Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

Lepidium cake as a feed stuff to pigs

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Abstract

The purpose of this study was to evaluate the effect of different levels of lepidium cake (LC) on palatability, coefficient of total tract digestibility (CTTAD), blood parameters and feeding behaviour in growing pigs. A total of 8 female growing pigs ((Land race x Yorkshire) × Hampshire) with an average initial body weight of 26.5±2.5 kg were used in the trail which lasted for 44 days. The pigs were fed four experimental diets (control, LC4: 4% of Lepidium cake + 96 % control; LC8: 8% of Lepidium cake + 92 % control; LC12: 12% of Lepidium cake + 88% control) in a double 4 × 4 Latin square design. The contol diet was composed of barley, wheat, soya bean protein, amino acids, premix and $Ti0_2$. Feed intake, faecal and blood samples, as well as feeding behavioral datas were collected and analysed. The result of current study showed that the total non-starch polysahharides (NSP) and dietary fiber (DF) content increased with increasing LC. The CTTAD of dry matter (DM), organic matter (OM), crude protein (CP) and gross energy (GE), decreased ($P < 0.01$) with inclusion of LC.This was mainly due to high proportion of indigestable fiber in the LC. The CTTAD of EE was increasing with inreasing inclusion levels of LC. Except for EE, the CTTAD of the basal diet was higher ($P<0.01$) than the diets with different inclusion levels of LC. Blood parameters, feed choice, and feeding time were not affected by inclusion levels of LC ($P>0.05$). However, feeding rate, rooting, searching, throwing and moving feed showed a tendency to be significant ($P = 0.01$). LC is palatable to growing pig, but lower digestibility due to high proportion of indigestible fiber in the diet. The regression model was poor to explain the source of variation. The lack of significant difference among test diets for blood, CTTAD and feeding behavior could be due to low levels of inclusion level of LC. The digestibility of LC at 100 % inclusion level is low (< 40%), this implies that the LC is poorly digestible by growing pig. Hence, strategies like dehulling of husk, supplementation of exogenous enzyme; alkali treatment such as sodium hydroxide (NaOH) could improve the CTTAD of LC. Furthermore, developing lepidium varieties having less fiber and glucosinolate content might sustainably improve the utilization of LC by growing pigs.

Key words; Lepidium cake, digestibility, growing pigs, feeding behaviour, glucosinolate, non-starch polysaccharides, blood profiles, sinalbin

Contents

1. Introduction

In the modern pig industry, feed cost is the main farm expense and constitutes about 70% of the total production costs (Niemi *et al*., 2010; Choi *et al*., 2015). Soybean and corn have globally been the major protein and energy sources in pig daily rations (Woyengo *et al*., 2014). Since, 2008 the prices of cereals and oil seeds, especially corn and soya bean have increased. The increase in price is partly due to the increasing demand of these crops for biofuel production (Tyner and Taheripour, 2007). Cultivation of soya bean are associated with several environmental issues such as land grabbing, use of pesticides, soil degradation due to mono-cropping and deforestation of rain forest (Biofuel Watch Center – Reporter Brazil, 2010). In the European Union, a large percentage of soya bean is imported especially from US and Brazil. Even though the imported soya bean serves as excellent source of amino acids, nitrogen (N) and phosphorous (P) to livestock, it creates an imbalance between imported nutrient in the soya bean and exported nutrient in the form of product (milk, egg, meat etc). This could contribute to nutrient load on environment-if nutrients are not effectively removed from the farm. Large on-farm surpluses can result in pollution of water bodies, and increment in green house gas due to volatilization of N as ammonia (Hristov *et al*, 206; Gourley *et al*., 2011). Beside this, several studies have shown that use of soya bean in pig diets is not cost effective (Woyengo *et al*., 2014& Hendricks *et al*., 2014). This implies that there is need for investigation on new oil seed by-products to be able to reduce feed cost and the dependency on soya bean.

Lepidium campestre is a wild plant, there is currently an interest in developing the plant for future use for oil production (food) and bio-diesel production, and the remaining cake is suggested as alternative protein sources to livestock (Andersson *et al*., 1999). *L. campestre* is biennial and tolerate cold better than winter rape seed (Andersson *et al*., 1999) which gives it potential to be an important future crop in the Nordic agriculture. Sown together with cereals *L. campestre* serves as a catch crop the first winter and as an oil crop the following season. An interdisciplinary research group at SLU, have been conducting intensive laboratory and field trials on domestication of lepidium and confirmed that *L. campestre* can be grown throughout Sweden (Andersson *et al*., 1999; Mistra-Biotech annual report, 2013).

A study conducted by Andersson *et al*. (1999) indicated that *L. campestre* is rich in crude protein, fat, crude fiber and minerals. The same study also revealed that *L. campestre* is rich in all essential amino acids which are crucial in the pig diet. But, it contains higher fiber (440 g kg^{-1}) and anti-nutritional factors such as erucic acid and glucosinolates, this could negatively influence the palatability, feed intake, digestibility and performance of the pig. Glucosinolates have been shown to cause toxic effects in animals (Sørensen, 1990). Its effect is manifested by dysfunction of thyroid gland and liver (Choi *et al*., 2015). Lepidium contains the same amount of glucosinolates as rapeseed, 123-138µmol g^{-1} and 150 µmol g^{-1}, respectively (Andersson *et al.,* 1999). This figure is from the old variety of rapeseed, but today subsequent rapeseed breeding program developed double 00- rapeseed or canola varieties which contain both lower glucosinolate and erucic acid (Przybylski *et al*., 2005). According to EU Regulation (EC) No 2316/1999 the maximum limit of glucosinolate in 00-rapeseed or canola meal is 25µmol g^{-1}. Diet contaning glucosinolate are reoported to negatively affect blood profiles (Choi *et al*., 2015), feed intake and feeding choice (Kyriazakis, and Emmans, 1992). However, the types of glucosinolates found in the rapeseed and *L. campestre* are totally different (Andersson *et al*., 1999). As a result Lepidium could have less tendency of toxicity than rapeseed, but a study conducted in rat revealed that the sinalbin which is the main glucosinolates in Lepidium lead to decrease palatablity, biological value and protein utilization (Bille, 1983). Besisde this, Andersson *et al*. (1999) reported that the whole seed of *L. campestre* contais about 40% fiber, which could reduce production performance such as feed intake and growth rate. However, the fiber could be beneficial from animal welfare point of view as it prolong satity and gut fill and reduce aggression beahviour of pigs (Brook, 2005). However, the palatability and utilization of lepidium cake (LC) as livestock feed has not been studied yet.

Objective

The aim of this study was to evaluate the effect of different levels of LC on palatability, apparent total tract digestibility, blood parameters and feeding behaviour in growing pigs.

Hypotheses

The hypotheses of this study were:

Inclusion of LC in the diet will have negative effect on apparent digestibility of dry matter, organic matter, crude protein, ether extract and energy, apparent digestible energy, protein utilization, blood profiles, palatability, feeding time and feeding choice of pigs.

2. Literature Review

2.1. Lepidium production and purpose

Lepidium campestre also called "field cress" belongs to *Brassicaceae* family having the same genus as mustard, cabbage and rapeseed (Gonzales, 2011; Ivarson *et al*., 2013). It is found in North America and Europe (Gonzales, 2011). There are many species of Lepidium. In Peru, *Lepidium meyenii* is used for different purposes; nutritional, fertility-enhancement and medicinal treatment (Chung *et al*., 2005; Gonzales *et al*., 2006). In Nordic countries, historically *Lepidium sativum* in Swedish "kryddkrasse'' have been used as anti-helmentic treatment for human and livestock (Waller *et al*., 2001). Beside this, field experiments have shown that lepidium can be sown as catch crop with cereals to improve seed yield and reduce ground water contamination due to leakage of mineral fertilizers (Merker & Nilsson, 1995; Merker *et al*., 2010; Mistra-Biotech, 2013). Importantly, *L. campestre* is an allelopathy crop that is a crop that produces chemical substance that hinders the growth and survival of other organisms. This could have advantage in suppressing weed growth when it intercrops with other crops that tolerate its effect.

In Sweden, the major economically oilseed crop is winter rapeseed, but it can only be grow in the southern part of the country (Mistra-Biotech annual report, 2013). Whereas, *L. campestre* is a perennial species with greater overwintering capacity than rapeseed and found to be more appropriate to grow under cold Swedish climate conditions even in the northern part of the country (Andersson *et al.*, 1999; Mistra-Biotech annual report, 2013). A study by Merker and Nilsson (1995) found that *L. campestre* can be developed to produce vegetable oils for food and automobile lubricant purposes (Merker and Nilsson, 1995). A study conducted by Andersson *et al*. (1999) suggested that *L. campestre* could be used as human and animal feed. As *L. campestre* is still a wild species and there is no commercial production anywhere in the world, but field trails reported that *L. campestre* produce 5-6 tons per hectare which is 30% higher yield than rapeseed (Anderson *et al*., 1995; Mistra-Biotech annual report, 2013).This characteristics gives the crop a great potential to become a novel crop in the Nordic countries which could be used for human food, biofuel and livestock feed (Andersson *et al*., 1995; Ivarson *et al*., 2013).

2.2. Protein and Amino Acid profile

The protein content of *L. campestre* and rape seed is 191 g/kg and 250 g/kg dry matter, respectively (Andersson *et al*., 1999). According to Anderson *et al*. (1999) and Mistra-biotech annual report (2013), the quantity of oil and protein are the desirable component of an oil crop, and the research group suggested that oil quantity and nutritional quality still needs to be improved. According to Andersson *et al*. (1999), *L. campestre* seed contains all essential amino acid in higher quantity than rapeseed. The predominant amino acids found in the lepidium seed are glutamic acid, arginine and aspartic acid (Andersson *et al*., 1999). Compared to soya bean, *L. campestre* have a higher content of methionine, cysteine and lysine which are the limiting amino acid in the pig diet (Table 1). This implies that lepidium could be a good source of amino acids to pigs.

Table1. The amino acid composition of whole crop L. campestre, soya bean and rapeseed in g kg^{-1} *CP*

Type of Amino Acid	*L. Campastre*seed[1]	Soya bean seed[2]	Rape Seed[3]
Methioine	12	5.3	20
Aspartic Acid	71	36.2	76
Threonine	34	13.5	48
Serine	39	15.4	44
Glutamic Acid	130	58.9	180
Proline	57	16.5	59
Cystine	25	5.4	25
Glycine	49	12.9	55
Alanine	40	13.9	47
Valine	47	16	55
Isolucine	38	16.2	43
Leucine	57	25.8	73
Tyrosine	31	11.4	31
Phenylalanine	40	17.8	43
Histidine	29	7.6	29
Lysine	63	17.3	63
Arginine	76	21.4	62

[1]Andersson *et al*. (1999); [2]Callaway (2004); [3]Feedepedia (www.feedipedia.com).

2.3. Oil and Fatty Acid profile

Nutritional studies by Anderson *et al*. (1999) and Eriksson (2009) found that *L. campestre* seed contains around 20% oil which is lower compared to rapeseed (45%). Beside this, the level of cholesterol was remarkably high. In *L. campestre* the main fatty acid was linolenic acid, followed by erucic acid and oleic acid (Andersson *et al*., 1999). The average linolenic acid (C18:3), oleic acid (C18:1) and linoleic acid (C18:2) content in the seed is 34.1%, 22-25%, 15.2% and 9.9% of total fatty acid (FAS), respectively (Andersson *et al*., 1999; Erickson, 2009). Linolenic acid was the most abundant fatty acid in the seed followed by erucic acid and oleic acid. Compared to whole rapeseed, the FAS profile in *L. campestre* seed had a lower content of oleic and linoleic acid, but a higher content of linolenic acid, however, this higher content of polyunsaturated fatty acid (linolenic and linoleic fatty acid) increases the risk of oxidative rancidity which in turn affects the odor, test and shelf-life of food (Andersson *et al*., 1999; Mistra-biotech report, 2013). On the other hand, consumers preference to foods rich in PUFAs and especially conjugated linolenic fatty acid (CLA) is increasing due to its health benefit; CLA *isomers* such as rumenic acid (*Cis*-9, *trans*- 11) and vaccenic acid which are biohydrogenation products of both linolenic and linoleic acid are identified as "functional food" components (Bauman *et al*., 2006). The concentration of these CLA isomers in milk and meat can be enhanced by the type of feed. Cows fed on lush pasture and vegetables oil was reported to produce milk and meat higher in PUFAs especially vaccenic acids and rumenic acid (Boumann *et al*., 2006). Study conducted in rat revealed that dietary supplementation of vaccenic or rumenic acid rich butter showed reduction in plasma cholesterol and had anticancerinogenic effect. By compromising the oxidation stability and health benefits of PUFAs; a research group is working to achieve ideal PUFAs level of lepidium (Mistra-Biotech annual report, 2013).

The same group is also working on a breeding program to improve the total oil content. So far they have considerably improved the total seed oil content from average of 23 % to 28%, but still they undertaking series of breeding programs aimed at developing *L. campestre* variety which contains 30% of oil. Erucic acid is the fatty acid with the second highest concentration in lepidium. Studies by Shahidi (1990) and Anderson *et al*. (1999) indicated that lepidium contains 22-25% of erucic acid which is higher than double-zero rapeseed (2%), but lower than old varieties of rapeseed and mustard which is 30% and 36-60 %, respectively. According to EU regulation of 24/2009, the amount of erucic acid in food or feed should not

be more than 5%. This is due to its side effect on human or animal health. Chicken and pigs supplemented with rapeseed containing high erucic acid showed low feed intake, growth rate and energy efficiency (Choi *et al*., 2015). The slow growth rate could be due to the lower feed intake (McDonald *et al*., 2002). The reduction in energy efficiency is due to the negative effect of erucic acid on nutrient metabolism (Clement & Renner, 1977).

2.4. Anti-nutritional Factors

Beside erucic acid, *L. campestre* also contains anti-nutritional factors such as glucosinolate and β-thioglucosidases (myrosinases) which are also commonly found in other oil crops such as mustard and rapeseed (Andersson *et al*., 1999; Alexander *et al*., 2008). Their concentration depends on crop varieties, agroecology, soil type and soil fertility (Wang and Daun, 2004).The major glucosinolates reported in rapeseed are progoitrin, sinigrin, gluconapin (Choi *et al*., 2015), whereas gluco-sinalbin was found to be the major glucosinolate in *L. campestre* (Andersson *et al*., 1999). Normally, glucosinolate and myrosinase serve as defence mechanism against herbivores and pathogens (Alexander *et al*, 2008). But, it has been shown to also cause anti-nutritional effects (Andersson *et al*., 1999; Eriksson, 2009). Pigs, poultry and young ruminant are among the most sensitive classes of animals to the adverse effects of glucosinolates (Alexander e al., 2008).This is due to their limited capacity to regulate glucosinolates (McDonald *et al*., 2002). During digestion, the glucosinolate undergo an enzymatic reaction catalyzed by myrosinase which results in the formation of hydrolyzed products mainly thiocyanates, oxazolidinethiones (Figure1). Thus, the hydrolyzed products causing toxicity in the animal by hindering iodine uptake and synthesis of thyroid hormone (T3 and T4) which results in dysfunction of the thyroid gland (Halkier and Gershenzon, 2006; Choi *et al*., 2015). Previous studies on Canola meal (Roth-Maier *et al.* 2004) and Juncea meal (Collins *et al.* 2011) using diets containing 0-5.5 µmol of glucosinolate per gram of diet showed that feed intake of growing pigs decreased in diets with greater than 2.0 µmol of glucosinolate per gram of diet.

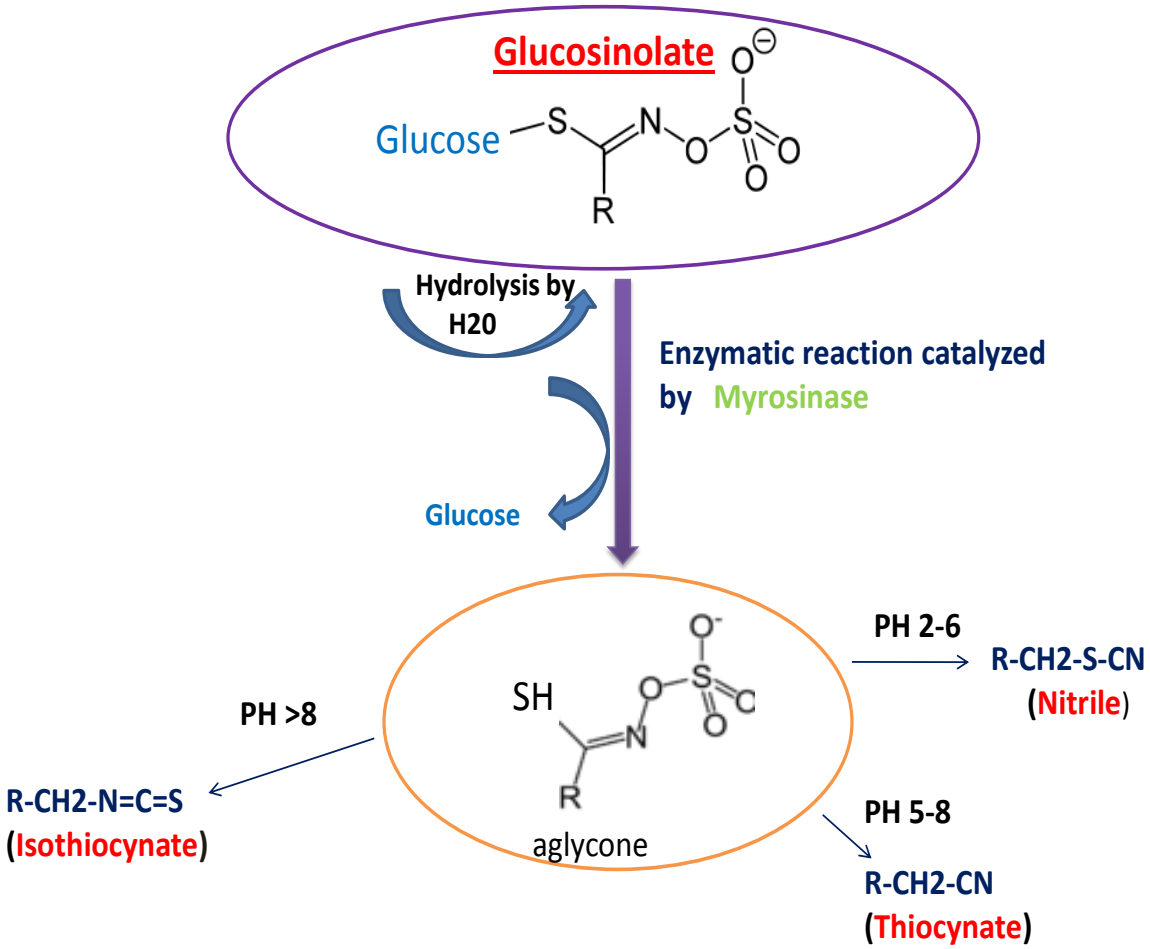

Figure 1. Chemical structure of glucosinolate and its hydrolyzed products (modified from; Chen and Andersson, 2001).

Beside this, glucosinolate and its hydrolyzed product lead to low feed intake due to its bitter taste and interfere with nutrient metabolism (Alexander *et al*., 2008; Choi *et al*., 2015).

Most oil seed cakes are processed using mechanical extraction which results in higher levels of glucosinolates then the entire seed which needs to be considered when used as animal feed at higher level. However, there are techniques to lower the glucosinolate content in feed including heat treatment; alkaline treatment; soaking in water; microwave irradiation and treatment with water and $CuSO_4$ solution (Barret *et al.*, 1998; Tripathi and Mishra, 2007; Alexander *et al*., 2008). To apply these methods you need scientific knowledge, availability of chemicals and equipment which may not be feasible to small holder farmer's where simple, safe and sustainable solution is needed.

2.5. Non-Starch polysaccharide (NSP)

Carbohydrates accounts two third of the total dry matter in the pig diet (Pluske *et al*., 2001) and is the single most abundant energy source in diets for growing pigs and sows comprising about 60% and 70%, respectively (Bach Knudsen *et al*., 2012). The total dietary carbohydrate ingested by pigs can be divided into starch and 'dietary fibre', where; starch is completely digestible by the enzyme secreted in the gastrointestinal tract (Pluske *et al*., 2001; Johnston *et al*., 2003). The term dietary fiber (DF) is wide and includes a complex mixture of carbohydrate (mono-, di-, oligo- and polsaccharides) and non-carbohydrate components mainly found in plant cell wall (McDougall *et al*., 1996). Different definitions by different scholars at different time have been proposed (Hipsley, 1953; Trowell, 1974; 1976). There was no universal definition of DF until 1990's when nutritionist agreed to define dietary fiber based on it's the physio-chemical effect. According to the physiological definition, DF is the dietary components which cannot be digest by endogenous enzymes secreted in the gastrointestinal tract, while chemically DF is defined as the sum of total non-starch polysaccharides (NSP) and lignin (Theander *et al*., 1994).

The analytical procudures and laboratory equipments of fiber analysis have evolved together with the fiber definition. The crude fiber (CF) method was developed in the middle of 19^{th} century and measures only a small portion of fiber component, mainly cellulose and lignin. Latter the detergent method with better percision was developed by Van Soest using neutral solutions (α-amylase and disodium ethylene diaminotetraacetate) and acid (H_2So_4) to determine the amount of fibre. Neutral detergent fiber (NDF), quantify the cellulose, hemicelluloses and lignin fraction whereas acid detergent fiber (ADF) measures the cellulose and lignin. Neutral detergent soluble is hemicellulose determined by subracting ADF from NDF and lignin. Lignin is the undigestible residue remaining after treating ADF with 72% of H_2SO_4 (McDonald *et al*., 2002). However, the NDF method was not sufficient as fiber analyse method for monogastrics since it does not show the water soluble fibers and water insoluble pectic substance which are lost during the NDF determination procedure. To be able to include the soluble dietary fibers enzymatic-chemical methods was developed. The method analyse individual sugars that builds up the NSP fraction and their degree of solublity- thus, the method provides a better indicator of nutrinal significance and utlization of fiber by the animal than CF and NDF (Bach Kundsen, 2001). NSP comprises a diverse group of polysaccharides with varying degrees of solubility, and structure; such complexities make NSP a difficult group to divide in clear-cut classes. Based on the physiological effect DF is

devided into water soluble and water-insoluble fractions (Theander *et al*., 1994). Accordingly, the water-soluble fiber fraction includes arabinose, galactose, gums, pectins, arabinoxylans, mucilages and some hemicelluloses and β-glucans. The water insoluble fiber fraction include cellulose, some hemicelulose (galactomannans, xylans and xyloglucans), and lignin (Bach Kundsen, 2001). However, Bach Knudsen (1997) analyzed the NSP content in a wide range of feedstuff and showed that there are both soluble and insoluble fractions of all sugars and arabinoxylans in most feedstuff.

NSP serves as structural component in plants (McDonad *et al*., 2002). Cellulose, hemi-cellulose as well as pectin are the most abundant NSP in the plant cell wall. Depending on the plant type and maturity of the plant; at matured stage cellulose account 70% of cell wall (Selvendran & Robertson, 1990; McDonald *et al*; 2002). The proportion of NSP in the cell wall depends on the degree of lignifications. As the plants grow the proportion of soluble nutrients (protein, soluble carbohydrates, and fatty acids) decreases, whereas the proportion of structural polysaccharides such as cellulose, hemicelluloses and lignin increase (McDonald *et al*, 2002). Consequently the palatability, dry matter intake and NSP digestibility also decrease.

Lignin is not a carbohydrate but a component of dietary fiber and it is insoluble by the animal, microbial enzymes and acid. It is impotent to consider in animal nutrition since it prevents hydrolysis of cell wall components and negatively affect digestibility (Bach Knudsen, 2001; McDonald *et al*., 2002).

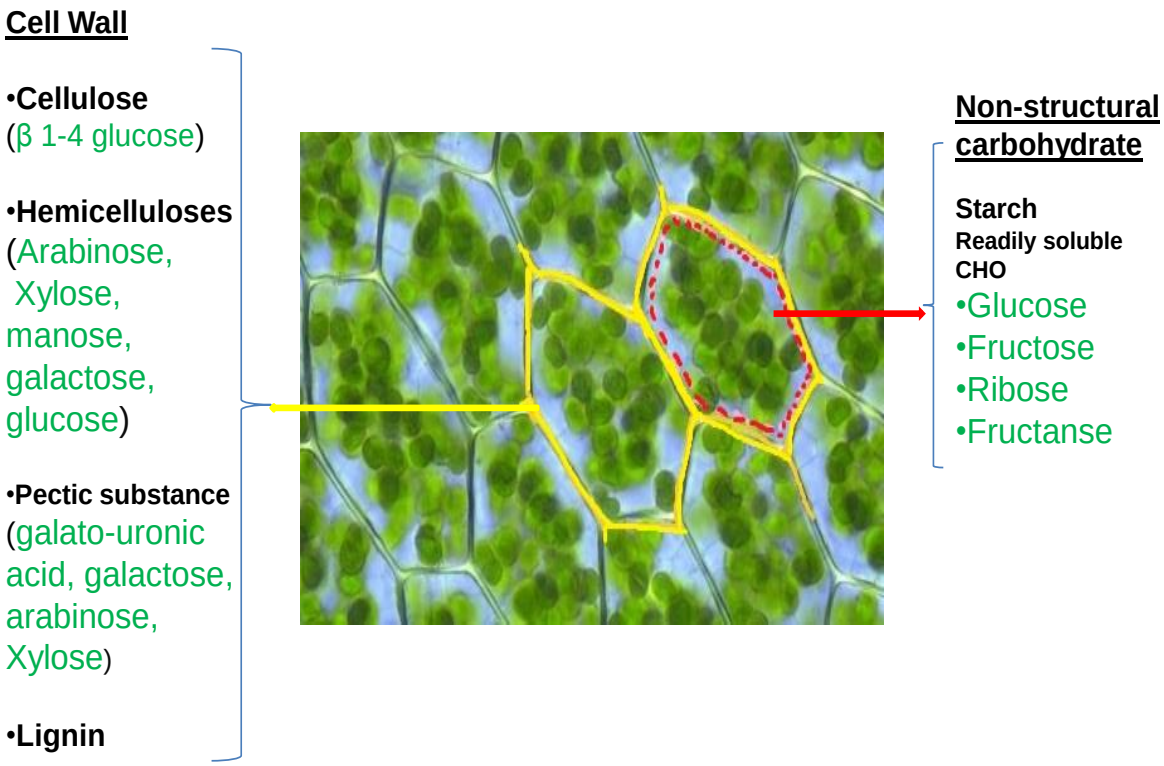

Figure2. Plant cell wall and its fiber fraction (modified from; Pamela, 2009)

The composition of the NSP and lignin varies largely between plant materials and plant tissue (Carpita and Gibeaut, 1993; McDougall *et al*., 1996). Arabinoxylans and β-glucan are the main component of cell walls in cereals grain (Bach Knudsen, 1997). But, the concentrations and types of NSP differ among cereal grains. For example, the predominant NSP in rye and barley are arabinoxylan and β-glucan, respectively (Smits & Annison, 1996).

A high content of NSP is also reported in legumes and oil seed cakes (Back kundsen, 2001; Aldwairji *et al*., 2014). Pectic substance is the main NSP in sugarbeet pulp and dicoteolydons like peas (McDougall *et al*., 1996; Bach Knudsen, 2001) whereas, oil seed cakes contains mainly lignin, uronic acid, insoluble sugars, galactose and low molecular weight sugars (Bach Knudsen and Li, 1991). According to Andersson *et al*. (1999), the content of dietary fiber in *L. campestre* is 414 g kg^{-1} which is much higher than found in rape seed (180 g kg^{-1}). The soluble and insoluble fiber content of *L. campestre* is 21 and 393 kg^{-1}, respectively (Andersson *et al*., 1999).

The proportion of fibre decreases from outer to inner part of the seed. The husk is mainly composed of insoluble NSP and lignin while the endosperm is rich in starch and soluble fiber (Fincher and Stone, 1986). The proportion of starch and DF is strongly influenced by the husk to endosperm ratio (Bach Knudsen, 2001). As shown in table 2, hulled cereals have lower concentration of NSP and lignin than dehulled counterparts (Bach Knudsen, 1997).

Table2. Fiber fractions of some pig feedstuffs in g kg^{-1} DM

	Wheat [1]		Barley [1]	Oat hull meal[1]	Linseed meal[1]	cotton seed cake[1]	Lepidium cake[2]	Soya bean cake [1]	Rape seed cake[1]	Peas[1]
	whole	bran	hulled							
Starch	651	222	587	213	27	18	na	27	15	454
Total sugar	19	53	21	14	42	66	na	137	90	88
β - glucan	8	24	42	14	na	na	na	na	na	na
NSP										
Arabinose										
Soluble	7	7	6	2	17	16	3	9	13	19
Insoluble	22	83	22	26	19	18	28	17	31	17
Xylose										
Soluble	9	10	6	0	38	6	1	2	2	1
Insoluble	38	138	50	212	28	54	13	17	15	12
Galactose										
Soluble	2	2	1	0	21	7	6	16	5	4
Insoluble	2	7	2	9	12	5	23	25	15	3
Glucose										
Soluble	4	8	39	8	10	6	2	6	3	5
Insoluble	7	27	8	12	27	2	95	1	5	31
Uronic acid										
Soluble	1	2	2	1	45	23	11	25	18	20
Insoluble	4	13	8	35	23	22	191	23	32	12
Total NSP	119	374	186	505	303	257	396	217	205	180
Klason lignin	19	75	35	148	119	83	123	16	90	12
Total Dietary fiber	138	449	122	653	423	340	519	233	295	192

[1] Bach Knudsen, 1997; [2]Lepidium cake used in the present study; na: not analysed

2.5.1. Inclusion of Dietary fiber in pig diets

Today, there is a growing interest to include dietary fiber in the daily ration of pig because of its positive effect on physiological function, gut health, gut microflora and welfare (Wenk, 2001; Jha *et al*., 2015). According to Mateos *et al*. (2006) growing pigs needs a minimum dietary fiber level of 6% of their diet.

Sugar beet and distillers dried grains with soluble (DDGS) are sugar and ethanol factory by-products, respectively. Both could be used as source of feed in pig diets. The sugar beet pulp was considered as high fibrous feed (737 g kg $^{-1}$DM) (Serena and Bach Knudsen, 2007) for pigs, but has high digestibility (0.80–0.85) even in growing pigs. Accordingly, it is recommended up to 15 % inclusion in grower and 20 % in finisher and sow diet (McDonald, *et al*., 2002).

DDGS is produced from the fuel ethanol industry and is available for inclusion in diets fed to pigs. The composition of distiller's grains depends on the starting materials. Grain distiller's grains have higher energy content than malt distiller's grains. Corn and barley are the main crops used for biofuel and brewery production.

The CP and DE content of distiller's grain is about 320 and 12 g kg-1 DM (McDoland *et al.*, 2002). Furthermore, Jaworski *et al*., (2015) reported that DDGS contains NSP and total dietary fiber of 192 and 322 g kg^{-1} DM, respectively .The low dry matter (DM) and high fibre contents limit the inclusion of distiller's grains in pig diets. Urriola and Stein *et al*., (2009) reported that inclusion of up to 30 % of distiller dried grain content in the diet negatively influence the feed intake, nutrient digestibility and performance of growing pigs. Such negative influence of dietary fiber depends on the age of the pig, fiber source, quantity, degree of solubility, proportion of lignin, and microbial composition of the gut of the pig (Bach Knudsen, 2001; Högberg and Lindberg, 2006). All monogastric animals lack digestive enzymes digesting NSP both soluble and insoluble. However, the microbes present throughout the gastro intestinal tract (GIT) but in higher concentration in the hindgut can digest both soluble and insoluble NSP, a higher extent of soluble, but also insoluble can be partly digested. The water soluble NSP (non-cellulose polymers and pectic substance) leads to increased viscosity of digesta in the small intestine (Noblet and LeGoff, 2001). This increased in viscosity of intestinal content might limit the rate of diffusion of substrates as well as

digestive enzymes, consequently, this leads to inefficient mixing of digesta with digestive enzyme in the gut and reduce absorption of nutrients (Cherbut *et al*., 1990; Bedford *et al*., 1991).

In *L. campestre*, klason lignin is the major part of the insoluble dietary fiber (Andersson *et al*., 1999). Lignin is a high molecular weight polymer and is not considered a functional dietary component because it is indigestible both by endogenous and microbial enzymes (Grieshop *et al*., 2001). Beside this, lignin influences the digestibility of other fibrous components of the diet by acting as physical barrier to microbial enzymes reaching to cellulose, hemicelulose and other nutrients (Jung and Deetz 1993).

Pigs can obtain energy from fiber, which derive mainly from the microbial fermentation of NSP in the hindgut generating short chain fatty acids such as acetic, propionic and butyric acid (Bach Knudsen, 2001). These fatty acids can supply 24-30% of the maintenance energy requirement for growing pigs (Varel and Yen, 1997; Montagne *et al*., 2003; Urriola *et al*., 2013). The pig's ability to utilize dietary fiber is positively related to age and weight of the pig (McDonald *et al*., 2002). Consequently, a wider range of fibrous feedstuffs may be appropriate for use in diets of late finishing and dry sows. Soluble fiber disolve in water and form gel-like substance which is responsible for increasing digesta viscosity- thus prevents mixing of digesta with digestive enzyme and absorpation of digested nutrients in small intestine (Bach Knudsen, 2001; El Khoury *et al*., 2012). On the other hand, the insoluble fiber induces an increased passage rate through the gut, this would result in a decreased digestion and nutrient absorption (Lattimer and Haub, 2010).

According to Jørgensen *et al*. (1996), increasing amount of dietary NSP reduces metabolizable energy (ME) intake and nutrient digestibility. Similarly, a 1% increase in the NSP content of the diet was found to decrease gross energy (GE) digestibility by 1.3% (Just *et al*. 1983). In contrast to this, experiment conducted by Longland (1994) on weaned pig using sugarbeet pulp which is high in soluble NSP (164 g NSP kg^{-1}) and cereal-based diet (75 kg^{-1}) showed that the digestibility of NSP (arabinose, mannose, glucose and uronic acids) was significantly higher ($P < 0.05$) in pigs fed high sugarbeet pulp than those who received cereal based diet. This could be attributed to that the fiber fraction of cereal is more insoluble and resistant to digestion than the soluble NSP in sugar beet (Vervaeke *et al*., 1991).

2.6. Determination and Utilization of Dietary Energy

Growing Pigs requires sufficient quantity and quality of nutrients to grow fast and attain slaughter weight. Among the required nutrients, energy and protein accounts the major proportion. When pigs consume dietary energy, part of it is absorbed in the body and the rest is excreted to the environment as faecal, urinary or heat energy. The part that is absorbed is then used for regulating body function (maintenance), growth, development of fetus, lactation and extra activities (McDonald *et al*., 2002; Kil *et al*., 2013). According to Black and de Lange (1995) and NRC (1998) growing pig use one third (33.3%) and two third (66.7%) of their total dietary energy intake for maintenance and protein or fat deposition, respectively.

There are different methods of expressing the dietary energy utilization as presented in figure 2. Gross (GE) or heat of combustion is the total energy content in the food or feed materials measured by complete burning of feed sample under bomb calorimeter (Larbier & Leclercq, 1994; Oresanya, 2005). Oxidation of dietary carbohydrates, protein and lipids yield an average GE values of 3.7, 5.6 and 9.4 kcal g^{-1}, respectively (McDonald *et al*., 2002; Oresanya, 2005).

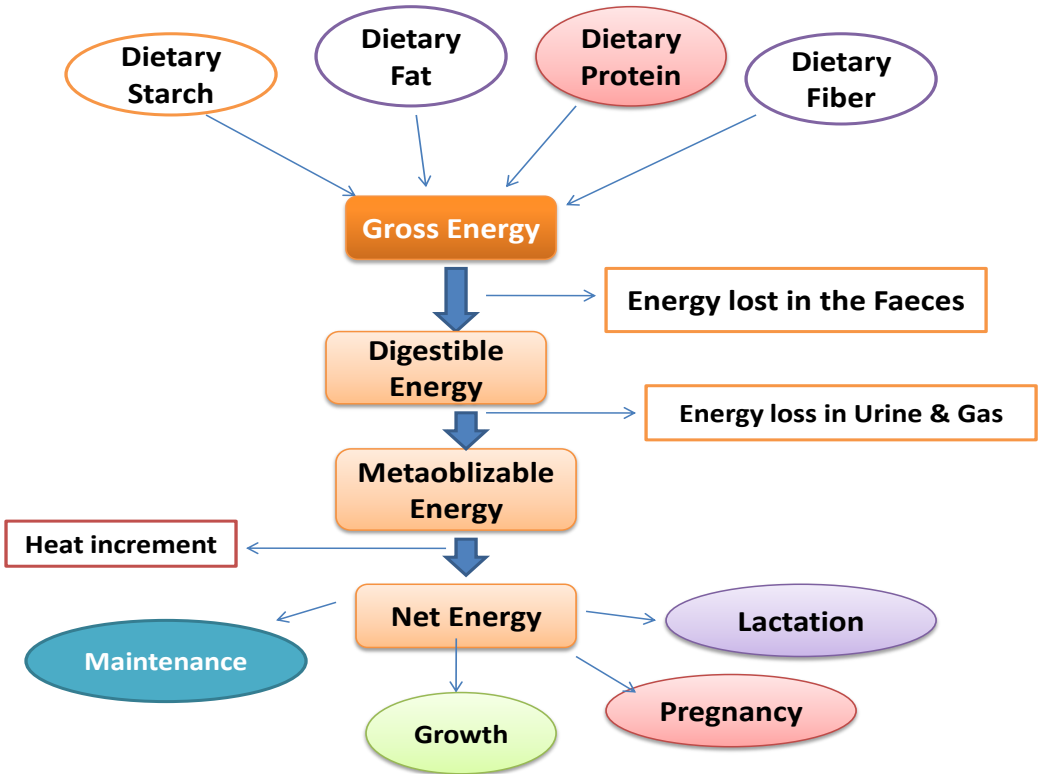

Figure 3. Dietary Energy Sources and Energy Use in Growing Pig (Modified from: Euken, 2012).

The potential energy in dietary feeds can be either stored in the body tissue in the form of glycogen or directly utilized for different biological functions as adenosine tri phosphate (ATP) (Emmans, 1999). However, GE fails to indicate the amount of energy which could be utilized by the animal (Oresanya, 2005). Beside to this, GE content in the faces is apparent (not true), since it contains endogenous secretion of energy from body fat which underestimate the energy value.

Digestible energy (DE) and metaoblizable energy (ME) have been commonly used systems for evaluating energy value of diets fed to pig (NRC, 1998; McDonald *et al*., 2002). DE is calculated by subtracting faecal GE from dietary GE. DE is assumed to be the quantity of energy which is absorbed in the animal body. Though DE is a better mean of energy utilization than GE, but fails to indicate the amount of energy lost in the form of urine, heat, and gas (McDonald *et al*., 2002). ME is defined as DE minus energy lost in the urine and gases like methane (CH_4). Methane accounts 11- 13 % and 0.1 - 3% of DE lost in ruminant and monogastric animals, respectively (Oresanya *et al*., 2008). ME is a better way of measuring energy utilization than GE and DE, however, it does not tell us how much energy is lost in the form of heat.

Net energy (NE) is determined by deducting heat increment from ME (NRC, 1998). The net energy (NE) content in diets for growing pigs is determined using a respiration chamber or published prediction equations (NRC, 1998; Milgen *et al*., 2000). Generally, GE, DE and ME represent potential energy, while NE represents the quantity of energy utilized by the animal for maintenance, growth, production and reproduction (Oresanya *et al.,* 2008) which is more covenant to deal with energy efficiency.

2.7. Digestibility

Digestibility is how big part of the diet that is digested and indicates the nutritive value of the feed. The readily degradable nutrients are easily digested in the stomach and small intestine by enzymes secreted in the wall of gut whereas the NSP part of the feed is digested by the help of fiber digesting microbes. In ruminant microbial digestion of fiber takes place mainly in the rumen, but also in the large intestine, while in pig and other monogastric animals, microbial fermentation of fiber is mainly held in the hindgut and yields end products like acetic acid, propionic acid, butyric acids, amino acids and vitamins. However, the digestion and absorption of NSP in the hindgut is not efficient due to short time stay of digesta in the

large intestine, for example, hind gut fermenting animals like rabbit re-eat their soft faces (rich in nutrient which is not absorbed in the gut) to compensate their nutrient requirement. Faeces contain non-dietary substances such as, saliva, enzymes, dead cell from the wall of gut, dead microbes and minerals which are endogenous origin; as a result the digestibility value obtained without excluding endogenous origin is apparent and underestimates the nutrient digestibility. Determination of ileal CP and amino acid digestibility by collecting digesta from the terminal ileum gives more accurate digestibility than coefficient of total tract digestibility. Accordingly, ileal digestibility is suitable technique to pig and other monogastric animals due to the following reasons; (1) it assumes that the easily digestible nutrient are already absorbed before the digesta reach ileum and digesta in the ileum could be considered as a part which cannot be digested by the animal enzyme; (2) end product of microbial fermentation in the hind gut fermentation have less contribution to support nutrient demand of the animal; (3) it also excludes the lower gut source of errors due to endogenous origin (McDonald *et al*., 2002).

Digestibility can be determined by total tract collection; marker technique; prediction equation through multiple regression and difference calculation as well as by *in vitro* techniques. Among these techniques, total collection is the most reliable method of digestibility measurement (Khan *et al*., 2003). Digestibility through total tract collection that is collection of all in and all out, require measures of the amount of nutrient consumed and amount lost in faeces. Metabolic create could be used to measure the faecal output of pigs (McDonald *et al.,* 2002). Total collection gives good estimation of DE. However, it takes time, more live animals are needed and it is labour intensive. The time and cost involved in digestion experiments can be minimized by the use of marker technique where spot samples of faces are collected for chemical analysis.

Markers are indigestible substance either added to the diet (external) or natural component (internal) of feed stuff (Khan *et al*., 2003). The most widely used external markers include titanium dioxide (TiO_2) and chromic oxide (Cr_2O_3) and the most common internal marker is acid detergent insoluble ash (ADIA) (Bodine *et al*., 2002; Christian, 2014). Chromium oxide (Cr_2O_3) is the widely applied marker in cattle digestion studies (Christian, 2014). Titgemeye (1997) and Christian (2014) reported that Cr_2O_3 recovery varies greatly among individual animals and using this technique is connected with health hazard due to its carcinogenic properties.

Compared to Cr_2O_3, TiO_2 marker is a rapid, cheap, has a consistent faecal recovery with better precision to predict nutrient digestibility and has low carcinogenic properties (Myers, 2004; Christian, 2014). As a result, TiO_2 has been introduced as an alternative marker to Cr_2O_3 in farm animals (Short *et al*., 1996; Titgemeyer *et al*., 2001).

Acid detergent insoluble ash (ADIA) is the residue after a sample has undergone acid detergent digestion by H_2SO_4 followed by ashing at 505 ^{0}C for 2 hours (Van Soest, 1994). It has a high 97.5-99.3% recovery in the faeces, especially in animals fed on forage alone (Bodine *et al*., 2002). ADIA was also found to give high accuracy to predict faecal output as well as dry matter digestibility (DMD) for cows on roughage diets (Kanani *et al*., 2014).

Protein deficiency is a constraint of practical significance and might limit the digestibility of NSP (Bediye *et al*., 1996; McDonald *et al*., 2002). Dietary protein will be a source of microbial protein for the fiber digesting microbes and is necessary for their multiplication and ability to digest the fiber. Bediye *et al*. (1996) suggested that supplementation of the diet with plant origin protein source from oil seed cake improves digestibility of fiber. However, the digestibility and utilization of most oil cakes is constrained by the presence of NSP and anti-national factors (example protease inhibitors, tannin, glucosinolates, gossypol and cianoglucosidase). Methods like de-hulling (McDonald *et al*., 2002), heat treatment (Hancock *et al*., 1990), extruding (Qiao *et al*., 2003), soaking (Ibrahim *et al*., 2002) and selective breeding (Andersson *et al*., 1999) could be alternative solutions to lower the level of anti-nutritional factors in the oil seed cakes. But, care should be given from overheating since amino acids especially lysine is susceptible to overheating (Willis, 2003).The digestibility of NSP is also influenced by type of DF, DM intake,site of sampling (ileal or total tract), laboratory analyis procudures (improper mixing of samples, boiling time, amount of regents added) .

2.8. Effect of feed on Feeding Behaviour and Feed Intake

2.8.1. Feeding Behaviour

For centuries, rations have been formulated based on the nutrient requirement of the animal. But recently, nutritionists and animal behavior scientists begin to consider the influence of diet on the behavior and welfare of the animal (Brooks, 2005).Feeding behavior is defined as the act of feeding which can be explain by reaction, interest of eating, ‘putting the head in the

trough' or 'chewing food' (Maselyne *et al*., 2015).Today many studies have been conducted in farm animals to understand feeding behavior (Watts *et al*., 2000; Brooks, 2005; Gonyou *et al*., 2012; Maselyne *et al*., 2015). Each of these studies includes different feeding behavior evaluating parameters such as palatability, feed intake, meal presentation, feeding frequency per day, time interval between feeding, time taken to finish and number of visits to the trough or feeder.

Palatability is an important factor that affects the feed intake of the animal (Gonyou *et al*., 2012). It is defined as the overall acceptance of the animal to consume on given feedstuff (Church, 1977). Behavioral studies showed that palatability is mainly associated with taste of the diet (Nelson and Sanregret, 1997; Gonyou *et al*., 2012). Since, taste of diet is an important factor that helps the animal to choice feed (Gonyou *et al*., 2012) and it aids the animal to identify whether the diet is toxic or not. Though the diet has unpleasant aroma or color, animals usually preferred to test.

Taste preference depends on innate behaviour, post-ingestive feedback or previous exposure (Gonyou *et al*., 2012; Provenza, 1996; Letarte *et al*., 1997). According to innate taste preference, new born animal have an innate preference to accept sweet and refuse bitter and sour (Provenza and Balph, 1990; Gonyou *et al*., 2012). Feed preference studies by Kennedy and Baldwin (1972) showed that pigs prefer to eat diets with sweet taste of sugars. Animals eat diets that contain anti-nutritional factors; however they limit their intake based on the level of the toxin in the diet (Provenza, 1995). A behavioral study by Kyriazakis and Emmans (1992) using conventional at (a low, 140 and a high, 300 g kg-1 of protein diets) and rapeseed meal (inclusion levels of 0 and 180 g kg^{-1}) showed that pigs preferred to eat conventional diets than rapeseed meal. This indicates that even though rapeseed is rich in amino acid and protein profile, the pig sense a bitter test, due to erucic acid present in rapeseed meal, and use it as a signal to change their preference.

Post-ingestive feedback is feedback or signals obtained from central nervous system following taste, flavour or ingestion of particular feed and is another mechanism animals use to select feed (Provenza, 1995). As a result of such interaction, animals adjust their feed preference. Taste preference develops through time as pigs eat on the same feed (Letarte *et al*., 1997). This implies that young pigs offered with novel diet could show less preference at

a time, but improved through time. Furthermore, feeding behavior of pigs is influenced by other various factors like type of feed, breed, stocking density, social environment, access to feed (*ad libitum* or restricted feeding), gut fill, feed presentation (pelleted, powder, dry or wet feed), and feeding time during the day (Watts *et al*., 2000; Brooks, 2005; Baumung *et al*., 2006; Cornou *et al.*, 2008).

De Leeuw *et al*. (2008) reported that pigs fed on fibrous diets showed longer satiety or less feeding motivation due to gut fill. On the other hand, a study by Bakare *et al*. (2014) revealed that pigs spent more eating time on fibrous diet like maize cobs compared to concentrate diet formulated from different proportion of yellow maize, soya bean, soya bean cake, wheat bran, sunflower, sunflower oil cakes. They emphasized that the longer eating time recorded on maize cobs is due to unsatisfied feeding motivation of the pigs to meet their nutrient requirement. Pigs that received dry feed took longer time to finish than those fed in wet form (Solà-Oriol *et al*., 2009). The content of amino acids and a deficiency in particularly lysine also have been reported to influence feeding behavior by reducing feed consumption and number of visit to feed trough (Maselyne *et al*., 2015). Illness negatively influences the feeding motivation of an animal (Weary *et al*., 2009). A behavioral studies done by Sowell *et al*. (1998) and Gonzalez *et al*. (2008) showed that diseased cows spent less time at the feed trough compared to healthy cows.

Comparing individually housed pigs and group housed pigs show that the physical and social environment in group housed pigs affects feeding behavior and production performance (Brooks, 2005; Steyn *et al*., 2012). Group housed pigs modify their feeding behavior by eating less frequently, but consume more food once-off at a faster rate than pigs penned individually (Nielsen *et al*., 1996; De Haer & Merks, 1992). This difference could be due to competition and social stress in the group or stress due to isolation (Steyn *et al*., 2012).

Generally, feeding behavior data provides a vital tool for farm management to better understand the factors that affects feed intake and to formulate diets based on their preference as well as to predict illness to improve housing and facilities and to maintain both the production performance and welfare of the pig (Cornou *et al.*, 2008; Brown-Brandl *et al*., 2013).

2.8.2. Feed intake

The commercial pork industry requires knowledge of pigs' voluntary feed intake to supply the animal with required amount and to avoid toxicity, wastage and cost associated with overfeeding (Schinckel *et al*., 2008). Feed intake refers to the quantity of feed consumed by the animal per day is often measured in g kg^{-1} DM. It determines quantity of nutrient intake of the pig for maintenance, growth, production and reproduction purpose (Nyachoti *et al*., 2004). Feed intake can be determined by subtracting amount feed refusal from amount feed offered (McDonald *et al*., 2002). Nyachoti *et al*. (2004) point out that understanding of the main factors that controls the dietary feed intake in pigs is very crucial for designing sound feeding strategies.

Feed intake of pig and other monogastric animals is regulated through concentration of metabolized nutrient in the blood, degree of 'gut fill' and ambient temperature, thus, sends signal to hypothalamus either to start or stop eating feed (McDonald *et al*., 2002). As animal grow, DM intake increases proportional to its metabolic body weight ($W^{0.75}$) (McDonald *et al*., 2002). Beside this, dietary factors such as bulkiness, nutrient density, processing and presentation are reported to influence feed intake (Nyachoti *et al*., 2004). According to McDonald *et al*. (2002) and Poppi and McLennan, (1995), a dietary feed containing below 7% CP is known to reduce the DM intake. Different supplementation strategies have been proposed to improve the nutrient density, DM intake, production and reproduction performance (Preston and Leng, 1986; Lenge and Devendra, 1995; McDonald *et al*., 2002). In this regard, oil industry by-products from linseed, sunflower, soya bean, peanut, groundnut and safflower have been widely studied on pig performance (Henry *et al*., 1992; Matthews *et al*., 2000; Qiao *et al*., 2003; De Vries, 2014). Most of the oil seed cakes contain 25-45% CP (McDonald *et al*., 2002). Reduction in DM intake was reported in pig fed on canola, juncea and rapeseed meal (Roth-Maier *et al*., 2004; Collins *et al*., 2011; Choi *et al*., 2015). This is due to the presence of glucosinolate in the meal (Lee *et al*., 1984). A study by Collins *et al*. (2011) showed that there is a decrease in feed intake when pigs are fed diets with more than 2.0 - 2.5 µmol total glucosinolates g^{-1} diet (Collins *et al*., 2011). Because, glucosinolate interfere with activities of digestive enzymes and absorption of nutrient, consequently, digestiblity and passage rate will be slow down there by pig will not be motivated to eat (Bill *et al*., 1983). Furthermore, a study by Adeniji and Azeez (2008) fed 5, 10 and 15% of cotton seed cake (CSC) to pigs and found that higher DM intake (1.45 kg) was recorded in pigs fed 5% of CSC than pigs fed 15% CSC (1.25 kg). Such gradual reduction in DM intake with

increasing proportion of CSC could be due to the CF content in the CSC. CSC contains 11 % crude fiber, hence, fiber is less digestible and fills the gut which in turn reduce DM intake by pigs (McDonald *et al*. 2002; Adeniji and Azeez, 2008).

DM intake is positively correlated with rate of digestion, this is because, the faster the rate of digestion, the more rapidly is the digestive tract emptied, and a signal is send to the hunger center through the central nervous system, then the animal starts to eat (McDonald *et al*., 2002).

2.9. Blood profile

Nutrition is one among the various factors which could positively or negatively affect the physiology and performance of farm animals (Ajao, 2013). Bamishaiye *et al.* (2009) emphasized that the nutritional status of an individual depends on the quality and quantity of dietary intake and it can be identified by either one or combinations of clinical sign, biochemical, hematological or dietary methods. Blood is medium of transport for oxygen, enzymes, hormones and digested nutrients to different parts of the body. In traditional (extensive) livestock production system, individual nutrient intake is unknown or difficult to assess, hence, nutritional status of the animal can be determined by analyzing the blood profiles of the animal (Chittavong *et al*., 2012). Nutritional studies revealed that the type and content of diet affects blood (example white blood cells, red blood cells, hemoglobin, cholesterol, glucose, urea nitrogen, pH, insulin, T3, T4 and electrolytes) profile of healthy animals (Spiegel *et al*., 1993; Kurtoğlu *et al.,* 2005; Choi *et al*., 2015).

Pigs and other monogastric animals are highly reliant on their dietary glucose as their major source of energy and average blood glucose level is 100g dL^{-1} (Pond, 2003). When the glucose level in the blood is lower than normal range is an indication of hypoglycemia (Olorunnisomo *et al*, 2012). Omotosho & Olufemi (2013) and Pond (2003) point out that the level of glucose in the blood increases after feeding and at stress conduction. When the blood glucose level is high, then insulin is released from the pancreas to regulate the elevated glucose level, by assisting the body to store as glycogen in the body tissues mainly in muscles and liver, but failure to regulate such high levels resulted diabetes (Omotosho & Olufemi, 2013). Blood urea nitrogen is an indicator not only for protein and amino acid utilization, but also for the capacity of the animal to retain dietary nitrogen in the body (Whang & Easter, 2000). It has been found that blood urea nitrogen is positively correlated to protein quantity in the diet, but negatively correlated to protein quality (Bassily *et al*., 1982; Whang & Easter,

2000). Other blood parameters such as sodium (Na^+), glucose, hematocrit (Hct) could be used as a sign of dehydration, under nutrition or loss of appetite (Buzzard *et al*., 2013). Lower level of glucose, Na^+, but higher level of hematocriate (Hct) and hemoglobin (Hb) have been reported in unhealthy pigs (Buzzard *et al*., 2013). The pH indicates the acidity or alkalosis associated to the feed, it might also play a role in examining toxicity like rumen acidosis in ruminant animals due to eating high concentrate (McDonald *et al*., 2002).

Beside this, blood parameters could be influenced by presence of anti-nutritional factors in the feed. Higher blood concentration of thyroid hormone T3 and T4 were found in growing pigs fed rapeseed, juncea and canola cake containing glucosinolates (Choi *et al*., 2015; Collins *et al*., 2011; Mejicanos *et al*., 2016). Rafiu *et al.* (2013) and Etim *et al.* (2014) reported that evaluating the blood profile of nutrient intake play great role in adjusting the inclusion level of certain nutrients in the animal diet. Hence, blood profiles serves us good indicators in monitoring nutrient metabolism, liver function, nutrient deficiency, feed toxicity, especially with dietary constituents that negatively affect the blood as well as the health condition of farm animals (Aro and Akinmoegun, 2012; Aro *et al.*,2013;Buzzard *et al*., 2013).

3. Material and Methods

3.1. Animal Ethics

Before the start of the experiment, all animal procedures such as housing, facilities and handling of pigs during data collection was first approved by the ethical committee of the Uppsala region.

3.2. Pigs and housing

A total of 8 female pigs ((Landrace x Yorkshire) × Hampshire) with an average initial body weight of 26.5±2.5 kg, 2 months age, were obtained from the pig herd at the Swedish Livestock Research Centre. The pigs were transported to VHC-building animal facilities at Ultuna campus, SLU. The pigs were kept in individual pens with nose contact with the neighbour pig. The pigs were adapted to the individual housing for one week before the start of the actual experiment. Each pen was supplied with individual heat lamp and nipple-drinkers. During the adaptation period, the pigs had access to straw, but at start of actual experiment, straw were replaced by rubber mat for easy collection of faeces and to minimize errors in the digestibility study as a result of eating straw. Pigs had *free access* to water through nipple drinkers. The pigs were healthy throughout the experimental period.

3.3. Experimental Design and Treatments

The experiment was performed with eight pigs in a double 4 × 4 Latin square, with a total of four diets and four periods, resulting in two pigs where fed each diet in each period. The pigs were randomly assigned to the treatment in period one. Each period lasting for a total of 11 days, including 7 days of adaptation followed by 4 days of sample collection. A total of 4 diets were formulated, a basal diet was formulated to meet the nutritional requirement of the pig and in the experimental diets, 4%, 8% and 12% of basal diet was substituted with LC. The LC was a residue after mechanical cold pressing to harvest oil from the lepidium seed. An indigestible marker of titanium-dioxide (TiO_2) was added in all diets at 2.5 g per kg and used for digestibility calculations. All diets were fed in a pelleted form through individual feeder. The compositions of the diets, as presented by the manufacturer, are presented in table 3.

Table 3. The nutrient composition and proportion of the concentrate mix (% DM) used in the feeding trial

Ingredients	Control	LC4[1]	LC8[2]	LC12[3]
Barley	52	49.9	47.8	45.7
Wheat	25	24	23	22
Wheat bran	10	9.6	9.2	8.8
Wheat middling	8	7.7	7.4	7
Soya bean protein	2	1.9	1.8	1.8
Limestone	1.7	1.6	1.6	1.5
Lysine	0.4	0.4	0.4	0.4
NaCl	0.4	0.3	0.3	0.3
Premix	0.4	0.4	0.4	0.4
Threonine	0.1	0.1	0.1	0.1
Methionine	0.1	0	0	0
Titanium dioxide (TiO2)	0.3	0.3	0.3	0.3
Lepidium cake	0	4	8	12

[1]LC4: 4% of Lepidium cake+ 96 % control; [2]LC8: 8% of Lepidium cake + 92 % control; [3]LC12: 12% of Lepidium cake+ 88% control.

The nutrient composition of the LC and experimental feeds used is presented in table 4.

Table 4. Nutrient composition of feeds in the current study in % of DM

Type of Nutrient	LC[1]	Control	LC4[2]	LC8[3]	LC12[4]
DM[a]	92.8	91	90.6	91.2	91.6
OM[b]	85	12	12.3	12.8	13
CP[c]	18.3	12	12.3	12.8	13
Ash	7.8	5.7	5.8	5.8	6.0
EE[d]	13.2	2.6	3	3.25	3.7
Essential Amino Acids of LC in g 100 g-1 protein as -is					
Lysine	1.2	na	na	na	na
Methionine	0.2	na	na	na	na
Treonin	0.6	na	na	na	na
Cystein + Cystine	0.5	na	na	na	na

[1]LC: Lepidium Cake; [2]LC4: 4% of Lepidium cake+ 96 % control; [3]LC8: 8% of Lepidium cake + 92 % control; [4]LC12: 12% of Lepidium cake+ 88% control. [a]DM: Dry matter; [b]OM: Organic matter; [c]CP: Crud protein; [d]EE: Ether extract; na: not analyzed.

Table 5. Dietary fiber composition of feeds in the current study in g kg-1 DM

	Control	LC4[1]	LC8[2]	LC12[3]
NSP				
Total	168	173	176	189
Indigestible	145	151	153	45
Arabinose				
Total	3	29	29	29
Insoluble	27	27	26	26
Xylose				
Total	58	54	50	50
Insoluble	53	51	47	46
Manose				
Total	6	6	6	6
Insoluble	5	6	5	6
Galactose				
Total	5	6	7	8
Insoluble	4	4	5	6
Glucose				
Total	60	62	61	64
Insoluble	48	50	49	52
Uronic Acid				
Total	9	15	23	30
Insoluble	7	13	20	28
Klason lignin	14	18	21	24
Total Dietary fibre	181	191	196	213

[1]LC4: 4% of Lepidium cake+ 96 % control; [2]LC8: 8% of Lepidium cake + 92 % control; [3]LC12: 12% of Lepidium cake+ 88% control; NSP: non-starch polysaccharides.

3.4. Data collection

3.4.1. Faeces

Following seven days of adaptation, faecal samples were collected every period for four days. Faecal spot samples were collected from carefully cleaned floor directly after defecation. The collected sample where mixed and 45 g of faeces per pig per day (a total of 180 g)were weighed in a petri dish, the samples for the four days collection were pooled and stored at -20 ^{0} C during collection period.

3.4.2. Blood sample

Blood samples were collected from jugular vein using 5 ml syringe at the end of every feeding period. The blood samples were analyzed immediately using a I-STAT analyzer to test pH, blood hematocrit glucose, hemoglobin (Hb), urea nitrogen, blood carbonate (HCO3-),

total carbon dioxide (TCO2), anion gap (AnGap), base excess (BE) and electrolyte (Na^+, k^+, and Ca^+).

3.4.3. Feed intake and Body weights

The pigs were weighed before start of every period, and feed allowance was set to 4 % of body weight, which was divided in two feedings per day at 7:00 and 15:00. At the end of feeding trail, the mean final body weight of the pigs was 52.5±4.5 kg.

3.5. Feeding behavior

A behavioural study was conducted to evaluate the effect of LC on pig feeding behaviour. The feeding behaviour of the pigs was assessed during the 1st and 8th day of each feeding period to determine how long time the pigs take to finish, amount of feed offered, weight of feed on floor and amount of feed left in trough. The frequencies of the behaviors rooting, searching, throwing and moving feed (Table 4) were recorded during the morning feeding for 2 hours (7:30-9:30 AM). For the statistical analysis the frequency of all these behaviors were summed up to a variable called FeedBeh.

A feed choice test was performed in the beginning and end of the trial to evaluate whether the pigs prefer control or treatment diets. Pigs were offered a small amount of control and treatment feed (LC12); one feed in the left and the other feed in the right corner of the trough. At the first and last day, 2 x 60 g were given to the pigs. The location of the two feeds was shifted at random between pens and rounds. The first feed choice (control or LC12) and the number of visits to each feed were continuously recorded for 5 minutes. This process was repeated once for each pig. After the second round it was recorded if there were any leftovers in the two corners.

Two eating parameters were analysed: eating time from the time the feed was offered to the pig until all feed was gone (in minutes) and feeding rate g min^{-1}. Feeding rate was calculated as amount of feed consumed / eating time. The first day some pigs did not eat all feed within the 2 hours they were studied. These pigs were given the value 121 minutes for eating time.

Table 6. Definitions of pig behaviors recorded during continuous observation

Type of Behaviour	Definition
Rooting	The pig's snout is moving in the feed trough
Searching	The pig seems to search for another feed
Throwing	The pig digs up the feed by its snout and spreads it to the floor.
Moving feed	The pig moves the feed in the through by its snout
Eating	The pig has its snout on the feed trough and consumes feed

3.6. Sample preparation and Analyses

At the end of the trial, the samples were freeze dried in (CD 8, Heto, Denmark) at 0.62mill bar, -91^{o}C for 3 days, milled at Cyclotec 1093 Sample Mill using1-mm screen size sieve before taken for nutrient analysis. The dry matter, ash, TiO_2, ether extract, crude protein and gross energy analysis were performed in the Animal nutrition laboratory at the Department of Animal Nutrition and Management, SLU and the analysis of non-starch polysaccharides were performed at the Food science Department laboratory, SLU.

DM was determined by drying 2 gram of feed and faecal samples in oven at 103 ^{0}C overnight. Ash content was determined by burning the feed sample under muffle furnace at 550°C for 3 hours. Nitrogen content was determined by the Kjeldahl method (Nordic committee on feed analysis, 2003). The CP value of feed and faecal samples was determined by multiplying the percent of nitrogen in each sample with a factor of 6.25. Crude fat was analysed according to the procedures of Official Journal of the European Communities (1984). Total and insoluble NSP, sugars and lignin content was determined according to a modified Uppsala method (Bach Knudsen, 1997). Gross energy of both feed and faeces were determined by bomb calorimeter (Parr 6300 Oxygen Bomb Calorimeter, USA). Total dietary fiber in the feed and faeces was calculated by adding of total NSP and lignin. Coefficient of apparent digestibility of the nutrient in the test diet (CTTAD) was estimated using TiO_2 as indigestible marker in the feed and in the faeces by using the following equation (Fastinger and Mahan, 2006) (Eq.1).

$CTTAD_T = 100-(Ti_F \times Nf)/(Tif \times N_F)$ (Equation 1)

Where, CTTAD = coefficient of apparent digestibility of the nutrient in the test diet,

TiF= TiO_2 concentration in the feed in g kg-1,

Nf = nutrient concentration in faeces in g kg-1,

NF = nutrient concentration in the feed (g/kg) and

Tif = TiO_2 concentration in faeces (g/kg).

The result of the TiO_2 of each sample was cheeked with standard curve and samples having CV higher than 5% were rerun.

The digestible energy (DE) content (MJ kg^{-1} of DM) was calculated as

$DE = GE_f \times$ CTTAD of GE (Equation 2).

Where GE_f is gross energy in the feed (MJ kg^{-1} of DM)

The digestibility of different nutrients in Lepidium cake was estimated both with regression and difference calculation. For the difference calculation, following equation was used (Bureau *et al.*, 1999):

$CTTAD_{LC} = CTTAD_{t.diet} + [(CTTAD_{t.diet} - CTTAD_{C.diet}) \times (0.88N_{c.diet} \times 0.12N_{t.diet})]$

However, for estimation of the CTTAD of Energy in lepidium cake following equation was used:

$CTTAD_{LCenergy} = [CTTADtdiet-(0,88*CTTADcdiet)]/0.12$

Where $CTTAD_{LC}$=Coefficient of apparent total tract digestibility of Lepidium cake; $CTTAD_{t.diet}$: Coefficient of apparent total tract digestibility of test diet; $CTTAD_{c.diet}$: Coefficient of total tract apparent digestibility of the control diet; $N_{c.diet}$: % nutrient in the control diet; $N_{t.diet}$: % nutrient in test diet. Only diets with 12 % inclusion of LC was used for the difference calculation.

3.7. Statistical analysis

The data on feed intake, body weight, apparent digestibility, blood profile and feeding behavior were analyzed using Proc Mixed procedure SAS (SAS Institute, USA, version 9.4). The model includes treatments (control, LC4, LC8, LC12) and periods (1, 2, 3, and 4) as fixed factor and the individual pig as random factor. The carry-over effects from previous period were tested as fixed factors in the models but were excluded if not significant.

The model used in the regression analysis was $yij = \beta 1 + xi\beta 2 + \varepsilon ij$ (Equation 3)

Where yij = the CTTAD at the LC inclusion level xi; $\beta 1$isintercept; xi is inclusion level of LC (%); $\beta 2$ is regression coefficient of the linear model; εij is residual error.

The model used to analyzed feeding behaviour was as follow:

$Y_{ij} = \mu + P_i + T_j + \varepsilon_{ij}$ (Equation 4)

Y_{ij} = *is* the behavioral parameters like eating time; μ is the mean value; P_i is the fixed effect of period (i = I, II, III, IV); T_i is the fixed effect of treatment (i= control, LC4, LC8, LC12) and ε_{ij} is residual error. The effect of week and dietary treatment on performance and feeding behavior is presented using least square means. Effects with P-values >0.05, <0.05 and < 0.01 are considered as non-significant, significant and highly significant, respectively.

4. Result

4.1. CTTAD and DE

Table 7.CTTAD of DM, OM, CP, GE, NSP, mean values ± pooled SEM

						P value	
	Basal	LC4[1]	LC8[2]	LC12[3]	SEM[4]	Period effect	Treatment effect
DM	0.81[a]	0.75[b]	0.74[b]	0.73[b]	0.005	P>0.05	P<0.01
OM	0.83[a]	0.77[b]	0.77[b]	0.76[b]	0.005	P>0.05	P<0.01
CP	0.81[a]	0.73[b]	0.74[b]	0.73[b]	0.008	P<0.05	P<0.01
GE	0.80[a]	0.75[b]	0.75[b]	0.74[b]	0.005	P>0.05	P<0.01
EE	0.62[b]	0.60[b]	0.66[ab]	0.73[a]	0.01	P>0.05	P<0.01
Total NSP	0.52[a]	0.42[b]	0.40[b]	0.41[b]	0.012	P>0.05	P<0.01
Arabinose	0.54[a]	0.46[b]	0.47[b]	0.49[b]	0.010	P>0.05	P<0.01
Xylose	0.56[a]	0.47[b]	0.49[b]	0.51[b]	0.011	P>0.05	P<0.01
Uronic Acid	0.40[a]	0.19[b]	0.15[b]	0.16[b]	0.031	P>0.05	P<0.01

[1]LC4: 4% of Lepidium cake+ 96 % control; [2]LC8: 8% of Lepidium cake + 92 % control; [3]LC12: 12% of Lepidium cake+ 88% control; NSP: non-starch polysaccharides; NSP: non-starch polysaccharides; [4]SEM: standard error of mean.

As shown in Table 7, the CTTAD of DM, OM,GE, EE, total dietary fiber, NSP, arabinose, xylose and uronic acid was not affected by period (P>0.05), and there was no carry-over effect but were significantly affected by treatment (P<0.01). Accordingly, the CTTAD of DM, OM, CP, GE, total dietary fiber, NSP, arabinose, xylose and uronic acid was significantly higher (P<0.01) in the control diet than the different inclusion levels of LC. Among the different inclusion levels of LC, there was no difference (P>0.05) in CTTAD of DM, OM, CP, EE and GE, total dietary fiber, NSP, arabinose, xylose and uronic acid. While, CTTAD of EE was increasing with increasing inclusion levels of LC. CTTAD of EE was lower (P<0.0001) in the control diet than diets containing 8 and 12 % of LC, but higher than LC4. The CTTAD of CP was affected by period (P=0.01), but there were no carry-over effect. Consequently, lower CTTAD of protein was documented in period I. The CTTAD of DE was higher (P<0.01) in control diet (13.05 MJ kg^{-1} DM) than different inclusion levels of LC. However there was no significant difference (P>0.05) among the inclusion levels of LC and the DE was 12.33, 12.35 and 12.32 MJ Kg^{-1} DM in LC4, LC8 and LC12, respectively.

Table 8. The regression analysis data of CTTAD of lepidium cake (LC) and CTTAD of LC estimated with difference calculation, mean values ±standard error

	β1	β2	R^2	Extrapolation at 100% LC Inclusion	CTTAD of LC estimated with difference calculation
DM	0.79	-0.005	0.62	0.31	0.24±0.035
OM	0.81	-0.006	0.65	0.23	0.27±0.028
CP	0.79	-0.005	0.39	0.27	0.35±0.029
GE	0.79	-0.006	0.55	0.17	0.28±0.045
EE	0.6	+0.009	0.54	1.54	0.88±0.024
DE	12.87	-0.057	0.39	7.17	ne
Total NSP	0.49	-0.019	0.43	0.4	0.07±0.059
TDF	0.42	-0.013	0.56	0.29	-0.12±0.059

β1: intercept; β2: inclusion level; R^2: R-squared. ne= not estimated.

As presented table 8, using extrapolation equation, as the inclusion level of LC was increased from 0 to 12 % the slope of DM, OM. CP, GE and DE decreased. On the other hand, the regression slope of the CTTAD of EE was found positive. The CTTAD of LC was higher for OM, CP and GE but lower for DM, EE; NSP and TDF when estimated with difference calculation compared to regression analysis.

4.2 Blood profile

Table 9. Mean values ± SEM of blood analysis

						reference interval	
	Control	LC4[1]	LC8[2]	LC12[3]	*P* value	Chittavong *et al.* (2012)	Cooper *et al.* (2014)
pH	7.4±0.04	7.5±0.04	7.4±0.04	7.4±0.04	P>0.05	7.26-7.34	na
Glucose (mmol/L)	6.3±0.21	6.5±0.21	6.3±0.21	6.3±0.23	P>0.05	5.7-7.1	7-13.9
Hb (g/L)	121±0.28	123±0.29	120±0.26	120.5±0.25	P>0.05	103-111	88-127
TCO2 (mmol/L)	33.8±0.8	33.5±0.8	31.6±0.8	33.3±0.9	P>0.05	na	na
Hct (%)	35.3±0.8	36.2±0.9	36.3±0.8	35.5±0.7	P>0.05	31-33	25.4-43.8
PCO2 (kPa)	6.9±0.5	6.7±0.5	6.5±0.5	6.3±0.5	P>0.05	na	na
HCO3 (mmol/L)	32.3±0.8	31.9±0.8	30.3±0.8	31.7±0.9	P>0.05	na	na
BE (mmol/L)	6.6±1.1	6.6±1.1	4.8±1.1	6.4±1.2	P>0.05	na	na
AnGap (mmol/L)	12.5±0.9	12.5±0.9	14.0±0.9	12.0±0.9	P>0.05	na	13-31
Na^+(mmol/L)	139.3±0.4	138.9±0.4	139.8±0.4	139.0±0.5	P>0.05	135.3-38.1	125-159
K^+ (mmol/L)	5.0±0.2	5.7±0.2	5.5±0.2	5.1±0.2	P>0.05	3.84-4.32	3.7-6.3
Cl^- (mmol/L)	99.1±0.6	100.1±0.6	101±0.6	100.3±0.6	P>0.05	na	90-112

[1]LC4: 4% of Lepidium cake+ 96 % control; [2]LC8: 8% of Lepidium cake + 92 % control; [3]LC12: 12% of Lepidium cake+ 88% control; na: not analysed

The blood electrolytes (Na+, K+, Cl-, AnGap and glucose), hematology (Hct and Hb) and blood gasses (pH, pCO2, TCO2, HO3-1 and BE) were not significantly different between the control diet and all inclusion levels of LC (P>0.05; Table 9).

4.3. Feeding Behaviour

In the first day of the experiment the pigs were given 683-715 g of feed in the morning. Three pigs did not finish eating within two hours; 1 control, 1 LC4 and 1 LC8. The leftovers were 10g, 235g and 268g respectively. They did not finish with 120 minutes, but were included in the analysis with a value of 121 minutes. The average eating time and feeding rate for different weeks are presented in Table 10.

Table 10: Mean, standev., min and max values of eating time and feeding rate per week

	Eating time, min				Feeding rate g min^{-1}			
Weak	Mean	Standev[1].	Min[2]	Max[3]	mean	Standev[1].	Min[2]	Max[3]
1	102.6	37.5	37	121	7	4	4	14
2	26.2	1.4	19	23	25	3	21	30
3	21.1	0.7	17	19	33	4	29	38
4	32.1	1.2	27	31	21	2	18	24
5	17.3	0.9	17	20	39	5	33	47
6	14.6	3.8	8	21	50	21	33	100
7	11.9	0.7	17	19	49	6	40	48
8	22.6	2.4	26	32	31	4	27	36

[1]Standev.; standared deviation; [2]min:minimum; [3]max: maximum; g min^{-1}: gram per minute

The frequencies of FeedBeh in different weeks are shown in Table 11.

Table11. Mean, mode, min and max values of FeedBeh in weeks

Week	Mean	Mode	Min	Max
1	2.0	1	1	6
2	2.5	2	0	6
3	2.1	2	0	5
4	1.8	4	0	4
5	1.1	0	0	3
6	1.1	0	0	6
7	1.0	1	0	3
8	0.5	0	1	2

Eating time was significantly affected by week (P< 0.001). Longer eating time was recorded in week 1 as compared to all other weeks (P<0.001), but there were no significant differences between the later weeks. Feeding rate was significantly affected by week (P<0.001). Slower

eating was recorded in week 1 as compared to all other weeks (P<0.01) and there were significant differences between several of the other weeks, but no clear pattern could be identified. FeedBeh was significantly affected by week (P<0.05). The pigs did more FeedBeh in week 2 than in week 8 (P<0.05) but there were no significant differences between the other weeks.

Average eating time and feeding rate for different treatments are presented in Table 12.

Table 12. Mean standev., min and max values of eating time and feeding rate per treatment

	Eating time, min				feeding rate g min-1			
Treatment	Mean	Standev.	Min	Max	Mean	Standev.	Min	Max
Control	34.6	33.1	15	121	28.3[b]	12.6	4	47
LC4	29.0	25.2	17	121	30.9[ab]	13.6	5	52
LC8	32.9	33.0	17	121	31.2[ab]	13.2	4	52
LC12	27.7	24.6	8	115	35.5[a]	22.5	5	100

Eating time and feeding rate for different treatments in different weeks is also illustrated in Figure 4. It should be remembered that the treatment curves include different pigs at different weeks.

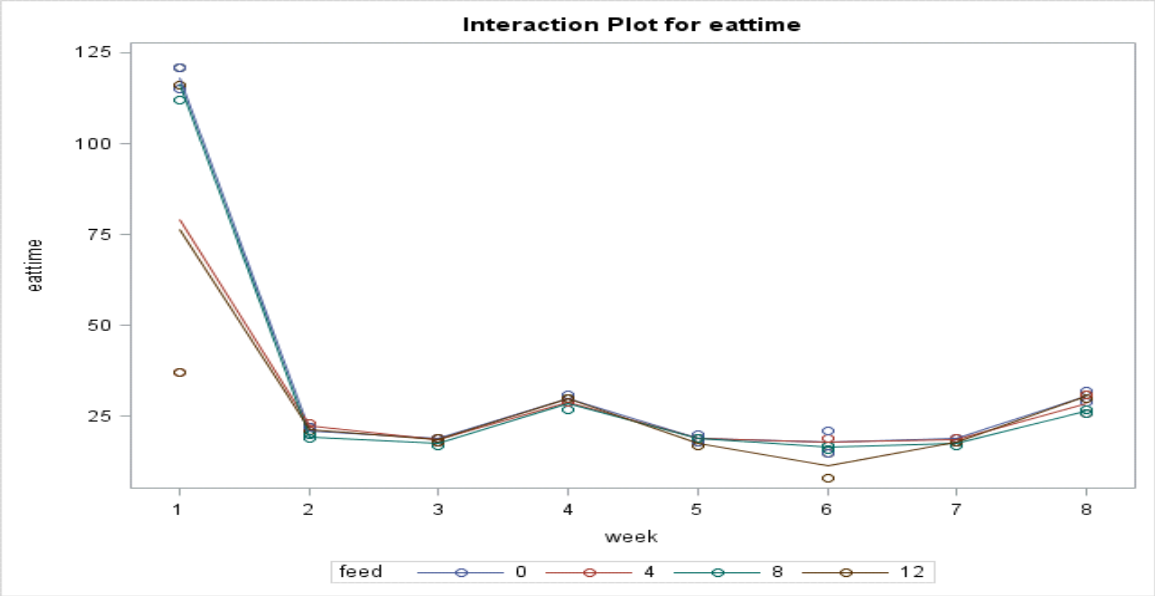

Figure 4.Eating time in minutes for the treatments control (blue), LC4 (red), LC8 (green) and LC12 (brown) from week 1 to week 8.

Eating time was not affected by treatment (P= 0.5). Feeding rate showed a tendency to be affected by treatment (P = 0.1). The pigs tended to eat the control feed slower than the LC12 feed (P = 0.1), but there were no significant differences between the other treatments. FeedBeh also tended to be affected by treatment (P<0.1). The frequencies of FeedBeh with different treatments are shown in Table 13. The pigs tended to do more FeedBeh when they ate control feed than when they ate LC4 (P<0.1) but there were no differences between the other treatments.

Table 13. Frequency of FeedBeh of different treatments

Treatment	Mean	Mode	Min	Max
Control	2.1 [a]	0	0	6
LC4	1.1[b]	0	0	3
LC8	1.4[ab]	0	0	5
LC12	1.6[ab]	1	0	6

The result of the choice test is presented in table 14 and 15. There pigs chose to start with LC12 feed as often as control feed. They ate small amounts of each feed and shifted repeatedly between the feeds. During the 2nd round of the first day 1 pig ate all feed, 2 pigs left equal amounts of control and LC12 feed, 2 pigs left only control feed and 3 pigs left only LC12 feed. During the 2nd round of the last day no pigs had any feed left.

Table 14: Mean, standev., min and max of first day feeding choice, including two rounds

	N=16	Mean	StdDev	Min	Max
Starts on Control	5				
Starts on LC12	11				
No. of visit to Control		8.1	3.1	3	15
No. of visit to LC12		7.8	3.2	3	16

Table 15.Mean, standev. min and max of last day feeding choice, including two rounds

	N=16	Mean	Standev.	Min	Max
Starts on Control	11				
Starts on LC12	5				
No. of visit to Control		8.0	2.6	4	11
No. of visit to LC12		7.4	3.1	3	12

5. Discussion

Inclusion of LC in the diet resulted in decrease in CTTAD of DM, OM, CP, GE and NSP. The lower CTTAD values could be mainly explained by the higher NSP content in the diets with LC then the control, a negative correlation between CTTAD of DM and NSP content have previously been reported by several authors (Sugiura *et al*., 1998; Sklan *et al*., 2004). In the current study, LC contains around 50 % of NSP which is higher than reported by Andersson *et al*. (1999; 393-442 g kg^{-1}), the difference is probably because Andersson analyzed the whole seed, and not the cake as in this study. To the best of our knowledge, no previous study has been done on feeding value of LC to farm animals, so there are no previous published CTTAD values to compare these CTTAD values of LC.

Furthermore, the glucosinolates present in LC could be other reason for the lower ($P<0.05$) CTTAD values documented in different inclusion levels of LC than control diets. A study by Choi *et al*. (2015) showed that pigs fed rape seed meal had a linear reduction in nutrient digestibility with increasing inclusion level (0, 3, 6, 9, and 12%) in the diet. Furthermore, reduction in feed intake and growth performance was reported in growing pigs fed on 5, 10 and 15 % rapeseed meal (Corino, 1991). They conclude that glucosinolate was the main factor for the negative effect of increasing inclusion levels. However the type of glucosinolates are not the same, the major glucosinolate in rapeseed are progoitrin, sinigrin and gluconapin (Choi *et al.*, 2015). A previous study conducted at SLU, by Andersson *et al*. (1999) confirmed that LC contains sinalbin as the main glucosinolate. According to Andersson *et al*. (1999), lepidium seed contains 123-138 µmol glucosinolate per gram of diet which is much higher than recommended level to growing pigs. Depending on the extraction method, oil seed cakes from mechanical extraction contain 12-20 % oil, but almost all glucosinolates remains in the cake and the content in the cake increases about 1.8 times which is 248 µmol g^{-1} of lepidium cake (Beltranena and Zijlstra, 2012). Form this it can be hypothesised that the LC contains more glucosinolate than the lepidium seed. Previous studies in rapeseed meal showed that the total glucosinolate tolerance level of growing pig fed rape seed ranges 2.0 – 2.4 µmol g^{-1} of diet (Roth-Maier *et al*., 2004). In the present study, the total quantity of glucosinolate in µmol g^{-1} of LC was not analyzed. But based on the theoretical calculations, the glucosinolate content in 4, 8 and 12% of LC was 9.92, 19.84 and 29.76 µmol g^{-1} of diet, respectively, which is still higher than the recommend level to growing pigs.

Bille *et al*. (1983) point out that the glucosinolate sinalbin interferes with the activity of digestive enzyme and absorption of nutrient. The anti-nutritional and toxic effects of sinalbin have previously been studied in a rat model with increasing dietary levels of sinalbin from 1-5 Mg g^{-1} DM (Bille *et al*., 1983). The authors concluded that inclusion of sinalbin at more than 1 Mg^{-1} DM affects the biological value, protein utilization and goitrogenic effect. We tried to analyses blood urea to investigate whether the glucosinolate sinalbin interferes with protein utilization, but in the current study, urea nitrogen in all treatment was below 1 and was excluded from further analysis. The blood urea of fattening pigs fed chestnut meal containing tannins ranged between 1.03 - 1.47 mmol urea L^{-1} (Lee *et al*., 2016). The low values in the current study might be because of the analyses method was not sensitive enough. The i-stat is a fast method of blood analysis, but not always the best. Hence, another method of analysis or a study at wider inclusion level (0, 10, 20 and 30 % of LC) is needed to confirm whether LC affects protein digestibility and utilization.

An interdisciplinary research group in SLU have been conducting intensive breeding programs to improve oil quantity, and quality, but effort to reduce the glucosinolate content seems to be forgotten. Hence, developing *L. campestre* variety with a lower glucosinolate content that meets the EU standard is needed to fully be able to utilize LC as livestock feed.

As shown in table 7, increasing the inclusion levels of LC in the diet negatively affect the apparent digestibility of crude protein. This agrees with previous studies (Bach Knudsen and Hansen, 1991; Wilfart *et al.,* 2007; László, 2010) who reported that increasing fibrous content in the diet reduce protein apparent digestibility. This might be due to fiber fermentation in the hindgut results in endogenous loss of nitrogen and amino acid as microbial origin in the faeces. Similarly, a study by Bindelle *et al.* (2009) on the influence of dietary fiber, using 10, 20, and 30% of sugar beet pulp, on microbial nitrogen showed that nitrogen loss in the faeces was positively increased with increasing inclusion levels of sugar beet pulp. In the current study CTTAD of crude protein were also affected by period. Accordingly, pigs showed lower protein digestibility in period 1 than in later periods. This suggests that in period I, the pigs might have immature digestive tract physiology with less protein digestive microbes, but in the later periods, the pigs get older and CP digestibility improved, due to improvement in microbial fermentation in the hindgut. Brooke (2010) and Nguyen *et al*. (2012) reported that young pigs have lower nutrient digestibility, but improves as they grow and matured.

Similarly, Ivarsson *et al*. (2011) investigated on the effect of fiber (chicory or ribwort forage) on growth, digestibility and microbiota and reported that the CTTAD of CP was higher ($P<0.05$) in week 5 than week 3 of the feeding trail.

The CTTAD of CP is subjected to underestimation of protein digestibility due to endogenous losses of nitrogen and amino acid is not corrected for, which also increases with high fiber content in the diet. Accordingly, the LC contains about 50% of fiber which could lead to high microbial activity in the hindgut and endogenous loss of nitrogen and amino acids. Therefore it is likely that the CTTAD of CP is under-estimated. In this regard, estimation of ileal digestibility and true ileal digestibility could give better estimation of protein digestibility. But in the current study we could not apply ileal protein digestibility since ileal samples requires cannualation or killing of the pig, which was not possible in this project. It should be remembered that this is as far as we know the first study on lepidium cake as a feedstuff to pigs. The major aim was to get a general picture about the effect of LC on nutrient digestibility and other parameters like blood and feeding behavior, therefore the total tract digestibility was used.

Fat contains 2.25 more energy than carbohydrate and play role in increasing the energy density in grower-finishing pig diets, reduce feed intake, improve feed conversion efficiency and growth rate of growing pigs (Santoma *et al*., 1987; De Rouchey, 2007, Collins *et al*., 2009). Supplementation of oil-rich diets increase the CTTAD of GE (Xiccato *et al*., 1995; Nizza *et al*., 1997). In the current study, the CTTAD of GE was not affeced by different inclusion levels of LC. However, the CTTAD of GE decreased with an increase of dietary fiber in the diet. This is in agreement with Urriola and Stein *et al*., (2010) who found that the digestibility of gross energy decreased when growing pigs were fed on fiber-rich diets containing 30 % distillers dried grains (DDGS) compared to a control diet based mainly on corn and soya bean meal.

According to Noblet *et al.,* (2006) DE measures the amount of energy in the feed available to the Animal. The CTTAD of DE was higher ($P<0.01$) in control diet (13.05 MJ kg^{-1} DM) than different inclusion levels of LC. However there was no significant variation ($P>0.05$) among the inclusion levels of LC which is 12.33, 12.35 and 12.32 MJ Kg^{-1} DM in LC4, LC8 and LC12, respectively.

In the present study the content of total fiber in the diet was negatively related to the CTTAD of DE content of the diet. The effect of dietary fiber on CTTAD of nutrient depends on fiber

source and the degree of solubility of the dietary fiber (Bach Knudsen, 1997; László, 2010). The major end products of the microbial fermentation of NSP in the hindgut are acetic, propionic and butyric acid, which provides about 30% of maintenance energy requirement of the pig (McDonald *et al*., 2002; Adesehinwa *et al.*, 2008) this implies that the pig may not satisfy their energy requirement from high fiber diets. There is not much literature about loss of microbial energy, but a study by Castiglia-Delavaud *et al*. (1998) showed that about 35% of the fermented sugar beet non-starch polysaccharide energy was found as faecal microbial energy. This implies that the CTTAD of GE and DE were mainly affected by higher indigestible NSP content of LC.

In the current study , the dietary fat in LC4, LC8 and LC12 were 3, 3.25 and 3.70 % of diet respectively and the CTTAD of EE in LC4, LC8 and LC12 was 0.6, 0.66 and 0.73 respectively, the increment of fat with increasing inclusion level of LC did not affect the CTTAD of energy ($P>0.05$), but increase CTTAD of EE ($P<0.05$), this in line with the finding of Santomá *et al*. (1987), Chen and Li (2008) and Brooke (2010), who concluded that CTTAD of EE increase with inclusion level of oil in the diet. The increasing CTTAD of fat with increasing inclusion level of LC might be due to increasing unsaturated fatty acid (UFA), which are efficiently absorbed in the small intestine. Accordingly, the CTTAD of EE was higher in LC12 followed by LC8. While, the CTTAD of EE in control and LC4 were almost similar, this could be due to similar fat content and net disappearance (Overland *et al*., 1994). Furthermore, diets with low inclusion levels of test diet are influenced by experimental error to a higher extent than those those with high inclusion level.

The increment in amount of oil due to different inclusion levels of LC did not affect the CTTAD of fiber. Thus, the correlation between dietary fat and fiber digestibility was not significantly different ($P>0.5$). Beside this, depending on the animal spices, inclusion of dietary fat >5% in the diet to ruminants negatively influence fiber digestibility by lowering the activity of fiber digesting microbes in rumen (McDonald *et al*., 2002). In the current study, the amount of fat in the diet was <5%. This implies that the quantity of oil in the diet was lower than to affect CTTAD of NSP. Except DE and CTTAD of EE, the CTTAD and the regression slope was linearly decreased with increasing levels of LC. The CTTAD of DM, OM, CP, GE, NSP and total dietary fiber at 100% of inclusion level is less than marginal (17-40%), this indicates that LC could not be fed alone to growing pigs. However, sows have large and well developed hindgut with better microbial population. Hence, fiberious diets like

LC may be better digested, and it might also reduce stereotypic behaviour by prolonging the feeling of satiety and reduce the feeling of hunger, which is a common problem for pregnant sows. Furthermore, LC could also be a fiber source to ruminant animal.

As shown in table 5, the amount of dietary fiber increased with increasing inclusion levels of LC. Beside this, in all dietary fiber parameters, the proportion of insoluble fiber was the major part of the total fiber in the LC. Klason lignin, uronic acid and glucose residues are the main components of the dietary fiber present in oil seed cakes where the majority are found in the husk (Bach Knudsen, 1997; Andersson *et al*., 1999). Removal of the husk lowers the total dietary fiber content and improves digestibility, and the nutritive value. For instance, the total dietary fiber in hulled and hulless barely was 35 and 9 g kg-1 DM, respectively (Bach Knudsen, 1997). The nutritive value of cotton seed cake decorticated and undecorticated was 231 and 457 g kg^{-1}CP; 248 and 87 g kg^{-1} total fiber, respectively. Beside this, the fiber digestibility coefficient of decorticated and undecorticated cotton seed cake was reported to be 0.20 and 0.28 respectively (McDonald *et al*., 2002). In the current study, the uronic acid was the main fiber component followed by Klason lignin and glucose. The amount of uronic acid, Klason lignin and glucose was higher than reported by Anderson *et al*. (1999). This might be explained by variation on soil were it grows, stage of maturity at harvest, storage time and the difference between the cake and the whole seed. Normally, whole seeds contain less fiber than the cake and in the study by Andersson *et al.* (1999) whole seeds where used and not cake. In the present study, the proportion of xylose was higher in the diets with inclusion of LC than in the cake alone; this could be explained by the additive effect of xylose from cereal gains in the control diet.

As shown in table 2, the NSP and total dietary fiber is higher than most oil seed cakes (soya bean cake, linseed, rape seed cake, and cotton seed cakes), cereals (wheat, barley) and legumes (pea), but lower than oat hull meal. The CTTAD of total NSP was 0.4 which is comparable with CTTAD of NSP of 0.38 reported in rapeseed cake (Schöne *et al.,* 1996), but lower than 0.58 reported in soyabean cake (Bach Knudsen, 2001). The lower CTTAD of NSP could be attributed to the higher proportion of indigestible NSP and lignin in the LC and a totally higher proportion of NSP in the LC diets. Högberg and Lindberg (2006) studied the effect of cereal based diets on fiber digestibility and reported that CTTAD of NSP was higher in pigs fed low levels of NSP (95-109 g Kg DM^{-1}) than diets with high level of NSP (160-203 g Kg^{-1} DM).

As shown in table 8, the regression model $yij = \beta 1 + xi\beta 2 + \varepsilon ij$ was poor to explain the source of variation. The regression model is based on four points (0, 4, 8, 12 % LC), which is the minimum amount of points required for the model. Moreover, the inclusion levels where quite narrow, and the highest level where only 12% which contribute to the poor explanation of the model. This might explain why the model gave an unrealistic estimation of CTTAD of EE. The equation for the difference calculation used in the present study is optimized for 30% inclusion of the test ingredient and 70 % of a basal diet. Lower inclusion levels, as in the present study, gives a less accurate prediction. The CTTAD of NSP and TDF gave lower values using the difference calculation compared to the regression model. However, the standard error was almost as high as the mean value, indicating a poor prediction with the difference calculation. But, the other values were in the same range when estimated with difference calculation and regression model. As stated before, this was the first study feeding lepidium cake to pigs, which is the reason why higher inclusion levels were not used. Hence, the CTTAD values estimated in this study can be used as indicative.

The blood electrolytes (Na^+, K^+, Cl^-, AnGap and glucose), hematology (Hct and Hb) and blood gasses (pH, pCO2, TCO2, $HO3^{-1}$ and BE) were not significantly different between the control diet and all inclusion levels of LC ($P>0.05$; Table9). The blood glucose level in this study is comparable with the result reported by Chittavong *et al.* (2012), but lower than Cooper *et al.* (2014). This might be due to the presence of high proportion (>80%) of insoluble glucose in the cake which might lead to lower supply of glucose to the pigs. Additionally, the method of analysis could be the other source of variation. I-Stat was used to analyse the blood sample in the resent study and in the study by Chittavong *et al.* (2012), while ADVIA 120 hematology system and a cobas 6000 C501 clinical chemistry analyzer were used by Cooper *et al.* (2014). Lower blood glucose was reported as a result of reduction in feed intake, malnutrion and dehydration (Buzzard *et al*., 2013). But in the current study, pigs received balanced diet, no refusal and free access to water.

As shown in table 9, the blood profiles gave significant information about blood-gas and acid-base balances (Verma and Roach, 2010; Güzel *et al.*, 2012). The normal blood pH range is 7.3 to 7.43; higher than 7.43 is alkalosis, lower than 7.3 is acidosis (Verma and Roach, 2010). In the present study, most treatment diets are characterized by having normal pH, but pH (7.5 ± 0.04) in LC4 was numerically slightly higher, but not significantly different from the other.

The anion gap (AnGap), bicarbonate ($HCO3^-$) and base excess (BE) reflects the metabolic component of the acid‑base balance. BE is the quantity of acid needed to restore a litter of blood to the normal pH, whereas, AnGap represents plasma cations (Na^+ and K^+) and the anions (Cl^- and $HCO3^-$) (Verma and Roach, 2010). In the current study the, AnGap, $HCO3^-$and BE were not affected by inclusion levels of LC. The levels of the Hct, AnGap, Na+, K+, Cl^- were within the normal range (Cooper *et al.*, 2014). Whereas, Hb, Na^+ and K^+ in the present study is higher than values reported in pigs managed under traditional feeding system in Lao PDR (Chittavong *et al*., 2012). Compared to the study by Chittavong *et al*. (2012), the higher Hb might be explained by the better Fe intake-as the pigs were fed on standardized rations having minerals to satisfy the daily requirement of the pigs.

The concentration of pCO_2 is an indicator of stress or respiratory disturbance which is the balance between metabolic production of CO_2 and ventilation excretion (Brouillette and Waxman, 1997). Even though pCO2 in the current study is higher than normal range (4.7-6. kPa) (Verma and Roach, 2010), but none of the pigs showed any symptoms of respiratory disorders. So the reason for such higher pCO2 is unknown. Hence blood profile studies should be supported by clinical studies such as heart rate, respiratory rate and temperature of the pigs.

For centuries, nutritionist have been formulating ration mainly based on the nutrient composition of the feed and daily nutrient requirement of the animal. But, considering feeding behaviour like pig preferences for different diets play vital role in the formulation of palatable diets that improve feed intake (Solà-Oriol *et al*., 2009). According to Gonyou *et al*. (2012) palatability of diet is influenced by innate taste avoidance behaviour or a post-ingestive effect which is a feedback sent from central nervous system following ingestion of feed to change or continue eating on the same diet. We hypothesized that inclusion of different level of LC negatively affected the palatability and feed intake of pigs. In the present study, the feed intake was the same for control and different inclusion levels of LC showing that the pigs found control and LC to be equally palatable. There was a low feed intake in the beginning of first day of the study. Similarly, Gonyou *et al*. (2012) found a decrease in feed intake in the first day when grower pigs where offered pea. If inclusion of LC caused negative post-ingestive feedback then a decrease in feed intake would have been observed on next consecutive days. In contrast to this, in the present study no difference in feed intake is observed between control and LC. Interestingly, pigs fed on control also showed a low fed

intake during the first day and there were no left over of feed from pigs fed on LC12. Similarly, no differences in feed intake were found when pigs fed diet 36 % of pea, containing anti-nutritional factor tannin, trypsin inhibitor and saponins, along with basal diet of soybean meal (Stein *et al*., 2004). This might be due to the lower inclusion rate of peas compare to recommended inclusion level (40%) to growing pigs (Grosjean *et al*., 1997). According to Rozin and Vollmecke (1986), neophobia is a mechanism by which animals avoid eating novel diets to avoid consuming toxic substances. In the current study, it is unlikely that the low feed intake during the first day is due to "neophobia" of pigs to novel LC diets, since pigs also seen to left feed on the control diet and no left over at all from pig fed on LC12. If lepidium cake contained anti-nutritive factors or toxins levels that affect palatability, the pigs should have avoided ingesting high amounts associated with the LC diet.

Eating time was not affected by treatment but there was significant variation between weeks. In the first day of feeding behavioural study, pigs eat less amount of feed but had longer eating time (102 minute). Later weeks pigs were given more feed but finished with shorter eating time (22 minute) (Figure 5).This indicated that the quantity of feed eaten per minute is lower at the beginning; this could be due to age of the pig and adaptation with the novel diets.

Eating time was not significant affected by treatment, however, feeding rate showed a tendency of significance ($P = 0.09$), accordingly LC12 showed higher ($P<0.001$) feeding rate and lower feeding rate was recorded in the control diet. LC8 and LC4 were not significantly different from LC12 but higher than control diet. Feeding rate for LC were compared to that of control diet, the pigs on LC diets ate more meals per minute. Accordingly, the highest and lowest feeding speed was recorded in LC12 and Control, respectively.

As shown in table10, the average feeding rate was higher than the values (23.6 g min^{-1}) reported by Hyun *et al.* (1997), but in agreement with the values (31.3 - 42.4 g min^{-1}) reported by Quiniou *et al*. (2000). A study by Kallabis and Kaufmann (2012) in growing pigs using standard diet, and two diets with inclusion of 6 % and 9% of lingo-cellulose fiber, showed longer eating time in pigs fed on fibrous diet than those fed standard diet. They conclude that the reason for longer eating time at 6 and 9 % was the fiber content which takes longer time to chew. Beside this, fiber is low in nutrient content so animals eat more and stay longer time to satisfy their nutrient requirement (McDonald *et al*., 2002). However, in the current study pigs fed on the standard diet containing low fiber showed longer eating time than different inclusion levels of LC, which contains higher fiber content. This contradicts with the previous

studies (Quiniou *et al.* 2000; Holt *et al*., 2006; Kallabis and Kaufmann, 2012). In the current study, the reason for pigs to show shorter eating time and higher eating rate min^{-1} in the LC could be because the pigs liked the LC.

In current study, control and LC12 were used to investigate the effect of diet on feeding choice and the feeding choice were found not to be affected by the feed type ($P>0.05$), in both rounds. Though we change the position of the feed in the second round, but the pig observed to prefer same side. Beside this, we observed that the number of FeedBeh were higher in specific pigs even at different treatment. This implies that the feeding choice in the current study was influenced by the individual behaviour of the pig. In day 1, when pigs where eating feed with LC12, they were observed stop eating for a while, chewing and open their mouth. This behaviour was not reflected in the recorded behavioural observations. The open mouth behaviour could be due to the bitter nature of the erucic acid present in the feed but as seen from palatability study it is temporal and does not affect the feed intake. In the current study the choice of feed might be affected by the position the animal stand (near to right or to the left) at the time feed is given.

Feeding choice could also be affected by inclusion rate, type, form, aroma, and texture or particle size of the diet (Solà-Oriol *et al*., 2009). Beside this, pigs have greater preferences for pellet compared to mash diets (Solà-Oriol *et al*., 2009). A study by Skoch *et al*. (1983) showed that pigs who received corn based diets either as pelleted or mash *ad libitum*, 85.5 of pigs preferred pelleted and 14.5 %, preferred mash. In the current study, both the control and test diet were pelleted, hence the feeding choice in the current study is not affected by form of diet.

The newly-weaned pig mostly depends on its oro-nasal sensing systems to evaluate feed, and the most palatable feed ingredients should be used at this stage (Forbes, 1995). Previous study done on diet selection of growing pigs, suggests given a choice between a low and a high protein diets, pigs are able to prefer a diet that meets their protein requirements and avoids, at least to a some extent eating on high protein diets (Kyriazakis and Emmans, 1991). In another study, growing pigs were offered a choice between two diets of similar nutrient content, but one based on soya and the other on rapeseed meal. The pigs showed a marked preference for the soya bean meal than rapeseed (Baidoo *et al*., 1986). This is due to the facts that rapeseed contains anti-nutritional factors, then the pigs could be expected to select less

from rapeseed. Beside this, the low number of pigs in this pilot study might be another reason to lack significant variation among treatment feeds on feeding time and feeding choice. Previous studies conducted of pigs by Jakobsen (2007), Solà-Oriol *et al*. (2009), Guanyu *et al*. (2012), Rudbäck (2013) and Clouard & Val-Laillet (2014) use 72, 912, 50, 60 and 32 pigs, respectively to investigate the effect of feed on feeding behaviour and performance.

In the current study, FeedBeh behaviour was higher the week 1, 2, 3 then linear decreases were observed in week 4, 5, 6, 7 and 8. According to Misslin and Cigrang (1986), an animal's natural initial response to novel feed or environment is neophobia followed by exploratory behaviour. This could be the reason for higher FeedBeh particularly in the beginning of the experiment. Importantly, the FeedBeh behaviour was common to certain pigs even at different diets, but the number of FeedBeh activity was decreased through time. The control feed showed higher ($P<0.001$) FeedBeh activities than different inclusion levels of LC. Compared to the control diet, the lower FeedBeh actives observed in different inclusion levels of LC might be suggested due to higher fiber content. And fiber improves the welfare of pigs, reduces hunger feeling due to improved gut fill and reduces stereotype behaviors (Brooks 2005; Holt *et al*., 2006).

6. Conclusions

Lepidium is a palatable feed ingredient to pigs and does not seem to cause any adverse health effects. However, the high level of insoluble fiber and glucosinolates results in low digestibility and energy values which limits its inclusion level.

7. Future Directions

- LC contains 50% NSP and growing pigs are not good enough to digest that much fiber. Sows or ruminant have a better capacity to digest fiber, and those animals would therefore likely digest the feed to a higher extent.
- Dehulling, plant breeding to produce less fiber LC varieties, alkaline treatment NaOH, supplementation of exogenous enzyme could be ways to improve the digestibility of LC.
- The lack of significance of inclusion level could be due to the inclusion levels of LC , further studies with higher inclusion level (0, 10, 20 and 30%) is needed.
- Further study on feed intake and growth rate to explore if the lower digestibility has impact on growth rate of growing pigs.
- Ileal digestibility to estimate true digestibility of amino acids.
- According to the secondary sources; the glucosinolate level in LC is above EU limit, hence plant breeding needs to develop *L. campestre* variety with lower glucosinolate level which is safe for animal feeding.
- It would have been good if feeding choice was studied in all the treatments and not only in control and LC12.
- To see the progress of their feed preference, it would have been good to test for three consecutive days in every period.
- Further research using flavoring agent needed if their feeding choice could be influenced by flavor.
- Use of scan sampling would give better precision and including more parameters example eating interval, time taking in every eating, standing, laying, drinking time, chewing as these parameters reflect the nature of the feed .
- Furthermore, higher number of pigs should be used to get real variation on behavioural parameters.

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