OPTIMIZING THE USE OF CANOLA MEAL IN SWINE DIETS FOR LIFETIME PERFORMANCE

By
Gustavo Adolfo Mejicanos

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Department of Animal Science
University of Manitoba
Winnipeg, Manitoba
Canada. R3T 2N2

ABSTRACT

The objective of this research was to study the optimization of the use of *Brassica napus* canola meal (CM) in swine diets. Chapter one had introduced the field of study, which helped to generate the research questions. Chapter two provided review information on means and techniques of improving the nutritive value of CM. Chapter three presents the hypothesis and objectives of the study. Chapter four studied the effect of high CM inclusion on growth performance, nutrient digestibility, and fecal bacteria in piglets fed corn or wheat-based diets. During phase-I, pigs fed wheat-CM had higher feed efficiency (G:F) than pigs fed corn-soybean meal (SBM) diet. The inclusion of CM influenced crude protein (CP), energy digestibility, and fecal microbial community, without affecting voluntary feed intake and body weight (BW) gain. Chapter five studied phosphorus (P) digestibility. Results indicated that feeding dehulled canola meal (DCM) increased apparent (ATTD) and standardized total tract digestibility (STTD) of P in pigs of different BW. For growing pigs, the ATTD and STTD of P were greater for DCM (42.4 and 46.1%) than for regular canola meal (RCM; 32 and 35.7%) and coarse canola meal (CCM; 24.5 and 28.4%). In chapter six, the effect of tail-end dehulling of CM on apparent (AID) and standardized ileal digestibility (SID) of amino acids (AA) when fed to growing pigs were determined. Dehulling increased the SID AA content of DCM compared to RCM by an average of 9%. Chapter seven investigated the effect of dietary supplementation of xylanase on a wheat-based diet containing CM. No effect on growth performance was found. However, a protein-xylanase effect on the ATTD of NDF ($P < 0.05$) was observed, and xylanase supplementation on RCM and DCM diets increased NDF digestibility. The ATTD of CP, P, Ca, ileum, and colon digesta pH was greater ($P < 0.05$) with xylanase supplementation. Xylanase supplementation increased ($P < 0.05$) the weight of the liver and spleen. A tendency ($P < 0.10$) for higher acetic

acid concentration in the colon digesta of pigs fed diets containing CCM was observed. In conclusion, dietary supplementation of xylanase increased nutrient digestibility and digesta pH but did not influence growth performance of weaned pigs fed wheat and CM-based diets over a 35-d period.

DEDICATION

I dedicate this thesis to my parents, Eduardo Mejicanos and Rosa Amalia de Mejicanos**,** to Reyna Leticia and our daughters María del Rosario, María Renée, and María Alejandra, to Marian, and my siblings, Hugo Eduardo, Luis Alberto, Edgar Leonel, Marco Antonio, and their appreciated families.

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A Nuestra Señora Maria Auxiliadora.

Glory to God forever.

FOREWORD

This thesis was prepared following a manuscript format and is composed of four manuscripts. Results from the manuscript I were presented as a poster at the 2016 ASAS-ADSA-CSAC-WSASAS Joint Annual Meeting, July 19-24, 2016, Salt Lake City, UT. Results from manuscript II and III were presented in the oral competition in the 2017 ADSA-ASAS Midwest Meeting, March 13-15, 2017, Omaha, NE, and the 2018 ADSA-ASAS Midwest Meeting, March 12-14, 2018, Omaha, NE, respectively. Abstract from manuscript IV was presented in the oral competition in the 2019 ASAS-CSAS Joint Annual Meeting, July 8-11, 2019, Austin, TX.

The thesis was written according to the Journal of Animal Science format. Manuscripts I, II, and III have been published as follows:

Manuscript I: Mejicanos, G. A., A. Regassa, and C. M. Nyachoti. 2017. Effect of high canola meal content on growth performance, nutrient digestibility, and fecal bacteria in nursery pigs fed either corn or wheat-based diets. Anim. Feed Sci. Tech. doi:10.1016/j.anifeedsci.2017.06.012

Manuscript II: Mejicanos, G. A., J. W. Kim, and C. M. Nyachoti. 2018. Tail-end dehulling of canola meal improves apparent and standardized total tract digestibility of phosphorus when fed to growing pigs. J. Anim. Sci. doi:10.1093/jas/sky040

Manuscript III: Mejicanos, G. A., and C. M. Nyachoti. 2018. Effect of tail-end dehulling of canola meal on apparent and standardized ileal digestibility of amino acids when fed to growing pigs. Anim. Feed Sci. Tech. doi.org/10.1016/j.anifeedsci.2018.07.011

Manuscript IV: Mejicanos, G. A., G. González-Ortiz, and C. M. Nyachoti. 2019. Effect of dietary supplementation of xylanase on a wheat-based diet containing canola meal on growth performance, nutrient digestibility, organ weight, and short-chain fatty acid concentration in

digesta, when fed to weaned pigs. The manuscript will be submitted to the Journal of Animal Science.

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LIST OF ABBREVIATIONS

AA	……..	Amino acids
ADF	……..	Acid detergent fiber
ADFI	……..	Average daily feed intake
ADG	……..	Average daily gain
AID	……..	Apparent ileal digestibility
Ala	……..	Alanine
ANF	……..	Anti-nutritional factors
Arg	……..	Arginine
Asp	……..	Asparagine
ATTD	……..	Apparent total tract digestibility
BCFA	……..	Branched-chain fatty acids
BW	……..	Body weight
CCM	……..	Coarse canola meal
CM	……..	Canola meal
CP	……..	Crude protein
Cys	……..	Cysteine
d	……..	Day

DCM	……..	Dehulled canola meal
DE	……..	Digestible energy
DM	……..	Dry matter
DMI	……..	Dry matter intake
EAL	……..	Non-specific endogenous loss
EE	……..	Ether Extract
EPL	……..	Endogenous phosphorous losses
g	……..	Gram
GE	……..	Gross energy
GSL	……..	Glucosinolates
Glu	……..	Glutamine
Gly	……..	Glycine
h	……..	Hour
His	……..	Histidine
Ile	……..	Isoleucine
K	……..	Potassium
kcal	……..	Kilocalorie
kg	……..	Kilogram

Leu	……..	Leucine
Lys	……..	Lysine
ME	……..	Metabolizable energy
Met	……..	Methionine
N	……..	Nitrogen
Na	……..	Sodium
NDF	……..	Neutral detergent fiber
NDICP	……..	Neutral detergent insoluble crude protein
NE	……..	Net energy
NRC	……..	National Research Council
NSP	……..	Non- starch polysaccharides
P	……..	Phosphorus
Phe	……..	Phenylalanine
Pro	……..	Proline
RCM	……..	Regular canola meal
SBM	……..	Soybean meal
Ser	……..	Serine
SD	……..	Standard deviation

SID	……..	Standardized ileal digestibility
SEM	……..	Standard error of the mean
STTD	……..	Standardized total tract digestibility
Thr	……..	Threonine
Tyr	……..	Tyrosine
Val	……..	Valine
VFA	……..	Volatile fatty acids
wk	……..	Week

CHAPTER ONE

GENERAL INTRODUCTION

Canola [*Brassica napus* (L.); *Brassica rapa* (L.); *Brassica juncea* (L.) Czern] belongs to the Brassica family (Gulden et al., 2008; Vaughan and Gordon, 1973). The genus *Brassica* contains many well-known plants, for example, radish, broccoli, and cauliflower. The most common canola is *B. napus* L. (Bell, 1984). Canola was originated from rapeseed. Rapeseed oil contains between 25-45% erucic acid, whereas the meal contains between 110-150 μmoles/g of aliphatic glucosinolates (Bell, 1993). Due to the high glucosinolate (GSL) content in rapeseed, which can be hydrolyzed by the enzyme myrosinase, releasing products that can cause adverse effects in animal performance (Mawson et al., 1994), plant breeders developed cultivars with low GSL in the meal and low erucic acid content in the oil (Stefansson et al., 1961; Bell, 1984). Those cultivars were classified as canola (Bell, 1984), which is a contraction of Canada and "ola," referring to "oil low acid" (Canola Council of Canada, 2011). The name canola aimed to differentiate the new cultivar from the high-GSL, high-erucic acid rapeseed. Canola includes seeds from the genus *Brassica* (*Brassica napus* L., *Brassica rapa* L. or *Brassica juncea* L.) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of anyone or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Canola Council of Canada, 2011). Canola is the principal oilseed crop in Canada with an annual production of 21.3 million tonnes in 2017, and 20.3 million tonnes in 2018 (Canola Council of Canada, 2014; Statistics Canada, 2018). Canola meal is derived from the crushing of canola seed for oil extraction. Canola seed yields 42% of oil, which is used for human consumption and 58% meal, which is used as a protein source in animal feed (Canola

Council of Canada, 2014). Canola meal (CM) is the second most abundant protein source in animal nutrition, after soybean meal (SBM), and it is widely utilized in the feed industry (Unger, 1990). Canola meal has a high protein content (35 to 45%) with good amino acid (AA) balance and a good source of sulfur AA (e.g., methionine and cysteine) (Canola Council of Canada, 2009), although it is limiting in lysine (Khajali and Slominski, 2012). The carbohydrate components of CM, such as simple sugars, sucrose, oligosaccharides, starch and non-starch polysaccharides, lignin with associated polyphenols, and proteins and minerals associated with the fiber fraction, account for roughly one-third of the meal (Khajali and Slominski, 2012).

Dietary inclusion levels of CM in swine diets have been limited due to the existence of anti-nutritional factors (ANF), particularly GSL (Khajali and Slominski, 2012). However, better processing procedures and advances in plant breeding of canola have been critical to the reduction of ANF content, resulting in the availability of CM with higher nutritive value for swine and poultry (Khajali and Slominski, 2012; Adewole et al., 2016). Canola meal with low levels of GSL (< 3.9 μmol/g; Rogiewicz et al., 2012) is available. The maximum inclusion level of GSL in growing pig diets has been indicated to be between 2.0 and 2.5 μmol per gram of diet (Bjerg et al., 1987; Bell, 1993 Schöne et al.,1997; Roth-Maier et al., 2004), which allows a higher inclusion of CM in swine diets. Another factor that affects the inclusion levels of CM in monogastric animals' diets is the higher level of fiber compared to SBM, which is the result of a large proportion of hulls relative to seed size (Bell, 1993). The hull represents 16.8% to 21.2% of the seed mass (Carré et al., 2015), but increases to around 30% of the meal weight after oil extraction. The higher level of fiber in CM has been associated with low levels of digestible energy (DE) and metabolizable energy (ME) in pigs (Bell, 1993).

Another ANF of importance in CM is phytic acid [myo-inositol (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate); de Lange et al., 1993; Angel et al., 2002; NRC, 2012]. It is the storage form of P in plant seeds (Khajali and Slominski, 2012). Phytic acid reduces the nutritional value of CM by binding to multivalent cations like Zn, Ca, and Fe, reducing their bioavailability (Al-Asheh and Duvnjak, 1994) and affecting animal performance by reducing nutrient digestibility through binding to nutrients, the digestive enzymes or both with increased endogenous loses of AA (Woyengo and Nyachoti, 2013). Overcoming the adverse effect of the ANF in CM would allow maximizing its utilization in swine diets and reduce feeding costs.

The first study presented in Chapter Four was developed from observations from several studies indicating that piglets can tolerate relatively high levels of CM inclusion. Sanjayan et al. (2014) included 25% CM in a wheat-SBM piglet diet without adverse effects on growth performance. Likewise, Mejicanos (2015) supplemented 15% of CM in corn-SBM basal diets, increasing feed efficiency, and BW. However, it is unclear if the tolerance to high CM inclusion and improved feed efficiency observed when CM was added to corn-SBM based diets depends on the cereal ingredient of the basal diet. The first study was directed to examine the effect of including CM in wheat or corn-based diets on growth performance, apparent total tract digestibility (ATTD), and fecal microbial communities compared with wheat- or corn-SBM based diets. Results indicated that in phase I, pigs fed the wheat CM diet had higher feed efficiency compared with those fed the corn-SBM diet (0.95 vs. 0.79). The improved feed efficiency in pigs that fed WCM in the present study might be attributed to the relatively higher abundance of commensal bacteria species such as Bifidobacterium and Clostridium cluster IV, which play a significant role in maintaining gut health and improving feed efficiency. Furthermore, results of the study showed that substitution of 200g/kg of CM for SBM in either wheat- or corn-based diet did not significantly

influence growth performance and that piglets can consume diets with high CM content right from the first day of weaning.

The second study presented in Chapter Five aimed to determine if the amount of fiber in CM affected P and AA digestibility. To achieve that purpose, CM was dehulled utilizing sieve size 355 µm, to produce two CM fractions, a dehulled fraction (DCM), and a coarse fraction (CCM). Tail-end dehulling of CM (dehulling after oil extraction) improves the ATTD and STTD of P in CM fed to growing pigs of two distinctive BW. The results from this study indicate that feeding high nutrient density DCM to growing and finishing pigs would decrease manure volume and P release into the environment, improving P use in swine diets.

The objective of the third study presented in Chapter Six was to determine the effect of the tail-end dehulling of CM on apparent (AID) and standardized (SID) ileal digestibility of AA when fed to growing pigs. Overall, the AID and SID of AA in CM were not affected by dehulling. Nevertheless, the content of ileal digestible AA was increased with tail-end dehulling of CM, which could be of importance in diet formulation. However, anti-nutritive factors present in DCM, such as phytate P and GSL, are also slightly enhanced, thus, affecting the AID and SID of some AA.

The final experiment presented in Chapter Seven studied the effect of dietary supplementation of xylanase on a wheat-based diet containing CM, on growth performance, nutrient digestibility, organ weight, pH, and concentration of short-chain fatty acids in the digesta of weaned pigs. Results indicated that the addition of xylanase to a wheat-based diet enhanced nutrient digestibilities, which were higher in the case of SBM and DCM. A two-way interaction of protein-xylanase for the ATTD of NDF was observed, and xylanase supplementation increased NDF digestibility from 28 to 31% and from 30 to 38% for RCM and DCM, respectively. No

increase in NDF digestibility was observed when pigs were fed diets containing SBM and CCM. A two-way interaction of enzyme-gender for the ATTD of NDF was also observed, and overall, supplementing xylanase to diets fed to gilts resulted in higher NDF digestibility. However, no effect on growth performance was observed. Furthermore, for colon pH, a three-way interaction for protein-xylanase-gender, and a tendency for a two-way interaction protein-gender were observed. Whereas, for ileal pH, a xylanase supplementation effect and a tendency for gender, were observed. The breakdown of fiber in the colon indicated by higher ATTD of Ca and P when diets were supplemented with xylanase (from 52 to 62% and from 45 to 49% respectively), could result in increased Ca absorption in the colon of pigs.

Additionally, higher ATTD digestibility of diet and DM observed when xylanase was supplemented without improved growth performance confirms the importance to determine, not just SID or AA, but also its effect on net energy, Ca, and P digestibility, when diets are supplemented with both, microbial phytase and xylanase. Additionally, values for net energy, ATTD, and STTD of Ca and P need to be determined, to properly formulate diets and to maximize growth performance of pigs, therefore, preventing adverse effects derived unbalance of Ca and P when diets are supplemented with phytase plus xylanase. Pigs fed diets containing SBM and RCM had heavier spleens than pigs fed diets containing DCM and CCM. Additionally, a tendency for the increased relative weight of the kidney due to dietary supplementation of xylanase and protein source was observed. Kidneys tended to have increased relative weight when DCM was the protein source.

The total SCFA production was not affected by the protein source, gender, or the addition of xylanase. The total concentrations of SCFA were in the range of 120.4 to 141.4 mM. A three-way interaction of protein-xylanase-gender in the concentration of acetic and propionic acid

indicates that the combined effect of protein source and xylanase will be different according to gender. Xylanase supplementation in CCM diets increased acetic acid concentration from 47.4 to 55.3 mM. However, no increase was observed in RCM diets, having similar NDF content, indicating that the type of fiber present in each CM fraction influences SCFA production. Xylanase tended to reduce butyric acid concentrations, which can be associated with increased pH with xylanase supplementation, as pH has a direct effect on bacteria composition in the gut. In conclusion, dietary supplementation of xylanase increased nutrient digestibility and digesta pH but did not influence growth performance of weaned pigs fed wheat and canola meal-based diets over a 35-d period.

CHAPTER TWO

LITERATURE REVIEW

Parts of the literature review were published in: Journal of Animal Science and Technology. doi:10.1186/s40781-016-0085-5. **G. Mejicanos,** N. Sanjayan, I. H. Kim, and C. M. Nyachoti. Recent advances in canola meal utilization in swine nutrition.

2.1 BACKGROUND

Canola (*Brassica napus* L., *Brassica rapa* L.; *Brassica juncea* L.) is an offspring of rapeseed, which belongs to the cabbage family or Brassicas. The genus *Brassica* also contains plants such as cabbage, radish, kale, mustard, and cauliflower (Bell, 1984; Gulden et al., 2008; Vaughan and Gordon, 1973). Rapeseed oil contains around 25-45% erucic acid, whereas the meal contains about 110-150 μmol/g of aliphatic glucosinolates (Bell, 1993). Rapeseed was cultivated more than 3,000 years ago in India and 2,000 years ago in China and Japan. The development of steam power resulted in the better industrial acceptance of rapeseed. It was introduced to Canada between 1936 and early 1940s as a method of diversifying crop production, especially for the Prairie Provinces (Bell, 1984; Classen et al., 2004; Oilseed Rape, 2007). The fuel shortage caused by World War II led to the increased production of rapeseed. However, with the switch to diesel engines, and the ban of the use of rapeseed for human consumption by the USA in 1956, its demand declined (USDA, 2012).

Rapeseed contains high levels of glucosinolates (GSL), which can be hydrolyzed by the enzyme myrosinase to release products with goitrogenic effects that interfere with iodine metabolism and therefore affect the functioning of the thyroid gland and consequently animal performance (Mawson et al., 1994). To address these effects, plant breeders worked to develop rapeseed cultivars with low glucosinolate content in the meal and low erucic acid content in the oil. The first low-erucic acid rapeseed was developed in Canada by Dr. Baldour R. Stefansson of the University of Manitoba (Stefansson et al., 1961; Downey and Craig, 1964). In 1968, the first low-erucic acid cultivars Tanka, Target, and Turret, were released and produced in Canada (Bell, 1984). However, high GSL content remained a concern, therefore following research focused on

the reduction of GSL in the meal, increasing the oil and protein content of the seed, and yield. By 1974, Dr. Stefansson released the first double-zero rapeseed, cv. Tower (Bell, 1984).

The name canola refers to “Seeds of the genus *Brassica* (*Brassica napus*, *Brassica rapa*, or *Brassica juncea.*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of anyone or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Canola Council of Canada, 2011). In the international community, canola is also known as “double-zero,” “zero-zero,” or “double-low” rapeseed. Canola is currently the leading oilseed crop in Canada with an annual production of 21.3 million tonnes in 2017, and 20.3 million tonnes in 2018 (Statistics Canada, 2018) and the importance of its meal as a protein supplement is second only to soybean meal. During crushing, canola seed yields 42% of oil, which is used as vegetable oil for human consumption and 58% meal, which is used as a protein source in animal feed (Unger, 1990). Rapeseed for industrial use is grown at a lower scale in Canada. The first high erucic acid rapeseed (HEAR) variety developed was R-500 (*B. rapa*). However, it produced a high glucosinolate meal. The second HEAR (*B. napus*) variety was Reston, developed and registered by the University of Manitoba, it contained medium GSL levels, since then, plant breeders have developed rapeseeds with high erucic acid and low GSL contents (Canola Council of Canada, 2017; Duncan et al., 2017).

2.2 CHEMICAL AND NUTRITIVE VALUE OF CANOLA MEAL

Earlier studies with different types of canola demonstrated that black and yellow seeds differ significantly in the chemical and nutritive composition, particularly in the contents of oil,

crude protein (CP), and fiber (Slominski et al., 2012). More recently, new varieties of black-seeded canola (*B. napus)* with high-protein and low-fiber content have been developed. The meal produced from these varieties contains higher protein and lower fiber content than the conventional canola meal (Berrocoso et al., 2015; Liu et al., 2016; Liu et al., 2018). As can be seen from Table 2.1, CP content of four different types of canola meal (CM) differed significantly, with high-protein *B. napus* showing the highest protein content of 45.03%, followed by *B. juncea* 42.3%, 41.0% in yellow-seeded *B. napus* and 36.9% in black *B. napus* (as-is basis). Notable differences can be observed between cultivars regarding neutral detergent fiber (NDF), acid detergent fiber (ADF), non-starch polysaccharides (NSPs), lignin and polyphenols, phosphorus (P), etc. Likewise, variations in the chemical and nutritive characteristics between Canadian processing facilities have been observed, especially regarding CP, dietary fiber, non-detergent insoluble crude protein (NDICP), GSL, and lysine content, moreover, it has been indicated that such variations are associated with differences in processing conditions, particularly heat treatment in the desolventizer-toaster (Adewole et al., 2016). The most common oil extraction process is pre-press solvent extraction using hexane, which requires the use of a wide range of temperature, moisture, and time, affecting the nutritional value of the meal (Canola Council of Canada, 2015). Moreover, the oil extraction process of the seeds would also affect the CP content with the oil-expelled meal containing 35.2%, while pre-press solvent extracted CM containing 37.5% (as-fed basis) (NRC, 2012). Other factors that affect the protein content of CM are the environmental conditions during the growing season. Tipples (1988) found that over the ten years, from 1978 to 1987, the CP content of CM ranged from 36 to 41%. Bell et al. (1999) found that location is another factor that can affect the mineral content of *B. napus, B. rapa,* and *B. juncea.*

Table 2.1 Chemical composition of meals derived from regular, high-protein, or yellow-seeded *B. napus* canola and canola quality *B. juncea* (%, as-is basis) [1]

Component	*B. napus* regular	*B. napus* high protein	*B. napus* yellow	*B. juncea* yellow
DM	91.30	89.40	92.00	90.70
CP	36.90	45.03	41.00	42.30
Fat	3.80	2.09	3.70	3.40
Ash	7.10	7.64	7.90	6.60
Sucrose	6.30	5.34	8.40	7.60
Dietary fiber fractions				
Acid detergent fiber	17.00	9.22	12.00	9.70
Neutral detergent fiber	23.60	15.10	16.40	15.90
Non-starch polysaccharides	17.00	-	21.10	19.40
Total fiber %	30.10	-	27.10	25.50
Lignin and polyphenols	10.30	3.73	2.70	4.00
Glycoprotein	2.80	-	3.20	2.10
Phosphorus (P)	0.95	1.20	1.25	1.04
Phytate P	0.56	0.87	0.80	0.58
Non-phytate P	0.39	0.33	0.44	0.46
Calcium	0.67	0.58	0.55	0.76
Glucosinolates, μmol/g [2]	9.20	10.20	13.50	12.2

[1]Adapted from Mejicanos (2015) and Liu et al. (2018); [2]Includes gluconapin, lucobrassicanapin, progoitrin, gluconapoleiferin, gluconasturtiin, glucobrassicin, and 4-hydroxyglucobrassicin.

2.3 PROTEIN AND AMINO ACID SOURCE

The newly developed black *B. napus* containing high-protein (45%, as-fed basis) also contains low fiber (Liu et al., 2018). However, through the dehulling process, CP in the regular *B. napus* can be increased above 40% (Hansen et al., 2017; Mejicanos et al., 2017). The complete removal of the hulls of canola would result in high protein-high energy meal with 47.8% protein, 10.8 % NDF, 6.6% ADF (Carré et al., 2015). The meal from yellow-seeded *B. juncea* and yellow-

seeded *B. napus* contains more CP (DM basis) in comparison with the conventional *B. napus*; 43.4 and 47.2 vs. 41.1%. (Radfar et al., 2015).

Canola meal contains a well-balanced amino acid (AA) profile, and when compared to soybean meal (SBM), it contains less lysine but more sulfur AA, such as methionine and cysteine (Newkirk et al., 2003a). Regular CM contains approximately 2% methionine as a percent of total protein, while SBM has 1.5%. However, CM also contains 10% lower available lysine compared to SBM (Sauer et al., 1982). Therefore, both meals complement each other when used in rations for livestock and poultry (Khajali and Slominski, 2012). A negative relationship between protein and the dietary fiber content of meals derived from black- and yellow-seeded *B. napus* canola has been reported (Slominski et al., 2012). Such differences will affect the percentage of AA content of the different cultivars. Removing fiber from the meal would translate into fractions with higher CP and AA content. For example, Mejicanos et al. (2015) evaluated the nutritive value of dehulled CM and observed that with the reduction of fiber, the CP and AA values were increased. Table 2.2 shows the AA composition of meals derived from black- and yellow-seeded *B. napus,* and yellow *B. juncea* and the corresponding dehulled fraction 1 produced by sieving; e.g., lysine increased from 2.02 to 2.26, from 1.91 to 2.34 and from 1.95 to 2.29% for black-seeded *B. napus*, yellow-seeded *B. napus* and *B. juncea* meal, respectively. Methionine increased from 0.68 to 0.81, 0.63 to 0.71, and 0.66 to 0.83% for black-seeded *B. napus*, yellow-seeded *B. napus*, and *B. juncea*, respectively. Conditions in the processing plants also affected the quality of CM, and in this regard variations in AA content ($P < 0.05$) of CM from different processing plants across Canada have been reported; e.g., arginine, lysine, methionine, and threonine averaged 2.22, 1.78, 0.52, and 1.07%, respectively, and ranged from 2.00 to 2.44% for arginine, 1.61 to 1.96% for lysine, 0.45 to 0.63% for methionine, and 0.94 to 1.34 % for threonine. The study also reported that the AA

content of the meal could be significantly reduced by pelleting (Adewole et al., 2015). Meal derived from seeds of high-protein canola has been incorporated in swine diets successfully (Liu et al., 2014; Liu et al., 2016; Berrocoso et al., 2014; Liu et al., 2018) As can be seen from **Table 2.3**, dehulled canola meal fraction 1 had a similar concentration of AA compared to meal from high-protein *B. napus.* Therefore, the dehulling process remains an important option to improve the nutritive value of CM.

Table 2.2 Amino acid composition of conventional black *B. napus* canola meal, yellow *B. napus* meal, and canola-type yellow *B. juncea* mustard meal, and their corresponding dehulled fraction 1 produced by sieving (%, as-is basis) [1]

Amino acid	Black *B. napus*		Yellow *B. napus*		Yellow *B. juncea*	
	Parent meal	Dehulled fraction 1	Parent meal	Dehulled fraction 1	Parent meal	Dehulled fraction 1
Indispensable AA						
Arginine	2.28	2.77	2.08	2.63	2.85	3.60
Histidine	1.18	1.37	1.10	1.35	1.31	1.51
Isoleucine	1.21	1.46	1.06	1.34	1.21	1.81
Leucine	2.43	2.92	2.31	2.86	2.76	3.52
Lysine	2.02	2.26	1.91	2.34	1.95	2.29
Methionine	0.68	0.81	0.63	0.71	0.66	0.83
Phenylalanine	1.40	1.69	1.31	1.61	1.53	1.98
Threonine	1.62	1.85	1.33	1.66	1.82	2.14
Valine	1.66	1.95	1.54	1.90	1.62	2.35
Dispensable AA						
Alanine	1.49	1.76	1.56	1.89	1.72	2.05
Aspartate	2.62	3.01	2.30	2.89	3.34	3.87
Cysteine	0.80	0.92	0.91	0.94	0.70	0.85
Glutamine	6.60	7.81	5.91	7.44	7.26	8.49
Glycine	1.85	2.19	1.45	1.85	2.16	2.56
Proline	2.54	2.89	2.44	2.85	2.77	2.93
Serine	1.69	1.93	1.63	1.99	1.94	2.18
Tyrosine	0.93	1.11	0.84	1.06	1.05	1.34

[1]Adapted from Mejicanos, (2015)

Table 2.3 Amino acid composition of regular *B. napus* canola meal and its dehulled fraction 1 produced by sieving, and high-protein *B. napus* (%, as-is basis) [1]

Amino acid	Mejicanos (2015)		Liu et al. (2018)	
	Regular *B. napus*	Dehulled *B. napus*	Regular *B. napus*	High protein *B. napus*
Indispensable AA				
Arginine	2.28	2.77	2.31	2.54
Histidine	1.18	1.37	1.01	1.12
Isoleucine	1.21	1.46	1.46	1.54
Leucine	2.43	2.92	2.67	2.84
Lysine	2.02	2.26	2.11	2.33
Methionine	0.68	0.81	0.73	0.83
Phenylalanine	1.40	1.69	1.52	1.66
Threonine	1.62	1.85	1.56	1.63
Tryptophan			0.53	0.62
Valine	1.66	1.95	1.86	2.04
Dispensable AA				
Alanine	1.49	1.76	1.66	1.80
Aspartate	2.62	3.01	2.55	2.74
Cysteine	0.80	0.92	0.90	1.07
Glutamine	6.60	7.81	6.66	7.65
Glycine	1.85	2.19	1.92	2.07
Proline	2.54	2.89	2.34	2.46
Serine	1.69	1.93	1.36	1.48
Tyrosine	0.93	1.11	1.07	1.07

[1]Adapted from Mejicanos, (2015) and Liu et al. (2018).

Results reported by Adewole et al. (2015) indicated that standardized ileal digestibility (SID) of arginine, lysine, methionine, and threonine in CM averaged 87.5, 78.8, 85.4, and 74.8%, respectively. Table 2.4 shows SID values of solvent-extracted CM and expeller extracted canola (EECM) fed to growing pigs as reported by Woyengo et al. (2010), Maison and Stain (2014), Seneviratne et al. (2010) and Sanjayan (2014). However, it's essential to determine if the dehulling process affects the apparent (AID) and standardized ileal (SID) digestibility of AA in CM.

Table 2.4 Standardized ileal digestibility of amino acids in canola meal fed to growing pigs (%).

	Expeller extracted			Solvent extracted		
Item	Woyengo[1]	Seneviratne[2]	Maison[3]	Woyengo[1]	Sanjayan[4]	Maison[3]
Indispensable AA						
Arginine	91.7	83.1	89.4	86.2	90.3	86.3
Histidine	84.7	81.7	83.8	78.1	87.1	82.0
Isoleucine	85.4	74.3	77.7	78.1	79.7	75.9
Leucine	87.2	78.8	81.6	79.0	80.3	79.3
Lysine	70.7	73.2	74.7	66.6	78.9	70.6
Methionine	87.4	83.9	87.1	84.1	84.2	84.5
Phenylalanine	90.4	78.0	81.1	90.4	70.8	78.2
Threonine	79.5	67.6	74.0	72.1	77.1	73.0
Tryptophan		83.9	83.4			82.6
Valine	83.8	70.5	75.9	76.7	78.5	74.4
Dispensable AA						
Alanine	85.1	72.1	80.2	76.3	78.2	75.8
Aspartate	82.2	72.0	77.8	75.0	77.8	71.8
Cysteine	80.1	72.7	72.9	79.3	79.8	73.2
Glutamate	91.6	84.3	85.9	86.9	88.3	83.4
Glycine	86.2	63.6	78.6	82.2	76.5	78.1
Serine	76.7	70.6	76.7	76.7	80.7	75.7
Tyrosine	98.2	75.1	75.6	93.3	78.7	74.7

[1]Woyengo et al., 2010; [2]Seneviratne et al., 2010; [3]Maison et al., 2014; [4]Sanjayan et al., 2014.

2.4 ENERGY SOURCE

One of the main factors that limit the nutritive value of CM is its low digestible energy content, which reflects its high crude fiber content (Thacker, 1990). Compared to soybean, canola contains a higher amount of oil, with many cultivars carrying between 40 and 45% oil on a dry matter basis (Dale, 1996). The energy content of CM can differ between samples obtained from different crushing plants due to the oil extraction process, i. e., canola expeller meal contains residual oil at average levels of 9.7%, compared to 3.2% for the pre-press solvent-extracted meal (NRC, 2012). The oil content of the meal from the pre-press solvent extraction process would also be affected by the amount of gums and soapstocks from oil added back to the meal following oil refining. As indicated by Bell (1993), gums and soapstocks may contain about 50% of canola oil, and such oil is expected to increase the metabolizable energy (ME) values of the meal.

Theodoridou and Yu (2013) evaluated the effect of processing conditions on the nutritive value of canola meal and reported significant differences between CM from black- and yellow-seeded *B. napus* for the basic nutrients, except ash. The differences between yellow- and black seeded canola included NDF, ADF, CP, and condensed tannins. Meal from yellow-seeded canola showed higher values for CP, total digestible CP, and lower fiber content (Bell, 1993; Slominski et al., 2012). The differences between CM from different cultivars of canola are illustrated in Table 2.1. Sucrose content for yellow-seeded *B. napus* was higher, and averaged 8.4%, while the mean values for *B. juncea*, regular *B. napus* and high-protein *B. napus* were 6.3, 7.6, and 5.34%, respectively. In the case of non-starch polysaccharides, meal from yellow-seeded *B. napus* showed higher values and averaged 21.1%, whereas values for meal from *B. juncea* and black *B. napus* averaged 19.4 and 17.0%, respectively. Total dietary fiber was lower in meal from *B. juncea* and averaged 25.5%; 27.1% for yellow-seeded *B. napus* whereas black *B. napus* had 30.1%. In the

case of the expeller meal, which contains an average 10.0% of ether extract (EE), the values reported for gross energy (GE), digestible energy (DE), ME and net energy (NE) averaged 4,873; 3,779; 3,540 and 2,351 kcal/kg, respectively. For pre-press solvent extracted CM, which contains less EE (3.2% on average), the values average 4,332; 3,273; 3,013 and 1,890 kcal/kg, respectively (NRC, 2012). However, a more recent study indicates that DE, ME, and NE content in solvent-extracted CM are 2,782, 2,629, and 2,106 kcal/kg, respectively (Kim et al., 2018). The values for yellow-seeded *B. napus* averaged 3,965; 3,248; 3,009 and 2,102 kcal/kg, respectively; whereas the values for yellow *B. juncea* averaged 4,037; 3,392; 3,224 and 2,340 kcal/kg, respectively (Heo et al., 2014).

Dehulling of canola can result in a higher energy meal, as shown by research on tail end dehulling of pre-press solvent extracted CM from black- and yellow-seeded *B. napus* and canola quality *B. juncea*. Dehulling resulted in the low-fiber high-protein fractions Fine 1 and Fine 2. Compared to their parent meals, the content of total dietary fiber in the fractions decreased from 30.1 to 21.4 and 26.7% for regular CM, from 25.5 to 15.3 and 18.7% for yellow-seeded CM, and from 27.1 to 21.6 and 23.4% for *B. juncea* meal, respectively (Mejicanos, 2015).

2.5 VITAMINS AND MINERAL SOURCE

Canola meal is a rich source of most of the minerals (Bell, 1993). Compared to SBM, CM has a relatively high amount of Ca, P, S, Mg, Mn, and Se. However, K and Cu contents are lower. Table 2.5 shows the chemical composition of CM compared to SBM (Bell, 1993; Simbaya, 1996; Khajali and Slominski, 2012); such values are in accordance with the National Research Council's Nutrient requirements of swine, 11th Rev. Ed. (NRC, 2012). However, the presence of phytic acid and high fiber in the meal reduces the availability of most of the minerals. In a study regarding the

chemical and nutritive characteristics of CM from Canadian processing facilities, differences (P<0.05) in total P content between processing facilities were found, with an overall mean of 1.12%. Whereas, non-phytate P contents were similar between processing facilities. However, the phytate-bound P ranged from 0.68 to 0.8%, with an average of 0.74% (Adewole et al., 2016). The dehulling process can increase total, non-phytate, and phytate P content of the meal to 1.27, 0.61, and 0.66%, respectively (Mejicanos et al., 2017). Such increases could mean increased P digestibility in dehulled CM with and without the use of exogenous enzymes.

Although the availability of most of the minerals is low in CM, it has high amounts of total Ca, Mg, and P compared to SBM, as shown in Table 2.5. Canola meal is also high in sulfur, with approximately 1.14% compared with 0.44% in SBM, from the total sulfur content in CM, only 20% comes from sulfur AA, compared to 75% in SBM (Summers, 1995). The high level of mineral in CM represents a challenge to the use of exogenous enzymes targeting phytate phosphorus and fiber due to the potential impact on the dietary electrolyte balance (dEB), which is essential for pigs to attain optimum growth performance. Jones et al. (2019) found that increasing dEB up to 243 and 199 mEq/kg in phase 1 and 2, respectively, in nursery pig diets, improved the growth performance of weanling pigs. No data is available on the effect of phytase and xylanase in diets containing CM on dEB. However, the addition of microbial phytase has shown to increase the apparent and standardized total tract digestibility of Ca in Ca supplements fed to growing pigs, and the ATTD of P in the diets (Gonzalez-Vega et al., 2015).

Furthermore, a recent survey analyzing 795 commercial pig and broiler diets from 2010 to 2015, it was found an excess of 0.22 percentage units of Ca in the diets. An excess of Ca in diets can have a detrimental effect on ADFI and ADG in finishing pigs when diets are balanced according to P requirements (Walk, 2016). Higher digestibility of Ca in swine diets supplemented

with phytase and xylanase can represent an excess of available Ca, which has been indicated as detrimental to the growth performance of pigs unless P is also included above the requirements (Merriman et al., 2017). The use of phytase reduces the endogenous loss of Ca in growing pigs and sows and increases the ATTD and STTD of Ca and P in the diets. Moreover, the addition of phytase plus xylanase can further increase the digestibility of Ca and P in diets containing CM as a protein supplement. Therefore, it is crucial to determine the impact of supplementing diets containing CM with microbial phytase and xylanase, on growth performance, the ATTD and STTD of Ca and P, to properly formulate diets and to maximize growth performance of pigs. Therefore, preventing adverse effects derived from excess Ca in diets (Lee et al., 2019).

Canola meal contains a considerably high phytate-bound P quantity, in proportion to total P, which ranges from 36% to over 70% (Khajali and Slominski, 2012). Due to this reason, the bioavailability of P has been estimated to be around 30 to 50% of the total P in CM (Enami, 2011). Compared to SBM, CM is a more abundant source of vitamins such as biotin, niacin, choline, thiamin, Vitamin B6, and niacin (Bell, 1993; Khajali and Slominski, 2012; Simbaya, 1996). However, the pantothenic acid content is lower in CM (Clandinin et al., 1959; NRC, 2012). Higher quantities of the total, non-phytate, and phytate P in dehulled canola meal resulted in higher ATTD and STTD of P in growing and finishing pigs (Mejicanos et al., 2018a).

Table 2.5. Chemical composition of canola meal compared to soybean meal (%, as-is basis).

Component	Canola meal	Soybean meal
DM	90.00	90.0
CP	36.50	45.60
EE	3.60	1.30
GE, MJ/kg	18.60	20.10
Carbohydrates		
Starch	2.50	0.70
Sucrose	6.00	6.20
Sugar	7.70	6.90
Oligosaccharides	2.50	5.30
Fiber		
Crude fiber	11.60	5.40
NSP	18.00	17.80
NDF	26.00	12.00
ADF	18.20	7.50
Total dietary fiber	31.70	21.80
Amino acids		
Arginine	2.04	3.23
Lysine	2.00	2.86
Threonine	1.57	1.74
Methionine	0.74	0.65
Cysteine	0.85	0.67
Tryptophan	0.48	0.64
Minerals		
Ca	0.70	0.30
P	1.20	0.70
Mg	0.60	0.30
Na	0.08	0.01
K	1.29	2.00
Vitamins, mg/kg		
Biotin	1.00	0.30
Folic acid	2.30	1.30
Niacin	169.50	29.00
Pantothenic acid	9.50	16.00
Riboflavin	3.70	2.90
Thiamine	5.20	4.50

Bell, 1993; Khajali and Slominski, 2012; Simbaya, 1996.

2.6. FACTORS AFFECTING THE NUTRITIVE VALUE OF CANOLA MEAL FOR SWINE

Several factors limit the use of CM, especially in monogastric animal nutrition. When compared with SBM, CM contains higher contents of dietary fiber, glucosinolates, sinapine, phytic acid, phenolic components such as tannins, lower metabolizable energy, with less consistent AA digestibility and less than optimum electrolyte balance due to high sulfur and low potassium contents (Khajali and Slominski. 2012). Among these, fiber, GSL, and phytic acid are considered to be the main antinutritional factors in CM.

2.6.1 Fibre

The fiber content in CM is 3 times higher than that in SBM (Bell, 1993), which is the result of a large proportion of hulls relative to seed size. The hull represents 16.8% to 21.2% of the seed mass (Carré et al., 2015), but increases to about 30% of the meal weight after oil extraction, which is the main reservoir for non-starch polysaccharides (NSP) and lignin. Low levels of DE and ME in CM is due to the high level of fiber (Bell, 1993). High protein soy and 44% soy with hulls added back, contain around 4% and 7.5% fiber, respectively, whereas CM has more than 10% crude fiber (Dale, 1996). Canola meal contains cellulose (4-6%), non-cellulosic polysaccharides (13-16%), lignin and polyphenols (5-8%), and proteins and minerals associated with the fiber fraction as the main fiber components (Slominski and Campbell, 1990). Previous studies demonstrated that meal derived from yellow-seeded canola has a low amount of fiber compared to meal derived from the black-seeded canola. For instance, ADF and NDF contents of *B. juncea* (9.7% and 15.9%) are lower compared to those (17.0% and 23.6%) of black-seeded *B. napus*, furthermore, the meal from

the high-protein, low-fiber *B. napus* contains 15.1 and 9.22% of NDF and ADF, respectively, as shown in Table 2.1.

Fiber present in CM contains NSP, lignin associated with polyphenols, polyphenols glycoprotein, and minerals associated with fiber (Simbaya, 1996). Non-starch polysaccharide components of CM are shown in Table 2.6. Pectic polysaccharides are present in CM as a non-cellulosic polysaccharide, which is indicated by the presence of uronic acids (Slominski and Campbell, 1990). Parts of the arabinose and galactose derive from arabinan and arabinogalactan. The presence of xylose indicates the presence of xylan and xyloglucans. Xyloglucans contain xylose, glucose, galactose, and fucose (Slominski and Campbell, 1990). Cellulose, arabinose, arabinogalactan, and pectins are the major NSP components in CM (Slominski and Campbell, 1990; Meng et al., 2005; Kiarie, 2008). Meng and Slominski (2005) reported that CM contained 174.5 mg/g total NSP, of which only 14.3 mg/g was water-soluble. The impact of high fiber in CM can be minimized through the dehulling process, as less fiber content would result in high nutrient density of the dehulled meal (Mejicanos et al., 2017). However, the use of fiber degrading enzymes could release starch, free sugars, and soluble NSPs encapsulated by the cell walls, increasing energy digestibility in CM. Fang et al. (2007), using in vitro techniques found improved DM and CP digestibility when xylanase was supplemented to corn-rapeseed meal diets.

Furthermore, ADG significantly increased when xylanase was supplemented. The combined supplementation of phytase-xylanase has been studied with inconsistent results, as shown by Woyengo et al. (2008) who found improved P and AA digestibility without observing an interaction between the two enzymes. However, Oryschak et al. (2002) found that dietary supplementation of carbohydrase (Xylanase + ß-glucanase) plus phytase was effective in improving energy and nitrogen digestibility; nonetheless, when phytase-only supplementation

decreased energy digestibility. Those conflicting results reaffirm the complexity of the interactions between fiber degrading enzymes, phytase, and digestibility of nutrients (Kim et al., 2005).

Table 2.6 Non-starch polysaccharides component sugars of canola meal (mg/g)[1]

Component	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*
Rhamnose	1.2	1.2	1.0
Fucose	1.0	0.8	0.8
Arabinose	22.9	24.1	24.8
Xylose	9.1	7.5	10.3
Mannose	2.6	1.5	2.1
Galactose	7.9	7.7	8.8
Glucose	29.6	27.6	27.2
Uronic acids	26.6	30.4	26.5

[1]Adapted from Slominski, 2012.

2.6.2 Glucosinolates

Glucosinolates (GSL) are sulfur-containing secondary plant metabolites found mainly in the order Capparales, also known as Brassicales, which contain plants of the family *Brassicaceae* that include the genus *Brassica* (rapeseed, mustard, and cabbage) (Chen and Andreasson, 2001; Khajali and Slominski, 2012). Intact GLS do not cause any harmful effects on animals. However, the breakdown products of GLS either by enzyme myrosinase or by non-enzymatic factors such as heat, low pH, and microbial activity could cause harmful effects to animals (Bell, 1993). Depending on the nature of GLS, reaction condition and concentration, the break down products-thiocyanate, isothiocyanate, oxazolidinethione (goitrin) and nitriles may be formed and impair not only feed intake (due to their bitter taste) and growth performance but also affect thyroid function by inhibiting thyroid hormone production and impair liver and kidney function (Bell, 1993; Campbell and Schöne, 1998; Mullan et al., 2000). Previous studies have shown that growing pigs

can tolerate a maximum of 2.0-2.5 μmol/g of glucosinolates in the diet (Bell, 1993; Schöne et al., 1997; Roth-Maier et al., 2004).

Glucosinolates are considered anti-nutritional factors present in CM. Rapeseed meal contained 110-150 μmol/g of GLS (Bell, 1993). However, through plant breeding techniques, new canola varieties have been developed with a low level of GLS (< 30 μmol/g). In a survey from crushing plants across Canada, the level of GLS in CM was reported to average 3.9 μmol/g (Rogiewicz et al. 2012). Canola meal contains two types of GLS, aliphatic (85%) and indolyl (15%) (Newkirk et al., 2003). Gluconapin, glucobrassicanapin, progoitrin, and napoleiferin are the primary aliphatic GLS present in CM of which progoitrin is the primary factor that is responsible for the anti-nutritional effect (Fenwick and Curtis, 1980; Simbaya, 1996).

A recent assessment of the chemical and nutritive characteristics of canola meal from Canadian processing facilities has shown that the content of aliphatic glucosinolates ranged from 1.59 to 7.14 μmol/g DM. The content of indoles ranged from 0.26 to 2.48 μmol/g DM. Whereas, the total GSL ranged from 1.9 to 9.7 μmol/g of GLS, with an overall mean of 4.6 μmol/g of GLS DM (Adewole et al., 2016). Reports from France show that the level of GLS in double-zero rapeseed averaged 10 μmol/g (Labalette et al., 2011). Additionally, Mejicanos (2015) reported GLS values of 9.2 and 12.2 μmol/g for conventional *B. napus* and *B. juncea*, respectively. The meal from the new high-protein *B. napus* contains 10.2 μmol/g GLS (Liu et al., 2018).

Table 2.7 shows the GLS content of black *B. napus* and *B. juncea* meals and its dehulled Fractions 1 and 2. As can be observed, dehulling did not increase the content of GLS significantly; however, the content of gluconapin was higher in *B. juncea* meal (10.1 μmol/g) compared to *B. napus* meal (2.1 μmol/g) which can affect palatability especially in weaned pigs. Landero et al. (2013) found that the level of GLS in *B. juncea* of 10.8 μmol/g decreased ADG as the levels of

inclusion of CM in the diet increased, which indicates that piglets are susceptible to GLS present in *B. juncea* meal. The reduced growth performance of weaned pigs could be the result of the high sensitivity of young pigs to GLS of *B. juncea* meal, especially gluconapin, which is the most abundant and responsible for growth depression in weaned pigs**.** Mejicanos (2015) found decreased feed efficiency in weaned pigs fed pre-starter diets containing dehulled CM from *B. juncea*, which can be attributed to increased amounts of glucosinolate gluconapin. In the same experiment, Mejicanos (2015) reported that when pigs were fed diets containing CM from black *B. napus*, feed efficiency increased compared to pigs fed diets containing *B. juncea* CM or diets containing the control SBM.

Table 2.7. Glucosinolates content of black *B. napus* and yellow *B. juncea* meals and their respective dehulled fractions 1 and 2 (µmol/g, as-is basis)[1]

Glucosinolate	Black *B. napus*			*Yellow B. juncea*		
	Parent Meal	Dehulled fractions		Parent Meal	Dehulled fractions	
		1	2		1	2
Gluconapin	2.1	2.6	2.3	10.1	11.2	11.2
Glucobrassicanapin	0.3	0.3	0.3	0.8	0.9	1.0
Progoitrin	5.1	5.7	5.3	0.8	0.9	1.0
Gluconapoleiferin	0.2	-	0.3	-	-	-
Glucobrassicin	0.4	0.3	0.4	0.1	0.1	0.1
4-Hydroxyglucobrassicin	1.2	0.8	1.1	0.3	0.3	0.4
Total glucosinolates	9.2	9.6	9.6	12.2	13.5	13.6

[1]Source: Mejicanos, 2015.

2.6.3 *Phytic acid*

Phytic acid [myo-inositol (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate)] is the storage form of P in grains and seeds. Although its role in animal nutrition is not entirely understood, it is considered an anti-nutritional factor (Khajali and Slominski, 2012). It is present in CM at levels of

4-6% and reduces its nutritional value by binding to multivalent cations like Zn, Ca, and Fe, thus reduces their bioavailability (Al-Asheh and Duvnjak, 1994). Phytic acid present in the diets decreases the apparent absorption of Ca, P, Mg, Fe, Zn, Cu, and Mn (Rimbach et al., 1995). Moreover, phytic acid could affect animal performance by reducing nutrient digestibility through binding to nutrients, the digestive enzymes, or both, which, in turn, would result in increased endogenous losses of amino acids (Woyengo and Nyachoti, 2013). A standard diet may contain 10 g/kg of phytic acid (2.8 g of phytate P/kg), and as much as 60% of this may be hydrolyzed by microbial phytase and absorbed by the terminal ileum (Adeola and Cowieson, 2011). Furthermore, it was also indicated in a study that dietary supplementation of phytase increased P and Ca digestibility in diets including calcium carbonate, fed to growing pigs (González-Vega et al., 2015). The basal endogenous losses of Ca were not affected when phytase was included in diets containing CM fed to growing pigs (González-Vega et al., 2013). However, microbial phytase reduces the basal endogenous loss of calcium in diets fed to gestating sows (Lee et al., 2019).

A recent study with CM from conventional *B. napus* and *B. juncea* by Adhikari et al. (2015), reported true total tract digestibility of phosphorus (TTTD) values of 33.3 and 32.0% respectively, while standardized total tract digestibility (STTD) values were reported to be 31.0 and 28.3%; the study reported endogenous loses of P averaging 665 ± 0.03 mg/kg DMI. Another study by Liu et al. (2014) found similar results comparing two diet types in the estimation of true digestibility using the regression method and reported values to be 30.19 and 27.22% for pigs fed semi-purified diet and practical diet, respectively. Mejicanos et al. (2017) indicated higher total P content (1.27%, as-is basis) of canola meal through tail-end dehulling. Higher total P in the dehulled meal is associated with higher non-phytate and phytate P. Therefore, the reduction in

fiber content and increase in CP with the corresponding increases in total, non-phytate, and phytate P would have implications in the improvement of P utilization in pigs.

2.6.4 Tannins

Tannins in canola are found mainly in the hulls; dark-colored hulls contain more tannins than yellow hulls (Durkee, 1971; Yapar and Clandinin, 1972; Theander et al., 1993). Insoluble tannins (i.e., proanthocyanidins) are predominant in canola and responsible for the dark color of the seeds. It has been demonstrated that adding soluble tannins to broiler diets resulted in growth depression (Mansoori and Acamovic, 2007). However, tannins present in canola are basically water-insoluble and are located in the hulls and thus may have minimal effect on the nutritive value of canola (Khajali and Slominski, 2012). Environmental growing conditions can affect the content of tannins (Naczk et al., 1998). Research on the effect of tannins on growth performance and intestinal ecosystem in weaned pigs has demonstrated some improvement in feed efficiency, which indicates that tannins may have beneficial effects, not just anti-nutritional effects (Biagi et al., 2010). Tannins have the potential to bind with protein and proteolytic enzymes in the gastrointestinal tract, thereby reducing the protein digestibility (Khajali and Slominski, 2012) and altering the intestinal absorption of amino acids, minerals, and simple sugars, however, they would have minor impact on the absorption capacity in the intestine as demonstrated in a broiler trial (Manssori et al., 2015).

2.6.5 Sinapine

Sinapine is the choline ester of sinapic acid (Butler et al., 1982), which is the most abundant phenolic ester in rapeseed. Sinapine is a bitter-tasting phenolic compound that is widely distributed

among plants of the Cruciferae family, and therefore it would contribute to the unpleasant and bitter flavor of glucosinolate-free rapeseed products, and its presence may limit feed intake (Nackz, 1998). Brand et al. (2007) reported differences in the sinapine content of different canola cultivars, with a mean value of 9.95 mg sinapine/g grain and values ranging from 7.72 to 11.53 mg sinapine/g grain. However, varieties grown in Australia have shown concentrations in the range of 10-18 g/kg (Rodney et al., 2008). Genetic improvement has been limited in the case of sinapine reduction due to reduced variation within *B. napus* cultivars. However, selection for lower levels can be achieved (Rodney et al., 2008). Nonetheless, research in Germany to reduce the levels of sinapine in rapeseed/canola by developing low-sinapine varieties with yellow-seeded and low-fiber characteristics has been performed (Norddeutsche Pflanzenzucht H. G. Lembke KG, 2010). Sinapine levels have been reduced up to 71%, and seeds with a content of 2.4 mg/g as compared to control with 7.5 mg/g are available (Emrani et al. 2015).

2.7 MEANS OF IMPROVING THE NUTRITIVE VALUE OF CANOLA MEAL FOR SWINE

2.7.1 Meal production procedure

Canola meal, a co-product of the canola oil crushing industry, is produced when oil is extracted using any one of the three main processes (Canola Council of Canada, 2011). These include solvent extraction (where oil is removed from the meal by physical expeller extraction followed by solvent washing), expeller pressed (where oil is physically extracted using heat), and cold-pressed (where oil is physically extracted without heat treatment; Mailer, 2004).

The most common and efficient method of oil extraction is pre-press solvent extraction; it results in a meal that has less than 5% residual oil (Spragg and Mailer, 2007). The solvent extracted

meal is placed into the desolventizer-toaster in which the solvent is removed by the use of steam, which provides heat to vaporize the hexane. During this process, the meal is heated to 95-115˚C and moisture content increases to 12-18%. The desolventized meal is then toasted on heated metal plates. The final products contain 10% moisture and less than 1% oil content (Mailer, 2004). In the processing plant, some of the canola oil refining products, including gums and soapstocks, are added back into the meal to increase the energy value and meal quality. Canola oil also can be extracted using expeller-pressed method where the oil and meal is physically extracted with added moisture of less than 12% and heat of up to 160˚C, but this method is less efficient and results in a meal with higher residual oil content (8-15%) (Spragg and Mailer, 2007; Canola Council of Canada, 2009).

The processing method used to extract canola oil would affect the quality of the meal, and in the case of solvent extraction, Newkirk and Classen (2002) demonstrated that prior to desolventizing/toasting, processing does not affect the AID of AA, except for cysteine and serine. However, it was found that meal desolventizing/toasting significantly decreases protein and AA digestibilities, especially lysine. Toasted CM had an average of total digestible AA content of 69.6 g 16 g^{-1}, while non-toasted CM had 77.6 g 16 g^{-1} (Newkirk et al., 2003a). Such detrimental effects are caused by the Maillard reaction, which would lead to the formation of aldose products of AA, which are not effectively utilized. Adewole et al. (2015) also indicated that the nutritive value of CM, particularly, digestibility can be enhanced or diminished by processing conditions, as excessive heating during pre-press solvent extraction may result in reduced digestibility of AA, particularly lysine. Likewise, it was indicated that dietary fiber and corresponding low glucosinolate content observed in some crushing plants could have been caused by CM overheating. The knowledge gained by studying CM from different processing facilities, especially

regarding processing conditions such as heat treatment has been useful to generate indicators of CM quality. A recent study concerning the effects of processing conditions on the quality of CM determined correlations between dietary fiber and: 1) NDICP contents; and 2) NDICP and GSL content. In the study, analyzed samples from processing facilities with CM low in lignin and polyphenols, and NDICP showed that CP, total P, sucrose, and NSP contents were also higher compared to samples with high lignin and polyphenols (Adewole et al., 2016). In a study evaluating toasting and AA availability of rapeseed meal in pigs was concluded that the improved acceptance of more extended heated meal with lower GLS content is compromised by decreased content of limiting AA such as lysine and correspondingly by more depressed SID of most AA (Schöne et al., 2015), which is consistent with findings by Adewole et al. (2016) who indicated that the processing facilities producing CM with the highest NDICP, lignin and polyphenols, and total dietary fiber contents, also had the lowest GSL content. Whereas, the processing facility producing CM with the lowest dietary fiber had the highest GSL content.

2.7.2 Dehulling procedure

Canadian canola seed production has increased from 12.7 MMT in 2010 (Canadian Oilseed Processors Association, 2017) to 21.3 MMT in the 2017-18 season (Statistics Canada, 2018), with the consequent increase in the availability of CM in the global market. However, limited research has been performed on practical and easy to implement technologies to increase protein and decrease fiber content in CM. Nevertheless, recently, the demand for protein alternatives in diet formulation has increased the interest for other options for canola processing that would allow the production of a meal with higher nutrient density. In that regard, in France, research on front-end dehulling (removal of the hull before oil extraction) has shown significant fat losses with the use

of the technology (Carré et al., 2016). Tail-end dehulling (removal of the hull after oil extraction) has been studied in Norway (Hansen et al., 2017). However, the suggested technology is complicated and challenging to implement at industrial-scale. In Canada, dehulling has been studied in Alberta, Manitoba, and Saskatchewan (Thakor et al., 1995; Clark et al., 2001; Beltranena and Zijlstra, 2012; Zhou et al. 2013; Mejicanos et al. 2017). However, further research is required to maximize the yield, the nutritive value of the meal, and its utilization in swine diets.

According to studies conducted by INRA, France, more than 70% of rapeseed fiber is present in the hulls; consequently, the removal of the hulls would improve the quality of the meal (Carré, 2009). Several seed dehulling processes have been developed. Reichert et al. (1986) developed a tangential abrasive dehuller device (TADD) consisting of an abrasive disk rotating horizontally, and a stationary lid with several grain cups over the rotating disk. The abrasive disk set to 80 degrees was found to be optimal for canola dehulling. Such a process, however, may require pre-conditioning of the seed to maximize the percentage of hull removal (Thakor et al., 1995). The French Institute for Oilseeds owns a patent for a dehuller that works based on a centrifugal propeller to separate the embryo and the hull fractions (Technical Feed Information, 2013). Dehulling can be done using an abrasive dehuller, which requires conditioning of the canola seeds, and the dehulling index is variable depending on the time of moistening and heating, which makes the commercial application unpractical (Ikebudu et al., 2000). Other methods for dehulling (i.e., rolling) have been described but have shown to be inefficient.

Clark et al. (2001) assessed the tail-end dehulling of CM in broilers. The method involved the addition of moisture up to 16%, milling using a disc mill with 200 µm gap, and sieving through a 70 mesh screen (250µm) in order to obtain 2 fractions, one being partially dehulled CM with high protein and reduced fiber contents and the other, a coarse fraction, with partly elevated fiber

and protein contents. Dehulling increased the protein and AA contents of dehulled meals. Crude protein and lysine increased in the range from 0.4 to 10.9% and 1.2 to 17.5%, respectively, with an average of 5% for both, whereas the increase in crude fat was 2.1 to 56% and averaged 23%. Kracht et al. (1999) observed that following dehulling, the CP content of CM increased from 39.6 to 42.4% (DM basis). It was also found that the amounts of AA per kg of meal increased following dehulling by 11%, with lysine rising by about 5% and methionine and cysteine by 26%. Mejicanos et al. (2014) evaluated tail-end dehulling using pre-press solvent-extracted meal, obtaining 2 dehulled fractions: Fine 1 and Fine 2. When the fractions were compared to the corresponding parent meal it was observed that the values of CP had increased from 36.8 to 42.0 and 39.6% for the conventional *B. napus* meal; from 41.0 to 43.6 and 43.0% for yellow-seeded *B. napus* meal; and from 42.3 to 47.9 and 46.8% for *B. juncea* meal (as-is basis). Table 2.2 shows that the AA contents of the dehulled fraction 1 were higher than those in the corresponding parent meals. Methionine increased from 0.68 to 0.81% for conventional *B. napus*, from 0.63 to 0.71% for yellow *B. napus*, and from 0.66 to 0.83% for *B. juncea*. Lysine also increased from 2.02 to 2.26% for conventional *B. napus*, from 1.91 to 2.34% for yellow *B. napus*, and from 1.95 to 2.29% for *B. juncea*. Mejicanos (2015) also indicated that GSL content was not significantly increased in the dehulled fractions, nonetheless in the case of *B. juncea* meal a different GSL profile was observed; gluconapin was reported as being 10.1 µmol/g (as-is basis) whereas conventional *B. napus* had 2.1 µmol/g (as-is basis). Table 2.7 shows the GSL content of black *B. napus* and *B. juncea* meals and their respective dehulled fractions 1 and 2.

Vibro-separation for CM classification has been studied in Alberta, Canada. Reducing the particle size by grinding of solvent-extracted *B. juncea* meal was effective in reducing the NDF content from 22.7% for fractions over 850 microns to 11.8% for fractions under 425 microns

(Beltranena and Zijlstra, 2011). Another method of tail-end dehulling is "air classification," which utilizes the difference in particle size/density (kg/m^3) between hulls and embryo (Thakor et al., 1995). The hulls of canola are rich in fiber which is denser than the oil-free cotyledons, so these seed components partially fractionate in a stream of air allowing air classification to separate CM into a low-fiber, light-particle fraction and a high-fiber, heavy-particle fraction which can be of interest for the feeding monogastric and ruminant species, respectively. Air classification increases apparent total tract digestibility coefficients (CATTD) of DM, GE, CP, and DE in pigs, but did not result in increases of ADFI, or ADG. Air classification had little effect on the growth performance of weaned pigs (Zhou et al., 2013). Hansen et al. (2017) demonstrated that with the combination of ball milling and sieving, the yield of the dehulled meal could increase to 42%. However, the methodology utilized was of difficult commercial application and suitable for laboratory usage. Therefore, an easy to implement procedure to achieve high yields of the dehulled meal using tail-end dehulling is critical to advance the commercial production of dehulled CM.

2.7.3 Enzyme supplementation

Few studies have been conducted to evaluate the effect of NSP-degrading enzymes on the digestibility and performance of pigs fed diets supplemented with CM. For instance, Thacker, (2001) fed barley-based diets containing CM and supplemented with muti-carbohydrase enzymes to growing pigs and found that enzyme did not affect growth performance and ATTD of nutrients. In a study with weaned pigs, Zijlstra et al. (2004) found that carbohydrase supplementation to wheat and CM based diet improved the ADFI and ADG but did not improve feed efficiency and ATTD of nutrients. They postulated that carbohydrase enzymes reduced the digesta viscosity, thereby increasing the passage rate, which led to an increase in ADFI. Zhang et al. (2014) reported

that when using exogenous multi-enzyme (EME) in piglets 35 to 65d of age, the values for ATTD of DM, CP, and GE were greater than when piglets were fed diets without EME supplementation. In the performance study, it was observed that the ADG, ADFI, and feed efficiency tended to be higher with the increasing levels of supplemented EME Additionally, it was found that inclusion of EME resulted in increased counts of *Lactobacilli* spp. and *Bacillus subtilis* spp. and reduced the population of *Salmonella* spp. and *Escherichia coli* spp. in the feces. The activities of amylase, lipase, and protease in the small intestine were enhanced with the inclusion of EME in the diets (Zhang et al., 2014).

The more significant impact of enzyme supplementation on nutrient utilization of CM has been observed with the use of phytase. It was reported that supplemental microbial phytase increased ATTD and STTD of P from 44.99 and 48.82% to 64.08 and 67.97% for CM; from 46.77 and 50.36% to 63.53 and 67.29% for 00-rapeseed; and from 44.83 and 48.60% to 69.18 and 72.99% for rapeseed expellers (Maison et al., 2015a). Additionally, in a study evaluating 2 types of CM and 3 levels of phytase (i.e., 0, 500 and 2,500 FTU/kg) Adhikari et al. (2015) observed that as the phytase level increased, the ATTD of P increased from 39.1 to 69.3, and 78.9% in treatments containing black *B. napus* meal, and from 46.0 to 71.4 and 78.0 in treatments containing yellow *B. juncea* meal fed to growing pigs. The STTD of P also increased in a similar way, as shown in Figure 2.1. Sanjayan et al. (2014) evaluated 20 and 25% inclusion levels of CM from *B. napus* and *B. juncea* with and without multi-carbohydrase supplementation and found that regardless of variety and inclusion level, there were no significant differences among treatments for ADG, ADFI, and G:F ratio for 4 weeks after weaning.

The use of phytase has been indicated to further increase the digestibility of AA by 1 to 3% (Kies et al., 2001). Xylanase has been utilized in swine diets with inconsistent results regarding

efficacy and growth performance. Fang et al. (2007) observed ADG of 878 g/d using xylanase on rapeseed meal containing diets, compared to 828 g/d when pigs were fed diets without xylanase. However, in a recent study, xylanase supplementation did not affect growth performance (Jang et al., 2017). Xylanase originating from *Bacillus subtilis* has shown to be more effective when applied to wheat-based diets, whereas xylanase from *Fusarium verticillioides* has shown better results in corn-based diets. The cereal-based diets with higher NSP content, promote a more diverse nutritional niche, resulting in higher microbiota diversity in the cecum (Zhang et al., 2018).

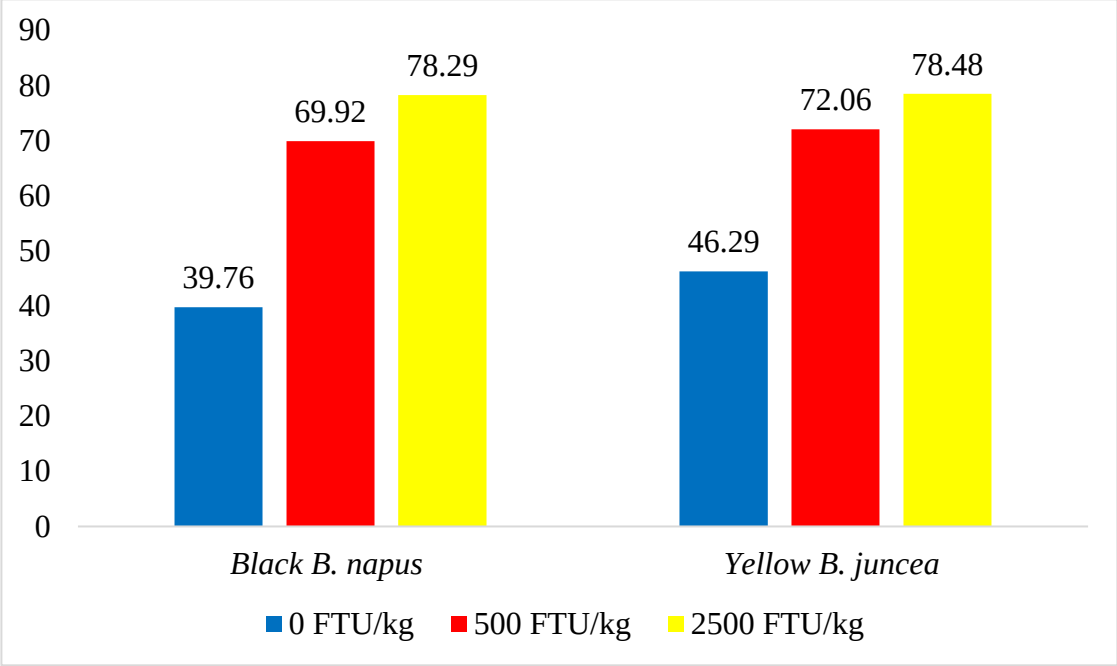

Figure 2.1. Effect of phytase supplementation on standardized total tract digestibility of phosphorus in two types of canola meal fed to growing pigs (Adapted from Adhikari et al. (2015).

The addition of phytase plus xylanase can further increase the digestibility of NSP, P, and Ca in diets containing canola meal as a protein supplement. Phytic acid and arabinoxylans present in most grains, and CM can be hydrolyzed (Bedford, 2000), alleviating adverse effects. Additionally, the decrease in pH between ileum and colon from the microbial breakdown of fiber may improve the solubility of minerals and increase their absorption; however, in some cases, no beneficial effect has been observed (Scholz-Ahrens and Schrezenmeir, 2007).

2.7.4 Fermentation

Solid-state fermentation (SSF) of CM using *Aspergillus ficuum* (Reichardt) has been used to increase the amount of protein and to reduce the amount of phytic acid (Nair and Duvnjak, 1990). Furthermore, Ebune et al. (1990) reported that phosphate and glucose concentration are essential factors to consider to maximize the production of phytases and the reduction of phytic acid content in CM during the SSF process using *A. ficuum.* The use of *Lactobacillus salivarius* in SSF of CM has resulted in a decrease in the amount of GSL, crude fiber (CF), and the increase of CP content (Ahmed et al., 2014).

Aljuobori et al. (2014) selected traditional foods fermented by microorganisms naturally present in food and isolated lactic acid bacteria (LAB). From the isolates obtained it was determined that most of them were *Lactobacillus*; 10 of them were selected to ferment CM, being *Lactobacillus salivarius* the most efficient LAB to reduce the total GSL and CF content of CM which reported reductions from 22.0 to 13.6% and from 12.0 to 10.1%, respectively. Such values are slightly lower than those published by Pal Vig and Walia (2001), in the research of solid-state fermentation in rapeseed meal using *Rhizopus oligosporus;* the study reported a reduction of GSL and CF by 43.1 and 25.5%, respectively. When compared fermented SBM products fed to pigs,

with canola expellers or fermented coproduct mixture containing canola, lower DE, and ME for both ingredients were observed (Navarro et al., 2015).

2.8 UTILIZATION OF CANOLA MEAL IN SWINE FEED

Canola meal can be used as a cost-effective protein substitute for other protein sources such as soybean meal in pig diets. Depending on its relative nutritive value and cost, it is economical to replace soybean meal partially or entirely with CM. The literature contains enough evidence that CM has been used for more than forty years in swine diets.

The energy system used to express requirements for pigs according to NRC 1971 was total digestible nutrients, then metabolizable energy; currently NRC (2012) expresses AA and nitrogen requirements as standardized ileal digestible and apparent ileal digestible basis, but also, they are shown on a total basis, which applies to corn-SBM based diets. In the same way, P requirements are listed on an STTD, ATTD, and total basis. Net energy is also used as the most accurate mean to predict the pigs' response to energy intake. It is assumed that if the diets are balanced according to SID of AA and net energy, similar performance will be achieved regardless of feedstuff used in the formulation. In that regard, recent research shows that CM can be included in pre-starter and starter diets at levels of 15, 20, and 25% without affecting pig performance. (Landero et al., 2011; Sanjayan, 2014; Mejicanos, 2015).

2.8.1 Starter pig diets

It appears that most of the studies on CM use in starter pig diets were mainly focused on growth performance. In the past, it was suggested that complete (McKinnon and Bowland, 1977) or partial (Castell, 1977) replacement of soybean meal with CM had adverse effects on pig

performance (Bell et al., 1991). It was also documented that increasing the inclusion of CM linearly reduced ADG and ADFI in weaned pigs (Baidoo et al., 1987). In a preference trial, weaned pigs were offered a choice between a SBM based control diet and CM at 5-20% inclusion level, results indicated that pigs preferred to eat the SBM based control diet more than any of the diets containing CM (Baidoo et al., 1986). There was also a significant reduction in the amount of feed consumed when CM inclusion level was increased from 5 to 20%. The possible reason for the low intake of a diet containing CM by starter pigs may be the influence of GSL breakdown products on thyroid function and the reduced palatability due to the presence of GSL and their break down products (McKinnon and Bowland, 1977).

However, recent findings are contrary to the results of past research. For instance, Seneviratne et al. (2010) reported that either solvent-extracted canola meal (SECM) or expeller-pressed canola meal (EPCM) at 150 g/kg inclusion level could partially replace SBM in weaned pig diets. In a 28 d study, Landero et al. (2011) fed 0, 50, 100, 150, and 200 g CM/kg in replacement for SBM to weaned pigs. It was found that increasing the inclusion of CM up to 20%, did not affect ADG, ADFI, and G:F. Although, increasing inclusion of CM reduced linearly the ATTD of energy, DM and CP and quadratically the DE content of the diets. Landero et al. (2012) also conducted another experiment to determine the effect of feeding increasing levels of EPCM up to 200 g/ kg diet to weaned pigs and found no significant differences in growth performance, although there were linear reductions in ATTD of DM, energy, and CP. In a more recent study, Sanjayan (2014) demonstrated that SECM from *B. napus* and *B. juncea* could be included in the weaned pig diets at up to 25% without adverse effect on the growth performance (Table 2.8). In another study, Mejicanos (2015) evaluated high levels of inclusion of parent and dehulled *B. napus* and *B. juncea* CM replacing SBM at 15% level and found increased growth performance when using Fine 2

dehulled CM. There were two possible explanations proposed for the improved performance of weaned pigs at high CM inclusion. Firstly, in the past, diets were formulated mainly based on CP and DE and not on SID AA or NE. Zijlstra and Payne (2007) suggested that formulating diets with by-products as alternative feedstuffs would minimize the risk associated with reductions in growth performance if the NE and SID AA systems were used. The second reason is that new cultivars of CM have comparatively low amounts of GSL compared to old varieties (Landero et al., 2011; Mejicanos, 2015; Adewole et al., 2016).

The type of diet can also affect the bacterial diversity and influence the ability of the pig to digest and absorb nutrients (Awati et al., 2005). Gut microbiota is subjected to changes because of factors such as diet, and the disruption of the gut microbiota would allow the establishment of pathogenic bacteria (Kim et al., 2011). In a study comparing ileum microbiota of pigs fed corn, wheat, or barley-based diets, Hill et al. (2005) observed that Lactobacillales-like sequences dominated the corn, barley, and wheat libraries while Clostridiales-like sequences were more prevalent in the corn library. The type of bacteria present in the gut can have beneficial or detrimental effects. Berg (1996) indicated that the indigenous gut microbiota synthesizes vitamin B_{12} and vitamin K, which will benefit the host; however, deconjugation of bile acids by bacteria in the upper bowel can decrease fat absorption leading to diarrhea and steatorrhea.

Table 2.8. Effect of dietary canola meal inclusion and canola meal type on nursery pig performance[1, 2]

Item	Control	Yellow *B. juncea*		Black *B. napus*	
	0 %	20 %	25 %	20 %	25 %
ADG, g/d	400	385	390	395	391
ADFI, g/d	617	607	620	622	618
G:F	0.63	0.64	0.63	0.64	0.63

[1]Adapted from Sanjayan et al. (2014).
[2]Piglets were fed canola meal containing diets in two phases for 28 days starting from weaning at 21 d of age. There was no effect of inclusion level or canola type.

2. 8.2 Grower-finisher pig diets

Previous studies reported that CM could be used to replace only up to 50% of the supplemental protein from SBM in grower pigs (McKinnon and Bowland, 1977). However, the replacement of 75% or complete replacement of SBM by CM significantly reduced the growth performance (Baidoo and Aherne, 1987). Sauer et al. (1982) indicated that lower DE and lysine contents in CM compared to SBM and the effect of GSL on feed intake and the metabolic process might be the possible reasons for the low performance in grower pigs. Thacker (1990) suggested that good performance could be achieved in grower pigs if CM supplies only one half of the supplementary protein in the diet. In a review on CM, Schöne et al. (1997) suggested that growing pigs can tolerate a maximum level of 2 μmol/ g of GSL in the diet. But the total GSL content of Canadian CM ranged from 1.9 to 9.7 μmol/g of GLS DM, with an overall mean of 4.6 μmol/g of GLS DM (Adewole et al., 2016; Newkirk et al., 2003); which implies a maximum level of 33% CM in growing pig diet.

Studies to determine the digestibility of nutrients of CM has been conducted, for instance, Bell et al. (1998) reported that black-seeded *B. napus* and yellow-seeded *B. juncea* had similar digestible protein and energy in finisher pigs. An experiment using toasted and non-toasted black and yellow-seeded *B. napus* and yellow *B. juncea* in grower pigs suggested that DE and NE content of yellow-seeded *B. napus* is higher than that of conventional black *B. napus* and *B. juncea* (Montoya and Leterme, 2009). National Research Council (2012) indicates NE value for CM from black *B. napus* to be 1,890 kcal/kg. Meanwhile, Heo et al. (2014) indicates that NE for yellow-seeded *B. napus* averaged 2,102 kcal/kg, while values for yellow-seeded *B. juncea* averaged 2,340 kcal/kg. Several studies have reported the SID of AA of CM and EPCM in grower pigs (Seneviratne et al., 2010; Woyengo et al., 2010; Maison, 2014; Sanjayan, 2014). In the mentioned studies, EPCM had greater digestible AA compared to CM, as can be seen in Table 2.3.

Previous studies also indicated that CM could be included in pig diets without affecting growth performance and carcass characteristics of the finisher pigs. A performance study was conducted in grower pigs with a decreasing amount of EPCM (22.5, 15, 7.5, and 0%) to validate the performance and carcass characteristics (Seneviratne et al., 2010). Increasing the inclusion level of EPCM did not affect carcass characteristics such as backfat thickness, loin depth, jowl fat, and fatty acid profile; however, ADG was reduced by 3 g/day per 1% inclusion of EPCM. Zanotto et al. (2009) fed 20, 40, 60, and 80% of CM, in replacement of SBM to growing-finishing pigs and found quadratic treatment effect on the weight gain. These authors found that the substitution level of 40% SBM resulted in increased weight gain and heavier carcass, whereas backfat depth was greater (Bell et al., 1981; Narendran et al., 1981). Canola meal feeding not only increased the proportion of unsaturated fatty acid in adipose tissue and muscle tissue, but it also didn't affect the carcass characteristics (Busboom, 1991).

CHAPTER THREE

HYPOTHESES AND OBJECTIVES

The studies tested the following hypotheses:

1. The cereal ingredient of the basal diet influences piglet response to high dietary canola meal (CM) addition.
2. Tail-end dehulling of CM affects P digestibility in the different fractions, and it differs when fed to growing pigs at 2 different body weights (BW).
3. Tail-end dehulling of CM influences apparent (AID) and standardized ileal digestibility (SID) of amino acids (AA) when fed to growing pigs.
4. Enzyme supplementation in wheat-based diets containing CM as a protein source could improve nutrient digestibility.

The overall objective was to explore and to determine data to allow the optimal utilization of canola meal in diets fed to weaned pigs.

The Specific objectives were:

1. To determine growth performance, apparent total tract digestibility (ATTD) of energy and protein, and the relative abundance of fecal microbial communities, in a corn-soybean meal (SBM) and wheat-SBM based diets with or without 20% inclusion of CM.

2. To determine the ATTD and standardized total tract digestibility (STTD) of P in the regular canola meal (RCM) and the 2 fractions obtained by tail-end dehulling; dehulled canola meal ((DCM) and coarse canola meal (CCM) fed to growing pigs of two distinct BW.
3. To determine the AID, the SID and standardized ileal AA content of RCM and the 2 fractions obtained by tail-end dehulling (DCM and CCM) fed to growing pigs.
4. To determine the effect of supplementing xylanase on RCM and the 2 fractions obtained by tail-end dehulling, in a wheat-based diet, and its impact on nutrient digestibilities, organ weight, pH in ileal and colon digesta, SCFA concentration in colonic digesta, and growth performance, when fed to weaned pigs.

CHAPTER FOUR

MANUSCRIPT I

Effect of high canola meal content on growth performance, nutrient digestibility and fecal bacteria in nursery pigs fed either corn or wheat-based diets

G.A. Mejicanos[a], A. Regassa[a] and C.M. Nyachoti [a]

[a]Department of Animal Science, University of Manitoba, 12 Dafoe Road, Winnipeg, MB, Canada R3T 2N2.

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4.1 ABSTRACT

In North-America, soybean meal (SBM) and canola meal (CM) are the most extensively used protein supplements in the feed industry, and corn and wheat are the primary sources of energy in swine diets. Recent studies show that piglets can tolerate relatively high levels of CM inclusion. However, it is unclear whether this ability depends on the cereal ingredient of the basal diet. This study was conducted to examine the effect of including CM in wheat or corn-based diet on growth performance, apparent total tract digestibility (ATTD), and fecal microbial communities compared with wheat- or corn-SBM based diet. Ninety-six pigs (Yorkshire-Landrace × Duroc) with an initial BW of 6.63 ± 0.028 kg (barrows) and 6.78 ± 0.036 kg (gilts) were used in this 28-d feeding study. There were 8 replicates per treatment, each with 3 pigs. Pigs were randomly allotted to one of the four dietary treatments: corn-SBM diet (CSBM), corn-SBM diet+20% CM (CCM), wheat-SBM diet (WSBM), and wheat-SBM diet+20% of CM (WCM). A two-phase feeding program was used (phase I, 1-14, and phase II, 15-28 d post-weaning). Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) were recorded weekly. Freshly voided fecal samples were collected on d 21 and 27 to determine ATTD of CP, energy, and fecal bacteria community. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS, and differences were declared significant at $P < 0.05$. No significant differences were observed in ADFI, ADG, and final-BW among treatments. During phase I, pigs fed the WCM diet had higher G:F compared with those fed the CSBM diet (0.95 vs. 0.79, $P < 0.01$). During phase II, pigs fed CSBM diet had higher ATTD of CP and energy compared with piglets fed the CCM, WSBM, and WCM diets (96.6 vs. 89.0, 90.9, and 87.2%; and, 95.3 vs. 89.6, 90.8 and 86.9%, $P < 0.01$). When compared to corn-based diets, wheat-based diets had a significant reduction in the relative abundance of *Lactobacillus* ($P < 0.05$). Likewise, the relative abundance of

Enterococcus was reduced ($P < 0.05$). However, wheat-based diets had a significantly higher relative abundance of Clostridium cluster-IV ($P < 0.05$). In conclusion, the inclusion of CM into either wheat- or corn-SBM based diet influences G:F, protein, and energy digestibility and relative abundance of the measured fecal microbial community without affecting voluntary feed intake and body weight gain.

Key words: canola meal, digestibility, fecal bacteria, growth performance, pig.

4.2 INTRODUCTION

Wheat is widely available in North America, and together with corn, they make up the most conventional sources of energy in swine diets (Statistics Canada, 2015; USDA-FAS, 2015; AAFC, 2016). Soybean meal (SBM) and canola meal (CM) are the primary sources of protein and are extensively used in the feed industry. Canola meal is derived from the crushing of canola seed for oil extraction (Canola Council of Canada, 2014). Canola meal has been used in swine diets for a long time; however, its dietary inclusion levels have been limited due to the presence of anti-nutritional factors (ANF), notably glucosinolates (GSL). However, due to advances in genetic improvements of canola that have led to the production of cultivars with significantly lower ANF content and improved processing procedures, CM with superior nutritive value for non-ruminant animals is now available (Khajali and Slominski, 2012; Adewole et al., 2016). The maximum GSL level that can be included in swine diets is 2.5 µmol per gram of diet (Bjerg et al., 1987; Bell, 1993). A recent study indicated that piglets could tolerate relatively high levels of CM without adverse effects on growth performance (Sanjayan et al., 2014). Accordingly, CM as higher as 250g/kg can be safely included in the wheat-SBM diet without adverse effect on piglet growth performance (Sanjayan et al., 2014). Likewise, increased feed efficiency (G:F) and final body

weight (BW) were reported when corn-SBM basal diets were supplemented with 150 g/kg of conventional or dehulled CM compared to corn-SBM alone (Mejicanos, 2015).

Wheat can be substituted by corn in diets for growing-finishing pigs without adverse effects on performance. However, higher G:F has been observed in wheat-based diets compared to corn-based diets (Han et al., 2005). The crude protein (CP) content and content of all essential and non-essential amino acids (AA), except leucine and alanine, are higher in wheat than in corn; but net energy, total dietary fiber, and ether extract are higher in corn (NRC, 2012). Crude protein and standardized ileal digestible (SID) AA concentration are higher in SBM than CM. However, CM has greater methionine and cysteine concentration. Thus, soybean meal and CM can complement each other when used together (Khajali and Slominski, 2012).

Dietary ingredients can affect the composition of the microbial community in the gut and influence the ability of the pig to digest and absorb nutrients (Awati et al., 2005). Gut bacteria benefit from the nutrients in the digesta, but also assist the host with nutrient digestion, vitamin synthesis, pathogen competitive exclusion and immune system development (Berg, 1996; Green et al., 2006; Looft et al., 2014). Furthermore, it has been indicated that short-chain volatile fatty acids (SCVFA) produced during carbohydrate fermentation by gut microbiota are a significant source of energy for the host's epithelial cells (Macfarlane and Macfarlane, 2003; Natarajan and Pluznick, 2014). Butyrate is one of the most important SCFA for colonocytes, and it stimulates the growth and development of intestinal cells (Ivarsson et al., 2014). Many factors can affect piglet response to diet composition. However, whether the dietary cereal ingredient of the basal diet influences piglet response to high levels of CM inclusion is not clearly understood, thus, this study was conducted to examine whether the cereal ingredient of the basal diet influences piglet response to high dietary CM addition and the relative abundance of fecal microbial communities.

4.3 MATERIALS AND METHODS

4.3.1 Animal care

The animal use protocol utilized in the present study was reviewed and approved by the University of Manitoba Animal Care Committee. Animals were managed according to procedures established by the Canadian Council on Animal Care (CCAC, 2009). The study was conducted at the T. K. Cheung Centre for Animal Science Research.

4.3.2 Animals and housing

A total of 96 piglets ([Yorkshire-Landrace] × Duroc; Genesus, Oakville, MB, Canada) weaned at 21 ± 1 days of age were obtained from Glenlea Swine Research Unit, University of Manitoba. Pigs were weighed and separated into groups of 3 according to gender and initial body weight (BW) then randomly assigned to pens in 2 rooms with 16 pens each, for a total of 32 pens. Barrows had an initial BW of 6.63 ± 0.028 kg (mean ± SD) whereas gilts weighed 6.78 ± 0.036 kg (mean ± SD). Pens with plastic cover and expanded metal floors were used and the space allowed was 0.6 m^2 per pig. Water and feed were provided ad libitum using nipple drinkers and stainless-steel feeding troughs throughout the experiment. Initial room temperature was set at 29 ± 1ºC and was gradually decreased by 1ºC every week. A 16-hour light (0600-2200h) and 8-hour dark cycle were provided. Body weight gain and feed disappearance were recorded weekly.

4.3.3 Diets

The experiment included 4 treatments and 8 replicates per treatment. Two wheat-soybean based diets and two corn-soybean based diets without or with 200 g/kg of CM were formulated to meet or exceed NRC (2012) nutrient requirements for growing pigs. The composition and nutrient content of the experimental diets are presented in Tables 4.1 and 4.2. Canola meal of conventional *B. napus* produced using the pre-press solvent extraction method was obtained from the Bunge Altona, MB, Canada processing plant. The wheat used in the experiment was a Hard Canada Western Red Spring (CWRS), corn, and all other ingredients were obtained from the local market.

4.3.4 Experimental procedures

On arrival at the T.K. Cheung Centre, piglets were fed the same creep feed as used to at Glenlea Swine Research facilities. On day 2, piglets were weighed and randomly assigned to one of the four diets in a 2-phase feeding program (Phase I, day 1 to 14, phase II, day 15 to 28). In preparation for fecal sampling, pens were thoroughly washed and rinsed using warm water. For the digestibility study, freshly voided fecal samples were collected by hand grab in sterile plastic bags (d 21 and d 27). Samples were carried on ice and stored in -20°C freezer. Fecal samples for analyzing bacterial community abundance were gathered on day 21. When possible, samples were collected directly from the rectum; nevertheless, often, feces fell to the floor. However, the part of the sample that was in direct contact with the floor was discarded; samples were stored in -80°C freezer.

Table 4.1. Composition and nutrient contents of Phase I diets[a] (%, as-fed basis).

Ingredient	CSBM	CCM	WSBM	WCM
Corn	48.78	41.27		
Wheat			60.45	50.60
Soybean meal	25.86	11.41	12.23	0.07
Fish meal	5.00	5.00	5.00	5.00
Dry whey	15.00	15.00	15.00	15.00
Canola meal		20.00		20.00
Spray-dried plasma protein	2.50	2.50	2.50	2.50
Canola oil		2.13	1.52	3.76
Calcium carbonate	1.03	0.88	1.13	0.98
Di-calcium phosphate	0.25	0.10	0.12	
L-Lysine	0.34	0.47	0.67	0.74
DL-Methionine	0.15	0.11	0.16	0.12
L-Threonine	0.09	0.11	0.21	0.20
L-Tryptophan		0.02	0.01	0.03
Vitamin premix [b]	0.50	0.50	0.50	0.50
Mineral premix [c]	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated composition				
Crude protein	23.00	23.00	23.00	23.00
Net energy (MJ/kg)	10.3	10.25	10.25	10.25
Calcium	0.85	0.85	0.85	0.85
Phosphorus (STTD)	0.45	0.45	0.45	0.45
Methionine + Cysteine [d]	0.82	0.82	0.82	0.82
Lysine [d]	1.50	1.50	1.50	1.50
Threonine [d]	0.88	0.88	0.88	0.88
Tryptophan [d]	0.26	0.25	0.25	0.25
Analyzed composition				
Crude protein	23.45	23.50	24.35	23.60
Neutral detergent fiber	8.07	11.68	6.37	12.62
Gross energy (MJ/kg)	16.42	17.13	16.84	17.41
Total phosphorus	0.65	0.75	0.67	0.74
Calcium	0.68	0.67	0.65	0.73

[a] CSBM, corn-soybean meal diet; CCM, corn-soybean meal diet + 20 % canola meal; WSBM, wheat-soybean meal diet; WCM, wheat-soybean meal diet + 20 % canola meal.

[b] Supplied per kg of diet: Vitamin A, 8250 IU; Vitamin D3, 825 IU; Vitamin E, 40 IU; Vitamin K, 4.0 mg; Thiamin (B1), 2.0 mg; Riboflavin, 10.0 mg; Pantothenate, 15 mg; Choline, 500 mg; Niacin, 22.5 mg; Vitamin B6, 4.5mg; Vitamin B12, 25 μg; Biotin, 200 μg; Folic Acid, 2.0 mg.

[c] Supplied per kg of diet: Cu, 150 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

[d] Standardized ileal digestible basis.

In preparation for analysis, feces were dried in a forced-air oven at 60°C for 5 days. Dried samples were mixed and ground using a heavy-duty blender Model CB15, Waring Commercial, Torrington, Connecticut, USA. Sub-sample was obtained by thoroughly mixing the ground feces, and before chemical analysis, fecal subsamples, CM, wheat, corn, and experimental diets were finely ground using a Foss sample preparation Cyclotec^{TM} 1093 mill (Foss Allé 1, DK-3400 Hilleroed, Denmark). Experimental diets (corn, wheat, SBM, and CM were analyzed for CP using a nitrogen analyzer, model TruSpec N (Leco Corp., St. Joseph, MI, USA). Crude protein was determined from the formula; CP = N × 6.25. The standard of AOAC (2005) procedures was used for dry matter (930.15) and ash determination (942.05). Calcium and P contents were determined using a Varian Inductive Coupled Plasma Mass Spectrometer (Varian Inc., Palo Alto, CA). Dietary gross energy content was measured using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL) calibrated using benzoic acid as a standard. Acid-insoluble ash was determined using the method described by McCarthy et al. (1974).

Table 4.2 Composition and nutrient contents of Phase II diets[a] (%, as-fed basis).

Ingredient	CSBM	CCM	WSBM	WCM
Corn	64.28	56.24		
Wheat			78.83	69.03
Soybean meal	31.79	17.47	14.13	1.96
Canola meal		20.00		20.00
Canola oil	0.50	3.05	3.04	5.26
Calcium carbonate	1.08	0.95	1.26	1.10
Di-calcium phosphate	0.80	0.64	0.59	0.45
L-Lysine	0.35	0.47	0.78	0.85
DL-Methionine	0.11	0.07	0.13	0.09
L-Threonine	0.09	0.11	0.24	0.24
L-Tryptophan				0.02
Vitamin premix [b]	0.50	0.50	0.50	0.50
Mineral premix [c]	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated composition				
Crude protein	20.00	20.00	20.00	20.00
Net Energy (MJ/kg)	1.01	1.01	1.01	1.01
Calcium	0.70	0.70	0.70	0.70
Phosphorus (STTD)	0.33	0.33	0.33	0.33
Methionine + Cysteine [d]	0.68	0.68	0.68	0.68
Lysine [d]	1.23	1.23	1.23	1.23
Threonine [d]	0.73	0.73	0.73	0.73
Tryptophan [d]	0.22	0.20	0.20	0.20
Analyzed composition				
Crude protein	19.13	19.51	18.99	19.02
Neutral detergent fiber	9.64	13.03	9.76	13.82
Gross Energy (MJ/kg)	16.40	17.06	17.06	17.74
Total phosphorus	0.58	0.67	0.62	0.65
Calcium	0.48	0.53	0.65	0.43

[a] CSBM, corn-soybean meal diet; CCM, corn-soybean meal diet + 20 % canola meal; WSBM, wheat-soybean meal diet; WCM, wheat-soybean meal diet + 20 % canola meal.

[b] Supplied per kg of diet: Vitamin A, 1560 IU; Vitamin D3, 180 IU; Vitamin E, 13.2 IU; Vitamin K, 0.6 mg; Thiamin (B1), 1.2 mg; Riboflavin, 3.0 mg; Pantothenate, 6.6 mg; Choline, 360 mg Niacin, 12.0 mg; Vitamin B6, 1.2 mg; Vitamin B12, 12.0 μg; Biotin, 200 μg; Folic Acid, 0.36 mg.

[c] Supplied per kg of diet: Cu, 4.8 mg; Zn, 72.0 mg; Fe, 72.0 mg; Mn, 2.4 mg; I, 0.168 mg; Se, 0.18 mg.

[d] Standardized ileal digestible basis

4.3.5 Extraction of Genomic DNA and quantitative real-time PCR

Bacterial genomic DNA was extracted from fecal samples using QIAamp® DNA Stool Mini Kit (QIAGEN, Canada) according to the manufacturer's instruction. Pairs of primers used for quantification of different bacterial groups were obtained from previously published works (Lee et al., 1996; Matsuki et al., 2002; Bartosch et al., 2004; Rinttilä et al., 2004; Guo et al., 2008b; Karlsson et al., 2011). Quantitative real-time PCR was performed in duplicate reactions including nuclease-free water, the forward and reverse primers, gDNA template, and SYBR Green as a detector using a CFX Connect ^{TM} Real-Time PCR Detection System (Life Science Research, Bio-Rad, Canada). Expression data for all bacterial groups were generated using the ΔΔCt method by normalizing the expression of the target bacterial group to that of total eubacteria, and the values were reported as fold changes of the expression of the target group in treatments compared with the control group. Table 4.3 shows the pair of primers used for the quantitative real-time qRT-PCR essay.

Table 4.3. Pairs of primers used for quantitative real-time qRT-PCR assay.

Name	Primer sequence		T °C [a]	Reference
	Forward	Reverse		
Lactobacillus	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG	62	Karlsson et al., 2011
Bifidobacterium	TCGCGTCYGGTGTGAAAG	CCACATCCAGCRTCCAC	63	Rinttilä et al., 2004
Enterococcus	CCCTTATTGTTAGTTGCCATCATT	ACTCGTTGTACTTCCCATTGT	60	Bartosch et al., 2004
Clostridium cluster IV	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAAC	56	Matsuki et al., 2002
Total Eubacteria	CGGYCCAGACTCCTACGGG	TTACCGAGGCTGCTGGCAC	58	Lee et al., 1996

[a]Annealing temperature

4.3.6 Calculations and experimental design

Apparent total tract digestibility (ATTD) of protein and energy was calculated using the following formula:

$$ATTD\ (\%) = [1 - (Nf \times AIAd)/ (Nd \times AIAf)] \times 100,$$

where Nf = nutrient concentration in feces (percentage of DM); Nd = nutrient concentration in diet; AIAf = acid insoluble ash concentration in feces; AIAd = acid insoluble ash concentration in diet (McCarthy et al., 1974).

Growth performance, nutrient digestibility, and gut microbial data were analyzed as a randomized complete block design using the Mixed Model Procedure of SAS software release 9.4 (SAS Institute, 2013). The model included block as a random factor. Treatment means were compared using Tukey's honesty significance difference, and all statements of significance are based on $P \leq 0.05$. The pen of 3 pigs served as an experimental unit. Treatments, gender, gender-treatment interaction, and block-treatment-gender interaction were considered as sources of variation. Because there was no gender effect, the gender-diet interaction effect was removed from the model. The main effects of protein source (SBM vs. CM), and energy source (corn vs. wheat) and their interactions were determined using contrasts. Significance was accepted at $P < 0.05$ and trends were observed at $P > 0.05 < 0.10$.

4.4. RESULTS

4.4.1 General observations

All pigs remained healthy and with good physical appearance throughout the study. The analyzed nutrient contents of the experimental diets were comparable to the calculated values. The

inclusion of CM at 20% increased NDF content (8.07 vs. 11.68% for the corn-SBM based diet, and 6.37 vs. 12.62% for the wheat-SBM based diet).

4.4.2 The effect of canola meal inclusion on growth performance

Dietary treatment had no effect on ADG, ADFI, or final BW over the entire 4-wk study period or final BW (Table 4.4). However, in phase I, pigs fed corn-based diets had a tendency for higher ($P < 0.10$) ADFI. A high difference was observed ($P < 0.05$) between pigs fed CSBM and WCM diets for G:F in phase-I and overall. Substitution of SBM with 20% of CM in wheat-SBM based diet resulted in an increased G:F compared to the CCM diet, but this difference was not significant (Table 4.4). In phase I, pigs fed diets containing CM as the primary protein source had higher G:F than those fed diets containing SBM as the main protein source ($P < 0.05$). Likewise, in phase I and overall, pigs fed wheat diets as the primary energy source had higher ($P < 0.005$) G:F compared with pigs fed corn as the main energy source (Table 4.4).

4.4.3 Digestibility

Pigs fed diets containing CM had lower ($P < 0.0001$) ATTD of protein than those fed diets containing SBM (88 vs. 93%). Likewise, pigs fed diets containing wheat had lower ($P < 0.0001$) ATTD of energy (89%) than those fed diets containing corn (92%) (Table 4.4).

Table 4.4. The effect of high dietary canola meal inclusion on growth performance and nutrient digestibility.

Item	ADG g/d/pig			ADFI g/d/pig			G:F ratio g gain / g feed			Final BW kg	Digestibility (%) Protein	Digestibility (%) Energy
	Phase I	Phase II	Overall	Phase I	Phase II	Overall	Phase I	Phase II	Overall	Overall	Phase II	Phase II
Treatment [a]												
CSBM	419	389	408	521	871	696	0.79 [b]	0.44	0.57[b]	18.0	95 [a]	95 [a]
CCM	421	375	400	523	867	698	0.84 [ab]	0.44	0.58[ab]	18.2	89 [c]	90 [b]
WSBM	425	384	407	470	885	671	0.92 [ab]	0.46	0.61[ab]	18.6	91 [b]	91[b]
WCM	424	403	416	445	865	654	0.95 [a]	0.46	0.63[a]	18.6	87 [d]	87 [c]
SEM [b]	25.88	14.46	12.61	36.21	6.91	23.23	0.014	0.018	0.111	0.1752	3.85	3.46
P-value	0.981	0.438	0.725	0.418	0.638	0.459	0.004	0.792	0.024	0.1267	0.0003	0.0003
Protein source												
Canola meal	423	396	409	485	870	678	0.90 [a]	0.45	0.60	18.2	88 [b]	88 [b]
Soybean meal	423	387	408	498	870	684	0.85 [b]	0.45	0.60	18.6	93 [a]	93 [a]
SEM [b]	18.88	8.74	9.76	23.43	10.95	16.70	0.011	0.011	0.006	0.1435	3.09	2.45
P-value	0.989	0.689	0.901	0.693	0.986	0.781	0.024	0.938	0.946	0.1644	<0.0001	<0.0001
Energy source												
Corn meal	420	385	401	526	869	697	0.82 [a]	0.44	0.57[b]	18.4	92 [a]	92 [a]
Wheat meal	426	399	413	460	871	664	0.93 [b]	0.46	0.63[a]	18.4	89 [b]	89 [b]
SEM [b]	18.88	7.73	9.75	23.42	10.77	16.68	0.114	0.010	0.006	0.1247	3.07	2.36
P-value	0.799	0.825	0.464	.076	0.934	0.168	<0.0001	0.347	0.0003	0.9612	0.0005	<0.0001

Means within a column with different superscript letters ($^{a–d}$) differ significantly ($P \leq 0.05$).

[a] CSBM, corn-soybean meal diet; CCM, corn-soybean meal diet + 20% canola meal; WSBM, wheat-soybean meal diet; WCM, wheat-soybean meal diet + 20% canola meal.

[b] Standard error of the mean

4.4.4 Effect of diet composition on the relative abundance of fecal bacteria

The relative abundance of *Lactobacillus* was different ($P < 0.05$) between dietary treatments (Table 4.5). Pigs fed CCM had a higher relative abundance of *Lactobacillus* than those fed the WCM diet. However, contrast analysis revealed that diets containing corn as the source of energy had a higher ($P < 0.05$) relative abundance of *Lactobacillus* than diets containing wheat as the source of energy. Differences ($P < 0.01$) in the relative abundance of *Enterococcu*s and Clostridium cluster IV were also observed between treatments (Table 4.5). The relative abundance of *Enterococcus* was higher in pigs fed CSBM than those fed CCM. Similarly, the relative abundance of *Enterococcus* was greater in pigs fed WSBM than those fed WCM. However, these differences were not significant. Contrast analysis indicated that pigs fed diets containing corn had a higher relative abundance of fecal *Enterococcus* ($P < 0.001$) compared to those fed diets containing wheat. The relative abundance of Clostridium cluster IV was different among dietary treatments ($P < 0.01$). The relative abundance of Clostridium cluster IV was higher in pigs fed the diet that contained CSBM compared to pigs fed CCM (Table 4.5). The relative abundance of Clostridium cluster IV was greater in pigs fed a diet that included WSBM compared to pigs fed WCM (Table 4.5). However, these differences were not significant. Contrast analysis indicated that the relative abundance of Clostridium cluster IV in pigs fed diets containing wheat as the source of energy was higher ($P < 0.05$) compared with pigs fed diets containing corn.

Table 4.5. The effect of high dietary canola meal inclusion on the relative abundance of selected fecal bacteria community.

	Lactobacillus	*Bifidobacterium*	*Enterococcus*	Clostridium Custer IV
Treatment [d]				
CSBM	1.07[ab]	1.05	0.88[a]	1.22[bc]
CCM	2.23[a]	0.55	0.53[ab]	0.76[c]
WSBM	1.02[ab]	0.44	0.11[b]	2.89[a]
WCM	0.50[b]	2.43	0.08[b]	2.26[ab]
SEM [e]	0.318	0.598	0.374	0.334
P-value	0.028	0.694	0.002	0.001
Protein source effect				
Canola meal	0.98	0.70	0.05	1.26
Soybean meal	0.97	0.33	0.24	1.72
SEM [e]	0.290	0.519	0.501	0.271
P-value	0.585	0.553	0.188	0.279
Energy source effect				
Corn meal	1.47[a]	0.70	0.45[a]	0.86[b]
Wheat meal	0.64[b]	0.33	0.03[b]	2.53[a]
SEM [e]	0.289	0.493	0.473	0.271
P-value	0.049	0.648	<0.001	0.032

Means within a column with different superscript letters ([a–c]) differ significantly ($P < 0.05$).

[d] CSBM, corn-soybean meal diet; CCM, corn-soybean meal diet + 20% canola meal; WSBM, wheat-soybean meal diet; WCM, wheat-soybean meal diet + 20% canola meal.

[e] Standard error of the mean.

4.5 DISCUSSION

4.5.1 Growth performance

Results of the present study showed that the substitution of 20% of CM for SBM in either wheat- or corn-based diet did not significantly influence growth performance (Table 4.4). These results were consistent with the findings of Landero et al. (2011) that indicated that substituting 20% of solvent extracted CM for SBM in wheat-based diets fed to weaned piglets did not affect BW gain and ADFI. During phase I, G:F was higher when 20% CM substituted SBM in the wheat-based diet compared with a corn-based diet, indicating that weaned pigs can utilize CM to support

growth. Similar results were observed by Wang et al. (2017) that found an increase in G:F during the first week post-weaning when 20% CM were substituted for SBM in a wheat-based diet. Improved G:F in weaned pigs was observed in a study replacing CM (15%, as-fed basis) for SBM in a corn-based starter diet (Mejicanos, 2015). Such results are consistent with the current findings showing that G:F can be improved during the starter period when CM substitutes SBM either in corn-based or wheat-based diet. Considering that all dietary treatments were isocaloric and isonitrogenous, the improved feed efficiency in pigs that fed WCM in the present study might be attributed to the relatively higher abundance of commensal bacteria species such as Bifidobacterium and Clostridium cluster IV which play a significant role in maintaining gut health and improving feed efficiency. The increased G:F might also be due to gut fill caused by enhanced fiber intake and reduced nutrient digestibility that enhances the mass of undigested residue in the gut (Jorgensen et al., 1996; de Lange et al., 2003).

Even when the objectives of the present study were not to compare corn-based diets against wheat-based diets, the findings of the present study agreed with other studies. Han et al. (2005) found improved G:F for wheat-based diets compared to corn-based diet, indicating that substituting wheat for corn would not have adverse effects on performance.

During phase I, pigs fed the corn-based diets consumed 66 g/day more than those on the wheat-based diet. Likewise, pigs fed SBM diets consumed 13 grams/day more than those fed CM diets (Table 4.4). The observed greater ADFI in the corn- and SBM diets without higher ADG can be an indication of increased availability of fermentable protein entering the large intestine (Goodband et al., 2014). All piglets in the experiment had similar ADG and remained healthy despite no use of any growth promoter. Skinner et al. (2014) in a study of nursery pigs feeding programs indicated that subsequent growth performance, carcass, and meat quality wouldn't be

affected when piglets are fed simple diets without the use of antibiotics. Furthermore, in the current study, improvements in G:F of 6.3 and 3.3% were obtained when CM was added to the corn-SBM and wheat-SBM based diets, respectively. However, substituting corn for wheat, and SBM for CM at 20% improved G:F by 20.3% in the pre-starter phase. Improved G:F when CM is added at 20% inclusion levels to the corn- and wheat-SBM based diets indicated that solvent-extracted CM had no adverse effects on growth performance. Piglets can utilize CM in pre-starter diets to support growth.

4.5.2 Digestibility

Energy and protein digestibility were significantly reduced due to the addition of 20% CM to the corn-SBM or wheat-SBM based diets, and this is attributed to the higher NDF content (Table 4.1) of the diets due to the inclusion of CM. However, because the diets were balanced according to SID AA and net energy, the negative effect was not observed on the growth performance of the pigs. This reduction in digestibility is consistent with results reported by Bakare et al. (2014), where growth performance was not compromised in pigs fed high fibrous diets. These results are also consistent with those of Wang (2017) where the digestibility of protein and energy decreased without an adverse effect on ADFI, ADG, and G:F when weaned pigs were fed a diet that contained 20% of CM. In another study, reduction in ATTD of CP and gross energy was observed when increasing amount of CM was added into a wheat-based diet without affecting ADG, ADFI, and G:F (Landero et al., 2011).

4.5.3 Fecal bacteria

Animals harbor gut microbial communities whose composition and relative proportions of dominant microbial groups vary among species. The gut microbiota is believed to influence many metabolic processes, nutrient absorption, and the state of health of the host (Richards et al., 2005). The microbiome of the gastrointestinal tract of the pig is not well understood, and many of its genera or species have not been characterized yet. These include gram-positive anaerobic bacteria such as Eubacterium and relatives, Clostridium and relatives, Bacillus (Lactobacillus, Streptococcus subdivision), and Peptostreptococcus and gram-negative bacteria correspond to the Bacteroidetes division (Leser et al., 2002; Guo et al., 2008a). Dietary ingredients can modify the composition of the microbial community in the gut and influence the ability of the pig to digest and absorb nutrients (Awati et al., 2005). Accordingly, the relative abundance of bacterial communities could vary with the dietary ingredients of a given diet, and the composition of microbiota may not be the same from different experiments. This study analyzed the effect of substituting 20% of CM for SBM in wheat or corn-based diets, on selected fecal bacteria in weaned pigs. The source of energy (wheat or corn) affected the relative abundance of *Lactobacillus, Enterococcus,* and Clostridium cluster IV. Feces of pigs fed the corn-based diets had higher numbers of *Lactobacillus* and *Enterococcus* than those fed the wheat-based diets. Substitution of 20% CM for SBM in corn diet resulted in a greater abundance of *Lactobacillus,* and replacement of 20% of CM for SBM in wheat diet led to a relatively higher abundance of *Bifidobacterium* (Table 4.5). However, although they are big, these differences were not statistically significant, and this could be attributed to the big variation among pigs as explained by high SEM (Table 4.5). *Lactobacillus, Bifidobacterium*, and Clostridium cluster IV are known commensal bacteria species, and their relative abundance can be related to improved gut health, feed efficiency, and

growth. The relative abundance of commensal Clostridium cluster IV was higher in the feces of pigs fed wheat-canola meal diet compared to those fed corn-canola meal diet. Clostridium cluster IV is composed of *Clostridium, Eubacterium, Ruminococcus and Anaerofilum* genera (Collins et al., 1994; Lopetuso et al., 2013) and is responsible for fermentation of resistant starch and dietary fiber in the colon and release of butyrate. Butyrate is the most important source of energy for colonocytes and is associated with gut health (Pryde et al., 2002; Giuberti et al., 2013; Lopetuso et al., 2013). Although there were no statistically significant differences between treatments, a higher relative abundance of *Bifidobacterium* was measured in pigs that were fed WCM, and this could explain in part the improved feed efficiency. The reduced relative abundance of *Lactobacillus* in the same group could be explained by the higher relative abundance of Clostridium cluster IV, which might have been favored by CM more than *Lactobacillus*.

4.6. CONCLUSION

The inclusion of CM into either wheat- or corn-SBM diet influences G:F, protein and energy digestibility and relative abundance of the measured fecal microbial community without affecting voluntary feed intake and body weight gain.

CHAPTER FIVE

MANUSCRIPT II

Tail-end dehulling of canola meal improves apparent and standardized total tract digestibility of phosphorus when fed to growing pigs

G. A. Mejicanos, J. W. Kim and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada

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5.1 ABSTRACT

Tail-end dehulling of canola meal (CM) has been shown to reduce dietary fiber and increase crude protein content in the dehulled meal. The application of this procedure also increased the total and non-phytate P content in the dehulled meal. However, it is unclear if dehulling affects P digestibility in the different fractions (i.e., the dehulled meal and the coarse fraction) and if it differs when fed to growing pigs at 2 different BW. Therefore, 2 experiments were conducted to determine the apparent (ATTD) and standardized (STTD) total tract digestibility of P in dehulled CM fed to growing pigs. Diets containing non-dehulled regular canola meal (RCM), and 2 fractions produced using sieve size of 355 µm: a low-fiber high protein fraction (DCM) and a high-fiber low protein fraction (CCM) as the only source of P were fed to growing pigs at 2 different BW. A total of 48 pigs were used for the 2 experiments. In Exp. 1, 24 barrows [(Yorkshire × Landrace) × Duroc] with initial BW of 24.5 ± 1.68 kg were individually housed in metabolism crates and fed the experimental diets for 10 d for total fecal collection. In Exp. 2, 24 barrows with an average initial BW of 73.8 ± 4.93 kg were used; experimental diets and fecal collection procedures were the same as in Exp. 1. Each experiment used 6 replicates per treatment. A P-free diet was used to determine basal endogenous losses of P (139.6 ± 10.7 and 150.89 ± 20.1 mg/kg of DMI for Exp. 1 and 2, respectively). Data were analyzed as a completely randomized design. In Exp. 1, the ATTD and STTD of P were greater ($P < 0.05$) for DCM (42.4% and 46.1%) than for the RCM (32.0% and 35.7%) and CCM (24.5% and 28.4%) diets. In Exp. 2, the ATTD and STTD of P were greater ($P < 0.05$) for DCM (38.7% and 42.8 %) than for the CCM diet (22.6% and 26.8%); whereas the values for RCM diet were intermediate (31.0% and 35.0 %) and not different from the DCM and CCM. In conclusion, dehulling canola meal increased ATTD and STTD of P in growing pigs of different BW; however, there was no effect of BW.

Key words*:* canola meal, dehulling, digestibility, phosphorus, pig.

5.2 INTRODUCTION

Phosphorus is the third most costly nutrient in swine diets, after energy and protein. The efficiency of its utilization is affected by the fact that 60 to 75% of the total P content in seeds is in the poorly available form of phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) (de Lange et al., 1993; Angel et al., 2002; NRC, 2012). Moreover, phytate P can't be digested by swine due to their negligible intestinal phytase activity (Pointillart, 1988; Jongbloed et al., 1992; Kies, 2005; Kim et al., 2017). It is estimated that the solvent-extracted canola meal (CM) from *Brassica napus* L. (*B. napus)* contains around 1.08% total P (as is basis). Approximately 40% is non-phytate P (NRC, 2012; Slominski et al., 2012; Adhikari et al., 2015; Mejicanos et al., 2016). However, in a recent study, Mejicanos et al. (2017b) found that through tail-end dehulling, the fraction 1 (particle size < 250 µm) increased the total P content to 1.27% (as is basis), of which approximately 48% was non-phytate P. Furthermore, a steady decrease in fiber content and a comparative increase in CP content was observed with the reduction in sieve size. Lower fiber and higher total and non-phytate P content in dehulled fractions of CM could improve P utilization by swine. However, it is unclear if the CM fractions obtained through tail-end dehulling will have different P digestibility compared to the parent meal. Higher apparent ileal digestibility (AID) of amino acids (AA) in sows fed CM compared to growing pigs, has been observed (Stein et al., 1999). Additionally, Bikker et al. (2016) found that basal endogenous phosphorus losses (EPL) in pigs increased with increasing feeding level, and BW. However, it is unclear if P digestibility would differ when fed to growing pigs of different BW. Furthermore, to effectively utilize the CM fractions obtained through the dehulling process, concerning dietary P supply, it is critical that its

standardized total tract digestible P content is determined and used in diet formulation (NRC, 2012). Therefore, the objective of this study was to determine the apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of P in the regular canola meal (RCM) and the 2 fractions obtained by tail-end dehulling; i.e., dehulled canola meal (DCM), and coarse canola meal (CCM) fed to growing pigs of two distinct BW.

5.3 MATERIALS AND METHODS

The animal use protocol utilized in the present study was reviewed and approved by the Animal Care Committee of the University of Manitoba. Pigs were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009). The study was conducted at the T. K. Cheung Centre for Animal Science Research.

5.3.1 Dehulling of canola meal

Based on results of tail-end dehulling of CM conducted by Mejicanos et al. (2017b), a sieve size of 355 µm was selected to produce 2 fractions: a low-fiber high-protein fraction (DCM) and a high-fiber low protein fraction (CCM). The 2 fractions were produced at the Canadian International Grains Institute, Winnipeg, MB, Canada, using a plansifter Model MPAR-8HK (Bühler AG, Uzwil, Switzerland). All other ingredients were obtained from the local market. The analyzed chemical composition of the CM used in the current study is presented in Table 5.1. The CM utilized in the present study was produced using the pre-press solvent extraction method.

Table 5.1. Analyzed chemical composition of non-dehulled (RCM), dehulled (DCM)and coarse (CCM) *B. napus* canola meals produced by sieving (%, as-is basis).

	Canola Meal[1]		
Item	RCM	DCM	CCM
GE, kcal/kg	4,323	4,345	4,252
DM	90.40	90.30	90.8
CP	36.20	39.50	35.40
Ether extract	3.50	3.70	2.60
Fiber fractions			
Neutral detergent fiber	26.20	17.80	30.30
Acid detergent fiber	19.40	12.10	21.10
Crude fiber	13.70	11.30	13.70
Total dietary fiber	33.00	24.57	37.25
Non-starch polysaccharides	20.54	16.97	20.99
Rhamnose	0.29	0.26	0.28
Arabinose	4.32	4.14	4.46
Xylose	1.67	1.69	1.86
Mannose	0.38	0.34	0.50
Galactose	1.53	1.38	1.66
Glucose	6.69	5.30	6.94
Uronic acids	5.66	3.86	5.29
Lignin and polyphenols	10.17	6.01	11.38
Glycoprotein (NDICP)	3.60	2.41	6.53
Ash	6.60	7.50	6.90
Total P	1.10	1.27	1.08
Phytate-bound P[2]	0.71	0.79	0.73
Non-phytate P[3]	0.39	0.48	0.35
Ca	0.67	0.60	0.67

[1]Dehulled *B. napus* (DCM, particle size < 355 μm) coarse *B. napus* (CCM, particle size > 355μm).

[2] Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[3] Non-phytate P was calculated as the difference between total P and phytate-bound P.

5.3.2 Animals and housing

A total of 48 barrows [(Yorkshire × Landrace) × Duroc; Genesus, Oakville, MB, Canada] were obtained from Glenlea Swine Research Unit, University of Manitoba. Pigs were subjected to dietary treatments in 2 separate experiments. In Exp. 1, 24 barrows with an initial BW of 24.53 ± 1.68 kg (mean ± SD) were used.

Table 5.2 Composition of experimental diets fed to growing pigs in Exp. 1 (%, as-fed basis).

	Diets			
Ingredients	RCM	DCM	CCM	P-Free
Non-dehulled canola meal	32.00	-	-	-
Dehulled canola meal	-	28.00	-	-
Coarse canola meal	-	-	32.00	-
Corn starch	23.26	26.48	23.00	47.28
Dextrose	10.00	10.00	10.00	-
Sucrose	20.00	20.00	20.00	20.00
Vegetable oil	4.00	4.00	4.00	4.00
Limestone	0.28	0.38	0.26	0.52
Solka floc	-	-	-	4.00
Vit-min premix[1]	1.00	1.00	1.00	1.00
Potassium carbonate	-	-	-	0.40
Magnesium oxide	0.01	0.02	0.01	0.10
Iodized salt	0.50	0.50	0.50	0.50
Pork gelatin	8.00	8.80	8.50	20.00
Lysine-HCl	0.24	0.23	0.22	0.25
DL-Methionine	0.09	0.09	0.09	0.35
L-Threonine	0.12	0.11	0.11	0.31
L-Tryptophan	0.07	0.08	0.06	0.16
L-Histidine	-	-	-	0.19
L-Isoleucine	0.06	0.10	0.06	0.26
L-Valine	0.16	0.06	0.03	0.21
L-Leucine	0.21	0.15	0.16	0.47

[1]Supplied per kg of diet, Vitamins: vitamin A, 1560 IU; vitamin D3, 180 IU; vitamin E, 13.2 IU; vitamin K, 0.6 mg; thiamin (B1): 1.2 mg; riboflavin, 3.0 mg; pantothenate, 6.6 mg; choline, 360 mg; niacin, 12 mg; vitamin B6, 1.2 mg; vitamin B12, 12 μg; biotin, 200 μg; folic acid, 0.36 mg. Minerals: Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg.

When Exp. 1 was completed, the barrows were moved to individual pens (1.7 m^2 per pig) with elevated plastic-coated metal flooring in a temperature-controlled room (20-22 °C) to reach the required BW for Exp. 2. A 16-h light (0600-2200h), and 8-h dark cycle was provided. Experiment 2 used 24 barrows with an initial BW of 73.83 ± 4.93 kg (mean ± SD). Pigs were randomly assigned to the experimental diets and housed individually in metabolic crates (1.8 × 0.6 m) featuring smooth, transparent plexiglass sides to allow visual contact between pigs in adjacent

crates. The floors consisted of plastic-coated expanded metal slatted sheets. The room temperature was set at 22 °C. Water from the city of Winnipeg was provided using nipple drinkers and was available for *ad libitum* intake throughout the Exp. The water quality test indicated 71 ppm of calcium carbonate, and 0.63 ppm total P (Winnipeg.ca, 2017). A 16-h light (0600 to 2200 h) and 8-h dark cycle was used.

Table 5.3 Calculated and analyzed nutrient content of experimental diets fed to growing in Exp. 1 (%, as-fed basis).

Item	Diets			
	RCM	DCM	CCM	P-Free
Calculated composition				
CP [1]	20.00	20.00	20.00	20.00
NE, kcal/kg	2,271	2,295	2,259	2,293
Ca	0.36	0.36	0.36	0.30
Total P	0.36	0.36	0.34	0.00
Potassium	0.50	0.50	0.50	0.40
Magnesium	0.10	0.10	0.10	0.10
Arginine	1.56	1.24	1.31	1.56
Histidine	0.34	0.34	0.36	0.34
Isoleucine	0.51	0.51	0.51	0.51
Leucine	0.99	0.99	0.99	0.99
Lysine	0.98	0.98	0.98	0.98
Methionine	0.35	0.35	0.36	0.53
Met + Cys	0.55	0.55	0.55	0.55
Threonine	0.59	0.59	0.59	0.59
Tryptophan	0.17	0.17	0.17	0.17
Phenylalanine + Tyr	1.63	1.28	1.30	1.63
Valine	0.64	0.64	0.64	0.64
Analyzed composition				
DM	92.00	91.90	92.18	92.18
GE, kcal/kg	4,084	4,156	4,156	4,108
CP	23.24	24.35	23.24	26.33
Ether extract	2.81	4.63	3.68	3.53
Ash	2.75	2.79	2.77	1.41
NDF	11.73	7.93	11.70	4.73
Ca	0.29	0.23	0.22	0.19
Total P	0.35	0.36	0.32	0.00

[1]All diets were formulated to contain the following quantities of the ileal digestible indispensable AA (%): Arg, 0.45; His, 0.34; Ile, 0.51; Leu, 0.99; Lys, 0.98; Met, 0.28; Met + Cys, 0.55; Phe, 0.59; Phe + Tyr, 0.92; Thr, 0.59; Trp, 0.17; Val, 0.64.

5.3.3 Experimental diets

Four mash corn starch-based diets were prepared for each Exp. Two diets contained the dehulled (DCM) and coarse fractions (CCM) obtained from the dehulling process, and one diet consisted of the regular canola meal (RCM) from *B. napus* as the only source of P. In the determination of EPL a P free diet was utilized (Petersen and Stein, 2006; Adhikari et al., 2015). Limestone was added to maintain a constant Ca:total P ratio in all diets. All diets were formulated based on standardized ileal digestible AA and supplemented with vitamins, AA and minerals (except P) to meet or exceed recommended specifications for growing pigs within the 25 to 50 kg (Exp.1) and 75 to 100 kg BW range (Exp. 2; NRC, 2012). Pigs were fed their respective diets at 4% of BW as recorded at the beginning of the Exp. The daily rations were offered in two equal meals at 0800 and 1600 h. Data for the daily feed supplied was summarized, and orts were subtracted to calculate total feed intake. The composition and nutrient contents of the diets used for Exp. 1 are shown in Tables 5.2 and 5.3. The composition and nutrient contents of the diets used for Exp. 2 are presented in Tables 5.4 and 5.5.

5.3.4 Experimental and analytical procedures

Pigs were assigned the experimental diets in a completely randomized design to give 6 replicates per diet. Experiment 1 had 12 d of experimental period divided into 7 d of adaptation to feeding and environment and 5 d of total fecal collection. When Exp. 1 was completed, pigs were fed a corn-soybean meal (SBM) based commercial diet until they reached the target BW (75kg) Experiment 2. had 10 d. of experimental period. The period of adaptation to feeding and environment was reduced to 5 d due to animal welfare concerns derived from the size of the animals and the limited space in the metabolic crates; the last 5 d were for total fecal collection.

Table 5.4 Composition of experimental diets fed to growing pigs in Exp. 2 (%, as-fed basis)

Ingredients	Diet 1 RCM	Diet 2 DCM	Diet 3 CCM	P-Free diet
Non-dehulled canola meal	32.00	-	-	-
Dehulled canola meal	-	28.00	-	-
Coarse canola meal	-	-	32.00	-
Corn starch	24.10	28.05	24.20	48.51
Dextrose	10.00	10.00	10.00	0.00
Sucrose	20.00	20.00	20.00	20.00
Vegetable oil	4.00	4.00	4.00	4.00
Limestone	0.27	0.39	0.27	0.52
Solka floc	-	-	-	4.00
Vit-Min premix[1]	1.00	1.00	1.00	1.00
Potassium carbonate	-	-	-	0.40
Magnesium oxide	0.01	0.02	0.01	0.10
Iodized salt	0.50	0.50	0.50	0.50
Pork gelatin	8.00	8.00	8.00	20.00
Lysine-HCl	0.07	-	-	-
$_{DL}$-Methionine	-	-	-	0.22
$_{L}$-Threonine	0.03	-	-	0.18
$_{L}$-Tryptophan	0.02	0.04	0.02	0.12
$_{L}$ Histidine	-	-	-	0.09
$_{L}$-Isoleucine	-	-	-	0.14
$_{L}$-Leucine	-	-	-	0.22
Total	100.00	100.00	100.00	100.00

[1]Supplied per kg of diet, Vitamins: vitamin A, 2000 IU; vitamin D3, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; thiamin (B1), 1.5 mg; riboflavin, 7.0 mg; pantothenate, 14 mg; choline, 350 mg; niacin, 21 mg; vitamin B6 2.5 mg; vitamin B12: 25 μg; biotin, 70 μg; folic acid, 1 mg. Minerals: Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg.

The total fecal collection was done as described by Ragland et al. (1998) and Woyengo et al. (2010). Briefly, on the morning of the first day of the experimental period (0800 h), each pig received 5 g of ferric oxide (Fisher Scientific, Ontario, Canada) as an indigestible marker in 100 g of feed; the remaining portion of feed was offered after all the marked feed was consumed. The fecal collection was initiated when the marker appeared in feces. On the morning of d 5 of the collection period, pigs were offered 100 g of marked feed containing ferric oxide, as indicated above, and fecal collection ended when the marker appeared in feces. Fecal samples for future

analyses were collected every morning from the trays underneath the metabolic crates, and crate floors into sealed sample plastic bags weighed and then stored frozen at –20°C.

Table 5.5. Calculated and analyzed nutrient content of experimental diets fed to growing pigs in Exp. 2 (%, as-fed basis).

Item	Diet 1 RCM	Diet 2 DCM	Diet 3 CCM	P-Free diet
Calculated Composition				
CP[1]	19.16	18.57	18.82	19.42
NE, kcal/kg	2,293	2,300	2,262	2,279
Ca	0.36	0.36	0.36	0.298
Total P	0.36	0.36	0.34	0.00
Potassium	0.50	0.50	0.50	0.40
Magnesium	1.00	1.00	1.00	1.00
Arginine	1.24	1.25	1.24	1.56
Histidine	0.36	0.35	0.36	0.25
Isoleucine	0.45	0.40	0.45	0.39
Leucine	0.82	0.82	0.82	0.74
Lysine	0.85	0.77	0.75	0.78
Methionine	0.26	0.26	0.26	0.4
Met + Cys	0.46	0.46	0.46	0.42
Threonine	0.51	0.47	0.48	0.46
Tryptophan	0.13	0.13	0.13	0.13
Phenylalanine + Tyr	1.28	1.23	1.26	1.63
Valine	0.60	0.56	0.60	0.44
Analyzed composition				
DM	91.83	91.94	92.13	92.08
GE, kcal/kg	4,108	4,108	4,132	4,251
CP	19.06	20.33	20.47	21.33
Ether extract	4.78	5.26	4.94	4.24
Ash	2.46	2.49	2.36	1.46
NDF	9.85	5.36	8.78	2.25
Ca	0.29	0.25	0.25	0.12
Total P	0.36	0.37	0.35	0.011

[1]All diets were formulated to contain the following quantities of the ileal digestible indispensable AA (g/kg): Arg, 0.33; His, 0.25; Ile, 0.39; Leu, 0.74; Lys, 0.73; Met, 0.21; Met + Cys, 0.42; Phe, 0.44; Phe + Tyr, 0.69; Thr, 0.46; Trp, 0.13; Val, 0.48.

In preparation for analysis, fecal samples were dried in a forced-air oven at 60 °C for 5 d. Dried samples were pooled for each pig and ground using a heavy-duty blender (model CB15, Waring Commercial, Torrington, CT), then a subsample was obtained after thoroughly mixing the ground feces for chemical analysis. Diets and CM were finely ground before analysis using a Foss sample preparation Cyclotec^{TM} 1093 mill (Foss Allé 1, DK-3400 Hilleroed, Denmark). Experimental diets and CM were subject to CP (N×6.25) analysis using an N analyzer, model TruSpec N (Leco Corp., St. Joseph, MI, USA). Standard AOAC (2005) procedures were used for DM (method 930.15), ether extract (EE; method 2003.06), and ash determination (method 942.05). Phytate-P was determined using the method described by Haug and Lantzsch (1983). Dietary fiber was determined by a combination of neutral detergent fiber (NDF) and detergent-soluble non-starch polysaccharide (NSP) measurements and was calculated as the sum of NDF and detergent soluble NSP (Slominski et al., 1994). The NDF was determined using an Ankom fiber analyzer (Ankom Technology, Macedon, NY) and according to AOAC (2005) method 2002.04. Total NSP were determined by gas-liquid chromatography (component neutral sugars) using an SP-2340 column and Varian CP3380 gas chromatograph (Varian Inc., Palo Alto, CA) and colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) and the procedure described by Englyst and Cummings (Englyst and Cummings, 1984; Englyst and Cummings, 1988) with some modifications (Slominski and Campbell, 1990). The content of NSP was measured in both the meals and the NDF residues. Neutral detergent soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue, and total dietary fiber was determined by the summation of NDF and NDF-soluble NSP. The contents of CP (N × 6.25) and ash in NDF residue were also measured. The value for lignin and associated polyphenols was calculated by finding the difference [NDF - (NSP + protein + ash)] (Slominski et al., 1994). Diets, fecal samples, and CM for Ca and P analysis were ash at 600^{o}C for 12 h, digested according to the AOAC (2005)

method 985.01, and determined using a Varian Inductive Coupled Plasma Mass Spectrometer (Varian Inc., Palo Alto, CA). The GE of the diets was measured using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL) calibrated using benzoic acid as a standard. The concentration of phytate-bound P in the CM was calculated as 28.2 % of analyzed phytate (Tran and Sauvant, 2004). Non-phytate P was calculated by subtracting phytate-bound P from total P.

5.3.5 Calculations and statistical analysis

The ATTD (%) of P in each of the 3 P-containing diets was calculated using the following equation:

$$ATTD = [(Pi – Pf)/Pi] \times 100$$

Where ATTD is apparent total tract digestibility; Pi is the total P intake (g) during the 5 d of fecal collection of each experimental period; Pf is the total fecal output of P originating from the feed intake during the collection period (g; Petersen and Stein, 2006). The ATTD of Ca was also calculated using the same equation.

The EPL were expressed relative to the DMI of the animals and calculated from pigs fed the P-free diet using the following equation (Petersen and Stein, 2006):

$$\text{Basal EPL (mg/kg of DM)} = [(Pf/Fi) \times 1{,}000 \times 1{,}000],$$

Where Fi is the total feed (g of DM) intake during the 5-d collection period. The endogenous losses for each of the P-containing diets were calculated by multiplying the calculated EPL per kilogram of DMI by the DMI of each pig for the 5-d collection period. The endogenous losses were then subtracted from the total fecal output of P, and the total amount of fecal P was

partitioned into P originating from endogenous losses and P originating from undigested dietary P.

The standardized total tract digestibility (STTD) of P for each ingredient was calculated by correcting ATTD values for the EPL, as described by Petersen and Stein (2006) using the following equation:

STTD (%) = {[Pi – (Pf – EPL)])/Pi}

Data were analyzed using the Mixed procedure of SAS (SAS software 9.4, SAS Institute, 2013) as a completely randomized design. Each pig was considered as an experimental unit. Means were separated using Tukey's honestly significant difference test. The PROC UNIVARIATE of SAS was used to confirm that variances were homogeneous and to analyze for outliers. Outliers were removed. All statements of significance are based on $P < 0.05$, and trends were observed at $0.05 < P \leq 0.10$.

Table 5.6. Basal endogenous phosphorus losses (EPL) in pigs fed P free diets.

	Experiment 1		Experiment 2	
Item	Mean	SE	Mean	SE
ATFI[1], g DM	5202	196	10835	736
P output[2], g	0.72	0.036	1.58	0.17
Average EPL, mg/kg DM	139.60	10.7	150.89	20.1

[1]Average total feed intake during the 5-d collection period (n=6)

[2]Total fecal output of P during the 5-d collection period (n=6)

5.4 RESULTS

5.4.1 Chemical composition of dehulled canola meal

The analyzed chemical compositions of the CM and fractions used in the formulation of the experimental diets are presented in Table 5.1. The total P was 1.1%, 1.27% and 1.08%; the non-phytate P was 0.39%, 0.48% and 0.35%; the phytate-bound P was 0.71%, 0.79% and 0.73%; for RCM, DCM and CCM, respectively. Whereas CP was 36.2%, 39.5% and 35.4%; total dietary fiber was 33.0%, 24.6% and 37.3%, for RCM, DCM and CCM, respectively.

5.4.2 Experiment 1

All pigs remained healthy and readily consumed their assigned diet throughout the Exp. The analyzed values for DM, GE, CP, Ash, and P were similar for all diets. However, NDF was lower for diets containing DCM compared to diets containing RCM and CCM (7.93% vs. 11.73% and 11.70%, respectively). Ether extract content was higher for diets containing DCM compared to diets with CCM and RCM (4.63% vs. 3.68% and 2.81%, respectively). Analyzed values for Ca were higher for diets containing RCM compared to diets containing DCM and CCM (0.29% vs. 0.23% and 0.22%, respectively). The determined basal EPL value was 139.6 ± 11 mg/kg of DMI (Table 5.6).

There were no differences ($P > 0.10$) among treatments for total feed and P intake (Table 5.7). However, Ca intake was greater ($P < 0.001$) for diets containing RCM than for diets containing DCM and CCM. Feeding DCM reduced ($P < 0.001$) total fecal output, reduced ($P < 0.05$) Ca output; and tended to reduce ($P < 0.10$) P output, compared to feeding RCM and CCM. Dehulled CM had greater ATTD of P ($P < 0.001$) compared to RCM and CCM (42.4% vs. 32.0% and 24.5%, respectively). Likewise, STTD of P was greater ($P < 0.001$) for DCM compared to RCM and CCM (46.1% vs. 35.7% and 28.4%, respectively). The ATTD of Ca was less ($P <0.001$)

for CCM compared to RCM and DCM (36.9% vs. 51.1% and 54.8%, respectively; Table 5.7). However, as limestone was added to the diets, the digestibility of Ca corresponds to a mix of CM and other sources.

Table 5.7 Apparent total tract digestibility (ATTD) P and Ca and standardized total tract digestibility (STTD) of P by growing pigs fed diets containing non-dehulled (RCM), dehulled (DCM) and coarse (CCM) canola meal from *B. napus* in Exp. 1.

	Canola Meal			SEM	*P*-value
Item	RCM	DCM	CCM		
Average BW (kg)	25.33	26.88	27.18		
Feed intake, g/d (as is)					
Feed intake	1,120	1,143	1,139	56.23	0.954
Ca intake	3.28[a]	2.61[b]	2.46[b]	0.126	0.001
P intake	3.88	4.00	3.76	0.187	0.607
Fecal output (DM basis)					
Total feces, g/d	92[a]	59[b]	103[a]	3.3	< 0.001
Ca in feces, %	1.71[b]	2.00[a]	1.50[b]	0.065	< 0.001
P in feces, %	2.84[b]	3.92[a]	2.71[b]	0.099	< 0.001
Ca output, g/d	1.60[a]	1.18[b]	1.55[a]	0.090	0.012
P output, g/d	2.7	2.3	2.7	0.15	0.090
Digestibility, %					
ATTD of Ca	51.1[a]	54.8[a]	36.9[b]	1.77	< 0.001
ATTD of P	32.0[b]	42.4[a]	24.5[c]	1.29	< 0.001
STTD[1] of P	35.7[b]	46.1[a]	28.4[c]	1.29	< 0.001

[a,b,c] Data within a row without a common letter are different ($P < 0.05$).

[1] Values for the STTD of P were calculated by correcting the ATTD values for the basal endogenous loss of P. The basal endogenous loss of P was estimated in pigs fed the P-free diet at 139.6 ± 11 mg/kg of DMI.

5.4.3 Experiment 2

The composition, calculated and analyzed nutrient contents of the experimental diets used in Exp. 2 are presented in Tables 5.4 and 5.5. The analyzed values for DM, GE, CP, Ash, and P

were similar for all the diets. However, NDF was lower for diets containing DCM compared to diets containing RCM and CCM (5.36% vs. 9.85% and 8.78%, respectively). Analyzed values for ether extract were higher for diets containing DCM compared to diets containing RCM and CCM (5.26% vs. 4.78% and 4.94%, respectively). Whereas, values for Ca were higher for diets containing RCM compared to diets containing DCM and CCM (0.29% vs. 0.25% and 0.25%, respectively). The determined basal EPL value was 150.89 ± 20 mg/kg of DMI (Table 5.6).

Table 5.8. Apparent total tract digestibility (ATTD) of P and Ca and standardized total tract digestibility (STTD) of P by growing pigs fed diets containing non-dehulled (RCM), dehulled (DCM) and coarse (CCM) from *B. napus* in Exp. 2.

	Canola Meal				
Item	RCM	DCM	CCM	SEM	*P*-value
Average BW (kg)	73.02	74.03	73.53		
Feed intake, g/d (as is)					
Feed intake	3,032	3,115	3,034	44.94	0.361
Ca intake	8.64[a]	6.15[b]	8.94[a]	0.112	< 0.001
P intake	10.35[b]	11.02[a]	10.11[b]	0.156	0.004
Fecal output (DM basis)					
Total feces, g/d	256[a]	158[b]	277[a]	11.551	<0.001
Ca in feces, %	1.89[b]	2.79[a]	1.82[b]	0.076	<0.001
P in feces, %	2.80[b]	4.27[a]	2.82[b]	0.067	<0.001
Ca output, g/d	4.87	4.45	5.04	0.357	0.511
P output, g/d	7.16	6.76	7.82	0.405	0.222
Digestibility, %					
ATTD of Ca	43.8[a]	27.6[b]	43.6[a]	4.89	0.049
ATTD of P	31.0[ab]	38.7[a]	22.6[b]	3.35	0.016
STTD[1] of P	35.0[ab]	42.8[a]	26.8[b]	3.35	0.018

[a,b] Data within a row without a common letter are different ($P < 0.05$).

[1] Values for the STTD of P were calculated by correcting the ATTD values for the basal endogenous loss of P. The basal endogenous loss of P was estimated in pigs fed the P-free diet at 150.89 ± 20 mg/kg of DMI.

There were no differences ($P > 0.10$) among treatments for total feed intake, Ca, and P output (Table 5.8). However, P intake was greater ($P < 0.01$) for diets containing DCM compared to diets containing RCM and CCM. Whereas Ca intake was greater ($P < 0.01$) for diets containing RCM and CCM compared to diets containing DCM. Feeding DCM resulted in less ($P < 0.001$) total fecal output than feeding RCM and CCM. However, feeding DCM resulted in higher ($P < 0.001$) concentration of Ca and P in feces compared to feeding RCM and CCM diets. No differences ($P > 0.10$) were observed for P and Ca output among diets.

The ATTD of Ca and P and the STTD of P are shown in Table 5.8. The ATTD and STTD of P were greater ($P < 0.05$) in DCM than in CCM. However, values for RCM were intermediate and not different from those of either DCM and CCM ($P > 0.05$). The ATTD of Ca in diets containing DCM was less ($P < 0.05$) than in diets containing RCM and CCM; but values of ATTD of Ca in RCM and CCM were not different ($P > 0.10$). However, as in Exp. 1, limestone was added to the diets. Therefore, the digestibility of Ca corresponds to a mix of CM and other sources.

5.5 DISCUSSION

Efficient use of P in swine diets is necessary to minimize feed cost and to decrease the potential environmental impact associated with P accumulation in soils and surface water bodies due to land application of swine manure (Baxter et al., 2003; Adhikari, 2013; Environment and Climate Change Canada, 2017). Several strategies have been developed to maximize the use of P in swine diets, and moreover, minimize P excretion, including phase feeding (Han et al., 2000), the use of microbial phytase (Jongbloed et al., 1992; Kies, 2005; Adhikari et al., 2016), replacing conventional ingredients with high available P varieties (Baxter et al., 2003), and the use of STTD

P in formulating swine diets (Petersen and Stein, 2006; NRC, 2012). Therefore, it is crucial to determine the STTD of P in different feed ingredients.

Dehulling of canola has been investigated to reduce fiber content, increase protein content, and enhance the nutritive value of the meal (Thakor et al., 1995; Hansen et al., 2017; Mejicanos et al., 2017b). Front-end dehulling (i.e., removal of hulls before oil extraction) and tail-end dehulling (i.e., removal of hulls from the meal after oil extraction), improve the quality of the CM (Mejicanos et al., 2017b). However, factors are preventing the crushing industry from implementing the suggested technologies. Among them: losses of oil during the front-end dehulling process, the excessive fineness of the dehulled meal, and the consequent difficulties with percolation of the miscella (Khajali and Slominski, 2012). Kracht et al. (2004) improved the nutritive value of CM using front-end dehulling, with a notable reduction in CF, NDF, and ADF contents (38, 28, and 25%, respectively); moreover, increasing CP and sugar by 7 and 14%, respectively. McCurdy and March (1992) defined a tail-end dehulling process for solvent-extracted CM, in which the meal was ground using a disc mill and sieved using a US standard mesh 70, producing a low fiber fraction. More recently, in Norway, a tail-end dehulling method has been developed, which combines ball milling with sieving technology, achieving high separation of hulls and endosperm (Hansen et al., 2017). Air classification has been utilized effectively to achieve tail-end dehulling of CM; this method is based on the difference in particle size and density between hulls and embryo (Beltranena and Zijlstra, 2011). Other factors affecting implementation of dehulling in CM include the high cost associated with air classification, and the variations in the methods currently suggested for tail-end dehulling (McCurdy and March, 1992; Kracht et al., 2004; Beltranena and Zijlstra, 2011; Hansen et al., 2017; Mejicanos et al., 2017b). Therefore, dehulled CM is not available at a commercial level. Moreover, there is no information available regarding ATTD and

STTD of P in dehulled CM. The information available considers mainly regular CM from *B. napus* and *B. juncea* (Adhikari et al., 2015; Maison et al., 2015a), and more recently, ATTD and STTD of P in high protein CM (She et al., 2017).

In the present study, it was determined that compared to RCM, DCM had 32% less NDF, 38% less ADF, 17% less NSP, 41% less lignin, and polyphenols and 26% less total dietary fiber. However, DCM had 9% more CP, 16% more total P, 23% more non-phytate P, 11% more phytate-bound P. Phytate-bound P levels in DCM are comparable to those found in high protein CM (Parr et al., 2015). Levels of total and phytate-bound P in RCM and DCM determined in the present experiment are consistent with values reported by Kracht et al. (2004). However, when compared to high protein CM and RCM, DCM had higher levels of non-phytate P (0.26 and 0.39 vs. 0.48%, respectively). Furthermore, high protein CM has almost twice the levels of glucosinolates (GSL) than regular CM (14.2 to 15.5 vs. 8.7 µmol/g, respectively; Parr et al., 2015). Higher GSL content in dehulled CM compared to its parent meal (9.6 vs. 9.2) has been observed (Mejicanos et al., 2017b). The DCM obtained by sieving in the present study had lower CP content than the high protein CM evaluated by Parr et al. (2015). However, they have similar values of GE, EE, NDF, and ADF. When compared to RCM, CCM had a reduction in CP of 2%, total P of 2%, non-phytate P of 10%. However, NDF increased by 15%, ADF by 9%, NSP by 2.2%, and lignin and polyphenols by 12%.

The two main concerns regarding tail-end dehulling are the yield of dehulled meal and marketability of the coarse fraction containing the hulls. In that respect, the tail-end dehulling procedure used in the present study was effective in producing a high nutrient density meal. Moreover, the yield obtained utilizing sieving technology (particle size < 355 µm) in CM from 8 Canadian processing facilities, was an average of 25.9% (Mejicanos et al., 2017b). The

combination of ball milling and sieving (sieve size 150 µm) can yield 42% dehulled meal (Hansen et al., 2017). However, as the yield of the dehulled meal increases, the fiber content of the fraction comprising the hulls also increases. In a study investigating CM fractionation, it was observed that total dietary fiber rose to 35.5% in the particle size of 355-600 µm, compared to 30.1% and 21.4% in the parent meal and the dehulled fraction Fine 1, respectively (Mejicanos et al., 2017b).

Regarding the marketability of the meals, regulations established by the Canadian Oilseed Processors Association, COPA (2016) indicated that the combined values for protein and fat in CM could not be below 37% and stipulate a maximum of 12% for crude fiber. In the present study, the combined values for CP and fat were: 39.70%, 37.98%, and 43.24% for RCM, CCM, and DCM, respectively. Moreover, despite crude fiber values in the parent meal being above the requirements for overseas export (13.7%), dehulling reduced crude fiber content in the DCM to 11.3%, which is within limits established by COPA.

Calcium intake was different between RCM, DCM, and CCM diets for both experiments. As Ca intake increased, ATTD of Ca increased, which is consistent with observations by González-Vega et al. (2013), indicating that the ATTD of Ca increased with increasing Ca level in the diets. The RCM, DCM, and CCM were the sole source of P in the experimental diets. In Exp. 1, the average analyzed total P in the diets was 0.34%. However, NRC (2012) recommends 0.60 %; likewise, the average analyzed Ca in the diets was 0.25%. However, NRC (2012) recommends 0.70%. It has been indicated that lower Ca:P ratios favor both Ca and P retention, and the optimal Ca:P ratio is valid only when the dietary levels of these two elements are supplied in the correct amount. Vipperman et al. (1974) found higher plasma P concentrations in pigs fed diets containing 0.25% Ca and 0.50% P, compared to pigs fed diets containing 0.50% Ca and 0.50% P; as the animal can draw from bones during periods of dietary shortage (Harrison and

Fraser, 1960; Campbell and Douglas, 1965). In the case of Exp. 2, the average analyzed Ca, and P were 0.26 % and 0.36 % %, respectively; however, NRC (2012) recommends 0.52% and 0.47%, respectively. It has been indicated that the Ca:P ratio is less critical if the diets contain excess P (Prince et al., 1984) and that a narrower Ca:P ratio could result in more efficient P utilization (Hancock et al., 1986; Jongbloed, 1987). Variations in P and Ca intake in research on ATTD and STTD of P in canola-rapeseed from different sources has been shown by Maison et al. (2015a).

When compared total fecal output on the pigs fed diets containing DCM and RCM, a reduction of 36 and 38% for Exp. 1 and Exp. 2, respectively, were observed. The decrease in fecal output can be attributed to higher nutrient density and lower fiber content in DCM compared to RCM. Using ingredients with low fiber content in place of high fiber ingredients has been shown to reduce manure volume considerable (Grandhi, 2001).

The ATTD of P in Exp. 1 and Exp. 2 were, for diets containing RCM, 32 and 31%, respectively; these values are in accordance with those indicated by NRC (2012) and Adhikari et al. (2015; 28% ± 4 and 30%, respectively), nevertheless higher than the values (24% ± 3) found by Rodehutscord et al. (1994). However, dehulling increased the ATTD of P in DCM compared to RCM from 32 to 42%, and from 31 to 39% for Exp. 1 and Exp. 2, respectively. Nevertheless, when comparing CCM to RCM, a reduction on ATTD of P from 32 to 25%, and from 31 to 23 % for Exp. 1 and Exp. 2, respectively, were observed. The increase in ATTD of P in DCM could be due to its higher non-phytate P content, which is known to be highly digestible (Jongbloed, 1987; Ravindran et al., 2000). Higher fiber content on CCM could be associated with lower ATTD of P. However, a P digestibility study in CM from *B. napus* and *B. juncea* found no differences in ATTD between the 2 meals despite distinct NDF content (24.2 vs. 16%, respectively). Still, the two meals had comparable phytate and non-phytate P contents (Adhikari et al., 2015). Furthermore, no effect

of fiber on ATTD was observed in a study on the effects of rapeseed meal fiber content on P digestibility in growing pigs (Bournazel et al., 2018). Diets in the present Exp. were formulated according to the total P content of the meal. However, non-phytate P content was higher for DCM compared to RCM and CCM (0.48 vs. 0.39, and 0.35%, respectively), which could be the main reason for differences in P digestibility, rather than fiber content of the meals.

The basal EPL found in the present study using the P-free diet were 139.6 ± 11 mg/kg DMI and 150.9 ± 20 mg/kg DMI for Exp. 1 and Exp. 2, respectively. The basal EPL was in accordance with values described by Petersen and Stein (2006); Almeida and Stein (2010); NRC (2012); Sulabo and Stein (2013); and Adhikari et al. (2015). However, EPL found in the present experiment was lower than the 499 mg/kg DMI value observed in gestating sows with initial BW of 201 kg (Bikker et al., 2016), which indicates comparable EPL in growing and finishing pigs, however, larger EPL would be observed in bigger pigs such as sows.

The STTD of P in Exp. 1 and Exp. 2 were: for diets containing RCM, 35.7, and 35.0%, respectively. The observed values are consistent with those reported by NRC (2012; 32% ± 6 SD). However, the STTD of P of RCM was lower than the values reported by Maison et al. (2015a) and She et al. (2017; 48.2 and 45.2%, respectively). It has been indicated that dietary CP level should be considered in P digestibility studies (Xue et al., 2017), as the ileal digestion of P could be limited by a deficiency in AA intake. However, the diets fed in the present study were balanced following ileal digestible AA requirements. Therefore, the observed ATTD and STTD of P were not affected by AA deficiencies.

The STTD of P for diets containing DCM were 46.1 and 42.8% for Exp. 1 and Exp. 2, respectively. These results are in accordance with a digestibility study feeding high protein CM to

growing pigs by She et al. (2017), indicating STTD of P of 48.78%. Higher STDD of P observed in DCM compared to RCM and CCM could be attributed to the chemical composition of the DCM which contains greater amounts of non-phytate P rather than lower fiber content (Adhikari et al., 2015; Bournazel et al., 2018). A reduction in STTD of P in CCM compared to DCM for Exp. 1 and Exp. 2, respectively, was observed. The reduction in P digestibility can be attributed to higher non-phytate P in DCM compared to CCM (0.48 vs. 0.35%, respectively). This is consistent with observations by Bournazel et al. (2018) who studied the effects of fiber content in rapeseed meal on P and Ca digestibility, and found that when using front-end dehulled rapeseed meal instead of whole rapeseed meal, or adding hulls, fiber content had no effect on ATTD of P. However, Partrige, (1978) observed a reduction in the apparent absorption of P when cellulose was included at high levels (90 g/kg), which suggests that the type of fiber can affect P digestibility.

In conclusion, tail-end dehulling of CM improves the ATTD and STTD of P in CM fed to growing pigs of two distinctive BW. The results from this study also indicate that feeding high nutrient density DCM to growing and finishing pigs would reduce manure volume and P discharge into the environment, improving P utilization in swine diets.

CHAPTER SIX

MANUSCRIPT III

Effect of tail-end dehulling of canola meal on apparent and standardized ileal digestibility of amino acids when fed to growing pigs

G. A. Mejicanos, and C. M. Nyachoti

Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada

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6.1 ABSTRACT

The aim was to determine the effect of tail-end dehulling (dehulling after oil extraction) of canola meal (CM) on apparent (AID) and standardized (SID) ileal digestibility of amino acids (AA) when fed to growing pigs. Three ileal cannulated barrows (initial BW 58 ± 3.6 kg) were assigned to the 3 experimental diets in a replicated 3×3 Latin square design using 6 periods to provide 6 replicates per treatment. The use of sieve size 355 µm resulted in the production of 2 CM fractions, a dehulled fraction (DCM), and a coarse fraction (CCM). Diets consisted of a cornstarch-based diet with either regular canola meal (RCM), DCM, or CCM as the only source of protein. All diets contained titanium dioxide (0.3%) as an indigestible marker. In general, there was no effect ($P > 0.10$) of dehulling on the AID of most AA. However, AID of Phe was higher ($P < 0.05$) in RCM, compared to DCM and CCM. The AID of Thr was greater ($P < 0.05$) in RCM and DCM compared to CCM. However, the AID of Ile and Leu was higher ($P < 0.05$) in RCM and CCM compared to DCM. The SID of indispensable AA was not affected ($P > 0.10$) by dehulling. However, the SID of Phe was greater for RCM ($P < 0.05$) compared to DCM and CCM. Whereas, SID for Thr was higher in RCM and DCM compared to CCM. By removing the fibrous component, dehulling increased ($P < 0.05$) the standardized ileal digestible AA content of DCM compared to RCM by an average of 9%. The standardized ileal digestible His and Lys contents were similar between RCM and CCM, whereas values for digestible Arg, Leu, Phe, and Thr contents were lower ($P < 0.05$) for CCM than for RCM and DCM. In conclusion, the results indicate that for most AA, the AID and SID in CM were not affected by dehulling. However, the content of ileal digestible AA can be increased with tail-end dehulling of CM.

Keywords: amino acid, canola meal, dehulling, digestibility, pig.

6.2 INTRODUCTION

The meal obtained from the crushing of canola seeds is used as a protein source for animal feeding, and it constitutes the second-largest protein supplement after soybean meal (SBM) (Canola Council of Canada, 2015). In 2016, the global rapeseed/canola meal consumption was 38.1 million metric tonnes, while soybean meal consumption was 225.1 million metric tonnes (Soystats, 2017). However, compared to SBM, canola meal (CM) has a lower concentration of most amino acids (AA), except Met and Cys (González-Vega and Stein, 2012; NRC, 2012; Mejicanos et al., 2016), and lower and less consistent AA digestibility than SBM (Khajali and Slominski, 2012; Liu et al., 2014a). Front-end dehulling (i.e., dehulling preceding oil extraction) can reduce total fiber and ADF content of the dehulled meal by 40 and 35%, respectively; additionally, the AA content can increase by 11% (Kracht et al., 1999). Tail end dehulling (i.e., dehulling after oil extraction) can increase crude protein (CP) by 13%, ether extract (EE) by 37%, total P by 21%, non-phytate P by 24.5%, and can decrease total dietary fiber by 29%, NDF by 37.3%, lignin and polyphenols by 53.4%, and non-starch polysaccharides (NSP) by 12.4% (Mejicanos et al., 2017b). Compared to CM, SBM has higher standardized ileal digestibility (SID) for most indispensable AA except for Arg and Trp (González-Vega and Stein, 2012). Air classification has been studied to achieve tail end dehulling; the system is based on differences in density between cotyledon and seed hull. The use of air streams partially separated these seed components based on size and density, to produce a low-fiber, light-particle fraction, and a high-fiber, heavy-particle fraction. Digestibility of AA of dehulled CM obtained using air classification of *B. napus* and *B. juncea* has been studied, and results indicated higher SID of protein and AA in the high-protein fraction compared to the high-fiber fraction. However, no differences in SID of AA among the dehulled high-protein fraction and the corresponding parent meal were observed

(Zhou et al., 2015). Therefore, the purpose of the present study was to determine the effect of tail-end dehulling of CM using sieving technology, on the apparent ileal digestibility (AID) and SID of AA when fed to growing pigs.

6.3 MATERIALES AND METHODS

6.3.1 *Animal care*

The animal use protocol utilized in the present study was reviewed and approved by the Animal Care Committee of the University of Manitoba. The pigs used in the experiment were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The study was conducted at the T. K. Cheung Centre for Animal Science Research.

6.3.2 Materials

Regular canola meal (RCM) was produced using the pre-press solvent extraction method and was obtained from the Bunge crushing plant, Altona, MB, Canada. The dehulled (DCM), and coarse (CCM) meal fractions were produced at the Canadian International Grains Institute, Winnipeg, Manitoba, Canada using a Plansifter, Model MPAR-8HK, Bühler AG, CH-9240, Uzwil. The use of sieve size 355 µm resulted in the production of 2 CM fractions, a dehulled canola meal (DCM), and a coarse fraction (CCM). The analyzed chemical composition of the meals used in the present experiment is shown in Table 6.1.

Table 6.1 Analyzed chemical composition of regular *B. napus* and its corresponding fractions produced by sieving (%, as-fed basis).

Item	Canola Meal[1]		
	RCM	DCM	CCM
DM	90.40	90.30	90.80
Ash	6.60	7.50	6.90
CP	36.20	39.50	35.40
EE	3.53	3.74	2.58
Fiber fractions			
NDF	26.20	17.80	30.30
ADF	19.40	12.10	21.10
Total dietary fiber	33.01	24.57	37.25
Non-starch polysaccharides	20.50	17.00	21.00
Lignin and polyphenols	10.17	6.01	11.38
Glycoprotein (NDICP)	3.6	2.41	6.53
Total P	1.10	1.27	1.08
Non-phytate P	0.39	0.48	0.35
Ca	0.67	0.60	0.67
Indispensable AA			
Arg	2.10	2.49	2.03
His	1.16	1.33	1.15
Ile	1.25	1.41	1.23
Leu	2.51	2.92	2.48
Lys	2.04	2.29	1.96
Met	0.47	0.48	0.48
Phe	1.44	1.63	1.39
Thr	1.59	1.80	1.58
Trp	0.42	0.43	0.42
Val	1.55	1.74	1.58
Dispensable AA			
Ala	1.73	2.03	1.72
Asp	2.78	3.17	2.72
Cys	0.59	0.78	0.67
Glu	6.57	7.61	6.39
Gly	1.80	2.09	1.78
Pro	2.42	2.61	2.25
Ser	1.80	2.05	1.76
Tyr	0.99	1.10	0.97

[1] RCM (regular canola meal); DCM (dehulled canola meal, particle size < 355 μm); CCM (coarse canola meal, particle size > 355 μm) .

6.3.3 Animals and housing

Three growing pigs [(Yorkshire-Landrace) × Duroc; Genesus, Oakville, MB, Canada] obtained from the University of Manitoba Glenlea Swine Research Unit were randomly assigned to the experimental diets according to a replicated 3×3 Latin square design using 6 periods (in lieu of 6 pigs) to provide 6 replicates per diet. Pigs had an initial average BW of 58 ± 3.6 kg. When pigs were approximately 25 kg BW, T-type cannulas were implanted in the distal ileum, following the procedure described by Nyachoti et al. (2002). However, pigs were used for another experiment before being assigned to the present study. Therefore a 2-week time-lag was applied before the commencement of the study. To prevent infections, reduce irritation and the risk of inflammation, the area where the cannula was fitted was cleaned every day using warm water and a mild detergent, then dried using a paper towel; at the end of the cleaning, zinc oxide-lanolin-based cream was applied around the cannula to reduce irritation. Pigs were housed individually in pens (1.7 m^2 per pig) with elevated plastic-coated metal flooring in a temperature-controlled room (20-22 ºC). A 16-h light (0600-2200 h) and 8-h dark cycle were provided.

6.3.4 Diets

Three diets containing RCM, DCM, and CCM as the only source of protein, were prepared. In the formulation of the diets, no fat was used, as dietary fat has been identified as a factor modifying endogenous nitrogen excretion (de Lange et al., 1989). However, the EE contents of the diets were 1.50, 1.89, and 1.71%, respectively. Pigs were fed a casein-based diet prior to the experiment, to estimate the non-specific endogenous loss of AA at the distal ileum (EAL). Vitamins and minerals were supplied in the diets to meet or exceed NRC (2012) requirements. All diets contained 0.3% of titanium dioxide (TiO_2) as an indigestible marker. The composition and

calculated nutrient content of the experimental diets are shown in Table 6.2. The analyzed nutrient content of the experimental diets is shown in Table 6.3.

Table 6.2 Composition and calculated nutrient content of cornstarch canola meal-based diets fed to growing pigs (%, as-fed basis).

Composition of diets	Diet[3]		
Item	RCM	DCM	CCM
Cornstarch	47.0	51.0	46.0
Regular *B. napus*	50.0		
Dehulled *B. napus*		46.0	
Coarse *B. napus*			51.0
Calcium carbonate	0.4	0.4	0.4
Monocalcium phosphate	1.0	1.0	1.0
Vitamin premix[1]	0.5	0.5	0.5
Mineral premix[2]	0.5	0.5	0.5
Iodized salt	0.3	0.3	0.3
Marker (Titanium oxide)	0.3	0.3	0.3
Total	100	100	100
Calculated nutrient content			
CP	18.10	18.17	18.05
NE (MJ/kg)	8.98	9.2	8.96
Ca	0.66	0.60	0.67
Total P	0.77	0.80	0.76
AA (total)			
Met + cys	0.57	0.58	0.54
Met	0.24	0.22	0.24
Lys	0.98	1.05	1.04
Thr	0.79	0.83	0.81
Trp	0.27	0.25	0.27
Val	0.79	0.64	0.80
Ile	0.62	0.65	0.64
Leu	1.24	1.34	1.28
Arg	1.02	1.15	1.07
His	0.58	0.61	0.59
Phe	0.70	0.75	0.73
Phe + tyr	1.18	1.26	1.24

[1]Supplied per kg of diet: vitamin A, 2000 IU; vitamin D3, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; Thiamin (B1): 1.5 mg; Riboflavin, 7.0 mg; Pantothenate: 14 mg; Choline, 350 mg Niacin, 21 mg; vitamin B6 2.5 mg; vitamin B12: 20 μg; Biotin, 70 μg; Folic Acid, 1 mg.

[2]Supplied per kg of diet: Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg. [3]Diet containing: RCM (regular canola meal); DCM (dehulled canola meal); CCM (coarse canola meal).

6.3.5 Feeding and sampling

Water and feed were provided using nipple drinkers and metal feed troughs. Pigs were fed 4% BW (corresponding to the calculated feed intake + wastage when allowed feed ad libitum) with the daily feed allowance offered in two equal portions at 0800 and 1600 h. Water was available *ad libitum* throughout the experiment. Individual pig weights were recorded at the beginning of each period to adjust the feed allowance. Each experimental period lasted 7 d, the first 5 d were for adaptation to the experimental diets, while the last two days were the collection period. During the collection period, digesta were collected through the cannulas by removing the caps and attaching plastic bags with hose clamps. Digesta were collected for a total of 12 h in the collection period. Collection bags contained 10 ml of 10% (v/v) formic acid to lessen bacterial activity. Digesta samples were stored in -20 ºC freezer.

6.3.6. Analytical procedure

Ileal digesta samples were thawed and pooled for each pig and period, then homogenized using a heavy-duty Blender LBC 15 Model CB15 (Waring Commercial, Torrington, Connecticut, USA. Serial No 575095). Feed and digesta samples of the 3 CM used in the experiment were ground to pass through a 1 mm screen using a Foss Sample preparation Cyclotec™ 1093 mill (Foss Allé l, DK-3400 Hilleroed, Denmark). Meals and diets were subject to dry matter (DM), CP, gross energy (GE), calcium (Ca), P, and TiO_2 analyses. Crude protein (Nx6.25) analysis was performed using a nitrogen analyzer, model TruSpec N (Leco Corp., St. Joseph, MI, USA). Standard AOAC (2005) procedures were used for DM (930.15), EE (2003.06), total P (965.17), and ash determination (942.05). Phytate P was determined using the procedure described by Haug and Lantzsch (1983). Titanium content was determined according to the methods described by

Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA).

Table 6.3 Analyzed nutrient content of cornstarch canola meal-based diets fed to growing pigs (%, as-fed basis).

Item	Diet[1]		
	RCM	DCM	CCM
Ash	5.70	5.30	5.60
DM	90.40	89.80	89.80
CP	17.90	17.70	17.90
NDF	13.80	7.80	13.10
ADF	8.50	4.90	9.20
GE (MJ/kg)	16.32	16.40	16.51
EE	1.50	1.89	1.71
Total P	0.72	0.73	0.75
Ca	0.76	0.63	0.66
Amino acids (total)			
Met + cys	0.72	0.71	0.76
Met	0.36	0.37	0.41
Cys	0.36	0.34	0.35
Lys	1.12	1.09	1.07
Thr	0.89	0.78	0.78
Trp	0.26	0.26	0.24
Val	0.91	0.85	0.89
Ile	0.71	0.66	0.69
Leu	1.44	1.39	1.35
Arg	1.16	1.18	1.10
His	0.68	0.62	0.59
Phe	0.81	0.79	0.76
Phe + tyr	1.34	1.29	1.27
Tyr	0.53	0.50	0.51

[1] Diet containing: RCM (regular canola meal); DCM (dehulled canola meal); CCM (coarse canola meal).

Gross energy was measured using an adiabatic bomb calorimetric (Model 6400, Parr Instruments, Moline, IL), which was calibrated using benzoic acid as a standard. Samples for AA analysis were prepared according to the AOAC procedures 994.12, alternatives 3 and 1 (sulfur AA), and then determined using an AA analyzer (S4300, Sykam GmbH, Eresing, Germany). The neutral and acid detergent fiber was determined using an Ankom fiber analyzer (Ankom

Technology, Macedon, NY, USA) and AOAC procedure 2002.04 and 973.18 (AOAC, 2005), respectively.

6.3.7 Calculations and statistical analysis

The non-specific endogenous loss of AA (EAL), coefficients of AID, and coefficients of SID of AA were calculated according to the following equations (Fan et al., 1995; Nyachoti et al., 1997a; Stein et al., 2007; Velayudhan et al., 2015):

$$AID = \{100 - [(AAd/AAf) \times Tif/Tid)]\} \times 100$$

Where AID, is the apparent ileal digestibility coefficient of an AA (%). AAd, is the AA content in ileal digesta (mg/kg DM). AAf is the AA content in the feed (mg/kg DM). Tif and Tid are the TiO_2 in the feed DM and the ileal digesta DM, respectively.

The non-specific losses of amino acids (EAL) were measured at the distal ileum after feeding a low protein (5%), casein-based diet to growing pigs and calculated according to the following equation.

$$EAL = AAd \times (Tif/Tid)$$

Where AAd is the concentration of that AA in ileal digesta (mg/kg DM); Tif, is the concentration of TiO_2 in the feed (mg/kg DM); and Tid is the concentration of TiO_2 in ileal digesta (mg/kg DM). In the calculation of the average EAL, in addition to data obtained in the present study, raw data from 3 different independent studies conducted in our lab (Velayudhan et al., 2015; Dadalt et al., 2016; Adewole et al., 2017) were considered.

Standardized ileal digestibility (SID) of AA were calculated using the following equation:

$$\%SID = AID + (EAL/AAf) \times 100$$

where EAL is the non-specific endogenous loss of AA at the distal ileum (mg/kg DM intake); AAf, the dietary content of the AA (mg/kg DM).

Data were analyzed using the mixed procedure of SAS (SAS software 9.4, SAS Institute, 2013). The effects of pig and period were not significant. Tukey's honesty significance difference separated means. All statements of significance were based on $P < 0.05$, and trends were observed at $0.05 < P \leq 0.10$.

6.4 RESULTS

6.4.1 Canola meal, corresponding fractions, and experimental diets

The analyzed chemical composition (as-fed basis) of the RCM and its corresponding fractions (DCM and CCM) produced by sieving is shown in Table 6.1. The composition of phase I diets is shown in Table 6.2. Whereas, the calculated and analyzed nutrient content of phase I experimental diets are shown in Table 6.3. The analyzed values for CP, AA, Ca, and P were similar to the calculated values of experimental diets. However, differences among diets containing RCM, DCM, and CCM were observed for NDF (13.8, 7.8, and 13.1%, respectively), ADF (8.5, 4.9, and 9.2%, respectively), and EE (1.5, 1.89 and 1.71%, respectively).

6.4.2 Apparent ileal digestibility

The coefficients of AID of most AA (Table 6.4) were not influenced by dehulling ($P > 0.05$). However, the AID of Phe and Ser was higher ($P < 0.05$) in RCM, compared to DCM and CCM, whereas the percentage for Thr was higher ($P < 0.05$) in RCM and DCM compared to CCM. A tendency for higher ($P \leq 0.10$) AID for Arg, Ala, Gly, and Pro, in RCM compared to DCM and CCM, was observed.

Table 6.4 Effect of dehulling on apparent ileal digestibility (AID) of AA in canola meal (CM) fed to growing pigs (%).

Item	Diet[1]			SEM	*P*-value
	RCM	DCM	CCM		
Indispensable AA					
Arg	82	79	79	1.6	0.060
His	58	55	52	3.0	0.107
Ile	74[a]	67[b]	70[ab]	1.7	0.025
Leu	77[a]	71[b]	74[ab]	1.4	0.004
Lys	71	72	71	1.9	0.933
Met	85	84	87	1.7	0.433
Phe	78[a]	74[b]	72[b]	1.7	0.016
Thr	67[a]	62[ab]	61[b]	1.5	0.015
Trp	74[a]	69[ab]	72[b]	1.4	0.036
Val	71	67	67	1.6	0.156
Dispensable AA					
Ala	75	71	73	1.5	0.095
Asp	65	64	63	1.4	0.306
Cys	64	66	64	2.4	0.917
Glu	82[a]	78[b]	80[ab]	0.9	0.021
Gly	64	59	59	1.8	0.086
Pro	70	66	65	1.9	0.061
Seri	69[a]	65[b]	65[b]	1.3	0.024
Tyr	74	73	71	1.7	0.143

[a,b]Means bearing different superscript letters within a row differ ($P < 0.05$)

[1]Diet containing: RCM (regular canola meal); DCM (dehulled canola meal); CCM (coarse canola meal)

6.4.3 Standardized ileal digestibility and standardized digestible amino acid content

The EAL values used to determine the SID of AA are shown in Table 6.5. The SID of AA (Table 6.6) was determined by correcting the AID for EAL. The SID of most AA were not influenced by dehulling. However, the SID of Phe was higher ($P < 0.05$) for RCM compared to DCM and CCM. The SID of Thr was higher ($P < 0.05$) for RCM than for CCM. The SID of Glu was higher ($P < 0.05$) for RCM than for DCM. Additionally, a tendency ($P \leq 0.10$) for a higher SID of Leu, Gly, and Ser, in RCM compared to DCM and CCM, was observed.

Table 6.5 Non-specific endogenous loss of AA (EAL[1]) at the distal ileum of growing pigs fed casein-corn starch diet (mg/kg of DM).

Amino Acids	Average[2]	CV	Range
Indispensable AA			
Arg	1077	82	880 - 1305
His	2386	186	1714 - 2725
Ile	871	93	619 - 1170
Leu	1098	75	871 - 1305
Lys	859	63	666 - 1024
Met	198	8	171 - 218
Phe	538	24	455 - 587
Thr	1217	84	925 - 1368
Trp[3]	133	-	-
Val	1138	111	818 - 1481
Dispensable AA			
Ala	897	53	720 - 1006
Asp	1698	98	1386 - 1966
Cys	428	19	361 - 468
Glu	2802	218	2097 - 3398
Gly	2226	183	1848 - 2858
Pro	5745	502	4441 - 7243
Ser	504	38	397 - 616
Tyr	1836	173	1281 - 2286

[1]Non-specific endogenous loss of AA (EAL) values used for determining standardized ileal digestibility (SID) of AA.

[2]n = 30

[3]Source: NRC (2012).

Table 6.6 Effect of dehulling on standardized ileal digestibility (SID) of AA in canola meal (CM) fed to growing pigs (%).

Item	Diet[1]			SEM	*P*-value
	RCM	DCM	CCM		
Indispensable AA					
Arg	88	86	85	1.1	0.118
His	79	77	74	3.1	0.152
Ile	79	75	76	1.4	0.143
Leu	82	78	78	1.1	0.060
Lys	76	77	76	1.8	0.862
Met	89	88	90	2.0	0.744
Phe	82[a]	77[b]	77[b]	2.0	0.021
Thr	75[a]	71[ab]	69[b]	1.6	0.037
Trp	77	77	76	1.8	0.991
Val	77	77	73	1.8	0.325
Dispensable AA					
Ala	81	78	79	1.1	0.270
Asp	72	71	70	1.5	0.423
Cys	70	72	71	2.4	0.889
Glu	86[a]	82[b]	84[ab]	0.9	0.047
Gly	76	72	72	1.8	0.093
Pro	97	94	92	2.3	0.160
Ser	79	75	75	1.6	0.067
Tyr	79	78	76	1.4	0.280

[a,b]Means bearing different superscript letters within a row differ ($P < 0.05$)

[1]Diet containing: RCM (regular canola meal); DCM (dehulled canola meal); CCM (coarse canola meal).

The standardized ileal digestible AA values are shown in Table 6.7. A higher quantity of total AA observed in DCM resulted in higher ($P < 0.05$) quantities of standardized ileal digestible AA, compared to RCM and CCM. However, no differences among meals for Met and Val were observed. Additionally, the standardized ileal digestible Ile was lower ($P < 0.05$) in CCM compared to RCM and DCM.

Table 6.7 Standardized ileal digestible AA in canola meal (CM) fed to growing pigs (%).

Item	Diet[1]			SEM	*P*-value
	RCM	DCM	CCM		
Indispensable AA					
Arg	1.85[b]	2.13[a]	1.73[c]	3.0	<0.001
His	0.91[b]	1.01[a]	0.87[b]	3.3	0.006
Ile	0.99[ab]	1.06[a]	0.93[b]	1.8	0.010
Leu	2.05[b]	2.24[a]	1.95[c]	3.3	<0.001
Lys	1.54[b]	1.72[a]	1.49[b]	2.6	0.001
Met	0.42	0.42	0.44	0.8	0.262
Phe	1.18[b]	1.26[a]	1.08[c]	2.5	<0.001
Thr	1.19[b]	1.28[a]	1.10[c]	2.4	0.001
Trp	0.41[b]	0.44[a]	0.35[c]	1.0	<0.001
Val	1.23	0.93	1.20	15.5	0.317
Dispensable AA					
Ala	1.40[b]	1.59[a]	1.36[b]	1.9	<0.001
Asp	2.00[b]	2.24[a]	1.90[b]	3.9	<0.001
Cys	0.41[b]	0.56[a]	0.47[b]	1.7	0.002
Glu	5.65 [b]	6.26 [a]	5.39[c]	6.0	<0.001
Gly	1.37[b]	1.51[a]	1.31[b]	3.2	0.007
Pro	2.33[b]	2.47[a]	2.09[c]	4.8	<0.001
Ser	1.42[b]	1.54[a]	1.33[c]	2.5	<0.001
Tyr	0.78[b]	0.86[a]	0.74[b]	1.3	0.001

[a,b,c]Means bearing different superscript letters within a row differ ($P < 0.05$)

[1]Diet containing: RCM (regular canola meal); DCM (dehulled canola meal); CCM (coarse canola meal).

6.5. DISCUSSION

6.5.1 Canola meals

The total AA and CP contents of DCM were higher than the corresponding parent meal; e.g., Lys rose 16%, Thr 13%, His 15%, Arg 19%, and CP increased by 9%. Additionally, total, non-phytate, and phytate P were higher by 15, 23, and 11%, respectively. Furthermore, compared to RCM, DCM had 41% less lignin and polyphenols, 38% less ADF, 32% less NDF, 26% less total dietary fiber, and 17% less NSP. Mejicanos (2015) reported 4% increase in glucosinolate content of DCM fraction 1 and 2, compared to RCM, without detrimental effect on growth

performance when CM was fed at 15% inclusion level to weaned pigs. However, higher total and non-phytate P content in DCM compared to RCM and CCM, resulted in increased apparent (ATTD) and standardized (STTD) total tract digestibility of P, reduced fecal and P output (g/day) when fed to growing and finishing pigs (Mejicanos et al., 2018a). The increases in Lys and CP contents were consistent with results by Clark et al. (2001) and Kracht et al. (2004), who indicated a 5 and 7% increase in CP in the dehulled meal compared to the parent CM, respectively. The AA content of the RCM used in the present study was comparable to the analyzed values reported for the parent *B. napus* CM by Zhou et al. (2015), who used air-classification for tail-end dehulling of CM. However, the quantity of most AA in DCM was higher than the values reported by Zhou et al. (2015) for the light fraction (high-protein and low fiber), except for Ile, Met, Val, and Cys. Additionally, the AA content in DCM was similar to the values for high protein CM reported by Liu et al. (2016). Although the contents of His, Leu, Ala, Asp, Gly, Pro, Ser, and Tyr, in DCM, were higher than values reported for high protein CM. The increase in CP content in DCM and the corresponding decrease in CCM are consistent with findings by Hansen et al. (2017) who used ball milling followed by sieving in the dehulling process, indicating an 18% increase in CP content for the high-protein fraction, whereas the high-fiber fraction had a reduction in CP of 7%. Results are also consistent with those by Li et al. (2017), who used a relatively new dehulling procedure in which cleaned seeds are dried to 40 g moisture/kg using sunlight. Then canola seeds get broken into fine particles (300 µm) using a spike roller, before the separation of kernels and hulls using the combination of vibrating screen and airflow. Finally, the kernels get moved into a twin-screw press to expel the oil and produce dehulled canola expellers containing 10% higher CP, compared to conventional expellers. Furthermore, total AA contents were 29.5% in the regular expellers, and 32.1 and 33.0%, in the dehulled canola seed expellers obtained from 2 different processing

facilities. Expeller extracted CM contains higher levels of EE than solvent-extracted CM (9.97 vs. 3.33; NRC, 2012). The increase in dietary fat levels from 3.2, 6.2, 9.2, and 12.2% resulted in a linear increase in the AID of most AA. Additionally, statistical differences in the SID of most AA between diets containing 3.2 and 12.2% canola oil, were observed (Li and Sauer, 1994). In practical diet formulation, fat is generally added. However, the experimental diets did not use vegetable oil. Nevertheless, the formulation of the diets followed similar net energy content. Greater digestibility of most AA in expeller extracted CM compared to solvent extracted CM has been observed (Maison et al., 2014).

6.5.2 Apparent ileal digestibility

The AID of AA in RCM was within the range reported for solvent-extracted CM. However, the AID of His was lower than the values reported in previous studies (NRC, 2012; Trindade Neto et al., 2012; Sanjayan et al., 2014; Berrocoso et al., 2015; Liu et al., 2016). Nonetheless, this was comparable to the value reported by Adewole et al. (2017). It is important to notice that differences of glycoprotein (NDICP) content between the parent and fractions could have influenced the AID of some AA. In the present study, the AID of Pro in RCM was higher than the values reported by Berrocoso et al. (2015) and Liu et al. (2016) for the regular CM and high protein CM. Differences in AA digestibilities between processing plants have been observed (Clark et al., 2001; Adewole et al., 2017).

No differences on AID among RCM, DCM, and CCM for most AA were observed, which is consistent with results by Liu et al. (2014a), Berrocoso et al. (2015) and Liu et al. (2016), showing no differences in AID between high protein CM and regular CM, for most AA. The AID observed in the present experiment is in disagreement with studies on dehulling by Zhou et al.

(2015) and Hansen et al. (2017), who found superior AA digestibility in the high-protein fraction compared to the high-fiber fraction. However, in both studies, the apparent AA digestibility in the high-protein fraction was not higher than the parent meal. In the present study, higher AID of Ile, Leu, Phe, and Thr, in RCM compared to DCM, was observed. Additionally, DCM had a higher AID for Arg, Lys, Phe than CCM. Such differences could be attributed to increased amounts of anti-nutritive factors in DCM, e.g., phytate P was 11% higher in DCM compared to the RCM. The use of phytase has been shown to improve the AID of AA (Selle and Ravindran, 2008; Cowieson et al., 2017). However, diet composition could be a factor, as some studies have shown that phytase supplementation did not improve the AID of CP and AA (Liao et al., 2005). Dietary phytate could reduce pig performance due to factors such as *de novo* binary protein-phytate formation, compromised intestinal uptake of AA, and increased endogenous losses of nutrients. Anti-nutritive factors present in the diet influences the secretion and reabsorption of endogenous nitrogen (Nyachoti et al.,1997b; Selle and Ravindran, 2008; Woyengo et al., 2012). In a study comparing the effect of feeding a wheat-barley and SBM diet and a high fiber diet containing a coarse fraction (20%) from air-classified CM and CM hulls (4%), lower coefficients of AID of all AA, except for Met when the high fiber diet was fed, were observed (Perez de Nanclares et al., 2017).

The values for NDF and ADF in the diets containing CCM were 68 and 75% greater than in the diets containing DCM. However, no differences in the AID of AA between DCM and CCM were observed, which indicates that the fiber content in CM did not affect the AID for most AA. However, for some AA, higher digestibility in RCM, compared to DCM, was observed. In a recent study, the AID and SID for all AA in CM from black *B. napus* and *B. juncea* were similar, albeit black *B. napus* containing 26% higher total dietary fiber (Sanjayan et al., 2014). Likewise, no differences were found in AID and SID for most AA when comparing two high protein CM and

the regular CM, regardless of NDF in regular CM being 37 and 40% greater than the 2 high-protein CM evaluated (Berrocoso et al., 2015). However, the type of fiber and fiber composition can affect CP and AA digestibility. In a feeding study using diets formulated to a similar level of fiber from wheat straw or powdered cellulose, a decrease in ileal digestibility of CP and most AA when feeding wheat straw was observed (Huisman et al., 1985). Additionally, in a study of the effect of dietary fiber source and starch source on ileal and fecal digestibility, it was found no impact on AID for most AA. However, ileal digestibility of His, Lys, and Trp was reduced by the inclusion of wheat bran (Wang et al., 2006).

6.5.3 Standardized ileal digestibility and standardized digestible amino acid content

The SID of AA in RCM, DCM, and CCM were within the range reported for solvent-extracted CM (NRC, 2012; Trindade Neto et al., 2012; Sanjayan et al., 2014; Berrocoso et al., 2015; Adewole et al., 2017). However, the SID for Phe and Glu were higher in RCM compared to DCM and CCM. Additionally, the SID of Thr was higher in RCM than in CCM. Moreover, in RCM, a tendency for a higher SID for Leu, Gly, and Ser, was observed. In the present study, His and Pro had higher SID compared to a study evaluating CM from 6 processing plants in Canada (Adewole et al., 2017). Higher NDF in diets containing CCM compared to DCM (68 %) did not result in lower coefficients of SID for most AA, which is consistent with studies by Berrocoso et al. (2015) and Liu et al. (2016) who did not find differences in SID of indispensable AA between high protein CM and conventional CM, despite high differences in dietary fiber content. Dehulling did not improve the SID in DCM, regardless of a 26% reduction in total fiber content compared to RCM. Such results disagree with findings by Zhou et al. (2015), who reported increased AA

digestibility in the light-particle fraction of air-classified CM fed to growing pigs, compared to the heavy-particle faction that had higher fiber content.

A higher concentration of total AA in DCM resulted in higher ileal digestible AA available for the pigs in the diets. Results are consistent with studies by Berrocoso et al. (2015) and Liu et al. (2016), who found more digestible AA in high protein CM compared to regular CM. However, no differences in digestible Met, and Val between RCM, DCM, and CCM, were observed. Furthermore, the CCM remained a meal with similar digestible AA content, compared to RCM, except for Arg, Leu, Phe, Thr, Glu, Pro, and Ser, which were lower in CCM. Differences in the standardized ileal digestible AA contents in CM from 6 crushing plants in Canada, have been observed (Adewole et al., 2017).

6.6 CONCLUSIONS

Tail-end dehulling did not influence the AID and SID of the meal for most AA. However, tail end dehulling provided a meal with an increased concentration of total and digestible AA content, which could be of importance in diet formulation. However, anti-nutritive factors present in DCM, such as phytate P and glucosinolates, are also slightly enhanced, thus, affecting the AID and SID of some AA.

CHAPTER SEVEN

MANUSCRIPT IV

Effect of dietary supplementation of xylanase in a wheat-based diet containing canola meal on growth performance, nutrient digestibility, organ weight, and short-chain fatty acid concentration in digesta, when fed to weaned pigs

G. A. Mejicanos[*], G. González-Ortiz†, and C. M. Nyachoti[*]

[*]Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada

†AB Vista, Marlborough, Wiltshire, SN8 4AN, United Kingdom

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7.1 ABSTRACT

This study was designed to determine the effect of dietary supplementation of xylanase on growth performance, nutrient digestibility, organ weight, digesta pH, and concentration of short-chain fatty acids (SCFA) of weaned pigs fed wheat-canola meal (CM) diets over a 35-d period. A total of 144 piglets weaned at 18 ± 2d of age, with an initial BW of 6.2 ± 0.7 kg, received one of 8 dietary treatments based on randomized complete block design. Body weight and feed intake were recorded weekly to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). Treatments consisted of a control wheat-SBM-based diet and wheat-regular (RCM), dehulled (DCM), or coarse (CCM) CM without and with 16,000 BXU/kg xylanase (Econase XT). All diets contained 500 FTU/kg of phytase (Quantum Blue 5G), and titanium-dioxide (0.3%). Apparent total tract digestibility (ATTD) of neutral detergent fiber (NDF), protein (CP), phosphorous (P), calcium (Ca) and dry matter (DM), and diet were determined. On d 35, one pig per pen was euthanized to evaluate the main factors of protein, xylanase supplementation, and gender on organ weight, ileal and colon digesta pH, and colon digesta concentrations of SCFA. The main factors did not affect growth performance. Xylanase supplementation improved nutrient digestibilities in all diets and increased ileal and colonic digesta pH, without affecting growth performance of weaned pigs fed wheat and canola meal-based diets. A protein-xylanase effect ($P < 0.05$) resulted in increasing the ATTD of NDF from 28 to 32% and from 29 to 37% for RCM and DCM, respectively. The ATTD of CP was greater ($P < 0.05$) with xylanase supplementation (75 vs. 70%). Xylanase supplementation increased ATTD of P and Ca. A three-way interaction ($P < 0.05$) for protein-xylanase-gender for colon pH, acetic and propionic acid in the colon digesta of pigs was observed. Xylanase supplementation increased ($P < 0.05$) the weight of the liver and spleen and tended ($P < 0.10$) to increase the size of the kidney. In conclusion, dietary

supplementation of xylanase increased nutrient digestibility and digesta pH but did not influence growth performance of weaned pigs fed wheat and canola meal-based diets over a 35-d period.

Key words: canola meal, dehulling, digestibility, pig, xylanase

7.2 INTRODUCTION

Canola meal (CM) is the second most abundant protein supplement after soybean meal (SBM; Canola Council of Canada, 2015). The Canadian canola seed production has increased from 12.7 MMT in 2010 to 21.3 MMT in 2017, increasing the availability of CM in the global market (COPA, 2017; Statistics Canada, 2018). However, the global rapeseed-canola industry has failed to process the meal to increase protein and decrease fiber content. Nevertheless, the need for protein alternatives in diet formulation has increased the interest for other options for processing in canola. Research in France on dehulling has been undertaken; however, significant fat losses have been observed (Carré et al., 2016). Dehulling also has been studied in Norway (Hansen et al., 2017), and in Canada, (Thakor et al., 1995; Clark et al., 2001; Beltranena and Zijlstra, 2011; Zhou et al. 2013; Mejicanos et al. 2017). Dehulling of canola increased the standardized total tract digestibility (STTD) of phosphorous (P; Mejicanos et al., 2018a) and increased the standardized ileal digestible (SID) amino acid (AA) content of the dehulled meal (Mejicanos et al., 2018b). Furthermore, the use of fiber degrading enzymes can improve nutrient digestibility, and growth performance of pigs fed diets containing rapeseed meal (Fang et al., 2007) and other fibrous plant-based feed ingredients (Kerr and Shurson, 2013).

Dehulling CM produces fractions with distinctive levels of non-starch polysaccharides (NSP), with the dehulled meal containing lower fiber and higher crude protein (CP; Mejicanos et al., 2017), which could affect the action of fiber degrading enzymes such as xylanase. Ndou et al. (2015) and Zhang et al. (2018) indicated that cereal-based diets with higher NSP content, promote a more diverse nutritional niche, resulting in higher microbiota diversity in the cecum. However, it is unclear if supplementing xylanase in a wheat-based diet containing regular CM or its fractions will affect growth performance, nutrient digestibility, or the concentration of short-chain fatty acids (SCFA) over a 35-d period. Therefore, the objective of this study was to determine the effect of xylanase supplementation in a wheat-based diet containing CM, and the influence on nutrient digestibility, organ weight, pH in ileal and colon digesta, SCFA concentration in colonic digesta, and growth performance, when fed to weaned pigs.

7.3 MATERIALS AND METHODS

7.3.1 Animal care

The animal use protocol utilized in the present study was reviewed and approved by the Animal Care Committee of the University of Manitoba. Pigs were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009). The study was conducted at the T. K. Cheung Centre for Animal Science Research.

7.3.2 Animals and housing

The current experiment used a total of 144 pigs [TNT70 (Large White × Landrace) × Tempo; Topigs Norsvin, Winnipeg, MB, Canada] obtained from Glenlea Swine Research Unit,

University of Manitoba. Piglets were weaned at 18 ± 2d of age, with an initial BW of 6.2 ± 0.7 kg (mean ± SD). On day one, piglets were weighed and randomly assigned to 1 of the 8 diets in a randomized complete block design. Pens with plastic-covered expanded metal floors were used (space allowed was 0.8 m² per piglet). Room temperature was initially set at 30 ± 1ºC and was gradually decreased by 1ºC every week. A 16-hour light (0600 to 2200h), and 8-hour dark cycle was provided. Water and feed were provided *ad libitum* using nipple drinkers and stainless-steel feeding troughs throughout the 5-wk study. Body weight and feed disappearance were monitored weekly. At the end of the study, one pig per pen was euthanized for organ weighing, ileal, and colonic digesta collection.

7.3.3 Dehulling of canola meal and ingredients

The dehulled (DCM) and coarse (CCM) meal fractions were produced at the Canadian International Grains Institute, Winnipeg, MB, Canada, using a sieve size of 355 µm and a plansifter Model MPAR-8HK (Bühler AG, Uzwil, Switzerland). The wheat used in the experiment was a Hard Canada Western Red Spring (CWRS). All other ingredients were obtained from the local market. The analyzed chemical composition of the wheat, SBM, regular CM, and the fractions used in the current study are presented in Table 7.1.

Table 7.1. Analyzed chemical composition of wheat, SBM and canola meal and its fractions used in the present experiment (%, as-is basis)

			Canola meals[1]		
Item	Wheat[3]	SBM[3]	RCM	DCM	CCM
DM	88.26	88.87	90.78	90.92	91.35
Ash	1.67	6.61	7.20	7.50	6.90
CP	11.86	47.60	39.52	41.85	37.62
Ether extract	1.97	0.99	3.49	4.89	6.06
Total fat	2.63	1.62	4.90	6.10	7.36
Starch	61.31	7.07	3.35	2.59	1.05
Sugar	3.23	8.37	7.68	7.35	8.74
Fiber fractions					
NDF	7.81	10.95	26.2	17.8	30.3
ADF	3.59	6.86	20.21	13.85	19.00
Total dietary fiber			33.01	24.57	37.25
Non-starch polysaccharides			20.54	16.97	20.99
Lignin and polyphenols			10.17	6.01	11.38
Glycoprotein (NDICP)			3.6	2.41	6.53
Total P	0.39	0.71	1.10	1.27	1.08
Non-phytate P[2]	0.11	0.33	0.39	0.59	0.31
Ca	0.06	0.33	0.67	0.60	0.67
Indispensable AA					
Arg	0.60	3.45	2.10	2.49	2.03
His	0.34	1.28	1.16	1.33	1.15
Ile	0.47	2.14	1.25	1.41	1.23
Leu	0.91	3.62	2.51	2.92	2.48
Lys	0.39	2.96	2.04	2.29	1.96
Met	0.22	0.66	0.47	0.48	0.48
Phe	0.64	2.40	1.44	1.63	1.39
Thr	0.40	1.86	1.59	1.80	1.58
Trp	0.17	0.66	0.42	0.42	0.42
Val	0.58	2.23	1.55	1.74	1.58
Dispensable AA					
Ala	0.47	2.06	1.73	2.03	1.72
Asp	0.71	5.41	2.78	3.17	2.72
Cys	0.33	0.70	0.50	0.78	0.67
Glu	3.88	8.54	6.57	7.61	6.39
Gly	0.57	1.99	1.80	2.09	1.78
Pro	1.36	2.53	2.42	2.61	2.25
Ser	0.60	2.36	1.80	2.05	1.76
Tyr	0.36	1.59	0.99	1.10	0.97

[1] RCM (regular canola meal); DCM (dehulled canola meal, particle size < 355 μm); CCM (coarse canola meal, particle size > 355 μm).

[2] Non-phytate P was calculated as the difference between total P and phytate-bound P

[3]Amino acid, total P, and Ca content data from NRC (2012).

7.3.4 Diets

The study included 8 diets consisting of a negative control wheat/SBM basal diet as well as the combination of 3 types of CM, regular canola meal (RCM), dehulled (DCM), and coarse (CCM), without and with 100 g/ton of xylanase equivalent to 16,000 BXU/kg (Econase XT 25P; AB Vista, Marlborough, Wiltshire, UK; 160,000 BXU/g). One BXU is defined as the amount of enzyme that produced reducing carbohydrates having a reducing power corresponding to one nmol xylose from birch xylan in one second under assay conditions (Baily and Poulanen, 1989). All diets contained 500 FTU/kg of an *Escherichia coli*-derived phytase (Quantum Blue 5G, AB Vista, Marlborough, Wiltshire, UK; 5,000 FTU/g). One FTU is defined as the amount of enzyme that liberates 1 micromole of inorganic P per minute from 0.0051 mol/l sodium phytate at 37° and pH 5.50 (AOAC, 2000). Diets were formulated to meet or exceed NRC (2012) nutrient requirements for weaned pigs. However, when balancing DCM containing pre-starter diets, the P contributions from the different ingredients exceeded the target, therefore, to maintain equal STTD P content in all diets, di-calcium phosphate was added to the other diets. All diets contained 0.3% titanium dioxide (TiO_2) as an indigestible marker to determine the apparent total tract digestibility (ATTD) of nutrients. A two-phase feeding program was used (phase I, 1-21 and phase II, 22-35 d post-weaning). Diets were balanced according to standardized ileal digestible (SID) amino acids, standardized total tract digestible P, and net energy (NE). The composition and nutrient contents of phase I diets are summarized in Table 7.2 and 7.3, respectively. In the formulation of phase II diets (d 22-35), CM or SBM were the sole protein supplement. However, synthetic AA were added to balance the diets and meet the essential nutrient requirements of pigs (NRC, 2012). The composition and nutrient contents of phase II diets are summarized in Tables 7.4 and 7.5, respectively. The diets were offered in a mash form and fed *ad libitum* for 35-d.

Table 7.2 Composition of Phase I diets fed to weaned pigs (%, as-fed basis)

Ingredient	Diets without xylanase[4]				Diets with xylanase[4]			
	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Wheat	47.31	42.40	45.36	44.28	47.31	42.40	45.36	44.28
SBM	15.00	-	-	-	15.00	-	-	-
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dry whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Regular canola meal	-	20.00	-	-	-	20.00	-	-
Dehulled canola meal	-	-	17.00	-	-	-	17.00	-
Coarse canola meal	-	-	-	17.40	-	-	-	17.40
Iodized salt	0.5	0.50	0.50	0.50	0.5	0.50	0.50	0.50
Canola oil	3.5	3.5	3.5	4.00	3.5	3.5	3.5	4.00
Calcium carbonate	0.78	0.63	0.79	0.62	0.78	0.63	0.79	0.62
Di-calcium phosphate	0.25	0.13	-	0.24	0.25	0.13	-	0.24
Lysine-$_{HCl}$	0.53	0.66	0.67	0.72	0.53	0.66	0.67	0.72
$_{DL}$-Methionine	0.17	0.20	0.19	0.20	0.17	0.20	0.19	0.20
$_{L}$-Threonine	0.16	0.17	0.18	0.21	0.16	0.17	0.18	0.21
$_{L}$-Tryptophan	-	0.01	0.01	0.03	-	0.01	0.01	0.03
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Enzyme premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Marker	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1] Supplied per kg of diet, Vitamins: vitamin A, 8250 IU; vitamin D3, 835 IU; vitamin E, 40 IU; vitamin K 4 mg; thiamin (B1), 2.0 mg; Riboflavin, 12 mg; pantothenate, 15 mg; choline 500 mg; niacin, 22.5 mg; vitamin B6, 4.5 mg; vitamin B12 25 µg; biotin, 200 µg; folic acid, 2 mg.

[2] Minerals: Cu,25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

[3] Enzymes: Phytase, 500 FTU/kg; xylanase, 16,000 BXU/kg, wheat used as a carrier.

[4] RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

Table 7.3 Calculated and analyzed nutrient content of Phase I diets fed to weaned pigs (%, as-fed basis)[1]

Item	Diets without xylanase[2]				Diets with xylanase[2]			
	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Calculated nutrient content								
CP	19.61	19.92	19.49	18.86	19.61	19.92	19.49	18.86
NE, kcal/kg	2483	2471	2507	2463	2483	2471	2507	2463
Ca, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Available P, %	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Methionine (SID, %)	0.45	0.47	0.45	0.47	0.45	0.47	0.45	0.47
Methionine + cysteine (SID, %)	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Lysine (SID, %))	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Threonine (SID, %)	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Tryptophan (SID, %)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Phytase (FTU/kg)	500	500	500	500	500	500	500	500
Xylanase (BXU/kg)	-	-	-	-	16000	16000	16000	16000
Analyzed nutrient content								
DM	90.38	90.88	90.82	91.10	90.60	91.02	91.34	90.93
Ash	4.18	4.54	4.76	4.37	3.93	4.67	4.45	4.49
CP, % (N x 6.25)	20.49	20.06	19.85	19.28	20.41	19.94	20.10	18.96
Ether extract	5.79	5.47	6.35	6.33	5.31	5.89	5.96	6.10
Total fat	6.74	6.43	7.35	7.33	6.27	6.85	6.96	7.10
Starch	35.19	33.61	32.32	32.82	37.54	32.07	34.06	33.95
Sugar	21.9	22.91	23.13	23.37	23.70	24.06	22.51	22.57
NDF	6.80	10.51	8.02	10.17	6.84	10.24	7.90	10.15
Ca	0.84	0.88	0.78	0.87	0.83	0.79	0.76	0.78
Total P	0.75	0.65	0.69	0.75	0.66	0.71	0.71	0.71
Phytase (FTU/kg)	528	630	567	999	1010	934	737	680
Xylanase (BXU/kg)	<2000	<2000	<2000	<2000	22800	21900	21000	23300

[1] All diets were formulated to contain the following quantities of the ileal digestible indispensable AA (%): Met, 0.39; Met + Cys 0.74; Lys, 1.35; Thr, 0.79; Trp, 0.22.

[2] RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

7.3.5 Experimental and analytical procedures

Pigs were assigned to the 8 experimental diets in a randomized complete block design to give 6 replicates of 3 pigs per experimental unit, in 2 separate rooms (blocks). In preparation for the ATTD sampling, pens were thoroughly washed and rinsed using warm water. Freshly voided fecal samples were collected by hand grab in plastic bags on d 30 of the study. Samples were carried on ice and stored at -20°C in the freezer until analyzed. Fecal samples were dried in a forced-air oven at 60°C for 5 d. Dried samples were pooled for each pen and ground using a heavy-duty blender (model CB15, Waring Commercial, Torrington, CT), then a subsample was obtained after thoroughly mixing and sieving for homogeneity (Sieve size 40, 420 µm) the ground feces for chemical analysis. Diets and CM samples were finely ground before analysis using a Foss sample preparation CyclotecTM 1093 mill (Foss Allé 1, DK-3400 Hilleroed, Denmark). Experimental diets and CM were subject to CP (N × 6.25) analysis using an N analyzer, model TruSpec N (Leco Corp., St. Joseph, MI, USA). Samples for AA analysis were prepared according to the AOAC procedures 994.12, alternatives 3 and 1 (sulfur AA), and then determined using an AA analyzer (S4300, Sykam GmbH, Eresing, Germany). Standard AOAC (2005) procedures were used for DM (method 930.15), fat (method 2003.06), and ash determination (method 942.05). Phytate-P was determined using the method described by Haug and Lantzsch (1983). Dietary fiber was determined by a combination of neutral detergent fiber (NDF) and detergent-soluble NSP measurements and was calculated as the sum of NDF and detergent soluble NSP (Slominski et al., 1994). NDF was determined using an Ankom fiber analyzer (Ankom Technology, Macedon, NY), and according to AOAC (2005) method 2002.04; NDF in fecal samples was determined using Ankom filter bags F58 due to significant pulverization of the sample during grinding. Total NSP were determined by gas-liquid chromatography (component neutral sugars) using an SP-2340

column and Varian CP3380 gas chromatograph (Varian Inc., Palo Alto, CA) and colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) and the procedure described by Englyst and Cummings (Englyst and Cummings, 1984; Englyst and Cummings, 1988) with some modifications (Slominski and Campbell, 1990). The content of NSP was measured in both the meals and the NDF residues. Neutral detergent soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue, and total dietary fiber was determined by the summation of NDF and NDF-soluble NSP. The contents of CP (N × 6.25) and ash in NDF residue were also measured. The value for lignin and associated polyphenols was calculated by difference [NDF - (NSP + protein + ash)] (Slominski et al., 1994). Diets, fecal samples, and CM for Ca and P analysis were ash at 600oC for 12 h, digested according to the AOAC (2005) method 985.01 and determined using a Varian Inductive Coupled Plasma Mass Spectrometer (Varian Inc., Palo Alto, CA). The concentration of phytate-bound P in the CM was calculated as 28.2 % of analyzed phytate (Tran and Sauvant, 2004). Non-phytate P was calculated by subtracting phytate-bound P from total P. Titanium dioxide concentration in feed and fecal samples was measured according to the revised protocol (Lomer et al., 2000), and submitted to analyses using a Varian Inductive Coupled Plasma Mass Spectrometer (Varian Inc., Palo Alto, CA). Diets were analysed for xylanase activity by ELISA method using Quantiplate Kits for Econase XT (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK) and phytase activity by an ELISA method, using Quantiplate Kits for Quantum Blue supplied by Envirologix (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK).

Table 7.4 Composition of Phase II experimental diets fed to weaned pigs (%, as-fed basis)

Ingredient	Diets without xylanase[4]				Diets with xylanase[4]			
	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Wheat	74.70	70.16	71.97	74.62	74.70	70.16	71.97	74.62
SBM	15.46	-	-	-	15.46	-	-	-
Regular canola meal	-	20.06	-	-	-	20.06	-	-
Dehulled canola meal	-	-	18.31	-	-	-	18.31	-
Coarse canola meal	-	-	-	15.00	-	-	-	15.00
Iodized salt	0.5	0.50	0.50	0.50	0.5	0.50	0.50	0.50
Canola oil	4.40	4.40	4.40	4.68	4.40	4.40	4.40	4.68
Calcium carbonate	1.20	1.08	1.24	1.07	1.20	1.08	1.24	1.07
Di-calcium phosphate	0.68	0.52	0.34	0.66	0.68	0.52	0.34	0.66
Lysine-$_{HCl}$	0.79	0.94	0.92	1.03	0.79	0.94	0.92	1.03
$_{DL}$-Methionine	0.19	0.22	0.20	0.24	0.19	0.22	0.20	0.24
$_{L}$-Threonine	0.27	0.29	0.29	0.35	0.27	0.29	0.29	0.35
$_{L}$-Tryptophan	0.01	0.03	0.03	0.05	0.01	0.03	0.03	0.05
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Enzyme premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Marker	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1] Supplied per kg of diet, Vitamins: vitamin A, 8250 IU; vitamin D3, 825 IU; vitamin E, 40 IU; vitamin K 4 mg; thiamin (B1), 2.0 mg; Riboflavin, 10 mg; pantothenate, 15 mg; choline 500 mg; niacin, 22.5 mg; vitamin B6, 4.5 mg; vitamin B12 25 µg; biotin, 200 µg; folic acid, 2 mg.

[2] Minerals: Cu,25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

[3]Enzymes: Phytase, 500 FTU/kg; xylanase, 16,000 BXU/kg, wheat used as a carrier.

[4] RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

Table 7.5 Calculated and analyzed nutrient content of Phase II diets fed to weaned pigs (%, as-fed basis)[1]

Item	Diets without xylanase[2]				Diets with xylanase[2]			
	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Calculated nutrient composition								
CP, %	17.23	17.41	17.34	15.78	17.23	17.41	17.34	15.78
NE, kcal/kg	2426	2415	2451	2412	2426	2415	2451	2412
Ca, %	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Phosphorus, (STTD)	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Methionine (SID, %)	0.4	0.42	0.39	0.42	0.4	0.42	0.39	0.42
Methionine + cysteine (SID, %)	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Lysine (SID, %))	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
Threonine (SID, %)	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Tryptophan (SID, %)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase (FTU/kg)	500	500	500	500	500	500	500	500
Xylanase (BXU/kg)	-	-	-	-	16000	16000	16000	16000
Analyzed nutrient content								
DM	89.16	89.20	89.29	89.60	88.91	89.34	89.45	89.36
Ash	3.25	3.49	3.57	3.40	3.25	3.48	3.53	3.03
CP, % (N x 6.25)	17.83	17.16	17.91	16.16	17.38	17.98	18.00	16.05
Ether extract	5.50	5.12	5.58	5.81	5.23	5.50	5.81	5.83
Total fat	6.61	6.20	6.68	6.94	6.34	6.57	6.90	6.95
Starch	49.85	45.93	46.77	47.05	49.40	45.51	46.27	48.80
Sugar	4.98	5.29	5.31	5.16	5.29	5.89	5.51	5.46
NDF	9.35	12.30	10.16	11.89	9.05	12.31	10.59	11.59
Ca	0.66	0.63	0.59	0.61	0.75	0.77	0.82	0.81
Total P	0.56	0.58	0.60	0.58	0.52	0.59	0.67	0.64
Phytase (FTU/kg)	696	744	956	1160	794	1020	846	991
Xylanase (BXU/kg)	<2000	<2000	<2000	<2000	18200	20100	18600	20900

[1] All diets were formulated to contain the following quantities of the ileal digestible indispensable AA (%): Met, 0.36; Met + Cys, 0.68; Lys, 1.23; Thr, 0.73; Trp, 0.20

[2] RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

7.3.6 *Short-chain fatty acids, digesta pH and organ weight*

On day 35 of the trial, one pig per pen was randomly selected, weighed, then sedated by an intramuscular injection of azaperone and xylazine (2.2 and 2 mg/kg, respectively; Elanco, Division Eli Lilly Canada Inc., Guelph, ON, Canada and Bimeda MTC Animal Health Inc., Cambridge, ON, Canada, respectively) and euthanized by penetration of captive bolt. Subsequently, pigs were eviscerated from sternum to pubis, the gastrointestinal tract and organs (heart and lungs without trachea) were removed, emptied from blood and digesta, and weighed. Approximately 20 ml of digesta samples from the ileum (30 cm immediately before the ileocecal junction) and the colon (medial colon, 30 cm) were collected for pH analysis using a digital pH meter (Accumet, Fisher Scientific, Hampton, NH, USA). Additionally, one gram of colonic digesta per pig was obtained for SCFA. Colonic digesta samples were sent to Alimetrics Diagnostics, Espoo, Finland, for SCFA analyses. Samples were analyzed by gas chromatography using pivalic acid as an internal standard.

7.3.7 Calculations and statistical analysis

The ATTD (%) of nutrients (CP, NDF, DM, P, and Ca) was calculated using the following equation:

$$ATTD\ (\%) = [1- (TiO_2\ \%\ diet / TiO_2\ \%\ feces) \times (Nutrient\ feces / Nutrient\ diet)] \times 100$$

Where TiO_2 % diet is the concentration of titanium dioxide in the diet TiO_2 % feces is the concentration of titanium dioxide in the excreta. Nutrient diet is the nutrient concentration in the diet. Nutrient feces is the nutrient concentration in the feces.

Data were analyzed using the Mixed Procedure of SAS (SAS software 9.4, SAS Institute, 2013). Treatments were randomly assigned to 2 rooms (blocks) with 24 pens per room, for a total of 48 pens. A pen of 3 pigs served as the experimental unit. Pig weight, feed intake, and disappearance were recorded weekly to calculate average feed intake (ADFI), feed efficiency (G:F), and average daily gain (ADG). Random effects of sex, room, sex-treatment interaction, and block-treatment-sex interaction were considered. The model included factorial effects as follows: protein source main effect, enzyme main effect, gender main effect, two- and three-way interactions from protein source-xylanase and gender. To determine the significance of factorial effects, contrasts were applied, and t-test for the main effect was used. The two- and three-way interactions were represented in figures generated by SAS 9.4. All statements of significance are based on $P < 0.05$, and trends were observed at $0.05 < P < 0.10$.

7.4 RESULTS

After two weeks of the study, one pen (3 pigs) was removed from the trial due to health concerns; all other pigs remained healthy for the duration of the study.

7.4.1 Chemical composition of canola meal and diets

The analyzed chemical composition (as-fed basis) of the wheat, SBM, RCM, and its corresponding fractions (DCM and CCM) used in the formulation of the experimental diets are presented in Table 7.1. The starch, gross energy (kcal/kg) and CP content of the wheat and was 61.31%, 3952 kcal/kg, and 11.86%, respectively. Whereas the non-phytate phosphorus and calcium were 0.11 and 0.06%, respectively. The soybean meal used in the experiment had 47.6% CP content. The other protein sources were RCM, DCM, and CCM. Lower total dietary fiber content was observed in DCM (24.57%) compared to CCM (37.25%) and RCM (33.01%)

respectively. Whereas, NSPs content were 20.54, 16.97, and 20.99% for RCM, DCM, and CCM, respectively. The nutrient composition, calculated and analyzed nutrient contents of the experimental diets fed to weaned pigs in phase I are presented in Tables 7.2 and 7.3, respectively. The total Ca to total P ratio for Phase I diets was 1.12 : 1; 1.35 : 1; 1.13 : 1 and 1.16 : 1 for diets without xylanase containing SBM, RCM, DCM and CCM. Whereas the Ca:P ratio was 1.26 : 1; 1.11 : 1; 1.07 : 1 and 1.1 : 1 for diets containing xylanase and SBM, RCM, DCM, and CCM, respectively. Additionally, starch was higher for diets containing SBM compared do diets containing RCM, DCM, and CCM.

The composition, calculated and analyzed nutrient contents of Phase II diets are presented in Tables 7.4 and 7.5, respectively. The total Ca to total P ratio was 1.18 : 1; 1.09 : 1; 0.98 : 1 and 1.05 : 1 for diets without xylanase containing SBM, RCM, DCM and CCM. Whereas the Ca:P ratio was 1.44 : 1; 1.31 : 1; 1.22 : 1 and 1.27 : 1 for diets containing xylanase and SBM, RCM. DCM and CCM, respectively. Additionally, starch was higher for diets containing SBM and CCM compared do diets containing RCM and DCM. The analyzed values for CP were lower in diets containing CCM compared to the other diets, whereas NDF was lower in diets containing SBM and DCM, compared to diets containing RCM and CCM. Analyzed enzyme activities in feed samples of phase I and II diets were all close to expected.

7.4.2 Effect of protein source and xylanase supplementation on growth performance, organ weight, nutrient digestibility, and digesta pH

The effect of protein source, xylanase supplementation, gender, and their two- and three-way interactions on the ADG, ADFI, and G:F ratio is shown in Tables 7.6, 7.7, and 7.8,

respectively. At the beginning of the Exp. barrows were heavier than gilts ($P < 0.05$; 6.32 vs. 6.19 kg), which resulted in gender effect ($P < 0.05$) and a two-way interaction ($P < 0.05$) for protein source and gender when the treatments were assigned. However, such differences were not maintained for the rest of the experiment. Protein source, xylanase supplementation, or gender did not affect ($P > 0.10$) ADG for the 5 weeks of the study. Likewise, protein source, xylanase supplementation, or gender did not affect ($P > 0.10$) on G:F ratio. However, a tendency ($P < 0.10$) for higher G:F ratio (0.66 vs. 0.63) with xylanase supplementation on week 4, was observed. Likewise, a tendency ($P < 0.10$) for higher G:F ratio when pigs were fed diets containing DCM as protein source in week 5, was observed. Protein source, xylanase supplementation, or gender did not affect ($P > 0.10$) on ADFI for the 5 weeks of the study. However, in the first week of the study, a significant two-way interaction ($P < 0.05$) for protein source and xylanase, was observed. Feeding diets containing DCM plus xylanase resulted in lower ($P < 0.05$) ADFI (118 g/day/pig) compared to feeding wheat-RCM, wheat-SBM or wheat-CCM without xylanase or wheat-CCM plus xylanase (161, 158, 157, 157 g/day/pig, respectively). Such differences were not maintained in the following weeks.

Table 7.6 Effect of protein source and xylanase supplementation the average daily gain (ADG) of weaned pigs.

Item	Initial BW (kg)	ADG g/day/pig					Final BW (kg)
		Week 1	Week 2	Week 3	Week 4	Week 5	
Least squares means for main effects							
Protein source effect[2]							
SBM	6.27	103	344	505	561	744	22.22
RCM	6.19	98	314	467	581	733	21.39
DCM	6.23	86	308	431	564	762	21.42
CCM	6.32	83	314	453	578	693	21.26
Pooled SEM	0.04	9.07	22.65	21.54	28.27	33.76	0.56
Xylanase effect							
With xylanase	6.26	92	322	453	570	713	21.55
Without xylanase	6.24	93	318	453	572	752	21.61
Pooled SEM	0.03	6.50	16.87	31.63	19.87	19.72	0.33
Gender effect							
Barrows	6.32[x]	92	319	455	567	719	21.42
Gilts	6.19[y]	94	322	473	574	747	21.73
Pooled SEM	0.03	5.96	15.65	14.21	19.87	19.72	0.34
Protein x xylanase interaction[2]							
SBM – no xylanase	6.28	105	349	526	558	729	22.62
RCM – no xylanase	6.17	100	302	456	597	776	21.31
DCM – no xylanase	6.15	92	301	399	554	782	21.66
CCM – no xylanase	6.26	76	319	475	579	720	21.88
SBM + xylanase	6.22	102	343	515	571	787	22.72
RCM + xylanase	6.28	96	325	454	567	691	21.12
DCM + xylanase	6.29	81	314	472	581	792	22.84
CCM + xylanase	6.10	90	311	450	578	665	21.17
Pooled SEM	0.07	12.12	31.16	19.61	41.77	58.14	0.86
Factors and significance[1]							
Protein source	0.242	0.393	0.638	0.210	0.942	0.643	0.695
Xylanase	0.663	0.940	0.852	0.909	0.939	0.330	0.927
Gender	0.013	0.830	0.876	0.442	0.803	0.492	0.645
Protein x gender	0.002	0.506	0.530	0.789	0.287	0.584	0.250
Xylanase x gender	0.614	0.205	0.546	0.211	0.729	0.301	0.248
Protein x xylanase	0.391	0.775	0.925	0.885	0.938	0.648	0.865
Protein x xylanase x gender	0.108	0.589	0.812	0.589	0.640	0.498	0.543

[1]Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, gender, two- and three-way interactions. Random effects included block and interactions of block with the three fixed effects.

[2]RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

[x, y] Main effect means sharing a common letter within a factor are not significantly different using a t-test ($P < 0.05$).

Table 7.7 Effect of protein source and xylanase supplementation on average daily feed intake (ADFI) of weaned pigs.

Item	ADFI (g/day/pig) g/day/pig				
	Week 1	Week 2	Week 3	Week 4	Week 5
Least squares means for main effects					
Protein source effect[2]					
SBM	151	417	746	853	1160
RCM	158	393	697	893	1162
DCM	137	384	670	877	1135
CCM	141	408	696	926	1190
Pooled SEM	6.20	31.56	23.12	35.29	46.39
Xylanase effect					
With xylanase	142	394	702	916	1153
Without xylanase	151	407	703	858	1171
Pooled SEM	3.69	26.95	13.70	23.18	27.91
Gender effect					
Barrows	146	406	700	894	1165
Gilts	148	395	705	881	1159
Pooled SEM	3.69	26.95	13.70	22.9	27.91
Protein x xylanase effect[2]					
SBM – no xylanase	160	425	778	839	1164
RCM – no xylanase	161	388	686	946	1195
DCM – no xylanase	159	396	629	933	1112
CCM – no xylanase	130	419	719	950	1226
SBM + xylanase	143	414	750	880	1215
RCM + xylanase	155	398	682	840	1124
DCM + xylanase	118	372	741	830	1175
CCM + xylanase	153	399	702	896	1156
Pooled SEM	10.11	41.79	37.78	61.72	90.01
Factors and significance[1]					
Protein source	0.187	0.748	0.294	0.584	0.913
Xylanase	0.234	0.568	0.955	0.149	0.745
Gender	0.750	0.643	0.854	0.733	0.924
Protein x gender	0.158	0.583	0.811	0.527	0.243
Xylanase x gender	0.678	0.195	0.433	0.459	0.458
Protein x xylanase	0.012	0.953	0.862	0.491	0.812
Protein x xylanase x gender	0.326	0.890	0.556	0.465	0.896

[1]Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, gender, two- and three-way interactions. Random effects included block and interactions of block with the three fixed effects.

[2]RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

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Table 7.8 Effect of protein source and xylanase supplementation on gain to feed ratio (G:F) of weaned pigs[1]

Item	G:F g gain/g feed				
	Week 1	Week 2	Week 3	Week 4	Week 5
Least square means for main effects					
Protein source effect[2]					
SBM	0.67	0.82	0.68	0.66	0.64
RCM	0.62	0.81	0.67	0.65	0.63
DCM	0.64	0.79	0.65	0.65	0.69
CCM	0.60	0.77	0.65	0.63	0.59
Pooled SEM	0.07	0.02	0.02	0.02	0.02
Xylanase effect					
With xylanase	0.61	0.81	0.66	0.66	0.63
Without xylanase	0.65	0.78	0.66	0.63	0.65
Pooled SEM	0.05	0.02	0.01	0.01	0.02
Gender effect					
Barrows	0.62	0.79	0.65	0.64	0.63
Gilts	0.64	0.80	0.67	0.65	0.65
Pooled SEM	0.05	0.02	0.01	0.01	0.02
Protein x xylanase interaction[2]					
SBM – no xylanase	0.65	0.82	0.69	0.66	0.64
RCM – no xylanase	0.63	0.79	0.66	0.63	0.63
DCM – no xylanase	0.60	0.76	0.62	0.61	0.71
CCM – no xylanase	0.58	0.76	0.67	0.61	0.60
SBM + xylanase	0.72	0.83	0.68	0.65	0.66
RCM + xylanase	0.61	0.82	0.67	0.68	0.62
DCM + xylanase	0.68	0.85	0.69	0.69	0.66
CCM + xylanase	0.61	0.78	0.64	0.64	0.58
Pooled SEM	0.09	0.04	0.03	0.02	0.02
Factors and significance[1]					
Protein source	0.809	0.291	0.785	0.642	0.054
Xylanase	0.513	0.151	0.747	0.054	0.345
Gender	0.796	0.471	0.438	0.348	0.513
Protein x gender	0.233	0.782	0.921	0.719	0.120
Xylanase x gender	0.119	0.064	0.427	0.730	0.520
Protein x xylanase	0.922	0.532	0.752	0.387	0.791
Protein x xylanase x gender	0.751	0.746	0.719	0.670	0.101

[1]Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, gender, two- and three-way interactions. Random effects included block and interactions of block with the three fixed effects.

[2]RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

The effect of protein source and xylanase supplementation on the ileal and colonic pH and the ATTD of nutrients is shown in Table 7.9. Ileum pH was higher ($P < 0.05$) in diets containing xylanase (6.66 vs. 6.27). Additionally, a tendency ($P < 0.10$) for higher ileal pH in diets fed to barrow, was observed. A three-way interaction for protein source-xylanase-gender for colonic digesta pH was observed (Figure 7.1), as can be seen, the means pH in digesta of pigs fed CCM, DCM, RCM and SBM behave in a different way in barrows compared to gilts, especially in the case of DCM and RCM. Furthermore, colonic pH was higher ($P < 0.05$) in diets containing xylanase (5.93 vs. 5.74). Protein source affected colonic pH, and diets containing DCM and SBM had higher pH ($P < 0.05$; 6.02 and 5.92, respectively) compared to diets containing RCM and CCM (5.68 and 5.73, respectively). A tendency ($P < 0.10$) for a two-way interaction protein-gender for colonic pH, was observed.

The addition of xylanase resulted in higher ($P < 0.05$) ATTD of protein, diet, DM, P and Ca, compared to diets without xylanase (75 vs. 70%, 80 vs. 78%, 80 vs. 77%, 49 vs. 45% and 62 vs. 52%, respectively). Protein source influenced ($P < 0.05$) diet, DM, and Ca digestibility. A tendency ($P < 0.10$) for higher protein digestibility when pigs were fed SBM containing diets was observed. A two-way interaction protein-xylanase ($P < 0.05$) for ATTD of NDF was observed, and xylanase supplementation to diets containing RCM and DCM resulted in increased ATTD of NDF from 28 to 31% and from 30 to 38%, respectively (Figure 7.2). Additionally, a two-way interaction xylanase-gender ($P < 0.05$) for ATTD of NDF, was also observed, and ATTD of NDF increased when xylanase was fed to gilts from 30 to 35%, whereas it decreased from 36 to 30% when fed to barrows (Figure 7.3). However, in general, diets containing SBM as protein source had higher digestibility coefficients than the others.

Table 7.9 Effect of protein source and xylanase supplementation on ileum and colon pH and apparent total tract digestibility (ATTD) of nutrients of weaned pigs.

	pH		Digestibility (%)					
	Ileum	Colon	CP	NDF	Diet	DM	P	Ca
Least square means for main effects								
Protein source effect								
SBM	6.35	5.92	75	37	82^{x}	81^{x}	51	66^{x}
RCM	6.36	5.68	71	30	78^{y}	76^{y}	44	52^{y}
DCM	6.50	6.02	72	33	79^{y}	77^{y}	47	57^{xy}
CCM	6.66	5.73	72	30	78^{y}	78^{xy}	46	54^{y}
Pooled SEM	0.14	0.11	0.98	2.40	0.71	1.07	1.76	2.92
Addition of xylanase effect								
With xylanase	6.66^{x}	5.93	75^{x}	33	80^{x}	80^{x}	49^{x}	62^{x}
Without xylanase	6.27^{y}	5.74	70^{y}	33	78^{y}	77^{y}	45^{y}	52^{y}
Pooled SEM	0.12	0.10	0.61	1.79	0.42	0.74	1.05	1.80
Gender effect								
Barrows	6.56	5.81	72	33	79	78	48	58
Gilts	6.37	5.87	72	32	79	78	47	55
Pooled SEM	0.12	0.10	0.58	1.79	0.42	0.74	1.02	1.80
Protein x xylanase interaction[2]								
SBM – no xylanase	6.08	5.73	73	37	80	79	52	64
RCM – no xylanase	6.18	5.78	68	28	76	74	42	49
DCM – no xylanase	6.25	5.76	70	30	77	75	41	48
CCM – no xylanase	6.54	5.65	70	36	78	77	44	46
SBM + xylanase	6.61	6.05	78	37	84	83	51	67
RCM + xylanase	6.55	5.78	73	31	79	77	47	56
DCM + xylanase	6.73	6.45	75	38	80	79	51	62
CCM + xylanase	6.76	5.80	73	24	79	80	48	63
Pooled SEM	0.16	0.12	1.54	3.19	1.21	1.51	2.82	3.49
Factors and significance[1]								
Protein source	0.105	0.018	0.059	0.159	0.007	0.038	0.137	0.045
Xylanase	0.002	0.020	0.002	0.920	0.015	0.011	0.042	0.007
Gender	0.092	0.376	0.985	0.770	0.770	0.825	0.396	0.385
Protein x gender	0.631	0.086	0.358	0.114	0.121	0.537	0.138	0.632
Xylanase x gender	0.360	0.503	0.397	0.029	0.184	0.108	0.762	0.640
Protein x xylanase	0.733	0.300	0.471	0.042	0.514	0.937	0.075	0.265
Protein x xylanase x gender	0.252	0.006	0.706	0.925	0.955	0.508	0.710	0.240

[1]Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, gender, two-and three-way interactions. Random effects included block and interactions of block with the three fixed effects.

[2]RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).

[x, y] Main effect means sharing a common letter within a factor are not significantly different using a t-test ($P < 0.05$).

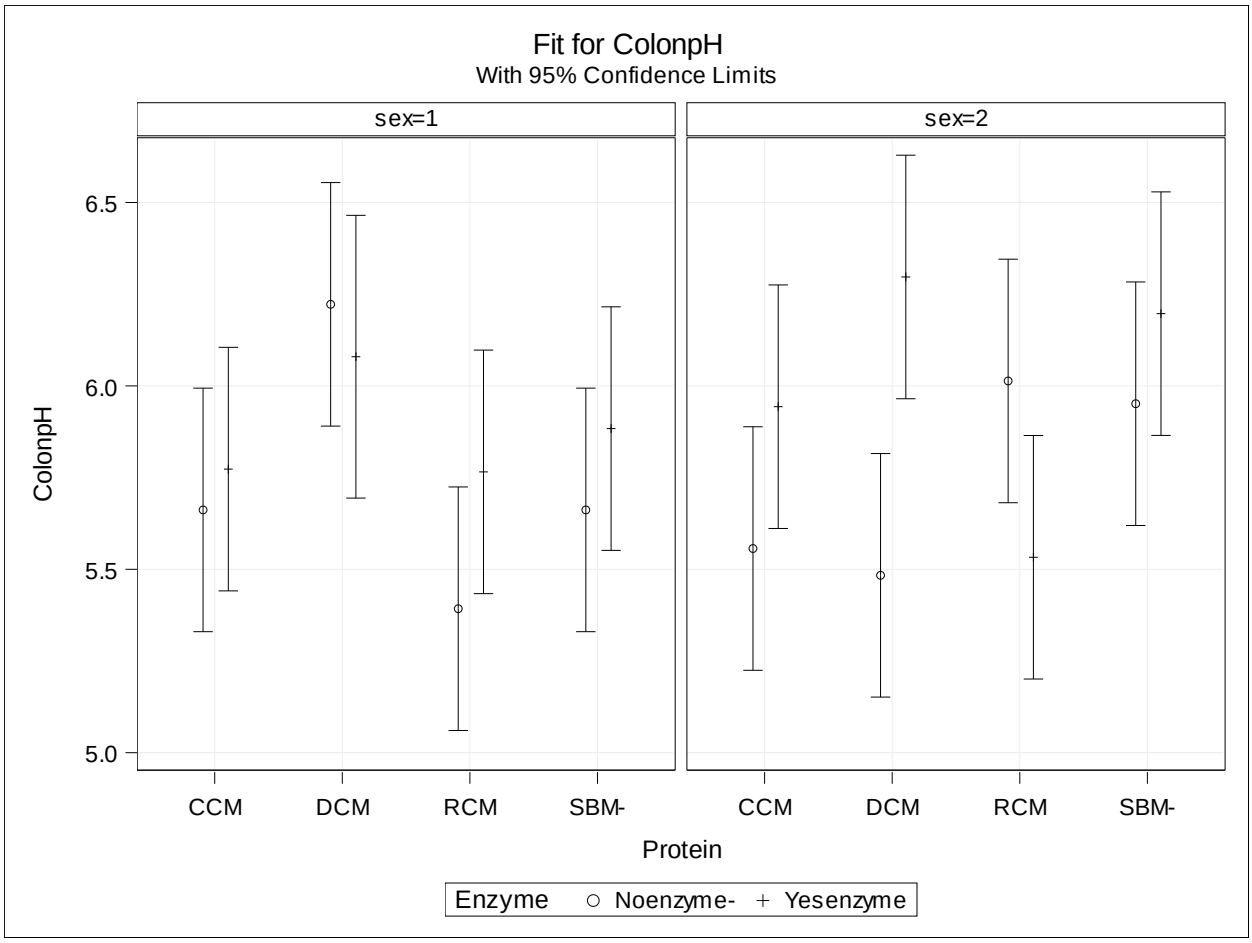

Figure 7.1. Three-way interaction for protein-xylanase-gender for colonic digesta pH of growing pigs fed wheat-based diets.
Sex 1 = barrows. Sex 2 = gilts.

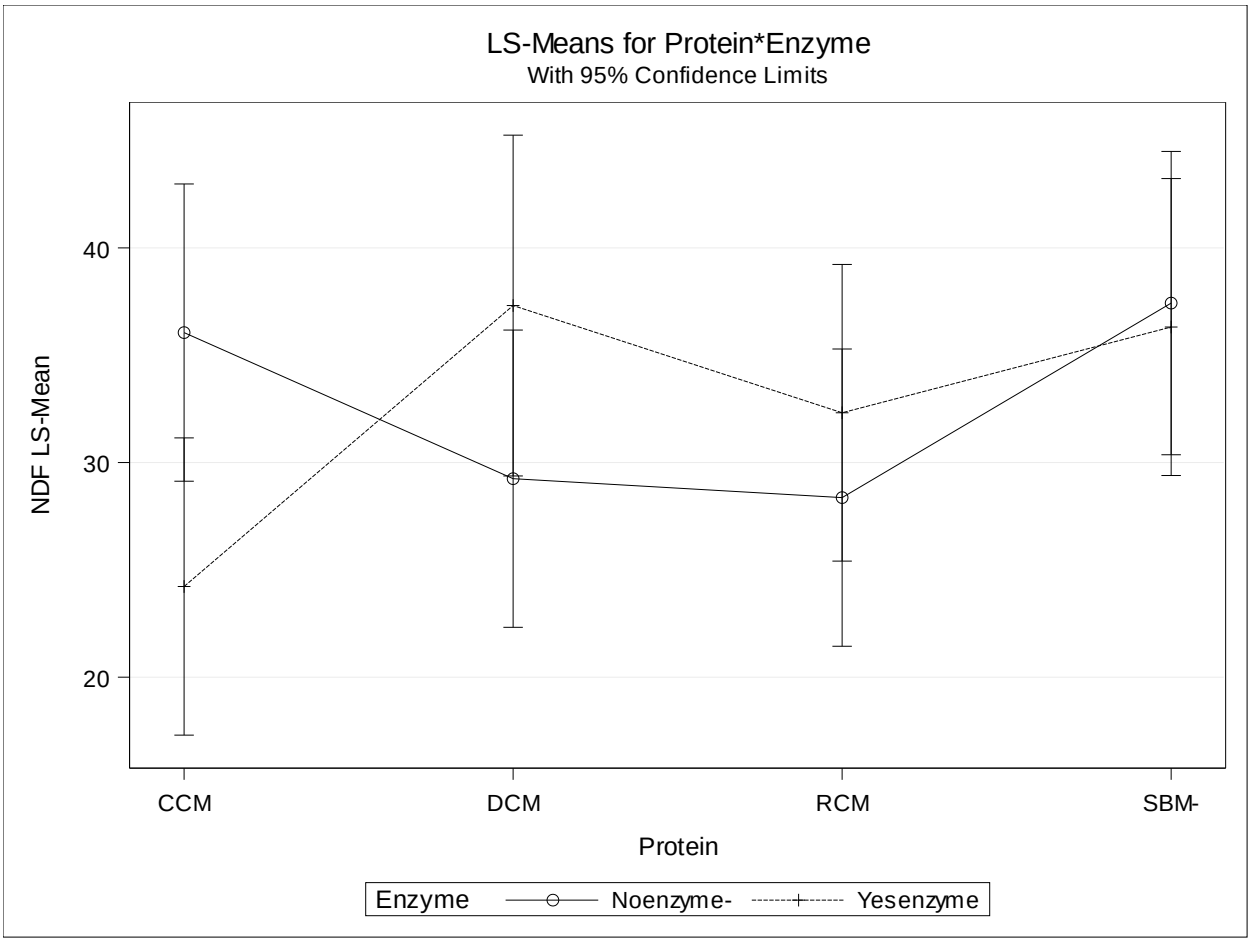

Figure 7.2. Two-way interaction for protein-enzyme for ATTD of NDF of growing pigs fed wheat-based diets.
Sex 1 = barrows. Sex 2 = gilts.

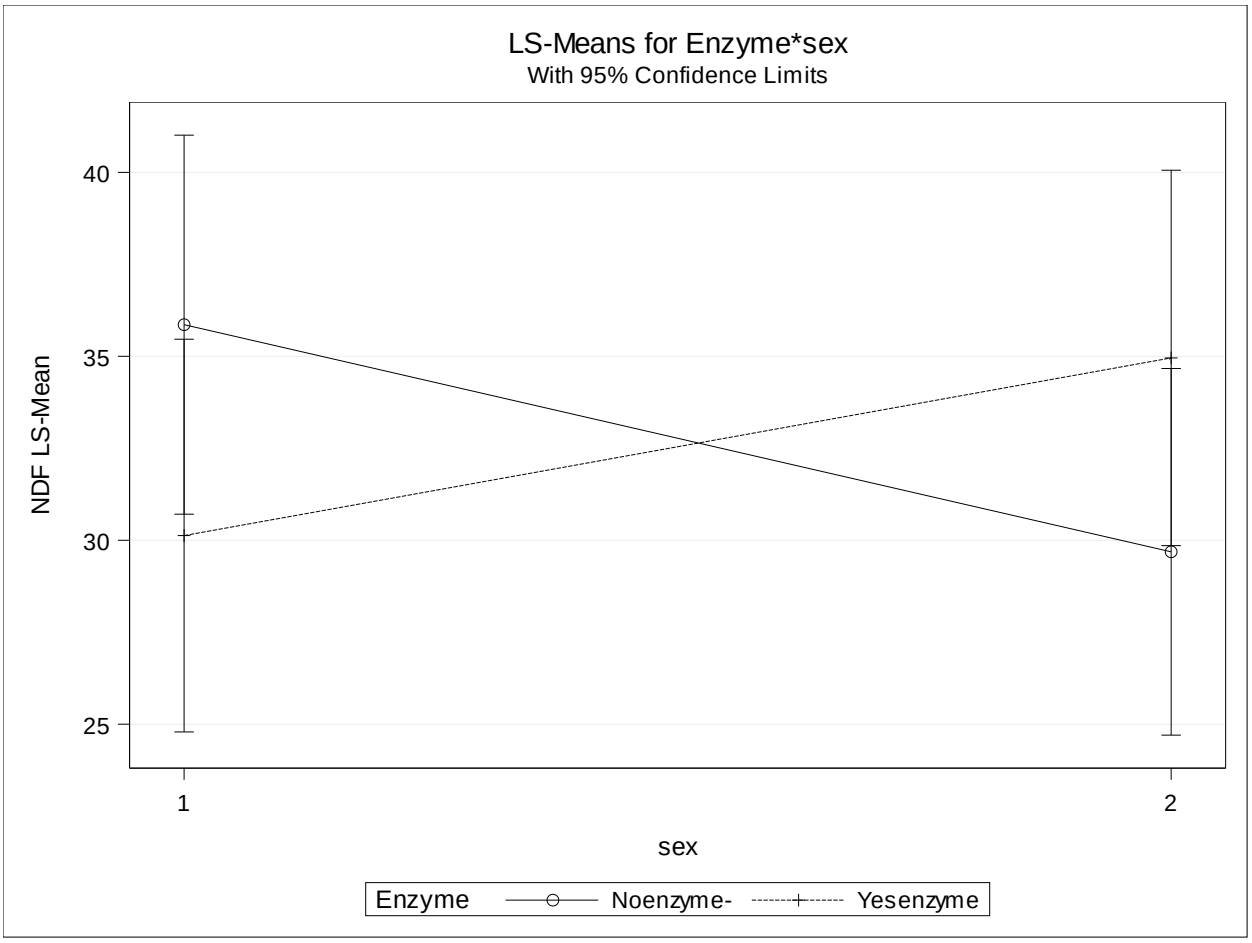

Figure 7.3. Two-way interaction for enzyme and sex for ATTD of NDF of growing pigs fed wheat-based diets.
Sex 1 = barrows. Sex 2 = gilts.

The effect of protein source and the use of xylanase on organ weights are shown in Table 7.10. Protein source had no effect ($P > 0.10$) on the relative weight of all organs measured, except ($P < 0.05$) liver and spleen. The liver of pigs fed RCM and DCM had higher ($P < 0.05$) relative weight (36.63 and 37.12 g/kg/BW) than pigs fed diets containing SBM and CCM (34 and 34.96 g/kg/BW). Whereas the relative weight of spleens in pigs fed diets containing SBM and RCM were 2.57 and 2.28 g/kg/BW, compared to 1.96 and 1.92 g/kg/BW in pigs fed DCM and CCM,

respectively. Additionally, protein source resulted in a tendency ($P < 0.10$) for higher relative weight of kidney when pigs were fed diets containing DCM compared to the other protein sources.

The relative weights of the liver and spleen were influenced by xylanase supplementation ($P < 0.05$), increasing the size of both organs. Xylanase supplementation tended to increase the relative weights of the kidney ($P < 0.10$; 6.39 vs. 6.02). Additionally, a tendency ($P < 0.10$) in the two-way interaction protein-xylanase for the relative weight of stomach, was observed, and as can be seen in Figure 7.4, the addition of xylanase to diets containing DCM increased the relative size of stomach from 8.03 to 8.41 g/kg/BW. Moreover, a tendency ($P < 0.10$) in the two-way interaction protein-gender for the relative weight of small intestine was observed, xylanase supplementation increased the relative weight of small intestine in barrows from 43.2 to 45.7 g/kg/BW, whereas in gilts small intestine decreased from 47.3 to 42.8 g/kg/BW (Figure 7.5).

Table 7.10 Effect of protein source and xylanase supplementation on the relative organ weights (g/kg/BW) of nursery pigs[1]

	Heart	Lungs	Thyroid gland	Liver	Spleen	Kidney	Stomach	Small Intestine	Large Intestine
Least squares means for main effects									
Protein source effect									
SBM	5.64	12.88	0.09	34.00^{y}	2.57^{x}	6.09	8.04	44.48	20.06
RCM	5.63	12.82	0.09	36.63^{x}	2.28^{x}	6.02	7.90	43.33	19.87
DCM	5.53	12.64	0.07	37.12^{x}	1.96^{y}	6.74	8.49	46.31	20.37
CCM	5.27	12.85	0.05	34.96^{y}	1.92^{y}	5.96	7.62	44.94	19.63
Pooled SEM	0.14	0.62	0.02	0.51	0.12	0.20	0.26	1.53	1.82
Addition of xylanase effect									
With xylanase	5.58	13.34	0.07	36.29^{x}	2.36^{x}	6.39	7.94	44.26	18.85
Without xylanase	5.45	12.26	0.07	35.06^{y}	2.01^{y}	6.02	8.09	45.27	21.12
Pooled SEM	0.09	0.37	0.01	0.34	0.08	0.14	0.15	1.03	1.54
Gender effect									
Barrows	5.50	13.11	0.09	36.01	2.21	6.17	8.11	44.44	20.20
Gilts	5.54	12.48	0.06	35.34	2.16	6.24	7.92	45.09	19.77
Pooled SEM	0.09	0.38	0.01	0.34	0.08	0.14	0.16	1.03	1.35
Protein x xylanase interaction[2]									
SBM – no xylanase	5.76	12.33	0.07	33.88	2.28	6.13	8.47	46.20	20.80
RCM – no xylanase	5.50	13.05	0.12	36.67	2.25	6.12	8.20	44.22	19.42
DCM – no xylanase	5.33	12.15	0.04	36.00	1.72	6.60	8.03	44.88	22.36
CCM – no xylanase	5.18	11.45	0.04	33.72	1.76	5.34	7.40	40.82	21.24
SBM + xylanase	5.69	13.25	0.10	33.52	2.87	5.92	7.55	42.79	18.99
RCM + xylanase	5.77	12.60	0.05	37.03	2.32	6.03	7.64	41.52	19.13
DCM + xylanase	6.08	15.18	0.08	37.26	2.20	6.70	8.41	45.31	19.80
CCM + xylanase	5.28	14.09	0.06	36.09	2.90	6.45	7.98	40.25	18.40
Pooled SEM	0.33	0.61	0.03	0.68	0.27	0.26	0.40	1.78	1.47
Factors and significance[1]									
Protein source	0.390	0.996	0.463	0.004	0.010	0.078	0.308	0.707	0.984
Xylanase	0.437	0.154	0.818	0.041	0.016	0.092	0.622	0.552	0.117
Gender	0.795	0.393	0.168	0.239	0.671	0.706	0.537	0.705	0.757
Protein x gender	0.046	0.555	0.684	0.363	0.085	0.206	0.245	0.664	0.915
Xylanase x gender	0.426	0.918	0.662	0.439	0.397	0.422	0.684	0.054	0.689
Protein x xylanase	0.845	0.494	0.252	0.175	0.484	0.132	0.093	0.330	0.954
Protein x xylanase x gender	0.306	0.753	0.621	0.298	0.114	0.584	0.299	0.849	0.669

[1] Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, gender, two- and three-way interaction. Random effects included block and interactions of block with the three fixed effects.
[2]RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal).
[x, y] Main effect means sharing a common letter within a factor are not significantly different using a t-test ($P < 0.05$)

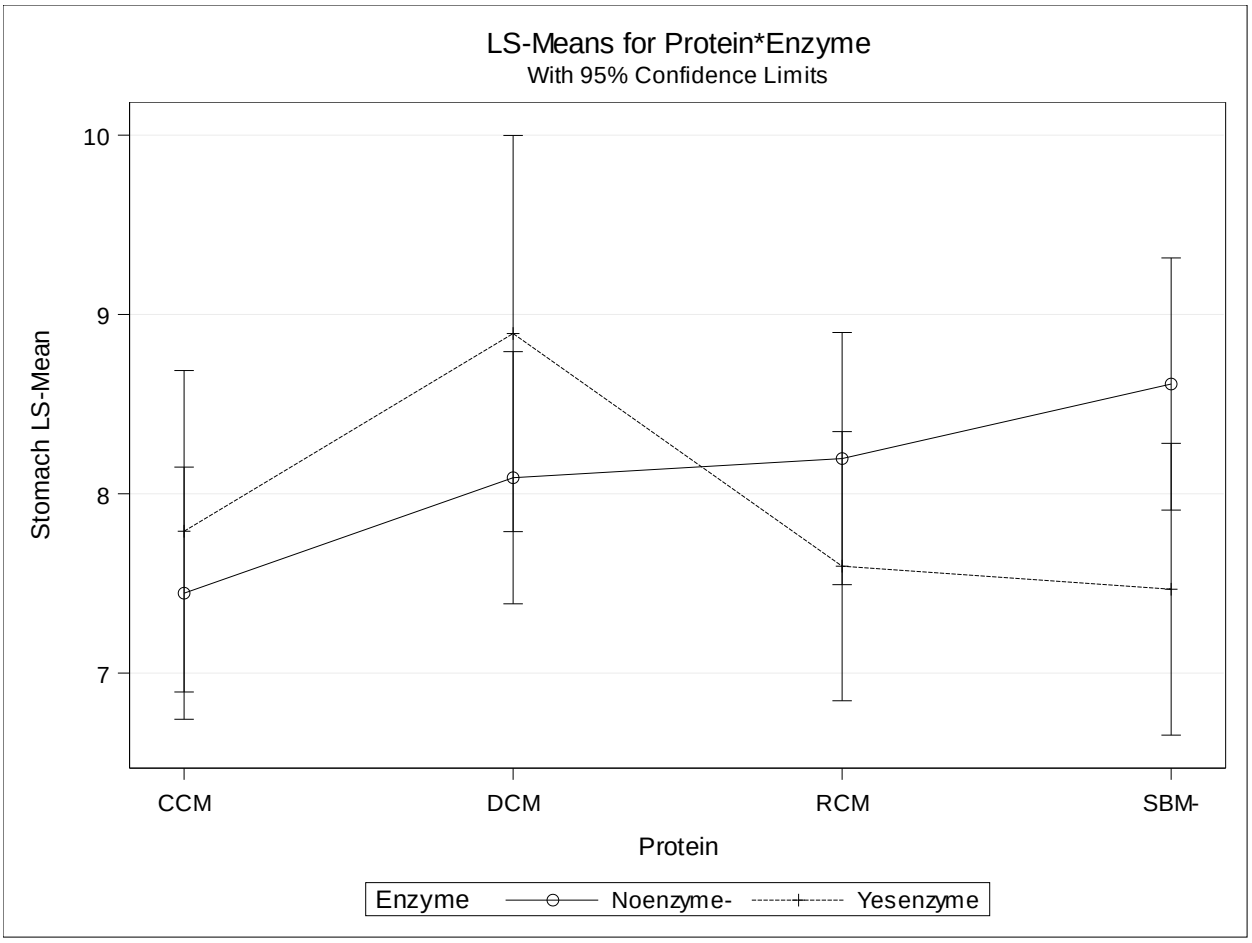

Figure 7.4. Two-way interaction for protein-enzyme for the relative weight of stomach of growing pigs fed wheat-based diets.

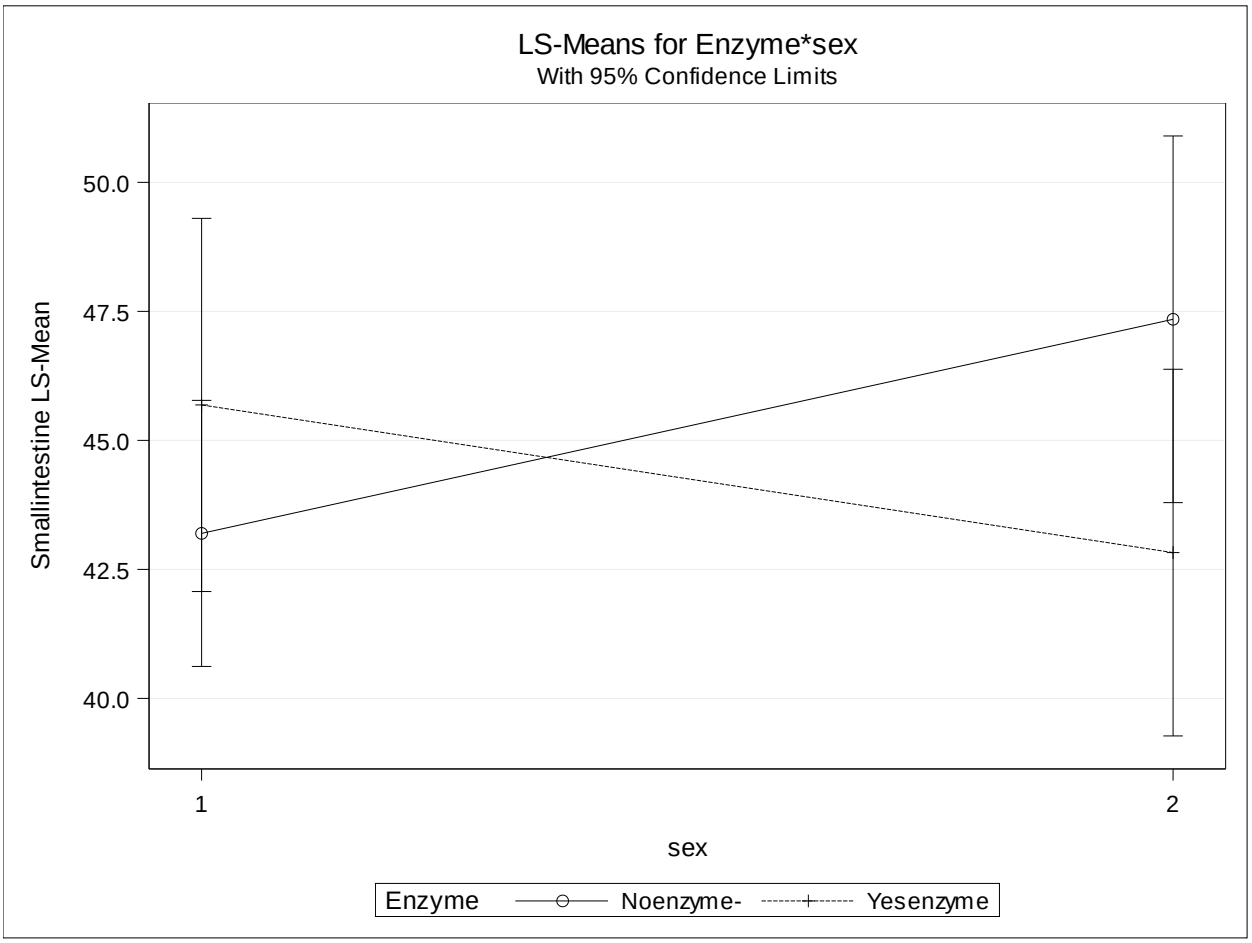

Figure 7.5. Two-way interaction for enzyme and sex for the relative weight of small intestine of growing pigs fed wheat-based diets.
Sex 1 = barrows. Sex 2 = gilts.

7.4.3 Effect of protein source and xylanase supplementation on the levels of short-chain fatty acids

The effect of protein source, gender, the use of xylanase, and their two- and three-way interactions on the levels of SCFA are shown in Table 7.11. A significant interaction for propionic acid was observed. Xylanase supplementation reduced ($P < 0.0001$) propionic acid in all diets, with higher ($P < 0.0001$) concentrations in the digesta of pigs fed RCM as the protein source. Two-way interactions ($P < 0.05$) for protein-gender and protein-xylanase were observed. Additionally, a three-way interaction protein-xylanase-gender for propionic acid was observed as can be seen in Figure 7.6, the concentration of propionic acid differs not just according to protein source and xylanase supplementation, it also differs according to sex, with the higher difference of media in the case of DCM in gilts. A tendency ($P < 0.10$) of the main factors and its two-way interactions for acetic acid was observed. However, a significant effect ($P < 0.05$) for the three-way interaction protein-xylanase-gender was observed, as can be seen in Figure 7.7. Valeric acid concentrations were influenced by protein source ($P < 0.01$). Pigs fed with RCM and DCM had higher concentrations; CCM had intermediate concentrations, while SBM had the lowest concentration (3.7 vs. 3.6 vs. 2.5 vs. 1.7 mM). Overall, xylanase supplementation reduced butyric acid concentration in diets (18.8 vs. 15.5 mM; $P < 0.10$).

Table 7.11 Effect of protein source and xylanase supplementation on the levels of short-chain fatty acids in mid colon of nursery pigs[1]

	Total SCFA[2]	Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid	Lactic acid[3]	BCFA[4]
Least squares means for main effects							
Protein source effect[6]							
SBM	135.2	54.4	36.2	17.6	1.7^{y}	5.2	2.3
RCM	138.9	56.3	41.0	17.5	3.7^{x}	3.0	1.5
DCM	126.0	50.2	36.2	16.3	3.6^{x}	5.0	1.7
CCM	125.3	53.8	32.9	17.2	2.5^{xy}	4.7	1.6
Pooled SEM	7.39	1.57	2.03	1.60	0.34	0.63	0.26
Addition of xylanase effect							
With xylanase	129.3	55.2	34.7	15.5	2.7	5.1	1.6
Without xylanase	133.4	52.2	38.4	18.8	3.1	3.9	1.9
Pooled SEM	6.36	0.94	1.97	1.0	0.21	0.38	0.20
Gender effect							
Barrows	132.8	51.7	37.0	18.4	3.1	4.2	1.7
Gilts	129.9	54.0	36.2	15.9	2.6	4.7	1.8
Pooled SEM	6.36	1.07	1.97	1.00	0.21	0.39	0.20
Protein x xylanase interaction[5]							
SBM – no xylanase	141.4	52.9	38.2	20.4	1.7	5.8	2.6
RCM – no xylanase	139.1	53.8	40.3	19.4	4.1	2.0	1.8
DCM – no xylanase	130.2	51.5	39.1	16.0	3.3	3.4	1.8
CCM – no xylanase	122.6	47.4	34.3	20.0	2.9	4.6	1.6
SBM + xylanase	129.1	56.2	32.6	14.3	1.5	5.3	2.0
RCM + xylanase	138.4	52.9	39.7	16.4	3.2	3.9	1.1
DCM + xylanase	120.4	49.3	32.3	16.0	3.4	6.8	1.6
CCM + xylanase	128.3	55.3	33.0	14.9	2.0	5.1	1.7
Pooled SEM	9.62	1.19	3.11	2.17	0.63	1.05	0.40
Factors and significance[1]							
Protein source	0.229	0.057	<0.0001	0.959	0.007	0.108	0.149
Xylanase	0.447	0.060	<0.0001	0.085	0.317	0.123	0.181
Gender	0.583	0.893	0.260	0.176	0.234	0.516	0.682
Protein x gender	0.647	0.064	<0.0001	0.963	0.354	0.063	0.697
Xylanase x gender	0.647	0.089	0.111	0.449	0.277	0.130	0.647
Protein x xylanase	0.647	0.089	0.018	0.449	0.277	0.698	0.650
Protein x xylanase x gender	0.111	0.015	<0.0001	0.174	0.113	0.339	0.1056

[1] Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, gender, two- and three-way interactions. Random effects included block and interactions of block with the three fixed effects.

[2]Short chain fatty acids;

[3] Non-volatile;

[4] Branched-chain fatty acids: includes isovaleric, isobutyric and 2-methyl-butyric acids;

[5]RCM (regular canola meal); DCM (dehulled canola meal,); CCM (coarse canola meal)

[x, y, z.] Main effect means sharing a common letter within a factor are not significantly different using a t-test ($P < 0.05$).

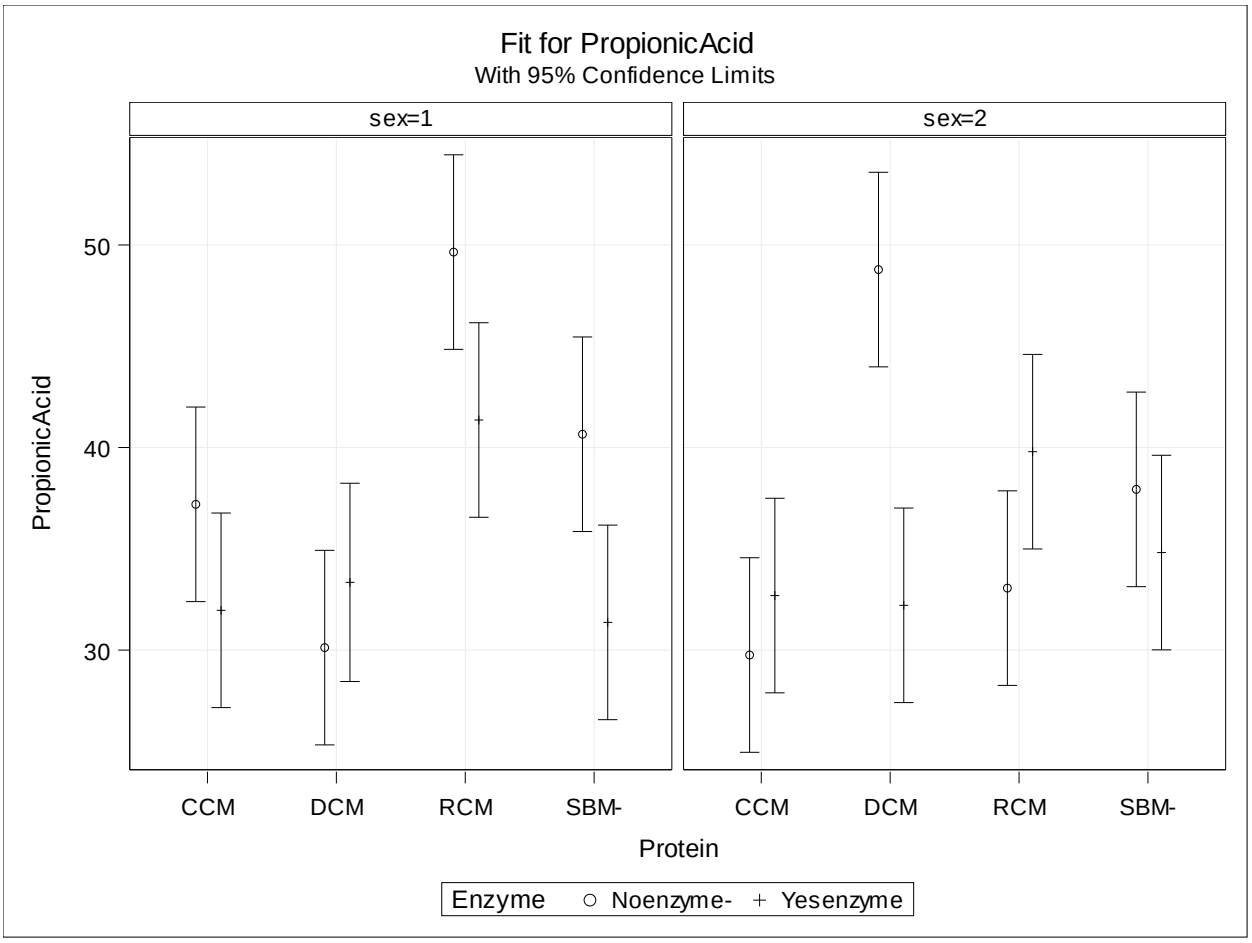

Figure 7.6. Three-way interaction protein-xylanase-gender for propionic acid concentration in colon digesta of growing pigs fed wheat-based diets.
Sex 1 = barrows. Sex 2 = gilts.

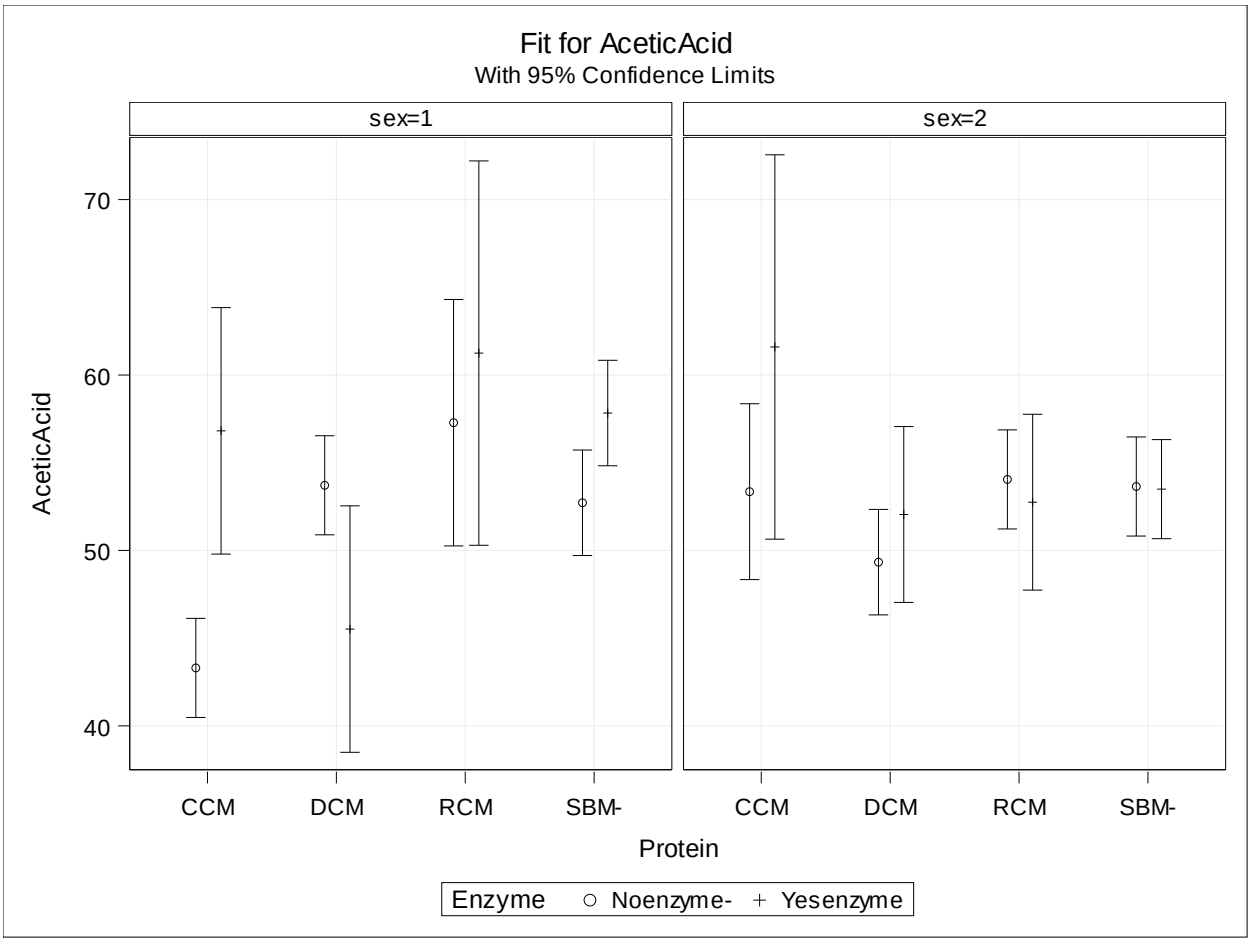

Figure 7.7. Three-way interaction protein-xylanase-gender for acetic acid concentration in colon digesta of growing pigs fed wheat-based diets.
Sex 1 = barrows. Sex 2 = gilts.

7.5 DISCUSSION

7.5.1 Chemical composition of canola meal and diets

The use of CM in monogastric animal nutrition has been limited due to the presence of anti-nutritive factors, among them, high dietary fiber content. It has been estimated that CM contains 3 times higher amount of fiber than SBM (Bell, 1993; Khajali and Slominski, 2012). Fiber includes NSP, lignin associated with polyphenols, polyphenols glycoproteins, and minerals (Simbaya, 1996). The fiber present in fibrous feed ingredients can reduce nutrient digestibility through encapsulation, resulting in lower feed efficiency and growth (Kerr and Shurson, 2013).

However, with the incorporation of NSP-degrading enzymes (NSPases) to the diet, those effects can be reduced. Nevertheless, the beneficial impact of NSPases, such as xylanase in swine diets has been inconsistent (Woyengo et al., 2008; Passos et al., 2015; Taylor et al., 2018). A systematic review and meta-analysis of the effect of feed enzymes on growth and nutrient digestibility in growing and finisher pigs found that G:F was improved in 32% of the studies, including growth data. Additionally, DM, and GE AID, and ATTD were improved by xylanase, xylanase + ß-glucanase, mannanase and protease dietary supplementation. Dietary supplementation of xylanase + ß-glucanase did not affect the ADG, ADFI, and G:F (Torres-Pitarch et al., 2019). Another noteworthy enzyme used in swine nutrition is phytase. The efficacy of phytase to increase the digestibility of P and reduce its excretion in feces has been widely documented, furthermore, the digestibility of other nutrients bound to phytate can also improve (Jongbloed et al., 1992, Kies et al., 2001; Kies, 2005, Adhikari et al., 2016). Therefore, given the extensive use of phytase in swine nutrition, all diets in the study contained phytase at 500 FTU/kg; therefore, any improvement in growth performance or digestibility of nutrients would be attributed solely to the use of xylanase. In the formulation of the diets for the current study, protein sources with distinctive nutrient value were utilized, such as the case of DCM, which was higher in CP, total P, non-phytate, and phytate P, and NSP contents, compared to the RCM and CCM. The total fiber content of the meals was 24.57%, 33.01%, and 37.25% for DCM, RCM, and CCM, respectively, which were high when compared to the dehulled, solvent-extracted SBM (16.71%; NRC, 2012). Higher efficiency derived from the use of xylanase has been shown for diets containing a high content of fiber and NSPs (Yin et al., 2000; Nortey et al., 2007; Ndou et al., 2015; Zhang et al., 2018).

7.5.2 Effect of protein source and xylanase supplementation on growth performance, organ weight, nutrient digestibility, and digesta pH

The protein source and the use of xylanase or gender did not affect pig growth performance for the 5 weeks of the trial. However, in week 2 a tendency ($P < 0.10$) in the two-way interaction xylanase-gender, was observed, and barrows fed diets supplemented with xylanase increased G:F from 0.755 to 0.824, whereas G:F in gilts went from 0.81 to 0.80. In week 4, a tendency for higher G:F when pigs were fed diets supplemented with xylanase, was observed. Additionally, in week 5, a tendency for higher G:F ratio when pigs were fed DCM as the protein source, was observed. Nevertheless, its important to mention that the piglets used in the current study were weaned at 18 ± 2 days of age, a very immature microbiota may imbalance the ability of the animal to handle the fermentation activity, which may have influenced the ability of the GIT microbiota of the piglets to face the fermentable oligosaccharides produced through the action of xylanase. We can speculate that if the piglets would have been weaned at 21 d of age or older, we may have observed effects on growth performance. Furthermore, the piglets were fed for only 35 days, and having the pigs on trial for a more extended period would help to know better about the potential of the enzyme on growth performance. Additionally, the diets met the nutrient requirements of swine, according to NRC (2012). However, even when an improvement in growth performance parameters was not observed, improvement in digestibility outcomes was observed. Improved nutrient digestibility, volatile fatty acid concentrations, and bacteria ratio in the large intestine resulted in improved growth performance when pigs were fed diets containing an enzyme complex containing amylase, protease, and xylanase (Yi et al., 2013)

Lower CP in diets containing CCM in phase II, had no adverse effect on growth performance, as all diets were formulated according to the SID of AA, STTD of P, and NE

contents. Improvement of growth performance was observed with the use of xylanase and ß-glucanase blend in nursery pelleted wheat- and barley-based diets deficient in digestible energy (Owusu-Asiedu et al., 2012). Furthermore, Kiarie et al. (2012) demonstrated that xylanase and ß-glucanase blend applied to mixed grain diets deficient in energy, improved growth performance, which is linked to an increase in nutrient digestibility.

In the present study, the use of xylanase resulted in a significant increase in the ATTD of protein (from 70 to 75%). It has been determined that higher CP digestibility is associated to higher AA digestibility, as can be observed from a study by Stein et al. (2001) that reported SID of CP and AA of 75.2% ± 2.6 and 83.6% ± 2.1, respectively, for wheat fed to growing pigs. However, SID of CP and AA increased to 82.8% ± 2.5 and 89.5% ± 2.7, respectively, when wheat was fed to gestating sows. Implicating that to maximize the use of phytase and xylanase in swine diets its essential to determine the SID of AA of the cereal-based diets as well as the protein used when supplemented with such exogenous enzymes, to properly formulate diets according to SID of AA.

A two-way interaction of protein-xylanase for the ATTD of NDF was observed, and xylanase supplementation increased NDF digestibility from 28 to 31% and from 30 to 38% for RCM and DCM, respectively. No increase in NDF digestibility was observed when pigs were fed diets containing SBM and CCM. A two-way interaction of enzyme-gender for the ATTD of NDF was also observed, and overall, supplementing xylanase to diets fed to gilts resulted in higher NDF digestibility.

Results in the present study are consistent with findings by Nortey et al. (2007), indicating increased energy, AA, and P digestibility with the use of xylanase and phytase in wheat byproducts. Weiland (2017) determined that the use of xylanase increased the ATTD of ADF in high fiber diets, increasing the hindgut disappearance of NDF, ADF, and hemicellulose, increased

the AID of DM, starch, and nitrogen, and tended to increase the AID of GE in low fiber diets. Passos et al. (2015), using xylanase on corn-SBM diets observed increases in apparent ileal digestibility of NDF, DM, and OM. The results are also consistent with findings by Dong et al. (2018) indicating increased nutrient digestibility with xylanase supplementation; however, the author also found that supplemental xylanase (2,000 U/kg) increased growth performance, decreased the richness of gut bacteria while diminishing the growth of pathogenic bacteria.

The pH of colonic digesta was affected by protein source and xylanase supplementation, furthermore, a three-way interaction for protein-xylanase-gender, and a tendency for a two-way interaction protein-gender were observed. Whereas, for ileal pH, a xylanase supplementation effect and a tendency for gender, were observed. The effects of xylanase increasing colonic pH is consistent with results by Taylor et al. (2018) that found higher pH values when diets were supplemented with 8,000 and 16,000 BXU/kg xylanase. However, the increase in pH in colon and ileal digesta with the use of xylanase is challenging to explain, since less Ca and P in solution would result in an increase in acidity rather than a decrease, because higher Ca and P in solution would have a buffer effect (Metzlr-Zebeli et al., 2010). The breakdown of fiber in the colon indicated by higher ATTD of Ca and P when diets were supplemented with xylanase (from 52 to 62% and from 45 to 49%, respectively), could result in increased Ca absorption in the colon of pigs. Calcium absorption could happen along the digestive tract. Zhao et al. (2019) studied the molecular distribution of porcine Ca sensing receptors (pCaSR); finding that pCaSR are distributed along the longitudinal axis of the digestive tract, but mostly located in the epithelia of the stomach, duodenum, jejunum, ileum, and colon. The pCaSR are responsive to changes in the extracellular Ca concentration, and it is involved in Ca homeostasis (Saidak et al., 2009). The small intestine accounts for approximately 90% of the Ca absorption (Wasserman, 2004; Schröder and Breves,

2006), while the stomach or the large intestine can take up 10% of the total Ca absorbed (Barger-Lux et al., 1989; Metzler-Zebeli et al., 2010; Zhao et al., 2019). However, the large intestine may become the main site for Ca absorption if NSP fractions are included in the diet that interferes with cation absorption in the small intestine (Metzler-Zebeli et al., 2010).

Additionally, higher ATTD digestibility of diet and DM observed when xylanase was supplemented without improved growth performance confirms the importance to determine, not just SID or AA, but also its effect on net energy, Ca, and P digestibility, when diets are supplemented with phytase plus xylanase. The addition of microbial phytase has shown to increase the apparent and standardized total tract digestibility of Ca in Ca supplements fed to growing pigs, and the apparent total tract digestibility of P in the diets (González-Vega et al., 2015). Higher digestibility of Ca in swine diets supplemented with phytase and xylanase as observed in the present study can represent an excess of available Ca, which has been indicated as detrimental to the growth performance of pigs unless P is also included above the requirements (Merriman et al., 2017). When dietary STTD P was above the requirements, the adverse effects on ADG and G:F of increased STTD Ca was observed if dietary STTD Ca exceeded 0.6% (Lagos et al., 2019).

Furthermore, a recent extensive survey analyzing commercial pig-broiler diets indicated an excess of 0.22 percentage units of Ca (Walk, 2016). An excess of Ca in diets can have a detrimental effect on feed intake and average daily gain in finishing pigs when diets are balanced according to P requirements. The addition of phytase plus xylanase in the present Exp. further increased the digestibility of Ca and P in diets containing CM or its fractions as a protein source. Therefore, it is crucial to determine the impact of supplementing diets containing CM with microbial phytase and xylanase, not just on growth performance, as well on the apparent and standardized total tract digestibility of Ca and P, to properly formulate diets and to maximize

growth performance of growing pigs, therefore, preventing adverse effects derived from excess Ca in diets (Lee et al., 2019). The STTD of Ca:STTD of P ratio required to maximize growth performance of 11 to 25 kg pigs has been determined to be less than 1.40:1. Furthermore, increased dietary Ca increases plasma Ca concentration and reduces the abundance of genes associated with transcellular absorption and transport of Ca in the duodenum, but may increase paracellular absorption (Lagos et al., 2019).

Protein source and the addition of xylanase affected the liver and spleen size without affecting growth performance. A significant increased in the relative size of liver and spleen when xylanase was added to the diets (from 35.06 to 36.29 and from 2.01 to2.36 g/kg BW, respectively) compared to unsupplemented diets regardless of protein source, was observed. Therefore, such increases can be attributed to the effect of xylanase in the release of anti-nutritive factors such as glucosinolates in CM, which can break down into toxic products such as thiocyanate, isothiocyanate, oxazolidinethione (goitrin) and nitriles, which may be formed and reduce feed intake and growth performance, also affect thyroid function by constraining thyroid hormone production and impair liver and kidney function (Bell, 1993). Higher GSL content in dehulled CM compared to its parent meal (9.2 vs. 9.6; Mejicanos et al., 2017) has been observed.

Pigs fed diets containing SBM and RCM had heavier spleens than pigs fed diets containing DCM and CCM. The oversized spleen can be related to over-activity of the spleen's function of destroying blood cells; however, the spleen also can store healthy erythrocytes, and serve as reservoir place for platelets (Clendening, 1930; Sherwood, 1997). Additionally, a tendency for increased relative weight of kidney due to dietary supplementation of xylanase and protein source was observed. Kidneys tended to have increased relative weight when DCM was the protein source. The amount of fiber in the diet has been related to increases in the relative weights of

organs. In a study feeding pigs high fiber diets, Anugwa et al. (1989) observed increased stomach, liver, and kidneys size. Likewise, Nyachoti et al. (2000) feeding high fiber diets to pigs found an increase in the relative size of liver, colon, and caecum, compared to pigs fed casein-corn starch diet. Feeding high-fiber diets would increase the secretion of digestive juices and enzymes, with implications on the increase in the workload of secretory organs, leading to hypertrophy (Agyekum et al., 2017). However, the tendency in the interaction between protein source and xylanase indicated that the relative weight of stomach of piglets fed DCM and CCM supplemented with xylanase were heavier.

7.5.3 Effect of protein source and xylanase supplementation on the levels of short-chain fatty acids

Short-chain fatty acids are the principal end-products of gut microbiota, and the main SCFA resulting from carbohydrate and AA fermentation are acetic, propionic, and butyric acid. The catabolism of branched-chain AA valine, leucine, and isoleucine are related to the production of formate, valerate, caproate, and branched-chain fatty acids (isovaleric, isobutyric and 2-methyl-butyric acids; Macfarlane and Macfarlane, 2003). The total SCFA production was not affected by the protein source, gender or the addition of xylanase. The total concentrations of SCFA were in the range of 120.4 to 141.4 mM, which is consistent with observations by Topping and Clifton (2001), indicating that depending on the diet, the total concentration of SCFA in the proximal colon decreases from 70 to 140 mM to 20 to 70 mM in the distal colon due to absorption. However, in the present study, total SCFA concentration was higher than those reported by Cardona et al. (2005) for piglets 35-d of age, raised indoors (30.3 to 75.5 mM). Moreover, xylanase supplementation has shown to increase the concentration of total SCFA, acetate, and propionate,

and the apparent ileal digestibility (AID) of total NSP in diets containing distiller's dried grain with solubles (Tiwari et al., 2018). In the present study, the concentration of SCFA in the colon of the pigs were in the range of 120.4 to 141.4 mM. Agyekum et al. (2016), found that the total SCFA in the ileum of pigs were between 20.93 to 28.28 mmol/L when feeding DDGS supplemented with multi-enzyme.

The main SCFA detected in the mid-colon were acetic, propionic, and butyric acid, which is consistent with findings by Nakatani et al. (2018), indicating acetate, propionate, and n-butyrate are the main SCFA in the hindgut of weaned pigs. The three-way interactions observed for acetic, and propionic acid showed the influence of protein source, gender, and xylanase supplementation in microbial fermentation.

Xylanase supplementation in CCM diets increased acetic acid concentration from 47.4 to 55.3 mM. However, this was not observed in RCM diets, having similar NDF content, indicating that the type of fiber present in each CM fraction influences SCFA production. Xylanase tended to reduce butyric acid concentrations, which can be associated with increased pH with xylanase supplementation, as pH has a direct effect on bacteria composition in the gut (Palfram et al., 2002) Butyrate has been indicated as the most significant source of energy for colonocytes, impacting gut health (den Besten et al., 2013; Giuberti et al., 2013). However, in the current study, it cannot be discarded an effect on butyric acid concentrations or another volatile fatty acid (VFA) compounds. The evaluation of any feed additive through its fermentation capacity by measuring VFA needs to be cautious by several reasons, as pointed by Gonzalez-Ortiz et al. (2019): 1) rate of production and absorption; 2) the turnover rate of VFA from the intestine into the blood is extremely fast; 3) the repeatability and reproducibility of such volatile parameters measured at one single point in time questions their relevance; and 4) measurements of the presence of enzymes

and genes involved in butyrate production would have helped to clarify the impacts of xylanase and different diets according to their fiber content on the intestinal microbiota and fermentability pattern.

In the present study, protein source affected valeric acid concentration in colonic digesta of pigs, and feeding diets containing RCM and DCM resulted in a higher concentration of valeric acid than feeding diets containing SBM, whereas CCM was intermediate, which suggests increased fermentation of branched-chain AA in the colon of the pigs. In the formulation of the diets, branched-chain AA did not exceed the recommended concentrations (NRC, 2012). The lower levels in valeric acid were observed in SBM as protein source, which can be related to the high digestibility of protein in SBM (75%); therefore, lower availability of protein for fermentation in the hindgut due to less protein arriving into the hindgut. Additionally, protein digestibility in RCM and DCM was 71 and 72%, respectively. However, the addition of xylanase to RCM and DCM resulted in increases in the ATTD of NDF from 28 to 31% and from 30 to 38%, respectively. Therefore, it is speculated that the breakdown of the fiber due to xylanase supplementation resulted in increased AA availability in the colon of pigs; thus, higher concentrations of valeric acid. Furthermore, the ATTD of protein in xylanase supplemented diets increased from 70 to 757%, which could have resulted in lower protein fermentability in the hindgut; therefore, lower concentration of BCFA with xylanase supplementation. Lower pH in ileum and colon when xylanase was supplemented to diets may be related to numerically lower total SCFA (129.3 vs. 133.4 for diets with xylanase and without xylanase, respectively). Higher absorption of butyric acid in xylanase supplemented diets fed to pigs can be speculated.

In conclusion, the use of xylanase improved nutrient digestibilities in all diets. However, a two-way interaction of protein-xylanase in the NDF digestibility indicates a greater influence of

the use of xylanase in wheat-RCM and wheat-DCM based diets. Xylanase supplementation increased ileal and colonic digesta pH without influencing the growth performance of weaned pigs fed wheat and canola meal-based diets for 35-days. All diets were balanced according to SID of AA, STTD of P, and NE requirements; therefore, equal growth performance was observed. Xylanase supplementation reduced the concentration of butyric acid in the colon of pigs. A three-way interaction of protein-xylanase-gender in the concentration of acetic and propionic acid indicates that the combined effect of protein source and xylanase will be different according to gender. However, supplementing xylanase increased the relative weight of the liver and spleen, and tended to increase the size of the kidney.

CHAPTER EIGHT

GENERAL DISCUSSION

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Canola meal (CM) is derived from the crushing of canola seed after oil extraction. Canola meal has been used in poultry and swine diets for a long time; however, its dietary inclusion has been restricted due to the occurrence of anti-nutritional factors (ANF) predominantly glucosinolates (GSL). However, better oil extraction processing techniques and genetic improvements of canola seeds, have led to new cultivars with reduced ANF content and superior nutritive characteristics currently available (Khajali and Slominski, 2012; Adewole et al., 2016). Canola meal at 25% inclusion level can be added in a wheat-SBM diet without a negative consequence on the growth performance of pigs (Sanjayan et al., 2014).

Chapter one presented served to introduce the current study laying the conceptual basis that helped to generate the research questions. Chapter two provided information about the nutritive value of CM and recent techniques (i.e., development of new canola cultivars, dehulling of CM and supplementation of feed enzymes and fermentation) which have been used to improve the nutritive value of CM and overcome the limitations encountered by the swine industry and its use as feedstuff. In chapter three the hypotheses and objectives were presented. In Chapter four, it was shown that the substitution of 20% of CM for SBM in either wheat- or corn-based diet did not significantly influence growth performance. Similarly, Landero et al. (2011) indicated that replacing 20% of CM for SBM in a wheat-based diet fed to weaned pigs did not affect BW gain and ADFI. It is essential to mention that in the present study, during phase I, G:F was higher when 20% CM replaced SBM in the wheat-based diet compared with the corn-based diet, demonstrating that piglets can utilize CM to support growth right after weaning. Similar results were observed by

Wang et al. (2017), who reported an increase in G:F during the first week post-weaning when 20% CM replaced for SBM in a wheat-based diet.

Moreover, better G:F in weaned pigs was observed in a study replacing CM (15%, as-fed basis) for SBM in a corn-based starter diet (Mejicanos, 2015). Such outcomes are consistent with the current findings showing that G:F can be enhanced during the starter period when CM substitutes SBM either in corn-based or wheat-based diet, however, the fecal bacteria composition from pigs fed wheat-CM diets was higher in Clostridium cluster IV, compared to corn-CM diets (0.76 vs. 2.26, respectively). Clostridium cluster IV is composed of *Clostridium, Eubacterium, Ruminococcus* and *Anaerofilum* genera (Collins et al., 1994; Lopetuso et al., 2013), and is responsible for fermentation of resistant starch and dietary fiber in the colon, and release butyrate, which is the most important source of energy for colonocytes and it is associated with gut health (Pryde et al., 2002; Giuberti et al., 2013; Lopetuso et al., 2013).

All diets were balanced according to net energy, STTD of P, and SID amino acid content. Consequently, the enhanced G:F in pigs fed wheat-CM detected might be attributed to the higher relative abundance of commensal bacteria species such as *Bifidobacterium* and Clostridium cluster IV which play an essential role in maintaining gut health and improving feed efficiency. The increased G:F could be attributed to gut fill caused by greater fiber consumption and reduced nutrient digestibility that improves the mass of undigested residue in the gut (Jorgensen et al., 1996; de Lange et al., 2003).

Energy and protein digestibility was decreased with the addition of 20% CM to the diets, and this could be due to the increased fiber content of the diets with the addition of CM. Nevertheless, diets were balanced according to SID AA and net energy. Consequently, no adverse effect on the growth performance of the pigs was observed. This decrease in digestibility is

consistent with results by Bakare et al. (2014) that showed that growth performance was not affected when pigs were fed diets with high fiber content. Likewise, in a study by Wang (2017) it was observed that the digestibility of protein and energy declined without a negative effect on ADFI, ADG, and G:F when piglets were fed diets containing 20% of CM. In a related experiment, decrease in ATTD of CP and gross energy was detected with an increased quantity of CM into a wheat-based diet without effect on feed efficiency, ADG, ADFI (Landero et al., 2011).

In Chapter five, it was hypothesized that the dehulling of CM affects P digestibility in the resulting meal from the dehulling process and that P digestibility differs when fed to growing pigs at 2 different BW. Phosphorus is one of the utmost costly nutrients in pig diets, following energy, and protein. (de Lange et al., 1993; Angel et al., 2002). Moreover, phytate P is poorly digested by swine due to their neglectable intestinal phytase activity (Pointillart, 1988; Jongbloed et al., 1992; Kies, 2005). Dehulling of canola reduces fiber and increase CP. However, the use of this technique correspondingly increases the P content in the dehulled fraction, as demonstrated in research on dehulling (Mejicanos et al., 2017b). The dehulling procedure of canola can increase the total P content to 1.27% (as-is basis), with roughly 48% being non-phytate P (Mejicanos et al., 2017b). Canola meal from black *B. napus* contains around 1.08% total P (as-is basis). However, approximately 40% is non-phytate P (Slominski et al., 2012). To optimize the use of the meals obtained by tail-end dehulling of canola, regarding dietary P supply, it is crucial to determine its STTD P content for diet formulation (NRC, 2012). Additionally, the current research responds to the need for information concerning ATTD and STTD of P in dehulled fractions of CM. Existing data refers principally to conventional CM from *B. napus* and *B. juncea* (Adhikari et al., 2015; Maison et al., 2015).

The focus of studies dealing with dehulling has been to decrease the fiber content and increase CP content (Thakor et al., 1995; Hansen et al., 2017; Mejicanos et al., 2017b). Front-end dehulling refers to the removal of hulls before the crushing of canola seed for oil extraction, while tail-end dehulling refers to the exclusion of hulls from CM after the oil has been extracted (Mejicanos et al., 2017b). Losses of oil during the front-end dehulling procedure, the unwarranted fineness of the dehulled meal and the problems with percolation of the miscella, are preventing the feed industry from applying dehulling of CM (Khajali and Slominski, 2012). Other issues influencing the adoption of dehulling in canola relates to the high cost of using techniques such as air classification (technique founded on the difference in particle size and density among hulls and embryo). Additionally, the unpredictable quality of the meals using the methods currently suggested for tail-end dehulling (McCurdy and March, 1992; Kracht et al., 2004; Beltranena and Zijlstra, 2011; Hansen et al., 2017).

Consequently, dehulled CM is not obtainable at a commercial level. Nevertheless, Mejicanos et al. (2017b) defined a tail end dehulling procedure that is fast and simple to apply, which was the basis of the tail end dehulling utilized in the present study, and that partially responded to the two main concerns regarding this process, the yield of dehulled meal and marketability of the coarse fraction comprising the hulls. In that regard, the tail-end dehulling process used in the current study succeeded in producing a high nutrient-density meal. Furthermore, when analyzing the data of yields of dehulled meal obtained using sieving technology (particle size < 355 μm) for CM from 8 processing facilities in Canada, an average of 25.9% was observed (Mejicanos et al., 2017b). However, in a study by Hansen et al. (2017), a yield 42% of the dehulled meal was obtained with the use of a combination of technologies, including ball milling and sieving, demonstrating that the combination of technologies can be

useful to increase the yield of the high-nutrient density dehulled fraction, however, a significant decrease on CP and increase on fiber contents in the high-fiber coarse fraction can be expected.

In the current study, compared to RCM, DCM had 32% less NDF, 38% less ADF, 17% less NSP, 41% less lignin, and polyphenols and 26% less total dietary fiber. Nevertheless, DCM had 9% more CP, 16% more total P, 23% more non-phytate P, 11% more phytate-bound P. Phytate-bound P levels in DCM were similar to those found in high protein CM by Parr et al. (2015) and NRC (2012).

Additionally, in Chapter five, the ATTD of P in Exp. 1 and. 2 were, for diets containing RCM, 32 and 31%, respectively, and consistent with values reported by Adhikari et al. (2015) and NRC (2012), nonetheless higher than the values found by Rodehutscord et al. (1994). Dehulling increased the ATTD of P in DCM compared to RCM from 32 to 42%, and from 31 to 39% for Exp. 1 and Exp. 2, respectively. When comparing CCM to RCM, a decrease on ATTD of P from 32 to 25%, and from 31 to 23 % for Exp. 1 and Exp. 2, respectively, were observed. The increase in ATTD of P in DCM could be due to its higher non-phytate P content, which is identified to be highly digestible (Jongbloed, 1987; Ravindran et al., 2000). The higher fiber content in CCM could be related with its lower ATTD of P. Nevertheless, in a P digestibility study in CM from *B. napus* and *B. juncea,* no differences in ATTD between the 2 meals were found, regardless of distinct NDF content (24.2 vs. 16%, respectively). Still, the two meals had similar phytate and non-phytate P contents (Adhikari et al., 2015). Additionally, in a study on the impact of rapeseed meal fiber content on P digestibility in growing pigs, no effect of fiber on ATTD was observed (Bournazel et al., 2018). Diets in the present Exp. were formulated according to the total P content of the meal. However, non-phytate P content was higher for DCM compared to RCM and CCM (0.48 vs. 0.39,

and 0.35%, respectively), which could be the main reason for differences in P digestibility, rather than fiber content of the meals.

The STTD of P in Exp. 1 and Exp. 2 in Chapter five were for diets containing RCM, 35.7, and 35.0%, respectively. The observed values are consistent with those described by NRC (2012;). Nonetheless, the STTD of P of RCM was lower than the values defined by Maison et al. (2015) and She et al. (2017). It has been shown that dietary protein level should be considered in P digestibility studies since a deficiency in protein consumption could limit the ileal digestion of P (Xue et al., 2017). To prevent protein and AA deficiencies, all diets fed in the present study were balanced on an ileal digestible AA basis (NRC, 2012) consequently, the observed ATTD and STTD of P were not affected by AA deficiencies. The STTD of P for diets containing DCM was 46.1 and 42.8% for Exp. 1 and Exp. 2, respectively. These results are consistent with a digestibility study feeding high protein CM to growing pigs by She et al. (2017), indicating STTD of P of 48.78%. High protein CM is the closest comparison to a dehulled CM available. Higher STDD of P observed in DCM compared to RCM and CCM could be attributed to the chemical composition of the DCM, which contains higher quantities of non-phytate P rather than lower fiber (Adhikari et al., 2015; Bournazel et al., 2018). A decrease in the STTD of P in CCM compared to DCM for Exp. 1 and Exp. 2, respectively, was observed. The decrease in P digestibility can be attributed to higher non-phytate P in DCM compared to CCM (0.48 vs. 0.35%, respectively). This is consistent with observations by Bournazel et al. (2018), who studied the effects of fiber content in rapeseed meal on P and Ca digestibility. Bournazel et al. (2018) found that when using dehulled rapeseed meal instead of whole meal, or adding hulls, fiber content did not affect the ATTD of P. However, Partrige, (1978) observed a reduction in the apparent absorption of P when cellulose was added at high levels, which indicates that fiber type has an effect on P digestibility.

In Chapter six, the effect of dehulling of canola on AID and SID of AA of the meal when fed to growing pigs was studied. It was hypothesized that tail-end dehulling of CM influences AID and SID of AA when fed to growing pigs. Overall, there was no effect of dehulling on the AID of most AA. The AID of AA in RCM was within the range described for solvent-extracted CM. Nevertheless, the AID of His was lower than the values reported in earlier studies (NRC, 2012; Trindade Neto et al., 2012; Sanjayan et al., 2014; Berrocoso et al., 2015; Liu et al., 2016). Nonetheless, this was similar to the value described by Adewole et al. (2017). The AID of Phe was higher in RCM, compared to DCM and CCM. The AID of Thr was higher in RCM and DCM compared to CCM. However, the AID of Ile and Leu was higher in RCM and CCM compared to DCM. Additionally, the AID of Pro in RCM was higher than the values described by Berrocoso et al. (2015) and Liu et al. (2016) for the regular and high protein CM.). It is important to notice that differences of glycoprotein (NDICP) content between the parent and fractions could have influenced the AID of some AA. Differences in AA digestibilities among processing plants have been observed (Clark et al., 2001; Adewole et al., 2017).

The values for NDF and ADF in the diets containing CCM were 68 and 75% higher than in those containing DCM. Nevertheless, no differences in the AID of AA between DCM and CCM were observed, indicating that fiber content in CM did not affect the AID for most AA. No differences in AID for most AA among RCM, DCM, and CCM, were observed, which is consistent with results by Liu et al. (2014), Berrocoso et al. (2015) and Liu et al. (2016), showing no differences in AID among high protein CM and conventional CM, for most AA. Similarly, no differences were found in AID and SID for most AA when comparing two high protein CM and the conventional CM, regardless of NDF in conventional CM being 37 and 40% higher than the 2 high-protein CM assessed (Berrocoso et al., 2015).

The SID of indispensable AA was not affected by dehulling. It was observed that the SID of AA in RCM, DCM, and CCM were within the range described for solvent-extracted CM (NRC, 2012; Trindade Neto et al., 2012; Sanjayan et al., 2014; Berrocoso et al., 2015; Adewole et al., 2017). Nevertheless, the SID of Phe was higher for RCM compared to DCM and CCM. Although, SID for Thr was higher in RCM and DCM compared to CCM. Higher NDF in diets containing CCM compared to DCM (68 %) did not result in lower coefficients of SID for most AA, which is consistent with studies by Berrocoso et al. (2015) and Liu et al. (2016) that did not find differences in SID of indispensable AA among high protein CM and regular CM, in spite of high differences in dietary fiber content. Dehulling did not improve the SID of AA in DCM, regardless of a 26% decrease in total fiber content compared to RCM. However, a higher concentration of total AA in DCM leads to higher ileal digestible AA content in the diets. Results are consistent with those by Berrocoso et al. (2015) and Liu et al. (2016), who found more digestible AA in high protein CM compared to conventional CM. Nevertheless, no differences in digestible Met, and Val between RCM, DCM, and CCM, were observed. Moreover, CCM had similar digestible AA content, compared to RCM, excluding Arg, Leu, Phe, Thr, Glu, Pro, and Ser, which were lower in CCM. By removing part of the fibrous component, dehulling increased the standardized ileal digestible AA content of DCM compared to RCM by an average of 9%.

In Chapter seven, it was hypothesized that enzyme supplementation in a wheat-based diet could improve the utilization of RCM and the 2 fractions obtained from the dehulling process, which produces meals with distinctive levels of NSPs, which could affect the action of degrading enzymes. Dehulled CM contains lower fiber, higher CP, higher total and non-phytate P. It is uncertain if the use of xylanase in a wheat-based diet containing CM will affect growth performance, nutrient digestibility, organ weight, ileal and colonic pH, or the concentration of

SCFA in colonic digesta. The fiber present in fibrous feed ingredients can decrease nutrient digestibility through encapsulation, resulting in lower feed efficiency and growth (Kerr and Shurson, 2013). Nevertheless, with the incorporation of NSP-degrading enzymes (NSPases) to the diet, those effects can be reduced. But, the beneficial impact of NSPases enzymes such as xylanase in swine diets has been inconsistent (Woyengo et al., 2008; Passos et al., 2015; Taylor et la., 2018). Phytase is extensively used in swine nutrition. The efficacy of phytase to increase the digestibility of P and decrease its excretion in feces has been broadly recognized. Additionally, the digestibility of other nutrients bound to phytate can be improved (Jongbloed et al., 1992, Kies et al., 2001, Kies, 2005, Adhikari et al., 2016). All diets in the study contained phytase at 500 FTU/kg; consequently, any enhancement in growth performance or digestibility of nutrients would be credited solely to the use of xylanase or the protein source.

All diets were formulated according to SID of AA, NE, and STTD of P; however, a P reduction was in place to take advantage of increased digestibility with the addition of phytase. It is noteworthy to mention that the analyzed values for CP and starch in phase I diets were higher for SBM diets, whereas NDF was lower for SBM and DCM diets, compared to RCM and CCM diets. For phase II diets CP was lower for diets containing CCM. Starch was higher for diets containing SBM, while NDF was lower for diets containing SBM and DCM. The protein source and the use of xylanase did not affect pig growth performance for the duration of the trial. Lower CP in diets containing CCM in phase II diets, had no adverse effect on growth performance, in the formulation of the diets, the SID of AA, STTD of P, and NE system was followed. Kiarie et al. (2012) verified that xylanase and ß-glucanase blend applied to mixed grain diets deficient in energy, enhanced growth performance, which is linked to an increase in nutrient digestibility. In the current study, the use of xylanase resulted in an overall increase in ATTD of CP from 70 to

75%. The use of xylanase improved ATTD of diet, DM, P, and Ca. A protein source and xylanase for the ATTD of NDF indicated that the use of xylanase in diets containing RCM and DCM had a higher impact on NDF digestibility compared to diets containing SBM and CCM.

Additionally, the use of xylanase improved the ATTD of Ca and P, which is consistent with results by Nortey et al. (2007), who found increased energy, AA, and P digestibility with the use of xylanase and phytase in wheat byproducts. Results are consistent with those of Weiland (2017) who found increased ATTD of ADF in high fiber diets with the use of xylanase, moreover, the hindgut disappearance of NDF, ADF, and hemicellulose increased, additionally, the AID of DM, starch, and nitrogen increased, and also the AID of GE in low fiber diets tended to increase. Passos et al. (2015) used xylanase on corn-SBM diets, observing increases in AID of NDF, DM, and OM.

Ileal and colonic digesta pH was affected by protein source and the use of xylanase, which is consistent with the results of Taylor et al. (2018) that indicated higher pH values when diets were supplemented with 8,000 and 16,000 BXU/kg xylanase. Nevertheless, the increase in pH in the colon and ileal digesta with the use of xylanase is challenging to explain, as lower Ca and P in solution would result in an increase in acidity rather than a decrease, as higher Ca and P in solution would have a buffering effect (Metzlr-Zebeli et al., 2010).

Protein source and the addition of xylanase increased the liver and spleen size without affecting growth performance. Liver of pigs fed diets containing xylanase were heavier than those of pigs fed diets without xylanase, which can be attributed to the effect of xylanase in the release of anti-nutritive factors such as glucosinolates which can break down into toxic products and affect thyroid function by constraining thyroid hormone production and impair liver and kidney function (Bell, 1993). Moreover, higher GSL content in dehulled CM compared to its parent meal (9.2 vs.

9.6; Mejicanos et al., 2017b) has been observed. The use of xylanase in SBM diets did not increase the relative weight of the liver. The amount of fiber in the diet has been related to increases in the relative weights of organs, Anugwa et al. (1989), feeding pigs high fiber diets observed increased in the stomach, liver, and kidneys. Nyachoti et al. (2000) also observed increased liver, colon, and caecum relative size in pigs fed high fiber diets compared to pigs fed casein-corn starch diet. The weight of spleen was also affected by the protein source and the addition of xylanase. Dietary supplementation of xylanase increased the relative weight of spleen. The oversized spleen can be related to over-activity of the spleen's function of destroying blood cells; however, the spleen also can store healthy erythrocytes and serve as reservoir place for platelets (Clendening, 1930; Sherwood, 1997). Increased comparative weight of liver and spleen did not affect growth performance or health status of pigs during the 35-d duration of the study.

The total concentrations of SCFA were in the range of 120.4 to 141.4mM, which is consistent with observations by Topping and Clifton (2001), indicating that depending on the diet, the total concentration of SCFA in the proximal colon decreases from 70 to 140 mM to 20 to 70 mM in the distal colon. However, in the present study, total SCFA concentration was higher than those reported by Cardona et al. (2005) for piglets 35-d of age, raised indoors (30.3 to 75.5 mM). Moreover, xylanase supplementation has shown to increase the concentration of total SCFA, acetate, and propionate, and the apparent ileal digestibility (AID) of total NSP in diets containing distiller's dried grain with solubles (Tiwari et al., 2018). Whereas, in a study by Agyekum et al. (2016), the total VFA in the ileum of pigs were between 20.93 to 28.28 mmol/L when feeding DDGS supplemented with multi-enzyme.

The main SCFA detected in the mid-colon were acetic, propionic and butyric acid, which is consistent with findings by Nakatani et al. (2018), indicating acetate, propionate, and n-butyrate

as the main SCFA in the cecal digesta of weaned pigs. The protein source influenced the production of propionic and valeric acid, tending to affect the production of acetic acid. Supplementing xylanase to diets containing CCM increased acetic acid concentration indicating that the high fiber content of the diet favors acetic acid production, as lower fiber content in DCM did not increase acetic acid concentration in the colon of the pigs. Xylanase tended to reduce butyric acid concentrations. Butyrate has been indicated as the most significant source of energy for colonocytes, impacting gut health (den Besten et al., 2013; Giuberti et al., 2013). However, in the current study, it cannot be discarded an effect on butyric acid concentrations or other volatile fatty acids (VFA) compounds.

Moreover, in the present study, there was an increase in the concentration of valeric acid when xylanase was added to diets containing DCM, which suggests increased fermentation of branched-chain AA in the colon of the pigs. In the formulation of the diets, branched-chain amino acids did not exceed the recommended concentrations (NRC, 2012).

A two-way interaction of protein-xylanase for the ATTD of NDF was observed, and xylanase supplementation increased NDF digestibility from 28 to 31% and from 30 to 38% for RCM and DCM, respectively. No increase in NDF digestibility was observed when pigs were fed diets containing SBM and CCM. A two-way interaction of enzyme-gender for the ATTD of NDF was also observed, and overall, supplementing xylanase to diets fed to gilts resulted in higher NDF digestibility. Therefore, it is speculated that the breakdown of the fiber due to xylanase supplementation resulted in increased AA availability in the colon of pigs; thus, higher availability of valeric acid. Diets containing RCM and DCM as protein sources resulted in higher concentrations of valeric acid compared to diets containing SBM or CCM. The addition of xylanase to diets containing RCM and DCM increased NDF digestibility.

CHAPTER NINE

SUMMARY AND FUTURE STUDIES

9.1. SUMMARY

The main points that can be drawn from the present research are:

1. Replacing soybean meal in wheat- or corn-based diet, with 20% of CM in nursery diets decreased nutrient digestibility without adverse effect on piglet growth performance.
2. Feed efficiency was significantly improved when 20% of CM was included in wheat-based diets.
3. Feeding wheat-based diets resulted in higher relative abundance of *Lactobacillus*, Enterococcus, and Clostridium bacteria species, compared to corn-based diets.
4. Wheat-based diets had a significantly higher relative abundance of Clostridium cluster-IV, which is related to gut health.
5. Tail-end dehulling of CM improves the ATTD and STTD of P in CM fed to growing pigs of two distinct BW.
6. Feeding high nutrient density DCM to growing and finishing pigs improved P utilization in swine diets and would reduce fecal output (DM) and P discharge.
7. The total AA and CP contents of DCM were higher than the corresponding parent meal.
8. For most AA, the AID and SID in CM were not affected by the dehulling process.
9. Dehulling increased the content of standardized ileal digestible AA of DCM compared to RCM.
10. The use of xylanase improved nutrient digestibilities without affecting growth performance.

11. The addition of xylanase increased the ATTD of CP, DM, diet, P, and Ca.
12. The three-way interaction between xylanase, protein, and gender affect pH in the colon of pigs.
13. The use of xylanase increased NDF digestibility in diets containing DCM and RCM.
14. The use of xylanase increased the NDF digestibility in gilts more than in barrows.
15. The addition of xylanase increased liver and spleen relative weight without affecting growth performance during the 35-d study.
16. Xylanase supplementation increased kidney size compared to non-supplemented diets.
17. A three-way interaction between protein source, xylanase, and gender for acetic acid and propionic acid was observed.

9.2. FUTURE STUDIES

The previous research expands the opportunities to optimize the use of CM in swine diets. However, more information is needed regarding net energy, STTD of P and Ca, and SID of AA in diets containing CM and supplemented with phytase and fiber degrading enzymes (NSPases). Furthermore, there are questions on factors affecting the optimal use of NSPases such as xylanase in swine diets, that need to be answered before we can translate the use of xylanase or other exogenous enzymes on increased growth performance, accordingly, the following research projects are suggested.

1. The effect feeding diets containing CM and NSPases to growing pigs, on ileal digestibility and colonic absorption of calcium, phosphorus, and other minerals.
2. The effect of the use of phytase and NSPases on the dietary cation-anion difference and voluntary feed intake in growing pigs fed diets containing canola meal.

3. The effect of NSPases on the dietary cation-anion balance and pH of ileal and colonic digesta when canola meal is fed to growing pigs.

4. The effect of using phytase and NSPases on the standardized ileal digestibilities of amino acids in canola meal fed to growing pigs.

5. Effects of supplementing NSPases-phytase to regular and dehulled canola meal, on apparent and standardized total tract digestibility of calcium and phosphorus when fed to growing pigs.

6. Effects supplementing NSPases-phytase to regular and dehulled fractions of canola meal, on digestible energy (DE), metabolizable energy (ME) and net energy (NE) when fed to growing pigs using indirect calorimetry (IC).

7. Effects of dietary supplementation of NSPases-phytase on growth performance, nutrient digestibility, organ weight, short-chain fatty acid concentration in digesta and carcass characteristics of pigs fed regular or dehulled fractions of canola meal

CHAPTER 10

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