CANOLA MEAL AS A MAJOR PROTEIN SOURCE FOR SOWS

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ABSTRACT

The objectives of this research were to determine the effects of high inclusion of canola meal (CM) in gestation and lactation diets on lactation performance, milk composition, energy and nutrient digestibility, and gut health in sows and suckling piglet performance. Standardized ileal digestibility (SID) of CP and AA and standardized total tract digestibility (STTD) of P in CM fed to gestating and lactating sows with or without a multi-enzyme complex (MC) was also determined. In Study 1, results showed no differences in sow and litter performance when soybean meal was replaced with increasing levels of CM in lactation diets. However, energy and nutrient digestibilities were reduced with increasing dietary CM inclusion, probably due to increased fiber content. Results indicated that inclusion of up to 30% CM in lactation diet could support satisfactory sow and suckling piglet performance. Results from Study 2 indicated that up to 30% CM could be included in sow diets as a sole protein source from early gestation until weaning without affecting sow and suckling piglet performance, energy and nutrient digestibility, along with an increase in the abundance of beneficial bacteria in the gut. Furthermore, enzyme supplementation improved P digestibility post-farrowing in sows fed CM-containing diets. From Study 3, SID of indispensable AA in CM in gestating and lactating sows were, respectively: Arg, 89.2 and 91.3%; His, 93.1 and 94.0%; Ile, 85.9 and 87.0%; Leu, 89.2 and 89.2%; Lys, 87.0 and 87.7%; Met, 92.2 and 93.2%; Phe, 89.2 and 87.8%; Thr, 84.3 and 82.7%; Trp, 88.1 and 91.5%; Val, 85.9 and 84.3%. When compared to the average SID coefficients of AA in CM fed to growing pigs from previous studies, the SID coefficients for AA in lactating sows were higher in the present study. Supplementation with MC improved the SID of some AA during lactation. The average STTD for P in CM in sows was 44.7 % and MC significantly improved STTD of P in CM during

lactation. Results imply that the use of nutrient digestibility values for CM in growing pigs would result in an underestimation of those for gestating and lactating sows.

DEDICATION

This thesis is dedicated to my son and daughter Ayaan and Sasha, my wife Chandini and my parents, Vanaja Velayudhan and Ettungalpadi Shanmughan Velayudhan.

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FOREWORD

The thesis was written in a manuscript format and it is composed of three manuscripts. Results from manuscript I and II were presented as poster at the 2016 ASAS-ADSA-CSAC-WSASAS Joint Annual Meeting, July 19-24, 2016, Salt Lake City, UT and 2018 ADSA-ASAS Midwest Meeting, March 12-14, 2018, Omaha, NE, respectively. The thesis was written according to the Journal of Animal Science format and manuscripts I and II have been published as follows:

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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
ATTD	Apparent total tract digestibility
BW	Body weight
CM	Canola meal
CP	Crude protein
d	Day
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
g	Gram
GE	Gross energy
h	Hour
K	Potassium
kcal	Kilocalorie
kg	Kilogram
kJ	Kilojoules
MEC	Multi-enzyme complex
ME	Metabolizable energy
Na	Sodium
NDF	Neutral detergent fiber
NE	Net energy
NSP	Non- starch polysaccharides
PUN	Plasma urea nitrogen
SD	Standard deviation
SID	Standardized ileal digestibility
SEM	Standard error of the mean
STTD	Standardized total tract digestibility

CHAPTER 1

GENERAL INTRODUCTION

Canola (*Brassica napus* L.), was developed through conventional breeding techniques to have low levels of erucic acid in the oil and low concentrations of glucosinolates in the meal, when compared to those from parent rapeseed varieties. In North America, the crop is generally known as canola; however, in other parts of the world such as Europe, China, and Australia, it is also known as double-low or double-zero (Spragg and Mailer, 2007; Newkirk, 2009), indicating the low erucic acid and glucosinolates. Hence, by definition, rapeseeds that contain low levels of erucic acid (< 2%) in oil and glucosinolates (< 30 μmol/g) in the meal are known as canola in North America. Global increase in the demand for livestock and poultry products has resulted in an increase in the demand of dietary protein sources for these food animals. Canola meal (CM) with a comparable protein content as soybean meal and with a balanced amino acid (AA) profile, could be an alternative protein source (Canola Council of Canada, 2015) to completely replace soybean meal in diets for swine and poultry. Though limiting in lysine, CM is an excellent source of sulfur containing AA, methionine and cysteine (Canola Council of Canada, 2015; Khajali and Slominski, 2012). Canola meal has a high P content, however, more than 60% of this P is bound to phytate. Though high in phytate, CM is a rich source of non-phytate P when compared to other oilseeds and cereal grains like soybean meal, cottonseed meal, wheat, wheat bran, corn, and barley (Khajali and Slominski, 2012). Apart from phytate, CM also contains some anti-nutritional factors such as glucosinolates, sinapine, and tannins (Slominski et al., 2012; Kasprzak et al., 2016). Nutrient composition of CM has been shown to vary depending upon the variety, climatic changes during growth, and harvesting conditions (Newkirk, 2011). Moreover, CM from different

processing plants has been shown to vary in their nutrient composition (Adewole et al., 2016). Oil extraction from canola seeds normally involves steps such as cleaning, drying, conditioning, flaking, expelling, cooking, solvent extraction, desolventization and toasting, drying and cooling (Newkirk, 2011) and processing conditions namely the temperature, moisture or the duration for each of the steps vary widely. Subsequently, the variations in temperature and moisture between different processing plants could result in a variation in the nutrient composition of the meal.

Over the years since the development of canola, the plant has undergone drastic variations in both qualitative and quantitative traits, the major being a significant reduction in the glucosinolates content in the meal. The tolerance level of dietary glucosinolates for growing pigs is between 2.0-2.5 μmol/g, whereas for sows it is below 4.0 μmol/g of diet. The current varieties of CM available in North America have low concentrations of glucosinolates (< 10 μmol/g), which allows a higher inclusion of CM in swine diets. However, the higher fiber content in CM has been shown to reduce energy and nutrient digestibility in pigs (González-Vega and Stein, 2012). Consequently, when using CM at high inclusion levels in swine diets, proper diet formulation needs to be considered (Newkirk, 2009). Diets formulated on standardized ileal digestible AA content and NE value have been shown to reduce the negative impact of feeding high fiber ingredients in swine diets (Zijlstra and Payne, 2007).

Lactation in sows is an energetically expensive phase which can result in the mobilization of body fat and body protein when nutrient intake fails to meet requirements. The mobilization of body tissue results in a negative energy balance which can have harmful consequences on health and reproduction, affecting piglet survivability (Mejia-Guadarrama et al., 2002). The low survival

rate of piglets from birth to weaning is a significant problem in the swine industry, worldwide. Canola meal is now widely being used as a protein source in pig diets. High inclusion of up to 25% CM in weaned pig diets did not have any adverse effect on growth performance when diets were formulated to contain equivalent NE value and standardized ileal digestible AA content (Sanjayan et al., 2014). In sows, using CM in concentrations of up to 20% in lactation diets did not show any adverse effects on production performance (King et al., 2001). However, the use of CM as a sole protein source in gestating and lactating sows has not been studied. Hence, it was hypothesized that optimal performance in lactating sows could be maintained when feeding diets containing high inclusion levels of CM if such diets are formulated on the basis of NE and standardized ileal digestible AA systems. Also, when using such advanced system of feed formulation, accurate estimation of the nutrient composition of the feed ingredients is necessary. Ileal digestibility coefficients determined for AA for various ingredients including CM in swine diets have been reported in numerous studies. However, the majority of these studies were done using weaned, growing or grower-finisher pigs (Fan and Sauer,1995; Fan et al.,1996; Grala et al., 1998; Woyengo et al., 2010; Kim et al., 2015). Hence not much data exists regarding the standardized ileal digestible AA content for feed ingredients for sows. Accordingly, studies involving AA metabolism in sows have to rely on digestibility values from growing pigs. Factors like age, BW, and feeding level have been shown to influence the ability of pigs to digest AA in a given diet (Moughan, 1993; Stein et al., 2001). Hence, ileal AA digestibility of an ingredient obtained in growing pigs may not always be similar to those corresponding values in gestating or lactating sows. Therefore, it was hypothesized that the standardized ileal digestibility (SID) of AA in CM in both gestating and lactating sows would differ from published values for growing pigs.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORY OF USE

Canola (*Brassica napus* L.), a group of rapeseed variety, is Canada's Cinderella crop that is now ranked among the top three oilseeds worldwide, after soybean and cottonseed (Daun, 2011). Canola belongs to one of the most widespread genera of the crucifer family called Brassicaceae. Crucifers are easily identified by their yellow flower with four petals forming the shape of a cross (Singh, 2006). The flower produces seed pods that contain around 20-30 tiny, round seeds which show a remarkable variation in seasonality, morphology, seed size, seed coat color, and chemical composition among the brassica varieties (Daun, 2011). The seeds contain approximately 40-44% oil and are used as a source of vegetable oil and the residual meal after oil extraction which contains 38-40% protein is used in livestock diets (Uppstrom, 1995; Newkirk, 2009).

The family Brassicaceae include common plants such as the model plant *Arabidopsis thaliana* L. (mouse-ear cress), the weedy relative *Sinapis arvensis* L. (wild mustard), vegetable crops such as *B. napus* (Rutabaga, Siberian kale), *B. rapa* (Chinese cabbage, bok choy, mizuna, Chinese mustard, broccoli rabe and turnip), *B. oleraceae* (cauliflower, cabbage, broccoli, brussels sprouts, kohlrabi, collards, kale) and *Raphanus sativus* L. (radish), and condiment crops such as *B. nigra* (black mustard), *B. carinata* (Ethiopian mustard), *B. juncea* (brown mustard), and *Armoracia rusticana* (horse radish) along with some other minor pot herbs and salad vegetables (Downey and Rimmer, 1993; Rakow, 2004). In general, Brassica varieties cultivated as oilseeds are referred to as "rapeseed," whereas those varieties where the seeds are used as spice are called "mustard." However, rapeseed and mustard may vary in their definition depending on the part of the world in which they occur. Within the Brassica family, canola is defined as Brassica species

with a seed oil that contain less than 2% erucic acid, and the residual solid component of the seed after oil extraction that contain less than 30 μmoles 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 (Daun, 2011; Canola Council of Canada, 2015). In other parts of the world, canola is also known as "double zero," "zero-zero" or "double low" rapeseed. Henceforth, rapeseed and canola are not terms to be used interchangeably. *Brassica nigra, B. oleracea* and *B. rapa* (campestris) are considered as the three-basic species, and hybridization between these species gave rise to the other species, namely *B. carinata, B. juncea,* and *B. napus* (Nagaharu, 1935). Due to its widest distribution, *B. rapa* is believed to be the oldest of the species with evidence suggesting its occurrence from Europe across to China and Korea and from Norway to India, over 2000 years ago (Daun, 2011). Since ancient times rapeseed has been used as a source of oil for food, lamps, soap, and lather for industrial purposes with its cultivation spreading over Asia, Europe, and Northern Africa. However, the Brassica family is mainly concentrated in temperate areas with maximum diversity in the Mediterranean.

The first probable use of Brassica plants was as potherbs and their seeds as spices and condiments because of their strong flavor. History suggests that the seeds of *B. nigra* could have been incorporated into agriculture as volunteer weeds in wheat and barley fields (Daun, 2011). The use of Brassica varieties as a source of oil possibly occurred much later than their use as a spice or potherb.

In Canada, the first recorded evidence of rapeseed production was in the year 1936 by an immigrant farmer from Poland, and the crop was later recognized as *B. campestris*, now denoted to as *B. rapa L*. species (Eskin, 2013). However, commercial production of rapeseed began in the Western Canadian provinces with the outbreak of the Second World War and subsequent blockage

of rapeseed oil import from Europe and Asia, since rapeseed oil was used as a lubricant for steam engines in Canada (Eskin, 2013). Consequently, *B. napus* seeds of Argentinean origin obtained from the USA, along with the Polish variety (*B. campestris*) were used to augment the rapeseed production in Canada. With the introduction of diesel engines during the 1950s, the demand for rapeseed oil as a lubricant started reducing, which caused a drastic drop in the Canadian rapeseed acreage and thus alternative markets were sought for rapeseed including its use in the food industry (Eskin, 2013). The major barriers that restricted the use of rapeseed oil and meal were the high erucic acid of around 25-45 % in the oil and high glucosinolate content of about 110-150 μmoles/g in the meal (Bell 1993). Erucic acid has been associated with cardiac injury in experimental studies in cattle and rodents (Burrows and Tyrl, 2012) while, hydrolyzed products of glucosinolates have shown to interfere with iodine metabolism and therefore affect the functioning of the thyroid gland and subsequently animal performance (Mawson et al., 1994ab; Do et al., 2017). Consequently, canola was bred from rapeseed through conventional plant breeding by Canadian scientists, Baldur Stefansson and Keith Downey, who were selecting rapeseed cultivars when looking for a crop that would produce a healthy, edible oil product (Eskin, 2013), thus earning them the title the 'fathers of canola.' The first low-erucic acid cultivars namely Tanka, Target, and Turret were released and produced in Canada in 1968 followed by the release of the first double zero rapeseed (low erucic acid and low glucosinolates), cv. Tower by Stefansson in 1974 (Bell 1984). Thus, in the year 1978, the term 'Canola' was registered as a trademark in Canada, with the name coming from "Can" as in Canada and "ola" as in oil.

2.2 CANOLA CROP IN CANADA

Canola is an annual or biennial species of Brassica developed from rapeseed using traditional plant breeding techniques and has grown to become one of Canada's most important crops with an average annual yield of 2.3 tonnes/hectare for the year 2017 (Canola Council of Canada, 2018). The transformation of rapeseed from an obscure inedible oilseed, to a genetically improved multibillion-dollar crop producing the healthiest edible oil on the market today, took over a quarter of a century in the making. Over these years canola production in Canada has witnessed a steady upward movement with a current land area utilization of 9.2 million hectares (Statistics Canada, 2017). Saskatchewan, Alberta, Manitoba and the Peace River region of British Columbia accounts for 99% of the total seeded area of canola in Canada. However, canola is also seeded in all other provinces except Newfoundland and Labrador (Statistics Canada, 2017). Canola grows particularly well on the prairies, where the nights are cold and days are hot, allowing the crop to develop its unique fatty acid profile. Factors influencing the growth of the canola plant include the temperature, moisture, light, nutrition, and variety (Zeleke et al., 2014). However, in the Prairies, studies have shown that water stress and temperature are the main environmental factors that regulate the growth and development of canola (Qaderi et al., 2012) and depending on location, growing season, date of seeding, and variety, the maturity of canola seeds shows considerable variation. In the year 1995, genetically modified versions of canola were commercially introduced in Canada. The modifications were mainly carried out to make the crop tolerant to specific herbicides, notably to glyphosate and to glufosinate (Brookes and Barfoot, 2015). Majority of the Canadian canola is now herbicide-resistant and hence controversy exists over its use, since it is derived from the genetically modified crop instead of from the conventional one. The canola oil from the herbicide-resistant varieties are reported to be similar to those

produced from conventional canola varieties, since the transgenic gene which is a protein is completely separated from the oil while processing (Canola Council of Canada, 2018)

Table 2.1. Canola Crop Areas in Canada Regions

Region	Hectares		
	2001	2006	2017
Canada	3,782,906	5,027,643	9,241,846
Saskatchewan	1,906,171	2,418,916	5,103,086
Alberta	1,076,670	1,646,468	2,804,471
Manitoba	757,744	922,134	1,262,619
British Columbia	23,548	26,018	38,445
Quebec	3,832	6,159	15,013
Newfoundland and Labrador	-	-	-
Prince Edward Island	-	64	-
Nova Scotia	-	10	-
New Brunswick	195	359	-
Ontario	14,746	7,517	-

Source: Statistics Canada, Census of Agriculture.

Canada exports 90% of its canola as seed, oil or meal to markets around the world and thereby contributes $26.7 billion to the Canadian economy each year (Canola Council of Canada, 2018). Over the years, apart from improvement in the nutritional profile of the oil and its meal, the conventional breeding as well as modern biotechnological tools have led to the improvement of various agronomically important quantitative and qualitative characters.

2.3 PROCESSING CANOLA

Canola oil has become third largest vegetable oil by volume after palm and soybean mainly due to its low saturated fatty acid content, high levels of monounsaturated fatty acids, and a significant level of n-6 and n-3 fatty acids (da Silva Filardi et al., 2005; Lauretti and Praticò, 2017), making it the oil of choice in many applications. Besides, oil from canola is also rich in phytosterols and vitamin E that may contribute to its nutritional effects. Moreover, with low erucic acid content, canola oil is considered to be one of the best nutritionally edible oil available.

Canola seed holds approximately 44% oil, and because of this high content and value of the oil, the primary focus has been to maximize the value of the seed through its oil content (Newkirk, 2009). About 45% of canola grown in Canada is processed locally (Canola Council of Canada, 2018) to extract the oil. After the oil is extracted, the remaining solid components of the seed are processed into a protein-rich meal that is an excellent addition to livestock feed. In general, three different methods are available for the extraction of oil, namely pre-press solvent extraction, expeller pressing, and cold pressing.

2.3.1 Pre-press Solvent Extraction

Canola seeds are traditionally processed using pre-press solvent extraction technique in order to separate the oil from the meal. The technique involves a combination of expelling and solvent extraction. In this two-stage oil extraction process, the seeds are pressed in a series of screw presses or expellers operating at a temperature of 100 to 120°C, resulting in the production of a seed cake with approximately 18-20% oil (Canola Council of Canada, 2015). The press-cake further undergoes solvent extraction using hexane, followed by a final desolventization and

toasting process at temperatures of 100 to 115°C (Spragg and Mailer, 2007) leading to the production of CM with less than 2% residual oil (Eklund et al., 2015). The pre-press solvent extraction process involves the following stages, namely seed cleaning, seed preconditioning and flaking, seed cooking, pressing, solvent extraction, desolventization and toasting, and drying, pelleting and storage (Spragg and Mailer, 2007; Newkirk, 2009).

2.3.1.1 Seed Cleaning

Canola seeds are usually stored for a couple of weeks in the processing plant prior to processing. Factors such as high chlorophyll content, high moisture, and seed damage have shown to influence the canola seed quality and the resulting oil and meal products (Unger, 2011) and thus, it is essential to store the seeds at a moderate temperature (usually below 18˚C in North America) to avoid insect infestations and heat damage. Hence, storage facilities are equipped with moisture and temperature control (Savic et al., 2009) and use temperature probes to monitor bulk seed temperature, sieve for insects, and aeration to promote uniform storage temperatures. The seed delivered to the processing plant may contain weed seeds, stems, pods and other materials termed "dockage," which are removed by cleaning operations prior to processing (Canola Council of Canada, 2015).

2.3.1.2 Seed Preconditioning and Flaking

Canola seeds are very fragile and are easily broken into smaller particles during the flaking process especially during the cold weather. Seed breakage can disrupt the oil release during the pressing stage, make solvent infiltration into the canola cell structure difficult, and prevent the leaching of oil bodies from the canola seed particles, which in turn results in reduced extraction

efficiency and poor process economics (Unger, 2011). Therefore, before the flaking process, the seeds are preheated to temperatures of 35°C for 30 to 45 minutes to achieve a moisture level of 6.5 to 7.5%. Thus, regulating the moisture content of the seed before any physical processing is crucial since it affects the oil recovery, solvent recovery from the meal post-processing, oil quality, and degumming efficiency (Unger, 2011). The flaking process involves passing the seed through a roller mill set with a narrow clearance to physically rupture the seed coat and flatten the cotyledons (McCurdy, 1990) which in turn permits the oil particles to move from the cellular structure to the outer surface of the flakes, where the liquid portion can be separated from the flakes. Flaking allows a better infiltration of the solvent into the cellular structure thereby percolating the oil, and also favours the outward flow of the solvent from the cellular structure during the desolventization process (Unger, 1990). To maximize the oil yield, an optimal flake thickness of 0.30 to 0.38 mm has been recommended (Canola Council of Canada, 2015; Mosenthin et al., 2016).

2.3.1.3 Seed Cooking

Cooking involves thermally rupturing the oil cells that have survived the flaking process, thereby reducing the oil viscosity, improving the coalescing capacity of oil droplets, increasing the diffusion rate of prepared oil cake and inactivation of enzymes such as myrosinase and lipase (Canola Council of Canada, 2015; Unger, 2011). Myrosinase enzyme is involved in the hydrolysis of glucosinolates, producing sulfur-containing derivatives such as isothiocyanates, nitriles, thiocyanates, or oxazolidinethiones that can negatively impact the oil and meal quality (Tripathi and Mishra, 2007; Unger, 2011). Cooking also helps in regulating the moisture content in the flakes, which is essential for an effective pressing operation. Flakes are cooked by passing through

a stack-type cooker or a horizontal rotary conditioner. The cooking cycle lasts 15–20 minutes, with the temperatures, usually ranging between 80 and 105°C, the optimum temperature being about 88°C (Canola Council of Canada, 2015).

2.3.1.4 Pressing

The cooked canola flakes are then passed through a series of screw presses or expellers, consisting of a rotating screw shaft within a cylindrical barrel equipped with flat steel bars that are placed edgewise around the border, and are spaced to allow the oil to flow between the bars whereas the cake is contained within the barrel. The screw pressing removes 60 to 70% of the oil from the canola flakes (Bredeson, 1983; Vadke and Sosulski, 1988; Unger, 1990). Moreover, screw pressing further ruptures the additional cellular structure that remained intact during the flaking process (Unger, 2011).

2.3.1.5 Solvent Extraction

The cake from the screw press contains around 18–20% oil (Canola Council of Canada, 2015), and to remove the remaining oil, the press-cake is solvent-extracted using hexane. A continuous belt extractor moves the canola cake and the miscella (hexane plus oil) in opposite directions to attain a continuous countercurrent extraction (Paraíso et al., 2008). The meal resulting from the solvent extraction process, referred to as marc (solvent saturated canola cake), has less than 1% of oil and the residual miscella holds around 25–30% oil and 70–75% hexane (Mosenthin et al., 2016). The hexane is removed by distillation from the oil and is recycled (Unger, 2011).

2.3.1.6 Desolventization and Toasting

The cake after solvent extraction process retains around 25 to 35% of the solvent which is removed using a desolventizer-toaster by means of evaporation and steam stripping for about 30 minutes. During this process, the meal is heated to 95–115°C and moisture increases to 12–18% and this is followed by cooling and drying of the meal by blowing air through it. The final meal after the desolventization and toasting process has a moisture content of approximately 12%. Apart from desolventization of the meal, the toasting process also inactivates the heat-labile anti-nutritional factors in the meal (Newkirk and Classen, 2002).

2.3.1.6 Drying, Pelleting and Storage

Canola meal obtained after the desolventization and toasting process is usually dried at a temperature of 100 to 120°C to bring down the moisture to about 10%. The meal is then granulated to a uniform consistency using a hammer mill and is either pelleted or sent directly to storage as a mash.

2.3.2 Expeller Pressing

Expeller pressing, also known as double pressing is seldom used as a processing technique in Canada mainly because of its low oil extraction efficiency when compared to the solvent extraction method. This method involves pressing the seed twice in an expeller to extract oil rather than using a solvent to separate the residual oil. In expeller pressing, seeds are first heated using steam up to 110°C. Following heat treatment, seeds are passed through an expeller twice to remove the oil (Leming and Lember, 2005; Spragg and Mailer, 2007), resulting in a meal called canola expeller meal with a higher oil content 8–11% (Toghyani et al., 2014) when compared to solvent

extracted CM. The reduced yield of oil from expeller pressing resulted in most crushing plants switching to solvent extraction technique to recover as much oil as possible (Adewole, 2017.

2.3.3 Cold Pressing

Cold pressing method of oil extraction involves mechanically pressing the cleaned canola seeds without the use of any heat or chemicals and extracting around 50 to 70% of the oil (Leming and Lember, 2005; Kaldmae et al., 2010; Kasprzak et al., 2016). The seeds are pressed at a slow pace to avoid temperatures above 60℃. However, temperatures have shown to rise to 65°C due to friction build-up in the press (Spragg and Mailer, 2007) or the barrel getting heated up, depending on the equipment. Thus, the nutritional quality of cold-pressed CM has shown to vary depending upon the processing conditions (Seneviratne et al., 2011). The oil recovery in cold pressing method is less efficient compared with solvent extraction or expeller pressing, and the resulting meal has a higher energy content due to more residual oil (Leming and Lember, 2005). Even though the cold-pressed CM and the expeller-pressed CM provides more dietary energy, they have a lower AA composition when compared to the solvent-extracted CM (Seneviratne et al., 2010; Woyengo et al., 2010).

2.3.4 Factors Affecting Canola Meal Quality During Processing

The physical conditions during the processing of canola seed, mainly the temperature and moisture content have a significant impact on the subsequent meal produced (Newkirk, 2009). Amino acids, particularly lysine and methionine have been susceptible to damage during any stages of processing in which heat is applied to the meal including, drying, preconditioning, cooking, expelling, desolventization and toasting or drying/cooling (Hurrell, 1984). However,

during drying and preconditioning, only a minimal amount of heat is used, which is unlikely to cause protein denaturation. Hence, the stages of processing that are most likely to cause protein damage would be cooking, and desolventization and toasting (Newkirk, 2002). Myrosinase, an enzyme present in canola seeds, breaks down the glucosinolates into their toxic metabolites in the animal's digestive tract which negatively impact growth performance. Deactivation of the myrosinase enzyme (30-70%) is attained during the cooking stage with minimum processing temperatures (Newkirk, 2009). Moreover, the rise in temperature while cooking should be done rapidly since the hydrolysis of glucosinolates will advance with increasing temperature until the enzyme is deactivated, and a slow rate of heating would support a higher level of glucosinolate hydrolysis. However, with higher temperatures and for longer durations, protein quality of the meal is reduced. The moisture content of the canola seeds during processing is another factor that influences meal quality. With moisture contents above 10%, hydrolyze the glucosinolates occur rapidly and if below 6%, the rate of inactivation of the enzyme myrosinase slows down (Newkrik, 2009) and thus the moisture content is maintained between 6–10% during processing of canola seeds.

Apart from temperature and moisture, protein in the canola cake are susceptible to degradation by several reactions during processing, the major being the Maillard reaction, (Newkrik, 2002). The Maillard reaction is a chemical reaction between AA and reducing sugars which results in the formation of brown pigments and several aromas while processing of proteins (Hurrell, 1984; Gerrard, 2002). The temperature primarily controls the rate of Maillard reactions, with slower reaction rates at room temperature, which increases exponentially with rising temperatures and occur commonly during the drying stage when the temperatures are over 100°C

(Adrian, 1974). Yet another factor that influences the rate of the Maillard reactions is the pH wherein, alkaline pH promotes Maillard reactions and acidic pH is inhibitory. However, Maillard reactions have shown to occur when the pH is above 3 (Adrian, 1974). The rate of Maillard reactions also varies with the moisture content in the cake. Maillard reaction products have shown to increase with increasing moisture levels, yet high levels of moisture inhibit the reaction (Newkrik, 2002). Consequently, the introduction of moisture during processing of canola seeds could enhance the production of Maillard reactions products which in turn affect the content and availability of AA in the meal (Newkrik, 2002; De Almeida, 2013).

2.4 CHEMICAL COMPOSITION OF CANOLA MEAL

In Canada, CM is a less expensive protein source for monogastric animals, but the presence of high dietary fiber has limited its use as a sole protein source. Nutrient composition in CM is determined by various factors namely the variety, environmental conditions during crop development, harvest conditions, and processing of the seed and meal (Barthet and Daun, 2011; Messerschmidt et al., 2014; Eklund et al., 2015). Yellow-seeded canola varieties have a higher concentration of protein and oil, and less fiber when compared to meal produced from black-seeded varieties (Slominski et al., 1994; Slominski et al., 2012; Trindade Neto et al., 2012). Likewise, CM from the solvent extraction technique has a higher concentration of protein and AA, and less concentration of oil when compared to expeller pressed CM (Sauvant et al., 2004; Spragg and Mailer, 2007; Newkirk, 2009; Seneviratne et al., 2010). The effect of excessive heat and moisture applied during processing and its effect on meal quality have been discussed in the previous sections. The basic nutrient composition of solvent extracted CM include protein, dietary

fiber, and fat, along with some anti-nutritive factors like glucosinolates, tannins, sinapine and phytic acid**.**

2.4.1 Protein and Amino Acids

Canola meal is a rich source of protein with a protein content varying between 40 and 44% (dry matter basis). The average protein and AA compositions of CM samples used in several previous studies in comparison to soybean meal are presented in Table 2.2. Compared to soybean meal, CM has a lower CP, however, with a balanced AA profile (Canola Council of Canada, 2015). Though limiting in lysine, CM is an excellent source of sulfur-containing AA (Woyengo et al., 2010; Li et al., 2015; Ivanova et al., 2016). Canola meal has a rather high concentration of methionine, cysteine, and threonine, but low levels of lysine and tryptophan when compared to soybean meal (Newkirk, 2009; Khajali and Slominski, 2012). Apart from different factors like the variety, environmental conditions, harvest conditions, and meal processing as discussed earlier, protein quality in CM could also vary between different crushing plants (Li et al., 2015, Adewole et al., 2016; Wang et al., 2017). In a study by Adewole et al. (2016) significant variation in arginine, lysine, methionine, and threonine concentration of CM was reported from different processing plants across Canada. Moreover, pelleting has been shown to reduce the digestible AA content of the meal (Adewole et al., 2017), probably due to the Maillard reaction products formed during the pelleting process.

Table 2.2 Chemical composition of canola meal (CM) compared to soybean meal, % dry matter

Item	Solvent extracted CM (black-seeded)	Solvent extracted soybean meal
CP	40.78	50.67
EE	3.67	1.44
GE, MJ/kg	20.67	22.33
Starch	2.78	0.78
Total fiber	35.22	24.22
NSP	20.00	19.78
NDF	28.22	13.33
ADF	18.00	8.33
Indispensable AA		
Arginine	2.52	4.20
Histidine	1.31	1.46
Isoleucine	1.43	2.62
Leucine	2.86	4.30
Lysine	2.30	3.46
Methionine	0.78	0.72
Phenylalanine	1.68	2.88
Threonine	1.73	2.13
Tryptophan	0.62	0.76
Valine	1.87	2.76
Dispensable AA		
Alanine	1.92	2.40
Aspartic acid	3.03	6.46
Cysteine	0.97	0.88
Glycine	1.97	2.37
Glutamic acid	7.32	10.20
Proline	3.14	3.16
Serine	2.02	2.69
Tyrosine	1.08	2.20
Ca	0.72	0.33
P	1.33	0.78
Mg	0.67	0.33
Na	0.09	0.11
K	1.43	2.22
Vitamins, mg/kg		
Biotin	1.11	0.33
Folic acid	2.56	1.44
Niacin	188.33	32.22
Pantothenic acid	10.56	17.78
Riboflavin	4.11	3.22
Thiamine	5.78	5.00

Adapted from Khajali and Slominski, 2012; Mejicanos et al., 2016

2.4.2 Fiber

Canola meal has relatively high fiber content mainly because of the hulls in the seeds remain with the meal (Newkirk, 2009; Barthet and Daun, 2011) and is one of the major factors that limit the use of CM as a primary protein source in diets for swine and poultry. In comparison to soybean meal, the NDF contents in CM is over 2 times higher (Adewole et al., 2016; Wang et al., 2017). Post-processing of canola seeds, the hull constitutes about 30% of the meal weight and is the major source for non-starch polysaccharides (NSP) and lignin (Slominski et al., 2012). The fiber component comprises of NSP, lignin and associated polyphenols, cell wall glycoproteins and minerals bound to fiber components (Simbaya, 1996; Jia et al., 2012). Based on solubility in water, dietary fibers are also grouped into water-soluble and insoluble. In CM, about 91.0% of the dietary fiber is water-insoluble, and around 9.0% is water-soluble (Wickramasuriya et al., 2015). Soluble fiber may increase digesta viscosity and prevent contact with endogenous enzymes thereby reducing nutrient digestibility (Jha and Berrocoso, 2015). Non-soluble fiber can accelerate digesta passage rate thereby resulting in reduced time for digestion and thus reducing nutrient utilization (Khajali and Slominski, 2012).

2.4.3 Fat

The residual oil in CM varies according to the processing techniques, with expeller press CM having a higher oil content compared to the solvent-extracted meal. The fat content of solvent extracted CM have shown to vary between 1.8 to 5.5 % (dry matter basis; Adewole et al., 2016). The higher efficiency of the solvent extraction technique results in the production of CM with more consistent oil content (Spragg and Mailer, 2007). However, the practice of adding back gums

and soapstocks from the refined oil back to the finished meal in certain processing plants results in higher oil content in the meal. Gums and soapstocks are the portion of phospholipid materials in crude canola oil which are removed while processing and are sometimes added back to the meal at the level of 0.5-2.0% with an intention to reduce the dustiness of the meal, increases the phospholipid content and the metabolizable energy content of the meal (Newkirk, 2009).

2.4.5 Minerals and Vitamins

The concentration of minerals in CM varies depending on the differences in soil concentration of minerals and seasonal variations (Bell and Keith, 1990; Mahan et al., 2005). Canola meal has higher concentrations of Ca, P, and Se ranging from 0.7 to 1.1%, 1.0 to 1.1%, and 1.1%, respectively, when compared to soybean meal (Bell, 1993; Newkirk, 2009; NRC, 2012). However, a majority of total P in CM is present as phytate-phosphorus and pigs are unable to digest phytate as they lack digestive enzymes that break it down and hence, the digestibility of P in CM fed to pigs is around 25-30% (Spragg and Mailer, 2007; Newkirk, 2009). Processing of canola has been shown to have no effect on the mineral concentration of the meal (Spragg and Mailer, 2007); however, the addition of soapstocks to the meal has been shown to affect the meal Na concentrations (Newkirk, 2009). Hardly any data exist on the vitamin concentration in CM, however, few studies have reported that CM contains high levels of biotin, choline, niacin, riboflavin, and thiamin, and low levels of folic acid and pantothenic acid when compared with soybean meal (Sauvant et al., 2004; Newkirk, 2009; NRC, 2012).

2.4.6 Anti-nutritional Factors

Anti-nutritional factors are defined, are those biological compounds present in the feed/food that reduce nutrient utilization or feed intake, thereby contributing to impaired gastrointestinal and metabolic performance (Dunlop, 2004). Among the factors that affect the nutritive value of CM for pigs and poultry include the presence of certain anti-nutritional compounds such as the glucosinolates, phytic acid, sinapine, and tannins (Khajali and Slominski, 2012).

2.4.6.1 Glucosinolates

Glucosinolates (alkyl aldoxime-O-sulphate esters with a β-D-thioglucopyranoside group) are a well-defined group of plant-produced sulfur-containing allelochemicals present in major oil- and protein-rich crops belonging to the plant order Capparales (Alexander et al., 2008; Khajali and Slominski, 2012). Glucosinolates are biologically inactive *per se*; however, they undergo hydrolysis either by myrosinase enzyme in the seed or by non-enzymatic factors such as heat and low pH (during meal processing), and hindgut microbial fermentation (Roland et al., 1996). Glucosinolate hydrolysis results in the production of a rage of undesirable products such as isothiocyanates, goitrin, nitriles, and thiocyanates, that interfere with the normal function of thyroid gland and negatively affect the performance in monogastric animals (McCurdy, 1990; Tripathi and Mishra, 2007; Khajali and Slominski, 2012). However, most of the myrosinase enzyme gets inactivated during various stages of solvent extraction technique, and moreover, the heat treatment during desolventizing and toasting process lowers the level of glucosinolates in the meal (Salunkhe et al., 1992; Jensen et al., 1995; Spragg and Mailer, 2007; Newkirk, 2009). The lack of desolventization process could be one of the probable reasons for a higher glucosinolates

content in expeller-pressed CM (Rahmani, 2017). Glucosinolates in canola are composed of two main types; aliphatic which comprise about 83% of the glucosinolates, and indole glucosinolates which account for the remaining 17% of glucosinolates in the meal (Adewole et al., 2016). Gluconapin, glucobrassicanapin, progoitrin, and napoleiferin are the major aliphatic glucosinolates present in CM of which progoitrin is bound to produce the anti-nutritional effect (Fendwick and Curtis, 1980; Simbaya, 1996). Among the CM varieties, those from *B. juncea* has a higher progoitrin content (10.1 μmol/g) when compared to the meal from *B. napus* (2.1 μmol/g; Mejicanos et al., 2016). In comparison to the parent rapeseed meal with a glucosinolate content of 110-150 μmol/g (Bell, 1993), the new canola varieties are developed has very low levels (<10 μmol/g) of glucosinolates (Labalette et al., 2011; Mejicanos, 2015). A recent survey from crushing plants across Canada, reported very low levels of glucosinolates in CM, with an average level of 3.9 μmol/g (Rogiewicz et al., 2012). The low glucosinolate content in current canola varieties was eventually attained through plant breeding techniques, thereby improving the meal quality (Weber et al., 2001; Newkirk, 2011).

2.4.6.2 Phytic Acid

Phytic acid [myo-inositol (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate)] is the storage form of P found in plant-derived feedstuffs. The phytic acid molecule is comprised of a central ring of myo-inositol with six ester bonds to phosphate groups, and each phosphate group can form two ionic bonds with positively charged ions like minerals or proteins (Newkrik, 2002). When phytic acid is bound to a mineral, it is known as phytate. When in phytate form, phosphorus is not bioavailable to non-ruminants because they lack the digestive enzyme, phytase that is required to separate phosphate from the inositol in the phytate molecule. However, ruminants are readily able

to digest phytate because of the phytase produced by rumen microorganisms (Winter et al., 2015). Canola meal has a relatively high concentration of P; however, around 85% of total P is in phytate form (Spragg and Mailer, 2007; Newkirk, 2009), making it inaccessible to non-ruminants and hence, inorganic P sources need to be added in feeds to meet P requirements. Canola meal has an average phytic acid content of 0.64% (on dry matter basis, Newkrik, 2009; Khajali and Slominski, 2012). Besides reducing P availability, phytate has shown to reduce the availability of other minerals such as Zn, Mg, and Ca as well as proteins (Cabahug et al., 1999). Phytic acid can also have a negative impact on animal performance by reducing nutrient digestibility by binding with nutrients or the digestive enzymes or both which consecutively result in increased endogenous loses of AA (Ravindran et al., 2000; Woyengo and Nyachoti, 2013). Phytic acid has shown to reduce energy digestibility, which could be attributed to reduction in energy-yielding substrates such as protein, starch and lipids, thereby reducing the energy available for growth (Nyachoti et al., 2018). Inclusion of exogenous phytase, an enzyme that catalyze the hydrolysis of phytic acid, is a common approach to improve the P digestibility and reduce P excretion in monogastric animals (Adeola and Cowieson, 2011, NRC, 2012, Nyachoti et al., 2018).

2.4.6.3 Sinapine

Sinapine is the choline ester of sinapic acid found in seeds of the cruciferous plants (Butler et al., 1982), and are responsible for the dark coloration, bitter taste, and astringency in CM (Kozlowska et al., 1990). Moreover, in laying hens sinapine present in CM can produce fishy taint in brown-shelled eggs (Butler et al., 1982), which is due to the presence of a compound known as trimethylamine in the egg yolk produced due to a genetic defect in Rhode Island Red breed of laying hens (Khajali and Slominski, 2012). Trimethylamine can be synthesized in the gut by

bacterial metabolism of its precursor, either sinapine or choline that are present in CM. However, CM has shown to contain around 1% sinapine, which is probably not high enough to produce a negative impact on animal performance (Newkrik, 2009). Besides, German researchers have been working on developing canola varieties with low sinapine and low-fiber content (Norddeutsche Pflanzenzucht H. G. Lembke KG, 2010).

2.4.6.4 Tannins

Tannins are phenolic compounds with a molecular weight ranging between 500 to 3,000 Da and are present in CM at a concentration of 1.5–3.0% (Newkrik, 2009; Khajali and Slominski, 2012). Tannins in canola are mainly concentrated in the hulls, with black-colored canola seeds having higher levels when compared to yellow-colored canola seeds (Durkee, 1971; Yapar and Clandinin, 1972). The amount of tannin in canola seeds are determined by the environmental growing conditions (Naczk et al., 1998). Tannins have shown to bind with protein and proteolytic enzymes in the gastrointestinal tract, thereby affecting the protein digestibility (Khajali and Slominski, 2012). Moreover, high tannin levels can cause astringency and bitter taste in CM (Simbaya, 1996). Supplementation with different levels of tannin-rich wood extract in young growing pigs, showed improved feed efficiency and a reduced proteolytic action by the gut microbes, indicating the beneficial effects of tannins, and not just anti-nutritive effects (Biagi et al., 2010).

2.5 CANOLA MEAL AS A PROTEIN SOURCE IN SWINE DIETS

Soybean meal has been used as a preferred protein source for livestock for an extended period of time. However, with the increasing cost of pig production, producers are looking for

alternative protein sources that are cost-effective. The use of less expensive protein source like CM is one of the ways to reduce the feed costs in livestock production. Moreover, its higher availability and a balanced AA profile (Spragg and Mailer, 2007), make CM a better substitute for soybean meal in swine diets. Although the meals from new varieties of canola are low in anti-nutritional factors, the presence of high dietary fiber has constrained its usage as a major protein source in swine. However, the use of more accurate feed quality evaluation systems for energy and AA, which include formulating diets based on NE and standardized ileal digestible AA content could counteract any performance reduction associated with CM utilization (Zijlstra and Payne, 2007).

2.5.1 Energy Content of Canola Meal Fed to Pigs

Canola meal is often considered to have a low digestibility of energy in swine, due to the high amount of fiber and a complex carbohydrate matrix (Newkrik, 2009, Mejicanos et al., 2016). The energy content of CM has been shown to differ between samples obtained from different processing plants due to the different oil extraction techniques being adopted, namely pre-press solvent extraction technique producing a meal with a lower amount of residual oil when compared to the meal from the expeller-press technique. A common practice in processing plants all over Canada is to add some oil refining products (1-2%) including gums and soapstocks back to the solvent-extracted meal, which increases the energy content of the meal since, the gums have been shown to contain around 50% of oil (McCuaig and Bell, 1981; Newkrik, 2009). The concentration of ME and NE in solvent extracted CM was reported to be 2,842 and 2,309 kcal/kg DM, respectively for growing pigs (Kim et al., 2018). Net energy values (2,102 and 2,341 kcal/kg for *B. napus* yellow and *B. juncia* yellow, respectively) reported in studies by Heo et al. (2014) were

also comparable to those reported by Kim et al. (2018) for growing pigs. The higher energy content for *B. juncia* could be due to the higher CP and starch content, and lower NDF content of *B. juncea* when compared to *B. napus*. However, expeller pressed CM has a higher NE value than solvent-extracted CM (2,550 kcal /kg DM Seneviratne et al., 2010), mainly due to its higher residual oil content.

2.5.2 Digestible Amino Acid Content of Canola Meal Fed to Pigs

Amino acid digestibility in CM for pigs is influenced by various factors namely the source of the meal, the temperature during the seed processing, and the age of pigs. The protein and AA content of canola seeds have been shown to vary due to the differences in the growing conditions (Bell and Keith, 1991; Adewole et al., 2016). In a recent study, significant variation in the content of asparagine, glutamine, proline, alanine, cysteine, tyrosine, phenylalanine and lysine in canola seeds have been reported between different crushing plants across Canada (Rahmani, 2017). Even the digestibility of AA among CM samples from 6 processing plants in Canada were found to be different (Adewole et al., 2017). Adewole et al. (2017) observed a difference in apparent ileal digestibility (AID) of methionine, cysteine, threonine, valine, glycine, and serine, SID of methionine and cysteine, and standardized ileal digestible content of all AA among the CM sources. Similar variation among CM sources was also reported by Fan et al. (1996), who observed variations in AID of arginine, isoleucine, leucine, methionine, cysteine, valine and glutamic acid in CM produced in western Canada. This discrepancy in AA digestibility was correlated with the variation in tannin content, fiber content and source, and hull content among different sources of CM (Fan et al., 1996). Yet another reason for the variation in AA digestibility among CM could be associated with the heat treatment while meal processing (Khajali and Slominski, 2012). The

negative effect of the heat during desolventization and toasting process on AA digestibility has been reported in previous studies (Newkirk et al., 2003; Khajali and Slominski, 2012; Adewole et al., 2016). Excessive heating during canola processing could result in the formation of Maillard reaction products which are suggestive of protein damage and would lead to reduction in protein digestibility (Slominski, 1997; Almeida et al., 2014). The products of early Maillard reaction (hexose amino acids) cannot be completely digested by the pig, and hence reduce the digestibility of AA (Hurrell, 1984). The oil extraction techniques used to separate oil from canola seeds may also have an impact on the digestibility values of CM. Expeller extracted CM has a higher SID of nitrogen, arginine, isoleucine, leucine, phenylalanine, glutamic acid, and proline than the solvent extracted CM (Woyengo et al., 2010). The ability of a pig to digest protein or AA have also been shown to be influenced by the age, BW, and the feeding levels (Moughan, 1993; Stein et al., 1999). Likewise, sows have a higher digestibility coefficient for energy and nutrients, due to a larger digestive tract and a better ability to ferment fiber (Shi and Noblet 1993ab; Le Goff and Noblet 2001). The SID of AA in CM fed to growing pigs from recent studies are summarized in Table 2.3. In a recent study, wherein CM from different sources were used to determine its effect on growth performance in pigs showed that the difference in the meal quality had a significant impact on the pig performance (Wang et al., 2017). Hence for sustainable use of feed ingredients, it is crucial to use the precise digestibility values for AA in practical feed formulation (Li et al., 2015; Ravindran et al., 2017).

Table 2.3. Standardized ileal digestibility (%) of amino acids in solvent extracted canola meal fed to growing pigs

Item	Stein et al., 2001	Woyengo et al., 2010	Gonzalez-Vega and Stein, 2012	Trindade Neto et al., 2012	Sanjayan et al., 2014	Adewole et al., 2016
Indispensable AA, %						
Arginine	86.9	86.2	88.0	91.0	90.3	87.5
Histidine	87.3	78.1	79.6	87.4	87.1	67.0
Isoleucine	81.3	78.1	76.4	82.7	79.7	80.9
Leucine	82.3	79.0	78.1	82.5	80.3	82.1
Lysine	83.6	66.6	67.7	81.7	78.9	78.7
Methionine	86.7	84.1	83.9	86.8	84.2	85.4
Phenylalanine	82.6	90.4	78.0	77.0	70.8	83.3
Threonine	78.5	72.1	70.5	78.8	77.1	75.0
Tryptophan	88.7	*NR*	85.8	*NR*	*NR*	*NR*
Valine	79.4	76.7	74.1	78.0	78.5	78.5
Dispensable AA, %						
Alanine	80.0	76.3	75.9	82.3	78.2	80.5
Aspartic acid	81.0	75.0	70.1	79.5	77.8	75.6
Cysteine	81.5	79.3	73.0	83.9	79.8	69.2
Glycine	77.5	82.2	75.8	79.0	76.5	79.3
Glutamic acid	82.7	86.9	83.8	90.9	88.3	87.2
Proline	97.7	101.9	115.0	84.6	*NR*	82.8
Serine	81.1	76.7	72.0	86.1	80.7	77.2
Tyrosine	81.9	96.3	75.7	78.0	78.7	80.1

NR = Not reported

2.5.3 Use of Canola Meal in Swine Diets

2.5.3.1 Nursery Pigs

The use of CM as a major protein source, especially in nursery pigs has been limited due to its high fiber content which has a negative impact on pig performance (Nyachoti et al., 2004). When given a choice, nursery pigs have been shown to consume soybean meal containing diets 2.5 to 7.0 times more, when compared to CM-containing diets (Baidoo et al., 1986), which could

be probably because of the glucosinolates in CM. Glucosinolate breakdown products have been shown to have a negative impact on thyroid function in pigs (McKinnon and Bowland, 1977; Tripathi and Mishra, 2007). Moreover, voluntary feed intake could be affected because of reduced feed consumption as a result of reduced palatability (due to glucosinolates), bitter taste (due to sinapine), and astringent effect in the mouth (due to tannin; Mawson et al., 1994a; Tripathi and Mishra, 2007; Choi et al., 2015). Earlier studies in young weaned pigs have reported reduction in growth performance when fed diets containing CM (Baidoo et al., 1986, 1987; McIntosh et al., 1986). However, newer varieties of CM with reduced fiber and low levels of anti-nutritive factors mainly glucosinolates have led to an increase in the usage of CM as a preferred protein source for pigs. Moreover, diets formulated based on NE values and standardized ileal digestible AA contents allow dietary inclusion of fibrous ingredients like CM at a higher level in pig diets without affecting performance (Zijlstra and Payne, 2007; Sanjayan et al., 2014). With diets formulated based on NE values and standardized ileal digestible AA contents, Seneviratne et al. (2011) reported that an inclusion of 15% solvent extracted CM in weaned pig diets did not affect the body weight gain. Similar results were observed with a higher inclusion level of 20% of solvent extracted CM in nursery pigs (Landero et al., 2011; Wang et al., 2017). Similarly, with up to 20% inclusion of expeller-pressed CM in nursery pig diets, no reduction in growth performance was reported (Landero et al., 2012). However, in both studies (Landero et al. 2011, 2012), increasing inclusion of CM (both solvent extracted and expeller-pressed) produced a linear reduction in total tract digestibility of energy and nutrients, which was mainly attributed to the increased fiber content in diets. Yet another study in weaned pigs in our lab reported that meals from both *B. napus* black and *B. juncea* yellow could be included at a level of 25% without compromising performance as long as the diets are formulated on NE content and standardized ileal digestible

AA basis (Sanjayan et al., 2014). In a recent study, it was observed that up to 30% inclusion of high protein CM or conventional solvent extracted CM in young weaned pig did not have any major impact on growth performance (Pedersen et al., 2016).

2.5.3.2 Growing-finishing Pigs

Increased fiber content in diets of finishing pigs can have a negative impact on carcass yield, since high dietary fiber increases the intestinal mass and thereby reducing lean yield, and also additional energy is needed to make up for lower nutrient availability (Pond et al., 1988; Pluske et al., 1998; Little et al., 2015). Moreover, glucosinolates in diets have been shown to cause enlargement of the thyroid gland in pigs, which in turn could result in muscle growth inhibition (Spencer, 1985; Busato et al., 1991; Mullan et al., 2000). Also growing pigs have shown to tolerate a total glucosinolates concentration of not more than 2 μmol/g of diet (Bell, 1993; Schone et al., 2001). However, total glucosinolates content in newer canola varieties in Canada is found to be under 10.0 μmol/g (Newkirk et al., 2003; Khajali and Slominski, 2012; Mejicanos, 2015), which allows an increased dietary inclusion of CM in growing pigs. Previous studies in growing-finishing pigs have shown that pigs can tolerate up to 30% CM in diets without affecting its growth performance (Busboom et al., 1991; Mullan et al., 2000; King et al., 2001; McDonnell et al., 2010). However, one of the major factors influencing the inclusion rate of CM in diets for growing-finishing pigs is the variation in the meal quality (Little et al., 2015). Roth-Maier et al. (2004) in their studies observed that growing pigs (up to 60 kg BW) could tolerate around 26% CM in diets without any negative impact on growth performance, however, in finishing pigs, an inclusion level of around 16% CM showed a significant reduction in performance. This reduction in performance was reported to be due to finishing pigs being more sensitive to glucosinolate content, even at

lower concentrations (Roth-Maier et al., 2004). Type of the meal used is yet another factor affecting the growth performance in pigs. In a study with increasing inclusion of expeller-pressed CM from 0 to 22.5% in growing-finishing pigs, resulted in a linear reduction in daily gain and feed intake (Seneviratne et al., 2010).

2.5.3.3 Gestation and Lactation Sow Diets

Energy and nutrient requirements in lactating sows are intended to maintain the body tissues, support milk production, along with maternal growth. When lactation diets fail to meet the energy and nutrient requirements, mobilization of body tissues take place to provide nutrients for milk production, mainly from fat stores (Wittemore, 1998). The excessive mobilization of fats and proteins often results in loss of BW, which can result in reproductive problems such as extended wean-to-estrus interval and smaller subsequent litter size (Boyd et al., 2002). There is a profound influence of gestation diets on lactation performance, since the nutrients supplied during gestation help to accommodate the deposition of the body reserves by laying down fat and protein tissue (Wittemore, 1998). Dietary protein concentrations have shown to influence the feed intake in sows. Mahan and Grifo (1975) observed a reduction in daily feed intake and consequent increase in sow BW loss during lactation with decreasing dietary protein levels. Similarly, the BW gain in piglets has shown to increase with an increasing dietary protein content (King et al., 1993; Yang et al., 2000; Manjarin et al., 2012), which could be related to an increase in the protein content of milk (Guan et al., 2004; Laspiur et al., 2009).

Canola meal with a balanced AA composition could be an exceptional protein source in sow diets with proper diet formulation. Early studies determining the effects of CM in sow diets

were carried out on varieties with high levels of glucosinolates, which resulted in delayed sexual maturity and reduced conception rates in gilts and sows (Manns and Bowland, 1963; Schuld and Bowland 1968; Marangos and Hill, 1977), reduced litter size and weight at birth (Manns and Bowland, 1963; Lee et al., 1985), and a high pre-weaning mortality (Schuld and Bowland 1968; Devilat and Skoknic´, 1971). Sows have shown to tolerate a daily intake of under 5 mmol of glucosinolate in gestation diets without affecting their reproductive performance, whereas the recommended glucosinolate content in lactation diets for sows is below 2 μmol/g (Quiniou et al., 2012). However, with glucosinolate levels being relatively low in new cultivars of canola, the negative impact of glucosinolates on reproductive performance in sows should not be of major concern even with high inclusion levels of the meal. Moreover, feeding high fiber diets during pregnancy has shown to reduce aggressive behavior in sows mainly due to reduced hunger (Ramonet et al., 1999; Danielsen and Vestergaard, 2001). Still, the highest recommended inclusion level of CM in diets of lactating sows based on available literature was up to around 20% (Flipot and Dufour, 1977; King et al., 2001; Quiniou et al., 2012).

2.5.4 Gap in Literature

Over the years, CM has evolved into a better protein source for livestock, being cost effective and readily available. The high fiber content in CM in comparison to soybean meal has restricted its use as a major protein source in different stages of pig production. Previous studies have shown that with diets formulated on similar NE content and standardized ileal digestible AA values, the risks associated with high inclusion levels of CM in growing pig diets could be avoided (Sanjayan et al., 2014). However, there is inadequate data to support the use of CM as a sole protein source in diets of gestating and lactating sows, considering its impact on lowering the feed price

by completely replacing soybean meal. Moreover, the ileal digestibility coefficients of AA for several ingredients including CM has never been estimated for sows. Hence research involving sows depend on AA digestibility values determined in growing pigs. The capability of a sow to digest protein or AA might differ from that of growing pigs since factors like age, BW, and feeding level influence the utilization of dietary AA (Moughan, 1993; Stein et al., 2001). Hence the ileal digestibility of AA in CM for sows should differ from those determined in growing pigs.

CHAPTER 3

HYPOTHESES AND OBJECTIVES

The studies tested the following hypotheses:

1. Optimal performance in lactating sows can be maintained while feeding diets containing a high inclusion level of CM if such diets are formulated on the basis of NE and standardized ileal digestible AA content.
2. With a longer adaptation period, CM could be used as a sole protein source in lactating sow diets with no adverse effects on nutrient digestibility and that enzyme supplementation could improve nutrient digestibility in high CM diets.
3. Standardized ileal digestibility of AA and STTD of P in CM in both gestating and lactating sows would differ from those published values for growing pigs.

The overall objective was to determine the effect of high dietary CM inclusion in lactating sows.

The specific objectives were:

1. To determine the effects of increasing dietary CM inclusion on lactation performance, milk composition and nutrient digestibility of sows.
2. To determine the effects of high CM inclusion from early gestation through to lactation on reproductive performance, milk composition, fecal bacterial profile and nutrient and energy digestibility of sows.
3. To determine the SID of CP and AA and the standardized total tract digestibility of P in CM fed to gestating and lactating sows with or without a multi-enzyme complex.

CHAPTER 4

MANUSCRIPT I

EFFECT OF INCREASING DIETARY CANOLA MEAL INCLUSION ON LACTATION PERFORMANCE, MILK COMPOSITION AND NUTRIENT DIGESTIBILITY IN LACTATING SOWS

4.1 ABSTRACT

The aim was to determine the effects of increasing dietary canola meal (CM) in substitution for soybean meal in lactation sow diets. Forty-five sows with an average parity of 1.8 (SD = 0.83) were randomly assigned to 1 of 3 dietary treatments (n = 15) consisting of a corn-based control diet and two diets with 15 and 30% CM formulated by replacing soybean meal with CM. Diets were formulated to be similar in standardized ileal digestible AA content and NE value and to meet or exceed NRC (2012) nutrient recommendations for lactating sows. Sows were moved to farrowing crates on d 111 of gestation and fed the experimental diets until weaning on d 21. Sows were fed 3.0 kg/d from d 111 of gestation until parturition. After farrowing, feed was gradually increased through d 6 after which the diets were offered on an *ad libitum* basis until weaning. Sows were weighed and backfat thickness measured on d 111 of gestation and also on d 0, 7 and 21 post-farrowing. Litters were weighed on d 0, 7 and 21. Weaning to estrus interval in sows was also recorded. Blood and milk samples were collected 2 h post-feeding from sows on d 0, 7 and 21 and analyzed for plasma urea nitrogen (PUN) and milk composition. Fecal samples were collected on d 10, 11 and 12 post-farrowing to determine energy and nutrient digestibility. There were no dietary effects on lactation feed intake, sow BW and backfat change, weaning to estrus interval and milk fat, protein, lactose, and urea composition. Also, there were no dietary effects on piglet

ADG (P > 0.10). Sows fed diets containing 15 and 30% CM had lower (linear, P < 0.05) PUN values compared with those fed the control diet on d 0, 7 and 21 post-farrowing. Apparent total tract digestibility (ATTD) of DM, CP and P were reduced linearly (P < 0.05) with increasing CM inclusion. In conclusion, up to 30% CM in lactation diet can support satisfactory sow and suckling piglet performance.

Key words: Canola meal, digestibility, milk composition, performance, sows, suckling piglets.

4.2 INTRODUCTION

Solvent-extracted canola meal, the main co-product of the canola seed crushing industry and a commonly used supplemental protein source for swine, has a lower CP and AA content (Woyengo et al., 2014) and approximately three times the fiber content (Bell, 1993) when compared to soybean meal. In recent years, the development of canola meal (CM) with low glucosinolate content has increased its usage in swine diets, but the higher fiber content has shown to reduce energy and nutrient digestibility in pigs (González-Vega and Stein, 2012). Therefore, when feeding CM as a major protein source for swine, proper diet formulation needs to be considered (Newkirk, 2009). Formulating diets based on standardized ileal digestible AA content and NE value would reduce the risk associated with reductions in growth performance when feeding high fiber ingredients in swine diets (Zijlstra and Payne, 2007).

Studies have shown that inclusion of up to 25% CM in weaned pigs did not have a negative effect on growth performance when diets were formulated to contain equivalent NE value and standardized ileal digestible AA content (Sanjayan et al., 2014). Dietary inclusion of CM in concentrations of up to 20% in lactating sow diets did not have adverse effects on production performance (King et al., 2001). During lactation in sows, excessive mobilization from body

tissues can occur because of inadequate nutrient intake; which can have detrimental consequences on sow reproduction and piglet survivability (Mejia-Guadarrama et al., 2002). However, there has been little, if any, research conducted to evaluate the effects of feeding high levels of CM on lactating sow and piglet performance. Hence, it was hypothesized that optimal performance in lactating sows can be maintained when feeding diets containing high inclusion levels of CM if such diets are formulated on the basis of NE value and standardized ileal digestible AA content. Therefore, the objectives of this study were to determine the effects of increasing dietary CM inclusion on lactation performance, milk composition and nutrient digestibility of sows.

4.3 MATERIALS AND METHODS

The experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

4.3.1 Animals, Housing and Diets

The experiment was conducted at the Glenlea Swine Research Unit, University of Manitoba. Forty-five (Yorkshire × Landrace) sows with an average parity of 1.8 (SD = 0.83) and their litters were involved in a 26-d experiment. The study was conducted over 3 consecutive farrowing groups, farrowing every 3 wk with 15 sows per group and 5 sows per treatment per group. Within farrowing group, sows were allocated to treatment based on parity and backfat depth. On d 111 of gestation, sows were moved from gestation pens and housed individually in fully slatted farrowing crates (2.30 × 1.70 m) equipped with a sow feeder, and a nipple drinker. Farrowing rooms were mechanically ventilated, and the temperature was maintained at

approximately 18 to 20°C. For piglets, each crate had a heat lamp and mat (Innovative Heating Technologies Inc., MB, Canada). Piglets were weaned at 21 d of age. Cross-fostering of piglets within treatment groups was performed within 48-h post-farrowing to adjust litter size to approximately 12 piglets per sow.

Experimental diets were formulated to contain similar standardized ileal digestible AA content and NE value and to meet or exceed NRC (2012) nutrient recommendations for lactating sows with an average post-farrowing BW of 210 kg, an expected average BW loss of 5.8 kg, and an expected piglet ADG of 230 g. The three experimental diets (Table 4.1) consisted of a corn-based control diet and two diets with 15 and 30% CM formulated by replacing soybean meal with CM (Diet A, B and C, respectively). Diets contained titanium dioxide (TiO_2) as an indigestible marker.

Table 4.1 Ingredient composition and analyzed nutrient content of experimental diets[1]

Items	Dietary CM inclusion level, %		
	0	15	30
Ingredients, % of diet			
Corn	64.00	64.89	58.69
Canola meal	-	15.00	30.00
Soybean meal, 46% CP	28.40	11.55	2.00
Vegetable oil	4.50	5.10	5.90
Limestone	0.85	1.10	1.32
Monocalcium phosphate	0.90	0.70	0.39
Iodized Salt	0.40	0.40	0.40
Vitamin- mineral premix[2]	0.65	0.65	0.65
L-Lysine	-	0.28	0.31
L-Tryptophan	-	0.03	0.04
Titanium dioxide	0.30	0.30	0.30
Calculated composition			
NE, kcal/kg	2,532	2,532	2,530
SID Lys, %	0.90	0.90	0.90
SID Met, %	0.27	0.26	0.29
SID Cys, %	0.29	0.28	0.31
SID Thr, %	0.61	0.53	0.55
SID Trp, %	0.20	0.18	0.18
Ca, %	0.69	0.69	0.69
Total P, %	0.61	0.60	0.60
Analyzed composition			
CP, %	17.60	15.90	16.90
Ca, %	0.63	0.70	0.72
Total P, %	0.58	0.61	0.64
NDF, %	4.90	7.05	9.89
Total Glucosinolates, μmoles/g	0.00	1.32	2.82

[1]as fed basis. All diets were formulated to contain 2.51 Mcal/kg of NE with 0.78, 0.21, 0.49 and 0.15% standardized ileal digestible lysine, methionine, threonine and tryptophan, respectively and 0.68 and 0.60% Ca and total P, respectively.

[2]Supplied the following per kg of finished feed: vitamin A, 6,058 IU; vitamin D, 805 IU; vitamin E, 66 IU; vitamin K, 6 mg; choline, 550 mg; pantothenic acid, 23 mg; riboflavin, 7 mg; folic acid, 1.65 mg; niacin, 33 mg; thiamin, 1.01 mg; vitamin B_6, 2.5 mg; biotin, 0.30 mg; vitamin B_{12}, 0.04 mg, Cu, 12 mg as copper sulfate; Zn, 122 mg as zinc oxide; Fe, 122 mg as ferrous sulfate; Mn, 15 mg as manganese sulfate; I, 0.4 mg as potassium iodate; Se, 0.3 mg as sodium selenite.

4.3.2 Sow and Litter Performance

On d 111 of gestation, sows were washed and moved into the farrowing crates. Sows were assigned randomly to 1 of 3 treatments to give 15 replicates per treatment based on parity and backfat depth according to a randomized complete block design. Sows were fed 3.0 kg/d from d 111 of gestation until parturition. After farrowing, feed was gradually increased from 3.0 kg/d (by approximately 0.5 kg/d) through d 6 after which the diets were offered on an *ad libitum* basis until weaning on d 21. Sows were fed twice daily at 0700 and 1400 h such that they had ad libitum access to feed and water. The quantity of feed provided and feed refusal per sow were recorded daily.

Sow BW and backfat depth were measured and recorded on d 111 of gestation, after farrowing (d 0), d 7 post-farrowing and at weaning (d 21). Backfat thickness was measured using an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco corporation, Minneapolis, MN) at the 10th rib and 6 cm off the midline. Values from the two measurements from both sides of the mid-line were averaged to obtain a single backfat measurement, according to the report of Wang et al. (2008). Total number born alive, stillborn, mummies and weaned piglets per sow were recorded and piglets were weighed on d 0, 7 and 21 to calculate ADG. Detection of estrus in sows was conducted post-farrowing to determine weaning to estrus interval. A sow was considered to be in estrus if the animal exhibited a standing response induced by a back pressure test when fence-line boar exposure is provided (Hossain et al., 2015). Sows were checked for estrus once a day using a 3 yr old boar.

4.3.3 Blood Collection and Analysis

Blood samples (5 mL) were collected from each sow via venipuncture of jugular vein into vacutainer tubes with heparin, 2 h after the morning feed on d 0, 7 and 21. Sows were restrained using a metallic snout snare around the upper jaw, caudal to the canines. Blood samples were centrifuged at 3,000 × *g* for 15 min at 4°C and plasma was pipetted into plastic screw-cap vials and frozen at −20°C until analyzed for plasma urea nitrogen (PUN) concentration. Plasma samples were thawed and then analyzed for PUN using a Nova Stat Profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA) as described by Jayaraman et al. (2015).

4.3.4 Milk Collection and Analysis

Colostrum on d 0 and milk samples on d 7 and 21 post-farrowing were collected soon after blood collection. To stimulate milk release, sows were administered an i.m. injection of 20 USP oxytocin (Rafter 8 products Inc., Calgary, AB, Canada) before collection. Milk samples from all the teats were collected manually and frozen at −20°C for analysis of fat, protein, lactose and urea content by Fourier transform infrared spectroscopy using CombiFoss 6000 (Foss Electric, Denmark) at Horizon Lab Ltd. (Winnipeg, MB, Canada).

4.3.4 Fecal Collection and Analysis

Fecal samples from all sows were collected once on d 10, 11 and 12 post-farrowing by grab sampling via rectal palpation. Feed and fecal samples were stored at −20°C until required for analysis. Fecal samples were thawed and oven dried at 50°C over a 5-d period and pooled within sow. Dried feces and experimental diets were ground to pass through a 1 mm screen before chemical analysis. Dry matter content was determined according to the AOAC (1990; method

925.09) by oven drying 5 g of sample at 102°C overnight. Gross energy was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL) which was calibrated using benzoic acid as a standard. Nitrogen content was determined using the combustion method (method 990.03; AOAC, 1990) using the LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI) and CP was calculated as nitrogen x 6.25. Neutral detergent fiber was analyzed according to the method of Van Soest et al. (1991) using the Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Samples for analysis of Ca and P were ashed for 12 h and digested according to AOAC (2005; method 985.01) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA). Titanium content was determined according to the procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Glucosinolates in the diets were determined according to the procedures described by Niu et al. (2015). Apparent total tract digestibility (ATTD) coefficients were calculated using the following equation: Apparent nutrient digestibility (%) = $100 - \{[(N_d/N_f) \times (Ti_f/Ti_d)] \times 100\}$, Where N_d = nutrient concentration in feces (mg/kg DM), N_f = nutrient concentration in feed (mg/kg DM), Ti_f = TiO_2 concentration in feed (mg/kg DM), Ti_d = TiO_2 concentration feces (mg/kg DM).

4.3.5 Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for a randomized complete block design. The individual sow was considered as the experimental unit. Models included the treatment as a fixed effect and block (farrowing group) as the random effect. Milk composition and PUN data collected repetitively were treated as repeated measures. Sow BW and backfat depth for d 0 was included in the model as a covariate to test their effects on response

variables. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing dietary inclusion levels of CM. Differences were considered significant when $P \leq 0.05$, and trends were noted when $0.05 < P < 0.10$.

4.4 RESULTS

Dietary treatments had no effect on sow BW loss, sow backfat change and ADFI during lactation (Table 4.2). Moreover, no significant difference in weaning to estrus interval was observed with increasing CM inclusion. There also was no difference in litter size at weaning, piglet BW at birth and weaning and piglet ADG among treatments (Table 4.3).

The composition of sow colostrum and milk are shown in Table 4.4. No differences were observed in fat, protein, lactose or milk urea N among the sows fed increasing levels of CM in diets. The PUN concentrations in sows fed diets containing CM were lesser (linear, $P < 0.05$) when compared with sows fed control diet (Table 4.4).

The ATTD of energy and nutrients are presented in Table 4.5. The ATTD of DM, GE, CP and P showed a linear ($P < 0.05$) reduction with increasing CM inclusion in diets. However, ATTD of Ca and NDF were similar among treatments.

Table 4.2 Effect of increasing dietary canola meal (CM) inclusion on lactation performance in sows[1]

Items	Dietary CM inclusion level, %			SEM	*P* value[2]
	0	15	30		Linear
Parity	1.7	2.1	1.8	0.25	0.876
Sow BW, kg					
d 111 of gestation	283.1	282.5	268.9	8.46	0.174
d 0	260.9	260.6	248.1	8.17	0.211
d 7	259.3	258.7	247.9	8.45	0.298
d 21 (weaning)	255.4	253.9	242.3	8.04	0.163
BW loss (d 0-d 21)	5.5	6.8	5.8	0.79	0.110
ADFI, kg/d/sow					
Lactation	7.4	7.2	7.6	0.36	0.813
Backfat thickness, mm					
d 111 of gestation	19.6	19.9	19.1	0.59	0.559
d 0	19.5	19.6	19.0	0.60	0.623
d 7	19.9	19.6	18.8	0.65	0.173
d 21 (weaning)	17.1	17.2	16.5	0.57	0.272
Backfat change (d 21-d 0)	-2.3	-2.5	-2.6	0.58	0.661
Wean-to-estrus interval, d	4.4	4.7	4.5	0.29	0.162

[1]A total of 45 sows (n=15) and their litters were used.
[2] Quadratic effects were not significant and hence their P-values were omitted from the tables.

Table 4.3 Effect of increasing dietary canola meal (CM) inclusion on suckling piglet performance

Items	Dietary CM inclusion level, %			SEM	*P* value[1]
	0	15	30		Linear
Litter, no.					
Piglets farrowed	16.0	15.1	15.6	1.05	0.702
Piglets born live	13.9	14.2	14.1	0.92	0.917
Piglets weaned	10.8	10.3	10.4	0.64	0.396
Piglet BW, kg/piglet					
d 0	1.46	1.50	1.44	0.05	0.873
d 7	2.86	2.99	2.97	0.14	0.695
d 21	6.6	6.7	6.6	0.25	0.862
ADG, g/d/piglet					
d 0 to d 7	202	219	216	13.2	0.616
d 7 to d 21	264	262	258	13.8	0.667
Overall (d 0 to 21)	243	247	244	11.9	0.899

[1]Quadratic effects were not significant and hence their P-values were omitted from the tables.

Table 4.4 Effect of increasing dietary canola meal (CM) inclusion on milk composition and plasma urea nitrogen in lactating sows[1]

Items	Dietary CM inclusion level, %			SEM	*P* value[2]
	0	15	30		Linear
Milk fat, %					
d 0	5.4	6.4	6.4	0.68	0.292
d 7	7.9	8.7	9.0	0.72	0.108
d 21	7.5	7.4	8.2	0.62	0.211
Milk CP, %					
d 0	9.7	9.0	8.6	0.67	0.133
d 7	5.2	5.3	5.1	0.21	0.659
d 21	4.9	5.1	4.9	0.15	0.743
Milk lactose oligosaccharide, %					
d 0	4.7	4.7	4.9	0.18	0.294
d 7	6.4	6.2	6.0	0.32	0.285
d 21	6.4	6.2	6.5	0.24	0.693
Milk urea N, mg/dL					
d 0	49.6	50.2	51.9	2.25	0.471
d 7	57.2	52.1	53.2	2.90	0.271
d 21	55.6	50.1	52.5	3.25	0.146
Plasma urea N, mmol/L					
d 0	3.7	3.1	3.2	0.19	0.019
d 7	5.1	4.2	4.1	0.20	0.005
d 21	5.3	4.3	4.4	0.20	0.004

[1]A total of 45 sows (n=15) and their litters were used.
[2]Quadratic effects were not significant and hence their P-values were omitted from the tables.

Table 4.5 Effect of increasing dietary canola meal (CM) inclusion on apparent total tract digestibility of nutrients and energy in lactating sows [1]

Items	Dietary CM inclusion level, %			SEM	*P* value[2]
	0	15	30		Linear
DM, %	88.4	85.3	82.2	0.34	< 0.001
GE, %	89.2	86.4	83.8	0.28	< 0.001
CP, %	91.0	87.0	84.4	0.38	< 0.001
Ca, %	30.8	29.2	30.0	1.12	0.756
P, %	42.6	38.5	35.5	1.62	0.001
NDF, %	32.6	27.7	31.2	2.91	0.159

[1]A total of 45 sows (n=15) and their litters were used.
[2]Quadratic effects were not significant and hence their P-values were omitted from the tables.

4.5 DISCUSSION

4.5.1 Sow and Litter Performance

The breeding of canola has resulted in cultivars with low concentrations of anti-nutritional factors (mainly glucosinolates), making it a conventional feedstuff for swine. Solvent-extracted CM, though a commonly used supplemental protein source for swine, only limited information is available on its feeding value in lactating sows. Performance data from the current study indicated that, CM could be well incorporated as a major protein source in lactating sow diets. Previous studies have shown that feeding CM-containing diets to pigs reduce growth performance (Baidoo et al., 1986, 1987; McIntosh et al., 1986). This could be because the diets in the above studies were formulated based on CP content and DE value, and the energy values of diets rich in protein or fiber are overestimated when expressed on DE basis (Velayudhan et al., 2015). Hence, NE system enables more effective utilization of high fiber ingredients like CM without affecting the animal performance. Results from the present study are in agreement with the contemporary research where in, it was observed that 15 to 25% CM could be included in weaned pig diets without any negative effects on growth performance (Landero et al., 2011; Seneviratne et al., 2011; Sanjayan et al., 2014; Parr et al., 2015). In sows, inclusion of 10% rapeseed meal in gestation and lactation diets had no deleterious effect on sow reproductive and suckling piglet performance (Flipot and Dufour, 1977; Quiniou et al., 2012). Quiniou et al. (2012) also observed no effect on ADFI in sows fed CM when such diets were formulated on similar NE values. Moreover, solvent-extracted CM when included at 202 g/kg in lactating sow diets had no adverse affect on sow performance (King et al., 2001). In the present study, the inclusion was further increased to 30%, replacing soybean meal.

Anti-nutritional factors in CM such as glucosinolates and fiber limit its utilization in swine diet (Schöne et al., 2001) even though the meal has a balanced AA profile (Khajali and Slominski, 2012). Growing pigs tolerate dietary glucosinolates levels between 2.0-2.5 μmol/g (Bell, 1993; Schöne et al., 1997; Roth-Maier et al., 2004). Parr et al. (2015) have observed a linear reduction in ADFI in weaned pigs fed diets containing increasing levels of CM, which could be attributed to increase in dietary glucosinolates. Sows can tolerate a maximum level of 4 μmol/g of total glucosinolates in diets, above which their reproductive performance is impaired (Mawson et al., 1994b). However, for lactating sows to avoid reduction in ADFI, a few studies have recommended to maintain the dietary glucosinolates concentration below 2 μmol/g (Spratt and Leeson, 1985; Schöne et al., 1999; Quiniou et al., 2012). With traditional plant breeding techniques, there has been a substantial reduction in the levels of glucosinolates (< 10 μmol/g) in CM (Khajali and Slominski, 2012). The CM used in the present experiment contained relatively low levels (7.9 μmol/g) of glucosinolates (Rogiewicz et al., 2014), which means the CM diets contained even lower (1.32 and 2.82 μmol/g for diets with 15 and 30% CM, respectively) contents of this anti-nutritional factor. Therefore, the lack of difference in ADFI between treatments in the current study could be due to the fact that lactating sows can tolerate a greater level (2.82 μmol/g for diets C) of glucosinolates in diets, which is in contrast with the previous studies.

4.5.2 Milk Composition and PUN

Nutrients absorbed during lactation in sows are primarily utilized by the mammary glands (Bauman and Currie, 1980); around 70% of the total energy requirement and 90% of the absorbed AA are used for milk production and mammary gland development (Boyd and Kensinger, 1998). When nutrient intake does not meet nutrient requirements, predominantly protein and energy,

protein from body tissues are mobilized to meet milk production needs (King, 1998). Furthermore, excessive mobilization of body tissues, mainly protein can result in post-weaning anestrus in sows (Jones and Stahly, 1999; Vinsky et al., 2006). Milk production in sows are relatively unaffected by marginal deficiencies in dietary protein and energy (De Bettio et al., 2016). In the present study, sows fed diets including CM had similar milk composition when compared to control probably because the diets were formulated to contain similar standardized ileal digestible AA contents. Average daily weight gain of piglets is positively correlated with the quantity and quality of milk produced by the sow, even though there are several factors associated with piglet weight gain (Skok et al., 2007). In the present study, the one reason for similar ADG of piglets could be due to no difference in the sow milk composition.

Urea is the main nitrogenous end product derived from catabolism of dietary protein not utilized by the body or from tissue protein turnover. Plasma urea N concentration in pigs has a weak but direct relationship with the dietary protein content and is inversely related to protein quality or the biological value of the protein (Eggum, 1970; Orok and Bowland, 1975; Bassily et al., 1982). However, PUN levels can also be influenced by factors apart from protein utilization (Cai, 1992). The nature of the dietary protein is much more important in determining the blood urea levels when compared to the amount of protein consumed (Makdani et al., 1988). Canola meal has a well-balanced AA profile and a high biological value when compared to soybean meal (Newkirk and Classen, 2002; Ghodsvali et al., 2005). This could be the probable reason for lower PUN values observed in the present study in sows fed CM when compared to those fed diets containing soybean meal.

4.5.3 Energy and Nutrient Digestibility

There has been an ongoing interest in considering the inclusion of CM as a major protein source for swine. In a cost-effective manner, soybean meal can be replaced with the conventional CM in diets for growing-finishing pigs (Woyengo et al., 2014). Sow and litter performance from the present study also indicate that CM could be used as a major protein source in lactating sows. On the contrary, the linear reductions in energy and nutrient digestibility values with increasing inclusion of CM could be likely attributed to increasing dietary fiber content. Though the increasing fiber content did not affect any of the growth indices in the present study, the lower nutrient digestibility coefficients were consistent with previous research in growing pigs fed diets containing higher inclusion levels of CM (Landero et al., 2011, 2012; Sanjayan et al., 2014). Furthermore, in the current study, a higher dietary inclusion of CM was restricted to lactation, which is a relatively short period in the sow reproductive cycle. Hence, there is a need for further investigation with long term feeding of higher levels of CM in diets from early gestation to lactation to determine the mechanisms underlying the nutrient digestibility depressing effects of CM in sows.

4.6 CONCLUSION

Results from the research showed no difference in sow and litter performance when soybean meal was replaced with increasing levels of CM in lactation diets. However, energy and nutrient digestibilities were reduced with increasing dietary CM inclusion, probably due to increased fiber content. Result indicates that inclusion of up to 30% CM in lactation diet can support satisfactory sow and suckling piglet performance.

CHAPTER 5

MANUSCRIPT II

EFFECT OF CANOLA MEAL INCLUSION AS A MAJOR PROTEIN SOURCE IN GESTATION AND LACTATION SOW DIETS WITH OR WITHOUT ENZYMES ON REPRODUCTIVE PERFORMANCE, MILK COMPOSITION, FECAL BACTERIAL PROFILE AND NUTRIENT DIGESTIBILITY

5.1 ABSTRACT

The goal of this research was to determine the effects of high canola meal (CM) inclusion in gestation and lactation diets on reproductive performance, milk composition, fecal bacterial profile and nutrient and energy digestibility of sows. Forty-five sows were randomly assigned to 1 of 3 dietary treatments consisting of a corn-soybean meal control diet, control diet containing 30% solvent-extracted CM with or without multi-enzyme complex (MEC). Sows were individually housed and offered the experimental diets from d 60 of gestation until weaning on d 21. Sows were weighed and backfat thickness measured on d 60 and 111 of gestation, and on d 0 and 21 post-farrowing. Litters were weighed on d 0 and 21. Weaning to estrus interval in sows was recorded. Blood and milk samples were collected 2 h post-feeding from sows on d 0 and 21 to determine the plasma urea nitrogen (PUN) content and milk composition. Fecal samples were collected from sows during lactation to determine energy and nutrient digestibility, and during gestation and lactation to determine the fecal bacterial profile. There were no dietary effects on lactation feed intake, sow backfat loss, weaning to estrus interval and milk fat, protein and lactose composition, and suckling piglet performance except for sow body weight (BW) loss during lactation, wherein sows fed CM-containing diets with MC had lower ($P < 0.05$) BW loss than those fed the control

diet. Sows fed diets containing CM (with and without MEC) had lower ($P < 0.05$) PUN values compared with those fed the control diet on d 21 post-farrowing. Apparent total tract digestibility (ATTD) of energy and nutrients showed no dietary effect except for ATTD of phosphorus (P), wherein sows fed CM-containing diets with MEC showed higher ($P < 0.05$) P digestibility compared to those fed CM-containing diets without MEC. Sows fed CM-containing diets with or without MEC had greater ($P < 0.05$) abundances of *Lactobacillus* and *Enterococcus* and lower ($P < 0.05$) abundances of *Firmicutes* when compared to those fed the control diet on d 90 of gestation. In conclusion, inclusion of up to 30% CM in gestation and lactation diet can support satisfactory sow and suckling piglet performance without affecting energy and nutrient digestibility, along with an increase in the abundance of gut lactic acid bacteria in sows. Moreover, enzyme supplementation reduced the sow BW loss and improved P digestibility in CM diets.

Key words: Canola meal, digestibility, milk composition, performance, sows, suckling piglets.

5.2 INTRODUCTION

Canola meal (CM) has become a widely used supplemental protein source for all phases of growth in swine. In comparison to soybean meal, CM contains a lower content of crude protein (CP) and amino acids (AA), with nearly three times the fiber content (Velayudhan and Nyachoti, 2017). Though recent varieties of CM are low in glucosinolate content, the presence of high dietary fiber has restricted its usage as a major protein source in swine (Sanjayan, 2013). However, formulating diets based on net energy (NE) value and standardized ileal digestible AA content has been shown to maintain optimal performance in pigs when fed fibrous ingredients (Agyekum et al., 2014; Sanjayan et al., 2014).

Lactation in sow is a catabolic phase, wherein lipid and protein catabolism occur from the body tissue to furnish the shortfall of nutrients for milk synthesis (Kim et al., 2013; Rosero et al., 2016). Henceforth, nutrients supplied during gestation must replenish the body reserves of the sow to meet the lactation requirements (Whittemore, 1998). Moreover, proper nutritional management is important in sows during gestation to maximize the number of piglets per sow per year, sow longevity, milk production and immunity among the herd heads (Hossain et al., 2015). Utilizing CM as a sole protein source in sow diets has not been studied. Available literature suggests an inclusion of 10-20% CM in gestation and lactation diets without affecting sow and litter performance (King et al., 2001; Clowes et al., 2003; Quiniou et al., 2012). Indeed, as reported in chapter 4, the inclusion of up to 30% CM in lactation sow diets formulated to be similar in standardized ileal digestible AA content and NE value was shown to support adequate sow and suckling piglet performance although energy and nutrient digestibility were reduced. Since, pigs have shown to adapt better with a longer period of feeding of fibrous diets (Varel, 1987; Agyekum and Nyachoti, 2017), it was hypothesized that with a longer adaptation period, CM could be used as a major protein source in sow diets without any negative effects on nutrient digestibility. However, there has been little, if any, research conducted to evaluate the effects of feeding high levels of CM during gestation, on lactating sow and piglet performance. Thus, it was hypothesized that optimal performance in lactating sows and suckling piglets could be maintained by feeding diets containing high inclusion levels of CM formulated on the basis of NE value and standardized ileal digestible AA content from early gestation through to lactation. Also, enzyme supplementation to diets containing high inclusion of CM could improve the energy and nutrient digestibility. Therefore, the objectives of this study were to determine the effects of a high dietary CM inclusion with or without a multi-enzyme complex (**MEC**) from early gestation through to

lactation on lactation performance, milk composition, potential gut health benefits and nutrient digestibility of sows and suckling piglet performance.

5.3 MATERIALS AND METHODS

The experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee, and sows and piglets were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

5.3.1 Animals, Housing, and Diets

The experiment was conducted at the Glenlea Swine Research Unit, University of Manitoba. Forty-five (Yorkshire × Landrace) sows with an average parity of 3.1 (SD = 0.75), and average initial backfat depth of 19 mm (SD = 1.7) and their litters were used in a 76-d experiment. The study was conducted over 3 consecutive farrowing groups, farrowing every 3 wk with 15 sows per group and 5 sows per treatment per group. Within the farrowing group, sows were allocated to treatment based on parity and backfat depth. From d 60 of gestation, sows were housed individually in gestation pens (2.10 × 0.69 m) equipped with a sow feeder, and a nipple drinker. On d 111 of gestation, sows were moved from gestation pens and housed individually in fully slatted farrowing crates (2.30 × 1.70 m) with a sow feeder and a nipple drinker. All rooms were mechanically ventilated, and the temperature was maintained at approximately 18 to 20°C. For newborn piglets, each crate had a heat lamp and mat (Innovative heating technologies Inc., MB, Canada). Piglets were weaned at 21 d of age. Cross-fostering of piglets within treatment groups was performed within 48-h post-farrowing to adjust litter size to approximately 12 piglets per sow.

Experimental diets (gestation and lactation) were formulated to contain similar standardized ileal digestible AA content and NE values and to meet or exceed NRC (2012) nutrient requirement recommendations for gestating and lactating sows. The three experimental diets (Table 5.1) consisted of a corn-soybean meal-based control diet, the control diet containing 30% solvent-extracted CM with or without a multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). All diets contained 3 g/kg of titanium dioxide (TiO_2) as an indigestible marker.

Table 5.1 Ingredient composition and analyzed nutrient content of experimental diets (as-fed basis)

Items	Diets[1]			
	Lactation		Gestation	
	Control	CM	Control	CM
Ingredient, % of diet				
Corn	64.00	60.63	65.01	61.35
Canola meal	-	30.00	-	30.00
Soybean meal	28.40		28.40	-
Vegetable oil	4.50	5.90	4.29	5.90
Limestone	0.85	1.32	0.65	1.10
Monocalcium phosphate	0.90	0.39	0.60	0.24
Iodized Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.65	0.65	0.65	0.65
L-Lysine	-	0.37	-	0.32
L-Tryptophan	-	0.04	-	-
Titanium dioxide	0.30	0.30	-	-
Calculated composition				
Net energy (MJ/kg)	10.6	10.5	10.6	10.6
Lysine[3], %	0.90	0.90	0.90	0.90
Methionine[3], %	0.27	0.29	0.27	0.29
Threonine[3], %	0.61	0.55	0.61	0.55
Tryptophan[3], %	0.20	0.18	0.20	0.14
Analysed composition				
Gross energy (MJ/kg)	16.9	17.4	15.7	17.3
Crude protein, %	18.10	16.80	17.90	16.20
Calcium, %	0.72	0.85	0.58	0.59
Total phosphorus, %	0.54	0.57	0.55	0.57
Neutral detergent fiber, %	8.50	14.90	8.80	13.20
Total glucosinolates (μmoles/g)	-	2.79	-	2.73

[1] Experimental diets consisted of a corn-soybean meal control diet, control diet containing 300 g/kg solvent-extracted canola mean (CM) with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada).
[2] Supplied the following per kg of finished feed: vitamin A, 6,058 IU; vitamin D, 805 IU; vitamin E, 66 IU; vitamin K, 6 mg; choline, 550 mg; pantothenic acid, 23 mg; riboflavin, 7 mg; folic acid, 1.65 mg; niacin, 33 mg; thiamin, 1.01 mg; vitamin B_6, 2.5 mg; biotin, 0.30 mg; vitamin B_{12}, 0.04 mg, Cu, 12 mg as copper sulfate; Zn, 122 mg as zinc oxide; Fe, 122 mg as ferrous sulfate; Mn, 15 mg as manganese sulfate; I, 0.4 mg as potassium iodate; Se, 0.3 mg as sodium selenite.
[3] Standardized ileal digestible basis.

5.3.2 Sow and Litter Performance

Sows were individually housed in gestation stalls from d 60 of gestation and were assigned randomly to 1 of 3 dietary treatments to give 15 replicates per treatment based on parity and backfat depth according to a randomized complete block design. On d 111 of gestation, sows were washed and moved into the farrowing crates. Sows were fed 3.0 kg/d from d 60 of gestation, and feed intake was increased to 3.5 kg/d during the last 10 d of gestation. After farrowing, the feed was gradually increased from 3.0 kg/d (by approximately 0.5 kg/d) through d 6 after which the diets were offered on an ad libitum basis until weaning on d 21. Post-farrowing, sows were fed twice daily at 0700 and 1400 h and had ad libitum access to water. The quantity of feed provided and the feed refusals per sow were recorded daily to determine the average daily feed intake (ADFI). Detection of estrus in sows was conducted post-weaning to determine weaning to estrus interval. A sow was considered to be in estrus if she exhibited a standing response induced by a back pressure test when fence-line boar exposure was provided (Hossain et al., 2015). Sow body weight (BW) and backfat depth were measured and recorded on d 60 and 111 of gestation, after farrowing (d 0), and at weaning (d 21). Backfat thickness was measured using an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco corporation, Minneapolis, MN) at the 10th rib and 6 cm off the midline. Values from the two measurements from both sides of the mid-line were averaged to obtain a single backfat measurement, according to the report of Wang et al. (2008). Total number born alive, stillborn, mummies and weaned piglets per sow were recorded, and litters were weighed on d 0 and 21 to calculate average daily gain (ADG).

5.2.3 Milk Collection and Analysis

Colostrum on d 0 and milk samples on d 21 post-farrowing were collected soon after blood collection. To stimulate milk release, sows were administered an intramuscular injection of 20 USP oxytocin (Rafter 8 products Inc., Calgary, AB, Canada) before collection. Milk samples from all the teats were collected manually and frozen at −20°C for fat, protein, and lactose oligosaccharide composition, determined by Fourier transform infrared spectroscopy using CombiFoss 6000 (Foss Electric, Denmark) at Horizon Lab Ltd. (Winnipeg, MB, Canada).

5.3.4 Blood Collection and Analysis

Blood samples (5 mL) were collected from each sow via venipuncture of jugular vein into vacutainer tubes with heparin, 2 h after the morning feed on d 0 and 21. Blood samples were centrifuged at 3,000 × *g* for 15 min at 4°C and plasma was pipetted into plastic screw-cap vials and frozen at −20°C until analyzed for plasma urea N (PUN) concentration. Plasma samples were thawed and then analyzed for PUN using a Nova Stat Profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA) as described by Jayaraman et al. (2015).

5.3.5 Fecal Collection and Determination of Energy and Nutrient Digestibility

Fecal samples from all sows were collected by grab sampling via rectal palpation on d 10, 11 and 12 post-farrowing. Feed and fecal samples were stored at −20°C until required for analysis. Fecal samples were thawed and oven dried at 50°C over a 5-d period and pooled within sow. Dried feces and experimental diets were ground to pass through a 1 mm screen before chemical analysis. Dry matter content was determined according to the AOAC (1990; method 925.09) by oven drying 5 g of sample at 102°C overnight. Gross energy was measured using an adiabatic bomb calorimeter

(model 6400, Parr Instrument, Moline, IL) which was calibrated using benzoic acid as a standard. Nitrogen content was determined using the combustion method (method 990.03; AOAC, 1990) using the LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI) and CP was calculated as nitrogen x 6.25. Neutral detergent fiber was analyzed according to the method of Van Soest et al. (1991) using the Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Samples for analysis of Ca and P were ashed for 12 h and digested according to AOAC (2005; method 985.01) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA). Titanium contents were determined according to the procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Glucosinolates in the diets were determined according to the procedures described by Niu et al. (2015). Apparent total tract digestibility (ATTD) coefficients were calculated using the following equation: Apparent nutrient digestibility (%) = $100 - \{[(N_d/N_f) \times (Ti_f/Ti_d)] \times 100\}$, Where N_d = nutrient concentration in feces (mg/kg DM), N_f = nutrient concentration in feed (mg/kg DM), Ti_f = TiO_2 concentration in feed (mg/kg DM), Ti_d = TiO_2 concentration feces (mg/kg DM).

5.3.6 Extraction of Fecal Bacterial Genomic DNA and Quantitative Real-time PCR

Fecal samples were collected from ten sows per treatment by rectal palpation on d 60 and 90 of gestation, and d 21 post-farrowing and were immediately stored at −80°C until required for analysis.

Bacterial genomic DNA was extracted from fecal samples using QIAamp DNA Stool Mini Kit (QIAGEN, Ontario, Canada) according to the manufacturer's instruction. Fecal samples were thawed before DNA was extracted. Briefly, 200 mg fecal samples were homogenized in stool lysis buffer, ASL, and heated at 95◦C for 5 min to lyse bacterial cells. After removal of potential

inhibitors by incubation with an InhibitEx tablet, the lysates were treated with proteinase K and buffer AL at 70◦C for10 min to remove protein and polysaccharides. The DNA was precipitated by ethanol, applied to a column provided in the kit followed by washes with buffers AW1 and AW2 and then dissolved in elution buffer. Quantity and quality of isolated DNA was determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Pairs of primers used for quantification of different bacterial groups were obtained from previously published works (Table 5.2). Quantitative real-time PCR was performed in duplicate reactions including nuclease-free water, the forward and reverse primers, template cDNA, 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.

5.3.7 Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for a randomized complete block design. The individual sow was considered as the experimental unit. Models included the treatment as a fixed effect and block (farrowing group) as the random effect. Milk composition and PUN data collected repetitively were treated as repeated measures. Sow BW and backfat depth for d 0 were included in the model as a covariate to test their effects on response variables. Least square means were compared using the Tukey test and differences were considered significant when $P \leq 0.05$, and trends were noted when $0.05 < P < 0.10$.

5.4 RESULTS

Dietary treatments had no effect on ADFI, sow backfat loss, weaning to estrus interval and milk fat, protein, and lactose composition, except for sow BW loss during lactation, wherein sows fed CM-containing diets with MEC had a significantly lower ($P < 0.05$) BW loss (Table 5.3). Also, there were no dietary effects on number of piglets born alive, stillborn, mummies, weaned piglets and piglet overall ADG. However, piglet ADG showed a tendency ($P < 0.10$) for improvement in sows fed CM containing diets on d 0 and 21 (Table 5.4).

The composition of sow colostrum and milk are shown in Table 5.5. No differences were observed in milk fat, protein and lactose composition among treatments. Sows fed diets containing CM (with and without MEC) had significantly lower ($P < 0.05$) PUN values compared with those fed the control diet on d 21 post-farrowing.

The coefficients of ATTD of energy and nutrients are presented in Table 5.6. Apparent total tract digestibility of DM, GE, CP, Ca and NDF showed no dietary effect except for ATTD of P, wherein sows fed CM-containing diets with MEC showed a significantly higher ($P < 0.05$) P digestibility.

The relative abundance of fecal bacteria is presented in Table 5.7. There were no differences in the relative abundance of any of the selected microbial community on d 60 of gestation. Sows fed CM-containing diets with or without MEC had greater ($P < 0.05$) abundances of bacteria belonging to *Lactobacillus* and *Enterococcus* and lower ($P < 0.05$) abundances of *Firmicutes* when compared to those fed the control diet on d 90 of gestation. However, there were no differences ($P > 0.10$) in the abundance of any of the microbial community on d 21 post-farrowing.

Table 5.2 Pairs of primers used for quantitative real-time PCR assay

Target bacterial group	Annealing temperature (°C)	Primer sequence		Reference
		Forward	Reverse	
Total Eubacteria	58	CGGYCCAGACTCCTACGGG	TTACCGAGGCTGCTGGCAC	Lee et al. (1996)
Lactobacillus	62	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG	Karlsson et al. (2011)
Enterobacteriaceae	63	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC	Bartosch et al. (2004)
Bifidobacterium	63	TCGCGTCYGGTGTGAAAG	CCACATCCAGCRTCCAC	Rinttilä et al. (2004)
Enterococcus	60	CCCTTATTGTTAGTTGCCATCATT	ACTCGTTGTACTTCCCATTGT	Bartosch et al. (2004)
Firmicutes	60	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC	Guo et al. (2008)
Clostridium cluster IV	56	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAAC	Matsuki et al. (2002)

Table 5.3 Effect of dietary canola meal (CM) inclusion with or without multi-enzyme complex (MEC) on lactation performance in sows[1]

Items	Diets[2]			SEM	P-value
	Control	CM (−)	CM (+)		
Parity	3.3	3.3	3.1	0.24	0.514
Sow BW (kg)					
d 60 of gestation	266.7	268.5	272.7	4.90	0.639
d 111 of gestation	306.6	308.9	309.4	5.12	0.887
d 0 of lactation	280.7	283.7	282.4	5.50	0.897
d 21 of lactation	274.6	278.3	280.2	5.75	0.696
Gestation BW gain (d 60 to 111)	41.1	40.0	38.9	1.04	0.111
Lactation BW loss (d 0 to 21)	6.0[a]	5.3[a]	3.3[b]	1.15	0.037
Lactation ADFI (kg)	7.05	7.6	7.3	0.39	0.173
Backfat thickness (mm)					
d 60 of gestation	19.5	19.2	19.4	0.51	0.271
d 111 of gestation	18.6	19.6	17.9	0.70	0.187
d 0 of lactation	18.4	18.9	17.8	0.72	0.139
d 21 of lactation	17.6	17.6	16.8	0.48	0.137
Lactation backfat loss (d 0 to 21)	1.1	1.2	1.2	0.11	0.331
Estrus interval (d)	4.7	4.5	5.1	0.49	0.500

Within a row, means with different superscripts ([a,b]) differ ($P < 0.05$).

[1]A total of 45 sows (n=15) and their litters were used.

[2] Experimental diets consisted of a corn-soybean meal control diet, control diet containing 300 g/kg solvent-extracted canola mean (CM) with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). +/– represents presence or absence MEC.

Table 5.4 Effect of dietary canola meal (CM) inclusion with or without multi-enzyme complex (MEC) on suckling piglet performance

Items	Diets[1]			SEM	P-value
	Control	CM (−)	CM (+)		
Litter					
Piglets farrowed	15.9	16.2	15.7	1.26	0.873
Stillborn	1.4	1.7	1.7	0.61	0.763
Mummies	0.4	0.1	0.2	0.22	0.409
Piglets born live	14.1	14.1	13.9	0.70	0.958
Piglets weaned	11.1	10.6	11.1	0.32	0.464
Piglet BW (kg)					
d 0	1.37	1.38	1.49	0.05	0.088
d 21	6.8	7.2	7.5	0.19	0.079
Overall ADG	0.260	0.280	0.285	0.011	0.189

[1] Experimental diets consisted of a corn-soybean meal control diet, control diet containing 300 g/kg solvent-extracted canola mean (CM) with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). +/– represents presence or absence MEC.

Table 5.5 Effect of dietary canola meal (CM) inclusion with or without multi-enzyme complex (MEC) on milk composition and plasma urea nitrogen in lactating sows[1]

Items	Diets[2]			SEM	P-value
	Control	CM (−)	CM (+)		
Milk fat, %					
d 0	5.3	5.5	4.9	0.49	0.693
d 21	6.9	7.4	7.5	0.39	0.117
Milk crude protein, %					
d 0	10.1	10.9	11.6	1.07	0.794
d 21	5.5	5.1	4.9	0.39	0.295
Milk lactose oligosaccharide, %					
d 0	4.8	4.4	4.3	0.35	0.606
d 21	6.7	6.7	6.6	0.12	0.788
Plasma urea nitrogen (mmol/L)					
d 0	4.1	3.5	3.4	0.35	0.249
d 21	6.4[a]	4.9[b]	4.4[b]	0.44	0.002

Within a row, means with different superscripts ([a,b]) differ ($P < 0.05$).

[1] A total of 45 sows (n=15) and their litters were used.

[2] Experimental diets consisted of a corn-soybean meal control diet, control diet containing 300 g/kg solvent-extracted canola mean (CM) with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). +/– represents presence or absence MEC.

Table 5.6 Effect of dietary canola meal (CM) inclusion with or without multi-enzyme complex (MEC) on apparent total tract digestibility (ATTD) coefficients of energy and nutrients in lactating sows[1]

Items	Diets[2]			SEM	P-value
	Control	CM (−)	CM (+)		
ATTD coefficients					
Dry matter	84.1	80.0	82.9	3.20	0.192
Gross energy	85.5	81.9	84.6	2.80	0.218
Crude protein	84.5	80.1	82.9	3.10	0.170
Calcium	39.4	34.8	39.1	6.30	0.481
Phosphorus	38.4^{ab}	32.6^{b}	41.7^{a}	3.40	0.050
Neutral detergent fiber	43.9	49.2	46.4	4.90	0.605

Within a row, means with different superscripts (^{a,b}) differ ($P < 0.05$).

[1] A total of 45 sows (n=15) and their litters were used.

[2] Experimental diets consisted of a corn-soybean meal control diet, control diet containing 300 g/kg solvent-extracted canola mean (CM) with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). +/– represents presence or absence MEC.

Table 5.7 Effect of dietary canola meal (CM) inclusion with or without multi-enzyme complex (MEC) on the relative abundance of selected fecal bacterial community[1]

Items	Diets[2]			SEM	P-value
	Control	CM (−)	CM (+)		
Lactobacillus					
d 60 of gestation	1.99	1.44	1.54	0.623	0.777
d 90 of gestation	1.49[b]	4.99[a]	5.46[a]	0.914	0.015
d 21 of lactation	1.45	2.97	2.74	0.808	0.445
Enterobacteriaceae					
d 60 of gestation	1.94	1.97	1.89	0.797	0.997
d 90 of gestation	1.54	1.41	0.65	0.468	0.379
d 21 of lactation	2.09	2.97	3.32	0.992	0.721
Enterococcus					
d 60 of gestation	1.54	1.68	1.58	0.665	0.986
d 90 of gestation	1.37[b]	4.41[a]	3.80[a]	0.664	0.019
d 21 of lactation	3.11	2.09	2.86	0.995	0.726
Fermicutes					
d 60 of gestation	1.02	1.11	0.94	0.108	0.426
d 90 of gestation	1.00[a]	0.79[b]	0.75[b]	0.051	0.008
d 21 of lactation	1.08	0.81	0.85	0.110	0.159
Bifidobacteriam					
d 60 of gestation	1.86	2.98	2.61	0.791	0.559
d 90 of gestation	1.88	3.08	3.61	0.909	0.119
d 21 of lactation	1.75	1.59	1.20	0.393	0.605
Clostridium Custer IV					
d 60 of gestation	1.06	1.21	0.95	0.227	0.654
d 90 of gestation	1.05	0.77	0.76	0.158	0.145
d 21 of lactation	1.12	0.78	0.76	0.138	0.127

Within a row, means with different superscripts ([a,b]) differ ($P < 0.05$).

[a] A total of 45 sows (n=15) and their litters were used.

[2] Experimental diets consisted of a corn-soybean meal control diet, control diet containing 300 g/kg solvent-extracted canola mean (CM) with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1 g/kg of diet) and phytase (Bio-phytase, 0.2 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). +/– represents presence or absence MEC.

5.5. DISCUSSION

5.5.1 Sow and Litter Performance

Over the recent years, CM has gained its importance as an economical protein source for swine. When compared to soybean meal, CM has a lower protein content. However, due to increased availability of CM, a high dietary inclusion in swine diets would result in a better utilization of the locally available feed ingredient, thereby reducing the feed cost. The inclusion of CM in swine diets as a major protein source has often been limited due to the high fiber content and the presence of glucosinolates. With diets formulated based on similar NE values and standardized ileal digestible AA content, no negative effects of CM inclusion were observed in the current study. The NE system of feed formulation has shown to improve the utilization of high fiber ingredients in swine diets (Zijlstra and Payne, 2007; Velayudhan et al., 2015). Results from the current study are in agreement with our previous research in lactating sows (chapter 4) wherein, it was observed that inclusion of up to 30% CM in lactation diet could support satisfactory sow and suckling piglet performance when diets were formulated based on NE system and standardized ileal digestible AA content. Quiniou et al. (2012) also reported no effect on daily feed intake in sows fed CM containing diets when such diets were formulated on similar NE values.

High level of glucosinolates in CM-containing diets has been shown to have a negative effect on the liver and thyroid functions in pigs (Tripathi and Mishra, 2007), thereby affecting the feed intake and BW gain. But sows have shown the ability to tolerate a higher level of glucosinolates in diets when compared to growing pigs without affecting its reproductive performance (Mawson et al., 1994b; Schöne et al., 1997; Roth-Maier et al., 2004). However, with advanced plant breeding techniques, canola with relatively low levels of glucosinolates have been

developed. The CM used in the current experiment had a glucosinolate content of 7.9 μmol/g, which could be one of the reasons for no difference in ADFI among treatments and is in agreement with results of our earlier study (chapter 4) wherein a 30% inclusion CM in lactation diets did not affect the sow performance. Previous studies concluded that a glucosinolate intake of less than 5 mmol/d in gestation diets would not affect the sow reproductive performance (Quiniou et al., 2012). However, in the current study up to 30% CM inclusion in gestation diets showed no negative effect on the reproductive performance of the sow and piglet performance, suggesting a higher glucosinolate tolerance level of up to 7.1 mmol/d (with an ADFI of 3.0 kg/d and with a glucosinolate content of 2.73 μmol/g diet at 30% inclusion rate of CM) in gestating sows. Also during lactation, the glucosinolate content in the diets had no effect on ADFI which is in agreement with previous studies wherein feeding diets containing less than 2 μmol/g of glucosinolates did not affect the daily feed intake in lactating sows (Schöne et al., 1999; Quiniou et al., 2012). However, with dietary levels above 2 μmol/g of glucosinolates, feed intake in lactating sows was reported to be reduced (Schöne et al., 1999). The corresponding glucosinolate content in diets were higher in the current study (2.79 μmol/g), and the lack of any negative effect on lactation feed intake could probably be due to the sows getting acclimatized to the high inclusion levels of CM fed from early gestation.

In the current study, it was recognized that the experimental design for measuring certain reproductive parameters such as the total number of piglets born, the number of piglets born alive, d 0 piglet BW, and the number of piglets weaned, were limited due to the small number of sows (n = 15) used in the study. The small number of replicates could have influenced the power of the study (for n = 15, the statistical power was 0.6). Assuming the average coefficient of variation for reproductive trails like the total number of piglets born to be 20%, to detect a difference of at least

10% with 80% power and a significance level of 5%, the number of sows required per treatment would be around 63 (Aaron and Hays, 2004). However, it should be noted that small number of sows, comparable to those in the current study have been used in previous studies to determine sow and suckling piglet performance (Nyachoti et al., 2006; Park et al., 2010; Flohr et al., 2014; Cheng et al., 2015). Since the number of sows used in the current study could have influenced the power of certain reproductive traits, to enhance the robustness of the study, future study designs could consider using a larger number of replications.

5.5.2 Milk Composition and PUN

Dietary AA in lactating sows is mainly utilized for the synthesis of milk protein depending on the animal's nutritional state, body condition and milk yield (King, 1998). Reproductive performance in young sows has shown to be compromised when the dietary supply of AA does not meet the maintenance requirement, milk production and tissue deposition (Tritton et al., 1996). In the current study, replacing soybean meal in the control diet with CM had no effect on the milk composition probably because the diets were formulated on standardized ileal digestible AA content. Moreover, minimal deficiencies in dietary protein has shown to have less effect on milk composition in sows (De Bettio et al., 2016). These results are consistent with our previous study (chapter 4) which showed no differences in milk composition in sows fed lactation diets containing 15 and 30% CM.

Canola protein has a higher biological value when compared to soybean meal (Ghodsvali et al., 2005). In spite of a lower AA digestibility, CM has a well-balanced AA composition (Newkirk and Classen, 2002). While, PUN levels can be affected by factors apart from protein utilization (Cai, 1992), the probable reason for a lower PUN content in the current study in sows

fed CM containing diets could be due to the inverse relationship with the protein quality or the biological value of the dietary protein to the PUN levels in pigs (Eggum, 1970; Orok and Bowland, 1975; Bassily et al., 1982). However, it should be noted that the biological value of dietary protein (a blend of protein from different feed ingredients), is determined by the complementarity of the ingredient protein and the synthetic AA added.

5.5.3 Energy and Nutrient Digestibility

The adverse effects of substituting soybean meal in swine diets with CM on animal performance could partly be explained by the higher fiber content in CM. Factors including dietary fiber composition (soluble vs. insoluble; Kritchevsky, 1988), processing of fiber post-harvesting (McDougall et al., 1996) and age and physiological status of the animal (Noblet and Shi, 1993) have a profound effect on nutrient digestibility in pigs fed fibrous diets. However, in the current study, a high fiber content in CM-containing diets did not affect any growth indices or energy and nutrient digestibility coefficients probably because the diets were formulated according to NE content and standardized ileal digestible AA. On the contrary, our previous study (chapter 4) showed a lower energy and nutrient digestibility in sows fed lactation diets containing 15 and 30% CM from d 115 of gestation till weaning presumably due to the short adaptation period to the high fiber content in CM-containing diets. Moreover, with a longer adaptation, sows can efficiently utilize the high dietary fiber as evident in the current study, probably due to an increase in hindgut fermentation because of an increased gut microbial mass (Varel, 1987).

Canola meal has a relatively higher phosphorus content when compared to soybean meal, most of which is in the phytate form and hence not available to pigs (Summers et al., 1989; Wickramasuriya et al., 2015). Hence exogenous phytase supplementation could improve the

availability of phosphorus in such diets as observed in the present study wherein supplementation of CM containing diets with MEC improved the phosphorus digestibility.

5.5.4 Relative Abundance of Fecal Bacteria

Diet is one of the major factors that help in shaping the mammalian gut microbial population which in turn influences digestion and absorption of nutrients (Awati et al., 2005; Frese et al., 2015). The dietary fiber, because of its physio-chemical properties (like viscosity, solubility, and water-holding capacity) has shown to alter the gut environment by influencing the growth and development of gut microbiome (Awati et al., 2005; Jha et al., 2010). Increased cellulolytic bacterial activity in hindgut of pigs due to fermentation of fiber has shown to reduce the pH (due to increased volatile fatty acid production), which in turn promotes the growth of probiotic species of bacteria in the gut. Similar observations were found in the current study wherein feeding CM-containing diets increased the abundance of lactic acid bacteria such as *Lactobacillus* and *Enterococcus* during later part of gestation. However, such variations were not observed during the end of lactation suggesting some kind of adaptation of the gut microbiome in sows which warrants additional studies to elucidate the link between dietary fiber and probiotic bacteria. Wellock et al. (2007) in his studies reported that water-insoluble non-starch polysaccharides have less influence on the growth of beneficial bacteria in the gut when compared to water-soluble non-starch polysaccharides. This could be the probable reason for not finding a major variation in the bacterial abundance in CM fed sows compared to those fed soybean meal based control diets, since CM contain around 144 g/kg of water-insoluble non-starch polysaccharides and only 14 g/kg of water-soluble non-starch polysaccharides (Newkirk, 2009).

5.6. CONCLUSION

Results of this study indicate that up to 30% CM can be included in sow diets as a sole protein source from early gestation until weaning without affecting sow and suckling piglet performance, and energy and nutrient digestibility, along with an increase in the abundance of beneficial bacteria in the gut. Furthermore, enzyme supplementation improved P digestibility post-farrowing in sows fed CM containing diets.

CHAPTER 6

MANUSCRIPT III

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY AND STANDARDIZED TOTAL TRACT PHOSPHORUS DIGESTIBILITY OF CANOLA MEAL FED TO GESTATING AND LACTATING SOWS

6.1 ABSTRACT

The study was conducted to determine the standardized ileal digestibility (SID) of CP and AA, and the standardized total tract digestibility (STTD) of P in canola meal (CM) fed to gestating and lactating sows with or without a multi-enzyme complex (MEC). Eight sows cannulated on d 40 of gestation were used for the study. Sows were assigned at random to the 4 dietary treatments in a replicated 4 × 4 Latin square design. The 4 diets included 2 cornstarch-based diets with solvent extracted CM as the only source of AA and P, with or without a multi-enzyme complex (MEC), a casein-cornstarch diet to determine ileal endogenous AA losses, and a P-free diet to determine the endogenous P losses. All diets contained 0.3% titanium dioxide as an indigestible marker. Sample collection was done over 3 phases; mid-gestation, late-gestation, and lactation. Each phase had 4 experimental periods lasting for 8 d each. In each period, after 5-d acclimation to the experimental diets, fecal samples were collected by grab sampling via rectal palpation on d 6. On d 7 and 8, ileal digesta samples were collected continuously for 12 h. Sows in lactation had higher ($P < 0.05$) AID for CP, and all AA, when compared to sows in mid- or late-gestation, except for AID of proline which tended ($P = 0.062$) to be higher in lactating sows. The MEC supplementation improved ($P < 0.05$) the AID of AA, namely histidine, lysine, methionine, valine and alanine, and showed a tendency ($0.05 < P < 0.10$) for improvement in AID of arginine, isoleucine, tryptophan,

and cysteine during the lactation phase. However, SID of most AA did not show any significant effect of the collection phase. The MEC supplementation improved ($P < 0.05$) the SID of arginine, histidine, lysine, methionine, and valine during lactation. Similarly, there were no differences in ATTD values for nutrients between the collection phases. Enzyme supplementation only improved ($P < 0.05$) the ATTD of DM and P, and STTD of P during lactation. The SID of indispensable AA in CM in gestating and lactating sows were, respectively: Arg, 89.2 and 91.3%; His, 93.1 and 94.0%; Ile, 85.9 and 87.0%; Leu, 89.2 and 89.2%; Lys, 87.0 and 87.7%; Met, 92.2 and 93.2%; Phe, 89.2 and 87.8%; Thr, 84.3 and 82.7%; Trp, 88.1 and 91.5%; Val, 85.9 and 84.3%. When compared to the average SID coefficients of AA in CM fed to growing pigs from previous studies, the SID coefficients for AA in lactating sows were higher in the current study. Supplementation with MEC improved the SID of some AA during lactation. The average STTD for P in CM fed in sows was 44.7 % and MEC significantly improved the STTD of P in CM during lactation. Results from the current study imply that the adoption of nutrient digestibility values for CM in growing pigs would result in an underestimation of those for gestating and lactating sows.

Key words: Amino acids, canola meal, digestibility, gestating sows, lactating sows, phosphorus.

6.2 INTRODUCTION

Solvent extracted canola meal (CM) is a commonly used feed ingredient in swine diets; however, its content of anti-nutritional factors such as glucosinolates and fiber has restricted its use as a major protein source (Schone et al., 2001; Newkirk, 2009; Barthet and Daun, 2011). Even though, the amino acid (AA) content in CM is reasonably high (NRC, 2012), high dietary inclusion has resulted in a reduction in feed intake, and energy and nutrient utilization in growing pigs (Nyachoti et al., 2004; González-Vega and Stein, 2012). However, newly developed canola

varieties have a reduced fiber content and low contents of glucosinolates (Slominski, 1997; Mejicanos, 2015). Recent studies have shown that a high inclusion of CM up to 25% in weaned pig diets (Sanjayan et al., 2014) and up to 30% in lactating sow diets (chapter 4) did not have a negative impact on performance when diets were formulated to have similar NE content and standardized ileal digestible AA values. Hence, determination of standardized ileal digestibility (SID) coefficients of AA of CM from new cultivars is indispensable for precise diet formulation, to achieve predictable performance in pigs.

Ileal digestibility of AA for feed ingredients including CM in pigs has been reported in various studies. However, the majority of these studies were done using either weaned, growing or finisher pigs (Fan and Sauer,1995; Fan et al.,1996; Grala et al., 1998; Woyengo et al., 2010; Kim et al., 2015). Hence not much data exists regarding ileal digestibility coefficients of AA for ingredients fed to sow. Accordingly, dietary supply of AA in studies involving sows have to rely on digestibility values from growing pigs. Factors like age, BW, and feeding level have shown to influence the ability of pigs to digest AA in a given diet (Moughan, 1993; Stein et al., 2001). Consequently, sows and growing pigs may digest dietary proteins differently. Also, Stein et al. (1999) reported that apparent total tract and apparent ileal AA digestibility of various ingredients obtained in growing pigs are not always similar to those corresponding values in gestating or lactating sows. It is also critical to optimize P utilization by sows to reduce the cost of feeding and potential for environmental pollution, for which the dietary P supply should not exceed the animal's requirement (NRC, 2012). However, there are no comparative data for P digestibility in CM fed to sows.

Thus, when utilizing CM as a main protein source for sows, proper diet formulation need to be considered. Therefore, it was hypothesized that the SID of AA in CM in both gestating and

lactating sows would differ from those for growing-finishing pigs. Thus, the objectives of this experiment were to 1) determine the SID of AA in CM fed to gestating and lactating sows; 2) determine the effect of enzymes on ileal digestibility of AA in CM in gestating and lactating sows; 3) determine the standardised total tract digestibility (STTD) of P in CM fed to gestating and lactating sows.

6.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee, and sows and piglets were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

6.3.1 Animals, Housing, and Diets

Eight gestating sows (Yorkshire-Landrace female × Duroc male; d 35 of gestation) with an average parity of 2.8 (SD = 0.83) were obtained from the University of Manitoba Glenlea Swine Research Unit and were fitted with a simple T-cannula at the distal ileum as described by Stein et al. (1998) on d 40 of gestation. Post-surgery, sows were housed individually in gestating pens (3.0 × 2.4 m) with smooth sides and plastic-covered, expanded metal sheet flooring, equipped with a stainless-steel sow feeder, and a nipple drinker. On d 112 of gestation, sows were moved from gestation pens and housed individually in fully slatted farrowing crates (2.30 × 1.70 m) with a stainless-steel sow feeder and a nipple drinker. All rooms were mechanically ventilated, and the temperature was maintained at approximately 18 to 20°C. The sows were allowed a recovery period of 10 d after surgery, during which they were fed a corn-soybean meal based diet before the commencement of the experiment.

The 4 experimental diets included 2 cornstarch-based diets containing solvent extracted CM as the only source of AA and P, with or without a multi-enzyme complex (MEC), a casein-cornstarch diet to determine ileal endogenous AA losses, and a P-free diet to determine the endogenous phosphorus losses. The MEC used was a mixture of carbohydrases (Superzyme-OM, 1.5 g/kg of diet) and phytase (Bio-phytase, 0.25 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada). All diets contained 0.3% titanium dioxide as an indigestible marker and were fed in mash forms.

Table 6.1 Ingredient composition and analyzed nutrient content of experimental diets (as-fed basis).

Item	Diets		
	CM[1]	LND[2]	PFD[3]
Ingredient, %			
Canola meal	31.30	-	-
Cornstarch	61.35	59.75	51.10
Casein	-	5.00	-
Dextrose	-	25.20	23.00
Pork gelatin	-	-	14.00
Vegetable oil	5.00	2.00	4.00
Limestone	1.00	0.40	1.40
Solka flock	-	4.00	3.500
Monocal phosphate	-	2.30	-
Iodized salt	0.40	0.40	0.40
Vitamine-mineral premix[4]	0.65	0.65	0.65
Lys-HCl	-	-	0.30
DL-Met	-	-	0.15
L-Thr	-	-	0.30
L-Trp	-	-	0.15
L-Ile	-	-	0.25
L-Leu	-	-	0.20
L-Val	-	-	0.30
Titanium dioxide	0.30	0.30	0.30
Analyzed nutrient content			
DM, %	89.0	90.0	88.0
CP, %	12.9	4.7	13.5
GE, kcal/kg	4,103	3,963	3,991
Ca, %	0.62	0.67	0.70
P, %	0.43	0.57	0.00

[1]CM = Canola meal containing diet with or without multi-enzyme complex (MEC). The MEC used was a mixture of carbohydrases (Superzyme-OM, 1.5 g/kg of diet) and phytase (Bio-phytase, 0.25 g/kg of diet) provided by Canadian Bio-System Inc. (Calgary, Alberta, Canada), and was added to the CM-containing diet (top dress) to make the second treatment.

[2]LND = Low nitrogen diet (casein-cornstarch diet).

[3]PFD = Phosphorus-free diet.

[4] Supplied the following per kg of finished feed: vitamin A, 6,058 IU; vitamin D, 805 IU; vitamin E, 66 IU; vitamin K, 6 mg; choline, 550 mg; pantothenic acid, 23 mg; riboflavin, 7 mg; folic acid, 1.65 mg; niacin, 33 mg; thiamin, 1.01 mg; vitamin B_6, 2.5 mg; biotin, 0.30 mg; vitamin B_{12}, 0.04 mg, Cu, 12 mg as copper sulfate; Zn, 122 mg as zinc oxide; Fe, 122 mg as ferrous sulfate; Mn, 15 mg as manganese sulfate; I, 0.4 mg as potassium iodate; Se, 0.3 mg as sodium selenite.

Table 6.2 Analyzed composition of canola meal (dry matter basis; CM)

Item	Solvent extracted CM
CP, %	41.49
EE, %	3.90
Ash, %	7.40
NDF, %	28.79
ADF, %	18.29
NSP, %	20.60
Ca, %	0.69
P, %	1.28
Phytate P, %	0.81
Non-phytate P, %	0.46
Glucosinolates, μmol/g	7.80
Indispensable AA, %	
Arginine	2.27
Histidine	1.18
Isoleucine	1.29
Leucine	2.57
Lysine	2.07
Methionine	0.68
Phenylalanine	1.51
Threonine	1.56
Tryptophan	0.56
Valine	1.68
Dispensable AA, %	
Alanine	1.73
Aspartic acid	2.73
Cysteine	0.87
Glycine	1.77
Glutamic acid	6.59
Proline	2.83
Serine	1.82
Tyrosine	0.97

6.3.2 Experimental Design and Procedures

After a 10-d recovery period, the eight sows were assigned at random to the dietary treatments in a repeated 4 × 4 Latin square design to give 8 observations per treatment. Gestating sows were fed 3.0 kg/d of the respective experimental diets, whereas, during lactation, sows had ad libitum access to their diets. Two equal meals were offered at 0700 and 1300 h as dry mash. Sample collection was done over 3 phases; mid-gestation, late-gestation, and lactation. Each phase had 4 experimental periods lasting for 8 d each. In each period, after 5-d acclimation to the experimental diets, fecal samples were collected by grab sampling via rectal palpation on d 6. On d 7 and 8, ileal digesta samples were collected continuously for 12 h (0700 to 1900 h) into plastic bags that were attached to the barrel of the ileal T-cannulas by a hose clamp as described by Nyachoti et al. (2002). Collection bags contained 10 mL of 10% (vol/vol) formic acid to minimize bacterial activity. Every 1 h or whenever the bags were three-quarters full, bags with digesta were removed and replaced with a new bag. Digesta and fecal samples were immediately frozen at −20°C until further processing.

6.3.3 Sample Preparation and Chemical Analyzes

Ileal digesta samples were thawed and pooled for each sow and period, homogenized in a heavy-duty blender (Waring Commercial, Torrington, CT), sub-sampled, and freeze-dried. Fecal samples were dried in an oven at 50°C for 5 d, and sub-sampled. Dried digesta, feces, and experimental diets were ground to pass through a 1 mm screen before chemical analysis. Ileal digesta, fecal, and diet samples were analyzed for DM, AA (digesta and diets only), CP, GE, Ca, P and titanium.

Dry matter content was determined according to the AOAC (1990; method 925.09) by oven drying 5 g of sample at 102°C overnight. Gross energy was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL) which was calibrated using benzoic acid as a standard. Nitrogen content was determined using the combustion method (method 990.03; AOAC, 1990) using the LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI) and CP was calculated as nitrogen x 6.25. Neutral detergent fiber was analyzed according to the method of Van Soest et al. (1991) using the Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). Samples for analysis of Ca and P were ashed for 12 h and digested according to AOAC (2005; method 985.01) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA). Titanium content was determined according to the procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). The AA content was determined as described in method 994.12 (AOAC, 1990), as modified by Mills et al. (1989). Briefly, a 100-mg sample was digested in 4 mL of 6 M HCl in vacuo for 24 h at 110°C. The digested mixture was neutralized with 4 mL of 6.25 M NaOH and allowed to cool at room temperature. The neutralized mixture was made up to a 50-mL volume with sodium citrate buffer solution (19.6 g/L; pH 2.2) and analyzed using an AA analyzer (Sykam, Eresing, Germany). Samples for analysis of S-containing AA (Met and Cys) were subjected to performic acid oxidation before acid hydrolysis. Tryptophan was determined according to the procedures described by Hugli and Moore, (1972). Briefly, 50-mg of sample was hydrolyzed with 25% NaOH at 120°C for 20 h and was analyzed using an AA analyzer (Sykam, Eresing, Germany).

6.3.4 Calculations and Statistical Analysis

Apparent ileal (AID) and apparent total tract digestibility (ATTD) coefficients were calculated using the following equation:

Apparent nutrient digestibility (%) = $100 - \{[(N_d/N_f) \times (Ti_f/Ti_d)] \times 100\}$,

Where N_d = nutrient concentration in digesta/feces (mg/kg DM), N_f = nutrient concentration in feed (mg/kg DM), Ti_f = titanium concentration in feed (mg/kg DM), Ti_d = titanium concentration in digesta/feces (mg/kg DM).

Standardized ileal digestibility of AA or STTD of P were calculated using the following Equations (Adewole et al., 2017; Kim et al., 2017):

% SID = AID + $[(EAL/AA_f) \times 100]$

% STTD = ATTD + $[(EAL/AA_f) \times 100]$

Where EAL = basal endogenous loss of AA/P (mg/kg DM intake), AA_f = dietary content of the AA (mg/kg DM), EAL for AA/P was calculated according to the following equation:

$EAL = N_{ex} \times (Ti_f/Ti_d)$

Where N_{ex} = concentration of AA in ileal digesta from sows fed casein-cornstarch/concentration of P in feces from sows fed P-free diet (mg/kg DM), Ti_f = titanium concentration in feed (mg/kg DM) and Ti_d = titanium concentration in ileal digesta/feces (mg/kg DM).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included treatment as the fixed variable and pen and period as the random variables. The effects of pen and period were not statistically significant, and thus the final model had treatment as the main effect. Results were considered significant at $P \leq 0.05$ and tendencies were observed at $0.05 < P < 0.10$.

6.4 RESULTS

The analyzed chemical compositions of the experimental diets and the solvent extracted CM samples are presented in Tables 6.1 and 6.2 The analyzed CP content of the diet were close to the calculated values.

6.4.1 Apparent Ileal Digestibility of Amino Acids

Apparent ileal digestibility values for DM, CP, and AA in CM are presented in Table 6.3. Sows in lactation had higher ($P < 0.05$) AID for CP, and all AA, when compared to sows in mid- or late-gestation, except for AID of proline which tended ($P = 0.062$) to be higher in lactating sows. The MEC supplementation improved ($P < 0.05$) the AID of AA, namely histidine, lysine, methionine, valine and alanine, and showed a tendency ($0.05 < P < 0.10$) for improvement in AID of arginine, isoleucine, tryptophan, and cysteine during the lactation phase. However, except glutamic acid showing higher ($P = 0.007$) AID and serine showing a tendency ($P = 0.071$) for improvement during mid-gestation, there was no enzyme effect in AID of AA during both mid- and late-gestation.

6.4.2 Standardized Ileal Digestibility of Amino Acids

Standardized ileal digestibility values for CP and AA in CM fed to gestating and lactating sows are presented in Table 6.4. The ileal endogenous N and AA losses determined using a low-protein diet are presented in Table 6.6. The reason behind the use a low protein diet instead of a protein-free diet in the current study is because the Animal care committee does not allow to feed 100% nitrogen-free diets and moreover, low protein diets does affect the protein or AA retention and subsequently does not upset the outcome of the study. No significant effect of the collection

phase was observed except for arginine, histidine, lysine, methionine, tryptophan, and serine which showed higher ($P < 0.05$) SID valued during lactation, and SID of glutamic acid, which tend ($P = 0.091$) to be higher in lactating sows. The MEC supplementation improved ($P < 0.05$) the SID of glutamic acid during mid-gestation, and SID of arginine, histidine, lysine, methionine, and valine during lactation. Moreover, during lactation SID of tryptophan and alanine showed tendency for improvement with enzyme supplementation.

6.4.3 Total Tract Digestibility of Nutrients

Total tract digestibility of energy and nutrients in CM fed to gestating and lactating sows are presented in Table 6.5. The endogenous P losses determined in sows fed a P-free diet are presented in Table 6.6. There were no differences in total tract digestibility values for nutrients between the collection phases (mid- and late-gestation, and lactation). Moreover, MEC supplementation only improved ($P < 0.05$) the ATTD of DM and P, and STTD of P during lactation. Also, ATTD of CP showed a tendency ($P = 0.054$) for improvement during lactation.

Table 6.3 Apparent ileal digestibility of DM, CP, and AA in canola meal (CM) fed to sows with or without multi-enzyme complex (MEC)[1]

Item	Phase									P-value			
	Mid gestation			Late gestation			Lactation						
	Diets[2]			Diets[2]			Diets[2]						
	CM (-)	CM (+)	SEM	CM (-)	CM (+)	SEM	CM (-)	CM (+)	SEM	Mid gestation	Late gestation	Lactation	Phase
DM, %	75.9	79.4	1.63	75.8	78.5	2.34	75.9	77.0	0.95	0.124	0.349	0.126	0.936
CP, %	65.2	65.9	0.83	65.6	65.6	0.96	67.0	69.4	0.95	0.554	0.999	0.135	0.004
Indispensable AA, %													
Arginine	75.8	75.3	0.90	77.3	75.9	0.74	83.5	86.1	0.71	0.699	0.279	0.059	<0.001
Histidine	35.8	35.3	0.58	37.8	37.2	0.75	53.2	57.2	0.39	0.611	0.218	<0.001	<0.001
Isoleucine	72.6	73.5	1.09	74.5	73.3	1.09	79.4	81.6	0.64	0.672	0.333	0.087	<0.001
Leucine	77.3	76.6	0.68	78.3	77.0	0.63	80.5	82.5	0.85	0.625	0.270	0.222	<0.001
Lysine	74.7	74.5	0.57	75.4	74.5	0.82	80.3	82.8	0.58	0.779	0.401	0.041	<0.001
Methionine	84.0	83.6	0.78	85.4	84.8	0.54	87.6	89.9	0.39	0.757	0.307	0.007	<0.001
Phenylalanine	76.3	76.8	1.35	77.7	77.2	0.63	79.9	82.5	1.02	0.764	0.650	0.123	0.003
Threonine	61.8	61.1	1.28	62.9	61.0	2.62	72.5	73.3	0.80	0.798	0.506	0.472	<0.001
Tryptophan	73.8	75.6	1.90	75.8	76.0	1.36	85.7	88.1	0.63	0.505	0.895	0.053	<0.001
Valine	72.4	72.1	1.06	73.0	72.6	0.61	74.5	81.1	0.79	0.853	0.720	0.001	<0.001
Dispensable AA, %													
Alanine	70.5	72.0	0.93	70.9	71.6	1.44	76.7	78.9	0.64	0.373	0.678	0.031	<0.001
Aspartic acid	59.1	59.4	1.10	59.2	59.5	2.36	71.1	71.0	1.19	0.871	0.884	0.954	<0.001
Cysteine	71.2	71.1	1.63	72.7	71.9	1.30	78.5	81.0	0.79	0.974	0.683	0.059	<0.001
Glycine	67.0	68.0	0.49	67.1	68.1	0.66	73.2	74.6	1.40	0.344	0.502	0.506	<0.001
Glutamic acid	81.4	83.5	0.49	83.5	83.9	0.42	86.6	87.8	0.68	0.007	0.352	0.158	<0.001
Proline	55.2	55.8	4.58	56.4	53.9	3.93	64.8	66.0	2.25	0.944	0.578	0.781	0.062
Serine	69.8	72.5	0.76	71.8	72.6	0.56	73.9	75.8	0.60	0.071	0.471	0.134	0.001
Tyrosine	69.5	70.2	1.13	70.5	70.4	1.41	78.1	78.6	1.09	0.740	0.979	0.772	<0.001

[1]n=8.

[2]CM = Canola meal containing diet. +/−Represents presence or absence multi-enzyme complex (MEC).

Table 6.4 Standardized ileal digestibility of DM, CP, and AA in canola meal (CM) fed to sows with or without multi-enzyme complex (MEC)[1]

Item	Phase									P-value			
	Mid gestation			Late gestation			Lactation						
	Diets[2]		SEM	Diets[2]		SEM	Diets[2]		SEM				
	CM (-)	CM (+)		CM (-)	CM (+)		CM (-)	CM (+)		Mid gestation	Late gestation	Lactation	Phase
CP, %	79.9	80.6	0.83	79.7	80.3	0.96	77.9	80.3	0.95	0.551	0.736	0.135	0.457
Indispensable AA, %													
Arginine	88.7	88.4	0.88	89.8	88.9	0.74	91.3	94.2	0.68	0.831	0.486	0.036	<0.001
Histidine	93.5	92.6	0.56	92.6	93.1	0.75	94.0	95.8	0.38	0.265	0.571	0.030	0.001
Isoleucine	85.9	86.7	1.09	86.0	86.6	1.09	87.0	88.9	0.65	0.675	0.636	0.150	0.127
Leucine	88.8	88.7	0.70	89.5	88.7	0.63	89.2	90.5	0.91	0.909	0.445	0.446	0.469
Lysine	86.9	86.8	0.59	87.0	86.7	0.82	87.7	89.9	0.58	0.881	0.781	0.045	0.005
Methionine	91.6	90.9	0.76	92.9	92.0	0.54	93.3	95.1	0.39	0.615	0.203	0.026	<0.001
Phenylalanine	88.7	88.0	1.36	89.6	88.7	0.63	87.8	89.9	0.99	0.686	0.416	0.253	0.819
Threonine	84.7	84.0	1.29	83.9	84.0	2.62	82.7	83.8	0.74	0.786	0.975	0.355	0.670
Tryptophan	87.5	89.1	1.92	88.8	89.8	1.36	91.5	93.8	0.63	0.548	0.574	0.059	0.006
Valine	85.8	84.7	1.00	86.1	84.9	0.61	84.3	89.3	0.78	0.512	0.328	0.004	0.281
Dispensable AA, %													
Alanine	85.8	85.6	0.93	87.5	85.7	1.44	84.9	86.7	0.69	0.894	0.277	0.068	0.561
Aspartic acid	84.8	84.8	1.15	82.5	85.3	2.36	84.7	83.9	1.17	0.982	0.192	0.751	0.676
Cysteine	87.0	86.3	1.51	87.5	86.5	1.30	87.2	88.5	0.80	0.812	0.615	0.215	0.665
Glycine	90.2	91.3	0.51	91.0	91.3	0.66	90.7	91.6	1.46	0.296	0.813	0.684	0.885
Glutamic acid	90.6	92.6	0.49	93.6	93.1	0.42	92.4	93.6	0.67	0.008	0.331	0.138	0.091
Proline	101.8	102.5	4.59	103.0	100.7	7.92	97.0	99.1	2.30	0.941	0.780	0.635	0.603
Serine	88.8	91.7	0.75	90.6	91.7	0.56	84.6	86.7	0.61	0.052	0.360	0.101	<0.001
Tyrosine	86.5	85.6	1.15	86.1	86.0	1.41	87.5	88.3	1.06	0.684	0.974	0.677	0.232

[1]n=8.

[2]CM = Canola meal containing diet. +/−Represents presence or absence multi-enzyme complex (MEC).

Table 6.5 Apparent total tract digestibility (ATTD) of nutrients and standardized total tract digestibility (STTD) of P in canola meal (CM) fed to sows with or without multi-enzyme complex (MEC)[1]

Item	Phase									P-value			
	Mid gestation			Late gestation			Lactation						
	Diets[2]		SEM	Diets[2]		SEM	Diets[2]		SEM				
	CM (-)	CM (+)		CM (-)	CM (+)		CM (-)	CM (+)		Mid gestation	Late gestation	Lactation	Phase
ATTD, %													
DM	84.0	83.8	1.32	83.6	85.0	1.24	84.5	86.2	0.56	0.896	0.589	0.024	0.383
CP	83.7	84.5	1.10	83.2	85.4	1.39	84.0	85.7	0.63	0.622	0.406	0.054	0.800
NDF	56.9	56.2	4.84	56.6	56.1	4.59	55.7	56.4	1.63	0.668	0.773	0.500	0.887
Ca	58.8	58.2	2.71	58.6	59.3	5.07	58.8	58.9	3.00	0.480	0.776	0.889	0.741
P	33.9	39.6	2.65	34.9	38.4	4.47	38.2	42.9	0.91	0.197	0.344	0.012	0.165
STTD, %													
P	44.5	50.4	2.65	45.5	49.2	4.47	44.2	48.9	0.92	0.188	0.326	0.011	0.904

[1]n=8.

[2]CM = Canola meal containing diet. +/−Represents presence or absence multi-enzyme complex (MEC).

Table 6.6 Non-specific endogenous Nitrogen, AA and P losses in sows[1]

Item	Non-specific endogenous losses[2], mg/kg DMI		
	Mid Gestation	Late Gestation	Lactation
Nitrogen	3206.6	3065.9	2382.8
P	614.1	609.1	343.0
Indispensable AA			
Arginine	797.9	769.4	502.7
Histidine	2091.9	1984.1	1479.2
Isoleucine	599.6	520.5	318.7
Leucine	868.4	842.6	600.1
Lysine	857.8	816.4	502.9
Methionine	201.3	198.8	148.1
Phenylalanine	520.1	503.8	306.8
Threonine	1094.8	900.5	501.2
Tryptophan	236.3	224.8	98.8
Valine	719.8	701.8	405.6
Dispensable AA			
Alanine	762.6	725.9	424.6
Aspartic acid	1971.2	1711.2	994.6
Cysteine	423.6	395.1	217.4
Glycine	1406.4	1448.7	1033.4
Glutamic acid	2033.2	2042.3	1289.9
Proline	3407.2	3254.1	2419.7
Serine	993.5	985.8	569.7
Tyrosine	466.9	428.7	264.1

[1]n=8.

[2]The non-specific endogenous loss of AA was estimated in sows fed the low nitrogen diet and the non-specific endogenous loss of P was estimated in sows fed the P-free diet.

6.5. DISCUSSION

The prime objective of this study was to determine the AA and P digestibility in CM in two different physiological stages in sows, namely gestation and lactation. The solvent extracted CM used in the current study had comparable concentrations of CP, NDF, AA, and minerals when compared with CM used in previous studies (González-Vega and Stein, 2012; Adewole et al., 2016, 2017). However, studies have shown that CM from different processing plants in Canada vary in their nutrient composition, mainly due to differences in growing and harvesting conditions, and also due to the variation in heat treatment during processing (Bell and Keith, 1991; Adewole et al., 2016)

6.5.1 Apparent Ileal Digestibility of Amino Acids

Studies associated with AA digestibility in CM fed to sows are limited, restricting the comparison between studies. The AID of CP in CM fed to gestating and lactating sows in the current study was found to be higher (46 and 24%, respectively for gestating and lactating sows) when compared to the digestibility coefficients in sows reported by Stein et al. (1999). Similarly, for AA, except for histidine, most of the AID coefficients observed in the current study in both gestating and lactating sows were found to be higher than those reported in sows by Stein et al. (1999). Protein and AA digestibility in CM fed to pigs has shown to be influenced by factors like the age of the pig (Maison and Stein, 2014), canola variety, and oil extraction technique used (Woyengo et al., 2010; Trindade Neto et al., 2012; Adewole et al., 2017). The AID coefficients of CM used in different studies has shown to vary consistently depending on the concentrations of tannin, NDF, and the hull in different varieties of canola being used (Fan et al., 1996). Moreover, the heat treatment during the meal processing has been reported to have a negative effect on AA

digestibility, mainly because of overheating (Parsons et al., 1991; Khajali and Slominski, 2012; Adewole et al., 2016).

Apparent ileal digestibility coefficients of CP and AA for lactating sows in the current study were found to be higher when compared to the corresponding values for growing-finishing pigs reported in previous studies (Stein et al., 1999; González-Vega and Stein, 2012; Maison and Stein, 2015b; Adewole et al., 2017). However, when compared with studies by Kim et al. (2015) and Liu et al. (2018) in growing pigs, similar AID coefficients in lactating sows were observed in the current study, except for AID of histidine, methionine, tryptophan, and proline. Moreover, AID of most of the AA in CM fed to gestation sows in the current study were either similar or even lower than those values in growing-finishing pigs as reported in the above-listed studies.

The major assumptions behind sows having a higher AID for protein and AA compared to growing pigs include a more efficient digestive system, due to a larger and more developed gastrointestinal tract in sows (Bridges et al., 1986). An increase in the intestinal volume could result in longer residence time for the digesta in sows (Varel, 1987; Low, 1993), resulting in better absorption of nutrients. Yet another factor that influences digestibility of nutrients is the feed intake by pigs. Lactating sows and growing pigs are usually allowed ad libitum feed intake, while gestating sows are maintained on restricted diets. Even though, ileal digestibility of AA in pigs are not directly affected by feeding levels (Haydon et al., 1984; Moter and Stein, 2004), a reduced feed intake has been shown to increase the digestibility of energy, crude fiber and fat (Cunningham et al., 1962; Everts et al., 1986; Shi and Noblet, 1993a). However, non-specific or the basal endogenous losses for AA (g/kg DM intake) are elevated with a reduction in feed intake (Moter and Stein, 2004; Adeola et al., 2016), and a higher excretion of non-specific endogenous AA results in a lower AID values of AA (Hess and Sève, 1999; Hodgkinson et al., 2000; Leterme and

Théwis, 2004; Adeola et al., 2016). The non-specific endogenous losses of AA are related only to the DM intake of the animal and are not influenced by the type of diet or the feed ingredient itself. Gestating sows in the current study showed an elevated endogenous AA loss when compared to lactating sows (Table 6.6). Moreover, the values for non-specific endogenous AA losses observed in the current study were higher for gestating sows and similar or even lesser for lactating sows for most of the AA when compared to those reported in growing pigs (Adeola et al., 2016; Adewole et al., 2017). The elevated non-specific endogenous losses of AA observed of gestating sows in the current study could be attributed to the restricted feed intake (3kg/d) which in turn could have resulted in a lower estimate of AID for AA in gestating sows when compared to lactating sows (average feed intake of 6.8 kg/d). Accordingly, for lactating sows with a similar endogenous losses as growing pigs is expected to have a similar AID for AA, however, in the current study, the high AID values in lactating when compared to growing pigs indicate an improved digestibility of AA. Similarly, gestating sows are expected to have a lower AA digestibility when compared to growing pigs, owing to a higher endogenous loss, but the fact that the results in the current study were not different from those in growing pigs from the previous studies could probably be due to a better AA digestibility in gestating sows.

6.5.2 Standardized Ileal Digestibility of Amino Acids

The SID of CP and AA in CM in gestating and lactating sows in the current study were found to be higher, but comparable to those SID coefficients for CM in sows reported by Stein et al. (2001). Lactating and gestating sows in the current study had similar SID coefficients for most AA, except for higher SID coefficients for arginine, histidine, lysine, methionine, tryptophan, and serine in lactating sows. The fact that SID of certain AA in lactating sows were higher than that in

gestating sows shows an improved AA digestibility. However, it should be noted that SID of AA are influenced by the level of feed intake (Stein et al., 2007), and hence SID coefficients obtained from pigs fed restricted diets may not be comparable to those obtained from pigs fed ad libitum diets (Moter and Stein, 2004). When compared to the average SID coefficients of AA in CM fed to growing pigs from previous studies (Stein et al., 2001; Woyengo et al., 2010; Gonzalez-Vega and Stein, 2012; Trinidade Neto et al., 2012; Sanjayan et al., 2014; Adewole et al., 2017), the SID coefficients for AA in lactating sows were higher in the present study. On the contrary, Stein et al. (2001) reported similar SID values for most of AA in CM in lactating sows and growing pigs.

The major site for fiber fermentation in pigs is the large intestine; however, a small but a significant amount of the soluble component of the dietary fiber is also being fermented in the distal part of the small intestine (Jha et al., 2010; Jha and Leterme, 2012). An improved fermentative capacity by the microbes in the small intestine of lactation sows when compared to growing pigs could be one of the probable reasons for a better AA digestibility in lactating sows. Since SID coefficients in lactating sows in the current study were higher when compared to those in growing pigs in previous studies, and the fact that both growing pigs and lactating sows were fed close to ad libitum, SID for AA in CM determined in growing pigs may not be representative for lactating sows.

6.5.3 Total Tract Digestibility of Nutrients

The ATTD of nutrients in the current study were found to be similar for gestating and lactating sows. On average, ATTD of protein in CM fed to sows was 83.6%, which was higher than that reported for CM in sows by Stein et al. (1999; 70.8 and 75.0% for gestating and lactating sows, respectively). Moreover, when protein digestibly in lactation sows were compared with

studies determining ATTD of protein in CM in growing pigs (Stein et al., 1999; Liu et al., 2018), it was observed that sows had a higher total tract digestibility coefficient. An improvement in digestive capacity in pigs with age has been observed in several studies, where they found an increased ATTD of CP in diets for sows when compared to growing pigs (Fernandez et al., 1986; Noblet and Shi, 1993; Etienne et al., 1997). Hindgut fermentation of fiber in sows is more efficient owing to an increased cellulolytic bacterial activity when compared to that in growing pigs (Varel, 1987; Fernandez et al., 1986). This could be the probable reason for a higher ATTD of NDF in the current study when compared to those reported of CM in growing pigs by Maison et al. (2015b; 56.4 *vs.* 51.9%).

To our knowledge, the STTD of P has not been previously reported for CM fed to gestating or lactating sows. In the current study P digestibility (both ATTD and STTD) were similar in both gestating and lactating sows, indicating that the physiological status of the sow had no influence on the digestibility coefficients. On average, the ATTD and STTD of P in CM in sows were 35.7 and 44.7%, respectively. Phosphorus excreted in the feces is comprised of non-digested P and endogenous P. The basal endogenous P losses measured in the current study varied between gestating and lactating sows, with gestating sows having a higher basal endogenous P loss. Bikker et al. (2016) reported a lower endogenous P loss for gestating sows (500 mg/kg DMI), which could probably be associated with a lower BW of sows (201 kg) when compared to the sows used in the current study (initial BW 260 kg). Studies have shown a direct relationship between BW and endogenous P losses in pigs (expressed as mg/kg DMI), wherein, P losses increase with increasing BW (Bikker et al., 2016, 2017). Similarly, endogenous P losses in growing pigs reported in previous studies (Adhikari et al., 2015; Kim et al., 2017; Mejicanos et al., 2018) were lower when compared to those in sows from the current study, which could also be attributed to a lower BW

in growing pigs. However, the current finding that the basal endogenous P loss was lower (343 vs. 611 mg/kg DMI) for lactating sows (fed close to ad libitum and with a lower BW) when compared to gestating sows fed restricted diet (higher initial BW) contradicts the finding by Bikker et al. (2016), where they observed an increase in endogenous P loss in pigs with increasing feeding levels. Hence it is true that variation in endogenous P loss in pigs is independent of the variation in BW and feed intake (Bikker et al., 2017).

The P digestibility in oilseeds fed to pigs vary according to the amount of phytate P (Selle and Ravindran 2008; Rodriguez et al., 2013). This could be one of the probable reasons for a wide variation in the STTD of P in CM (30.0 to 50.0%) fed to growing pigs in previous studies (Akinmusire and Adeola, 2009; Rodriguez et al., 2013; Adhikari et al., 2015; Maison et al., 2015a; Mejicanos et al., 2018). The STTD of P in the current study (44.7%), though was within the range observed in growing pigs, it is difficult to make a comparison due to the wide variation in P digestibility of CM in growing pigs. The phytate content in CM is influenced by the plant variety and P availability in soil (Uppström and Svensson, 1980). Consequently, the phytate P content in CM may vary depending on the canola varieties, the environmental conditions, and the area where the canola is grown (Rodriguez et al., 2013). Hence, while comparing the P digestibility of CM among studies, the phytate P content of the meal should also be taken into consideration.

Calcium digestibility was similar in both gestating and lactating sows, with an average ATTD of 58.7% for CM. Apart from CM, limestone was also a source of Ca in the current study. On comparison with published values in growing pigs, ATTD of Ca in CM in sows were found to be higher than those reported by Adhikari et al. (2015) and Mejicanos et al. (2018) and lower than those reported by Rodriguez et al. (2013). However, comparable values for ATTD of Ca in CM were observed in studies by Maison et al. (2015a).

6.5.4 Enzyme Supplementation

Oilseeds, when used in high inclusion rates in the swine diet, may significantly contribute to the total quantity of non-starch polysaccharides (NSP) and phytate in the diet. High-NSP diets have been shown to reduce AA digestibility in pigs (Torre et al., 1991; Myrie et al., 2008), mainly by physical entrapments of nutrients, thereby creating a barrier for absorption (Bedford and Schulze, 1998), or due to higher flow of endogenous AA directly caused by the fiber component (Le Goff and Noblet, 2001; Bartelt et al., 2002). Phytate, on the other hand, could bind to six phosphate groups, making P unavailable, since non-ruminant animals lack the enzyme phytase to break these bonds.

The use of a mixture of carbohydrases that target different NSP has shown to produce more significant benefits than the use of individual NSP-degrading enzymes since one enzyme may facilitate the activity of the other (Olukosi and Adeola, 2013). Moreover, the use of carbohydrases and phytase combination, results in hydrolysis of the cell wall by carbohydrases, which improves the contact of phytase and phytate, thus enhancing phytase activity (Parkkonen et al., 1997; Simon, 1998).

The use of exogenous enzymes in sow diets has not been studied much probably because sows can utilize fibrous feedstuffs more effectively than growing pigs. The stage of reproduction has a significant influence on the effect of enzyme supplementation in sows (Olukosi and Adeola, 2013). Nyachoti et al. (2006) observed an improvement in P digestibility during late-gestation when compared to early gestation in sows supplemented with phytase. On the contrary, in the current study, phytase supplementation only improved the P digestibility during lactation. This finding is in agreement with Kemme et al. (1997), who reported that phytase has a higher efficacy in lactating sows when compared to gestating ones. It should also be noted that the effect of phytase

on P utilization also varies depending upon the hydrolyzing activity of phytase which in turn varies depending on the source (Paditz et al., 2004; Nyachoti et al., 2006). However, considering the effect of phytase on P digestibility of CM, several studies in growing pigs have reported consistent results, wherein phytase supplementation improved the STTD of P (Akinmusire and Adeola, 2009; Rodriguez et al., 2013; Adhikari et al., 2015; Maison et al., 2015).

The efficacy of multi-carbohydrases in degradation of cell wall polysaccharide of CM has been verified by Meng et al. (2005) in an in-vitro study. However, with in-vivo studies, the results of multi-carbohydrases supplementation in pigs have been inconsistent. Studies involving the use of Superzyme OM (multi-carbohydrase used in the current study), did not improve the AA digestibility in growing pigs (Sanjayan, 2013). However, in the current study, improvement in nutrient digestibility with MEC supplementation was predominantly observed during the lactating phase, the probable reason being lactating sows fed ad libitum feed compared to gestating sows fed restricted diets.

6.6. CONCLUSION

In conclusion, SID of essential and non-essential AA in CM in gestating and lactating sows were determined and when compared to the average SID coefficients of AA in CM fed to growing pigs from previous studies, the SID coefficients for AA in lactating sows were higher in the present study. Supplementation with MEC improved the SID of some AA during lactation. The average STTD for P in CM fed in sows was 44.7 % and MEC significantly improved to STTD of P in CM during lactation. The results imply that the adoption of nutrient digestibility values for CM in growing pigs would result in an underestimation of those for gestating and lactating sows.

CHAPTER 7

GENERAL DISCUSSION

Solvent extracted canola meal is a co-product of canola crushing industry and could be a cost-effective protein source in lactating sow diets. However, the high fiber and high glucosinolate content in the CM have been a major concern in using CM as a sole protein source in sows. However, the current cultivars of canola have low concentrations of glucosinolates, leading to production of CM with lower glucosinolates content which in turn allows higher inclusion levels in diets for sows. Hence, the overall objective of this study was to determine the effects of including solvent extracted CM as a sole protein source in lactating sow diets on reproductive performance, milk composition, suckling piglet performance, gut health and nutrient digestibility. Standardized ileal digestibility of AA and STTD of P in CM fed to gestating and lactating sows were also determined. The findings from the studies conducted to define the above objectives are discussed here.

In manuscript I, effects of increasing dietary CM in substitution for soybean meal in lactation sow diets were determined. Lactation performance data from the current study showed that CM could be well incorporated as a major protein source in lactating sow diets. Previous studies have shown that feeding CM-containing diets to pigs reduce growth performance (Baidoo et al., 1986, 1987; McIntosh et al., 1986). This could be attributed to the fact that the diets in the above studies were formulated based on CP content and DE value, and that the energy values of diets rich in protein or fiber are overestimated when expressed on DE basis (Velayudhan et al., 2015). Hence, NE system enables more effective utilization of high fiber ingredients like CM without affecting pig performance. The recommended dietary glucosinolate concentration for

lactating sows is below 2 μmol/g, so as to avoid any reduction in feed intake (Spratt and Leeson, 1985; Schöne et al., 1999; Quiniou et al., 2012). The CM used in the present experiment contained relatively low levels (7.9 μmol/g) of glucosinolates and hence the diets with 15, and 30% inclusion of CM contained 1.32 and 2.82 μmol/g of glucosinolates, respectively. Therefore, the lack of difference in daily feed intake in sows between treatments in the current study could be because lactating sows can tolerate a higher level (2.82 μmol/g for 30% CM inclusion) of glucosinolates in diets. In manuscript I, sows fed CM containing diets had similar milk composition when compared to those fed control diet probably because the diets were formulated to contain similar standardized ileal digestible AA contents. The quantity and quality of milk produced by the sow has an influence on the BW gain in piglets (Skok et al., 2007) and one reason for a similar BW gain of piglets in the current study could be due to no difference in the sow milk composition. Plasma urea N concentration in pigs has a direct relationship with the dietary protein content and is inversely related to protein quality or the biological value of the protein (Eggum, 1970; Orok and Bowland, 1975; Bassily et al., 1982). The probable reason for lower PUN values observed in the present study in sows fed CM when compared to those fed diets containing soybean meal could be due to the fact that CM protein has a high biological value when compared to soybean meal (Newkirk and Classen, 2002; Ghodsvali et al., 2005). Moreover, the CP content of the corn soybean meal based control diets were higher when compared to those diets containing CM, resulting in a higher PUN values in sows fed control diets. However, increasing inclusion of CM in this study resulted in a linear reduction in energy and nutrient digestibility in lactating sows and could be likely attributed to increasing dietary fiber content.

In manuscript II, it was hypothesized that with a longer adaptation period, CM could be used as a sole protein source in sow diets with no adverse effects on nutrient digestibility and that enzyme supplementation could improve nutrient digestibility in high CM diets. In this study, the effects of

CM inclusion as a sole protein source with or without enzymes from early gestation through to lactation on reproductive performance, milk composition, fecal bacterial profile and nutrient and energy digestibility in sows were determined. Lactation performance showed no negative effects of using CM as the only protein source in sows when diets formulated on similar NE values and standardized ileal digestible AA content were fed from early gestation. Also, it was recognized that the experimental design for measuring specific reproductive parameters such as the total number of piglets born, the number of piglets born alive, day 0 piglet BW, and the number of piglets weaned, were limited due to the small number of sows (n = 15) used in the study. Milk composition data and PUN content were consistent with results from manuscript I. Also, with a longer adaptation, sows were able to efficiently utilize the high dietary fiber in CM containing diets without any reduction in energy and nutrient digestibility, probably due to an increase in hindgut fermentation because of an increased gut microbial mass (Varel, 1987). Moreover, enzyme supplementation improved P digestibility in CM-containing diets. Fermentation of fiber has shown to increase the cellulolytic bacterial activity in hindgut of pigs and the resulting production of volatile fatty acid reduces the gut pH, which in turn promotes the growth of probiotic species of bacteria in the gut. Similarly, in the current study feeding CM-containing diets increased the abundance of lactic acid bacteria such as *Lactobacillus* and *Enterococcus* during the later part of gestation. Such variations in the abundance of beneficial bacteria in the gut were not observed during the end of lactation suggesting some kind of adaptation of the gut microbiome in sows which warrants additional studies to explain the association between dietary fiber and probiotic bacteria.

Accurate knowledge of the standardized ileal digestible AA and standardized total tract P contents would be essential for a more precise and cost-effective diet formulation and in the

ranking of feedstuffs. Therefore, for manuscript III, the primary objective was to determine SID of AA and STTP of P in CM fed to gestating and lactating sows and to compare with the corresponding published values for growing pigs. The solvent extracted CM used in the current study had comparable nutrient composition when compared with CM used in previous studies. However, it should be noted that CM from different processing plants could vary in their nutrient composition, mainly due to differences in growing and harvesting conditions, and due to the variation in heat treatment while processing (Bell and Keith, 1991; Adewole et al., 2016). Only limited studies have determined the AA digestibility of CM in sows, restricting the comparison between studies. The AID of CP and most of the AA in CM in gestating and lactating sows in the current study were found to be higher when compared to those digestibility coefficients for CM in sows reported by Stein et al. (1999). Moreover, lactating sows had a significantly higher AID for most of the AA when compared to sows in gestation. When compared to growing-finishing pigs (Stein et al., 1999; González-Vega and Stein, 2012; Maison and Stein, 2015; Adewole et al., 2017), AID coefficients of CP and AA in CM for lactating sows in the current study were found to be higher, however, AID coefficients of AA for gestating sows were either similar or even lower. The high non-specific endogenous losses of AA observed in gestating sows in the current study when compared to lactating sows could have resulted in a lower estimate of AID for AA in gestating sows. Accordingly, for lactating sows with similar endogenous losses as growing pigs is expected to have a similar AID for AA, however, in the current study, the elevated AID values in lactating sows when compared to growing pigs from previous studies indicate an improved digestibility of AA. The SID of CP and AA in CM in gestating and lactating sows in the current study were found to be higher, but comparable to those SID coefficients in sows reported by Stein et al. (2001). However, lactating and gestating sows in the current study had similar SID coefficients for AA,

except for higher SID coefficients for arginine, histidine, lysine, methionine, tryptophan, and serine in lactating sows. The fact that SID of certain AA in lactating sows were higher than gestating sows suggests an improved AA digestibility. When compared to the average SID coefficients of AA in CM fed to growing pigs from previous studies (Stein et al., 2001; Woyengo et al., 2010; Gonzalez-Vega and Stein, 2012; Trinidade Neto et al., 2012; Sanjayan et al., 2014; Adewole et al., 2017), the SID coefficients for AA in lactating sows were higher in the present study. Microbes in the distal part of small intestine in pigs have been shown to utilize smaller amounts of dietary fiber. An improved fermentative capacity by the microbes in the small intestine of lactating sows when compared to growing pigs could be one of the probable reasons for better AA digestibility in lactating sows. Since SID coefficients in lactating sows in the current study were higher when compared to those in growing pigs in previous studies, and the fact that both growing pigs and lactating sows were fed close to ad libitum, SID for AA in CM determined in growing pigs may not be representative for lactating sows. The ATTD of nutrients in the current study were found to be similar for gestating and lactating sows. Hence, on average, the ATTD and STTD of P in CM in sows were 35.7 and 44.7%, respectively. The P digestibility in oilseeds fed to pigs vary according to the amount of phytate P (Selle and Ravindran 2008; Rodriguez et al., 2013). This could be one of the probable reasons for a wide variation in the STTD of P in CM (30.0-50.0%) fed to growing pigs in previous studies (Akinmusire and Adeola, 2009; Rodriguez et al., 2013; Adhikari et al., 2015; Maison et al., 2015a; Mejicanos et al., 2018). The STTD of P in the current study, though was within the range observed in growing pigs, it is difficult to make a comparison due to the wide variation in P digestibility of CM in growing pigs. Also, enzyme supplementation improved the SID of some AA and STTD of P during lactation.

CHAPTER 8

CONCLUSIONS AND FUTURE STUDIES

8.1. Conclusions

The following conclusions can be drawn from the present research:

1. Replacing soybean meal with increasing levels of CM in lactation diets showed no effect on sow and suckling piglet performance.
2. Solvent extracted CM up to 30% in lactation diet can support satisfactory sow and suckling piglet performance.
3. With high inclusions of CM during lactation, energy and nutrient digestibility were reduced, probably due to increased fiber content and a shorter adaptation period to diets.
4. With longer adaptation period, up to 30% CM in lactation diet can support satisfactory sow and suckling piglet performance, without any adverse effects on energy and nutrient digestibility.
5. Feeding CM containing diets increased the abundance of beneficial bacteria in the gut.
6. Phytase supplementation improved P digestibility in CM during lactation period.
7. Standardized ileal digestibility coefficients for AA in CM in lactating sows were higher compared to those published for growing pigs.
8. Supplementation with a mixture of carbohydrases and phytase improved the SID of some AA and STTD of P in CM during lactation.
9. Standardized ileal digestibility for AA in CM determined in growing pigs may not be representative for lactating sows.

8.2. Future Studies

1. Determine the effect of CM inclusion as a sole protein source on reproductive performance and nutrient digestibility in consecutive reproductive cycles in sows.
2. To repeat the study in a commercial facility with larger number of sows to determine the cost efficiency in replacing soybean meal with CM.
3. Since the number of sows used in the current study to determine lactation performance was less, to enhance the robustness of the study, future study designs could consider using a larger number of replications.
4. To determine the ileal digestibility values simultaneously in growing pigs and sows to validate the current findings.

CHAPTER 9

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