9 015
Highlight	11 072	12 809	13 341	12 099	7 262
Marrowfat	535	2 773	3 909	4 636	6 637
SW Parade	MV	MV	MV	2 636	6 393
Profi	1410	2 378	5 358	10 024	6 229
Eclipse	MV	MV	MV	529	6 223
Eiffel	445	1 102	4 055	723	5 051

Source: Morrow, 2003.

MV, Missing values indicate that crop was not yet produced commercially.

nutritional factors in most peas is considered to be lower and accepted as having negligible effects on pig performance (Stefanyshyn-Cote, 1998).

With the increased production of peas, there is also a growing demand for alternative feedstuffs mainly due to the expanding hog industry in Canada, the high cost related to imports, and the need for nutrient management. The unprecedented supply of peas into the animal feed market has provided swine producers with a great way to reduce feed costs. The amount of peas used in swine/livestock feed has increased over the years (Table 1).

The purpose of this thesis is to review the value of peas as a protein source for swine and to explore means with which to optimize the use of amino acids in peas when fed to pigs, focusing on true ileal amino acid measurements.

2.2 PEAS AS A FEEDSTUFF FOR PIGS

In recent years, there has been an increase in pea production in Canada, and in 1998 over 2 million tonnes of peas were produced (Skrypetz and Oleson, 1998). The plans for the future are to increase feed pea supplies to 4 million tonnes by the year 2010 (Gowans et al. 2000). As production of peas increases, so does the interest in using peas as a protein supplement in swine diets, in place of the often expensive and imported soybean meal (Bazylo, 2001).

Peas are locally grown, providing a more economical and dependable source of protein. When comparing feeding peas instead of soybean meal in a simple 0.7% lysine diet for finisher pigs, it brings a savings of \$2.50/pig (Gowans et al. 1998). In most

studies comparing peas to soybean meal, pig performance has been equivalent or greater in the pigs fed the pea diet (Saskatchewan Pulse Growers, Undated). Another positive attribute of peas is that they are easy to handle in the feed mill with good grinding and pelleting qualities, and because of the hull, the peas are protected against environmental conditions preventing it from deteriorating during storage (Mateos et al. 1998).

Furthermore, peas have an excellent nutrient profile for feeding to swine/livestock.

2.2.1 Chemical and nutrient profile of peas

The average chemical and nutrient composition of peas is presented in Table 3. The composition of peas, however, can vary rather widely. The two main components that differ in composition are the hulls and the kernel or cotyledon fraction. In mature seeds the hulls make up 70-140 g/kg of the seed and consist mainly of NSP (Jansman and Verstegen, 2002). The kernel or cotyledon fraction is the main fraction (860-930 g/kg) consisting mainly of starch, protein and smaller amounts of ash, crude fat, fibre and low molecular weight carbohydrates (Jansman and Verstegen, 2002). Peas are also good sources of many important elements, however swine diets containing peas are usually supplemented with minerals and vitamins. Levels of trace minerals are considered to be similar to levels found in many cereal grains (NRC, 1998).

Peas like any other feed ingredient are valued for their ability to supply energy and protein in the proportions and levels required by the animal. Currently, in the United States the most common ingredients in swine diets are corn and soybeans. In commercial swine diets corn is considered to be a highly valuable feed ingredient for its high energy content, whereas soybeans are highly valued for their level of protein. Compared to corn,

Table 3. Chemical and nutrient composition of peas (DM basis).

Nutrient		Nutrient	
Dry Matter %	89.0	DE – kcal/kg	3435
Crude Protein %	22.8	ME – kcal/kg	3210
Legumin % CP ^a	28.6	NE – kcal/kg	2195
Vicilin % CP ^a	30.9	Ca %	0.11
Albumin % CP ^a	40.6	P %	0.39
Starch % ^a	50.0	Availabilty of P % ^b	47
Soluble sugars % ^a	5.1	Iron mg/kg	65
Ash % ^a	3.4	Mn mg/kg	23
Crude Fibre % ^a	7.0	Zn mg/kg	23
ADF %	7.2	Mg %	0.12
NDF %	12.7	K %	1.02
Water insoluble cell walls % ^a	14.7	Na %	0.04
Proportion hull % ^a	0.85	Se mg/kg	0.38
Crude Fat %	1.2	Cu mg/kg	9
Linoleic Acid %	0.47	Vitamin E mg/kg	0.2
TIA U/g ^a	5.1	Biotin mg/kg	0.15
Lectins mg/g ^a	3.3	Choline mg/kg	547
Amylose % starch ^a	32.0	Vit. B1-Thiamine mg/kg	4.6
Oligosaccarides % ^a	6.7	Vit. B2-Riboflavin mg/kg	1.8
Condensed tannins % ^a	0.007	Vit. B5-Pantothenic Acid mg/kg	18.7

Source: NRC, 1998; Grosjean et al. 1999^a; Stefanyshyn-Cote, 1998^b.

the average digestible energy content of peas is similar (3550 kcal/kg for corn and 3400 kcal/kg for field peas) (Zijlstra et al. 2002). Peas have approximately half the amount of protein that soybeans have (16.7-25.5 % for field peas compared to 42.7-52.7 % for soybean meal) (Zijlstra et al. 2002). In Western Canada, wheat and barley (digestible energies of 3425 and 3100 kcal/kg, respectively) are often used in swine feed formulation instead of corn due to price and availability (Zijlstra et al. 2002).

2.2.1.1 *Pea carbohydrates*

The three main NSP residues in peas are glucose, uronic acid, and xylose. These sugars are found in the cotyledon cell walls, which are high in arabinose-containing pectic substances, along with xyloglucans and cellulose. The pea hull cell walls are rich in insoluble residues, made up of cellulose, acidic xylans, pectic polysaccharides (uronic acids) and lignin (Canibe et al. 1997). Pea cultivars are relatively high in starch contents (Gâtel and Grosjean, 1990; Igbasan et al. 1997), which is the main source of dietary energy. Igbasan et al. (1997) found that most cultivars that were high in starch, were also relatively low in protein content. However, the differences in starch and protein were not fully explained by this relationship.

2.2.1.2 *Pea protein*

Pea protein is made of two main fractions. Globulins or storage proteins make up 70-80% of the protein while the other 20-30% of protein in peas are albumins, which consist of biologically active proteins like enzymes, protease inhibitors and lectins (Le Guen et al. 1993). Globulins can be further subdivided into legumins and vicilins (Fan

and Sauer, 1999). Legumins, vicilins, and albumins all have different amino acid profiles, so depending on the proportion of each fraction, the amino acids content in peas will vary (Fan et al. 1994). An example of this is that the sulphur-containing amino acids and tryptophan levels are low in vicilins and high in albumins (Fan et al. 1994).

Lysine content and amino acid digestibility vary substantially among field pea samples (Table 4). Protein content only explains 28 to 69 % of the variation in total lysine content (Zijlstra et al. 2002). Analysis or prediction of total amino acid content is thus more useful than protein content. The protein and lysine content ranges substantially for peas in Western Canada, but prediction equations for digestible amino acid content have not been developed.

2.3 LIMITATIONS OF PEAS AS A FEEDSTUFF

Peas are a high quality feed ingredient that can be used for all classes of swine from young stock to market and breeding animals. However, their use in practical swine diets requires that inclusion levels are limited (Table 5). Reasons for this are that peas are highly variable in energy and crude protein and true ileal amino acid digestibilities are not readily available for diet formulation. Furthermore peas, like all legumes, may contain anti-nutritive factors (ANFs) like trypsin inhibitors, lectins, tannins, and fibre.

2.3.1 Variation in nutritive value

The commercial feed industry has not yet fully accepted peas as the sole source of supplemental protein. Largely, this is due to the wide variation in energy and crude

Table 4. Ranges in composition and apparent ileal digestibilities of amino acids in peas.

Amino Acid	Composition as fed (%)	Apparent Ileal Digestibility (%)
Indispensable		
Arginine	1.31 – 2.90	81 – 91
Histidine	0.46 – 0.67	70 – 82
Isoleucine	0.81 – 1.11	73 – 92
Leucine	1.30 – 2.00	73 – 82
Lysine	1.55 – 2.00	76 – 85
Methionine	0.18 – 0.30	70 – 80
Phenylalanine	0.75 – 1.25	68 – 84
Threonine	0.57 – 1.18	60 – 77
Tryptophan	0.20 – 0.27	53 – 70
Valine	0.94 – 1.29	68 – 76
Dispensable		
Alanine	0.82 – 1.14	63 – 74
Aspartic acid	2.15 – 3.11	72 – 81
Cysteine	0.26 – 0.39	59 – 66
Glutamic acid	3.20 – 4.74	76 – 86
Glycine	0.73 – 1.18	55 – 68
Proline	0.80 – 1.03	60 – 78
Serine	0.11 – 1.33	67 – 77
Tyrosine	0.32 – 0.83	68 – 80

Sources: Leterme et al. 1990; Fan et al. 1994; Fan and Sauer, 1999; Grosjean et al. 2000;

Owusu-Asiedu et al. 2002.

Table 5. Practical inclusion levels of peas for pigs, % of diet.

For Pigs	Peas
Prestarter 5-15 kg	5
Starter 15-25 kg	15
Grower 25-50 kg	40
Finisher 50-110 kg	40
Breeding Stock	30

Source: Stefanyshyn-Cote and Belanger, 2002.

protein content seen among different cultivars and within a cultivar grown in different growing conditions (i.e., location, season, etc.) (Reddy et al. 1979). Canibe et al. (1997) found the year of harvest had an effect on the digestibility of some pea NSP residues fed to rats. The wide variation in composition of peas can be attributed to both genetic and environmental factors (Pandy and Gritton, 1975; Savage and Deo, 1989). Igbasan et al. (1997) found starch to be negatively correlated with protein content, which accounts for some of the protein differences seen among cultivars. They also saw decreases of indispensable amino acid concentrations as the level of protein increased in peas. The average crude protein content of peas is 24% with a range of 15.6-32.5% (Fan and Sauer, 1999).

Another limitation preventing widespread use of peas as a feedstuff is the insufficient characterization of ileal amino acid digestibilities. Although some cultivars of European peas have prediction equations developed for estimating the nutritive value of the pea protein based on apparent digestibilities, there has not been prediction equations developed based on true ileal digestibilities. Available equations only predict the content of total amino acids as opposed to available amino acids. A measure of total amino acids is inadequate for predicting nutritive value (e.g., Sauer and Ozimek, 1986). Furthermore, there has been little or no work done on Canadian cultivars, and in particular Manitoba grown cultivars.

2.3.2 Anti-nutritive factors

Like any other pulse and legume crops, peas contain a number of different ANFs (Savage and Deo, 1989). These include trypsin inhibitors, lectins, tannins, and fibre.

Tannins are only found in *averse* (dark coloured flower) varieties (Grosjean and Gâtel, 1989). Trypsin inhibitor activity has been found to be greater in winter compared to spring and smooth compared to wrinkled pea cultivars (Valdebouze et al. 1980). Most Canadian peas, however, are spring seeded, white-flowering cultivars, and characteristically, have low levels of ANFs with little anti-nutritional significance (Slinkard and Tyler, 1993). In practical diets the low levels of ANFs results in an accepted, negligible impact on pig performance (Stefanyshyn-Cote, 1998).

2.3.2.1 *Trypsin inhibitors*

Found in leguminous seeds, especially raw soybeans, trypsin inhibitors are capable of forming stable, inactive complexes with the enzymes trypsin and chymotrypsin (Tamminga et al. 1995). Trypsin and chymotrypsin are enzymes secreted from the pancreas and are important for protein digestion. Trypsin inhibitors in swine feed result in lower apparent ileal digestibilities of amino acids (Li et al. 1998). Trypsin inhibitor activity is independent of protein content, only 10% is found in the hulls and 90% in the cotyledons, proportionate to the respective weight of the whole seed (Stefanyshyn-Cote, 1998). Barth et al. (1993) found that the addition of 3.0 g Kunitz trypsin inhibitors to soybean diets increased the amount of undigested endogenous nitrogen leaving the small intestine of pigs. Caine et al. (1998) found that when feeding an unprocessed diet compared to an autoclaved diet (substantially reducing soybean trypsin inhibitors) the endogenous and exogenous nitrogen recoveries were 22 and 78% compared to 10 and 90%, respectively. Caine et al. (1998) suggested that soybean trypsin inhibitors negatively affect the activity of pancreatic enzymes, but do not result in an increase in

their secretion. However, trypsin inhibitors in peas are Bowman-Birk type and should not be compared with trypsin inhibitors in soybeans, which are mainly Kunitz type and accessory Bowman-Birk (van Amerongen et al. 1998). The main difference that has been widely accepted between the Bowman-Birk and Kunitz trypsin inhibitors is that the Bowman-Birk trypsin inhibitors are heat-stable while the Kunitz trypsin inhibitors are heat-labile. Also, trypsin inhibitor activity is comparatively lower in peas than soybean meal. Jansman and Verstegen (2002) found the trypsin inhibitor activity content of peas to be 1-7 mg/g compared to 20-40 mg/g trypsin inhibitor activity of raw soybeans. Other studies have also shown peas to contain low levels of trypsin inhibitor. For example, Canibe et al. (1997) found the trypsin inhibitor activity in dried Solara peas to be 0.40-0.62 TIU/mg DM, and after toasting no activity was detected. Furthermore, Fan and Sauer (1999) found no correlation between trypsin inhibitor activity and amino acid digestibility values in peas. However, Grosjean et al. (2000) measured trypsin inhibitor in 13 different pea samples and found a range of 2.3 to 11.8 TIU/mg DM. A negative relationship exists between trypsin inhibitor activity and ileal digestibility of amino acids in feed pea samples (Bengala-Freire et al. 1991; Huisman et al. 1992; Grosjean et al. 2000).

2.3.2.2 *Lectins*

Present in legume seeds, are lectins, also known as haemagglutinins. They are glycoproteins that bind to specific sugars or proteins. Lectins also bind to the mucosa of the intestinal lumen causing damage shedding of the epithelial cells and depressing nutrient absorption, reducing brush border enzyme activity (Jansman and Verstegen,

2002). This can lead to scouring and may further reduce weight gain and feed conversion efficiency (Huisman and Van der Poel, 1994). Schulze et al. (1995b) found that the addition of soya-lectins to diets increased the amount of undigested endogenous nitrogen leaving the small intestine of pigs. However, compared to raw soybeans, peas have low levels of lectins. Jansman and Verstegen (2002) found peas to have 2-5 mg/kg lectins compared to the 8-13 mg/kg in raw soybeans.

2.3.2.3 *Tannins*

Tannins are water soluble polyphenolic compounds, that are capable of precipitating proteins and because of this they may interact with feed protein as well as digestive enzymes, affecting both apparent and true digestibilities (van Leeuwen et al. 1995). White-flowering cultivars of peas have tannin levels that are quite low (Huisman and Jansman, 1991). Gabert et al. (1996) studied two white-flowering pea cultivars (i.e. Radley and Ascona) and found both to contain low tannin levels (0.04 % catechin equivalents for both cultivars) compared to soybean meal and faba beans (0.08 and 0.50 % catechin equivalents, respectively). In some cultivars of pea (i.e. Solara) tannins are not even at detectable levels (Canibe et al. 1997). Jansman and Verstegen (2002) found the level of tannins in peas to range from 0 to 10 mg/g. Consequently, with such low levels of tannins in peas, the amount of tannins in a complete pea-based diet is very low.

2.3.2.4 *Fibre*

Cellulose, hemicellulose, pectin, gums, mucilages, algal types, and lignin are all part of what is considered to be dietary fibre. The level of endogenous nitrogen and

amino acids in ileal digesta is subject to the amount and source of dietary fibre (Schulze et al. 1994). There is a close relationship between the amount of neutral detergent fibre (NDF) in the diet and the amount of daily ileal dry matter flow (Schulze et al. 1995b). Not only does the fibre content affect the passage rate of digesta through the gastrointestinal tract, but fibre may also have water holding and gel forming properties that can affect the digestion and absorption of nutrients (Tamminga et al. 1995). Canibe et al. (1997) found the cultivar Solara to vary in dietary fibre from 121 to 132 g/kg DM (Klason lignin 3-4 g/kg DM and cellulose 41-42 g/kg DM). Another study with Solara peas found the level of NDF (cell walls) to be 11.8% DM (Leterme et al. 1990). Differences in NDF are partially responsible for the variation in digestibility values for protein in peas (Fan and Sauer, 1999). This is partially explained by the endogenous secretions being dependant upon not only the dry matter intake, but also the content and type of fibre in the ration (Green et al. 1987; de Lange et al. 1989a).

2.4 PROMOTING THE USE OF PEAS IN SWINE FEEDS

For a long time peas have been recognized as a rich source of protein with high lysine content and energy for animal feed (Table 6). With proper characterization of locally grown cultivars in terms of nutritive value, accurate feed formulation can be achieved. To further enhance the accuracy of feed formulation, nutrient digestibility coefficients should be available.

Table 6. Ranges in digestible energy (DE), crude protein, and total lysine in feed ingredients (DM basis).

Ingredient	DE content (kcal/kg)	Crude protein (%)	Total lysine (%)
Corn	3140 – 3740		
Barley	2680 – 3130	8.4 – 16.4	0.35 – 0.41
Wheat	3020 – 3640	10.5 – 18.9	0.37 – 0.50
Field peas	3100 – 3730	16.7 – 25.5	1.40 – 1.79
Canola meal	3000 – 3270 ¹	31.8 – 39.2	1.92 – 2.15
Soybean meal	3180 ² – 3675	42.7 – 52.7	2.68 – 3.18

Source: Zijlstra et al. 2002; Hickling, 1997¹; NRC, 1998².

With respect to amino acid supply in the diet, true ileal digestible amino acids should be used. True ileal digestibility coefficients are better estimates of availability and are more additive in a mixture of feedstuffs, therefore, allowing for more accurate feed formulation (Imbeah et al. 1988; Nyachoti et al. 1997b, 2002; NRC, 1998). If the feed does not contain a balanced amino acid profile, it has the potential to contribute to environmental pollution via excess nitrogen excretion (de Lange et al. 1999). The development of a method to rapidly predict true ileal digestible amino acid content in peas from simple chemical components would be beneficial to swine producers for the anticipated reduction in cost and the improved pea product consistency (van Barneveld, 2001).

2.5 DIETARY SUPPLY OF AMINO ACIDS IN SWINE DIETS

There are numerous feedstuffs included in swine diets for the purpose of supplying essential amino acids and other nutrients. Protein sources are often the most expensive ingredients in diets for swine; therefore their use should be optimized for minimizing the cost of production. To optimize protein utilization, an understanding of amino acid digestibility coefficients is required for precise feed formulation.

2.5.1 Ileal vs. fecal digestibility coefficients

Before researchers started measuring ileal digestibility coefficients, fecal digestibility coefficients were used. Fecal digestibility coefficients are based on the difference between dietary nutrient intake and fecal nutrient output. However, fecal

digestibilities were found to be inadequate for the determination of amino acid digestibilities as more research was completed. The reason the fecal method was found to be inadequate is because of the modifying action of the microflora in the cecum and large intestine of pigs on amino acid metabolism (Zebrowska, 1973). Furthermore, the nitrogen that is absorbed from the hindgut is not available for body nitrogen metabolism (Zebrowska, 1973; Just et al. 1981). Ileal digestibility coefficients are based on the difference between nutrients going in from the diet and nutrients coming out at the terminal ileum. When ileal digestibilities are used, the modifying effects of bacterial metabolism of amino acids in the hindgut are removed (Sauer and Ozimek, 1986). Currently, ileal rather than fecal amino acid digestibility coefficients are used in pig feed formulation.

2.5.2 Apparent vs. standardized vs. true ileal digestibilities

Apparent ileal digestibility is the direct measurement of the difference between nutrients going in from the diet and nutrients coming out at the terminal ileum. With apparent ileal digestibility, the non-digested dietary amino acids are not distinguished from the non-reabsorbed endogenous amino acids in ileal digesta. Another limitation of apparent ileal digestibilities is that there are variations in the digestibility coefficients in different samples of the same feed ingredient (Rademacher et al. 2000). For example, in two separate studies Fan et al. (1994) and Fan and Sauer (1999) found ileal digestibilities of pea protein to range from 70 to 76% and 75 to 80%, respectively. This difference could be due to the use of different pea cultivars as well as the different growing conditions. Furthermore, apparent digestibilities, unlike true ileal digestibilities are not additive in a

mixture of feed ingredients, and additivity is important for accurate feed formulation (Imbeah et al. 1988; Nyachoti et al. 1997b, 2002; NRC, 1998).

Standardized ileal amino acid digestibilities are apparent ileal digestibilities corrected for minimum endogenous amino acid losses derived from pigs fed a protein-free diet (AmiPig, 2000; Rademacher et al. 2000). Therefore, ileal amino acid digestibility coefficients are corrected for the endogenous protein loss related to dry matter intake and not the specific dietary factors such as fibre and other ANFs (Rademacher et al. 2000). Standardized ileal amino acid digestibilities will therefore be higher compared to apparent ileal amino acid digestibilities (Owusu-Asiedu et al. 2002). Furthermore, Stein et al. (2001) found that when apparent ileal amino acid digestibilities for growing pigs and lactating sows were corrected for using standardized ileal amino acid coefficients the previously reported differences in digestibility were no longer present. Fan and Sauer (1997) reported that the true ileal digestibility values, found using regression analysis (therefore considered standardized ileal digestibility), of amino acids were not as variable as their respective apparent ileal digestibility values in soybean meal. They also found the true ileal amino acid digestibilities to be independent of the level of amino acids in the diet. In general, standardized ileal amino acid digestibilities should be used for practical feed formulation over apparent digestibilities, as they are more additive in mixtures of feed ingredients (Rademacher, 2001).

True ileal digestibility coefficients are derived by correcting the apparent ileal amino acid digestibilities for the amount of endogenous amino acid losses (Rademacher et al. 2000). True ileal digestibility can only be estimated when endogenous amino acids in the ileal digesta are accurately measured. Unlike standardized ileal amino acid

digestibilities, true ileal amino acid digestibilities are measured using specific endogenous amino acid losses related to the feed ingested by the animal, making them more specifically related to the dietary factors such as fibre and other ANFs. Therefore, standardized ileal amino acid digestibilities are easier to determine than true digestibility values, as standardized ileal amino acid digestibilities can be calculated from previously determined book values rather than requiring experimental measurements. In a study done by Huisman (1999) the apparent ileal digestibilities of nitrogen of two pea cultivars (i.e. Finale and Firjaune) were found to be statistically different (79% and 74%, respectively), while the true ileal digestibilities of nitrogen measured with the ^{15}N isotope dilution technique were found to be similar (95% and 93%, respectively).

2.5.2.1 Endogenous gut nitrogen losses

Endogenous gut nitrogen losses (ENL) are essentially nitrogen from enzymes, mucoproteins, desquamated cells, serum albumin, peptides, amino acids, amides, and amines, and may contain bacteria and swallowed body hair (Nyachoti et al. 1997a). They are secreted as saliva, gastric secretions, bile, pancreatic juice, sloughed off epithelial cells and mucins along the gastro-intestinal tract (Tamminga et al. 1995). Not all of the endogenous nitrogen secreted into the gut is lost in excreta; it is digested and reabsorbed along with dietary nitrogen. It has been estimated that 70-80% is reabsorbed before the terminal ileum (Souffrant et al. 1986, 1993).

The secretion and re-absorption of endogenous nitrogen is affected by numerous factors, such as body weight, dietary protein quality and content, dietary fibre content, dry matter intake, and dietary ANFs content and type (Jansman and Verstegen, 2002;

Nyachoti et al. 1997a). A study done by Jondreville et al. (2000) found dietary fibre to be the main factor affecting ENL losses for soybean meal. By minimizing ENL, the contribution that pigs make to environmental pollution can be reduced (Nyachoti et al. 1997a). The amounts of ENL also have an impact on the maintenance energy and amino acid requirements of the pig and can have an effect on the overall performance of the pig (Nyachoti et al. 1997a).

2.6 TECHNIQUES FOR MEASURING ENL

There are a number of methods that have been developed to quantify the amount of endogenous amino acid losses in pigs. The traditional methods of feeding nitrogen free diets and mathematical regression have been criticized for underestimating the amount of ENL, and overestimating availability of the amino acids (Tamminga et al. 1995; Boisen and Moughan, 1996; Nyachoti et al. 1997a). Different studies have shown that the endogenous losses of amino acids from the small intestine of monogastrics is higher following dietary intake of protein or peptides (Darragh et al. 1990; de Lange et al. 1990; Moughnan and Rutherford, 1990; Butts et al. 1991, 1993). Alternative methods that have been developed and shown to provide more reliable results include the isotope dilution technique, the peptide alimentation ultrafiltration method (a.k.a. enzymatically hydrolyzed casein method (EHC)), and the homoarginine technique. Recently another method has been developed, the reactive lysine method, potentially providing true ileal digestibility estimates based on feed analysis.

2.6.1 Protein-free diets

The protein-free method involves feeding an animal a diet which is void or nearly void of protein, therefore all of the protein present in the digesta collected must be of endogenous origin (Hodgkinson et al. 2000). The simplicity of this method is a favourable attribute, however, there are a number of criticisms with regards to the accuracy of the technique. It has been shown that pigs fed peptide-containing diets have more endogenous nitrogen and amino acid flows than pigs fed a nitrogen free or low protein diet (Moughan et al. 1992). The greater endogenous nitrogen and amino acid flows in the animals fed peptides may be attributable to the stimulatory effect dietary peptides have on the endogenous secretions (Boisen and Moughan, 1996). When fed protein-free diets, animals mobilize body protein, in particular muscle protein, to supply the needed amino acids for critical metabolic functions. Feeding nitrogen free or low protein diets disrupt the digestive process and results in animals with abnormal physiological states (Low, 1980; Tamminga et al. 1995). Furthermore, the protein-free method makes the assumption that the amount of endogenous protein and the amino acid composition of endogenous protein is constant and unaffected by the amount or type of protein, or other factors in the diet (Carlson and Bayley, 1970). Studies have demonstrated that these assumptions are questionable (Souffrant et al. 1986; de Lange et al. 1989a). For instance, Fan and Sauer (1997) found large differences in the estimates of endogenous amino acid levels (i.e. between different ranges of graded dietary levels of crude protein; 4, 8, 12, 16, 20, and 24 %), although not significantly different, due to the large standard errors. This is important as these differences can affect the estimation of true ileal amino acid digestibilities.

2.6.2 Regression analysis

The regression analysis uses mathematical equations to estimate the recovery of endogenous protein and each of the individual amino acids under relatively normal conditions of protein (amino acid) supply (Fan and Sauer, 1997). Graded levels of dietary amino acids are fed to pigs to provide the data points needed for the calculations. There are two linear relationships that have been developed and used for the estimation of endogenous amino acid losses with the regression analysis technique. An estimation using a linear relationship between the contents of the apparent ileal digestible and total amino acids was developed by Furuya and Kaji (1986) and the extrapolation method that uses the linear relationships between the ileal outputs and the dietary inputs of amino acids was developed by Carlson and Bayley (1970) and Taverner et al. (1981). However, if different ranges of graded dietary amino acid levels are used in the digestibility assay, the results from the assay may be affected, decreasing the repeatability of the analysis. Fan and Sauer (1997) found the ranges of graded dietary protein levels of 4-24, 4-20, 4-16, and 4-12% to give the most reliable estimates of endogenous losses. There also remain unanswered questions in regards to the effect that graded dietary amino acids have on the level of amino acid recovery and amino acid composition of endogenous crude protein. This is because the regression method assumes that there are no interactions between endogenous amino acid losses and protein intake.

2.6.3 Isotope dilution technique

The isotope dilution technique involves the use of ^{15}N to label either the animal's nitrogen pool or the dietary nitrogen pool (Souffrant et al. 1986). The technique was

introduced by Souffrant et al. (1986) and allows the differentiation between undigested dietary and endogenous protein. The estimate of endogenous protein is based on the ratio of ^{15}N -enrichment in ileal digesta to ^{15}N -enrichment in an appropriate precursor pool, making the assumption that the ^{15}N -enrichment is similar between pools of nitrogen in an animal. Scientists are not clear as to whether or not this assumption is accurate.

When the ^{15}N -isotope dilution technique is used, the estimates of endogenous protein are up to twice the amount observed when using protein free and regression methods. Estimates with the ^{15}N -enrichment method range from 25 to 35 g/kg dry matter intake (de Lange et al. 1990; Huisman et al. 1992; Souffrant et al. 1993) compared to results obtained with the protein free method of 13 to 17 g/kg dry matter intake (de Lange et al. 1989b; Fan et al. 1995; Fan and Sauer, 1997).

There are still a number of criticisms as to the accuracy of the ^{15}N technique. This technique is criticized for the difficulty of achieving a steady state and in choosing the right precursor pool (Nyachoti et al. 1997a; Moughan et al. 1992). Also, results by Lien et al. (1997) suggest that the ^{15}N -isotope dilution technique may contain factors (i.e. enrichments in the TCA-soluble pool not providing accurate estimates of actual enrichment) in its methodology that underestimate the enrichment of the precursor pool for endogenous N secretion, potentially resulting in an overestimation of the recovery of endogenous protein in ileal digesta. Isotope dilution is not commonly used due to the intensive process and high costs (Gabert et al. 2001). Furthermore, with this technique only the total endogenous protein and not the levels of each individual amino acid can be determined (Fan et al. 1995; Souffrant et al. 1986). Assumptions still have to be made with regard to the amino acid composition of the endogenous protein, thus making the

reliability of individual amino acid values calculated in this way questionable, as the amino acid composition of endogenous protein is variable (Boisen and Moughan, 1996).

2.6.4 Enzymatically hydrolyzed casein method

The enzymatically hydrolyzed casein (EHC) method is another method used to determine endogenous amino acid flows at the terminal ileum and was first introduced by Moughan et al. (1990). The EHC method is performed by feeding EHC as the sole source of dietary protein, and is limited to semipurified diets (Schulze et al. 1995a; Gabert et al. 2001). Digesta is collected from the distal ileum of the animal and the endogenous protein (mol wt > 10 000) is separated out by centrifugation and ultrafiltration. The precipitate along with the high molecular weight fraction (mol wt > 10 000) make up the estimate of endogenous protein.

When the EHC method is used the estimates of endogenous lysine are greater than when using the protein free method. Moughan et al. (1990) found using 15 kg pigs that the endogenous flow of lysine using the EHC method was 461 mg/kg DM intake, while using the protein free method they found the endogenous flow of lysine to be 312 mg/kg DM intake. A study with rats found the endogenous flow of lysine using the EHC method to be 293 mg/kg DM intake, while the endogenous flow of lysine using the protein free method was 219 mg/kg DM intake (Butts et al. 1991). However, Mavromichalis et al. (2001) found endogenous lysine flows using the EHC method similar to those reported for the protein free method. Using two different digestibility markers they found the endogenous flow of lysine to be 221 and 236 mg/kg DM intake,

which could also be attributed to the fact that smaller pigs (17-d old) were used (Mavromichalis et al. 2001).

Another positive attribute of this method is that it is direct and does not rely on isotopes or other markers. Also, with this method the influence of dietary peptides is present (Moughan et al. 1990; Butts et al. 1991). It has been shown to give estimates of endogenous amino acids losses comparable to those found directly, after ingestion of whole protein (Moughan and Rutherford, 1990; Butts et al. 1993). Compared to the protein free method, the EHC method has been found to give significantly higher endogenous ileal amino acid flows (Moughan et al. 1992). However, one disadvantage of the EHC method is that it cannot effectively be applied to diets containing fibre and ANF's from plant ingredients (Schulze et al. 1995b,c). Another limitation of the method is that some free endogenous amino acids and peptides are discarded in the low molecular weight (mol wt < 10 000) thus leading to an underestimation of the endogenous amino acid excretions. This can be a significant loss, research in both rats and pigs have shown that free endogenous amino acids and peptides make up 11-21% of total digesta nitrogen (Moughan et al. 1990; Moughan and Schuttert, 1991; Butts et al. 1992). Therefore, the method is a valuable tool and provides a relatively accurate estimate for amino acid flows in swine fed diets with protein from animal sources. The results from the EHC method have been found to be similar to those obtained with the ¹⁵N-isotope dilution method (Schulze et al. 1995a).

2.6.5 Homoarginine method

The homoarginine method, first introduced by Hagemester and Erbersdobler (1985), is a relatively simple and quick method for determining ENL. The technique is carried out by chemically converting lysine into its synthetic derivative homoarginine (2-amino-6-guanidinohexanoic acid) via a guanidination reaction. The homoarginine method is based on a number of assumptions: that the chemical transformation does not affect either the digestion or the absorption of the dietary protein, that the absorption of homoarginine is similar to that of lysine, and that homoarginine is not incorporated into endogenous secretions (Imbeah et al. 1996). These assumptions have been validated (Schmitz et al. 1991; Marty et al. 1994; Siriwan et al. 1993; Ravindran et al. 1998; Nyachoti et al. 2002). Uniform guanidination of lysine is needed in a protein source if homoarginine is to be used effectively as the marker for determining endogenous recoveries of amino acids. Even distribution of homoarginine can be obtained when feeding relatively simple protein (i.e. casein), but it is questionable whether even distribution can be expected in complex proteins (Caine et al. 1997). Depending on the protein source and the guanidination conditions, the extent of lysine conversion is known to vary considerably (Imbeah et al. 1996; Maga, 1981; Rutherford and Moughan, 1990). It is important to have a high level of conversions of lysine to homoarginine to ensure that a representative proportion has been transformed (Siriwan et al. 1993). Therefore, optimal conditions for the guanidination of lysine in individual feedstuffs should be determined to enhance the accuracy of this technique.

One downfall of the homoarginine technique is that since homoarginine is not used for protein synthesis, a lysine deficiency can occur if used over a prolonged period of

time (Nyachoti et al. 1997a). Endogenous protein recovery has been found to be similar using the homoarginine method, 25 g/kg dry matter intake (Caine et al. 1997), compared to the ^{15}N -isotope dilution technique, 25.5 g/kg dry matter intake (de Lange et al. 1990). Nyachoti et al. (1997b; 2002) and Roos et al. (1994) also found ileal amino acid digestibility results using the homoarginine technique to be in close agreement with the values found by de Lange et al. (1990) for barley and canola meal, using the ^{15}N -isotope dilution technique. The results from Caine et al. (1997) also showed higher endogenous protein recoveries than were determined using the conventional protein-free diet method (de Lange et al. 1989b; Fan et al. 1995; Fan and Sauer, 1997). The homoarginine method, although comparable to the ^{15}N -isotope dilution technique, and therefore also comparable to the EHC method, may still underestimate true digestibilities to some degree, although this is believed to be negligible. Assumptions still have to be made, like for the ^{15}N -isotope dilution technique, with regard to a standard amino acid composition of the endogenous protein. Dietary factors may not only cause changes in the amount of endogenous losses, but also in the amino acid composition. Specifically, the amino acid composition in digesta may be affected by differences in available nitrogen for bacterial growth (Caine et al. 1999).

2.6.6 Reactive lysine method

Reactive lysine is the amount of lysine in the feed that is nutritionally available to the animal. In swine diets, lysine is often the first limiting amino acid. Therefore, for the most accurate feed formulation, the level of reactive lysine should be used as opposed to using the amount of total or digestible lysine. The total amount of lysine in the diet

includes lysine that has reacted with other compounds in the feed thus rendering it nutritionally unavailable. Previously used methods to measure the ileal digestibility of lysine have not been accurate in measuring available lysine in heat-treated proteins. This is because lysine will undergo chemical reactions like the Maillard reaction rendering some of the lysine nutritionally unavailable to the animal (Rutherfurd et al. 1997a, b). These chemical reactions will take place during processing or from prolonged storage. Also, some lysine derivatives can revert to lysine during the acid hydrolysis step in conventional amino acid analysis because of being acid labile (Rutherfurd et al. 1997b). This will lead to an overestimation of the content of nutritionally available lysine, as this reversion does not take place in the digestive tract of the animal.

The reactive lysine method has the potential to provide a rapid means of measuring the available lysine of feed ingredients prior to incorporation into diets. The reactive lysine technique has the potential to reduce costs of production and improve production consistency by providing a simple means to measure the available lysine content of heat processed feed ingredients (van Barneveld, 2001). Reactive lysine can further be used to assess the extent of heat damage and storage on lysine availability. Before that can be done in practical situations, however, digestibility trials using the reactive lysine method need to be completed to determine the digestibility of the reactive lysine. By combining the guanidination technique with the traditional true ileal amino acid digestibility assay it is possible to determine the ileal digestibility of reactive lysine (Moughan and Rutherfurd, 1996). The ileal digestibility assay is conducted in the same manner as if to find the apparent ileal digestibility, and the guanidination technique is used to measure the reactive lysine content of both the diet and the digesta. From the reactive lysine

measurements of the diet and the digesta it is possible to calculate the true ileal reactive lysine digestibility coefficient and to determine the available lysine (Moughan and Rutherford, 1996).

2.7 METHODS FOR COLLECTING ILEAL DIGESTA

In order to measure the endogenous losses of protein in the pig, a method to collect ileal digesta is required. It is important that whatever method is used to collect the digesta, a representative sample of digesta is collected between feeding intervals (Jørgensen et al. 1997). There have been a number of cannulation techniques developed for this purpose along with the ileo-rectal anastomosis technique, and the slaughter technique, and they all are useful depending on the research and results desired. The types of canulas include re-entrant canulas, T-canulas, post-valvula T-caecum canulas.

The re-entrant canula, post-valvula T-caecum canula, and the ileo-rectal anastomosis technique all require surgery that disrupt normal intestinal function (i.e. complete transection of the small intestine, caecumectomy, and colonectomy, respectively) (Nyachoti et al. 1997a). The T-cannulation technique is most commonly used as the surgery is less invasive than that required for the previously mentioned techniques (Nyachoti et al. 1997a). The other alternative is to apply the slaughter technique, collecting digesta after the killing of an animal. With this method, the major downfall is that the digesta can only be sampled at one point in time and the animals cannot be used as their own controls. However, when the slaughter technique was compared directly with the simple T-cannulation technique no differences in ileal digestibilities were observed (Donkah et al. 1994).

2.8 CONCLUSION

The production of peas in Western Canada has increased over recent years. With this growth there has also been increased interest in utilizing peas as a feedstuff for swine in place of the imported and expensive soybean meal. This interest is expected to flourish as the swine industry in Manitoba expands. Peas have a nutrient profile that provides them with the potential to become highly valued as a feed ingredient for swine. Two of the most important nutrients sought after in feed ingredients are energy and protein, for which peas are an excellent source. Furthermore, the most limiting amino acid in swine diets is lysine, which peas are relatively high in compared to other sources of protein for pigs. However, peas have not to date been accepted by the commercial feed industry as the sole source of supplemental protein, despite the available information indicating that peas could completely replace soybean meal in grower-finisher pigs. This is mainly due to the variability in energy and crude protein content seen among different cultivars and within a cultivar grown in differing conditions. There is also the issue that exports pay a higher price than our feed industry is willing to pay.

In order to optimize the use of peas in the swine industry, a means to ascertain the content and availability of nutrients (especially energy and amino acids) in individual batches prior to feeding is needed. Little work has been done with cultivars grown in Canada, and in particular Manitoba. Determining the true ileal amino acid digestibility coefficients in peas offer an excellent opportunity to enhance utilization of locally grown peas as source of protein in swine diets.

Whether done by ^{15}N -isotope dilution, enzymatically hydrolyzed casein, or the homoarginine technique, true ileal amino acid digestibilities are more accurate than

apparent or standardized ileal amino acid digestibilities, as they are measured using specific endogenous amino acid losses related to the feed ingested by the animal, making them specifically related to the dietary factors such as fibre and other ANFs. However, the ^{15}N -isotope dilution method is too complex and expensive for regular use as a method of measuring true ileal amino acid digestibility and due to the fact that peas contain complex plant proteins the enzymatically hydrolyzed casein method would not be the best choice for analysis. The homoarginine method is relatively quick and simple, and has been shown repeatedly to give similar results to the other two aforementioned methods. Therefore, in practice the homoarginine method is the most useful technique for the estimation of true ileal amino acid and protein digestibilities.

Developing conditions that will allow for the routine use of the homoarginine technique in assessing the ileal amino acid digestibility in peas will promote both the use of feed peas in commercial diets and also serve as a tool for the fast screening of new pea cultivars, thus aiding in cultivar development.

The focus of this project is to promote the use of locally-grown peas in the commercial swine feed industry through an improved understanding of their nutritive value. Further strategies can then be developed to deal with the variation in nutrient content to enhance precision in swine diet formulation when peas are used. This not only has the potential to reduce feed costs, but also to provide the additional benefit of minimizing nutrient excretion in pig manure, enhancing the competitiveness of the Manitoba Pork Industry. Furthermore, the promotion of peas through greater use as a feed ingredient in swine diets will allow peas to compete with conventional protein sources, helping develop a stable market for Manitoba-grown peas.

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3.0 MANUSCRIPT I

Chemical composition of Manitoba grown peas and suitable conditions for guanidination of lysine in peas (*Pisum sativum*).

3.1 ABSTRACT

Nine pea cultivars (yellow- and green-seeded) were evaluated for chemical composition. Two of those cultivars (one yellow- and one green-seeded) were used to establish suitable conditions for guanidination of lysine in peas. The chemical evaluation involved analyses for dry matter, protein, fat, NDF, ADF, starch and NSPs. The cultivars showed a wide range of protein (20.0-24.2%) and starch (331-428 mg/g) contents. The cultivars were low in fat (0.5-1.2%) and relatively high in starch (331-428 mg/g) and NSP (75-104 mg/g). Conditions tested for the guanidination of lysine in peas were O-methyl-iso-urea (OMIU) concentration (0.4 or 0.5 M) and reaction period (4, 5 or 6 d) at pH 10.5. Using 0.4 M OMIU solution for 4, 5 or 6 d gave guanidination rates of 91.9, 94.2, and 95.2% for Carneval (a yellow-seeded pea) and 92.6, 95.1, and 95.4% for Keoma (a green-seeded pea), respectively. Using 0.5 M gave 94.3, 95.6, and 96.3% for Carneval and 94.8, 95.8, and 96.9% for Keoma, respectively. The present results show that peas are highly variable in their composition, which should be taken into account when formulating diets containing peas. An OMIU solution of 0.4M and a 5-d reaction period are recommended for converting lysine to homoarginine in peas.

3.2 INTRODUCTION

In recent years the production of peas (*Pisum sativum*) has increased and in 2003 total world supply is expected to increase 13% to 10.8 Mt due to the higher production in Canada, the European Union, and Australia (Skrypetz and Oleson, 2003). As production of peas increases so does the interest in using peas as a protein supplement in swine diets, in place of the often expensive and imported soybean meal. The commercial feed industry has not yet fully accepted peas as the sole source of supplemental dietary protein. Largely, this is due to the wide variation in energy and crude protein content seen among different cultivars. The average crude protein content of peas is 24% with a range of 15.6-32.5% (Fan and Sauer, 1999).

In order to optimize amino acid utilization in swine diets true ileal digestibility coefficients should be used. However, true ileal digestible amino acid contents in different peas has not been determined. Of the many techniques for determining true ileal amino acid digestibilities in feedstuffs for pigs, the homoarginine technique has been suggested as a suitable method. The homoarginine technique involves a chemical process that uses O-methyl-iso-urea (OMIU) to transform the lysine in the test protein to homoarginine (2-amino-6-guanidinohexanoic acid) in a guanidination reaction (Hagemeister and Erbersdobler, 1985). In order for the method to be used effectively a uniform guanidination of lysine into homoarginine is required in the test protein (Nyachoti et al. 1997b). The uniformity of lysine conversion into homoarginine increases with increasing guanidination rates (Siriwan et al. 1994).

The rate of lysine conversion is known to differ among and within feedstuffs (e.g. Maga, 1981; Rutherford and Moughan, 1990; Imbeah et al. 1996). Previously, different

researchers have used a variety of different reaction conditions including the molarity of OMIU, the length of reaction period, and the pH. Imbeah et al. (1996) tested molarities of OMIU at 0.4M and 0.6M with a reaction period of 24 hours, and a pH of 10.5, and found lysine guanidination rates of between 91.6-100% in casein. Nyachoti et al. (2002) tested molarities of OMIU at 0.4M, 0.5M and 0.6M with reaction periods of 4 and 6 days, and a pH of 10.5 and found that increasing the molarity of OMIU above 0.5M did not increase the conversion rate in barley and canola meal. However, when the reaction period was increased from 4 to 6 days the conversion rates increased for barley but not for canola meal with a 0.6M OMIU solution. Rutherford and Moughan (1990) used a 0.6M OMIU solution, with a reaction period of 6 days to evaluate the effect of pH of reaction mixture between 10.2-11 and found that the highest conversion rates in isolated soy protein occurred at a pH of 10.8. Clearly, suitable guanidination conditions differ among feedstuffs and thus creating the need to establish optimal guanidination conditions for a wide variety of feedstuffs used in pig feeds.

3.3 OBJECTIVES

1. To examine the chemical composition of Manitoba-grown peas.
2. To establish suitable conditions for converting lysine in peas into homoarginine for determining true ileal amino acid digestibilities and estimating available lysine.

3.4 MATERIALS AND METHODS

Nine pea cultivars grown in southern Manitoba were evaluated in this study. Seed samples for the nine cultivars were kindly supplied by Dr. D. J. Bing, Agriculture and Agri-Food Canada Research Station, Morden, Manitoba. Each pea sample was subsampled for chemical analyses. About 0.5 kg of seeds from each cultivar were ground to pass through a 1-mm sieve prior to chemical analyses. Samples were analyzed for DM, and hexane extract (fat) by standard methods of analysis (AOAC, 1990). Nitrogen was determined by the Kjeldahl procedure (AOAC, 1990) and converted to crude protein content using a conversion factor of 6.25.

The NDF component was determined using a refluxing apparatus, Tecator Equipment (Laboratory Construction Co., Kansas City, MO) according to a procedure outlined by Van Soest and Wine (1967) and modified by Robertson and Van Soest (1977) with the addition of α -amylase enzyme (Termamyl; Novo Nordisk A/S, Bagsvaerd, Denmark). The procedure for the determination of ADF was similar to that of NDF, except that acid detergent buffer solution was used rather than neutral detergent buffer. Non-starch polysaccharides (NSP) and uronic acids were determined using gas liquid chromatography according to a procedure outlined by Englyst and Cummings (1984) and modified by Slominski and Campbell (1990). Starch was determined using the NSP procedure of Englyst and Cummings (1984) and calculated by difference between the total glucose content and the glucose present in the NSP residue following hydrolysis with α -amylase. All analysis were performed in duplicate.

Guanidination of two pea cultivars (i.e. Carneval and Keoma) in duplicate was carried out using OMIU. One litre of OMIU solution (0.4M OMIU) was made by

dissolving 68.9 g O-methyl-iso-urea-hydrogen sulfate in 500 mL of glass distilled water. 126.2 g barium hydroxide octahydrate was added to the solution and stirred with a magnetic stir plate for 30 minutes. The resulting mixture was centrifuged at $1500\times g$ for 10 minutes. The clear supernatant was siphoned into another container and the pH was adjusted to 10.8 with 2M NaOH (Rutherford and Moughan, 1990). In a volumetric flask the volume of the solution was brought to 1L with glass distilled water. To make the 0.5M solution 86.12 g O-methyl-iso-urea-hydrogen sulfate was used with 157.75 g barium hydroxide octahydrate.

Imbeah et al. (1996) tested different ratios of lysine:OMIU and found that using soybean protein a ratio of 1:12 resulted in $78 \pm 0.7\%$. A lysine to OMIU ratio of 1:16 resulted in a slightly higher conversion rate of $79.8 \pm 0.3\%$. Even though the reaction only requires a ratio of 1:1, OMIU is needed in large excess for complete guanidination of complex protein (Imbeah et al. 1996). The ratio of 1:12 was used to determine the amount of OMIU that would be used to guanidinate the pea protein, as the higher ratio only resulted in minor increases in conversion rate and the cost of OMIU is high. An average lysine content of 1.5% in peas was used for calculations (NRC, 1998).

A 20g sample (previously ground through a 1-mm screen) of peas was added to 72 mL of glass distilled water and then 72 mL of the OMIU solution was added and stirred with a magnetic stir plate for 60 minutes. The pH was adjusted to 10.8 and checked daily for 4, 5, or 6 days. The reaction mixture was stored throughout the reaction period at 4°C . After the reaction period, the pH was adjusted to 4.5, the isoelectric point of pea protein, by the addition of 1M HCl to precipitate out the protein (Le Guen et al. 1993). The mixture was then centrifuged for 10 minutes at $1500\times g$ and the supernatant

discarded. The precipitate was then washed by re-suspension in glass-distilled water (pH 4.5) 3 times and re-centrifuged for 10 minutes at 1500×g to rinse out any unreacted OMIU. The samples were kept frozen prior to freeze drying. After freeze-drying the samples were analyzed for lysine and homoarginine content using the method of AOAC (1990).

3.4.1 Calculations

The conversion rate of lysine into homoarginine was calculated as follows:

$$\text{Extent of guanidination (\%)} = \frac{\text{mol homoarginine}}{\text{mol homoarginine} + \text{mol lysine}} \times 100$$

Guanidination rates and chemical composition data are presented as means ± standard deviation.

3.5 RESULTS AND DISCUSSION

The proximate analysis and chemical composition of the nine different pea cultivars are shown in Tables 7 and 8. The dry matter content ranged from 88.99 to 90.94%, which is within the range of values found by other researchers (Fan et al. 1994; Gabert et al. 1996; Canibe et al. 1997; Fan and Sauer, 1999). The cultivars showed a broad range of crude protein contents of 19.98 to 24.24%, however the ranges found by previous studies showed similar results (Igbasan et al. 1997; Fan and Sauer, 1999). Five yellow

Table 7. Chemical composition of selected pea cultivars grown in Manitoba.*

Item	Pea cultivar									Average
	AC Melfort	Eclipse	CDC Mozart	Century	Carneval	Radley	AC Advantage	Nitouche	Keoma	
Cotyledon Color	Yellow	Yellow	Yellow	Yellow	Yellow	Green	Green	Green	Green	N/A
Dry Matter (%)	90.5 ± 0.6	89.4 ± 0.0	89.1 ± 0.2	89.6 ± 0.4	89.1 ± 0.2	90.9 ± 0.1	89.6 ± 0.1	89.2 ± 0.2	89.0 ± 0.2	89.5 ± 0.6
Protein (%)	24.2 ± 0.3	23.8 ± 0.1	23.1 ± 0.4	22.8 ± 0.9	21.2 ± 0.3	23.0 ± 0.2	20.8 ± 0.0	20.3 ± 0.3	20.0 ± 0.8	22.1 ± 1.6
Fat (%)	0.7 ± 0.1	0.9 ± 0.0	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	0.7 ± 0.0	1.2 ± 0.1	0.9 ± 0.2
NDF (%)	8.3 ± 0.2	MV	8.3 ± 0.0	MV	8.5 ± 0.4	10.3 ± 0.2	MV	9.5 ± 0.5	11.5 ± 0.2	11.0 ± 4.6
ADF (%)	7.4 ± 0.1	5.4 ± 0.1	6.5 ± 0.3	5.9 ± 0.0	6.7 ± 0.0	8.2 ± 0.0	5.2 ± 0.3	7.4 ± 0.3	7.4 ± 0.1	6.7 ± 1.0
Starch (mg/g)	383 ± 4	398 ± 4	410 ± 9	411 ± 0	369.3 ± 9	359 ± 10	428 ± 8	372 ± 3	331 ± 11	385 ± 30
Uronic Acids GA Content (mg/g)	19.8 ± 0.3	19.1 ± 0.2	19.4 ± 0.7	18.1 ± 0.3	22.1 ± 0.9	23.0 ± 0.5	18.4 ± 0.1	23.5 ± 1.1	20.0 ± 0.8	20.4 ± 2.0

*Values are mean ± standard deviation, n=2.

MV – missing value.

Table 8. Non Starch Polysaccharides (NSP) content of selected pea cultivars grown in Manitoba (mg/g).

Item	Pea cultivar									Average
	AC Melfort	Eclipse	CDC Mozart	Century	Carneval	Radley	AC Advantage	Nitouche	Keoma	
Cotyledon Color	Yellow	Yellow	Yellow	Yellow	Yellow	Green	Green	Green	Green	N/A
Ramnose	0.0	0.8	0.0	0.0	0.6	0.0	1.2	1.2	0.0	0.4
Arabinose	23.2	27.7	17.7	24.6	20.7	15.9	23.9	24.1	22.1	22.2
Xylose	10.0	7.5	9.0	7.0	11.8	10.8	7.2	10.5	11.4	9.5
Mannose	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.9
Galactose	4.8	4.8	4.4	4.6	4.6	4.6	4.4	4.8	4.3	4.6
Glucose	51.3	38.4	48.9	47.2	51.1	63.9	38.7	63.5	53.4	50.7
Inositol	44.8	44.6	44.7	44.8	44.8	45.2	44.8	44.5	44.7	44.8
Average total NSP	89.9	77.3	81.0	84.3	89.4	96.1	75.4	104.3	91.7	87.7

and four green pea cultivars were included in this study and the variations in crude protein contents did not appear to be related to seed colour.

The fat (hexane extract) content was low in the pea cultivars tested, however, similar results have been reported previously by Igbasan et al. (1997), NRC (1998), and Fan and Sauer (1999). The tested pea cultivars were rich in starch (Table 7) and NSP (Table 8). Starch values were similar to those reported by Igbasan et al. (1997), NRC (1998), and Fan and Sauer (1999). The amount of neutral detergent fibre (NDF) found within the selected cultivars for the current study were slightly lower than the values found by Igbasan et al. (1997) and Fan and Sauer (1999), who found a range of 14.6-18.2%. However, the NRC (1998) value of 12.7% is similar to the average NDF content (11.0%) found in the current study. The ADF content (6.7%) found in the current study agrees with previous findings (NRC, 1998; Grosjean et al. 1999).

The conversion rates of lysine into homoarginine are shown in Table 9. Between the two cultivars of peas selected for having a large acreage grown locally, the conversion rates were found to be similar. The extent of lysine conversion in Carneval and Keoma peas tended to be slightly greater with the 0.5M OMIU solution (94 to 97%) compared to the 0.4M OMIU solution (92 to 95%). As the reaction period length got longer, the extent of lysine conversion in Carneval and Keoma peas tended to become greater. However, the increase in conversion rate from a 5d reaction period to a 6d reaction period was relatively small, as was the increase from 0.4M to 0.5M with a 5d reaction period. The guanidination rates for peas (range from 91.2 to 96.9%) were substantially higher than those reported for canola meal (72.3 to 86.0%, Nyachoti *et al.* 2002) and soybean meal (78%, Marty et al. 1994; 68.6%, Siriwan et al. 1994; 79.8%,

Table 9. Lysine conversion into homoarginine (%) from guanidination using different reaction periods and concentrations of O-methyl-iso-urea (OMIU).*

OMIU	Carneval			Keoma		
	Reaction Period					
	4d	5d	6d	4d	5d	6d
0.4M	91.9 ± 0.011	94.3 ± 0.000	95.3 ± 0.004	92.6 ± 0.009	95.1 ± 0.003	95.4 ± 0.006
0.5M	94.3 ± 0.004	95.6 ± 0.001	96.3 ± 0.001	94.8 ± 0.003	95.8 ± 0.006	96.9 ± 0.001

*Values are mean ± standard deviation, n=2.

Imbeah et al. 1996). Imbeah et al. (1996) reported conversion rates (using a ratio of 1:12, lysine: OMIU) in casein and soybean meal of 92.8 and 78.0%, respectively. Clearly, conversion rates of lysine into homoarginine differ among feedstuffs, just as the suitable conditions for guanidination differ among feedstuffs.

Maga (1981) found the optimum reaction period and OMIU concentration to be 4d and 0.5M using a 5% by weight lysine solution. However, he measured the two parameters separately and did not look at the effect of increasing reaction period and OMIU concentration together. In the current study the conversion rates with a 4d reaction period and 0.5M concentration of OMIU were the same as the conversion rates with a 5d reaction period and 0.4M concentration of OMIU. Since the cost of OMIU is high, and the availability of OMIU is sometimes limited, a method using 0.4M OMIU is preferred over a method using 0.5M OMIU.

3.6 CONCLUSIONS

From the results it can be concluded that there is a high variability in the chemical composition of peas, and this should be taken into consideration when formulating diets containing peas.

The most suitable conditions for converting lysine in peas into homoarginine need to maximize the conversion rate. Since the conversion rates with a 4d reaction period and 0.5M concentration of OMIU were the same as with a 5d reaction period and 0.4M concentration of OMIU, the more economical conditions are recommended. OMIU is an

expensive solution and therefore the 5d reaction period with the 0.4M concentration of OMIU is recommended when using the homoarginine technique for guanidinating peas.

4.0 MANUSCRIPT II

Apparent, standardized, and true ileal amino acid digestibilities of peas (*Pisum sativum*) fed to growing pigs determined with the homoarginine and the reactive lysine methods.

4.1 ABSTRACT

Apparent, standardized and true ileal amino acid digestibilities of peas fed to growing pigs were determined. Four barrows with an average initial body weight of 24.4 ± 1.8 kg were fitted with a simple T-cannula at the distal ileum and used to determine ileal amino acid (AA) digestibilities in four pea-based diets according to a 4 × 4 Latin square design. The pea cultivars used were Profi (18.3% CP), Swing (19.8% CP), Croma (21.3% CP) and a mixture of AC Melfort, CDC Mozart, and Eclipse (23.7% CP). Peas were the only protein source in a cornstarch-based diet formulated to contain 14.5% CP. Chromic oxide and titanium oxide were used as digestibility markers. Pigs were fed three times daily at 08:00, 16:00, and 24:00 h. Experimental periods lasted 8 days and digesta was collected for two 24 h periods, starting at 08:00 on days 6 and 8, respectively. On the morning of day 8 pigs were fed a diet in which half of the peas were replaced with guanidinated peas for the determination of true ileal amino acid digestibilities with the homoarginine technique. Digesta samples were pooled by pig and by 24 h period to give four observations per diet. Apparent, standardized, and true ileal crude protein digestibilities determined with the homoarginine technique and reactive lysine methods

were similar ($P > 0.05$) among diets. Endogenous lysine losses were also similar ($P > 0.05$) among diets. Apparent, standardized, and true ileal lysine digestibilities determined with the homoarginine technique and reactive lysine methods differed ($P < 0.05$) among diets. The true ileal digestibilities were estimated for other amino acids. The overall average of apparent ileal AA digestibilities (74.3%) were less than the overall average standardized ileal digestibility of the AA (80.8%), which were lower than the overall average true ileal digestibilities of the AA using the homoarginine technique (97.0%) and the reactive lysine method (99.8%). Apparent ileal digestibilities were more variable among diets compared with standardized and true ileal digestibilities. The present results show that correcting apparent ileal AA digestibilities for minimum endogenous AA losses diminishes the differences in ileal AA digestibilities among pea cultivars.

4.2 INTRODUCTION

Pea (*Pisum sativum*) production in Canada has increased considerably within the last few years. In 1998, it was estimated that over 2 million tonnes of peas were produced in Canada (Skrypetz and Oleson, 1998). Peas are potentially an excellent source of energy and protein for swine (Jansman and Verstegen, 2002) and for this reason, there has been an increased interest to utilize peas in swine diets in Western Canada. The role of peas in pork production is expected to increase as the swine industry in Manitoba expands. However, peas have not to date been accepted by the commercial feed industry as the sole source of supplemental protein, despite the available information indicating that peas could completely replace soybean meal in grower-finisher pigs (e.g.

Kehoe et al. 1991). This is mainly due to the variability in energy and crude protein content seen among different cultivars and within a cultivar grown in differing conditions.

With respect to dietary amino acid supply in pig diets, ileal digestibility coefficients should be used (Sauer and Ozimek, 1986). Furthermore it has been suggested that true as opposed to apparent ileal digestibilities should be used in diet formulation as the former are more additive in a mixture of feed ingredients (Imbeah et al. 1988; Nyachoti et al. 1997b, 2002; NRC, 1998). However, true ileal amino acid digestibilities in pea cultivars grown in Canada have not been well characterized.

Ideally, the content of amino acids that is actually available for use by the pig should be determined and used to formulate swine rations. Traditional means for determining the bioavailability of amino acids in pig feed ingredients are expensive and time consuming (van Barneveld, 2001). Recently, it has been suggested that availability of amino acids can be estimated from the measurement of the so called 'reactive lysine' content of feedstuffs (Rutherford et al. 1997a,b). Only limited studies have used this approach to quantify lysine availability in pea feed ingredients.

Determining true ileal digestible amino acid content or reactive lysine content in pig feed ingredients involve additional processes than what is needed for determining apparent ileal digestible amino acid content. Consequently, it has been suggested that standardized ileal digestible amino acid, which are generated by correcting apparent ileal amino acid digestibilities for basal endogenous amino acid losses, should be used in commercial feed formulation (Rademacher, 2001).

The objectives of the study reported herein were to determine the true ileal amino acid digestibilities and the available lysine content in peas fed to growing pigs using the homoarginine technique and the reactive lysine method, respectively. Apparent and standardized ileal amino acid digestibilities were also determined.

4.3 MATERIALS AND METHODS

4.3.1 Animals and housing

Four castrated Costwold (Platinum) pigs with an average initial body weight of 24.4 ± 1.8 kg were obtained from Glenlea Research Farm and housed in individual metabolism crates (118 cm \times 146 cm) with smooth transparent plastic sides and tender foot floors in the Animal Science Research Unit. After a 7 day adjustment period, pigs were surgically fitted with simple T-cannulas at the distal ileum according to procedures adapted from Sauer et al. (1983). After surgery, pigs were immediately returned to their metabolism crates and allowed 17 ± 1 days to recover and regain their pre-surgery appetite. During this period pigs were fed thrice daily increasing amounts of a commercial grower diet and had unlimited access to water. One day prior to and three days after surgery each pig received excenel (Upjohn company, Orangeville, Ontario, Canada) intramuscularly at a dose of 1 ml per 17 kg liveweight. Pigs were washed twice daily with Stanhexidine (Novopharm, Toronto, Canada), dried with paper towels and the skin smeared with zincoderm (Rhone Merieux Canada Inc.) to minimized skin irritation due to the emission of digesta around the cannula.

The University of Manitoba Animal Care Committee approved the use of pigs and experimental procedure in the current study and pigs were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

4.3.2 Experimental diets

Three pea cultivars namely, Swing, Croma and Profi were obtained from a local farm while AC Melfort, CDC Mozart and Eclipse were obtained from Agriculture and Agri-Food Canada, Research Station, Morden. The three cultivars from Agriculture Canada were combined prior to feed mixing so as to obtain a pea protein with 23.7% CP. Four cornstarch-based experimental diets containing peas as the only source of protein were used (Table 10). Three of the diets contained individual cultivars (Profi, Swing, Croma) and a fourth diet contained a mixture of peas (21% AC Melfort, 35% CDC Mozart, 44% Eclipse). However, all diets were formulated to contain 14.5% crude protein and to meet or exceed the minimum mineral and vitamin requirements for the growing pig (NRC, 1998). Peas were ground through a 5 mm screen prior to diet formulation. Chromic oxide (0.4%) was included as an indigestible marker for determining apparent ileal amino acid digestibilities.

For the estimation of true ileal amino acid digestibilities and endogenous amino acid flows, samples of the four pea-protein sources were guanidinated before diet preparation. The guanidination was achieved using the procedure described by Imbeah et al. (1996) with the modifications established in a preliminary study (Manuscript I). The homoarginine diets were prepared by replacing 50 % of the protein source in each diet (Table 10) with their respective guanidinated samples. Titanium oxide was used in place

Table 10. Composition (%) of experimental diets.

Item	Diet/Pea Cultivar			
	Profi	Swing	Croma	Mixture*
Ingredients				
Peas	79.3	73.3	68.1	61.3
Cornstarch	2.3	8.3	13.5	20.3
Vegetable Oil	3.0	3.0	3.0	3.0
Vit-Min Premix ¹	5.0	5.0	5.0	5.0
Sucrose	10.0	10.0	10.0	10.0
Chromic Oxide	0.4	0.4	0.4	0.4
Calculated Nutrient Composition				
Crude Protein (%)	14.52	14.52	14.51	14.53
Crude Fat (%)	4.0	4.0	3.8	3.7
Lysine (%)	1.2	1.2	1.0	0.9
Ca:P	1.4	1.4	1.4	1.5
Digestible Energy (Kcal/kg)	3458	3458	3522	3560

*Mixture of peas contains AC Melfort, CDC Mozart, and Eclipse cultivars.

¹Premix provided per kg of diet: 9000 mg Calcium, 4250 mg Phosphorus, 3000 mg Salt, 1200 mg Sodium, 125 mg Magnesium, 35 mg Manganese, 152.5 mg Iron, 137.5 mg Zinc, 125 mg Copper, 0.75 mg Iodine, 11750 iu Vitamin A, 1500 iu Vitamin D3, 50 iu Vitamin E, 1.75 mg Vitamin K, 750 mg Choline Chloride, 38 mg Niacin, 35.75 mg Calcium Pantothenate, 10 mg Riboflavin, 1 mg Thiamine, 1 mg Pyridoxine, 27.5 mg Vitamin B12, 100 mcg Biotin, 0.5 mg Folic Acid, and 0.3 mg Selenium.

of chromic oxide (0.4 %) as an indigestible marker, so as to have a marker unique for the homoarginine diets. The ileal digestibility of reactive lysine was determined by combining the guanidination technique with the traditional true ileal amino acid digestibility assay. Reactive lysine content is considered to be equivalent to the amount of homoarginine present after guanidination. Both diet and digesta samples were guanidinated and from the reactive lysine measurements the true ileal reactive lysine digestibility coefficient and the available lysine were calculated according to Moughan and Rutherford (1996).

4.3.3 Experimental design and general conduct of study

The experiment was designed and carried out according to a 4×4 latin square design, with each experimental period lasting 8 days. Diets were fed in mash form and pigs received their daily feed allowance in three equal portions at 08:00, 16:00, and 24:00 h. Feed refusal and spillage were recorded and used to determine actual dry matter intake. Daily feed allowance was fixed at $2.6 \times$ maintenance energy requirements based on body weight at the beginning of each experimental period. Pigs had unlimited access to water at all times throughout the experiment.

During any one period, pigs were fed their respective diets from day 1 to 5 followed by a 24-h ileal digesta collection period starting at 08:00 h on day 6 for the determination of apparent digestibilities in the regular, non-guanidinated diets. On day 8, a meal of the diets with guanidinated protein was fed at 08:00 h followed by a second 24-h ileal digesta collection period starting at 08:00 h. During the 24-h collection periods, sample bags were attached to the cannula with a hose clamp and bags were changed every 2 h. Each bag had

10 mL 10% formic acid solution to minimize microbial activity. Upon removal of bags digesta samples were immediately frozen at -18°C until they could be freeze dried at a later date.

4.3.4 Sample analysis

Digesta samples were freeze dried and then pooled for each pig within a collection period. Pooled samples were then ground in a Wiley mill through a 1 mm screen and then thoroughly mixed. Along with diet samples, the digesta were analyzed for dry matter, crude protein, chromic oxide, titanium oxide, and amino acids. All analyses were done in duplicate.

Dry matter was determined by weighing out 5 g of sample in a pre-weighed silica dish and dried to a constant weight at 102°C for 18 h (AOAC, 1990). The samples were then removed, cooled in a dessicator and re-weighed. Crude protein ($\text{N} \times 6.25$) content of the diets and ileal digesta samples was determined using Leco NS 2000 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI., USA).

Chromium concentration in digesta and diets was determined according to the procedure described by Williams et al. (1962). Briefly, a 0.4 g or 1 g sample of digesta or feed, respectively, was weighed into a crucible and ashed at 600°C for 12 h. After cooling, 3 mL of 7.6% w/v manganese sulfate monohydrate and 4 mL of 4.5% w/v potassium bromate solution were gently added, and the crucible was covered with a watch glass, placed on a hot plate and digested until no effervescence was observed and the solution changed to a purple colour. After cooling, the solution was carefully transferred into a 200 mL volumetric flask containing 100 mg calcium as CaCl_2 . The flask was made up to

marked volume using deionized water. Chromium was then determined using atomic absorption spectrophotometry (Perkin-Elmer, model 603 A) at 357.9 nm against 5 standards (0, 2, 4, 6, 8, 10 μg chromium/mL) that were used to prepare the standard curve.

Titanium concentration in digesta and diets were determined according to AOAC (1990). Briefly, a 1 g sample of digesta or feed was weighed into a silica dish and ashed at 450°C for 12 h. After cooling, the sample was carefully transferred to a 150 mL conical flask and 3.0 g of anhydrous sodium sulfate, 20 mL of concentrated sulphuric acid, and 5 to 6 anti bumping granules were added. A glass funnel was placed in the conical flask before gently boiling the mixture on a hotplate for 45 to 60 minutes. After cooling, the funnel and contents of the 150 mL flask were rinsed with de-ionized water into a 200 mL conical flask and made up to mark. Colour development was achieved by transferring 5 mL of the above solution and 0.2 mL of 30% hydrogen peroxide into a 10 mL test tube and mixing well. Titanium oxide was then determined using a Pharmacia Ultrospec 2000 spectrophotometer at 408 nm against 5 working standards of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg titanium oxide that were prepared as the standard curve.

Amino acid determination was carried out by weighing a 100 mg sample and preparing the sample 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. A stopper was placed on the tube and then evacuated for at least 30 seconds before sealing. This was then placed on a pre-heated block and heated for 24 h at 110°C. The sample was removed the next day and quickly cooled in ice, before adding 4 mL of 25% w/v sodium hydroxide and thoroughly mixing. The sample was quantitatively washed into a 50 mL volumetric flask

and made up to mark using a sodium citrate sample dilutor (pH 2.2). Then 10 mL of the mixture was filtered and frozen till ready for analysis. 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 corresponding internal standard. Peak areas were recorded and calculated by Hewlett-Packard Integrator. Methionine and cystine were not determined, as they require performic acid oxidation prior to hydrolysis. Tryptophan was not measured because it is destroyed by acid hydrolysis.

4.3.5 Calculations and statistical analysis

Apparent and true ileal amino acid and protein digestibilities were calculated as previously described (Nyachoti et al. 1997b). The endogenous flow of lysine was determined directly according to Nyachoti et al. (1997b). The endogenous flow of amino acids other than lysine were calculated from the observed flows of endogenous lysine and the amounts of other amino acids relative to lysine as reported by de Lange et al. (1989b), except for tyrosine and leucine, for which ratios reported by Boisen and Moughan (1996) and Rademacher et al. (2000) were used, respectively. Endogenous flows with the reactive lysine method were calculated as described by Rutherfurd and Moughan (1997). Standardized ileal digestibilities (SID) were derived by correcting apparent ileal amino acid

digestibilities (AID) for basal endogenous amino acid losses (ENL) according to the following equation (Rademacher et al. 2000):

$$\text{SID} = \text{AID} + (\text{ENL}/\text{AA}_f) \times 100\% \quad [1]$$

Where SID represents the standardized ileal digestibility coefficient (%) of an amino acid, AID is the apparent ileal digestibility coefficient (%) previously calculated, ENL is the nonspecific endogenous loss of that amino acid measured at the distal ileum (mg/kg DMI) using the same averages of published values that were used for the true ileal amino acid digestibilities previously mentioned, and AA_f is the dietary content of the amino acid (mg/kg diet DM). The same equation was used to calculate the SID for protein.

Reactive lysine was derived by combining the guanidination technique with the traditional ileal amino acid digestibility assay (Moughan and Rutherfurd, 1996). The ileal digestibility assay was conducted to find the apparent ileal digestibility, and the guanidination technique was used to measure the reactive lysine content of both the diet and the digesta. Diet and digesta were both guanidinated and analyzed for homoarginine. The amount of homoarginine determined was considered equivalent to the amount of reactive lysine. From the reactive lysine measurements of the diet and the digesta the true ileal reactive lysine digestibility coefficients were calculated according to the following equation (Moughan and Rutherfurd, 1996), and using the same averages of published values for endogenous loss that were used for the true ileal amino acid digestibilities previously mentioned:

$$\begin{aligned} \text{True reactive lysine digestibility} = & [\text{dietary reactive lysine} - \\ & (\text{ileal reactive lysine flow} - \text{endogenous lysine} \\ & \text{flow})] / \text{dietary reactive lysine intake} \times 100 \end{aligned} \quad [2]$$

Analysis of variance was carried out using the general linear model (SAS Institute Inc., 1988). Duncan's multiple range test was used to compare and separate treatments when a significant F-test was obtained in the model. The α -level for significance was $p \leq 0.05$. The model used was $y_{ijk} = \mu + d_i + p_j + a_k + e_{ij(k)}$. Where y_{ijk} = the digestibility of the k^{th} pig fed diet i in the j^{th} period; μ = the population mean; d_i = the effect of the i^{th} diet; p_j = the effect of the j^{th} period; a_k = the effect pig k ; and $e_{ij(k)}$ is the residual error.

4.4 RESULTS AND DISCUSSION

The pigs remained healthy throughout the experiment, readily consumed their daily feed allowance, and grew normally. The dry matter and crude protein (Table 11) of the diets did not differ, although the crude protein was slightly higher than expected from the formulation. This, however, should not have an impact on the results, as the level of protein was not significantly different between the four diets. The amino acid composition of Profi, Swing, and Croma diets were similar, while the Mixture diet had a different amino acid profile. This may be because the first three were grown in the same year, with the same environmental conditions, while the Mixture pea diet contained peas grown in a different year and at a different location. Other studies have found that the wide variation in composition of peas can be attributed to both genetic and environmental factors (Pandy and Gritton, 1975; Savage and Deo, 1989).

Apparent ileal crude protein digestibilities were not different among diets ($P > 0.10$) and averaged 70.2% (Table 12). Of all the indispensable amino acids, significant differences in apparent ileal digestibilities were observed for histidine with Profi having the lowest ($P < 0.05$) apparent ileal digestibility compared with Swing and Croma, whose

Table 11. Dry matter, crude protein, and amino acid content (%) of pea-based diets (DM basis).

Item	Diet/Pea Cultivar			
	Profi	Swing	Croma	Mixture*
Dry matter	92.2	92.1	91.7	92.2
Crude protein	14.8	13.8	14.5	14.1
Indispensable amino acids				
Arginine	1.5	1.3	1.5	0.5
Histidine	0.5	0.5	0.5	0.5
Isoleucine	0.7	0.7	0.7	0.3
Leucine	1.3	1.3	1.3	1.0
Lysine	1.4	1.3	1.4	1.0
Phenylalanine	0.8	0.8	0.8	0.6
Threonine	0.8	0.8	0.7	0.5
Valine	0.8	0.7	0.8	0.4
Dispensable amino acids				
Alanine	0.8	0.7	0.7	0.6
Aspartic acid	2.2	2.0	2.2	2.0
Glutamic acid	3.2	3.1	3.0	2.8
Glycine	0.8	0.7	0.7	0.6
Proline	1.0	0.8	0.7	0.8
Serine	1.0	1.0	1.0	0.9
Tyrosine	0.5	0.5	0.5	0.5

*Mixture of peas contains AC Melfort, CDC Mozart and Eclipse cultivars.

Table 12. Apparent ileal protein and amino acid digestibilities (%) in pea-based diets fed to growing pigs (DM basis).

Item	Diet/Pea Cultivar				Average	SEM**
	Profi	Swing	Croma	Mixture*		
Crude protein	70.2	70.2	70.7	69.8	70.2	0.8
Indispensable amino acids						
Arginine	89.0 ^a	90.7 ^a	88.8 ^a	71.2 ^b	84.9	2.3
Histidine	61.7 ^b	80.1 ^a	76.8 ^a	79.2 ^a	74.4	2.3
Isoleucine	76.3 ^a	78.3 ^a	74.5 ^a	53.0 ^b	70.6	3.0
Lysine	82.6 ^{ab}	84.0 ^a	81.3 ^{ab}	77.9 ^b	81.5	0.9
Leucine	79.1 ^a	79.1 ^a	76.4 ^{ab}	72.3 ^b	76.7	1.2
Phenylalanine	78.8	79.9	75.8	72.5	76.7	1.2
Threonine	70.0 ^a	71.6 ^a	66.1 ^{ab}	57.3 ^b	66.3	1.9
Valine	73.0 ^a	73.8 ^a	70.6 ^a	52.5 ^b	67.5	2.6
Dispensable amino acids						
Alanine	72.6	73.1	69.0	64.3	69.8	1.7
Aspartic acid	78.6	78.7	77.0	77.8	78.0	0.8
Glutamic acid	82.0	84.6	81.0	80.9	82.1	0.9
Glycine	68.4	69.2	64.7	62.3	66.1	1.5
Proline	74.6 ^a	74.6 ^a	67.0 ^b	67.6 ^b	71.0	1.3
Serine	67.6	74.3	72.3	71.4	71.4	2.0
Tyrosine	76.7	79.0	73.8	78.8	77.1	1.1

*Mixture of peas contains AC Melfort, CDC Mozart and Eclipse cultivars.

**SEM, pooled standard error of the mean.

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

apparent ileal digestibility were in turn similar ($P > 0.10$). In general, apparent ileal digestibilities of essential amino acids were lower in the mixture diet compared with the other three diets and these differences were significant for arginine, isoleucine, and valine ($P < 0.05$). The average apparent ileal digestibilities of indispensable amino acids ranged from 66.3% for threonine to 84.9% for arginine (Table 12). Among the dispensable amino acids, significant differences were only observed for proline with Profi and Swing diets having higher ($P < 0.05$) values than Croma and Mixture diets (74.6 vs. 67.0%). On average, apparent ileal digestibilities of dispensable amino acids ranged from 66.1% for glycine to 82.1% for glutamic acid (Table 12). The present results for apparent ileal protein and amino acid digestibilities are within the range of values previously reported for peas, (Leterme et al. 1990; Fan et al. 1994; Fan and Sauer, 1999).

Standardized ileal crude protein digestibilities were not different among diets ($P > 0.10$) and averaged 78.8% (Table 13). Of all the indispensable amino acids, significant differences in standardized ileal amino acid digestibilities, like the apparent ileal amino acid digestibilities, were observed for histidine with Profi having the lowest ($P < 0.05$) standardized ileal digestibility of histidine than Swing and Croma whose standardized ileal digestibility for histidine were in turn similar ($P > 0.10$). In general, standardized ileal digestibilities of essential amino acids were lower in the Mixture diet compared to the other three diets and these differences were significant for arginine, isoleucine, and valine ($P < 0.05$). Standardized ileal digestibilities of indispensable amino acids ranged from 77.6% for valine to 89.1% for arginine (Table 13). There were no significant differences for any of the dispensable amino acids among the diets ($P > 0.10$). On

Table 13. Standardized ileal protein and amino acid digestibilities (%) in pea-based diets fed to growing pigs (DM basis).

Item	Diet/Pea Cultivar				Average	SEM**
	Profi	Swing	Croma	Mixture*		
Crude protein	78.5	79.2	78.8	78.6	78.8	0.8
Indispensable amino acids						
Arginine	91.9 ^a	94.0 ^a	91.6 ^a	79.0 ^b	89.1	1.8
Histidine	69.8 ^b	84.5 ^a	81.2 ^a	83.4 ^a	79.7	1.9
Isoleucine	82.6 ^a	84.6 ^a	80.8 ^a	66.4 ^b	78.6	2.3
Lysine	84.8 ^{ab}	86.3 ^a	83.5 ^{ab}	80.8 ^b	83.8	0.9
Leucine	82.7	82.9	80.1	77.1	80.7	1.1
Phenylalanine	82.8	84.2	80.0	78.1	81.3	1.2
Threonine	80.5 ^{ab}	82.2 ^a	77.3 ^{ab}	72.4 ^b	78.1	1.6
Valine	81.3 ^a	82.4 ^a	78.9 ^a	67.8 ^b	77.6	2.0
Dispensable amino acids						
Alanine	79.6	80.6	76.3	73.1	77.4	1.6
Aspartic acid	83.3	83.8	81.7	82.8	82.9	0.8
Glutamic acid	85.7	88.4	84.8	85.2	86.0	0.9
Glycine	78.6	80.0	75.2	74.1	77.0	1.5
Proline	80.9	81.9	75.4	75.6	78.4	1.2
Serine	80.8	81.1	79.3	79.1	80.1	1.1
Tyrosine	80.5	83.2	77.8	82.6	81.0	1.1

*Mixture of peas contains AC Melfort, CDC Mozart and Eclipse cultivars.

**SEM, pooled standard error of the mean.

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

average, standardized ileal digestibilities of dispensable amino acids ranged from 77.0% for glycine to 86.0% for glutamic acid (Table 13).

The standardized ileal protein and amino acid digestibilities found in the current study (78.5 to 79.2% and 67.8 to 94.0%, respectively) agree closely with the results of Leterme et al. (1990) (78.0 to 80.0% and 70.5 to 87.9%, respectively) obtained with the nitrogen free method. Carlson and Bayley (1970) also found similar results for soybean meal using the protein free method, ranging from 80.4% to 91.8% for the amino acid digestibilities. Fan et al. (1995) and Fan and Sauer (1997) completed two studies using regression analysis for soybean diets fed to growing pigs and found the crude protein digestibilities ranged from 87.3 to 93.0% and 87.2 to 92.8%, respectively and the amino acid digestibilities ranged from 81.6 to 96.8% and 82.2% to 96.8%, respectively. Furuya and Kaji (1986) also found similar results to the current study with regression analysis for soybean diets fed to growing pigs with the crude protein digestibility at 82% and the amino acid digestibilities ranging from 55% to 91%. Finding that the standardized ileal digestibilities for protein and amino acids are similar to findings with the protein free method and the regression analysis would be expected as the standardized ileal digestibilities are based on averages of endogenous protein when either feeding a protein free diet or regression analysis. The smaller SEM with the standardized digestibilities compared to the apparent digestibilities implies that endogenous amino acid losses account for part of the differences in apparent ileal amino acid digestibilities. Fan and Sauer (1997) reported that the true ileal digestibility values of amino acids were not as variable as their respective apparent ileal digestibility values in soybean meal. They also found the true ileal amino acid digestibilities to be independent of the level of amino acids in the diet.

Total and endogenous lysine flows, measured using the homoarginine technique and the reactive lysine method, were not different among diets ($P > 0.10$) and are presented in Table 14. Endogenous lysine flows measured using the reactive lysine method are slightly greater than those measured using the homoarginine technique. The endogenous lysine flows at the distal ileum of pigs fed pea-based diets in the current study were comparable to values found by Nyachoti et al. (1997b, 2002) in pigs fed barley-canola meal diets. The total and endogenous flows were slightly greater when the reactive lysine method was used compared with the homoarginine technique thus, explaining the differences between the digestibilities found with each method.

Endogenous lysine flows were the basis for the calculation of true ileal amino acid digestibilities with both the homoarginine technique and the reactive lysine method. Flows of amino acids other than lysine were determined based on their ratios to lysine in endogenous protein as reported in previous studies (de Lange et al. 1989b; Boisen and Moughan, 1996; Rademacher et al. 2000). Therefore, there are still inaccuracies when calculating true ileal amino acid digestibilities using homoarginine and reactive lysine methods, as there are variations in reported ratios of other amino acids relative to lysine in endogenous gut protein. The amino acid ratios of endogenous protein that are available are largely derived from studies feeding protein free diets and, therefore, are not specific for pea diets (de Lange et al. 1989b; Boisen and Moughan, 1996; Rademacher et al. 2000). It has already been shown in a number of different studies that the endogenous losses of amino acids from the small intestine of monogastrics are higher following dietary intake of protein or peptides (Darragh et al. 1990; de Lange et al. 1990; Moughan and Rutherford, 1990; Butts et al. 1991, 1993) than when protein-free diets

Table 14. Total and endogenous lysine flows, measured using the homoarginine technique and the reactive lysine method, at the distal ileum of pigs fed pea-based diets.

Item	Diet				SEM**
	Profi	Swing	Croma	Mixture*	
Total flow					
mg/kg DMI	2379.3	2098.7	2588.8	2303.2	104.5
mg/day	2517.3	2220.4	2738.9	2436.8	110.6
Endogenous flow – homoarginine technique					
mg/kg DMI	1954.9	1570.4	1817.2	1860.7	107.9
mg/day	2068.3	1661.5	1922.6	1968.6	114.1
Endogenous flow – reactive lysine method					
mg/kg DMI	2166.2	1822.6	2088.5	2055.0	106.7
mg/day	2291.9	1928.3	2209.6	2174.2	112.9

*Mixture of peas contains AC Melfort CDC Mozart, and Eclipse cultivars.

**SEM, pooled standard error of the mean.

are fed. Therefore, endogenous losses are affected by dietary intake of protein and peptides and the amino acid composition of endogenous losses differs with different protein feedstuffs.

True ileal crude protein digestibilities determined using the homoarginine technique were not different among diets ($P > 0.10$) and averaged 92.8% (Table 15). Of all the indispensable amino acids, significant differences in true ileal amino acid digestibilities determined with the homoarginine technique were observed for lysine, phenylalanine, and valine. Croma had the lowest ($P < 0.05$) true ileal digestibility of lysine compared to Profi, Swing, and Mixture whose true ileal digestibility coefficients were in turn similar ($P > 0.10$). Croma had a lower ($P < 0.05$) true ileal digestibility of phenylalanine than Profi, and Swing and Croma had lower ($P < 0.05$) values for valine than Profi and Mixture diets (97% vs. 102%). The general trend in the essential amino acid apparent digestibilities of the Mixture diet having a lower digestibility was not seen in the true ileal amino acid digestibilities found with the homoarginine technique. On average, true ileal amino acid digestibilities of indispensable amino acids ranged from 91.7% for histidine to 104.4% for threonine (Table 15). Among the dispensable amino acids, significant differences were only observed for alanine and tyrosine with Croma having a lower value ($P < 0.05$) for alanine compared to the Profi diet and Croma having a lower value ($P < 0.05$) for tyrosine compared to the Mixture diet. On average, true ileal digestibilities determined with the homoarginine technique of dispensable amino acids ranged from 93.7% for aspartic acid to 101.0% for tyrosine (Table 15).

The true ileal crude protein digestibilities found in the current study agree with those found in previous studies using peas, (Huisman et al. 1992; Jansman and Verstegen,

Table 15. True ileal protein and amino acid digestibilities (%) in pea-based diets fed to growing pigs using the homoarginine technique (DM basis).

Item	Diet/Pea Cultivar				Average	SEM**
	Profi	Swing	Croma	Mixture*		
Crude protein	92.3	93.9	91.6	93.2	92.8	0.8
Indispensable amino acids						
Arginine	99.0	99.9	98.0	97.0	98.5	0.9
Histidine	90.1	92.4	91.1	93.0	91.7	0.7
Isoleucine	98.4	96.0	94.9	97.3	96.6	0.9
Lysine	96.9 ^a	96.0 ^a	94.4 ^b	95.8 ^a	95.8	0.4
Leucine	96.9	93.9	93.3	94.8	94.7	0.7
Phenylalanine	98.6 ^a	96.8 ^{ab}	94.9 ^b	98.3 ^{ab}	97.2	0.8
Threonine	106.7	101.3	102.3	107.2	104.4	1.3
Valine	101.9 ^{ab}	97.7 ^b	97.4 ^b	103.2 ^a	100.0	1.1
Dispensable amino acids						
Alanine	97.1 ^a	94.0 ^{ab}	92.7 ^b	93.7 ^{ab}	94.4	0.7
Aspartic acid	94.9	93.0	92.2	94.5	93.7	0.5
Glutamic acid	95.1	95.4	93.6	95.0	94.8	0.6
Glycine	103.9	99.5	98.9	101.6	101.0	1.9
Proline	96.6	95.0	94.5	93.9	95.0	1.3
Serine	97.5	93.4	94.8	97.0	95.7	0.8
Tyrosine	101.5 ^{ab}	101.4 ^{ab}	97.9 ^b	102.2 ^a	100.8	1.0

*Mixture of peas contains AC Melfort, CDC Mozart and Eclipse cultivars.

**SEM, pooled standard error of the mean.

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

2002) and barley-canola (Nyachoti et al. 1997b; 2002). True ileal amino acid digestibilities have not yet been published for peas. However the true ileal amino acid digestibilities found in the current study using the homoarginine technique are similar to the true ileal amino acid digestibilities of a barley-canola-based diet (Nyachoti et al. 1997b; 2002). True ileal crude protein digestibilities determined using the reactive lysine method were not different among diets ($P > 0.10$) and averaged 92.1% (Table 16). Of the indispensable amino acids, significant differences in true ileal amino acid digestibilities determined with the reactive lysine method were observed for lysine, threonine, and valine. Croma had a lower true ileal lysine digestibility ($P < 0.05$) compared to Profi and Swing whose true ileal digestibility were in turn similar ($P > 0.10$). The true ileal digestibility of threonine determined with the reactive lysine method was higher in the Mixture diet compared with the Swing diet. Also, the mixture diet had a higher true ileal digestibility for valine ($P < 0.05$) when determined with the reactive lysine method compared to the Swing and Croma diets whose true ileal digestibilities for valine were in turn similar ($P > 0.10$). Again, like the true ileal digestibilities determined with the homoarginine technique, the general trend of lower essential apparent and standardized amino acid digestibilities in the Mixture diet was not seen in the true ileal digestibilities determined with the reactive lysine method, indicating that the true digestibilities of amino acids are less variable compared to apparent and standardized ileal amino acid digestibilities. On average, true ileal digestibilities of indispensable amino acids ranged from 97.0% for leucine to 109.2% for threonine (Table 16). For the dispensable amino acids there were no differences observed among the diets ($P > 0.10$). On average, true

Table 16. True ileal protein and amino acid digestibilities (%) in pea-based diets fed to growing pigs using the reactive lysine method (DM basis).

Item	Diet/Pea Cultivar				Average	SEM**
	Profi	Swing	Croma	Mixture*		
Crude protein	91.5	93.2	91.4	92.4	92.1	0.9
Indispensable amino acids						
Arginine	100.1	101.4	99.3	99.7	100.1	0.8
Histidine	93.2	94.4	93.2	94.4	93.8	0.7
Isoleucine	100.8	98.8	97.9	101.9	99.8	1.0
Lysine	98.4 ^a	97.9 ^a	96.4 ^b	97.6 ^{ab}	97.6	0.4
Leucine	98.8	96.3	95.8	97.1	97.0	0.7
Phenylalanine	100.8	99.6	97.8	101.0	99.8	0.7
Threonine	110.6 ^{ab}	106.1 ^b	107.7 ^{ab}	112.5 ^a	109.2	1.2
Valine	105.0 ^{ab}	101.6 ^b	101.4 ^b	108.5 ^a	104.1	1.2
Dispensable amino acids						
Alanine	99.7	97.3	96.2	96.8	97.5	0.6
Aspartic acid	96.6	95.3	94.5	96.3	95.7	0.5
Glutamic acid	96.6	97.1	95.4	96.4	96.4	0.6
Glycine	107.7	104.3	104.1	105.7	105.5	1.8
Proline	99.0	98.2	98.6	96.7	98.1	1.1
Serine	101.3	98.2	101.2	101.5	98.6	0.8
Tyrosine	104.2	105.0	101.5	104.7	103.9	1.0

*Mixture of peas contains AC Melfort, CDC Mozart and Eclipse cultivars.

**SEM, pooled standard error of the mean.

^{a,b} Within a row, means without a common superscript letter differ ($P < 0.05$).

ileal digestibilities determined with the reactive lysine method of dispensable amino acids ranged from 95.7% for aspartic acid to 105.5% for glycine (Table 16).

In general, the homoarginine and reactive lysine methods give similar results while the standardized and apparent digestibility methods provide noticeably lower digestibilities (Table 17). Indicating that the two true ileal digestibility methods provide equally valid results.

4.5 CONCLUSION

The apparent ileal amino acid digestibilities of peas are highly variable. Standardized ileal digestibilities are a simple and inexpensive means for estimating true ileal digestibilities in pigs fed practical feed types like peas. The homoarginine technique and the reactive lysine method are more expensive, but give less variable digestibilities than the standardized digestibility coefficients. Whether the digestibilities are standardized calculations, or estimated from the homoarginine or reactive lysine methods, assumptions have to be made about the amino acid composition of the endogenous protein. For practical feed formulation true rather than apparent ileal digestibilities should be used, and until subsequent research can be done to define true ileal digestibilities of all of the common feedstuffs used in swine diets, standardized digestibilities should be used.

Table 17. Comparison overall average digestibilities (%) found using different digestibility methods (DM basis).

Item	Digestibility Method			
	Apparent	Standardized	Homoarginine	Reactive Lysine
Arginine	84.9	89.1	98.5	100.1
Histidine	74.4	79.7	91.7	93.8
Isoleucine	70.6	78.6	96.6	99.8
Lysine	81.5	83.8	95.8	97.6
Leucine	76.7	80.7	94.7	97.0
Phenlalanine	76.7	81.3	97.2	99.8
Threonine	66.3	78.1	104.4	109.2
Valine	67.5	77.6	100.0	104.1
Crude Protein	70.2	78.8	92.8	92.1
Compared to				
Apparent	100%	112%	132%	131%
Compared to				
Standardized	89%	100%	118%	117%
Compared to				
Homoarginine	76%	85%	100%	99%
Compared to				
Reactive Lysine	76%	86%	101%	100%

5.0 MANUSCRIPT III

Effect of increasing dietary pea (*Pisum sativum*) levels on nursery pig performance.

5.1 ABSTRACT

The objective of the present study was to evaluate the effect of feeding increasing levels of peas on nursery pig performance as indicated by feed intake, average daily gain and gain:feed. Ninety-six 17-d old weaned Costwold (Platinum) pigs (6.05 ± 1.08 kg initial body weight) were fed a commercial starter diet (crumbled) for a 4 d adjustment period before being randomly assigned to one of five diets during three consecutive periods (Phase I, 21 to 28 d, Phase II, 29 to 42 d, Phase III, 43 to 56 d). The five different diets consisted of a corn-soybean-meal-based control and four pea-based diets containing 15, 20, 25, or 30% peas. Individual body weights and pen feed disappearance were monitored weekly. Average daily gain, feed intake, and gain:feed were similar ($P > 0.05$) in Phase I, Phase II, Phase III, and overall. Pigs tended to do worse when fed the 25% pea-based diet compared to the other four diets, however, the differences were not significant ($P > 0.05$). The results demonstrate that peas can be fed up to 30% to weaned pigs without significantly affecting performance.

5.2 INTRODUCTION

Peas (*Pisum sativum*) are a high quality feed ingredient that can be used for all classes of swine from young stock to market and breeding animals. Two of the most important nutrients sought after in feed ingredients are energy and protein, for which peas

are an excellent source (Igbasan et al. 1997). Also, compared to other protein sources for swine, peas contain a relatively high content of lysine, which is often the first limiting amino acid in swine diets. The production of peas in Western Canada has increased over recent years, peaking in 2000. In 2003 total world supply is expected to increase 13% to 10.8 Mt due to the higher production in Canada, the European Union, and Australia (Skrypetz and Oleson, 2003). This increase in pea production, however, has not led to the same level of increase in utilization of peas in livestock feed, especially in diets for nursery pigs. The use of peas in practical swine diets does require that inclusion levels are limited, current recommendations for practical inclusion levels of peas for pigs are 5% of the diet for prestarter pigs (5-15 kg) and 15% of the diet for starter pigs (15-25 kg) (Stefanyshyn-Cote and Belanger, 2002). The main reason for this is that peas, like all legumes, may contain anti-nutritive factors (ANFs) like trypsin inhibitors, lectins, tannins, and fibre, which have been shown to reduce pig performance (Savage and Deo, 1989; Le Guen et al. 1995). Castell et al. (1996) reported lower performance when piglets were fed diets containing more than 15% peas, compared with those fed no peas. Also, feed efficiency of young pigs (4.5 to 10 kg BW) was lower when fed a corn based diet containing 19% peas compared to a control (Owusu-Asiedu, 2002). However, in 10 to 20 kg pigs, the overall performance was similar between pigs fed the pea-based diet and those fed the corn-soybean based diet (Owusu-Asiedu et al. 2002).

The objective of the present study was to measure the effect of feeding increasing levels of peas to nursery pigs on feed intake, average daily gain, and gain:feed. Swing peas were used, as they were the most available out of the three cultivars used in a previous study.

5.3 MATERIALS AND METHODS

The experiment was carried out in the Animal Science Research Unit, University of Manitoba, Canada. Ninety-six Costwold (Platinum) piglets with a mean initial body weight of 6.05 ± 1.08 kg (mean \pm standard deviation) and weaned at 17 d of age were used in the study. Four pigs, balanced for body weight and gender, were randomly assigned to each of the 24 pens (1.06×1.72 m). The pigs came in on two separate dates due to availability. On the first day, 68 pigs came in and were randomly assigned to 17 pens with two male and two female pigs per pen (average pen weight $6.32 + 0.04$ kg per pig), and on the second day, 28 pigs came in and were randomly assigned to 7 pens with two male and two female pigs per pen (average pen weight $5.39 + 0.02$ kg per pig). Pigs were fed a commercial corn-soybean-meal-based starter diet (crumbled) for a 4-d adjustment period before assigned to experimental diets.

Five dietary treatments, which included a corn-soybean-meal control diet, and four pea-based diets containing 15, 20, 25, or 30% peas were used. Swing peas (18.85% CP) were obtained from a local farm for use in the experiment. Peas were ground through a 5 mm screen prior to diet formulation. Published values (NRC, 1998) were used for chemical composition of ingredients in diet formulation, with the exception of pea crude protein. Nitrogen was determined by the Kjeldahl procedure (AOAC, 1990) and converted to crude protein content using a conversion factor of 6.25. Diets were formulated to similar nutrient levels and to meet NRC (1998) nutrient requirements for pigs from 21-28 d (Phase I, Table 18), 29-42 d (Phase II, Table 19), and 43-56 d (Phase III, Table 20) and fed as pellets. The four pea diets were fed to five replicate pens while the control was fed to only four due to space limitation. Dietary treatments were

Table 18. Composition (%) of phase I diets.

Ingredients	Diet				
	Control	15% Peas	20% Peas	25% Peas	30% Peas
Corn	21.76	10.83	7.26	3.58	0
Soybean meal, 48%	19	15	13.6	12.3	10.9
Peas	0	15	20	25	30
Fishmeal	5	5	5	5	5
Vegetable Oil	5	5	5	5	5
Spray dried porcine plasma	7	7	7	7	7
Whey	20	20	20	20	20
Oat Groats	20.1	20.1	20.1	20.1	20.1
Vit-Min Premix ¹	2	2	2	2	2
Lysine-HCl	0.14	0.07	0.04	0.02	0
Calculated Chemical Composition					
Crude Protein (%)	24.6	24.6	24.6	24.6	24.6
Crude Fat (%)	8.5	8.1	8.0	7.8	7.7
Lysine (%)	1.8	1.8	1.8	1.8	1.8
Ca (%)	1.05	1.05	1.05	1.05	1.05
P (%)	0.86	0.86	0.86	0.86	0.86
Ca:P	1.2	1.2	1.2	1.2	1.2
Digestible Energy					
(Kcal/kg)	3502	3485	3479	3473	3468

¹Premix provided per kg of diet: 3600 mg Calcium, 1700 mg Phosphorus, 1200 mg Salt, 480 mg Sodium, 50 mg Magnesium, 14 mg Manganese, 61 mg Iron, 55 mg Zinc, 50 mg Copper, 0.3 mg Iodine, 4700 iu Vitamin A, 600 iu Vitamin D3, 20 iu Vitamin E, 0.7 mg Vitamin K, 300 mg Choline Chloride, 15.2 mg Niacin, 14.3 mg Calcium pantothenate, 4 mg Riboflavin, 0.4 mg Thiamine, 0.4 mg Pyridoxine, 11 mg Vitamin B12, 40 mcg Biotin, 0.2 mg Folic Acid, and 0.12 mg Selenium.

Table 19. Composition (%) of phase II diets.

Ingredients	Diet				
	Control	15% Peas	20% Peas	25% Peas	30% Peas
Corn	25.84	14.92	11.35	7.77	4.1
Soybean meal, 48%	24	20	18.6	17.2	15.9
Peas	0	15	20	25	30
Wheat	30	30	30	30	30
Fishmeal	5	5	5	5	5
Vegetable Oil	2	2	2	2	2
Whey	10	10	10	10	10
Vit-Min Premix ¹	3	3	3	3	3
Lysine-HCl	0.16	0.08	0.05	0.03	0
Calculated Chemical Composition					
Crude Protein (%)	21.9	21.9	21.9	21.9	21.9
Crude Fat (%)	4.9	4.5	4.4	4.3	4.2
Lysine (%)	1.4	1.4	1.4	1.4	1.4
Ca (%)	0.98	0.98	0.98	0.98	0.98
P (%)	0.83	0.83	0.83	0.83	0.83
Ca:P	1.2	1.2	1.2	1.2	1.2
Digestible Energy					
(Kcal/kg)	3502	3485	3479	3473	3468

¹Premix provided per kg of diet: 5400 mg Calcium, 2550 mg Phosphorus, 1800 mg Salt, 720 mg Sodium, 75 mg Magnesium, 21 mg Manganese, 91.5 mg Iron, 82.5 mg Zinc, 75 mg Copper, 0.45 mg Iodine, 7050 iu Vitamin A, 900 iu Vitamin D3, 30 iu Vitamin E, 1.05 mg Vitamin K, 450 mg Choline Chloride, 22.8 mg Niacin, 21.45 mg Calcium pantothenate, 6 mg Riboflavin, 0.6 mg Thiamine, 0.6 mg Pyridoxine, 16.5 mg Vitamin B12, 60 mcg Biotin, 0.3 mg Folic Acid, and 0.18 mg Selenium.

Table 20. Composition (%) of phase III diets.

Ingredients	Diet				
	Control	15% Peas	20% Peas	25% Peas	30% Peas
Corn	34.85	23.92	20.35	16.67	13.1
Soybean meal, 48%	28	24	22.6	21.3	19.9
Peas	0	15	20	25	30
Wheat	30	30	30	30	30
Vegetable Oil	2	2	2	2	2
Vit-Min Premix ¹	5	5	5	5	5
Lysine-HCl	0.15	0.08	0.05	0.03	0
Calculated Chemical Composition					
Crude Protein (%)	20.2	20.3	20.2	20.3	20.2
Crude Fat (%)	4.8	4.4	4.3	4.2	4.1
Lysine (%)	1.2	1.2	1.2	1.2	1.2
Ca (%)	1.02	1.02	1.02	1.02	1.02
P (%)	0.83	0.83	0.83	0.83	0.83
Ca:P	1.2	1.2	1.2	1.2	1.2
Digestible Energy (Kcal/kg)	3445	3428	3422	3416	3410

¹Premix provided per kg of diet: 9000 mg Calcium, 4250 mg Phosphorus, 3000 mg Salt, 1200 mg Sodium, 125 mg Magnesium, 35 mg Manganese, 152.5 mg Iron, 137.5 mg Zinc, 125 mg Copper, 0.75 mg Iodine, 11750 iu Vitamin A, 1500 iu Vitamin D3, 50 iu Vitamin E, 1.75 mg Vitamin K, 750 mg Choline Chloride, 38 mg Niacin, 35.75 mg Calcium pantothenate, 10 mg Riboflavin, 1 mg Thiamine, 1 mg Pyridoxine, 27.5 mg Vitamin B12, 100 mcg Biotin, 0.5 mg Folic Acid, and 0.3 mg Selenium.

randomly assigned to pens. Feed and water were available at all times from self-feeders and low pressure water nipples, respectively. Weekly body weight per pig and feed intake (disappearance) per pen were recorded. The measurements of average daily gain and average daily feed intake were used to calculate feed efficiency (gain:feed). Room temperature was maintained at 30 ± 1 °C. The University of Manitoba Animal Care Committee approved the use of pigs and experimental procedure in the current study and pigs were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Data were analyzed with the general linear model of SAS (SAS Institute Inc., 1988). The α -level for significance was $p \leq 0.05$. The model used was $y_{ijk} = \mu + d_i + p_j + a_k + e_{ij(k)}$. Where y_{ik} = the performance (ADG, ADFI, or gain:feed) of the k^{th} pig shipped on day i in the j^{th} pen ; μ = the population mean; d_i = the effect of the i^{th} shipping day; p_j = the effect of the j^{th} pen; a_k = the effect of the k^{th} pig; and $e_{ij(k)}$ is the residual error. Preliminary assessment suggested shipping day was important for some data and was therefore incorporated into the model. Interactions with shipping day could not be assessed because of insufficient data.

5.4 RESULTS AND DISCUSSION

Pigs remained healthy throughout the experiment and grew normally. Average daily gain, feed intake, and gain:feed for phases I, II, III and overall (days 21 to 56) are shown in Table 21. Feed intake, average daily gain, and feed efficiency (gain:feed) were similar ($P > 0.05$) among dietary treatments in the three phases and overall. However, piglet

Table 21. Performance of nursery pigs fed diets with increasing levels of peas.*

Item	Diet				SEM ¹	P ²	
	Control	15% Peas	20% Peas	25% Peas			30% Peas
Phase I – days 21-28							
ADG ³ , kg/d	0.15	0.12	0.14	0.11	0.12	0.013	0.114
ADFI ⁴ , kg/d	0.22	0.19	0.20	0.17	0.17	0.012	0.069
Gain:feed	0.70	0.64	0.73	0.61	0.71	0.058	0.564
Phase II – days 29-42							
ADG ³ , kg/d	0.33	0.29	0.29	0.27	0.33	0.020	0.157
ADFI ⁴ , kg/d	0.46	0.38	0.41	0.38	0.43	0.022	0.123
Gain:feed	0.72	0.75	0.70	0.69	0.77	0.027	0.217
Phase III – days 43-56							
ADG ³ , kg/d	0.54	0.52	0.48	0.46	0.50	0.027	0.234
ADFI ⁴ , kg/d	0.81	0.76	0.73	0.66	0.74	0.037	0.102
Gain:feed	0.67	0.68	0.65	0.69	0.68	0.033	0.902
Overall – days 21-56							
ADG ³ , kg/d	0.38	0.36	0.33	0.31	0.35	0.017	0.104
ADFI ⁴ , kg/d	0.55	0.50	0.50	0.45	0.50	0.021	0.054
Gain:feed	0.69	0.70	0.68	0.69	0.72	0.025	0.719

*LS means.

¹ SEM, pooled standard error of the means; because of slight numeric unbalance, SEM of treatment errors differed, average SEM shown here.

² P, level of significance.

³ ADG, average daily gain.

⁴ ADFI, average daily feed intake.

performance was numerically poorer for the 25% pea-based diet compared with the other four diets, but this difference was not significant ($P > 0.05$). This numerical difference in performance may have been due to differences in mean pen weights across treatments resulting from the fact that pigs were delivered in two batches. Indeed, two of the five pens assigned to the 25% pea-based diet were from the second, lighter batch of pigs and three of the four pens assigned to the control diet were from the first, heavier batch of pigs. This also may explain why for the 30% pea-based diet fed pigs performed numerically better than the 25% pea-based diet fed pigs as four of the five pens assigned to the 30% pea-based diet were from the first, heavier batch of pigs. The present results for Phase III (11 to 18 kg pigs) agree with those found by Owusu-Asiedu et al. (2002) in pigs (10 to 20 kg) fed a diet containing 19% raw peas. Similarly, Van Nevel et al. (1999) found no effect of feeding diets containing up to 40% peas from 0 to 49 days postweaning. Zivkovic et al. (1987) found that pigs with an average initial body weight of 12 kg, fed a diet containing 30% peas had a lower average daily gain ($P < 0.05$) compared to pigs fed a diet without peas, this difference however was not seen when methionine was supplemented to the 30% pea diet. Gâtel et al. (1989) reported that pigs with an average initial body weight of 11 kg fed a diet containing 24% peas had a greater feed:gain ratio ($P < 0.05$) compared to pigs fed a diet without peas, this difference however was not seen when tryptophan was supplemented to the 24% pea diet.

It is generally recommended that dietary pea levels for young pigs be restricted to 5-15% (Castell et al. 1996; Stefanyshyn-Cote and Belanger, 2002). Indeed performance of phase I and II piglets has been shown to decline if dietary pea levels exceeding 15% are used (eg. Bengala-Freire et al. 1989). However, the results of the present study suggest that

phase I and II nursery pigs can effectively utilize diets containing up to 30% peas. The response to pea containing diets seems to be dependant on diet formulation and in particular the supply of the amino acid methionine, which is often limiting in peas and other pulses.

In the present study diets were balanced for methionine and other amino acids to meet or exceed NRC (1998) recommendations for young pigs, which may explain the lack of effect of pea diets on piglet performance. This observation has a significant implication to the swine and pulse industry as it suggests that higher levels of peas can be used in weanling pig diets with proper formulation.

Growing pigs (prior to ~ 60 kg) have been fed up to 50% pea diets without adverse effects when the diet is supplemented with methionine and threonine. In the finishing period diets contain relatively less protein, therefore making it less likely to include high levels of peas in the diet. However, large amounts of pea consumption may influence the carcass characteristics. For example, feeding finishing pigs diets containing 36% peas has been shown to increase significantly the amount of saturated fat in the carcass (Matre et al. 1990).

Czarnecki et al. (1988) fed boars a diet containing 20% peas and did not observe any adverse affects on semen quality. The results reported for females in the breeding herd are conflicting. Ogle and Hakansson (1988) found that less than 20% peas in gestation and lactation diets may have a negative effect on birth and weaning weight. On the other hand, Gâtel et al. (1988) fed diets containing 16 and 24% peas to gestating and lactating sows without any significant effects on reproductive performance.

5.5 CONCLUSION

In conclusion, peas can be fed to nursery pigs at levels of up to 30% of the diet without affecting growth performance, when diets are balanced for amino acids.

6.0 MANUSCRIPT IV

Economic value of peas (*Pisum sativum*): shadow prices, inclusion levels, and savings from the use of peas in commercial swine diets.

6.1 ABSTRACT

The economic worth of peas was evaluated in the current study by determining the shadow price, inclusion levels, and savings from the use of peas in commercial swine diets. Prices for corn, wheat, barley, peas, soybean meal 47%, canola meal 35%, and animal fat in February and August, from 2000-2003 were used for analysis in a linear feed formulation program. The starter, grower, and finisher diet shadow prices for peas ranged from \$146.40 to \$220.30, \$150.50 to \$227.40, and \$150.50 to \$212.90/metric tonne, respectively. The actual price of peas ranged from \$128.67 to \$201.67/metric tonne, which is generally lower than the shadow prices indicating that the inclusion of peas in most diets will reduce the total cost of the complete feed. The starter, grower, and finisher feed costs were all decreased with the increase in pea inclusion level, except for February 4, 2002, where the feed cost increased as the level of pea inclusion increased. The optimum inclusion levels (i.e. most economical, lowest complete feed cost) of peas in the starter, grower, and finisher diets included peas at levels from 36 to 44%, 56 to 69%, and 32 to 35% peas, respectively. In conclusion, peas generally have a good economic value and where appropriate they can be used to reduce the cost of complete swine feeds.

6.2 INTRODUCTION

Peas have a nutrient profile that provides them with the potential to become highly valued as a feed ingredient for swine. The production of peas in Western Canada has increased over recent years, sparking great interest within the feed industry and among swine producers to find economical ways to use peas in pork production. With the increased production of peas, there is a growing demand for alternative feedstuffs mainly due to the expanding hog industry in Canada, the high cost related to imports, and the need for nutrient management. The unprecedented supply of peas into the animal feed market has provided swine producers with a great way to reduce feed costs. Peas are locally grown, providing a more economical and dependable source of protein. When comparing feeding peas instead of soybean meal in a simple 0.7% lysine diet for finisher pigs brings a savings of \$2.50/pig (Gowans et al. 1998).

Peas are a high quality feed ingredient that can be used for all classes of swine from young stock to market. However, the inclusion level of peas in practical swine diets are limited: prestarter (5-15 kg), 5% of diet; starter (15-25 kg), 15% of diet; grower (25-50 kg), 40% of diet; and finisher (50-110 kg), 40% of diet (Stefanyshyn-Cote and Belanger, 2002). Reasons for this are that peas are highly variable in energy and crude protein and true ileal amino acid digestibilities are not readily available for diet formulation. Furthermore peas, like all legumes, may contain anti-nutritive factors (ANFs) like trypsin inhibitors, lectins, tannins, and fibre.

In a previous experiment (Manuscript III) it was determined that nursery pigs (6-20 kg) could be fed peas at levels of up to 30% of the complete diet. However, the optimum inclusion rate for peas based on efficiency and cost may not coincide with the maximum

rate that can be used by the pigs. The objective of the current study is to determine the economic value of including peas in starter, grower, and finisher pig diet up to 30% of a complete diet.

6.3 MATERIALS AND METHODS

Prices of common feed ingredients fed to swine (i.e. corn, wheat, barley, peas, soybean meal 47%, canola meal 35%, and animal fat) were determined for the years 2000 to 2003 (Table 22). The other ingredients used included ground limestone, calcium phosphorus, salt, DL methionine, L lysine, L threonine, choline 60%, and a micro premix; for which costs were fixed at, \$70, \$473, \$170, \$4110, \$2400, \$5750, \$1600, and \$370/metric tonne, respectively. Two points in each year (the first Monday in February and the first Monday in August) were arbitrarily chosen for evaluation, as prices are constantly changing and it would be repetitious to evaluate prices for each month in the year. All prices were determined for starter (7-25 kg), grower (25-50 kg), and finisher (50-105 kg) pig diets. The nutrient specifications were maintained constant for metabolizable energy, calcium, available phosphorus, total tryptophan, choline, and digestible methionine, methionine and cystine, lysine, and threonine according to specifications published in the NRC (1998). Feed Formulation Linear Program (FFLP) © 1985-1999 by General Systems Inc. (Version 1.43 MS-DOS revised September 9, 1999) was used for all calculations. Shadow prices (defined as the value at which an ingredient would be added to the complete feed based on least cost formulation) were determined by entering the price of peas as \$999, so the peas would not be included in the

Table 22. Prices of feed grains in Winnipeg Manitoba, February and August, from 2000-2003 (\$/metric tonne).

Ingredient	Feb. 7, 2000	Aug. 7, 2000	Feb. 5, 2001	Aug. 6, 2001	Feb. 4, 2002	Aug. 5, 2002	Feb. 3, 2003
Corn	110.00	102.00	137.00	130.00	142.00	172.00	158.00
Wheat	100.35	103.45	112.95	111.35	164.00	189.00	186.00
Barley	93.35	93.34	106.00	134.86	150.85	169.75	172.00
Peas*	N/A	128.67	152.33	161.00	201.67	185.67	193.33
Soybean meal 47%	246.50	249.00	275.00	313.00	279.00	341.50	306.00
Canola meal 35%	180.00	160.00	194.00	242.50	204.00	225.00	235.00
Animal fat	430.00	420.00	420.00	410.00	420.00	435.00	480.00

*Price from Saskatoon, Saskatchewan

Source: Pelletier, 2003

N/A not available

formulation, but the value at which peas would be added to the complete feed (shadow price) was calculated by FFLP. Diets were then re-formulated with the corresponding price of peas (i.e. depending on the price date) to determine the maximum inclusion level of peas in commercial diets. Finally, diets were formulated with 0, 5, 10, 15, 20, 25, and 30% inclusion of peas to determine the price of the complete feed and whether or not the inclusion of peas in the diet would result in monetary savings for swine producers.

6.4 RESULTS AND DISCUSSION

The shadow prices for pea inclusion in commercial swine diets are presented in Table 23. The starter, grower, and finisher diet shadow prices for peas ranged from \$146.40 to \$220.30, \$150.50 to \$227.40, and \$150.50 to \$212.90/metric tonne, respectively. The actual price of peas (Table 21) ranged from \$128.67 to \$201.67/metric tonne, which is generally lower than the shadow prices indicating that the inclusion of peas in most diets will reduce the total cost of the complete feed. Figures 1, 2, and 3 illustrate that, in general, the higher the inclusion level of peas, the lower the cost for complete feed. Even a small reduction in the cost of a metric tonne of swine feed can add up to large savings for the producer, as numerous tonnes of feed are required for the production of swine. However, it is important that the lower cost feed does not result in decreased performance, as the savings from the feed may not outweigh the cost of poor performance.

The starter, grower, and finisher feed costs were all decreased with the increase in pea inclusion level, except for February 4, 2002, where the feed cost increased as the

Table 23. Shadow pricing for field pea inclusion in commercial diets, February and August, from 2000-2003 (\$/metric tonne).

Diet	Feb. 7,	Aug. 7,	Feb. 5,	Aug. 6,	Feb. 4,	Aug. 5,	Feb. 3,
	2000	2000	2001	2001	2002	2002	2003
Starter 7-25kg	149.50	146.40	168.30	176.90	184.00	220.30	198.50
Grower 25-50 kg	152.50	150.50	169.60	178.20	185.10	227.40	206.50
Finisher 50-105 kg	150.50	151.60	167.40	176.90	185.50	212.90	209.80

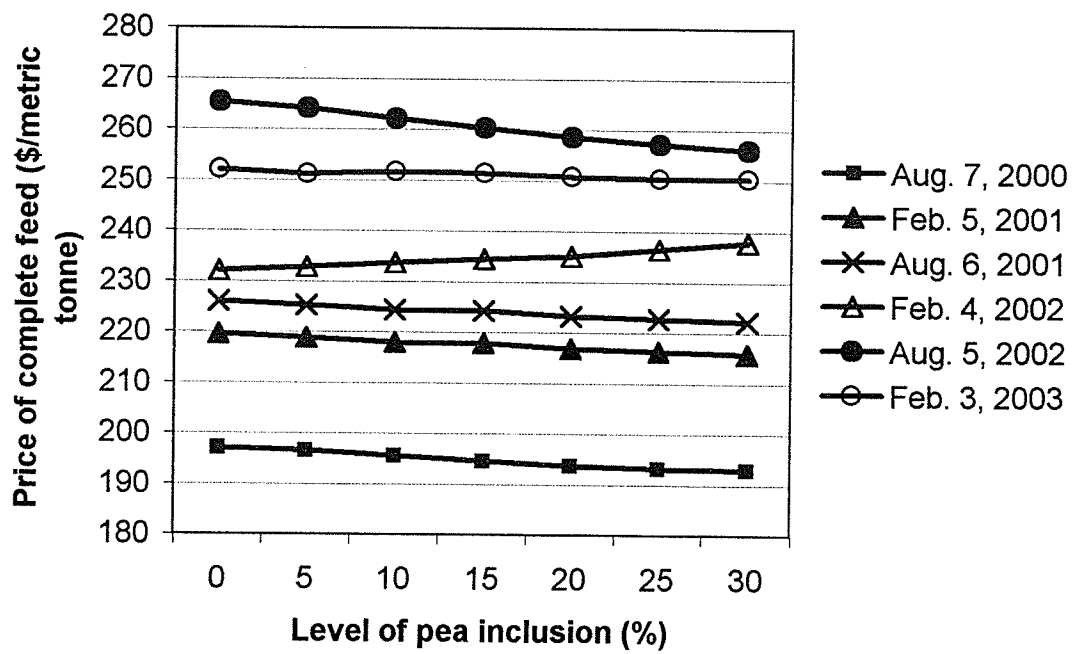


Figure 1. Price of starter (7-25 kg) complete feed depending on the level of pea inclusion.

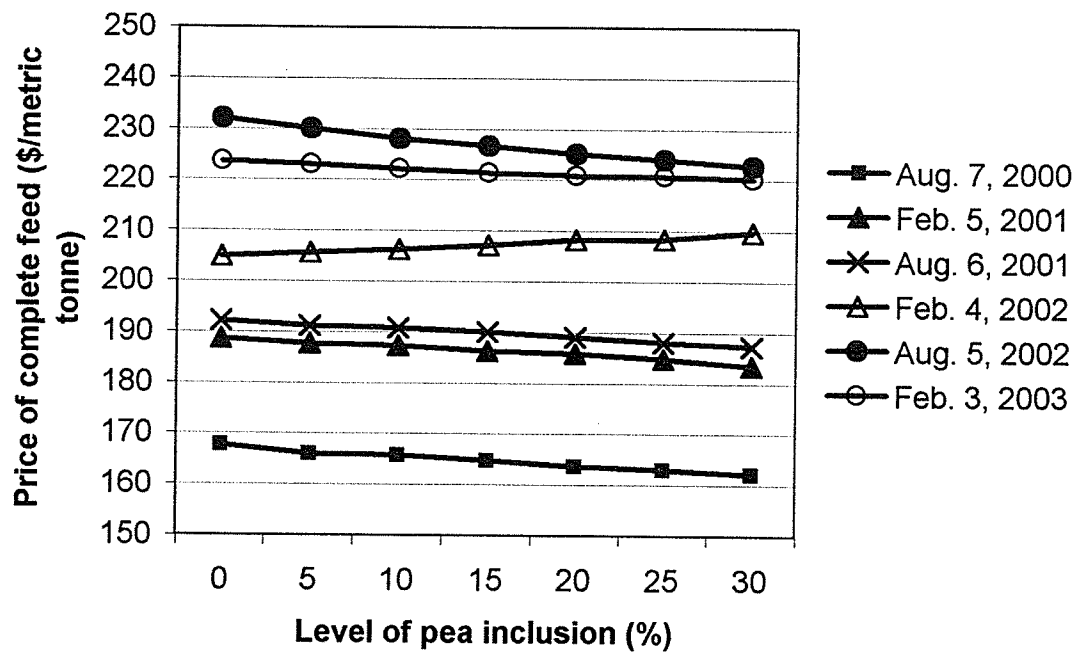


Figure 2. Price of grower (25-50 kg) complete feed depending on the level of pea inclusion.

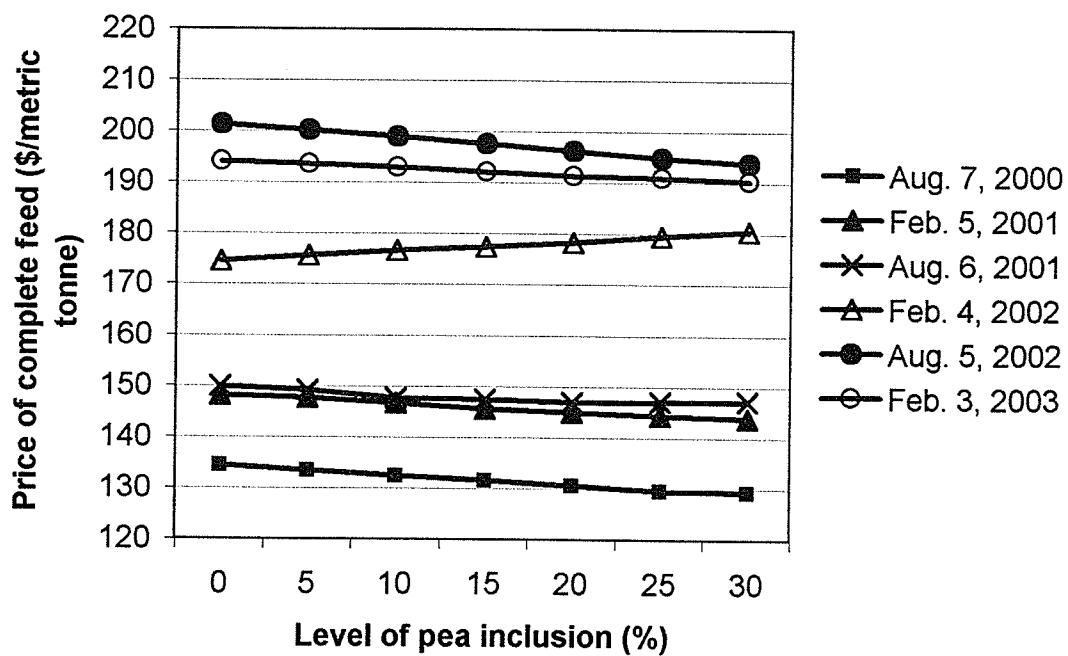


Figure 3. Price of finisher (50-105 kg) complete feed depending on the level of pea inclusion.

level of pea inclusion increased. This was due to the shadow price for the starter, grower, and finisher diets for February 4, 2002 being greater than the actual price of peas on February 4, 2002. Also, the slopes of the lines in Figures 1, 2, and 3 were increased when the difference between the shadow price and the actual price was greater.

The maximum inclusion levels (i.e. most economical, lowest complete feed cost) of peas in commercial diets based on least cost formulation are presented in Table 24. The starter diet includes peas at levels from 36 to 44%. The grower diet has higher levels of pea inclusion from 56 to 69%. The finisher diet includes peas at levels similar to the starter diet, ranging from 32 to 35%. All of the maximum inclusion levels are greater than the 30% level tested in Manuscript III, and the current study. However, including levels of up to 70% peas in the grower diet would most likely result in decreased performance. Depending on how poorly the pigs performed, the savings from the reduced feed cost may not be beneficial overall. Therefore, further experimentation should be done with higher levels of pea inclusion, possibly up to 70% peas.

6.5 CONCLUSION

Peas generally have a good economic value, reducing the cost of complete feed in most cases. However, least cost feed formulation should always be done prior to feed manufacturing to determine if peas are economically viable at that point in time. Animal performance should also be considered; as least cost feed is not necessarily the most economical when animal performance is included in the equation.

Table 24. Maximum inclusion levels (%) of field peas in commercial diets formulated on least cost basis, February and August, from 2000-2003 (%).

Diet	Feb. 7, 2000	Aug. 7, 2000	Feb. 5, 2001	Aug. 6, 2001	Feb. 4, 2002	Aug. 5, 2002	Feb. 3, 2003
Starter 7-25kg	N/A	37.18	44.42	44.42	MV	37.18	36.19
Grower 25-50 kg	N/A	68.85	56.14	56.14	MV	68.83	68.83
Finisher 50-105 kg	N/A	34.71	31.61	33.11	MV	34.88	34.32

N/A, not available

MV, missing value due to price

7.0 GENERAL DISCUSSION

There is a need for expanding the ingredient opportunities in Western Canada and Manitoba in particular to cater to the growing pork industry. Peas could be an excellent protein and energy source for pigs but the commercial feed industry has yet to accept them as the sole source of protein in pig feeds. One of the main reasons for this is that peas tend to be very variable in their nutrient profile among and within cultivars (Fan and Sauer, 1999). It is therefore important to characterize the pea cultivars grown locally. The proximate analysis and chemical composition of the nine different pea cultivars from Manuscript I showed peas to be highly variable. The dry matter and crude protein contents ranged from 90 to 91% and 20 to 24%, respectively. These values are similar to those reported previously (Fan et al. 1994; Gabert et al. 1996; Canibe et al. 1997; Igbasan et al. 1997; Fan and Sauer, 1999). Five yellow and four green pea cultivars were included in this study and the variations in crude protein contents did not appear to be related to seed colour.

The fat (hexane extract) content was low in the pea cultivars tested, however, similar results have been reported previously by Igbasan et al. (1997), NRC (1998), and Fan and Sauer (1999). The tested pea cultivars were rich in starch and NSP (starch values were similar to those reported by Igbasan et al. (1997); NRC (1998); and Fan and Sauer (1999)). The amount of neutral detergent fibre (NDF) within the selected cultivars for the current study are slightly lower than the values found by Igbasan et al. (1997) and Fan and Sauer (1999), who found a range of 14.6-18.2% NDF content. However, the NRC (1998) value of 12.7% is similar to the average NDF content (11.0%) found in the

current study. The ADF content (6.7%) found in the current study agrees with previous findings (NRC, 1998; Grosjean et al. 1999).

According to NRC (1998), true ileal digestible amino acid coefficients should be used in formulating pig feeds, as these are more additive in a mixture of feed ingredients than apparent digestibilities (Imbeah et al. 1988; Nyachoti et al. 1997b, 2002). However, true ileal amino acid contents in Canadian pea cultivars have not been reported. In the current studies, the homoarginine technique was used to determine true ileal amino acid digestibilities in peas (Profi, Swing, Croma, and a mixture of AC Melfort, CDC Mozart and Eclipse cultivars). Apparent and standardized ileal amino acid digestibilities were also determined. The conversion rates of lysine into homoarginine found for peas in Manuscript I (range from 91.2 to 96.9%) were substantially higher than those reported for canola meal (72.3 to 86.0%, Nyachoti *et al.* 2002) and soybean meal (78%, Marty et al. 1994; 68.6%, Siriwan et al. 1994; 79.8%, Imbeah et al. 1996). Imbeah et al. (1996) reported conversion rates (using a ratio of 1:12, lysine: OMIU) in casein and soybean meal of 92.8 and 78.0%, respectively. Clearly, conversion rates of lysine into homoarginine differ among feedstuffs, thus suggesting the need to determine suitable conditions for different feedstuffs. As the guanidination rates obtained with a 4-d reaction period and 0.5M OMIU solution were similar to those obtained with a 5-d reaction period and 0.4M OMIU solution, the latter conditions are recommended for lysine guanidination in peas. The reason for this is that OMIU is expensive, and its availability may sometimes be limited.

Apparent ileal amino acid digestibilities are confounded by the presence of endogenous amino acids in ileal digesta. This has been suggested to be responsible for

the differences in apparent ileal amino acid digestibilities among feed ingredients (e.g. Nyachoti et al. 1997a). In the present study, significant differences were observed among the pea-based diets in apparent ileal amino acid digestibilities for all indispensable amino acids except phenylalanine (Manuscript II). However, the apparent ileal amino acid digestibility coefficients observed in the current study were similar to those reported previously (Leterme et al. 1990; Fan et al. 1994; Fan and Sauer, 1999). In general, the apparent ileal amino acid digestibilities in the Mixture diet were lower than in the diets based on single pea cultivars but the reason for this is not readily obvious.

It has been suggested that for practical feed formulation, standardized ileal amino acid digestibilities, which are apparent digestibilities corrected for basal (minimum) endogenous amino acid losses, should be used (Rademacher et al. 2000). The standardized ileal protein and amino acid digestibilities found in the current study ranged from 78.5 to 79.2% and 67.8 to 94.0%, respectively, which falls within the range of previously reported values (Carlson and Bayley, 1970; Leterme et al. 1990; Fan et al. 1995; Fan and Sauer, 1997). Using endogenous amino acid losses determined with the protein free method or the regression method provide similar standardized ileal amino acid coefficients. This is because these two methods provide similar values for basal endogenous amino acid losses in pigs (Boisen and Moughan, 1996). The smaller SEM associated with the standardized digestibilities compared with the apparent digestibilities implies that endogenous amino acid losses account for part of the differences in apparent ileal amino acid digestibilities. Fan and Sauer (1997) reported that the true ileal digestibility (determined using regression analysis) values of amino acids were not as variable as their respective apparent ileal digestibility values in soybean meal. They also

found the true ileal amino acid digestibilities to be independent of the level of amino acid in the diet.

True ileal digestibility coefficients are derived by correcting the apparent ileal amino acid digestibilities for the amount of endogenous amino acid losses (Rademacher et al. 2000). True ileal digestibility can only be estimated when endogenous amino acids in the ileal digesta are accurately measured. Unlike standardized ileal amino acid digestibilities, true ileal amino acid digestibilities are measured using specific endogenous amino acid losses related to the feed ingested by the animal, making them more specifically related to a dietary factor such as fibre and other ANFs. The endogenous lysine flows at the distal ileum of pigs fed pea-based diets in Manuscript II were comparable to values found by Nyachoti et al. (1997b, 2002) in pigs fed barley-canola meal diets. The total and endogenous flows were slightly greater when the reactive lysine method was used compared with the homoarginine technique thus, explaining the differences between the digestibilities found with each method.

Endogenous lysine flows were the basis for the calculation of true ileal amino acid digestibilities with both the homoarginine technique and the reactive lysine method. Amino acid flows other than lysine were determined based on ratios of amino acids in endogenous protein found in previous studies (de Lange et al. 1989b; Boisen and Moughan, 1996; Rademacher et al. 2000). Therefore, there are still inaccuracies when calculating true ileal amino acid digestibilities using homoarginine and reactive lysine methods, due to the inconsistencies in determining the correct amino acid ratios in endogenous gut protein. The amino acid ratios of endogenous protein that are available are largely derived from studies feeding protein free diets and, therefore, are not specific

for pea diets (de Lange et al. 1989a,b; Boisen and Moughan, 1996; Rademacher et al. 2000). It has already been shown in a number of different studies that the endogenous losses of amino acids from the small intestine of monogastrics are higher following dietary intake of protein or peptides (Darragh et al. 1990; de Lange et al. 1990; Moughnan and Rutherford, 1990; Butts et al. 1991, 1993) than when protein-free diets are fed. Therefore, endogenous losses are affected by dietary intake of protein and peptides and the amino acid composition of endogenous gut protein differ with different protein feedstuffs.

The true ileal crude protein digestibilities found in Manuscript II agree with those found in previous studies using peas, (Huisman et al. 1992; Jansman and Verstegen, 2002) and barley-canola (Nyachoti et al. 1997b; 2002). To the best of my knowledge, true ileal amino acid digestibilities have not yet been published for peas.

The commercial feed industry currently limits the amount of peas included in swine diets, especially those for weaned piglets, as their digestive systems are not fully developed and, therefore, unable to fully digest many diets. It is generally recommended that dietary pea levels for young pigs be restricted to 5 to 15% (Castell et al. 1996; Stefanyshyn-Cote and Belanger, 2002). In the current study piglets were fed diets containing up to 30% peas with no effect on performance (Manuscript III). The results from Manuscript III for Phase III (11 to 18 kg pigs) agree with those found in previous studies (Van Nevel et al. 1999; Owusu-Asiedu et al. 2002). However, Zivkovic et al. (1987) found that pigs fed a diet containing 30% peas with an average initial body weight of 12 kg had lower average daily gain ($P < 0.05$) compared with pigs fed a diet without peas, but this difference was not seen when methionine was supplemented to the 30% pea diet. Gâtel et al. (1989) reported that

pigs fed a diet containing 24% peas from an average initial body weight of 11 kg had a greater feed:gain ratio ($P < 0.05$) compared to pigs fed a diet without peas, this difference however was not seen when tryptophan was supplemented to the 24% pea diet.

Indeed performance of phase I and II piglets has been shown to decline if dietary pea levels exceeding 15% are used (eg., Bengala-Freire et al. 1989). However, the results found in Manuscript III suggest that phase I and II nursery pigs can effectively utilize diets containing up to 30% peas. The response to pea containing diets seems to be dependent on diet formulation and in particular the supply of the amino acid methionine, which is often limiting in peas and other pulses. In Manuscript III diets were balanced for methionine and other amino acids to meet or exceed NRC (1998) recommendations for young pigs, which may explain the lack of depression of pea diets on piglet performance. This observation has a significant implication to the swine and pulse industry as it suggests that higher levels of peas can be used in weanling pig diets with proper formulation.

The optimum inclusion rate for peas may not coincide with the maximum rate that can be used by the pigs. In general, the higher the inclusion level of peas, the lower the cost of the complete feed (Manuscript IV). Even with a small reduction in the cost of a metric tonne of swine feed, this can add up to large savings for the producer, as numerous tonnes of feed are required annually for the production of swine. However, it is important that the lower cost feed does not result in decreased performance, as the savings from the feed may not outweigh the cost of poor performance.

The maximum inclusion levels (i.e. most economical, lowest complete feed cost) of peas in commercial diets based on least cost formulation were determined in Manuscript IV. The starter diet included peas at levels from 36 to 44%. The grower diet

has higher levels of pea inclusion from 56 to 69%. The finisher diet includes peas at levels similar to the starter diet, ranging from 32 to 35%. All of the maximum inclusion levels are greater than the 30% level tested in Manuscript III, and IV, therefore a significant saving can be had at 30% inclusion of peas. However, including levels of up to 70% peas in the grower diet would most likely result in decreased performance.

Depending on how poorly pigs perform, the overall savings from the feed cost may not be justified. Therefore, further experimentation should be done with higher levels of pea inclusion.

8.0 SUMMARY AND CONCLUSIONS

1. From the chemical composition results it can be concluded that there is a high variability in the chemical composition of peas, and this should be taken into consideration when formulating diets containing peas.
2. OMIU is an expensive solution and therefore the 5d reaction period with the 0.4M concentration of OMIU is recommended when using the homoarginine technique for guanidinating peas.
3. The apparent ileal amino acid digestibilities of the peas are highly variable.
4. For practical feed formulation, true rather than apparent ileal digestibilities should be used. The homoarginine technique and the reactive lysine method are more expensive, but give less variable digestibility coefficients than the standardized calculations produce.
5. Whether the digestibilities are standardized calculations, or estimated from the homoarginine or reactive lysine methods, assumptions have to be made about the amino acid composition of the endogenous protein.
6. Standardized digestibilities are a simple and inexpensive means for estimating true ileal digestibilities in pigs fed practical feed types like peas. Until subsequent

research can be done to define true ileal digestibilities of all of the common feedstuffs used in swine diets, standardized digestibilities should be used.

7. Peas can be fed to nursery pigs at levels of at least up to 30% of the diet without affecting the performance of the pigs.

8. Peas generally have a good economic value, reducing the cost of complete feed in most cases. However, least cost feed formulation should always be done prior to feed manufacturing to determine if peas are economically viable at that point in time. Animal performance should also be considered; as a least cost feed is not necessarily the most economical when animal performance is included in the equation.

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