THE NUTRITIONAL VALUE OF FLAXSEED MEAL FOR SWINE

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ABSTRACT

The nutritional value of flaxseed meal (FSM), a by-product of the flax crushing industry, has not been evaluated properly for use within swine rations. A series of experiments were conducted to determine the nutritional profile of this novel feed ingredient for pigs.

The analysis of FSM revealed that it contains, on a dry matter (DM) basis, 133 g/kg ether extract (EE), 345 g/kg crude protein (CP), 60 g/kg ash, 164 g/kg ADF, 250 g/kg NDF, 102 g/kg crude fibre, 14 g/kg starch and 9 g/kg phosphorus. The gross energy (GE) content of the meal was 5.2 Mcal/kg DM. The ether extract fraction was characterized by, as a percent of total fat, 46.6% α-linolenic acid, an omega-3 fatty acid. Palmitic, stearic, oleic and linoleic acids accounted for 9.5, 4.8, 20.7 and 18.4% of the total fat content respectively. The crude protein content was well balanced for all amino acids with the exception of lysine (4.1% of CP), the level of which falls below that of the requirements for growing pigs (5.3% of CP for pigs 20-50 kg). The apparent digestibility of DM, nitrogen, ash, EE and GE as well as determination of the DE and NE content of FSM was determined for both growing pigs (32 pigs, initial weight 70 ± 3 kg) and gestating sows (26 pigs, parities 2 – 4). Animals were fed wheat/barley based diets containing 0, 10, 20 or 30% FSM. Faecal grab samples were collected for 3 days after a dietary adaptation period. The apparent digestibility of nutrients in FSM was determined both by regression and by difference calculations. As calculated by difference, the apparent digestibility coefficients for DM, nitrogen, ash, and GE were 63.0, 60.8, 22.3 and 60.5% respectively for growing pigs. The values obtained for sows were 64.1, 58.8, 20.8, 94.9 and 65.4% for DM, nitrogen, ash, EE and GE respectively. The DE content was 3.37 Mcal/kg for growing pigs and 3.52 Mcal/kg for sows. Net energy was then estimated by use of a prediction equation to be 2.34 and 2.44 Mcal/kg for growing pigs and sows.

An experiment was conducted to evaluate the growth performances and carcass fatty acid profiles of pigs fed with graded levels of FSM. A total of 200 pigs (100 barrows, 100 gilts; initial weight 32 ± 4 kg) were blocked by gender and housed in groups of 5 pigs per pen. The experiment was divided into three phases for pigs 32-60 kg, 60-85 kg and 85-115 kg. Each group was assigned to one of four dietary treatments containing 0, 5, 10 or 15% FSM at the expense of wheat and soybean meal. At the time of market, 6 pigs per treatment group were randomly selected for carcass fatty acid analysis, and backfat and rib-end loin samples were collected. The average daily gains, average daily feed intakes and gain to feed ratios were not affected by dietary treatment ($P > 0.05$). Inclusion of 15% dietary FSM increased the ALA content from 11 to 47 (± 0.8) mg/g of backfat ($P < 0.001$) and

from 5 to 10 (± 0.4) mg/g of loin tissue ($P < 0.001$). Increasing dietary FSM decreased the saturated fatty acid content of backfat ($P < 0.01$).

The final experiment was designed to determine the availability of phosphorus in semi-synthetic diets containing FSM, and to determine the effects of microbial phytase inclusion of this availability. Five treatment groups, 8 barrows (45 ± 4 kg initial weight) each, were fed a diet containing 30% FSM with increasing levels of phytase (0, 575, 1185, 2400 and 2570 FTU/kg). Apparent P digestibility increased from 20.6 to 61.3% with the inclusion of up to 2570 FTU/kg microbial phytase ($P < 0.001$), and followed a quadratic response pattern with an R^2 value of 0.96. A broken-line analysis estimated the optimal phytase inclusion level to be 1415 FTU/kg of diet. Inclusion of just 575 FTU/kg accounted for half of the response, improving the apparent P digestibility by 20% and reducing P excretion by 850 mg/kg dry matter intake.

Key Words: flaxseed meal, pig, digestibility, growth performance, omega-3 fatty acid, phosphorus, phytase

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DEDICATION

I would like to dedicate this Thesis to my family. To my parents, Elaine and Steve, I am grateful to have such wonderful parents; my life wouldn't be what it is today without you. To my grandmother, Freda, your continual encouragement means the world to me. To my siblings Nicky, Mark, Steve and Leslie, I don't know what I would do without you; you are always there to support me and to give me a laugh when I need it. I love all of you very much, and couldn't be more grateful to be a part of such a wonderful family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ArA	Arachidonic Acid
ADF	Acid Detergent Fibre
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AID	Apparent Ileal Amino Acid Digestibility
ALA	α-Linolenic Acid
ADF	Acid Detergent Fibre
Ca	Calcium
DE	Digestible Energy
DHA	Docosahexaenoic Acid
DM	Dry Matter
DRI	Dietary Reference Intake
EPA	Eicosapentaenoic Acid
EU	European Union
FSM	Flaxseed Meal
G:F	Gain to Feed Ratio (Feed Conversion Ratio)
IL	Interleukin
LT	Leukotriene
NDF	Neutral Detergent Fibre
NE	Net Energy
P	Phosphorus
PG	Prostaglandin
SID	Standardized Ileal Amino Acid Digestibility
TNF	Tumour Necrosis Factor

1 INTRODUCTION

Within the current agriculture industry, the cost of producing livestock is rising for several reasons, one of which is the rise in feed grain prices around the world. For this reason, the pork industry is continually seeking alternative ingredients for use in swine diets in order to either 1) diversify rations, thus helping to reduce the cost of feeding or 2) to achieve a final pork product that meets certain specifications, such as the enrichment of the pork product with omega-3 fatty acids, which can be sold within a niche market and hopefully obtain a premium price.

The use of flaxseed and its related products is becoming a growing interest within the swine industry as it possesses properties which make it unique as a feed ingredient. Flaxseed is rich in α-linolenic acid, an omega-3 fatty acid, giving it a highly desirable lipid fraction. Although flaxseed is a high priced oilseed, there is potential for the use of flaxseed meal (FSM), the by-product remaining after the oil has been extracted, within the swine industry. FSM will be less costly than flaxseed, and may still contain up to 12% oil, thus allowing for the potential to enrich the carcass with α-linolenic acid.

Before flaxseed meal will be viewed favourably by the pork industry a number of factors need to be considered, the most important of which is a well-defined nutrient profile. The nutrient profile of any ingredient available to the pork industry must address the key traditional formulation variables, including digestible energy (DE), net energy (NE) and amino acid content. It is also important to ensure that inclusion of any novel ingredient does not adversely impact growth performance and feed conversion ratios. In the case of FSM, it would also be important to understand the impact of dietary inclusion on the carcass fatty acid profile.

The use of FSM by the pork industry will not occur until it can be used effectively for all classes of swine. Limited bin capacity in feed mills will always be used for ingredients with the broadest application and those ingredients in which producers have the greatest confidence. Currently, little information is available on the use of FSM for pigs and thus this study was designed to determine the nutritional value of FSM for pigs and the effects on carcass fatty acid profiles.

This study was divided into four major sections, each designed to look at a separate aspect of the use of FSM in swine rations. The first part of the study was to conduct a full chemical analysis of flaxseed meal and determine its nutrient contents. Part 2 of the study was to determine the DE and NE content of FSM for both gestating sows and growing pigs, as well as to determine the apparent digestibility of nutrients such as crude protein, ether extract and energy. The third section of the study was performed to determine the effects of dietary inclusion on pig performance throughout the grower/finisher stages, and to determine if FSM inclusion could alter the carcass fatty acid profile. The final component of the study was to determine the availability of phosphorus in FSM, and the effects of microbial phytase inclusion on this availability.

2 LITERATURE REVIEW

2.1 Introduction

Flaxseed is a cool, temperate annual crop which is also known as linseed in many countries. Flaxseed is one of the oldest cultivated plants in the world; in fact, its scientific name *Linum usitatissimum* means "of greatest use". Originally bred thousands of years ago for its fibre (linen) and for the medicinal properties of the seed, it is now cultivated mainly for its oil. Canada is a leading producer of flaxseed in the world, producing ± 1 M t/year which accounts for 30-40% of total world production (Bhatty, 1995; Flax Council of Canada, 2008). The majority of flax produced in Canada is found in the prairie provinces of Alberta, Saskatchewan and Manitoba. As well as being a leading flaxseed producer, Canada is also a major flax exporter, exporting approximately 90% of its total production (Flax Council of Canada, 2008). According to the Flax Council of Canada, Canada currently ships 60% of its total exports to the EU and 30% to the United States of America. Belgium is the leading EU importer of Canadian flaxseed. Despite being a leading producer of flaxseed, Canada does not have a large crushing industry and thus there is very little FSM currently available to Canadians.

The flax seed is composed of approximately 41% oil, more than 70% of which is polyunsaturated (Flax Council of Canada, 2008). These high levels of polyunsaturates allow flax oil to be used by industry in the production of many products including, but not limited to, paints, soaps, detergents, linoleum, vinyl and printing inks. In Europe, the defatted meal which remains after oil processing is recycled into animal nutrition, principally into rations for dairy and beef cattle. Whole flaxseed is increasingly becoming more popular in human nutrition due to both the high fibre content and high polyunsaturated oil content, specifically a high α-linoleic acid (ALA) content.

2.2 Production of Flaxseed Meal

Flaxseed meal is the by-product remaining after the oil has been extracted from the seed. There are many different processing methods available for the extraction of oil, the main goal being to maximize the amount of oil extracted while minimizing damage that may occur to both the oil and solid fractions and reducing the amount of impurities, thus also producing a residual meal product which is as consistent as possible (Carr, 1989). Common oil extraction methods include use of a hydraulic expeller or screw press (either in a cold or warm environment), the use of high pressure, or utilization of a solvent such as hexane after grinding, dehulling, flaking or cooking of the seed (Carr, 1989). Essentially, the type of extraction system utilized depends primarily on the oil content of the seed. Mechanical pressing is commonly used with seeds containing more than 20% oil, while solvent extraction is applied to seeds or press cakes which contain less than 20% oil (Carr, 1989). Both soybeans and canola are primarily solvent extracted.

In the case of flaxseed, solvent extraction is often applied after the initial press in order to maximize the amount of oil extracted. This method is referred to as prepress solvent extraction (Carr, 1989). The solvent extraction method removes more oil from the seed than does a press; however, the traditional extraction method most common in EU countries such as Belgium is the screw press. Companies such as Vandeputte S.A. (Mouscron, Belgium) utilize a double screw pressure technique in which the first is cold pressure providing a pure first grade oil, followed by a warm press allowing for removal of the maximum amount of oil. This method of double screw pressing also produces a relatively consistent FSM product. The variables present in the processing industry vastly influence the final composition of the meal product (Bell, 1989). As an example, FSM produced from prepress solvent extraction contains approximately 2-3% oil (Batterham *et al*., 1991; NRC, 1998), whereas FSM produced by companies such as Vandeputte has approximately 12% oil, thus vastly affecting the energy value of the meal.

2.3 Chemical Composition of Flaxseed Meal

Flaxseed meal has many different components including the major nutrients such as protein and oil, as well as many other compounds, some of which are anti-nutritional factors. Compounds found in FSM include non-starch polysaccharides (called mucilage), cyanogenic glycosides, phytic acid, trypsin and chymotrypsin inhibitors, linatine (a vitamin B_6 antagonist), lignans (phyto-estrogens), minerals and vitamins. The proximate composition of FSM will vary considerably

depending not only on the flax crushing methods utilized, but also on the growing conditions, the cultivar and the laboratory analysis techniques.

2.3.1 Protein and Amino Acid Composition

Flaxseed meal contains on average 30-35% crude protein, as shown in Table 2-1. There is little information available in the literature on the amino acid profile of FSM itself; however, crushing of full fat flaxseed will concentrate the amount of protein, but will not significantly alter the amino acid profile. Table 2-1 shows the amino acid profile of FSM which was determined by combining the information available from the few papers on FSM composition along with those available on full fat flaxseed composition.

It is important to note that flaxseed, and thus FSM, proteins are distinguished by a low lysine content (approximately 3.7% of crude protein). Growing swine 25-50 kg in weight require a diet containing a lysine content of 5.3% of total crude protein (NRC, 1998), and thus it is important to account for this when formulating diets. Although flaxseed and FSM protein is low in lysine, it has one of the highest levels of tryptophan of all feed ingredients currently used in animal nutrition (INRA, 2004), as well as containing levels of threonine and sulphur-containing amino acids which meet or exceed the nutrient requirements of growing swine.

Despite the fact that lysine is by far the main limiting factor for use of FSM in swine rations, this problem can be overcome relatively easily by including ingredients which can balance the lysine content such as peas or synthetic lysine, as long as it is economically justified.

2.3.2 Oil Content

The main reason for the recent interest in the use of flaxseed and its related products within the animal production industry is its oil content and profile. Flaxseed contains approximately 41% oil, 57% of which is the omega-3 fatty acid α-linolenic acid (Flax Council of Canada, 2008). In fact, flaxseed contains the largest amount of ALA of all other land based sources. As discussed below, the omega-3 fatty acids, including ALA, have many potential implications in terms of human and animal health. Although FSM is a defatted product, it may still contain levels of

Table 2-1: Average chemical composition values of flaxseed meal compiled from the literature

Chemical Composition (% Dry Matter)	Average	SD	Minimum	Maximum
Crude Protein	35.9	2.8	30.9	39.0
Ether Extract	5.6	2.3	2.0	8.3
Ash	7.6	3.3	4.8	13.8
Crude Fibre	9.6	1.3	7.4	11.7
NDF	26.4	2.9	23.4	29.1
ADF	16.9	1.9	14.2	18.3
Ca	0.4	0.1	0.4	0.5
P	0.9	0.1	0.8	1.1
Amino Acids (% of Crude Protein)				
Arginine	9.0	1.5	7.5	11.1
Histidine	2.5	0.5	1.8	3.1
Isoleucine	4.3	0.8	3.1	5.2
Leucine	6.0	0.9	5.1	7.1
Lysine	3.7	0.4	3.2	4.3
Methionine	1.8	0.5	0.9	2.5
Phenylalanine	4.7	0.7	3.6	5.3
Threonine	3.9	0.8	3.0	5.1
Tryptophan	1.5	0.2	1.4	1.7
Valine	4.8	0.9	3.8	5.8
Cysteine	2.2	1.1	1.2	4.3
Fatty Acids (% of Oil)				
Palmitic Acid	6.4			
Stearic Acid	3.4			
Oleic Acid	18.7			
Linoleic Acid	14.7			
α-linolenic Acid	54.2			

Sources: Bell, 1989; Hossain and Jauncey, 1989; Bhatty and Cherdkiatugumchai, 1990; Batterham *et al*., 1991; DeClercq *et al*., 1992; Bell and Keith, 1993a; Hasan *et al*., 1997; Hossain *et al*., 1997; NRC, 1998; Rodriguez *et al*., 2001; Hosseinian *et al*., 2004; INRA, 2004; Flax Council of Canada, 2008.

up to 12% oil, and can thus still have potential benefits from its fatty acid profile. The actual quantity of oil remaining in the defatted product will vary with the oil extraction methods implemented. When the oil is extracted with solvents, the residual content does not exceed more than 3% of dry matter (DM); however, when extracted by expeller, the more traditional method of extraction for flaxseed, the meal will contain anywhere from 8-12% oil.

Similar to the amino acid profile, there is limited information in the literature as to the exact fatty acid profile of FSM. The published French tables (INRA, 2004) appear to be the only place where fatty acid data is presented specifically for FSM, and are thus the source of the values presented in Table 2-1. FSM contains only five main fatty acids. These include palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3).

2.3.3 Minerals

Flaxseed meal contains appreciable amounts of many minerals, especially of both calcium and phosphorus as shown in Table 2-1. Currently there is little information on the availability of these nutrients in FSM but it is expected that the majority of phosphorus will be bound in phytic acid, and a large portion of calcium would be bound to the phytate molecule as a chelate. Similar to most other plant sources of phosphorus, it is estimated that up to 70% of phosphorus in FSM is bound in the phytate form and is thus unavailable to animals lacking the phytase enzyme such as pigs (Bhatty and Cherdkiatgumchai, 1990). INRA (2004) indicates that 65% of phosphorus is bound in the form of phytic acid.

2.3.4 Mucilage

The seed coat of flaxseed is primarily composed of a non-starch polysaccharide called mucilage. This mucilage will also be present in FSM. Mucilage is a thick, water-soluble, glutinous polysaccharide which accounts for up to 39% of the total flax seed (Wanasundara and Shahidi, 1997). Arabinoxylans make up approximately 75% of the mucilage, while the other 15% is composed of two fractions of pectin-like substances which are composed primarily of uronic acid, galactose and rhamnose (Warr *et al*., 2003). The mucilage fraction of flaxseed, and thus FSM, varies greatly depending on the genetic origin of the plant as well as the growing conditions (Diederichsen *et al*., 2006). High levels of dietary mucilage has been shown to reduce nutrient availability by increasing

gastric viscosity in broiler chickens, leading to a decrease in body weight with increasing levels of FSM (Bhatty, 1993).

The fermentation of non-starch polysaccharides such as mucilage in the gastrointestinal tract of pigs has been receiving attention in the last several years. This increased attention is primarily due to the fact that the use of antibiotics as growth promoters has been banned in EU countries. Essentially, researchers are looking for feed ingredients which contain prebiotic properties – fermentable carbohydrates that aid in the development of health promoting bacteria such as *Lactobacilli* and *Bifidobacteria* at the expense of pathogenic bacteria such as E. *Coli* and *Salmonella*. Several preliminary studies have shown that flaxseed based diets can increase the *Lactobacilli* population in the digestive tracts of monogastric animals (Alzueta *et al*., 2003; Smith, 2005); however, further work is required before any conclusions can be made as to whether or not flaxseed mucilage can be considered to have prebiotic properties.

2.3.5 Cyanogenic Compounds

Cyanogenic glycosides are anti-nutritional factors which are present in flaxseed and FSM. The primary cyanogenic glycosides found in Canadian flax cultivars are linustatin and neolinustatin, with very few samples containing linamarin (Oomah *et al*., 1992). The concentration of the cyanogenic compounds can vary greatly between cultivars (Bhatty, 1995). Disruption of the cell wall following processing leads to the enzymatic hydrolysis of the cyanogenic compounds causing the subsequent release of hydrogen cyanide (HCN) or prussic acid (Bhatty, 1995). In the wild, it is thought that this release of HCN is to prevent the consumption of the plant by herbivorous animals, and is thus a defensive mechanism developed by the plant. It is not likely that the amounts of cyanogenic compounds would have detrimental effects on pigs for two reasons; 1) inclusion of FSM into the diets of pigs will generally be limited to a maximum of 15% and 2) any form of heat processing of the diet such as pelleting, and of the FSM itself during oil extraction will result in a decrease in the release of HCN which can be explained by a decrease of the glycosidase activity (Feng *et al*., 2003; Krech and Fieldes, 2003).

2.3.6 Lignans

Lignans are estrogen-like compounds which are found in many plant species. Kuijsten *et al*. (2005) reported that flaxseed contains the highest level of lignans known in the plant kingdom, and thus it is possible to estimate that FSM also contains a high lignan concentration. There are two main types of lignans found in flaxseed, secoisolariciresinol diglycoside and matairesinol, which are contained primarily in the seed coat (Sicilia *et al*., 2003). These compounds are converted by intestinal colonic bacteria into two mammalian type lignan molecules, enterodiol and enterolactone, which can mimic the activity of endogenous hormones by binding to the estrogen receptors within cells (Sicilia *et al*., 2003). The flaxseed lignans as well as the molecules enterodiol and enterolactone can be metabolized by the body and excreted in the urine of mammals (Setchell *et al*., 1980).

High levels of estrogens are required during the gestation period to ensure embryo implantation and to enhance uterine growth, and are thus essential in maintaining pregnancy. The high levels of endogenous estrogen are controlled by the presence of other hormones, and this helps to prevent toxicity to the foetus (Tou *et al*., 1998). It is possible that providing additional estrogens to gestating animals may have detrimental effects on the pregnancy by increasing the estrogen levels above the toxic threshold, justifying a concern of providing high levels of lignans to pregnant animals. Some potential effects of exogenous estrogens may include reductions in feed intake and thus weight gain, which in turn can lead to dystocia (Tou *et al*., 1998). It is important to note that the estrogenic activity of lignans is much lower than that of endogenous estrogens, and a study by Farmer *et al*. (2007) showed that diets containing 10% flaxseed had no mammary gland development effects in gilts.

2.3.7 Trypsin Inhibitors

Trypsin inhibitors are anti-nutritional factors which form an indigestible complex with trypsin. This prevents trypsin activity and thus decreases trypsin concentration in the small intestine. The pancreas of the animal compensates for this by secreting more trypsin and thus increases the protein requirements of the animal for these increased secretions. Although trypsin inhibitors are present in FSM, their activity level appears to be much lower than the trypsin inhibitors found in both soybean meal and canola meal (Bhatty, 1993). As shown by Bhatty (1993) the trypsin inhibitor activity (TIA) level of soybean meal is 1650 units of TIA, canola meal is 99 units of TIA and FSM ranges from 14 to 51 units of TIA depending on cultivar and processing. Since the TIA level of FSM

is much lower than other common ingredients used in swine nutrition, it is unlikely that negative effects will be noted due to trypsin inhibitors.

2.3.8 Linatine

For many years it has been known that under experimental conditions vitamin B_6 deficiency symptoms could be induced in pigs by feeding diets containing FSM (Chick *et al*., 1938). These symptoms include appetite loss, poor performance, anaemia, and nervous disorders. It was not until approximately 30 years later that a vitamin B_6 antagonist called linatine was isolated from FSM by Klostermann *et al*. (1967). Linatine is a dipeptide of serine and glutamine. It is present at levels of approximately 100 mg/kg of FSM (Bhatty, 1995) and when fed at high levels can induce vitamin B_6 deficiency symptoms. In growing pigs, induction of moderate vitamin B_6 deficiency has not been reported until FSM inclusion levels have reached 30% (Bishara and Walker, 1977; Batterham *et al*., 1991). Bishara and Walker (1977) found no signs of vitamin B_6 deficiency in rats with 30% inclusion. Since inclusion of FSM into the diets of grower pigs is unlikely to reach levels greater than 15% inclusion, concerns regarding the effects of linatine are moderate.

2.4 Use of Flaxseed Meal for Swine

There is limited information available in the literature on the use of FSM in swine rations; however, there have been several studies looking at growth performance of pigs and the digestibility of nutrients. The usefulness of any oilseed meal, including FSM, is affected by several different factors including, but not limited to, digestibility and availability of nutrients (especially amino acids), palatability of the ingredient as well as the presence of anti-nutritional factors or toxic compounds.

2.4.1 Nutritional Value

The nutritional value of FSM for pigs can be described by looking at the digestible and net energy contents as well as amino acid and mineral digestibility, especially in terms of phosphorus. In 1993, Bell and Keith studied the digestibility of FSM in terms of energy, crude protein and fibre contents. The percentage of ether extract in the FSM used ranged from 5.4 to 7%. They found that the apparent digestibility of energy was approximately 73%, that of crude protein was approximately 75% and that of crude fibre varied from 13-26% depending on the variety of flaxseed used.

Increasing dietary levels of FSM also reduced the digestibility of energy. Unlike other oil meal products for which apparent oil digestibility is low (30-32% for canola meal and soybean meal), the oil digestibility of FSM is high (46-68%; INRA, 2004).

As shown in Table 2-2, the DE value of FSM varies not only among reports, but also within studies. These variations can be attributed to both variations in the oil content of the FSM (between reports) and the cultivar of flaxseed (within studies). Oil is a concentrated form of energy and thus high oil FSM will have a greater DE value when compared to low oil FSM. For example, the values reported by Bell and Keith (1993a) exceeded those reported by Batterham *et al*. (1991). The FSM used by Batterham and colleagues was produced by pre-press solvent extraction and contained only 3% total oil, thus severely reducing the energy value of the meal. It is also interesting to note that the DE values reported in the tables are on average 4% higher for sows than those obtained for growing pigs (3.65 to 3.84 Mcal/kg DM; INRA, 2004), as sows are generally able to utilize nutrients more efficiently than growing pigs.

Currently, there is no information available on the bioavailability of phosphorus (P) in FSM. Similar to other plant sources of phosphorus, approximately 70% is bound by phytic acid (Bhatty and Cherdkiatgumchai, 1990), thus rendering it unavailable to monogastric animals. It could be expected that the bioavailability of P would be similar to values of other common meal products fed to swine such as canola meal and soybean meal, whose P availability is 21 and 31% respectively (NRC, 1998).

2.4.2 Growth Performance

Despite all of the information available on the use of FSM in swine diets and the potential benefits of its incorporation, producers will be much more willing to include FSM into their diets if evidence demonstrates that growth performance is not compromised. There have been several studies looking at the inclusion of full fat flaxseed on pig performance; however, it appears that there are only three journal articles showing the effects of feeding FSM on growth performance (Table 2-3).

Batterham *et al*. (1991) showed that pigs from 20-45 kg live weight grew more slowly on 30% FSM diets when compared to soybean meal-based diets. They also found that the pigs fed FSM had lower gain to feed ratios. The FSM utilized in this study was produced by prepress solvent extraction and contained an oil level of 3%. Diets were formulated to contain

Table 2-2: Nutritional value of flaxseed meal; DE, NE and apparent ileal digestibility of amino acids

	NRC, 1998 (2% Oil)	INRA, 2004 (8% Oil)	Batterham *et al.*, 1991 (3% Oil)	Bell and Keith, 1993a (5.5% Oil)
DE (Mcal/kg DM)				
Growing Pigs	3.06	3.32	2.15 – 2.35	2.68 – 3.14
Sows	-	3.46	-	-
NE (Mcal/kg DM, estimated)				
Growing Pigs	1.84	2.00	-	-
Sows	-	2.09	-	-
Amino Acids (AID, %)				
Arginine	86	-	-	-
Histidine	72	-	-	-
Isoleucine	75	-	-	-
Leucine	68	-	-	-
Lysine	70	-	-	-
Methionine	76	-	-	-
Phenylalanine	78	-	-	-
Threonine	63	-	-	-
Tryptophan	75	-	-	-
Valine	74	-	-	-

similar estimated DE contents; however, it is possible that the authors overestimated the actual amount of available DE and thus the FSM diets may have contained lower levels of energy than estimated. It is also possible that inclusion levels of 30% FSM could reduce palatability of the diets due to high fibre levels, thus decreasing the feed intakes of the pigs as noticed by the authors. The authors also discussed the presence of anti-nutritional factors as a possible reason for the decreased performance of the pigs.

Bell and Keith (1993a) also found significant decreases in average daily gain when feeding pigs (23-57 kg) with increasing levels of FSM from 0-18%. The pigs in this study were unaffected by increasing levels of FSM during the finishing stage (57-100 kg). Feed intake tended to decrease with increasing FSM in the diet; however, this was not statistically significant. Gain to feed ratios also decreased with increasing FSM. Pig responses to increasing FSM did not become apparent until the diets reached 12% FSM inclusion. Since the decrease in pig performance could not be attributed to a decrease in feed intake, it is possible that it could be due to a decrease in the total DE content of the diets as well as a change in the amino acid supply. In this experiment, FSM was incorporated into the diets at the expense of soybean meal, which has a higher DE value when compared with FSM (Batterham *et al.*, 1991). The diets were also only corrected with minimal amounts of lysine, and it is thus possible that lysine was limiting the growth performance of the pigs. In a second experiment, Bell and Keith (1993b) found no significant affect on pig performance (23-100 kg) in terms of average daily gains and feed intakes with the addition of up to 5% FSM. The FSM was incorporated with either wheat or hulless barley.

2.5 Flaxseed Meal as a Source of Omega-3 Fatty Acids

Flaxseed is the richest land-based source of the omega-3 fatty acid α-linolenic acid, or ALA (Connor, 1999). The flaxseed contains on average 41% oil, 56% of which is ALA (Flax Council of Canada, 2008). Although FSM is a defatted by-product of the flax processing industry, it may still contain up to 12% total oil depending on the processing method utilized. The fatty acid profile of the meal product remains unchanged and thus approximately 56% of the total 12% oil content is ALA.

Table 2-3: Effects of flaxseed meal inclusion on growth performances of pigs with different bodyweights

(A) Bell and Keith (1993a)	**0% FSM**	**4% FSM**	**8% FSM**	**12% FSM**	**16% FSM**
23-57 kg Pigs					
ADG (g)	677^{ab}	702^{a}	650^{abc}	634^{bc}	604^{c}
ADFI (g)	1700	1750	1640	1670	1610
G:F	0.40^{a}	0.40^{a}	0.40^{a}	0.38^{ab}	0.38^{b}
(B) Bell and Keith (1993b)	**0% FSM**	**5% FSM**			
23-57 kg Pigs					
ADG (g)	624	606			
ADFI (g)	1640	1660			
G:F	0.38^{a}	0.36^{b}			
57-100 kg Pigs					
ADG (g)	741	754			
ADFI (g)	2620	2660			
G:F	0.28	0.28			
(C) Batterham *et al.* (1991)	**0% FSM**	**30% FSM**			
20-45 kg					
ADG (g)	793^{a}	695^{b}			
ADFI (g)	1690^{a}	1540^{b}			
G:F	0.47	0.45			

Values in the same row with differing subscripts are statistically different ($P<0.05$)

Diets: (A) 55% barley, 28% wheat, 10 to 0% soybean meal; (B) 88 to 74% wheat, 0 to 8% barley hulls, 4 to 12% canola and soybean meal mix; (C) 73 to 54% wheat, 25 to 6.5% soybean meal

2.5.1 What are Omega-3 Fatty Acids?

Omega-3 fatty acids can be defined as a family of long-chain polyunsaturated fatty acids which include α-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) as shown in Figure 2-1. These three fatty acids have three or more double bonds (carbon-carbon), the first of which is located three carbons from the methyl end of the fatty acid (Holub, 2002). ALA is the parent compound of the omega-3 fatty acids; it is from ALA that EPA and DHA are formed through a series of desaturation and elongation reactions (Whelan and Rust, 2006).

Unfortunately, mammalian cells lack the enzymes required to add double bonds to (desaturate) fatty acids beyond the ninth carbon from the carboxyl end of the molecule (Whelan and Rust, 2006) and thus the omega-3 fatty acids are termed 'essential fatty acids' meaning they must be provided in the diet. The body does have some ability to convert the parent compound ALA into both EPA and DHA; however, the conversion rates appear to be low. Several review papers have been published looking at the conversion rates of ALA to EPA and DHA. Holub (2002) states that this conversion occurs with about 10-15% efficiency in humans, whereas Whelan and Rust (2006) state that the ALA conversion to DHA occurs with less than 1% efficiency. Although the numbers are not in total agreement, it is apparent that the conversion of ALA into the other omega-3 fatty acids is not a very efficient process but is able to occur within the body.

The omega-3 fatty acids have several specific metabolic and structural roles within the body. ALA is one of the most available fatty acids for normal β-oxidation, leading to the production of energy (Cunnane and Anderson, 1997). EPA competes with the omega-6 fatty acid arachidonic acid (ArA, Figure 2-1) for both the inclusion into phospholipid membranes as well as for the cyclooxygenase used during eicosanoid synthesis (Cunnane, 2003). DHA is an important component of complex neuronal membranes as well as in photoreceptor cells (Crawford *et al*., 1999).

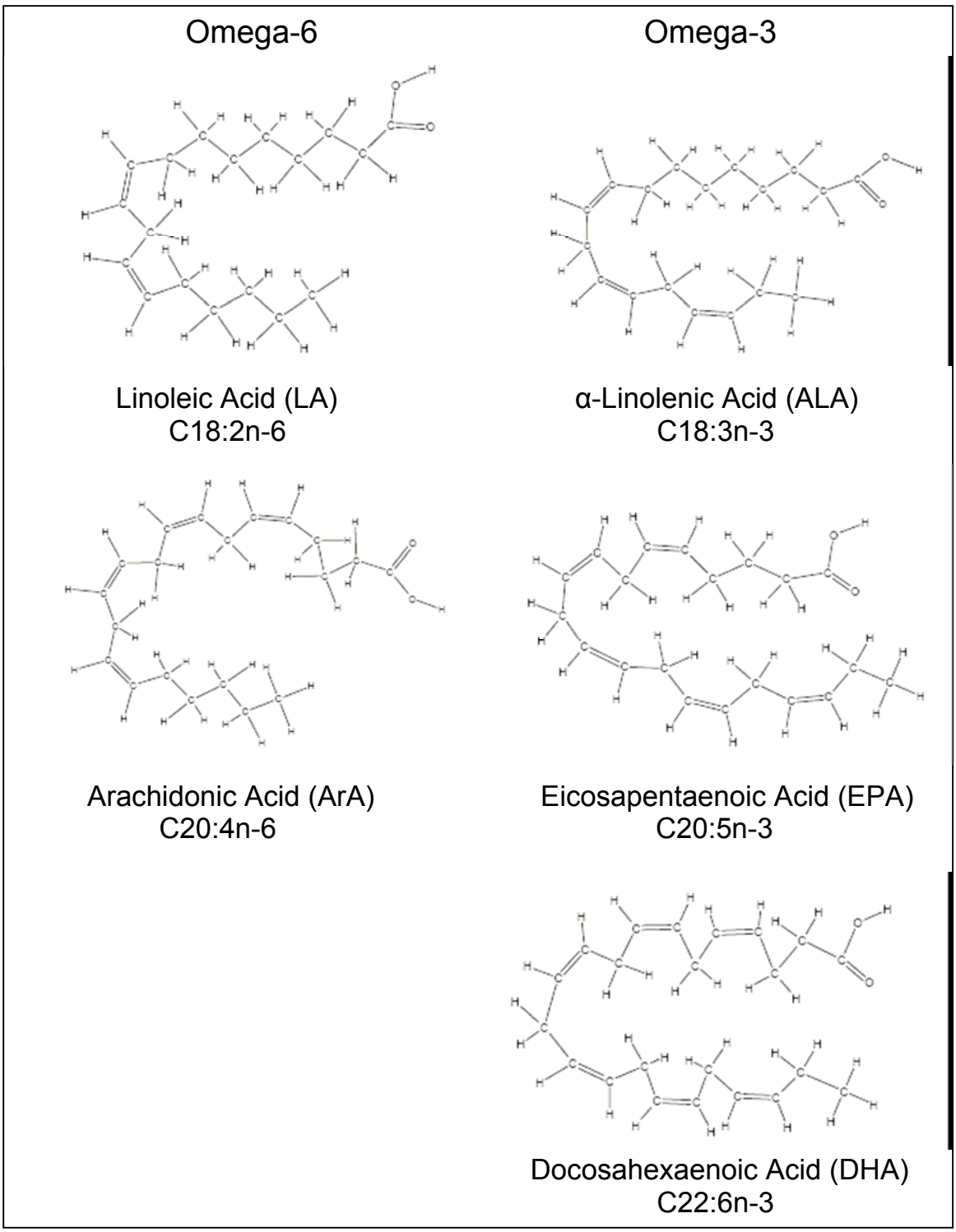

Figure 2-1: Chemical structures of linoleic acid (n-6), α-linolenic acid (n-3), arachidonic acid (n-6), eicosapentaenoic acid (n-3) and docosahexaenoic acid (n-3);
Modified from Holub (2002)

2.5.2 Omega-3 Fatty Acids and Health

Aside from the specific functions of the omega-3 fatty acids discussed above, recent evidence indicates that the omega-3 fatty acids have many potential health benefits for humans and animals.

2.5.2.1 Immunologic and Anti-inflammatory Properties

The immune system is an extremely complex system which contains many molecules involved in both the immunological and anti-inflammatory responses within the body. Dietary fatty acids can influence immunity by regulating the production or suppression of many of the immune molecules. Some of the key mediators of inflammation are the eicosanoids, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), both of which are derived from the
omega-6 fatty acid ArA. EPA, and thus indirectly ALA, can compete with enzymes required to convert ArA to PGE_2 and LTB_4, and in turn, lead to a reduction in numbers of these pro-inflammatory molecules (James *et al*., 2000). Other key mediators in the inflammation process are tumour necrosis factor α (TNFα) and interleukin 1β (IL-1β), which are inflammatory cytokines, and play a key role in the inflammatory disorder rheumatoid arthritis as well as in the cellular pathology of atherosclerosis (Caughey *et al*., 1996). A review article published by Calder (2001) discussed many studies showing that both dietary ALA and EPA can reduce production of TNFα and IL-1β, can reduce the chemotaxis of neutrophils and monocytes, and can reduce production of reactive oxygen species.

Although both inflammatory and immune responses are beneficial during acute phase injury or illness, these responses can become detrimental to the body over long periods of time. It may be beneficial for people with chronic immune or inflammatory responses to increase consumption of the omega-3 fatty acids in order to aid in the reduction of certain immune and pro-inflammatory molecules. The exact mechanisms by which dietary fatty acids can influence the immune system are not fully known; however, it may involve alterations in both gene expression and membrane lipid composition (Field *et al*., 2002; Lessard *et al*., 2003).

2.5.2.2 Anti-cancer Properties

There are several studies showing the potential benefits of omega-3 fatty acids in terms of reducing the risk of cancer (Dolecek, 1992; Johnston, 1995). Baro *et al*. (1998) showed that patients with colorectal cancer tended to have lower plasma levels of ALA, and Klein *et al*. (2000) showed

that elevated levels of both ALA and DHA in breast tissues are associated with reducing the risk of breast cancer. It is important to note however, that there are also studies published with opposite findings, indicating that increased levels of ALA can lead to increased risks of breast and prosthetic cancer (Vatten *et al*., 1993; Newcomer *et al*., 2001). The mechanisms involved in developing cancer are extremely complex, and can be affected significantly by diet, thus it is difficult to determine if dietary omega-3 fatty acids are beneficial or detrimental due to complex interactions. For example, a major dietary source of ALA for many people is meat, and studies have shown that high intakes of meat may increase the risk of certain cancers (Gionvannucci *et al*., 1993). Any negative affects seen by increased ALA could potentially be due to the source of the fatty acid, as opposed to the fatty acid itself. One of the main mechanisms proposed in the reduction of cancer risk is the reduction of several pro-inflammatory eicosanoids derived from arachidonic acid (Cunnane, 2003) as described previously.

2.5.2.3 Cardio-protective

Over the past several years there has been a growing interest in the effects of omega-3 fatty acids on prevention and management of cardiovascular disease. In large part, the basis for this interest stems from information based on epidemiological studies showing that consumers of diets rich in fish and fish oils show decreased mortality and morbidity from cardiovascular disease when compared with those who do not consume high levels of fish (Holub, 2002). The omega-3 fatty acids which are implicated in reducing the risk of cardiovascular disease are primarily EPA and DHA, both of which are found in high levels in many fish species. Although ALA is not believed to be directly responsible for the cardio-protective properties of the omega-3 fatty acids, it is important to remember that some dietary ALA can be converted into DHA and EPA and thus may have an indirect effect.

Holub (2002) summarized the various mechanisms by which EPA and DHA can be cardio-protective. He proposed several different mechanisms: 1) EPA and DHA enriched cardiac lipids can reduce malignant ventricular arrhythmias; 2) reduction in blood platelet reactivity can lead to a reduction in thrombotic effects as well as a reduction in plasma viscosity; 3) increased levels of HDL leading to a reduction of LDL and VLDL, thus potentially reducing the risk of atherosclerosis; 4) increased vasodilation due to enhancement of nitric oxide pathways; 5) reductions in inflammation and atherosclerosis through altering eicosanoid synthesis and reduced expression of cell adhesion

molecules; and 6) a reduction in the production of inflammatory cytokines such as interleukins and tumour necrosis factor.

2.5.2.4 Improved Cognitive and Visual Acuity

Over the past several years large amounts of research have been conducted to determine the effects of the omega-3 fatty acids on neural development and visual acuity. The mammalian brain contains large amounts of DHA (up to 35% of fatty acids) found primarily in membrane lipids (Innis, 2007). The amount of DHA found varies greatly with dietary intakes of the omega-3 fatty acids, as well as with age (levels increase with development but decrease as age increases) (Uauy *et al*., 2006). Although the exact mechanisms as to how DHA functions within the brain are unknown, several hypotheses have been proposed. Since DHA is present at high levels within brain membranes, and due to the fact that DHA is a polyunsaturated fatty acid, it is believed that this delivers a high degree of flexibility within the membrane thus increasing interactions between membrane proteins and, in turn, increases the speed of the neurotransmission processes (Chalon, 2006). Bazan (2006) and Kitajka *et al*. (2002) have proposed that unesterified DHA plays a role in regulating gene expression and the activity of ion channels. A study conducted in 2006 by Kawakiya and collaborators showed that DHA also plays an important role in neurogenesis. These are just some of the mechanisms proposed as to how DHA can improve cognitive ability in young mammals.

Along with improving brain function, it has been shown that supplementing dietary omega-3 fatty acids can improve visual acuity. Both pre-term (Carlson *et al*., 1991) and full-term (Carlson *et al*., 1996) infants fed milk containing supplemented DHA show improved visual acuity when compared to non-supplemented infants. In the second study by Carlson *et al*. (1996), DHA was supplemented at a level of 0.1% and significant differences in visual acuity were noted by 2 months of age. As mentioned above, DHA may help to increase the speed of neurotransmission, which may play a role in the improved vision. In a recent study by Bazan (2006), the roles of DHA in reducing oxidative stress-induced induction of inflammation and apoptosis in photoreceptor cells have been discussed, indicating another possible mechanism through which DHA may improve retinal function.

2.5.2.5 Reproduction

Reproductive processes are extremely complex and involve many different hormones and molecules. In general, the hormones play major roles in everything from estrous cyclicity to

maintenance of pregnancy and induction of parturition. Some of the major reproductive hormones are prostaglandins (PG), including $PGF_{2\alpha}$ and $PGF_{3\alpha}$. Prostaglandins are lipid molecules which contain 20-carbon unsaturated fatty acids such as ArA or EPA. Similar to the conversion of ALA to EPA, the omega-6 fatty acid linoleic acid can be converted into ArA through a series of desaturation and elongation reactions. ArA is the precursor fatty acid for the F-2 series of prostaglandin hormones while EPA is the precursor for the F-3 series of hormones. Competition between ArA and EPA for the desaturation and elongation enzymes could result in a decrease in $PGF_{2\alpha}$ and an increase in $PGF_{3\alpha}$ with increasing levels of dietary ALA or EPA.

The role of $PGF_{2\alpha}$ is to trigger luteolysis during estrus and pre-parturition. Luteolysis must be prevented in order for an animal to remain pregnant, and thus throughout pregnancy, the production of $PGF_{2\alpha}$ must be removed, reduced or re-routed. There are many signals, hormones and other molecules which are involved in this complicated process; however, it may be possible to aid in the maintenance of pregnancy by increasing the level of $PGF_{3\alpha}$ produced at the expense of $PGF_{2\alpha}$ (Ambrose *et al*., 2006). Recently, there have been several studies looking at the effects of increasing dietary omega-3 fatty acids through the addition of flaxseed on the reproductive performances of dairy cattle (Ambrose *et al*., 2006; Petit & Twagiramungu, 2006). These studies have shown that flaxseed intake can reduce abortion rates as well as increase the diameter of the ovulatory follicles. This is a fairly new area of research, and currently there appears to be very few scientific publications looking at the effects of omega-3 fatty acid intake on sow reproduction performance.

2.5.3 Consumer Trends and Demands for Omega-3 Enriched Products

Due to the many potential benefits of dietary omega-3 fatty acids, consumer demands for omega-3 enriched products are increasing. According to the dietary reference intakes (DRIs), the daily omega-3 fatty acid requirement for adult males is 1.6 g and for adult females is 1.1 g (National Academy of Sciences, 2005). The major dietary sources of ALA include vegetable oils such as soybean and canola oil. Flaxseed oil is the best land based source of ALA; however, intakes tend to be much lower for flaxseed oil when compared to other vegetable oils (Whelan and Rust, 2006). Nuts, seeds, vegetables, grains, legumes and fruits can also contribute to the overall ALA intake. Both EPA and DHA are not found in plant food sources, and are primarily found in fish, although terrestrial animals do contribute to the overall EPA and DHA intakes. In general, consumption of the omega-3 fatty acids is much lower than the DRI levels (Whelan and Rust, 2006), and thus consumers

are seeking new ways to incorporate these fatty acids into their diets without significantly changing their ingredient intake.

Many products are now produced to contain enriched levels of omega-3 fatty acids. These products include omega-3 enriched eggs, breads, pastas, dairy products, infant formula, baby food, milk, juices, cereals, salad dressings and meat, all of which are available for purchase at most grocery stores. Some of the most recognizable products include Neilson Dairy-Oh® Milk and Nestlé Good Start® baby formula. As consumer demand increases for these omega-3 enriched products, producers will be continuing to search for the most cost effective production methods.

2.5.4 Omega-3 Fatty Acid Enrichment of Pork

The ability to produce an omega-3 enriched pork product is becoming of great interest to many producers, as well as to consumers, as discussed above. In monogastric animals such as pigs, dietary fatty acids are absorbed from the gastrointestinal tract with relatively few changes. Essentially, the carcass fatty acid profile directly reflects the fatty acid profile of the animals diet. This is different to foregut fermenting animals such as cattle, in which the dietary fatty acids undergo changes due to microbial fermentation and biohydrogenation prior to absorption from the gastrointestinal tract. It is for these reasons that altering the carcass fatty acid profile of monogastric animals is simpler than altering the carcass fatty acid profile of ruminant animals. Enrichment of the carcass fatty acid profile of pigs can, in turn, lead to a greater consumption of omega-3 fatty acids by the consumer. Since flaxseed has a desirable fatty acid profile, many producers are interested in including it into their finisher pig rations with the goal of improving the fatty acid profile of meat.

Several studies have been conducted to determine first, if flaxseed can be used to enrich the swine carcass with ALA, and second, how much and for how long does flaxseed need to be fed to obtain optimal ALA levels in the meat products. Many of the studies also looked at the physical and sensory characteristics of the meat products as high levels of polyunsaturated fatty acids can cause off flavours and soft, runny fat.

One of the first studies looking at the effects of dietary flaxseed on carcass lipid profiles was conducted by Cunnane *et al*. (1990). Piglets were fed creep diets containing 5% flax from 2 weeks of age until 10 weeks of age. The piglets had significantly higher levels of ALA in their liver, kidney, heart, skin, subcutaneous fat and muscle, and significantly higher levels of DHA and EPA in the liver, kidney, and heart. This study provided evidence that inclusion of dietary flaxseed can improve the omega-3 fatty acid profile of pig carcasses.

After this, more research was conducted to determine the optimal dietary inclusion level of flaxseed and the appropriate length of time to provide a carcass enriched in omega-3 fatty acids without having negative impacts on carcass quality. Romans *et al*. (1995a,b) conducted two studies with the goal of answering these questions. In the first study pigs were fed diets containing 0, 5, 10 or 15% ground flaxseed for 25 days prior to slaughter. The authors observed a linear increase in the ALA content of both the backfat and bacon of the animals. The levels of ALA in the backfat of the pigs were 10, 23, 37 and 53 mg/g tissue for dietary inclusion of 0, 5, 10 and 15% flaxseed respectively. In this study they observed no negative effects on the physical characteristics of the carcass. Trained taste panellists were able to detect off-flavoured bacon from the pigs fed 10 and 15% flaxseed but were unable to detect differences in taste in the loin samples of the pigs. It is for this reason that the authors concluded that flaxseed should not be fed to finisher pigs above a level of 15% inclusion.

In the second study by Romans *et al*. (1995b), pigs were fed levels of 15% flaxseed, but the length of dietary treatment varied. Pigs were fed for 7, 14, 21 or 28 days prior to slaughter. ALA content of the backfat increased significantly in a linear manner with increasing length of time on the flaxseed diet. Significant effects of flaxseed on ALA content were noted after inclusion for just 7 days, but the level continued to increase with time. Actual ALA levels detected in the inner backfat layer were 3.9, 6.4, 11.0, 14.0 and 15.0 mg/g tissue for 0, 7, 14, 21 and 28 days respectively. It is also important to note that the diets used in the two studies by Romans *et al*. were corn based diets. Corn is rich in the omega-6 fatty acid linoleic acid, and thus the effects of flaxseed inclusion might not be as good as when the diets were wheat and barley based. Fontanillas *et al*. (1998), Matthews *et al*. (2000), Enser *et al*. (2000) and Thacker *et al*. (2004) are just some of the other studies showing similar effects of dietary flaxseed inclusion on the carcass fatty acid profile of finisher pigs.

It is also important to consider, when formulating diets containing ingredients with high levels of long chain unsaturated fatty acids such as flaxseed and/or flaxseed meal, the possibility of fatty acid oxidation. Fatty acid peroxidation occurs when an oxygen free radical species interacts with a fatty acid, of which polyunsaturated fatty acids are highly susceptible as it is easier to remove a hydrogen molecule from a double bond than from a single bond (Halliwell and Chirico, 1993). Hydrogen is removed from the fatty acid by the free radical, leaving behind a fatty acid radical which can interact with an oxygen molecule forming a lipid peroxyl radical. This process can also become self propagating if multiple double bonds are available (Halliwell and Chirico, 1993). This oxidative damage can result in the rancidity of dietary fats leading to off-flavours and the destruction of the fats. If this process is not prevented or stopped, the feed ingredient will affect palatability and will

reduce the amount of the polyunsaturated fatty acids found in the diets of the animals. It is thus important to ensure that when including ingredients with high levels of polyunsaturated fatty acids into swine rations, antioxidants are also included to help prevent this process from occurring. Antioxidants function by either reducing the number of reactive oxygen species present or stopping the oxidative damage process (Halliwell and Chirico, 1993). The most common antioxidants that can be included into the diets of pigs are vitamin E, selenium, butylated hydroxytoluene (BHT) and ethoxyquin.

Recently, in 2007, Prairie Orchard Farms in Manitoba began to market omega-3 enriched pork products. This is the first enriched pork product available on the market to Canadians. Little information is available as to how the company has achieved a consistent enrichment which has allowed them to market the product as a source of omega-3 fatty acids; however, their product also contains increased levels of antioxidants to help combat oxidative damage to the meat product. The Canadian Food Inspection Agency requires a product to contain 0.3 g or more of omega-3 fatty acids per reference serving amount in order to be labelled as a "source of omega-3 polyunsaturated fatty acids" (CFIA, 2003); thus the Prairie Orchard Farms pork products must contain at least 0.3g of omega-3 fatty acids per 100 g reference serving size. In fact, their loin cuts contain 1g of omega-3 fatty acids per 100 g reference size. At this enrichment level, a 100 g pork loin chop would meet 63% of the adult male, and 91% of the adult female daily omega-3 fatty acid requirement.

Although it is now well known that flaxseed can improve the fatty acid profile of pork, it is fairly costly for flaxseed to be included on a regular basis into finishing rations for swine. Flaxseed is an expensive feed grain, and thus many producers may not be inclined to include it in their rations. As previously mentioned, FSM contains much less total oil than full fat flaxseed; however, the fatty acid profile remains the same. It may be possible to create an omega-3 enriched pork product with dietary inclusion of FSM, which will be much more cost effective for producers due to the fact that it is a by-product feed ingredient.

2.6 Summary

Flaxseed meal is the by-product of the flaxseed processing industry. The total oil content of the meal product varies depending on the oil extraction method used, and this will affect the energy value for the feed. It contains a fatty acid profile which is similar, if not identical, to that of flaxseed. Approximately 56% of the total oil content is the omega-3 fatty acid ALA. The nutritional value of FSM for pigs is defined by a low lysine content which must be corrected for during feed formulation.

Flaxseed meal contains appreciable levels of certain amino acids such as tryptophan, methionine and cystine, as well as certain minerals such as phosphorus. There are several anti-nutritional factors found in FSM, including cyanogenic compounds, trypsin inhibitors, phytate and linatine. These anti-nutritional factors are unlikely to cause significant effects with the fairly low dietary inclusion levels that would be expected. FSM contains low levels of DE when compared with other meal products; however, it is potentially possible to include FSM into swine rations if diets are properly balanced for digestible lysine as well as for net energy. Previous studies have shown some negative impacts on growth performance with the inclusion of FSM into swine rations; however, the diets used in these studies contained levels up to 30% FSM, and were not formulated to meet both the digestible lysine content and net energy.

Overall, there is very little information available on the use of FSM for pigs. Accurate determination of digestible energy values and estimation of net energy for FSM will be key in proper dietary formulation. Determination of the availability of minerals such as phosphorus as well as for the amino acids will also be extremely important in allowing for accurate diet formulation. If all of the necessary information is available for proper diet formulation, it may be possible to incorporate FSM into swine rations without having negative impacts on growth performance. It may also be possible to take advantage of the fatty acid profile in terms of health benefits for the pigs, and as a means of enriching pork with the omega-3 fatty acid ALA.

3 NUTRITIONAL VALUE OF FLAXSEED MEAL FOR SWINE

3.1 Introduction

This study was conducted with the goal of determining the nutritional value of a novel feed ingredient for use within the swine industry. Prior to the adoption of a new ingredient into swine production systems, both the producers and their nutritionists must be comfortable with what an ingredient has to offer, and feed mills will be unwilling to give up valuable space to an ingredient which can not be utilized for broad applications.

In Europe, there is a continually increasing amount of flaxseed meal becoming available due to a continually growing flax crushing industry. The majority of this meal product is currently utilized by the ruminant industry. As more of this product becomes available, new outlets must be found for its use, and thus the rational behind determining its nutritional value for swine.

The overall objective of this project was to evaluate the nutritional value of flaxseed meal for pigs. The project was divided into four major components in order to evaluate the specific experimental objectives. The first component was designed to determine the complete chemical composition of flaxseed meal. The second component involved the determination of the energy value and apparent nutrient digestibility in growing pigs and gestating sows. The objective of the third component was to evaluate the growth performances of grower/finisher pigs fed graded levels of flaxseed meal, and to analyze the carcass fatty acid profile. The final component of this study was designed to determine the availability of phosphorus in FSM and to determine the effects of microbial phytase inclusion on this availability.

3.2 Materials and Methods

Flaxseed meal, supplied by Vandeputte s.a. (Mouscron, Belgium) was utilized for all experiments. Prior to laboratory analysis, the FSM was ground through a 1 mm mesh screen (Retsch Mill ZM1, Newtown, PA), and prior to inclusion into dietary rations the FSM was ground through a 1/8 screen using a hammer mill.

All laboratory analyses were conducted at the Department of Animal and Poultry Science, University of Saskatchewan, unless otherwise stated and all diet and faecal samples were ground through a 1 mm mesh screen (Retsch Mill ZM1, Newtown, PA) prior to analysis. All samples were analyzed in duplicate.

All animals utilized for these experiments were PIC Camborough Plus genetics (Camborough Plus females x C337 sires, PIC Canada Ltd. Winnipeg, MB) and were housed within their thermoneutral zones on a 12 hour light:dark cycle. The pigs were cared for according to the Prairie Swine Centre Inc. standard operating procedures, and animal use was approved by the University of Saskatchewan Committee of Animal Care and Supply protocol number 970019.

3.2.1 Chemical Analysis of Flaxseed Meal, Diets and Faecal Samples

Two separate batches of flaxseed meal (shipped several months apart) were analyzed for their chemical composition. The proximal analysis included measures of dry matter (method 930.15, AOAC 1990), ash (method 942.05, AOAC 1990), nitrogen (method 968.06, AOAC 1990 using a LECO FP-528 analyzer, St. Joseph MI, USA), ether extract (method 920.39, AOAC 1990 using a Soxhlet apparatus and petroleum ether), crude fibre (method 978.10 using crucibles, AOAC 1990), neutral detergent fibre (method 2002.04 using crucibles, AOAC 2002), acid detergent fibre (method 973.18 using crucibles, AOAC 1990) and gross energy by adiabatic calorimetry (IKA Calorimeter C5003, Wilmington NC, USA). Additional nutrient analyses included measures of total phosphorus content (dry ash method using ammonium metavanadate colorimetry; Soil and Plant Analysis, ICARDA, 2001), amino acid content (method 982.30E abc, AOAC 1990, University of Missouri-Columbia, Columbia MO), starch (Megazyme International kit, Wicklow, Ireland) and the fatty acid profile (GC analysis of fatty acid methyl esters adapted from O'Fallon *et al*., 2007) of the FSM.

Experimental diets and faecal samples were also analyzed for dry matter (method 930.15, AOAC 1990), gross energy by adiabatic calorimetry (IKA Calorimeter C5003, Wilmington NC, USA), nitrogen (method 968.06, AOAC 1990 using a LECO FP-528 analyzer, St. Joseph MI, USA),

acid insoluble ash (insoluble ash by gravimetry after treatment with 3N HCl) and ether extract (method 920.39, AOAC 1990 using a Soxhlet apparatus and petroleum ether). Diets were also analyzed for neutral detergent fibre (method 2002.04 using crucibles, AOAC 2002), acid detergent fibre (method 973.18 using crucibles, AOAC 1990), ash (method 942.05, AOAC 1990) and starch (Megazyme International kit, Wicklow, Ireland).

3.2.2 Energy Value and Apparent Nutrient Digestibility of Flaxseed Meal in Growing Pigs and Sows

3.2.2.1 Animals and Housing

A total of 32 barrows with initial weights of 70 ± 3 kg (mean ± SD) were randomly assigned to one of 4 dietary treatments containing 0, 10, 20 or 30% FSM. The barrows were housed in individual pens measuring 0.91 x 1.83 m (1.67 m^2), located within an intensive room at the Prairie Swine Centre. Each pen consisted of fully slatted, pre-cast concrete floors and solid PVC planked sides. Each pen had a 7.5 cm wide opening on the back wall, allowing for pig-to-pig contact. Single space dry feeders were located at the front of each pen, and nipple drinkers with *ad libidum* water access were located on the centre of the rear wall.

A total of 26 gestating sows were used for this experiment. Sows were between parities 2 and 4, with weights ranging from 190 to 300 kg (average of 245 kg). All sows were housed in gestation crates at the Prairie Swine Centre. Each crate measured 0.58 x 2.16 m (1.25 m^2), and consisted of partially slatted, pre-cast concrete floors and had a single space feeder located at the front of each sow. Water was provided *ad libidum* through nipple drinkers located beside the feeders. Sows were blocked by body weight for the purpose of ensuring adequate feed intake; sows weighing between 190 and 220 kg were fed 2.7 kg of feed/day, sows weighing between 220 and 260 kg were fed 2.8 kg/day and sows weighing more than 260 kg were fed 2.9 kg of feed per day.

3.2.2.2 Diets

Diets utilized for each digestibility study were the same. A total of four diets with increasing levels of FSM (0, 10, 20 and 30%) were prepared at the University of Saskatchewan using a horizontal mixer. The basal diet was composed of barley, wheat and soybean meal, and FSM was included at the expense of the basal diet, with the exception of mineral and vitamin supplements (Table 3-1). Celite® 545 (Celite Corporation, Lompoc CA, USA) was included into the diets as a source

Table 3-1: Dietary composition for determination of the energy value and apparent nutrient digestibility of flaxseed meal in growing pigs and gestating sows

% Flaxseed Meal	0%	10%	20%	30%
Diet Composition (g/kg As Fed)				
Barley	561	503	444	386
Wheat	250	224	200	175
Soybean Meal	150	135	120	105
Flaxseed Meal	-	100	200	300
Limestone	8.0	9.0	9.0	9.0
Dical-P	14.0	12.0	10.0	8.0
PSC Minerals[1]	4.0	4.0	4.0	4.0
PSC Vitamins[2]	4.0	4.0	4.0	4.0
Salt	5.0	5.0	5.0	5.0
Celite®[3]	4.0	4.0	4.0	4.0
Formulated Analysis (g/kg As Fed)				
DE (Mcal/kg)	3.07	3.11	3.16	3.20
Crude Protein	147.8	164.4	181.0	197.6
Fat	16.4	26.1	35.8	45.6
Calcium	6.8	7.2	7.3	7.3
Available P	4.3	4.3	4.3	4.3

[1]Provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite.

[2]Provided (per kg of diet): Vitamin A, 8250 IU; Vitamin D, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.

[3]Celite® 545, Celite Corporation, Lompoc CA, USA

of insoluble ash for use as an internal marker for digestibility calculations. All non-bulk ingredients were mixed with a Hobart mixer prior to adding to the bulk ingredients to ensure that all ingredients were mixed thoroughly. Gestating sows and barrows were randomly assigned to one of the four dietary treatments, for a total of 6 sows and 8 barrows per diet.

3.2.2.3 Sample Collection

All animals were on test for a total of 12 days: the first 9 days were used as an acclimation period followed by a 3 day faecal collection period. Faecal samples were collected by grab sampling directly from the pig, ensuring no faecal to floor contact occurred during collection. At the conclusion of the 12 day experimental period, all animals were able to re-enter normal barn production. Two days into the acclimation period, one sow was removed from trial after aborting her litter and replaced with another sow.

Faecal samples were kept frozen at -18 °C until the time of analysis. For each pig the faecal grab samples were pooled, mixed and an aliquot prepared for freeze-drying. After freeze-drying the samples were ground through a 1 mm mesh screen prior to laboratory analysis.

3.2.2.4 Calculations

The apparent digestibility of dry matter, energy, nitrogen, ether extract and ash were calculated for each pig based on the presence of the indigestible marker using the equation:

$$AD = \{1 - [(IA_d/IA_f)/(N_d/N_f)]\} \times 100 \quad \textbf{(3.1)}$$

where AD is the apparent digestibility, IA_d and IA_f are the insoluble ash levels in the diets and faeces, respectively, and N_d and N_f are the nutrient (dry matter, energy, ether extract or ash) levels in the diets and faeces.

Digestible energy (DE) content of the diets were then calculated using the equation:

$$DE = D_{GE} \times GE \quad \textbf{(3.2)}$$

where D_{GE} is the apparent digestibility of energy and GE is the gross energy content in the diets.

The apparent digestibility of each of the nutrients in FSM alone was then calculated both by use of regression equations, extrapolating for 100% FSM, and by means of a difference equation for each of the diets containing 10, 20 and 30% FSM. The average value of the results obtained by difference was then calculated. The difference equation utilized is as follows:

$$AD_{FSM} = [AD_{FSM\text{-based diet}} - (AD_{basal\ diet} \times \%\ nutrient_{basal\ diet})]/\ \%\ nutrient_{FSM} \quad \textbf{(3.3)}$$

where AD_{FSM} is the apparent digestibility of a nutrient for FSM alone, $AD_{FSM\text{-based diet}}$ is the apparent digestibility of the nutrient for the diet containing specified amounts of the basal diet and FSM (adjusted for the proportions of the nutrients in both the basal diet and FSM), and $AD_{basal\ diet}$ is the apparent digestibility of the nutrient for the basal diet alone.

The net energy (NE) value of FSM was then calculated by means of a prediction equation (Noblet *et al*., 1994):

$$NE = 0.7 \times DE + 1.58 \times EE + 0.48 \times Starch - 0.91 \times CP - 0.87 \times ADF \quad \textbf{(3.4)}$$

where NE is the net energy, DE is the digestibility of energy, EE is the ether extract, CP is the crude protein and ADF is acid detergent fibre content of the FSM.

3.2.2.5 Statistical Analysis

The apparent digestibility of nutrients in diets containing 0, 10, 20 and 30% FSM in both growing pigs and gestating sows were analyzed statistically using a completely randomized design one-way ANOVA model (SAS/STAT Version 9.1, SAS Institute, 2002) using the PROC MIXED function. Both linear and quadratic regression contrasts were included in the analysis. P values less than 0.05 were considered to be significant and all values are reported as the mean ± SEM.

3.2.3 Effects of Dietary Flaxseed Meal Inclusion on Performance and Carcass Fatty Acid Profile

3.2.3.1 Animals and Housing

In order to determine the effects of FSM inclusion on pig performance, a total of 200 growing pigs were housed in the grow/finish wing of the Prairie Swine Centre. A total of 40 pens were used for this experiment, each holding 5 pigs grouped by gender. Each pen measured 1.7 x 2.4 m (4.1 m^2) and consisted of fully slatted, pre-cast concrete floors and sturdy PVC planked walls. Each pen contained a single space feeder located on the side wall and a water nipple drinker located on the back wall.

The 200 pigs were divided evenly by gender (100 barrows and 100 gilts) and had an initial weight of 32 ± 4 kg (mean ± SD). Each pen was randomized within gender to contain approximately equal starting weights. For this experiment, four treatment groups were used, each with increasing levels of dietary flaxseed intake as described below. There were a total of 50 pigs per treatment group and thus there were 10 pens per treatment, 5 pens of barrows and 5 of gilts. Each pen was considered the experimental unit.

3.2.3.2 Diets

The four dietary treatments contained graded levels of FSM (0, 5, 10 and 15%). Each diet was further divided into three phases to better meet the requirements of the pigs as they grew, and to more closely mimic normal production practices in swine herds. The three phases were for pigs 32-60 kg, 60-85 kg and 85-115 kg. Diets were formulated to be balanced for both net energy content and digestible essential amino acids (Tables 3-2 and 3-3). As shown in Table 3-2, FSM was included at the expense of both wheat and soybean meal and peas were included into the rations to help balance the diets in terms of both net energy and lysine content (peas are high in NE and lysine where FSM is low in both of these nutrients). Within each phase the level of peas remained constant to ensure any effects of dietary treatment noticed would be due to the presence of FSM and not due to the presence of peas.

Diets for phase 1 were prepared at the PSC Elstow Feed Mill, whereas diets for both phase 2 and phase 3 were prepared at Coop Feeds (Saskatoon, SK). Each phase was prepared as they became required, thus ensuring that no diet was stored for long periods of time prior to feeding. Diets were stored in bins throughout the course of the experiment.

Table 3-2: Dietary composition of diets utilized to determine growth performance and carcass fatty acid profile

	Phase 1 (32-60 kg)				Phase 2 (60-85 kg)				Phase 3 (85-115 kg)			
FSM in Diet (%)	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
Diet Composition (g/kg As Fed)												
Barley	100	100	100	100	150	150	150	150	300	300	300	300
Wheat	595	572	543	514	572	547	518	498	464	435	405	376
Peas	150	150	150	150	180	180	180	180	150	150	150	150
Soybean Meal	125	100	80	60	75	50	30	0	60	40	20	0
Flaxseed Meal	0	50	100	150	0	50	100	150	0	50	100	150
Premix*	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Limestone	5.5	5.6	5.7	5.9	3.7	4.0	3.5	3.5	3.7	3.1	2.9	2.5
Dical-P	5.7	4.0	2.3	0.8	3.2	2.5	1.1	1.0	2.0	1.7	1.4	1.0
L-Lysine HCl	2.2	2.4	2.6	2.8	1.0	1.5	1.6	1.7	0.2	0.3	0.4	0.5
L-Threonine	0.8	0.8	0.8	0.8	0.3	0.5	0.4	0.4	-	-	-	-
DL-Methionine	0.3	0.2	0.2	0.2	0.2	-	-	-	-	-	-	-
Canola Oil	-	-	-	-	-	-	-	-	5.0	5.0	5.0	5.0

*PSC Mineral Premix[1], PSC Vitamin Premix[2] and Plain Salt (1:1:1)

[1]Provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite.

[2]Provided (per kg of diet): Vitamin A, 8250 IU; Vitamin D, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.

Table 3-3: Chemical analysis of diets utilized to determine growth performance and carcass fatty acid profile

	Phase 1 (32-60 kg)				Phase 2 (60-85 kg)				Phase 3 (85-115 kg)			
FSM in Diet (%)	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
Analysis (g/kg As Fed)												
Fat	19.0	18.0	26.0	34.0	19.0	23.0	33.0	38.0	21.0	30.0	31.0	42.0
NDF	139.0	144.0	157.0	151.0	114.0	129.0	137.0	152.0	126.0	143.0	144.0	149.0
DE (Mcal/kg)*	3.23	3.23	3.24	3.24	3.22	3.22	3.24	3.24	3.21	3.22	3.23	3.25
NE (Mcal/kg)*	2.32	2.32	2.32	2.32	2.32	2.33	2.33	2.34	2.35	2.35	2.35	2.35
SID Lysine*	8.3	8.3	8.4	8.5	6.7	6.6	6.7	6.6	5.4	5.4	5.4	5.4
SID Threonine*	5.3	5.3	5.2	5.3	4.4	4.3	4.3	4.3	3.8	3.8	3.8	3.9
SID Sulphur AA*	4.8	4.7	4.8	4.9	4.3	4.2	4.3	4.3	4.0	4.1	4.2	4.3
SID Tryptophan*	1.6	1.6	1.7	1.8	1.4	1.4	1.5	1.6	1.3	1.4	1.4	1.5
Calcium*	5.4	5.3	5.2	5.1	5.0	5.0	5.0	5.1	4.8	4.7	4.8	4.8
Total P*	3.9	3.9	4.0	4.0	4.3	4.4	4.5	4.7	4.0	4.3	4.6	4.8
Available P*	3.0	3.1	3.2	3.2	2.9	3.0	3.1	3.2	2.9	3.0	3.2	3.3
Fatty Acids (mg/g As Fed)												
Palmitic Acid	8.3	8.1	8.2	8.9	7.4	7.7	8.0	8.3	7.9	8.1	8.2	8.7
Stearic Acid	1.0	1.1	1.4	1.8	0.9	1.5	1.4	1.6	1.0	1.2	1.4	1.7
Oleic Acid	7.4	8.0	9.2	10.5	7.0	8.2	9.3	10.3	9.7	10.7	11.6	13.5
Linoleic Acid	15.9	15.5	15.9	17.0	14.4	14.3	15.7	16.3	15.8	15.9	16.3	17.6
α-Linolenic Acid	5.4	7.0	9.8	12.6	5.0	7.2	10.0	12.2	5.3	7.7	9.9	13.1

*Calculated Values, SID is standardized ileal AA digestibility

3.2.3.3 Sample & Data Collection and Analysis

Pigs were randomly selected and assigned to treatments one week prior to the start of data collection, allowing pigs to acclimate to their new environment and diets. Throughout the trial all pigs were weighed bi-weekly and average pen weights were determined. Feeder weigh-backs were recorded at the time of bi-weekly weighing for each pen and the amount of all feed added was recorded for the duration of the experiment. Average daily feed intakes for each pen were determined on a bi-weekly basis. Pen ADFI's were then divided by the number of pigs in the pen to obtain ADFI's for each pig per pen. As the average pen weight for each group of pigs reached the cut-off weight for one phase, they were changed onto the next phase of the same diet. As individual pigs reached a target market weight of 115 kg, they were removed from the pen and marketed weekly (Mitchells Gourmet Foods, Saskatoon, SK and Maple Leaf, Brandon, MB). At the time of market, 6 pigs per treatment group (total of 24 pigs) were randomly selected and sent to Drake Meat Processors (Drake, SK) where both backfat (inner and outer layers) and rib-end loin samples (*longissimus dorsi*) were collected for fatty acid analysis. All records of pig weights, feed intakes, feeder weigh-backs, calculations and the time at which pigs were removed from trial were kept electronically using Microsoft Excel.

Performance calculations for pigs were determined for each dietary phase (32-60 kg, 60-85 kg and 85-115 kg) and for the overall time period of the experiment (32-115 kg). These calculations included the average daily gains, average daily feed intakes and gain to feed ratios. Records were also kept on the number of pigs lost from test throughout the experiment. A total of 21 pigs were lost throughout the duration of the trial, dispersed amongst all treatment groups. Thirteen of the pigs were lost due to an outbreak of Circo Virus. These pigs were removed from test at the onset of symptoms to reduce the risk of spreading the disease. The other 8 pigs were removed from test either due to lameness or due to tail-bite.

Diet and faecal samples were collected and analyzed as previously described. Diets were also analyzed for their fatty acid profile (O'Fallon *et al.,* 2007). Loin samples were analyzed for their total ether extract content (AOAC 920.39 using a Soxhlet apparatus and petroleum ether) as well as for their specific fatty acid profile (O'Fallon *et al.,* 2007).

The fatty acids analyzed in this study were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1*n*-9), linoleic acid (C18:2*n*-6) and α-linolenic acid (C18:3*n*-3). These fatty acids were selected for analysis as they are the five major fatty acids found in FSM. Carcass, diet and FSM fatty acid composition was determined by gas chromatography using an Agilent 6890 system with Agilent

ChemStation software (Agilent Technologies, Mississauga, ON). Loin samples were trimmed of any visible backfat, and de-boned prior to analysis. Both loin (rib end) and backfat (both layers) samples were uniformly ground using a food processor (Moulinex DPA2, France) in the frozen state, and diets and FSM were ground through a 1mm mesh screen. Direct fatty acid methylation was then performed according to the procedure described by O'Fallon *et al*. (2007), with few minor differences. The samples were vortex-mixed using a single tube vortex instead of a multi-tube vortex, and samples were centrifuged (Beckman Coulter J6-MC Centrifuge, Mississauga, ON) for 5 minutes at 1500 rpm. Non-methylated C13:0 (Sigma-Aldrich, Inc., St. Louis, MO) was used as the internal standard, and all other chemicals used were of GC grade, and obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Pressurized helium, air and hydrogen were purchased from Praxair Canada Inc. (Mississauga, ON).

Fatty acid methyl ester samples were then compared to palmitic, stearic, oleic, linoleic and α-linolenic acid methylated reference samples (Nu-Chek Prep Inc., Elysian, MN) using the following GC program, slightly modified from O'Fallon *et al*. (2007): The machine was set for a 1.0 µl injection, split at a ratio of 30:1. The injector set points were a temperature of 260^{o}C, pressure of 25 psi and a total flow of 23.4 ml/min. The initial oven temperature was set to 140^{o}C and held for 5 minutes. The temperature was ramped up at a rate of 20^{o}C per minute to a maximum of 240^{o}C and held for 25 minutes. The total run time for analysis was 35 minutes. The stationary phase was a Supelco fused silica capillary column SP 2560 (Sigma-Aldrich, St. Louis, MO).

3.2.3.4 Calculations

For each phase of the experiment and for the overall experimental period, average daily gains, average daily feed intakes and feed conversion ratios were calculated for each pen of pigs as pen was considered to be the experimental unit.

Pen average daily gains were calculated by first determining the average daily gain for each pig within a pen over a certain time period (for example, phase 1 of the experimental period). This was determined by calculating the total weight gained for each pig over the time period and then dividing by the total number of days. The average value of each of the pigs in a pen was determined and considered to be the average daily gain per pig per pen for that period of time.

Average daily feed intakes were determined at the same time as each ADG calculation. These values were determined by calculating the total amount of feed added to a pen over a certain time period and subtracting the weight of any feed remaining at the end of the period. This value was then divided by the number of days providing the average daily feed intake for the entire pen, which was

further divided by the number of pigs in the pen providing the ADFI value on a per pig basis for each pen.

Feed conversion ratios (G:F) were determined for each period of time by dividing the ADG value per pig per pen by the ADFI value. All calculations could be made as frequently as every two weeks based on the bi-weekly schedule; however, values for the overall experimental period and for each phase of dietary treatment were the primary calculations. Frequent weighing provided the opportunity to improve observations on pig health.

3.2.3.5 Statistical Analysis

The effects of FSM inclusion on pig performance and carcass fatty acid profiles were analyzed using the PROC MIXED function of SAS (SAS/STAT Version 9.1, SAS Institute, 2002) as a randomized complete block two-way ANOVA design with gender as the block. Due to the fact that FSM inclusion is a continuous variable and not a completely separate treatment, means separation was not included in the analysis but linear and quadratic regression contrasts were. Average daily gain, average daily feed intake, gain to feed ratios and each fatty acid in the loins and backfat were analyzed using the same model. The total amount of fat in the loin samples was also analyzed with the same model to determine if dietary treatment had an effect on total fat content.

All data is presented with a P value for the diet, diet x gender interaction as well as linear and quadratic main effects. If a diet x gender interaction was present the data was then analyzed within each gender and the results for barrows and gilts presented. P values of less than 0.05 are considered significant in all cases, and all values reported are in the form mean ± SEM. Mean values reported in all cases are LS means.

3.2.4 Phosphorus Availability in Flaxseed Meal and the Effects of Microbial Phytase Inclusion

3.2.4.1 Animals and Housing

This experiment had a total of 5 dietary treatment groups, each with 8 barrows. The barrows were housed in individual pens at the Prairie Swine Centre as previously described in section 3.2.2.1. Each pen contained a single space feeder which was modified into a trough (without a feed drop) by blocking off the back opening with a sturdy plastic board. This allowed the use of feeders designed for the pens when feeding controlled amounts of wet feed. Pigs were an average of 46 ± 4.5 kg in weight at the start of this trial which lasted for a total of 15 days.

3.2.4.2 Diets

All diets fed throughout this experiment were semi-synthetic diets containing 30% of FSM as the only source of phosphorus. Increasing levels of microbial phytase (Phyzyme XP 5000G; EC 3.1.3.26, Danisco Animal Nutrition, Marlborough, UK) were added to the diets in order to determine the effects of phytase on phosphorus availability from FSM. Each diet contained Celite® as a form of insoluble ash for digestibility calculations. The dietary formulation for the 30% FSM diet is shown in Table 3-4. The diet was then divided into 5 separate treatments containing microbial phytase at levels of 0, 575, 1185, 2400 and 2570 FTU/kg diet (expected levels were 0, 500, 1000, 1500 and 2000 FTU/kg diet).

Ingredients used for the semi-synthetic diets included pea starch (Parrheim Foods, Saskatoon, SK), Solka-Floc® (International Fiber Corporation, North Tonawanda, NY) as a source of powdered cellulose, calcium caseinate (Burt Lewis International Corp., Chicago, IL) and dextrose (Tate & Lyle, Decatur, IL). All diets were prepared at the University of Saskatchewan.

Table 3-4: Dietary formulation for the determination of phosphorus availability

Composition	**g/kg As Fed**
Pea Starch	492
Flaxseed Meal	300
Solka-Floc®	38
Casein	60
Dextrose	60
Canola Oil	18
Salt	4.6
PSC Mineral Premix[1]	4.6
PSC Vitamin Premix[2]	4.6
Limestone	5.5
L-Lysine HCl	4.6
DL-Methionine	0.7
Celite®[3]	7.4
Formulated Analysis	**g/kg As Fed**
DE (Mcal/kg)	3.41
Crude Protein	145.0
Calcium	4.8
Available P	1.0

[1]Provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite.

[2]Provided (per kg of diet): Vitamin A, 8250 IU; Vitamin D, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.

[3]Celite® 545, Celite Corporation, Lompoc CA, USA

3.2.4.3 Sample Collection and Analysis

Throughout the experimental period, pigs were blocked by weight and then randomly assigned to one of the dietary treatments. Pigs were blocked by weight only to ensure that feed intakes were adjusted based on the size of the animals. The pigs were acclimated to their assigned diets for a period of 12 days prior to the start of faecal collections. Faecal grab samples were collected directly from the pig for a period of 3 days, each collection occurring after feeding. The experimental period thus lasted for 15 days. Each meal was mixed with water to increase palatability and offered twice daily (0800 and 1500) at a level of 90 g DM/kg $W^{0.75}$/day (3x the maintenance requirement).

Faecal samples were kept frozen for storage at -18^{o}C. For each pig the faecal grab samples were pooled, mixed and an aliquot prepared for freeze-drying. After freeze-drying the samples were ground through a 1mm mesh screen prior to laboratory analysis.

Diet samples were analyzed as previously described. Faecal samples were analyzed for dry matter (method 930.15, AOAC 1990), insoluble ash (insoluble ash, by gravimetry after treatment of the sample with HCl 3N) and total phosphorus (dry ash method using ammonium metavanadate colorimetry; Soil and Plant Analysis, ICARDA, 2001). Diets were also analyzed for their phytase activity level (analyzed by Danisco Animal Nutrition, Marlborough, UK).

3.2.4.4 Calculations

Apparent faecal digestibility values of the phosphorus in the experimental diets were calculated using the indigestible marker and the following equation:

$$AD = \{1 - [(IA_d/IA_f)/(P_d/P_f)]\} \times 100 \quad \textbf{(3.5)}$$

where AD is the apparent digestibility, IA_d and IA_f are the insoluble ash levels in the diets and faeces respectively, and P_d and P_f are the phosphorus levels in the diets and faeces.

The apparent digestibility of phosphorus for diets containing 30% FSM with added microbial phytase was then plotted and a regression equation calculated for the relationship between phytase level and phosphorus digestibility.

3.2.4.5 Statistical Analysis

The apparent faecal digestibility of dry matter and phosphorus in diets containing 30% FSM with added levels of microbial phytase in growing pigs were analyzed statistically using a completely randomized design one-way ANOVA model (SAS/STAT Version 9.1, SAS Institute, 2002) with the PROC MIXED function. Both linear and quadratic regression contrasts were included in the analysis. P values less than 0.05 were considered to be significant, and all values are reported as the mean ± SEM. The regression model for the effect of phytase inclusion on phosphorus availability was then plotted using Microsoft Excel. A broken-line regression analysis was performed using the PROC NLIN function in SAS (Version 9.1) to determine the optimal level of microbial phytase inclusion in terms of the apparent P digestibility. The single-slope model utilized was $y = L + U \times (R - x)$, where $(R - x)$ is defined as zero when $x > R$, as discussed by Robbins *et al*. (2006).

3.3 Results

3.3.1 Chemical Analysis of Flaxseed Meal

The proximate analysis and fatty acid profile of flaxseed meal utilized in these experiments are shown in Table 3-5. The flaxseed meal contained 133 g/kg ether extract on a dry matter basis which was characterized by a high α-linolenic acid content (64.7 mg/g of dry flaxseed meal, 47% of the total fat content). The fibre content of the flaxseed meal was also high, with the NDF fraction accounting for 250 g/kg of the dry matter. When compared to full fat flaxseed, the crude protein content was much more concentrated for flaxseed meal, accounting for 344 g/kg of the dry matter content.

The amino acid profile of the flaxseed meal is shown in Table 3-6, and is expressed as both g/kg of dry matter and as a percent of crude protein. The amino acid profile of flaxseed meal is well balanced when compared to the requirements of swine with the exception of lysine which falls well below the requirement of 5.3% of crude protein for growing pigs 20-50 kg (NRC 1998). This amino acid deficiency of flaxseed meal should not be an issue as long as it is taken into consideration when formulating swine diets.

Table 3-5: Analyzed chemical composition of two batches of flaxseed meal

Chemical Analysis (g/kg of Dry Matter)	Average	Standard Deviation
Moisture (%)	8.4	0.1
Crude Protein	343.5	2.8
Ether Extract	132.8	3.4
Ash	57.6	2.4
ADF	164.4	5.8
NDF	250.1	13.1
Crude Fibre	101.9	7.2
Starch	14.1	4.2
Phosphorus	8.8	0.1
Gross Energy (Mcal/kg)	5.2	0.01
Fatty Acids (g/kg of Dry FSM)		
Palmitic Acid (C16:0)	13.1	0.37
Stearic Acid (C18:0)	6.7	0.31
Oleic Acid (C18:1)	28.7	0.89
Linoleic Acid (C18:2)	25.5	0.09
α-Linolenic Acid (C18:3)	64.7	1.54

Table 3-6: Amino acid profile of flaxseed meal

Essential Amino Acids	**g/kg of Dry Matter**	**% of Crude Protein* (DM Basis)**
Arginine	31.4	9.10
Histidine	7.4	2.15
Isoleucine	14.4	4.18
Leucine	20.7	6.00
Lysine	13.3	3.85
Methionine	6.4	1.86
Met + Cys	11.8	3.42
Phenylalanine	17.0	4.93
Phe + Tyr	26.3	7.64
Threonine	12.6	3.65
Tryptophan	5.7	1.66
Valine	17.2	4.99
Non-Essential Amino Acids		
Alanine	15.5	4.50
Aspartate	31.5	9.14
Cysteine	5.4	1.56
Glutamate	63.0	18.27
Glycine	20.1	5.84
Proline	13.3	3.85
Serine	13.4	3.88
Tyrosine	9.3	2.71
Total	**317.6**	**92.14**

* Flaxseed meal contains 34.4% crude protein on a dry matter basis

3.3.2 Energy Value and Apparent Nutrient Digestibility of Flaxseed Meal in Growing Pigs and Sows

The apparent faecal digestibility of dry matter, nitrogen, ash, ether extract and energy for diets containing 0, 10, 20 and 30% flaxseed meal are shown in Table 3-7. In terms of the growing pig, with the exception of the nitrogen, the digestibility of the nutrients was different for each diet ($P < 0.05$). A linear effect ($P = 0.003$) in the digestibility of each nutrient, for which diet had a significant effect, occurred as the level of flaxseed meal increased in the diet. The apparent digestibility of the ether extract fraction increased linearly ($P < 0.001$) from 0.0 to 41.1% (± 0.79), whereas the digestibility of dry matter ($P = 0.005$), ash ($P < 0.001$) and energy ($P < 0.001$) all decreased linearly.

Similar to that of growing pigs, the apparent faecal digestibility of nutrients for sows differed for each diet ($P < 0.001$) with the exception of ash ($P = 0.182$). A linear effect ($P < 0.001$) was found for dry matter, nitrogen, ether extract and energy digestibility. For sows, the apparent digestibility of dry matter went from 82.0 to 77.2% (± 0.5) with increasing levels of flaxseed meal; the digestibility of nitrogen decreased from 84.3 to 78.2% (± 0.9) and the digestibility of energy went from 84.7 to 79.0% (± 0.5). Ether extract digestibility increased from 18.8 to 41.7% (± 1.6) with increasing levels of flaxseed meal in the diet.

The apparent digestibility of dry matter was higher for sows than for growing pigs. A linear decrease in nitrogen digestibility occurred within sows which did not occur with growing pigs; however, a linear decrease in ash was noticed for growing pigs and not for gestating sows. The digestibility of ether extract was higher for sows in diets containing 0, 10 and 20% of flaxseed meal; however, the EE digestibility for the 30% diets was similar between the two groups of pigs. The apparent digestibility of energy was also slightly higher for sows than for growing pigs.

Table 3-7: Coefficients of apparent digestibility (%) of the experimental diets in growing pigs (n=32) and gestating sows (n=24)

	% Flaxseed Meal in Diet					P Value		
	0	**10**	**20**	**30**	**SEM**	**Diet**	**Linear**	**Quadratic**
Growing Pigs								
Dry Matter	78.6	76.1	77.5	74.4	0.79	0.005	0.003	0.696
Nitrogen	75.6	72.3	76.1	73.6	1.09	0.063	0.666	0.691
Ash	47.0	37.6	44.3	36.5	1.54	<0.001	0.001	0.603
Ether Extract	0.0	18.9	41.3	41.1	6.14	<0.001	<0.001	0.105
Gross Energy	78.4	75.4	76.9	73.3	0.74	<0.001	<0.001	0.678
Gestating Sows								
Dry Matter	82.0	79.7	79.8	77.2	0.57	<0.001	<0.001	0.773
Nitrogen	84.3	81.7	78.3	78.2	0.90	<0.001	<0.001	0.179
Ash	36.7	31.3	33.5	31.8	1.80	0.182	0.141	0.321
Ether Extract	18.8	25.8	35.3	41.7	1.63	<0.001	<0.001	0.834
Gross Energy	84.7	81.8	81.5	79.0	0.53	<0.001	<0.001	0.756

The apparent digestibilities of the different nutrients, as well as the DE and NE contents, were calculated for flaxseed meal alone both by regression and by difference, and in all cases the proportion of the nutrient in both the basal diet and in the flaxseed meal were accounted for. The results are shown in Table 3-8. As calculated by regression, the digestibility of dry matter for flaxseed meal did not differ between growing pigs and gestating sows (72.0 vs. 68.0%). The apparent digestibility of nitrogen, and thus of crude protein, was higher for growing pigs than for sows, as was the digestibility of ash. The apparent digestibility of energy was slightly higher for sows than for growing pigs. The apparent ether extract digestibility was higher for growing pigs than for gestating sows. There were no differences in the digestible energy and net energy values for the gestating sows and for growing pigs.

As shown in Table 3-8, there were no major differences for apparent nutrient digestibility, DE or NE values of flaxseed meal alone for growing pigs or gestating sows. A slight difference between the two groups occurred in the apparent digestibility of energy, in which sows had a higher digestive capacity at 73.4% when compared to that of growing pigs at 70.8% (when calculated by regression). This difference in the digestibility of energy also carried forth to slight differences between growing pigs and gestating sows in terms of both DE and NE contents. The values determined through difference calculations were similar to those calculated by regression. In general, the apparent nutrient digestibility, DE and NE values obtained through regression calculations tended to be larger than those obtained by difference.

Table 3-8: Apparent digestibility of nutrients (%), DE and NE contents of flaxseed meal alone calculated by regression and by difference

	Growing Pigs (n=32)		Gestating Sows (n=24)	
	Average	**Std. Deviation**	**Average**	**Std. Deviation**
By Regression				
Dry Matter	72.0	2.4	68.0	2.2
Nitrogen	75.8	3.6	56.2	3.3
Ash	28.3	6.1	21.6	4.7
Ether Extract	67.4	26.3	48.7	9.6
Gross Energy	70.8	2.4	73.4	2.3
Digestible Energy (Mcal/kg)	3.52	0.10	3.58	0.06
Net Energy* (Mcal/kg)	2.46	-	2.49	-
By Difference				
Dry Matter	62.6	21.7	64.5	9.7
Nitrogen	57.9	33.9	51.5	15.8
Ash	30.6	20.7	18.7	14.6
Ether Extract	58.9	26.3	44.8	8.9
Gross Energy	62.4	17.7	67.5	8.3
Digestible Energy (Mcal/kg)	3.51	0.44	3.54	0.34
Net Energy* (Mcal/kg)	2.43	-	2.44	-

*Estimated by the prediction equation

NE = 0.7 x DE + 1.58 x EE + 0.48 x Starch – 0.91 x CP – 0.87 x ADF (Noblet *et al*., 1994), where the DE value used was the average DE value reported in the table.

3.3.3 Effects of Dietary Flaxseed Meal Inclusion on Performance and Carcass Fatty Acid Profile

The results of dietary FSM inclusion on pig performance in terms of average daily gain, average daily feed intake and gain to feed ratios are shown in Table 3-9. The results are divided into each phase of the experiment; phase 1, 32-60 kg pigs; phase 2, 60-85 kg pigs and phase 3, 85-115 kg pigs, and also includes the overall experimental values for pigs 32-115 kg. For each phase and for the overall experimental period, diet had no effect ($P > 0.05$) on ADG, ADFI or G:F. There were also no significant linear or quadratic main effects within phases or overall ($P > 0.05$). Diet by gender interactions were found within phases 1 and 2 as well as overall. In phases 1 and 2 the interactions were present for both ADG and G:F ratios, and for the overall experiment an interaction was found for ADG. The P values for these interactions are found in Table 3-9. The diet by gender interactions are detailed in Table 3-10.

As shown in Table 3-10, diet was not a significant factor for gilts at any point. The gender interactions occurred only within the barrow group, for which the dietary inclusion of FSM impacted ADG and G:F ratios in phase 1, and ADG for phase 2 as well as the overall experiment. Within phase 1 a quadratic effect was found ($P < 0.05$) for both ADG and G:F for barrows. A linear effect was present in terms of ADG and G:F for phase 2 ($P < 0.05$). Similar to phase 1, the ADG of barrows for the overall experiment had a quadratic effect ($P < 0.05$). In general, the presence of flaxseed meal in the diets of growing and finishing pigs did not have any detrimental impact on performance for gilts, but did have some minor effects for barrows in terms of average daily gains and thus feed conversion ratios.

The effects of dietary flaxseed meal inclusion on carcass fatty acid profiles are shown in Tables 3-11 through 3-15. The five fatty acids of interest were palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid as they are the five main fatty acids found in FSM. Table 3-11 includes the effects of diet, any diet x gender interactions as well as linear and quadratic main effects on the fatty acids found in backfat. Inclusion of up to 15% dietary FSM caused linear reductions ($P < 0.01$) for palmitic acid and stearic acid. Linear increases ($P < 0.01$) were present for both linoleic acid and α-linolenic acid. The primary fatty acid of interest was the omega-3 fatty acid α-linolenic acid, the level of which increased from 11 to 47 (± 0.8) mg/g backfat ($P < 0.001$). A diet by gender interaction was noted for linoleic acid, the details of which are shown in Table 3-12. In this table, it is apparent that diet did not significantly affect the linoleic acid content of barrows and gilts separately. The fact that significance is present in Table 3-11 but not in 3-12 can be explained by the number of

Table 3-9: ADG, ADFI and G:F ratios of pigs fed with graded levels of flaxseed meal (n=200)

	% FSM in Diet					P Value			
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Diet x Gender**	**Linear***	**Quadratic***
32-60 kg									
ADG (g)	897	847	837	873	21.3	0.206	0.029	0.395	0.052
ADFI (g)	2043	2003	2077	2012	55.9	0.782	0.380	0.940	0.824
G:F	0.44	0.42	0.41	0.44	0.014	0.258	0.038	0.517	0.078
60-85 kg									
ADG (g)	1003	995	1000	954	22.0	0.369	<0.001	0.158	0.394
ADFI (g)	2669	2756	2806	2779	99.1	0.784	0.924	0.398	0.569
G:F	0.38	0.37	0.36	0.35	0.014	0.410	0.009	0.095	0.943
85-115 kg									
ADG (g)	982	1031	989	985	24.4	0.458	0.105	0.764	0.286
ADFI (g)	3054	3144	3298	3130	102.3	0.406	0.704	0.410	0.216
G:F	0.33	0.33	0.31	0.32	0.014	0.590	0.684	0.434	0.863
32-115 kg									
ADG (g)	948	937	909	923	15.0	0.301	<0.001	0.135	0.412
ADFI (g)	2657	2648	2787	2670	65.9	0.419	0.442	0.550	0.419
G:F	0.36	0.36	0.33	0.35	0.011	0.238	0.089	0.249	0.341

* Linear and quadratic P values shown are for main effects. Diet by gender interactions (when present, indicated by $P < 0.05$ in diet x gender column) are shown in Table 3-10.

Table 3-10: ADG and G:F ratios for barrows and gilts where diet x gender interactions were present (n=200; 100 per gender)

	% FSM in Diet					P Value		
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Linear**	**Quadratic**
Phase 1 (32-60kg)								
ADG (g) – Gilts	868	818	876	824	26.9	0.335	0.548	0.971
ADG (g) – Barrows	926	876	798	922	32.9	0.049	0.550	0.018
G:F – Gilts	0.42	0.43	0.43	0.41	0.020	0.841	0.913	0.412
G:F - Barrows	0.47	0.42	0.38	0.46	0.019	0.013	0.418	0.003
Phase 2 (60-85kg)								
ADG (g) – Gilts	910	912	926	948	32.3	0.830	0.389	0.761
ADG (g) – Barrows	1096	1078	1074	960	29.7	0.020	0.007	0.126
G:F – Gilts	0.34	0.33	0.34	0.34	0.016	0.932	0.936	0.763
G:F - Barrows	0.41	0.41	0.37	0.35	0.022	0.248	0.055	0.757
Overall (32-115kg)								
ADG (g) – Gilts	894	898	896	864	22.2	0.670	0.367	0.428
ADG (g) – Barrows	1002	976	922	982	20.3	0.071	0.227	0.050

Table 3-11: Fatty acid content of backfat (mg fatty acid/g backfat) in pigs fed graded levels of flaxseed meal (n=6)

	% FSM in Diet					P Value			
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Diet x Gender**	**Linear**	**Quadratic**
C16:0	242	182	177	165	12.6	0.002	0.706	<0.001	0.072
C18:0	184	136	120	116	13.4	0.008	0.954	0.002	0.111
C18:1	293	295	293	284	5.5	0.555	0.905	0.284	0.353
C18:2	67	70	71	76	2.2	0.053	0.015	0.008	0.690
C18:3	11	21	34	47	0.8	<0.001	0.207	<0.001	0.130

Table 3-12: Fatty acid content of backfat (mg fatty acid/g backfat) for barrows and gilts where diet x gender interactions were present (n=6; 3 per gender)

	% FSM in Diet					P Value		
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Linear**	**Quadratic**
C18:2								
Gilts	69	77	75	81	3.3	0.171	0.065	0.724
Barrows	65	63	69	75	2.8	0.173	0.064	0.311

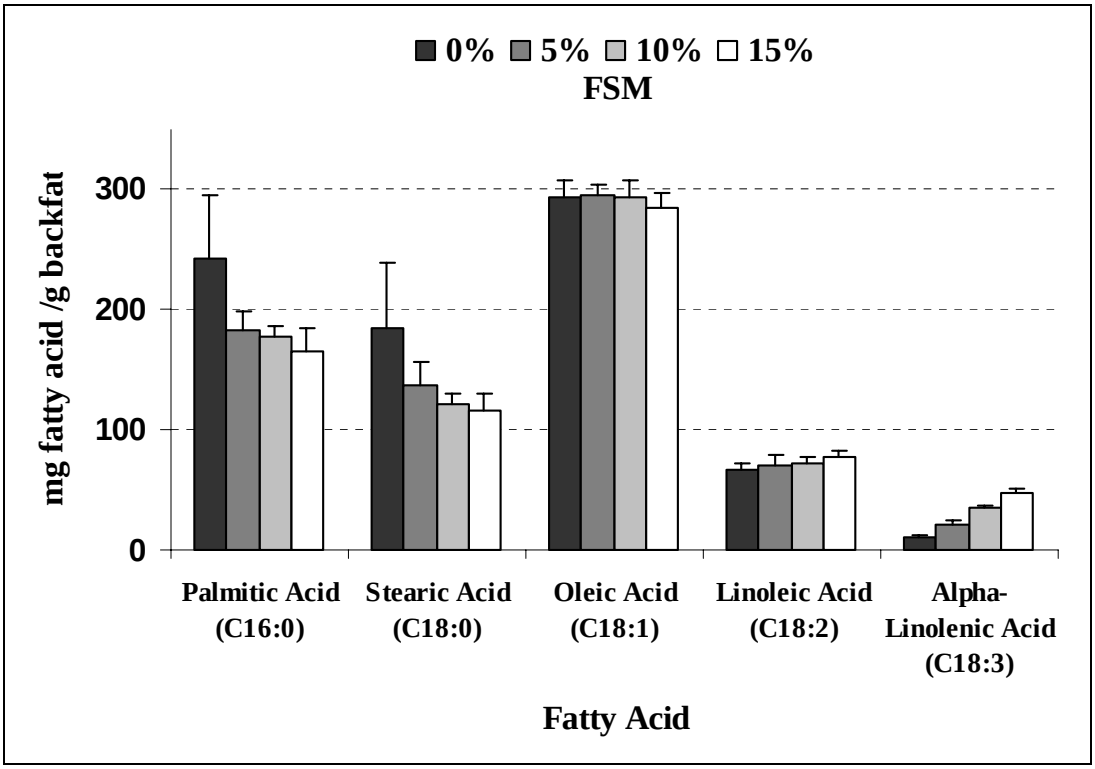

Figure 3-1: Fatty acid content in the backfat of pigs fed with increasing levels of flaxseed meal (selected fatty acids are the five main ones found in flaxseed meal)

pigs. The values presented in Table 3-11 are for 6 pigs total, whereas the values presented in Table 3-12 are for 3 pigs per gender, which is not enough to convey significance. The profile of the backfat fatty acids is shown in Figure 3-1.

The effects of dietary flaxseed meal inclusion on rib-end loin (*longissimus dorsi*) fatty acids are shown in Tables 3-13 and 3-14. Table 3-13 shows the effect of diet, any diet x gender interactions and the linear and quadratic main effects. Although the P values for the effect of diet on palmitic, stearic and oleic acids were greater than 0.05, they all fell between 0.05 and 0.1 indicating a tendency towards being different. A diet x gender interaction was also present for each of these three fatty acids in the loin samples, the details of which are shown in Table 3-14. A quadratic effect was found for each of the three fatty acids for barrows ($P < 0.05$); however no effect was noted for gilts ($P > 0.05$). Table 3-13 also shows that dietary FSM inclusion had effects on both linoleic acid ($P = 0.02$) and α-linolenic acid ($P < 0.001$). A linear increase occurred for linoleic acid from 13 to 16 (± 0.8) mg/g loin ($P = 0.019$), as well as for α-linolenic acid from 5 to 10 (± 0.4) mg/g loin ($P < 0.001$). The profile of loin fatty acids are shown in Figure 3-2.

Due to the fact that the main fatty acid of interest, α-linolenic acid, increased in the loin samples as dietary flaxseed meal increased, the total amount of fat in the loin samples was also analyzed to determine if the increase in C18:3 was due to an increase in total fat or due to an actual loin enrichment. The results of this analysis are presented in Table 3-15. Although the total fat in the loin samples ranged from 12 to 15% of ether extract (± 1.5), these values were not different due to the dietary treatment group ($P < 0.05$).

Faecal samples were collected during the third phase of the trial and analyzed for dry matter, gross energy, nitrogen and acid insoluble ash. The apparent digestibilities of these nutrients, as well as the DE content for these diets are shown in Table 3-16. The digestibility of DM, nitrogen and energy all decreased linearly with increasing levels of dietary flaxseed meal. The DE content for each diet was calculated to be 3.7 ± 0.01 Mkcal/kg of diet.

Table 3-13: Fatty acid content of loins (mg fatty acid/g tissue) in pigs fed graded levels of flaxseed meal (n=6)

	% FSM in Diet					P Value			
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Diet x Gender**	**Linear**	**Quadratic**
C16:0	39	38	48	36	3.0	0.057	0.036	0.963	0.098
C18:0	28	28	36	27	2.4	0.054	0.013	0.647	0.076
C18:1	59	59	75	57	4.8	0.064	0.047	0.619	0.086
C18:2	13	14	17	16	0.8	0.020	0.202	0.019	0.178
C18:3	5	6	9	10	0.4	<0.001	0.280	<0.001	0.478

Table 3-14: Fatty acid content of loins (mg fatty acid/g tissue) for barrows and gilts where diet x gender interactions were present (n=6; 3 per gender)

	% FSM in Diet					P Value		
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Linear**	**Quadratic**
C16:0								
Gilts	36	28	45	36	4.4	0.141	0.428	0.950
Barrows	42	48	51	36	4.1	0.125	0.432	0.037
C18:0								
Gilts	24	18	34	28	3.8	0.095	0.153	0.933
Barrows	31	37	38	25	3.1	0.055	0.256	0.014
C18:1								
Gilts	54	43	72	61	6.9	0.091	0.146	0.996
Barrows	64	75	78	53	6.8	0.118	0.393	0.032

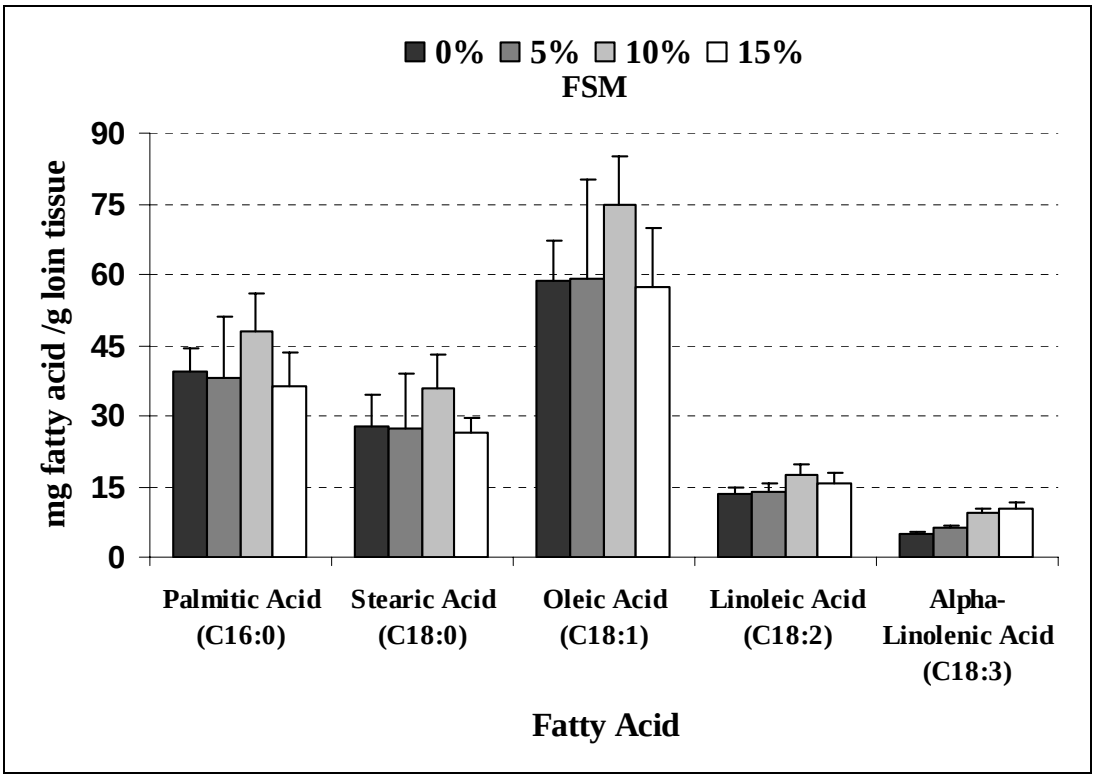

Figure 3-2: Fatty acid content in the loins of pigs fed with increasing levels of flaxseed meal (selected fatty acids are the five main ones found in flaxseed meal)

Table 3-15: Total fat content of loins (% ether extract) in pigs fed graded levels of flaxseed meal (n=6)

	% FSM in Diet					P Value			
	0	**5**	**10**	**15**	**SEM**	**Diet**	**Diet x Gender**	**Linear**	**Quadratic**
Total Fat	12	13	15	15	1.5	0.334	0.707	0.113	0.528

Table 3-16: Apparent Digestibility (%) of the phase 3 experimental diets in growing pigs (n=24)

	% Flaxseed Meal in Diet					P Value		
	0	**10**	**20**	**30**	**SEM**	**Diet**	**Linear**	**Quadratic**
Dry Matter	85.3	84.9	82.8	82.8	0.24	<0.001	<0.001	0.523
Nitrogen	84.5	83.4	81.2	80.9	0.63	0.002	<0.001	0.570
Energy	85.6	85.4	83.4	83.3	0.24	<0.001	<0.001	0.865

3.3.4 Phosphorus Availability in Flaxseed Meal and the Effects of Microbial Phytase Inclusion

Laboratory analysis of the five dietary treatments, each containing 30% flaxseed meal with added levels of microbial phytase, showed that the phytase activity levels were 127, 701, 1313, 2530 and 2696 FTU/kg diet. The diet containing 127 FTU/kg diet contained no exogenous phytase and thus the activity level was present due to endogenous phytase found in FSM itself. The diet contained 30% FSM, so by calculation, FSM alone has an endogenous phytase activity level of 423 FTU/kg. When accounting for this endogenous activity, the amount of microbial phytase added to each of the diets was 0, 575, 1185, 2400 and 2570 FTU/kg.

Table 3-17 shows both the apparent DM and P digestibility values for diets containing 30% flaxseed meal with increasing levels of microbial phytase. The effect of diet was significant for both nutrients ($P < 0.001$), as increasing levels of microbial phytase improved not only the P digestibility from 20.6 to 61.3% (± 2.5), a 40% increase, but also increased the digestibility of DM from 82.0 to 86.1% (± 2.5).

Both linear pattern and quadratic patterns were found for the apparent digestibility of DM and P. In the case of DM, the linear relationship was more significant ($P < 0.001$) when compared to the quadratic relationship ($P = 0.036$). In the case of apparent P digestibility, both the linear and quadratic relationships had $P < 0.001$, indicating that both relationships were significant. In order to determine which relationship was a better fit, linear and quadratic regressions were plotted and R^2 values were determined. A polynomial (quadratic) regression equation was determined to have the best fit with an R^2 value of 0.96 ($y = -7E\text{-}06x2 + 0.0347x + 17.942$) as compared to a linear R^2 value of 0.84 ($y = 0.0126x + 26.809$). Figure 3-3 shows both the quadratic and linear regression relationships between microbial phytase inclusion and apparent phosphorus digestibility in diets containing 30% flaxseed meal.

A broken-line regression analysis was conducted to determine the optimal level of microbial phytase inclusion for the improvement of phosphorus digestibility, where optimal is defined as the point at which additional phytase inclusion would not significantly improve the apparent digestibility of phosphorus. Figure 3-4 shows the graphic representation of this analysis, and as indicated by the dashed line, shows the microbial phytase inclusion level which was determined to be optimal through statistical analysis. The most effective level of exogenous phytase inclusion for improving P digestibility in diets containing 30% FSM is 1542 FTU/kg ($P = 0.05$); however, accounting for the 127 FTU/kg diet of endogenous phytase activity, the optimal exogenous inclusion level would be 1415 FTU/kg.

Table 3-17: Apparent digestibility (%) of DM and P, and P excretion (g P/kg DM intake) from pigs consuming diets containing 30% flaxseed meal with added levels of microbial phytase (n=40)

	Exogenous Phytase Level (FTU/kg diet)						P Value		
	0	**575**	**1185**	**2400**	**2570**	**SEM**	**Diet**	**Linear**	**Quadratic**
DM Digestibility	82.0	85.4	84.0	84.3	86.1	0.39	<0.001	<0.001	0.036
P Digestibility	20.6	41.0	49.9	53.5	61.3	2.52	<0.001	<0.001	<0.001
P Excretion	2.9	2.1	1.9	1.7	1.4	0.09	<0.001	<0.001	<0.001

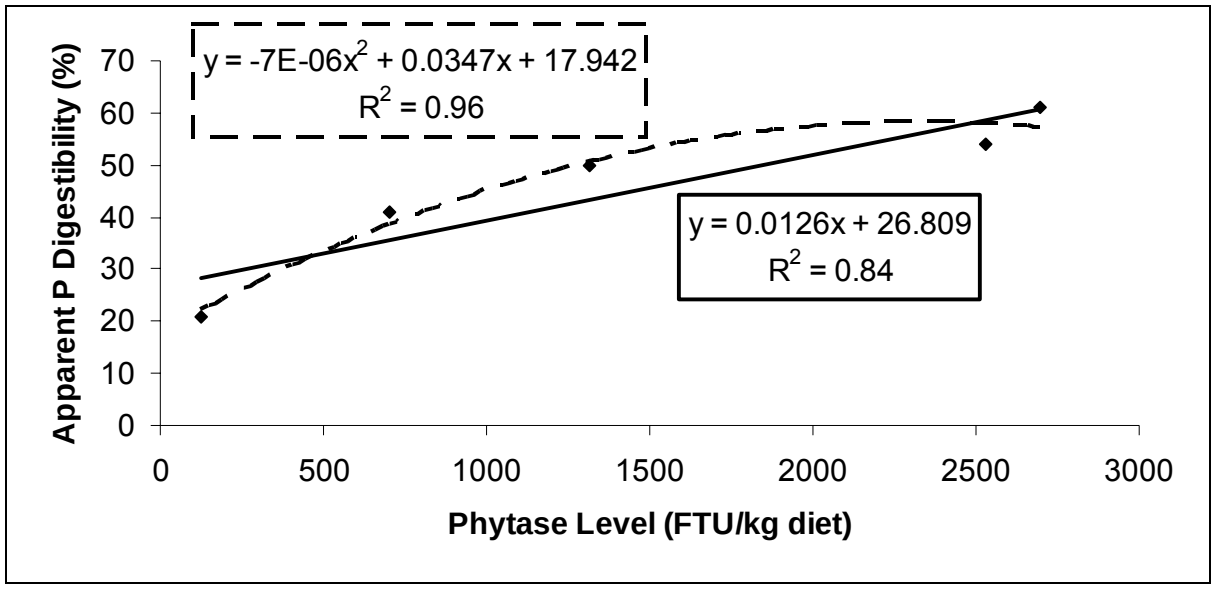

Figure 3-3: Apparent P digestibility in pigs fed a diet containing 30% flaxseed meal supplemented with increasing levels of microbial phytase

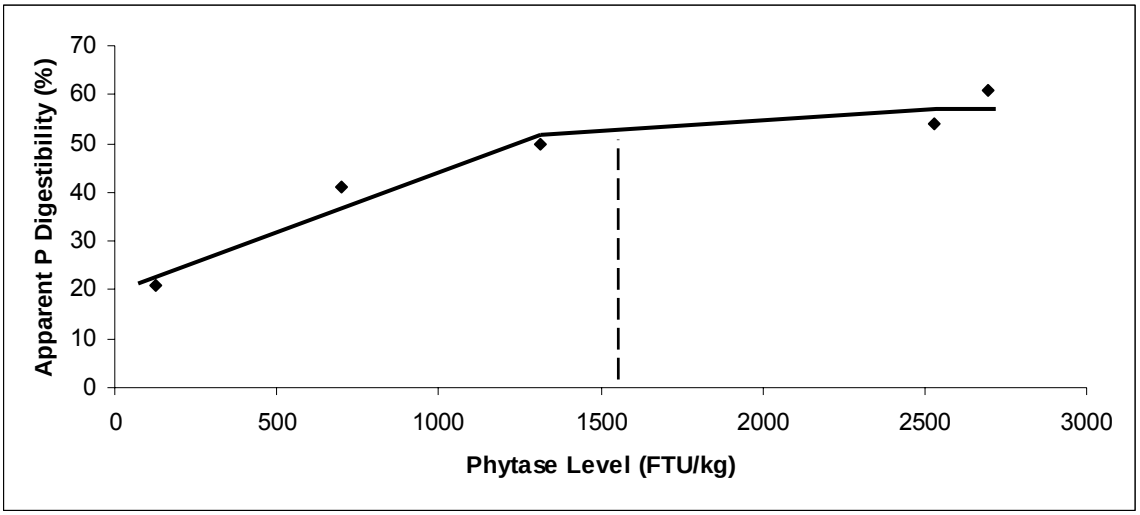

Figure 3-4: Point of optimal phytase inclusion as determined by broken-line analysis

The increase in exogenous microbial phytase from 0 up to 2570 FTU/kg of diet resulted in an increase in phosphorus absorption from 0.74 to 1450 mg per kg of dry matter intake ($P < 0.001$), and decreased phosphorus excretion into the environment from 2940 to 1450 mg/kg dry matter intake for a total of 1490 mg/kg dry matter intake reduction ($P < 0.001$). Both linear and quadratic relationships exist ($P < 0.001$) for the effect of microbial phytase inclusion on P excretion. Figure 3-5 shows the linear and quadratic relationships as well as the regression equations and correlation coefficients.

Inclusion of just 575 FTU/kg of dry matter intake accounted for half of the improvement in the apparent digestibility of phosphorus (20 percentage points) and increased the apparent P absorption by 0.65 g/kg of dry matter intake. This phytase inclusion level also reduced P excretion by 850 mg/kg of dry matter intake which is equivalent to 1.48 mg P/kg DM intake per unit of phytase activity.

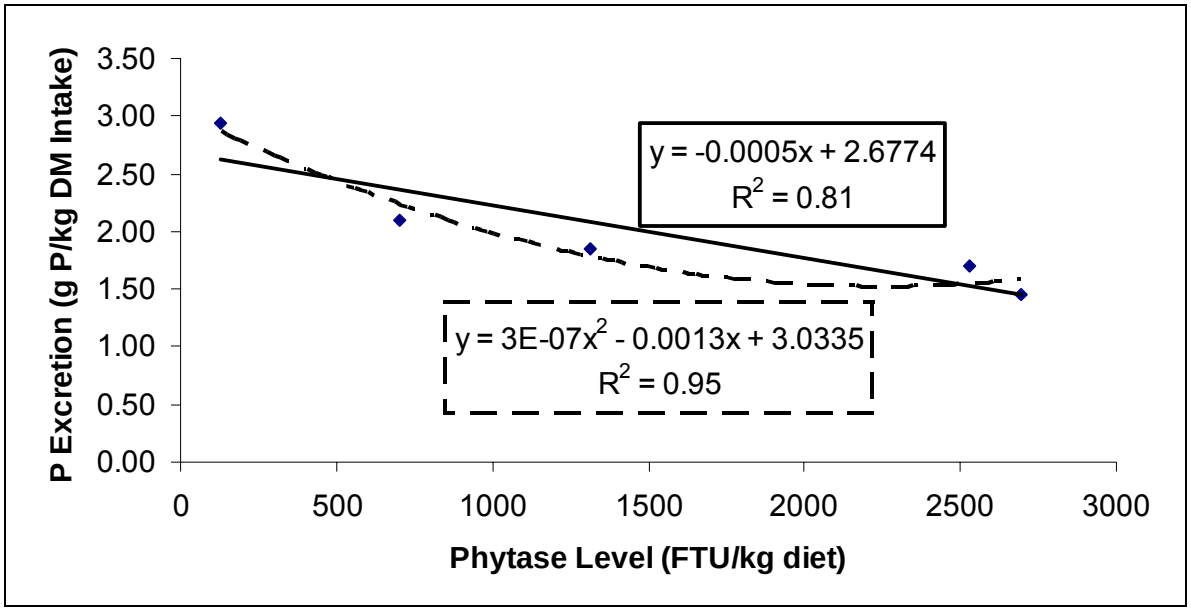

Figure 3-5: Phosphorus excretion from pigs fed a diet containing 30% flaxseed meal supplemented with increasing levels of microbial phytase

3.4 Discussion

3.4.1 Chemical Analysis of Flaxseed Meal

The chemical analysis of the flaxseed meal utilized throughout the course of this project was consistent with the average values reported in the literature (Batterham *et al*., 1991; Bell and Keith, 1993a; INRA, 2004). This indicates that the FSM provided by Vandeputte s.a. (Mouscron, Belgium) is consistent in terms of crude protein, ash, crude fibre, NDF, ADF and P content with other flaxseed meal products. The amino acid profile of the experimental FSM was also determined to be highly similar to reported values. The major difference between this FSM and those reported in the literature is the ether extract content. The average ether extract content in the literature is 560 g/kg on a dry matter basis, with minimum and maximum values of 20 and 83 g/kg respectively, as discussed in Table 2.1. The FSM utilized in these experiments contained 133 g/kg ether extract on a dry matter basis, as it was processed via press extraction without the use of solvents. When the fat content was calculated by summing the individual fatty acids analyzed, it was determined to be 139 g/kg, which is slightly higher than that determined by ether extract analysis. This can be explained by the fact that the fatty acid analysis procedure uses a more complete fatty acid extraction step than the basic ether extract analysis.

Despite the difference in processing methods, the overall chemical composition of flaxseed meal was unaffected. Increased levels of oil did not alter the chemical composition of the meal; however, it did have an effect on the gross energy content. As reported by INRA (2004), the gross energy content of flaxseed meal containing 30 g/kg oil was 4.1 Mcal/kg DM, and that of FSM with 80 g/kg oil was 4.4 Mcal/kg DM. The GE content of the experimental FSM was 5.2 Mcal/kg DM.

The chemical composition of FSM can also be compared to other oilseed by-products commonly utilized within the swine industry, such as canola meal. On a DM basis, flaxseed meal contained 344 g/kg CP, 164 g/kg ADF and 250 g/kg NDF. The NRC (1998) reports that solvent extracted canola meal contains, on a dry matter basis, a crude protein content of 396 g/kg, an ADF content of 191 g/kg and an NDF content of 236 g/kg. These chemical fractions are all similar to the values obtained for FSM, although the crude protein content is slightly higher. Within the protein fraction, canola meal contains a much higher lysine content than that of FSM which should also be considered when FSM is to be included as a dietary ingredient.

Overall, the chemical composition of FSM is relatively consistent across studies. This information will aid the swine industry in gaining confidence in a novel by-product as a feed

ingredient. Despite the fact that this product can be produced through many different processing techniques, it still provides a highly consistent chemical profile. In terms of the chemical composition, the only potential pitfall for the use of flaxseed meal by the swine industry is that the amino acid profile is characterized by a low lysine content (4.3% of crude protein) when compared to the NRC (1998) requirement level of 5.3% for growing pigs. This issue can be overcome by combining FSM with ingredients that contain high lysine levels such as peas, or by including synthetic lysine when deemed to be cost effective.

3.4.2 Energy Value and Apparent Nutrient Digestibility of Flaxseed Meal in Growing Pigs and Sows

In order to determine the DE content of flaxseed meal for pigs, an indirect method of determination was performed. When determining the DE content of a cereal grain, the ingredient can be fed as a sole ingredient and DE can be calculated directly, using an indigestible marker. Non cereal ingredients such as FSM can not generally be fed alone due to problems with palatability, or due to imbalances in protein or high fibre contents, and must be mixed with other ingredients. Two different indirect calculation methods can be employed to determine the DE content of an ingredient: regression or by difference, both of which were utilized in this study. In order to calculate by regression, graded levels of the test ingredient must be fed; in this case, graded levels of flaxseed meal (0, 10, 20 and 30%). To calculate the DE content of FSM by difference, only two diets are required, a basal diet containing 0% of the test ingredient, and the test diet containing the basal diet and the largest portion of the test ingredient possible.

For the difference calculations, it was extremely important to include as much FSM as possible into the diet to reduce any estimation errors that would occur when extrapolating for 100% FSM. Large inclusion levels of a test ingredient, however, can interfere with the digestion of ingredients found within the basal diet. In this study, inclusion of the graded levels of FSM not only allowed for the calculation of DE by regression, but also indicated the maximum level of FSM inclusion that could be used for DE calculations by difference. A plot of the dietary DE contents for both growing pigs and gestating sows yielded a decreasing linear relationship as dietary FSM increased ($P < 0.05$). A sharp change in this curve, indicated by a significant quadratic relationship, would be an indicator of an interaction occurring between the ingredient digestibility and that of the basal diet. Any inclusion level beyond a sharp curve change would not be adequate for calculating the DE content of a dietary ingredient, either by regression or by difference. This is also true when calculating the digestibility of nutrients such as dry matter, nitrogen and ether extract. In this study,

no quadratic effects were found for the apparent digestibility of different nutrients including DE, indicating that FSM did not interact with the basal diet at any inclusion level utilized. This allowed for the inclusion of values obtained for the 30% FSM diets into both the regression and difference calculations.

In general, both regression and difference calculation methods are adequate for the estimation of nutrient digestibility in a test ingredient; however, if large amounts of the test ingredient can be incorporated at the expense of the basal diet, calculations by difference reduce the costs associated with the experiment. As previously mentioned, difference calculations require only two diets whereas regression calculations require several more. In this study, the values obtained for the apparent digestibility of the different nutrients in FSM were slightly greater when calculated by regression than by difference; however, these differences were minor showing that both calculation methods provide similar results (Table 3-8). Since similar results can be obtained by either method, the difference method is more cost effective, and wherever possible, should be the method chosen for digestibility studies.

The apparent faecal digestibility of dry matter was not different for the two groups of pigs. The faecal digestibilities of ash and of nitrogen were greater for growing pigs than for sows; however, the digestibility of energy was slightly greater for sows. The apparent faecal digestibility of ether extract was greater for the growing pigs than for the sows; however, it is possible that problems in the faecal ether extract analysis in growing pigs may have occurred since the digestibility of energy and the DE and NE contents of FSM were greater for sows. If, in fact, the digestibility of either extract was greater for the growing pigs, the energy value associated with the meal product should also have been greater for that group.

The values obtained for the apparent digestibility of energy are similar to those reported in the literature. Bell and Keith (1993a) reported that digestibility of energy in FSM was 73%. The digestibility of energy in the present study was on average 65%. Consistency between values obtained in this study and the literature provide more evidence of the consistency of the chemical composition of the meal product, despite differences in processing methods. The faecal digestibility of DM (72% when calculated by regression) was also similar to that reported for canola meal (70%; INRA, 2004). Unlike the other common meal products for which the digestibility of ether extract is low (31% for canola meal; INRA, 2004), the oil digestibility of FSM was determined to be greater than 45%. Due to the fact that the oil digestibility varied greatly between sows and growing pigs, the value obtained should be confirmed in future studies. It is also important to understand that oil

digestibility values reported within the literature are highly variable, making comparisons between products with different oil contents difficult.

A major component in determining the nutritional value of an ingredient for use within the swine industry is the determination of the digestible energy and net energy contents. The DE content of FSM determined in this study ranged from 3.5 to 3.6 Mcal/kg depending on the calculation method and the age of the pigs. The NE content was then predicted using the DE content and the chemical composition of the meal. The NE content ranged from 2.4 to 2.5 Mcal/kg. Both the DE and NE contents of FSM did not differ between the growing pigs and sows.

It was unexpected to find that there was no difference in either the DE or NE content of FSM between the two ages, as sows generally have a greater capacity to digest and utilize nutrients from an ingredient. The growing pigs utilized for this study had initial weights of 70 (± 3) kg, thus providing an explanation for this lack of difference between the two groups of pigs. Growing pigs of this age would have well developed gastrointestinal tracts relative to young pigs, the digestive capacity of which would be comparable to that of sows. A large portion of the digestive capacity of an animal is related to the dietary retention time in the gastrointestinal tract. The longer a diet or ingredient can be retained within the GIT, the greater the time available for digestion. Although values of mean retention times (MRT) reported in the literature are highly variable, Le Goff *et al*. (2006) reported that adult sows have a MRT of 81 hours and Van Leeuwen *et al.* (2006) reported that pigs between 50 and 120 kg body weight had an average MRT of 75 hours. This is significantly different from the MRT reported for young pigs (33 kg body weight), which was 33 hours (Le Goff *et al*., 2002). Since the MRT between the two groups of pigs used for this study is comparable, it would be expected that the DE and NE contents of FSM for the two groups would also be similar. Recent findings by Noblet *et al*. (2008) also show that there is no difference between growing pigs weighing 60 kg and sows in terms of the DE and NE contents of ground and extruded flaxseed.

The DE content of FSM for both growing pigs and sows is approximately equal to the reported DE values of canola meal (2.8-3.0 Mcal/kg) and soybean meal (3.5-3.7 Mcal/kg) as reported by INRA (2004). The NE values determined in this study exceed the NE values reported for other oilseed meals. Canola meal is reported to have an NE content of 1.5-1.6 Mcal/kg, whereas soybean meal has an NE content of 1.9-2.0 Mcal/kg (INRA, 2004).

The net energy system assigns an energy value which takes into account not only the energy lost in the faeces, but also considers the energy cost associated with feeding a specific ingredient. DE overestimates the real energy value of an ingredient containing high levels of crude protein and dietary fibre but underestimates that of ingredients high in fat. These differences can be explained by

looking at the metabolism processes involved in utilizing an ingredient as an energy source. Fibrous feeds are digested largely through microbial fermentation processes. During this process, a large part of the ingredient disappearance can be attributed to the production of gases such as methane and carbon dioxide, which are lost to the atmosphere, and can not be utilized by the animal for energy. In the case of fat, the animal is able to absorb this readily from their GIT. Once absorbed, fat can be used directly for energy production through β-oxidation and the Krebs cycle, or can be easily incorporated into body tissues and stored as a future energy source. There are smaller energy losses from the animal associated with feeding dietary fats when compared to fibrous feeds.

The FSM utilized in these studies contained 133 g/kg fat on a dry matter basis (>45% of which is digestible), whereas canola and soybean meals generally contain up to 2 or 3% (with only 30% digestibility). In terms of the actual energy that FSM provides to the pig, the net energy, the nutritional value of the product is greater than that of other common oilseed meals utilized by the industry.

3.4.3 Effects of Dietary Flaxseed Meal Inclusion on Performance and Carcass Fatty Acid Profile

3.4.3.1 Growth Performance

Throughout the course of the experimental period, overall average daily gains were not affected by the inclusion of dietary FSM ($P > 0.05$); however, minor decreases in gains were found, which could be of concern for producers. In the weeks just prior to the start of this study, an outbreak of Circo Virus occurred within the barn. It is highly possible that the shifts in gains could be attributed to this virus outbreak. Several pigs were lost throughout the course of the trial due to suspected virus contraction. Pigs were affected across all treatment groups; however, 70% of those lost were from the 5 and 10% FSM treatment groups, the two groups which appear to have greater shifts in their average daily gains during phase 1. Calculations of pen average daily gains and average daily feed intakes had to be adjusted based on the losses of these pigs. Also, it is largely possible that many pigs, although not symptomatic, had sub-clinical infections which could easily affect weigh gains. Both sub-clinical and clinical infections can contribute significantly to reductions in weigh gain and to reductions in feed conversion ratios as the body is working towards clearing the infection instead of towards growth production (Le Floch and Sève, 2000). Many metabolism processes are involved in fighting an infection. The energy cost associated with these metabolic processes involved

in the recruitment and production of immune cells is extremely large, thus reducing the energy available to the animal for non-essential processes including growth. The average daily feed intakes were similar between all treatment groups, and thus inclusion of up to 15% FSM did not reduce diet palatability for pigs between 32 and 115 kg of weight, and the suspected virus did not have significant impacts on feed intakes.

Previous studies have shown that inclusion of dietary flaxseed meal beyond 10% had negative impacts on the growth performance of the animals, as discussed in Table 2.3. In the case of Batterham *et al*. (1991), who utilized FSM containing 30 g/kg oil, there were several potential reasons for this impact on performance. First, it is possible that the authors overestimated the amount of energy actually available in FSM for the pigs as the diets were formulated using the DE system. This study also utilized an inclusion level of 30%, which presented palatability issues since the feed intakes decreased. Bell and Keith (1993a) also noticed a decrease in pig performance with increasing dietary FSM (containing 55 g/kg oil) inclusion. That experiment incorporated the FSM at the expense of soybean meal without properly correcting for the amino acid supply or DE content of the feedstuffs, thus potentially affecting animal performance which was attributed to the inclusion of the FSM. It was essential then, to ensure that the dietary formulations utilized in the growth performance trial of this project were balanced in terms of NE and digestible essential amino acid contents. At the time of dietary formulation, the NE content of the FSM containing 133 g/kg oil had not yet been determined, and thus an estimate was made based on the NE value of FSM containing 80 g/kg oil presented by INRA (2004), assuming that the oil content was 100% digestible.

Inclusion of dietary FSM beyond 15% is not likely to occur for several reasons. First, inclusion may be limited due to the presence of some anti-nutritional factors as previously discussed. Second, and by far the main reason for limited inclusion, is the difficulty involved in formulating diets with high inclusion rates to meet the nutrient requirements of the pigs. Flaxseed meal contains both a low lysine content and a low NE content. In order to ensure that growth performance is not compromised, it is extremely important that the diet meets all of the nutrient requirements of the animals. Lysine is the most limiting nutrient for swine, and thus it will be difficult to include high levels of an ingredient in a diet with a low lysine content such as FSM. Diets must also be formulated to meet the energy requirements of the animals for their stage of production, whether is be for maintenance, growth and lean meat deposition or for reproduction. Flaxseed meal has a low NE content and will therefore be limited in its inclusion when a diet is formulated properly to be balanced for its energy level. Maximum levels of FSM inclusion will occur if diets are formulated to

contain an ingredient which complements FSM such as field peas, which contain high levels of both NE and lysine.

3.4.3.2 Carcass Fatty Acid Profile

As consumer demands are rising for health conscious products such as those which contain the presence of omega-3 fatty acids, the swine industry has been seeking ways to enrich the pig carcass with these beneficial fatty acids. A product which provides an additional benefit beyond its purely nutritive value can be sold within a niche market, and can be considered as a functional food source for consumers. The use of full fat flaxseed as a means of enriching the swine carcass with α-linolenic acid has been under investigation for several years now. Many studies have been successful in this endeavour; however, inclusion levels beyond 12 or 15% have negative impacts on meat quality and taste (Romans *et al*., 1995). Although FSM is a defatted by-product, the potential to enrich the swine carcass with ALA remains. As previously discussed, the FSM utilized throughout this experimental period contained 13% oil on a dry matter basis. This means that a diet containing 15% FSM is equivalent in terms of oil content to a diet containing approximately 4-5% flaxseed, and would be unlikely to affect the quality and taste of the meat product.

Results from our study indicate that inclusion of up to 15% FSM in the diets of finishing pigs significantly impacted the carcass fatty acid profile. The ALA content increased linearly in both the loins and backfat of the pigs with increasing FSM levels ($P < 0.05$). In the loin samples, a 15% FSM diet provided 1 g of ALA per 100 g sample, which is sufficient in terms of CFIA labelling standards for an omega-3 enriched product, the requirement of which is 0.3 g of omega-3 per reference sample size (100 g in the case of pork loin). It is important to note however, that this study analyzed only six pigs per treatment group which is not likely to be enough to determine the consistency of the fatty acid profile. The loin samples of pigs consuming 10% FSM had larger quantities of palmitic, stearic, oleic and linoleic acids when compared to pigs fed 0, 5 and 15% FSM. A study with more pigs would be able to determine if these results are a true representation of the fatty acid profile, or if these results were due to the small number of pigs analyzed. This study can be used to show that FSM contains great potential to create an omega-3 enriched pork product, but more work should be conducted utilizing large numbers of pigs to determine the optimal level and length of dietary FSM inclusion to obtain consistent carcass enrichment. Flaxseed meal inclusion within this experiment also reduced the amount of saturated fat found in the backfat samples from the pigs; however, this

was not noticed for the loin samples. It is possible that an effect on saturated fatty acid content could be found in a larger study was performed.

Another major concern present when determining the effects of an ingredient on the fatty acid content of meat is the potential of increasing the total fat content of the product. This study revealed that a slight increase in total loin fat did occur with increasing dietary FSM; however, this increase was not statistically significant ($P > 0.05$). When looking at the individual fatty acids, the total fat content decreased in the loin samples as the level of dietary FSM increased. This appears to be contradictory to the rise in total ether extract content which occurred with increasing FSM. In the individual fatty acid analysis, only the five main fatty acids found in FSM were analysed in the loin samples; however, several other fatty acids would also be present in the loins which were not analyzed, and could account for this discrepancy between total fat when determined by summing the individual fatty acids or by looking at the ether extract contents of the loins. Again, it is also recommended that a larger scale study be conducted to determine the effects on total fat content as six pigs per treatment group may not show all of the potential differences that could occur.

Overall, up to 15% FSM can be incorporated into the diets of growing and finishing pigs without having adverse affects on pig growth performance or feed intakes, and also can lead to an enrichment of the final pork product with the omega-3 fatty acid ALA. This information will aid producers in developing an acceptance for use of this novel product within the swine industry. There is the potential to utilize this product not only to diversify rations and prospectively reduce costs, but also to produce a product which can be sold within a niche market.

3.4.4 Phosphorus Availability in Flaxseed Meal and the Effects of Microbial Phytase Inclusion

In order to determine the bioavailability of phosphorus and the effects of phytase inclusion, it was important that the diets utilized contain only one organic P source, namely the FSM. This was achieved by feeding a semi-synthetic diet, with the only plant ingredient being the FSM. The use of a typical diet with added FSM would prevent the calculation of P availability from the flaxseed meal alone, as the dietary P would be coming from several ingredients. There are issues that can arise with feeding semi-synthetic diets, a major one being a reduction in diet palatability. To improve palatability and improve feed consumption, the diets were mixed with water just prior to feeding. These diets contained a high level of purified starch, which, if not mixed with water would be difficult for the pigs to consume. Also, in order to ensure that diet consumption was not a concern

during the faecal collection period, pigs were acclimated to their diets for a period of 15 days, and were fed at a level corresponding to 90% of *ad libidum* feed. By day 5 of the acclimation period, all feed was being consumed by all pigs on trial.

As determined in this study, FSM contains an endogenous phytase activity level of 420 FTU/kg. Common feed ingredients utilized in Western Canada such as wheat and barley contain 460 and 540 FTU/kg of endogenous phytase respectively, whereas common oilseed by-products such as canola meal and soybean meal contain, respectively, only 10 and 20 FTU/kg (INRA, 2004). This high level of phytase activity may improve the digestibility of P in FSM when compared to other oilseed meals.

The response of apparent P digestibility in FSM to graded levels of exogenous phytase inclusion, as determined in this study, follows a quadratic response. Although both a linear and quadratic pattern was found to be significant, the quadratic regression curve had a more significant R^2 value. Traylor *et al*. (2001) found a similar quadratic response when looking at the effects of graded phytase inclusion on the digestibility of P in semi-purified soybean meal based diets. It would be expected that this type of response pattern exists due to the fact that there will become a point at which the addition of a certain level of the enzyme will no longer increase the availability of phytate bound P, either because there is no more phytate bound P to release or because the molecular structure may be limiting the access points for efficient enzyme hydrolysis. The point at which the curve begins to plateau could be considered the level of optimal inclusion, as anything beyond that point will not have any major effects for the animal.

The broken-line analysis conducted for this experiment indicated that the optimal inclusion level of exogenous phytase for diets containing 30% FSM is 1415 FTU/kg of diet. This estimate is not the exact value at which the breakpoint occurs due to the fact that the line after the breakpoint still has a slightly increasing slope. If this second line had a slope of zero, the estimated value should correspond directly to the breakpoint of the line. In this experiment, only 5 treatment groups were utilized. If just one of these data points were skewed, the estimate obtained from the break-point analysis would also be skewed. As shown in Figures 3-3 and 3-4, the final two points on the curve were close together in terms of the phytase activity levels. The expected phytase activity levels were 1500 and 2000 FTU/kg; however, the actual levels were 2400 and 2570 FTU/kg. It is possible that one of these points is an outlier; however, without the presence of more treatment groups it is not possible to determine which of the two points could be incorrect. The apparent digestibility of P appears to plateau between 1185 and 2400 FTU (4% increase), but a 7% increase occurs between 2400 and 2570 FTU/kg. It is possible that the final data point is skewing the data upwards, which

would increase the estimate for optimal inclusion levels, or that the fourth data point is skewing the data down, reducing the estimate for optimal inclusion. Based on the information that apparent P availability follows a quadratic pattern in response to graded levels of phytase inclusion (Traylor *et al*., 2001), it is likely that the fifth data point from this study is slightly higher than would be expected, or that a laboratory error occurred when determining the phytase activity level, causing an increasing slope in the second line fragment of the broken-line regression, leading to a slight overestimation of the optimal phytase inclusion level.

The apparent digestibility of P in diets containing 30% FSM is relatively low at 21%. The addition of 2570 FTU/kg diet increased the apparent digestibility of phosphorus by 40%, reducing P excretion by 1490 mg/kg dry matter intake, a 50% decrease. The inclusion of the first level of microbial phytase, 575 FTU/kg, accounted for half of this increase in digestibility. Similar results for improvements in P digestibility have been reported in the literature. In 2001, Traylor *et al*. found that the addition of 1000 FTU/kg improved the apparent digestibility of P by 30% in semi-purified diets containing soybean meal (30% inclusion) as the only phosphorus source. The authors also noted that the majority of the improvement in P digestibility occurred at the first supplementation level of 500 FTU/kg. Similar improvements in P digestibility from corn-soybean meal based diets have also been reported by Jongbloed *et al*. (1992), Lei *et al*. (1992) and Cromwell *et al* (1995). The results obtained for the effects of microbial phytase inclusion on the P digestibility from FSM based diets corresponds to the literature available on common oilseed meals utilized in the swine industry.

When considering the estimate produced by this experiment for the optimal exogenous phytase inclusion level for diets containing 30% FSM, it is possible to determine, in general, if a dietary cost benefit is present. Determining if it is cost effective for a producer to include microbial phytase into a diet would depend highly on cost of both inorganic phosphorus and microbial phytase. The following is a theoretical situation using the dietary formulation for the 30% FSM diet shown in Table 3-1. This diet contained a level of 8 g/kg diet of inorganic P (dical/P). In February 2008, the approximate cost of Dical/P was $520/tonne and the cost of microbial phytase, although highly variable among brands, is estimated at $3000/tonne. The cost of inorganic P, at an inclusion level of 8 g/kg diet, for 1000 kg of diet would be approximately $4.15. Inclusion of 1400 FTU/kg diet improved P digestibility in FSM by 30%, thus reducing the need for inorganic P by 30%. A 30% reduction in dietary inorganic P corresponds to an inclusion of 5.6 g/kg diet (reduced from 8 g/kg), which has a cost of $2.91. The phytase activity level of 1400 FTU/kg corresponds to approximately 215 g/kg diet for a cost of $0.65 per 1000 kg of diet. The cost associated with dietary P for the diet containing the phytase enzyme is $2.91 for inorganic P plus $0.65 for the enzyme for a total of $3.56

per 1000 kg diet. The cost without the enzyme was $4.15. Based on this theoretical scenario, inclusion of 1400 FTU/kg diet could save the producer approximately $0.60 per 1000 kg of diet. Since prices fluctuate regularly, these savings may not always be present; however, dietary phytase inclusion will always reduce the amount of P excreted into the environment, thus reducing the environmental footprint left behind from hog farming. In many European countries legislation is in place to limit the amount of phosphorus per pig (or barn) that can be placed back onto the land. This is also recently becoming a topic of interest in Canada. In March 2008 the Manitoba government put forth legislation banning the construction of new or expanded hog operations due to the potential of future phosphorus contamination of water bodies from hog farms. It is important to note however, that this legislation did not include bans for cattle or poultry operations, and has no impact on urban development.

3.5 General Discussion and Conclusions

This study has provided a large portion of the information required for the swine industry to be comfortable with incorporating FSM into the diets of their pigs. It has provided information on the chemical composition, apparent nutrient digestibility, growth performance, phosphorus availability and on the carcass fatty acid profile of pigs fed with FSM. It has also provided an overview of both the advantages and disadvantages associated with the use of FSM.

As a meal by-product, it is easy to compare FSM to canola meal, another by-product which is used widely in swine rations within Western Canada. Meal products are often included as a dietary protein source. The amount of protein in FSM is similar to that of canola meal; however, the amino acid profile of FSM falls below that of canola meal. Flaxseed meal is lower in lysine, the most limiting amino acid in swine nutrition, but is also lower in terms of methionine, cysteine, leucine, histidine and threonine content. Flaxseed meal however, contains much higher levels of tryptophan. Despite the fact that several of the amino acids are lower than that found in canola meal, the only one of large concern is lysine, which does not meet the requirements of growing pigs. The first limiting factor for the inclusion of meal products into a swine ration is the energy content, and the NE content of FSM is much larger than that of canola meal. Overall, when compared to canola meal, FSM is superior in terms of energy value but falls behind in terms of protein quality.

Flaxseed meal contains several anti-nutritional factors which may be of concern. These anti-nutritional factors include the cyanogenic compounds linustatin, neolinustatin and linamarin, as well as trypsin inhibitors and linatine (a vitamin B_6 antagonist). The presence of cyanogenic compounds

and linatine are not likely to have major impacts on the pigs due to the fact that inclusion rates of FSM will generally be 15% or less, and in the case of cyanogenic compounds, any form of dietary heat processing will reduce the release of HCN. The trypsin inhibitor activity level of FSM (14 to 51 units of activity (TIA)) is much lower than that of both soybean (1650 TIA) and canola (99 TIA) meals (Bhatty, 1993), and thus should also not be a major concern when including FSM into swine rations. Although these anti-nutritional factors were not evaluated directly in this study, inclusion of up to 15% FSM into the diets of growing and finishing pigs did not have any adverse effects on weight gain and overall pig performance, indicating that the presence of these anti-nutritional compounds was not problematic.

No adverse impacts on feed intake were found throughout the growing or finishing stages, indicating that at 15% inclusion, diet palatability was not reduced. This is important for several reasons. First, it indicates that the presence of the anti-nutritional factors did not reduce the palatability of the ingredient. Second, and perhaps more importantly, rancidity due to the high level of unsaturated fatty acids in FSM was not a problem in these diets. As discussed previously, rancidity can occur when dietary fatty acids react with free radicals and undergo oxidation. Fatty acid rancidity will reduce palatability of a diet due to the generation of off flavours and smells. All diets utilized in these studies contained both vitamin E and selenium, which act as anti-oxidants, preventing and/or suspending the oxidation process as described by Halliwell and Chirico (1993). Fatty acid analysis of the diets utilized in the growth trial showed that the level of polyunsaturated fatty acids increased with increasing FSM, indicating that oxidation of the unsaturated fatty acids was not a problem in these diets. It is extremely important to ensure that diets containing FSM include an anti-oxidant in order to prevent palatability problems associated with fatty acid rancidity.

The presence of phytic acid in FSM is a problem which can be dealt with relatively easily. Although approximately 65-70% of the phosphorus in FSM is bound in phytic acid, the inclusion of microbial phytase in the diet can significantly reduce the need for inorganic P supplementation by improving the availability of the P from the FSM itself. The inclusion of microbial phytase into diets is common practice within the swine industry, and thus the issue of phytic acid in FSM is not a major concern.

One of the most promising aspects of including FSM into swine rations is related to its fatty acid profile. As shown in this study, the presence of increasing quantities of ALA in the diets of finisher pigs can improve the fatty acid profile of the final pork product. The omega-3 fatty acids have many potential benefits, including but not limited to, reductions in the risk of myocardial

infarctions and certain cancers. Providing a product enriched with omega-3 fatty acids is thus a major interest for producers, especially as consumer demands for these products increases.

Although this study has provided information on many components of the nutritional value of FSM, there is one important area which should still be addressed. Essential amino acid digestibility is a key parameter in diet formulation and thus in order to complete the nutritional profile of FSM for use in swine, a study should be carried out to determine the ileal amino acid digestibility values for FSM.

Overall, this project utilized several different analysis techniques to evaluate the nutritional profile of flaxseed meal for pigs. The values obtained from the apparent nutrient digestibility studies in growing pigs and gestating sows are consistent with those found in the literature. The values from these studies were also calculated by two separate techniques, with no major differences found between the methods. Nutrient digestibility values obtained from this study appear to be reliable, and based on the experimental methodology employed, should be repeatable. Although a certain level of experimental variation can be expected when using live animals, all means possible were employed to reduce this variation as much as possible, thus improving the quality of the results obtained. As an example, within the digestibility studies, it was extremely important to collect faecal samples directly from the pigs to prevent faecal to floor contact. This would reduce the chance of sample contamination, helping to reduce any possible variability between samples.

Although no dietary significance was found, the results obtained throughout the growth performance study were affected slightly by the barn outbreak of circo-virus. This should be taken into consideration when interpreting the growth performance data, especially throughout the first phase of the experiment. In terms of the carcass fatty acid evaluation, a larger sampling group would have provided more information on the variability of the effects of FSM inclusion. The fatty acid analysis methodology employed for this experiment was new to the laboratory and thus it was essential that this method be validated to ensure that the results obtained were accurate. The method validation results for this technique are presented in Appendix A.

In conclusion, the nutritional value of FSM, as determined within this study, indicates that dietary inclusion of the product into swine rations can be done without concern, as long as key formulation issues are addressed and the diets are balanced in terms of NE and lysine contents. Swine producers should feel comfortable with using this alternative ingredient within their growing and finishing stages, and potentially within their sow herd. Due to the low lysine and energy content however, FSM would not be a good candidate for inclusion into the diets of nursery pigs.

4 IMPLICATIONS

The use of alternative feed ingredients within swine production herds is always of interest for producers and nutritionists. Within the current agriculture industry, the cost producing livestock is rising for several reasons, one of which is the rise of feed grain prices around the world. As the cost of feeding continues to increase, producers will be more willing to accept a novel ingredient for use within their herds, especially if it proves to be cost effective and/or can create a functional food product which can attract a premium selling price within a niche market. The use of plant oils within the fuels and chemicals industries is continuing to increase, leading to an increase in the amount of 'meal' products available to the livestock industry. Gradually, a shift in the plant products available to producers is occurring, more by-products are becoming available and the availability of feed grains is decreasing, primarily due to the rising costs.

There are several potential advantages as well as disadvantages when including FSM as an ingredient in swine rations. Some of the disadvantages include the low lysine and NE contents which will limit the inclusion level to approximately 15% and the high level of phytate bound phosphorus. Despite the disadvantages, the fact that FSM contains 13% oil, of which 6.2% is the omega-3 fatty acid ALA, is a major advantage. This property of FSM allows for the potential to create an ALA enriched carcass through feeding a by-product of the flax crushing industry. Flaxseed meal also contains a high level of fibre, which can be viewed as either a disadvantage or as a potential advantage. The high fibre level reduces the NE value of the product, contributing to the limitation of feeding to growing and finishing pigs; however, it is possible that this high fibre level can be of benefit when feeding sows to aid in the gut fill effect. A large portion of the fibre is mucilage, a soluble fibre, which may also function as a pre-biotic, an area of research which still needs to be assessed. Additional 'functional' properties of the omega-3 fatty acid content in terms of pig health and reproductive performances should also be addressed.

The nutritional value of FSM, as determined within this study, can be easily compared to canola meal, a common oilseed by-product used widely by the industry. Flaxseed meal is similar to canola meal in terms of crude protein content, as well as NDF and ADF contents. It is extremely important to note however, that the crude protein content of FSM is characterized by a low lysine

content, which will be a major concern when adopting this ingredient into rations. This issue can be corrected for by either feeding synthetic lysine, where cost allows, or by including FSM along side an ingredient containing high levels of lysine such as field peas. The ether extract content of FSM is greater than that of canola meal, providing FSM with a greater NE content due to the fact that oils are readily digested by the pig and are easily utilized as an energy source within the body. This ether extract content, containing high levels of ALA, allows for the production of an omega-3 enriched product, a property not present from feeding canola meal. Although canola oil contains a high level of ALA (11%) when compared to many other plant sources, it is still much lower than that found in FSM (57%). Also, the amount (3%) and apparent digestibility (31%) of oil in canola meal are both significantly lower when compared to FSM (13% oil, 95% digestible). Due to the consistency of the chemical composition of FSM and its nutritional value for pigs, it is relatively easy to formulate into rations, as long as the low lysine content is accounted for. With properly balanced diets in terms of NE and digestible essential amino acids, the inclusion of up to 15% FSM does not affect the growth performances of pigs, and thus can be included into rations with confidence.

Flaxseed meal is a partially defatted by-product, and based on its chemical composition and nutritional value, it would be expected that it could compete with canola meal in terms of price; however, in January 2008, the price of FSM in Europe was approximately $150/t CDN more than canola meal, and was more comparable to soybean meal. Flaxseed meal does provide producers with an opportunity to produce a niche market product in a cost effective manner when compared to feeding full fat flaxseed or fish products to obtain an omega-3 fatty acid label.

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APPENDIX A

A large component of this thesis project involved the analysis of fatty acids in flaxseed meal, diets and in carcass samples. A new, direct method for fatty acid methylation described by O'Fallon *et al*. (2007) was adopted, with minor adaptations, for this procedure and is detailed below. Prior to actual sample analysis, the method was validated both for use with the University of Saskatchewan Animal and Poultry Science Laboratory, and for use with the specific samples of interest in this project. The components of method that were tested during method validation included precision, repeatability, stability and accuracy for each of the five fatty acids of interest (palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid). This appendix is designed to present the data obtained during the method validation process.

A.1 Materials and Methods

Loin, backfat and certified reference material (CRM) samples were prepared for analysis in the frozen state. Visible sections of backfat were removed from the loin samples prior to the direct fatty acid methyl ester (FAME) synthesis procedure. Samples were uniformly ground using a food processor (Moulinex DPA2, France), and approximately 1 g was placed into a 16 x 125 mm screw-cap Pyrex culture tube (exact sample weights were recorded for calculations). A total of 1 ml of non-methylated C13:0 internal standard (0.5 mg of C13:0/ml of MeOH) was added to each sample, followed by 0.7 ml of 10 *N* KOH in water and 5.3 ml of MeOH. The tubes were then placed into a water bath and incubated at 55^{o}C for 1.5 hours. The samples were subject to vigorous handshaking for 5 s every 20 minutes throughout incubation. Samples were then removed and cooled below room temperature by placing into a cold tap water bath. An additional 0.58 ml of 24 *N* H_2SO_4 in water was added and the samples mixed by inversion immediately. The tubes, which now contained the K_2SO_4 precipitate, were incubated again in a 55^{o}C water bath for 1.5 hours with vigorous handshaking for 5 s every 20 minutes. Samples were again cooled below room temperature in a cold tap water bath and 3 ml of hexane was added to each. Tubes were vortex mixed using a single tube vortex and then centrifuged (Beckman Coulter J6-MC Centrifuge, Mississauga, ON) for 5 minutes at 1500 rpm. The hexane layer containing the FAME was removed and placed into a GC vial, capped, covered with parafilm and stored at -18^{o}C until analysis.

The C13:0 was obtained from Sigma-Aldrich, Inc. (St. Louis, MO) and all other chemicals were of GC grade, also obtained from Sigma-Aldrich, Inc. (St. Louis, MO).

The fatty acids analyzed in this study were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1*n*-9), linoleic acid (C18:2*n*-6) and α-linolenic acid (C18:3*n*-3). Carcass, diet and FSM fatty acid composition was determined by gas chromatography using an Agilent 6890 system with Agilent ChemStation software (Agilent Technologies, Mississauga, ON). Fatty acid methyl ester samples were then compared to methylated reference samples (Nu-Chek Prep Inc., Elysian, MN) using the following GC program: the machine was set for a 1.0 µl injection, split at a ratio of 30:1. The injector set points were a temperature of 260^{o}C, pressure of 25 psi and a total flow of 23.4 ml/min. The initial oven temperature was set to 140^{o}C and held for 5 minutes. The temperature was ramped up at a rate of 20^{o}C per minute to a maximum of 240^{o}C and held for 25 minutes. The total run time for analysis was 35 minutes. The stationary phase was a Supelco fused silica capillary column SP 2560 (Sigma-Aldrich, St. Louis, MO).

A.2 Precision

Precision was measured by running multiple injections of the same sample vial into the GC sequentially. After sample preparation, a loin sample was selected randomly and injected 9 times into the machine. A hexane blank sample was run at the start and after every 3 samples to ensure no carry over effect was occurring. The chromatograms produced from each hexane blank did not show any evidence of fatty acid carry over. The amounts of each of the 5 fatty acids for the 9 subsequent injections are shown in Table A-1. This data indicates that there is no difference in the amounts of the fatty acids for each of the nine injections from the same vial, thus indicating that this method meets the requirements for analytical precision.

A.3 Sub-Sampling Repeatability

Sub-sampling repeatability is measured by preparing multiple sub-samples from the same initial sample. For this procedure, 12 sub-samples were prepared from a single loin sample and analyzed sequentially on the GC. Since loin samples are composed of lean tissue and fat, this section of method validation is used to ensure that 1) adequate sub-sampling is occurring and 2) that there are no major differences in terms of analysis for each subsample. The data collected for each of the five fatty acids for the 12 sub-samples of a single loin is shown in Table A-2. Variability is expected to be larger for sub-sampling repeatability than for precision testing; however, extremely large

Table A-1: Precision data for nine injections of a single sample vial with five separate fatty acid amounts (mg/g tissue)

Injection #	C16:0	C18:0	C18:1	C18:2	C18:3
1	54.0	38.3	82.0	18.4	10.0
2	54.1	38.4	82.3	18.3	10.0
3	55.1	39.0	83.7	18.4	9.9
4	55.3	39.2	84.0	18.6	10.1
5	55.0	38.8	83.4	18.3	9.9
6	54.8	38.7	83.1	18.3	9.9
7	55.0	38.8	83.4	18.3	9.9
8	55.1	39.1	83.7	18.5	10.1
9	55.2	39.1	83.9	18.4	10.0
Average	54.8	38.8	83.3	18.4	10.0
Std. Deviation	0.47	0.31	0.71	0.12	0.06

Table A-2: Sub-sampling repeatability data for twelve sub-samples from a single loin with five separate fatty acid amounts (mg/g tissue)

Sub-Sample #	C16:0	C18:0	C18:1	C18:2	C18:3
1	56.2	41.5	83.2	18.4	9.9
2	66.9	64.5	75.6	17.3	9.4
3	85.4	85.2	91.5	20.5	11.0
4	53.5	39.1	79.6	18.4	10.0
5	50.2	36.1	75.4	17.0	9.3
6	66.6	58.0	88.5	19.7	10.7
7	45.5	32.8	69.2	15.6	8.6
8	66.5	57.1	82.9	18.6	10.0
9	71.3	58.5	95.1	22.1	11.7
10	57.1	39.8	86.5	18.7	10.0
11	60.2	41.8	91.4	19.8	10.5
12	54.1	37.8	81.4	18.1	9.7
Average	61.1	49.4	83.4	18.7	10.1
Std. Deviation	10.85	15.47	7.67	1.70	0.81

variations are indicative of inadequate sub-sampling procedures. It is also evident that the standard deviations of samples are greater for the fatty acids which are present in larger quantities. Based on the data obtained for sub-sampling repeatability, the method utilized in this procedure was adequate.

A.4 Sample Stability

All samples used throughout the fatty acid analysis procedure were stored in 2ml GC vials as fatty acid methyl esters dissolved in hexane. When not in use, the sample vials were covered in para-film and stored at -18^0C. After preparation, a single vial was randomly selected and analyzed immediately and then analyzed two subsequent times, the first one week after initial sample analysis and the second three weeks after the initial analysis. This component of method validation ensures that a sample will not significantly change under the storage conditions for a period of time. Data obtained for the sample stability tests are shown in Table A-3.

A.5 Accuracy

The accuracy of sample analysis relies not only on the accuracy of the analytical equipment such as the GC, but also on the accuracy of the sample preparation procedure. In order to validate the method for its accuracy, a certified reference material (CRM) can be purchased and prepared in the same manner as all of the other samples. This material can then be analyzed and the obtained values compared back to the certified values which are included with the reference material. The certified reference material obtained for this part of the method validation was SRM 1546 – Meat Homogenate (National Institute of Standards and Technology, Gaithersburg MD, USA). The meat homogenate was prepared in the same manner as the carcass, flaxseed and diet samples, and was analyzed using the same GC program in duplicate. The data obtained from the analysis of the CRM is presented in Table A-4.

A.6 Summary

Overall, the method validation procedure performed for the analysis of fatty acids from diets, flaxseed and carcass samples was sufficient to confirm that the fatty acid methylation procedure adapted from O'Fallon *et al.* (2007) was adequate for the needs of this experiment. The method validation confirmed that this procedure was accurate, precise and repeatable, as well as provided sample stability during storage.

Table A-3: Stability data for three injections of a single sample vial over time with five separate fatty acid amounts (mg/g tissue)

Analysis Time	C16:0	C18:0	C18:1	C18:2	C18:3
Time 0 (T0)	50.6	35.7	76.2	17.1	9.3
T0 + 1 week	56.2	41.5	83.2	18.4	9.9
T0 + 3 weeks	54.0	38.3	82.0	18.4	10.0
Average	53.6	38.5	80.5	17.9	9.7
Std. Deviation	2.77	2.90	3.76	0.74	0.37

Table A-4: Fatty acid analysis of a Certified Reference Material (CRM) and comparison to the accepted 'true' values

	C16:0	**C18:0**	**C18:1****	**C18:2**	**C18:3*****
CRM #1	43.6	21.4	71.1	21.8	5.0
CRM # 2	44.5	22.9	73.1	22.1	5.4
CRM Average	44.0	22.2	72.1	22.0	5.2
CRM 'True' Value	45.6	21.7	82.0	19.6	1.2
Std. Deviation*	1.11	0.33	7.01	1.68	2.68

*Std deviation is the standard deviation of the average value of the two CRM samples to that of the CRM true value

**The CRM handbook stated that a possible co-elution of another fatty acid occurred with C18:1 during the National Institute of Standards and Technology analysis, thus increasing the deviation of the analyzed value to the true value

*** The CRM true value for C18:3 is a reference value only, this was not analyzed by the National Institute of Standards and Technology