

**NUTRITIONAL STRATEGIES FOR OPTIMIZING CANOLA MEAL
UTILIZATION IN SOW DIETS**

By
Xiaoxiao Zhang

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

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ABSTRACT

The objective of this research was to optimize the use of canola meal in gestating and lactating sow diets to fully replace soybean meal without causing adverse effects on reproductive performance or piglet outcomes. In manuscript I, the effects of including 300 g/kg solvent-extracted canola meal (**SCM**) in late-gestation and lactation diets, with or without *Saccharomyces cerevisiae* product supplementation, on sow and litter performance were evaluated. Results showed that the 300 g/kg SCM-containing diet had no effect on sow or litter growth performance but compared to the control diet, reduced the apparent total tract digestibility of energy, dry matter, and crude protein in gestating sows. Lactating sows fed the canola meal-containing diet exhibited improved digestibility of NDF, and *Saccharomyces cerevisiae* product supplementation increased the digestibility of calcium and phosphorus. Additionally, milk fat content at weaning was higher in sows fed the canola meal supplemented with *Saccharomyces cerevisiae* product, demonstrating that probiotic supplementation can enhance nutrient digestibility and milk composition in canola meal-based diets. In manuscript II, the effects of including 300 g/kg SCM and expeller-extracted canola meal (**ECM**) with or without *Saccharomyces cerevisiae* product supplementation on sow and litter performance and microbiome were evaluated. Results showed that ECM diets reduced sow body weight post-farrowing and decreased crude protein digestibility in gestating sows, while *Saccharomyces cerevisiae* product supplementation improved nutrient digestibility and mitigated the negative effects of ECM. In addition, SCM diets did not adversely affect sow performance, and probiotics increased milk fat content on d 19 post-farrowing. Thus, SCM can replace 300 g/kg soybean meal in sow diets without adverse effects on sow reproductive performance, while ECM inclusion may require *Saccharomyces cerevisiae* product supplementation to ensure optimal performance. In manuscript III, the study aimed to evaluate the long-term effects of SCM inclusion

as the main protein source in gestation and lactation diets on sow and litter performance over two reproductive cycles. Results showed that sow body weight and backfat thickness were unaffected by diet but varied between cycles, with greater body weight and backfat thickness observed in the second cycle. Litter performance, including weight and size, was unaffected by dietary treatment, though litter weights at birth and after cross-fostering were higher in the second cycle. The inclusion of canola meal did not adversely affect colostrum or milk composition, serum metabolites, or nutrient digestibility, demonstrating that canola meal can replace soybean meal without compromising sow or litter performance over multiple parities.

DECICATION

My dear grandfather Haifeng

My dear parents Huanyong and Fengling

My partner Wenye

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FOREWORD

This thesis was prepared following a manuscript format and is composed of three manuscripts.

Manuscript I was accepted by *Translational Animal Science*.

Manuscript III was submitted to the *Animal* journal.

Manuscript II and the peer-reviewed section in Chapter 2 are under preparation for publication.

In addition, results from the manuscript I were presented as a poster at the ASAS-CSAS, June 26-30th, 2022, OKC, US and the ANCC, May 9-11th, 2023, Montreal, CA, respectively

Results from manuscript II were presented as a poster in the 2023 IRC 16th International Rapeseed Congress, September 24-27th, Sydney, AU.

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LIST OF ABBREVIATION

AA	amino acid
ADF	acid detergent fiber
ADFI	average daily feed intake
ADG	average daily gain
ATTD	apparent total tract digestibility
BW	bodyweight
Ca	calcium
CCM	corn- 300g/kg canola meal-based diet
CCM-P	CCM diet with <i>S. cerevisiae</i> (Actisaf® Sc 47)
CEC	control diet with soybean meal replaced by 300 g/kg expeller-extracted canola meal
CEC-P	CEC diet with <i>Saccharomyces cerevisiae</i> supplementation
CON	corn-barley and soybean meal control diet
CP	crude protein
CSB	soybean meal control diet
CSB-P	CSB diet with <i>Saccharomyces cerevisiae</i> supplementation
CSC	control diet with soybean meal replaced by 300 g/kg solvent-extracted canola meal
CSC-P	CSC diet with <i>Saccharomyces cerevisiae</i> supplementation
CSCM	corn-barley and canola meal treatment diet
CTRL	corn-soybean meal-based diet
DE	digestible energy

DM	dry matter
ECM	expeller-extracted canola meal
GE	gross energy
GGT	gamma-glutamyl transferase
K	potassium
ME	metabolizable energy
Mg	magnesium
Na	sodium
NDF	neutral detergent fiber
NE	net energy
NSP	non-starch polysaccharides
P	phosphorus
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SCM	solvent-extracted canola meal
SID	standardized ileal digestibility

CHAPTER 1

GENERAL INTRODUCTION

The nutritional management of gestating and lactating sows is important for sustainable pork production, ensuring optimal reproductive performance, sow longevity, and growth of their suckling piglets (Solà-Oriol and Gasa, 2017). Gestation requires enough nutrients to support fetal development, placental growth, and maternal tissue maintenance, while during lactation, the metabolic demands of sows increase dramatically to support milk production, which is critical for piglet survival and immune function (Noblet and Etienne, 1987; Theil et al., 2022). Failure to meet these nutritional demands can lead to poor sow body condition, reduced milk production, lower piglet growth rates, and compromised reproductive performance in subsequent cycles. Soybean meal, traditionally the primary protein source in swine diets due to its high protein content, superior amino acid profile, and digestibility, has faced increasing challenges in recent years as global demand escalates driven by its extensive use in livestock. Additionally, the increased demand, coupled with fluctuating prices in the market, has raised the cost of soybean meal, making it a less economically viable option for swine producers (Kim et al., 2019). Thus, the need for sustainable feeding strategies dictates the search for alternative protein sources that can reduce reliance on soybean meal while maintaining productivity.

Canola meal, a by-product of canola oil extraction, has emerged as a promising alternative protein source for swine diets, with abundant production in regions like North America and Europe where canola is widely cultivated, ensuring a steady and cost-effective supply (Khajali and Slominski, 2012). Compared to soybean meal, canola meal offers a competitive amino acid profile,

particularly in lysine, methionine, and cysteine, which are essential for gestating and lactating sows. Canola meal is available in two primary forms based on the extraction method: solvent-extracted canola meal (**SCM**) and expeller-extracted canola meal (**ECM**), each with distinct chemical compositions. For example, SCM has a lower residual oil content, resulting in a higher protein concentration, while ECM retains more oil due to the low efficiency of expeller extraction method (Mejicanos et al., 2016). However, few studies have investigated the utilization of ECM in swine diets, especially in sows, and therefore it is necessary to compare the reproductive outcomes of sows fed the SCM and ECM. Despite its potential, the inclusion of canola meal in swine diets has historically been limited due to the presence of anti-nutritional factors such as glucosinolates, which can impair thyroid function and reduce feed intake, and phytic acid, which binds minerals and limits their bioavailability (Khajali and Slominski, 2012). Additionally, the relatively high fiber content of canola meal can lower energy density and reduce nutrient digestibility. However, advances in seed breeding, feed processing, and enzyme supplementation strategies have significantly mitigated these limitations of canola meal. However, recent advances in canola seed breeding have led to the development of low-glucosinolate canola varieties, and improved processing methods such as heat treatment and enzyme supplementation have helped mitigate the negative effects of anti-nutritional factors.

The fiber content in canola meal, while posing challenges for energy and nutrient digestibility, also provides functional benefits. For instance, fiber increases satiety, reduces stereotypic behaviors, and helps manage body weight in gestating sows, while also supporting better farrowing performance and enhancing feed intake during early lactation (Guillemet et al., 2007; Quesnel et al., 2009; Guillemet et al., 2010). When combined with probiotics, such as live yeast, the fermentation of dietary fiber is improved due to increased microbial enzymatic activity

that breaks down complex carbohydrates into fermentable monosaccharides and oligosaccharides. These substrates are then metabolized by microbes, leading to increased production of short-chain fatty acids, such as acetate, propionate, and butyrate, which in turn support the growth of beneficial microbiota and enhance gut health (Price et al., 2010; Ogbuewu et al., 2019). The produced microbial metabolites provide energy, support gut health, and enhance overall nutrient utilization. This synergistic effect not only improves sow performance but also positively impacts piglet outcomes by enhancing milk production and the transfer of beneficial microbes (Tsai et al., 2016).

Although several studies have shown that sows fed diets containing canola meal exhibited lower nutrient digestibility compared to those fed soybean meal (Velayudhan and Nyachoti, 2017; Velayudhan et al., 2018), sows are known to have a greater fiber utilization compared to young pigs. This suggests that prolonged exposure to high fiber ingredients like canola meal may allow physiological adaptation over time. Thus, it is necessary to study the long-term effects of canola meal on maintaining body condition and nutrient utilization more effectively over multiple parities, thereby supporting reproductive longevity.

Therefore, the main objective of this project was to evaluate nutritional strategies for optimizing canola meal utilization in sow diets. Based on the findings, canola meal may be optimized in future feeding strategies.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND

Canola, derived from *Brassica napus* L., *Brassica rapa* L., and *Brassica juncea* L., is a prominent oilseed crop that originated from rapeseed. Rapeseed belongs to the Brassicaceae family, which also includes well-known plants like cabbage, radish, kale, mustard, and cauliflower (Wanasundara, 2011). Canola was developed through selective breeding to remove undesirable compounds found in rapeseed, notably high levels of erucic acid and glucosinolates (Canola Council of Canada, 2024). Historically, rapeseed has been cultivated for more than 3,000 years in India and about 2,000 years in China and Japan, where it was primarily utilized as a source of oil for cooking, lighting, soap production, and various industrial applications (Raboanatahiry et al., 2021).

The introduction of rapeseed to North America, particularly in Canada during the 1930s and 1940s, aimed to diversify agricultural production (Juska and Busch, 1994). However, the traditional rapeseed oil's high erucic acid content (25-45%) and the residual meal's glucosinolate concentration (110-150 $\mu\text{mol/g}$) limited its use for human and animal consumption due to potential health concerns (Slominski, 1997). Studies have associated erucic acid with cardiovascular issues in animals, while glucosinolates, when hydrolyzed by the enzyme myrosinase, produce goitrogenic compounds that interfere with iodine metabolism, impair thyroid function, and consequently inhibit animal growth and performance (Khajali and Slominski, 2012; Radfar et al., 2017; Gołębiewska et al., 2022). In the 1950s, Canadian scientists recognized the need to develop an oilseed that would be safer and more suitable for both human and animal consumption by reducing

levels of erucic acid and glucosinolates (Juska and Busch, 1994). This led to the development of the first “double-zero” cultivars, characterized by low concentrations of erucic acid and glucosinolate (Slominski, 1997). These cultivars were named "canola," a term derived from "Canada" and "ola" (oil low acid), to distinguish them from traditional high-erucic rapeseed (Slominski, 1997; Khajali and Slominski, 2012). The release of double-zero canola varieties transformed the crop into a staple of Canadian agriculture. Today, canola is one of Canada’s most economically significant crops, contributing substantially to agricultural exports. Globally, canola is among the most widely used vegetable oils, second only to soybean oil in consumption (Lin et al., 2013). In Canada, canola is primarily cultivated in the western provinces of Saskatchewan, Alberta, and Manitoba, where it plays an essential role in crop rotation systems (Canola Council of Canada, 2024). Canola has become one of Canada’s most important crops, generating about one-quarter of all farm crop receipts with an annual production of over 15 million tonnes (Canola Council of Canada, 2024). Farmers reported planting 3.3 million acres of canola-seeded area in Manitoba in 2024.

Canola seeds contain approximately 42% oil, commonly utilized as a vegetable oil for human consumption, and 58% meal, which serves as a significant protein source in animal feed (Khajali and Slominski, 2012). In addition, the advance in canola breeding through last decades have resulted in yellow-seeded varieties that provide improved nutrients over black-seeded canola meal. Yellow-seeded canola seeds are generally larger, which increases the proportion of embryo in the whole seed resulting in higher crude protein content (Khajali and Slominski, 2012). In addition, the thinner hull in yellow-seeded canola meal reduces fiber content in the meal to approximately 24–27%, compared with 30–34% in black-seeded canola meal (Simbaya et al., 1996; Canola Council of Canada, 2024). More importantly, yellow-seeded canola meal contains

approximately 3.9 $\mu\text{mol/g}$ glucosinolates, whereas traditional rapeseed meal contained about 155 $\mu\text{mol/g}$ (Slominski et al., 2012; Bell, 1993). These nutritional improvements have contributed to the widespread adoption of canola meal in animal feed (Simbaya, 1996).

In swine nutrition, canola meal represents a valuable protein source, as its amino acid (AA) composition includes high concentrations of essential amino acids such as lysine, methionine, and cysteine, which are crucial for growth and reproductive performance in pigs (Khajali and Slominski, 2012). However, the suitability of canola meal as a feed ingredient for sows is limited by factors like residual glucosinolate content, fiber levels, and overall digestibility. These factors can influence feed intake, nutrient absorption, and performance, particularly in sensitive groups such as lactating and gestating sows (Velayudhan et al., 2018). Recent research has concentrated on enhancing the digestibility and nutritional value of canola meal through various processing methods and dietary supplementation, including the use of enzymes and probiotics to mitigate anti-nutritional compounds and improve nutrient availability (Mejicanos et al., 2017).

This review seeks to summarize recent advancements in the application of canola meal in swine diets, specifically focusing on its benefits in sow nutrition. Topics addressed include the nutritional strengths and limitations of canola meal, factors affecting its utilization in pig diets, and innovative strategies to optimize its inclusion, such as enzyme and probiotic supplementation. By exploring these areas, this review aims to provide a comprehensive evaluation of canola meal's potential as a protein supplement for sows, contributing to ongoing efforts to reduce feed costs and enhance the sustainability of swine production systems.

2.2 CANOLA MEAL PRODUCTION PROCEDURE

Canola seeds contain around 42% oil, making oil extraction the primary focus in maximizing the seed's economic value (Leming and Lember, 2005). Canola oil ranks as the second most widely produced industrial product from oilseeds on a global scale. Once the oil is extracted, the remaining coproduct—a protein-rich meal—serves as a valuable ingredient in livestock feed due to its high protein content. Oil extraction from canola seeds can be achieved using one of three primary methods: pre-press solvent extraction, which involves pressing the seed before using a solvent to extract the remaining oil; expeller pressing, a mechanical method that uses pressure to release the oil; and cold pressing, which extracts oil without heat to retain more of its natural properties (Kovari, 2004).

2.2.1 Solvent extraction

The solvent extraction method is the most widely used and effective technique for removing most of the oil from canola seeds. This process involves multiple stages, beginning with seed handling and cleaning, followed by air aspiration at an exit temperature of approximately 52°C as shown in **Fig 2.1**. The seeds are then conditioned at 75–78°C for 30–40 minutes to prepare them for further processing (Newkirk et al., 2003a). After conditioning, the seeds are flaked and cooked at temperatures ranging from 75–120°C, with an optimal temperature of about 88°C for 15–40 minutes (Anderson-Hafermann et al., 1993). Following cooking, the seeds undergo expelling to remove some of the oil and then proceed to solvent extraction at 50–60°C for 90 minutes. The desolventiser-toasting stage uses steam injection to eliminate any remaining solvent, operating at temperatures of 95–160°C for 30–60 minutes. The meal is then cooled, air-blown, granulated, and finally stored as mash or processed into pellets. In colder climates, crushing facilities may pre-heat

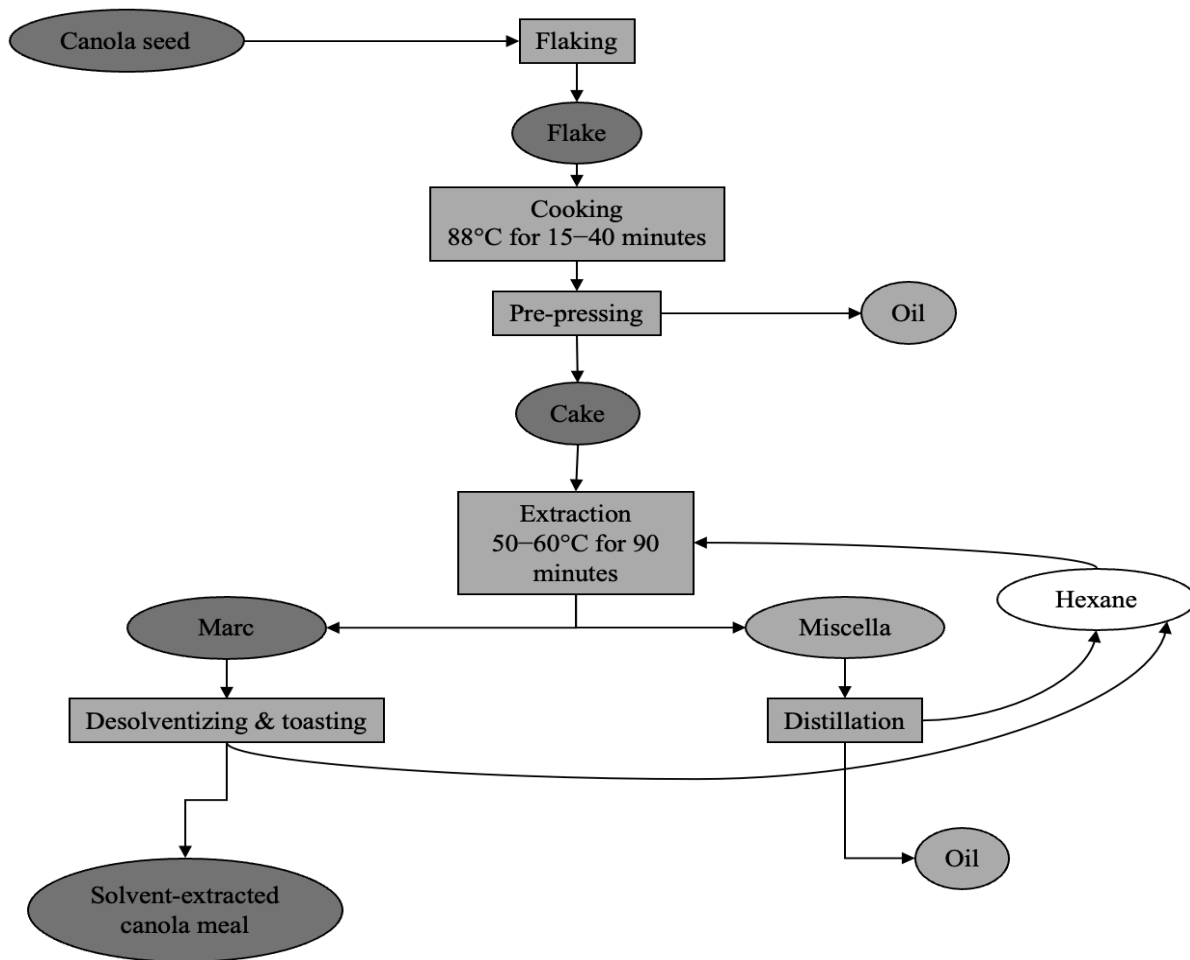
the stored seeds to around 35°C before flaking, using grain dryers to prevent the seeds from shattering during the flaking process (Anderson-Hafermann et al., 1993). During the cooking stage, seed flakes pass through steam-heated drum or stack-type cookers. This heating ruptures oil cells, reduces the oil's viscosity, and promotes the coalescence of oil droplets. Phospholipids removed from the crude extracted oil, commonly referred to as “gum” can be reintroduced into the meal at a rate of 1–2% after the desolventiser-toasting stage. The final product, known as solvent-extracted canola meal (**SCM**), is subjected to a hot solvent extraction to recover residual oil (Mejicanos et al., 2016). The finished SCM contains approximately 10% moisture and less than 1% residual oil.

2.2.2 Expeller extraction

Canola oil could also be extracted via expeller pressing, which involves pressing the seed twice to extract oil (Sey et al., 2020). Double-press expelling has lower capital costs than solvent-extraction and is common practice by smaller refineries, biodiesel plants, or in regions with limited canola access (Toghyani et al., 2014). Even though solvent extraction is more efficient with oil extraction, expeller extraction is more accessible in some local or small-scale processing facilities. Expeller extraction avoids the use of organic solvents such as hexane, unlike solvent extraction. Rather than using a solvent to separate the residual oil, in expeller pressing, high pressure in the compression sections led to oil extraction and the formation of hard cake. Canola seeds are first heated using steam up to 110°C, and seeds are then passed through a set of dies under high-pressure heat with steam following heat treatment, seeds are passed through an expeller twice to remove the oil as shown in **Fig 2.2**. (Leming and Lember, 2005), resulting in a meal called expeller-extracted canola meal (**ECM**) with 36.8% protein and 8–15% oil content 8–11%. Even though solvent extraction

is more efficient with oil extraction, expeller extraction is more accessible in some local or small-scale processing facilities.

Fig 2.1 Solvent-extracted canola meal production procedure¹



¹Adapted from Canola Council of Canada, 2024

2.2.3 Cold pressing

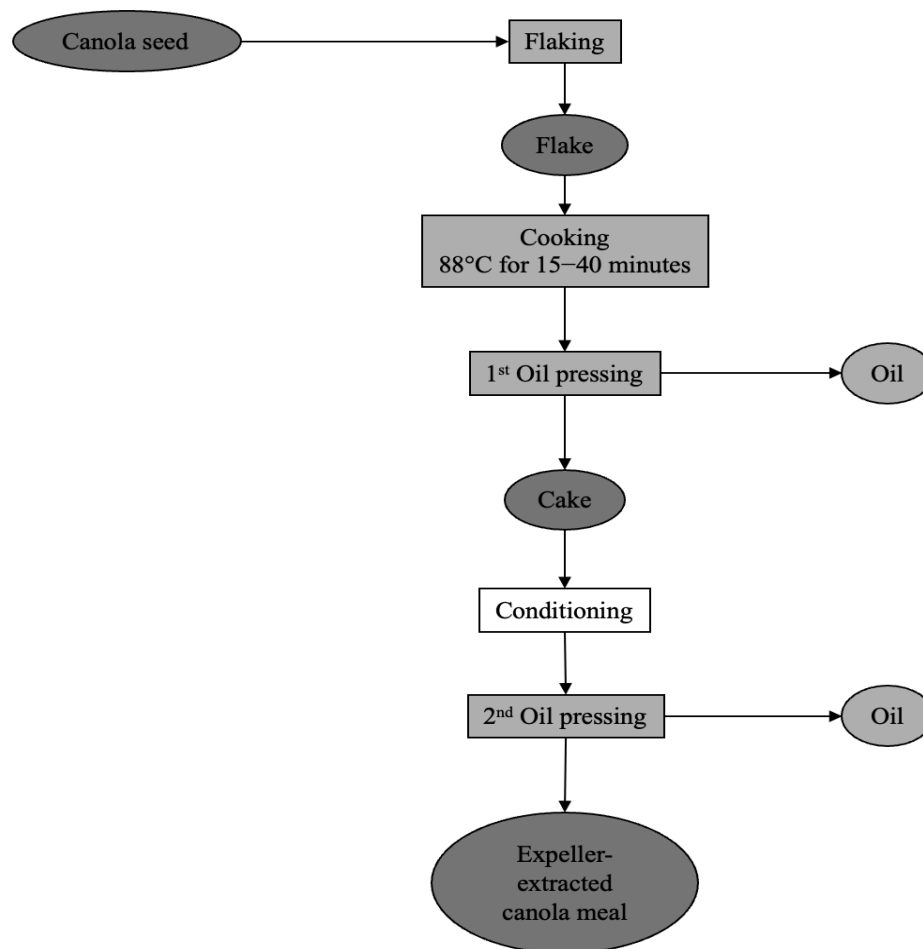
Cold pressing method of oil extraction involves mechanically pressing the cleaned canola seeds without the use of any heat or chemicals and extracting around 50 to 70% of the oil (Leming and Lember, 2005). During cold-press oil extraction, seeds are mechanically pressed at low heat ($\leq 65^{\circ}\text{C}$) from frictional forces in the barrel (Mejicanos et al., 2016). However, temperatures have shown to rise to 65°C due to friction build-up in the press or the barrel getting heated up, depending on the equipment (Seneviratne et al., 2010). Thus, the nutritional quality of cold-pressed canola meal has shown to vary depending upon the processing conditions (Seneviratne et al., 2010). 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).

2.3 CHEMICAL COMPOSITION OF CANOLA MEAL

Canola meal, a by-product of oil extraction from *Brassica napus* and *Brassica rapa* species, is recognized for its potential as a protein-rich feed ingredient. Canola meal has been used as an inexpensive alternative protein source to reduce feed cost in swine production because it is not only a good source of protein but also of vitamins such as biotin, choline, folic acid, niacin, riboflavin and thiamine (Mejicanos et al., 2016). Nutrient composition in canola meal is determined by various factors namely the variety, environmental conditions during crop development, harvest conditions, and processing of the seed and meal (Newkirk et al., 2003a). For example, dry seasonal conditions create lower oil content and higher levels of protein content (Seneviratne et al., 2010). Furthermore, canola meal quality is influenced by the type of oil extraction process as mentioned. Level of heating duration contributes to the degradation of heat

sensitive amino acids such as lysine. Expeller-pressed canola meal contains more residual oils than SCM (Seneviratne et al., 2010). Even though the cold-pressed canola meal and the ECM provide more dietary energy, they have a lower AA composition when compared to the SCM (Seneviratne et al., 2010; Woyengo et al., 2010). Therefore, understanding its complete chemical composition is essential for developing optimized feed formulations that address species-specific nutritional requirements.

Fig 2.2 Expeller-extracted canola meal production procedure¹



¹Adapted from Leming and Lember, 2025.

2.3.1 Protein content and amino acid profile

Dietary protein content in canola meal is highly influenced by the protein content of canola seeds, which varies yearly due to differences in growing and harvest conditions. Besides, protein quality is directly related with processing method (Newkirk and Classen, 2002). For instance, SCM typically has a crude protein content of 42% on a dry matter basis, while ECM, produced through mechanical pressing without solvents, retains 8-12% residual oil. This residual oil results in a slightly lower crude protein content of 32-38%. The differences in crude protein levels between SCM and ECM may be attributed to heat treatments applied during oil extraction, as well as the subsequent desolventizing and toasting of canola seeds (Jia et al., 2009). Studies have demonstrated that heat treatments reduce soluble protein content, which can negatively impact protein deposition, ileal-digestible lysine retention, and growth performance in monogastric animals (Khajali and Slominski, 2012). In comparison, SBM, the most common protein source for livestock, contains 44-52% crude protein on a dry matter basis. While soybean meal is favored for its superior protein and AA digestibility, the protein content of canola meal positions it as a potential alternative protein source to partially or fully replace soybean meal in animal diets.

Table 2.1 Chemical composition of solvent-extracted and expeller-extracted canola meal and soybean meal (DM basis)

Component, %	SCM	ECM	Soybean meal
Reference	Canola Council of Canada (2024) ¹	Canola Council of Canada (2024)	Rodrigue et al. (2020)
Crude protein	42.00	38.65	51.50
Indispensable AA ¹			

Arginine	2.49	2.38	3.54
Histidine	1.23	1.05	1.29
Isoleucine	1.57	1.62	2.36
Leucine	2.71	2.72	3.86
Lysine	2.32	2.26	3.12
Methionine	0.78	0.70	0.67
Phenylalanine	1.52	1.55	2.59
Threonine	1.63	1.55	1.98
Valine	1.83	2.07	2.44
Dispensable AA ¹			
Alanine	1.79	1.69	2.15
Aspartate	2.83	2.76	5.58
Cystine	0.73	0.88	0.71
Glutamate	7.07	6.99	8.93
Glycine	1.97	1.95	2.08
Proline	2.83	2.41	2.40
Serine	1.50	1.38	2.35
Tyrosine	1.02	1.00	1.87
Ash	7.30	6.96	7.54
Ether extract	3.20	10.96	0.64

¹Mean values calculated from Woyengo et al. (2010) and Agyekum and Woyengo (2022)

The AA content of SCM, ECM and soybean meal differs significantly due to their distinct protein profiles and processing methods as shown in **Table 2.1**. SCM and ECM provide a well-

balanced AA profile, particularly rich in sulfur-containing amino acids like methionine and cysteine (Newkirk et al., 2003b). However, the lysine levels in SCM and ECM are lower compared to SBM, which is notable for its superior lysine content. ECM retains slightly more residual oil than SCM, which may dilute its AA concentration but contributes additional energy to the diet. Despite having lower lysine content than soybean meal, both SCM and ECM remain valuable protein sources, especially when formulated with other feed ingredients to balance the AA profile for optimal animal performance (Khajali and Slominski, 2012). Additionally, some amino acids, particularly lysine, can become biologically unavailable during heat processing or the pelleting process in the extraction of SCM and ECM (Kim et al., 2012).

2.3.2 Carbohydrate components

Canola meal contains several complex carbohydrate fractions, which contribute significantly to its fiber content (**Table 2.2**). The fiber components in canola meal include lignin and associated polyphenols (8%), cellulose (4-6%), and non-cellulosic polysaccharides (13-16%), predominantly consisting of pectic substances (Slominski and Campbell, 1990). Other key components of canola meal include oligosaccharides (2.2%), glycoproteins (5%), such as arabinogalactan-protein and cell wall proteins, phytate (3.3%), and minerals associated with the fiber fraction (1%) (Bell, 1993). Additionally, gums represent about 4% of canola meal. The carbohydrate content of both SCM and mechanically ECM is relatively high, however, variability in carbohydrate composition may arise due to factors such as analysis methods, genetic differences, harvest management, and environmental conditions during plant growth (Bell, 1993). In comparison to soybean meal, canola meal contains over twice the neutral detergent fiber (**NDF**) content (Adewole et al., 2016; Wang et al., 2017). The fiber fraction in canola meal is comprised of non-starch polysaccharides (**NSP**),

lignin, associated polyphenols, cell wall glycoproteins, and minerals bound to fiber components (Jia et al., 2012). Dietary fibers in canola meal can be classified based on their solubility in water into water-soluble and water-insoluble fractions. Approximately 90% of the dietary fiber in canola meal is water-insoluble, while the remaining 10% is water-soluble (Wickramasuriya et al., 2015).

Table 2.2 Carbohydrate components of solvent-extracted and expeller-extracted canola meal and soybean meal (as fed basis)

Component, %	SCM	ECM	Soybean meal
	Canola Council of Canada, (2024)	Omosho et al. (2024) ¹	Khajali and Slominski, (2012)
Starch	0.43	0.30	0.70
Monosaccharides	1.55	0.17	6.90
Disaccharides	5.58	3.78	6.20
Oligosaccharides	2.23	1.44	5.30
NSP	20.15	18.20	17.80
ADF	16.32	17.83	7.50
NDF	25.51	25.80	12.00
Total dietary fiber	34.53	32.75	21.80

¹Data represent the mean of five samples collected from five processing plants across Western Canada

2.3.3 Energy content

The energy supply from canola meal is directly related to the residual oil content in the meal. However, canola meal generally provides lower metabolizable energy (**ME**) compared to soybean

meal (Newkirk et al., 2003a), primarily due to its higher fiber content, which dilutes the energy value. Additionally, the digestibility of the meal plays a crucial role in energy availability, with soybean meal typically being more digestible due to its superior protein quality and AA profile, making it a more energy-dense feed ingredient compared to canola meal. Several factors influence the ME content of canola meal, including its fiber, protein, and oil content, all of which are affected by seed variety, quality, and feed processing methods (Mejicanos et al., 2016). Among the different forms of canola meal, the more efficient solvent extraction technique used in SCM results in a low, consistent oil content, whereas ECM retains more oil, leading to a higher ME than SCM (Woyengo et al., 2010). Furthermore, plant breeding has contributed to improved ME content in canola meal by reducing glucosinolate levels (Bell, 1993). The dehulling process also enhances the ME levels of canola meal by reducing its fiber content, thereby increasing its digestible energy value. For example, the digestible energy (**DE**) in canola meal is approximately 3,346 kcal/kg, whereas dehulled canola meal contains about 4,063 kcal/kg DE due to the reduction of fiber in the hulls, which comprise about 12–16% of canola seeds (Slominski et al., 2006).

2.3.4 Minerals and vitamins

Canola meal is considered a high-quality source of essential minerals, which varies depending on the differences in soil concentration of minerals and seasonal variations (Bell, 1993) (**Table 2.4**). Several studies have indicated that canola meal is a relatively good source of essential minerals, particularly calcium (**Ca**) and phosphorus (**P**), with higher concentrations of these minerals than soybean meal (Simbaya, 1996). However, it is important to note that approximately 65% of the phosphorus in canola meal is present in the phytate form, which is poorly digestible by monogastric animals due to their limited ability to break down phytate (Slominski and Campbell, 1990). Despite

the reduced bioavailability of phosphorus, canola meal remains a more effective source of Ca and P compared to soybean meal, which has lower mineral content overall. Furthermore, canola meal is recognized for its relatively high selenium content, an essential trace mineral with antioxidant properties that plays a vital role in immune function and reproduction (Gjerlaug-Enger et al., 2014; Lauridsen et al., 1999). In terms of processing, it has been shown that standard canola processing methods do not significantly alter the mineral concentrations in the meal.

In terms of vitamin content, canola meal has a greater concentration of certain vitamins compared to soybean meal (**Table 2.4**). However, there is limited information available regarding the vitamin composition of ECM, making it difficult to draw direct comparisons between the different forms of canola meal. Despite this, several studies have reported that canola meal contains higher amounts of B vitamins, including biotin, folic acid, niacin, riboflavin, and thiamine, when compared with SBM (Canola Council of Canada, 2004; Batal et al., 2010). These vitamins are essential for various metabolic processes, such as energy production, cell growth, and immune function, and their higher concentrations in canola meal make it a valuable feed ingredient.

Table 2.3 Mineral composition of solvent-extracted and expeller-extracted canola meal and soybean meal (as fed basis)

Component	SCM	ECM	Soybean meal
	Canola Council of Canada, (2024)	NRC (2012)	Batal et al. (2010) ¹
Calcium, %	0.65	0.69	0.31
Phosphorus, %	0.99	1.15	0.67
Sodium, %	0.07	-	0.01

Chlorine, %	0.10	-	0.05
Potassium, %	1.13	-	2.05
Sulphur, %	0.63	-	0.44
Magnesium, %	0.54	60.30	0.28
Copper, mg/kg	4.70	5.40	15.00
Iron, mg/kg	162.00	232	172.00
Manganese, mg/kg	58.00	52	41.00
Zinc, mg/kg	47.00	72.00	48.00
Selenium, mg/kg	1.10	-	-

Dashes (-) indicate that no data was available

¹Data represent the mean of 16 samples collected from five processing plants from US harvest

Table 2.4 Vitamin content of solvent-extracted canola meal and soybean meal (as fed basis)

Component, mg/kg	SCM	SBM
	Canola Council of Canada, (2024)	NRC (2012)
Vitamin E	18.50	4.47
Pantothenic acid	9.40	15.00
Niacin	160	22.00
Choline	6700	2700
Riboflavin	5.80	2.9
Biotin	1.08	0.32
Folic acid	1.55	1.3

Pyridoxine	7.10	5.0
Thiamin	5.20	3.2

2.4 ANTI-NUTRITIONAL FACTORS IN CANOLA MEAL AND SOYBEAN MEAL

Soybean meal and canola meal contain several anti-nutritional factors that can adversely affect the health and performance of monogastric animals. Traditionally, the inclusion of canola meal in pig diets has been limited due to its high fiber content and the presence of anti-nutritional factors, including glucosinolates, erucic acid, sinapine, phytic acid, and trypsin inhibitors (Newkirk and Classen, 2002). However, advancements in plant breeding and genetic engineering have resulted in the development of new canola cultivars with significantly reduced levels of glucosinolates, erucic acid, and phytic acid, thereby improving the nutritional profile of canola meal (Mejicanos et al., 2016). Anti-nutritional factors are known to decrease feed palatability, lower voluntary feed intake, and impair growth rates. They can also negatively impact the physiological health of birds, often leading to liver damage, developmental abnormalities, and increased mortality rates (Mejicanos et al., 2016). Additionally, canola meal contains secondary compounds that can hinder the bioavailability and utilization of essential nutrients such as proteins, carbohydrates, phosphorus, Ca, and sodium (**Na**), potentially resulting in nutrient deficiencies and suboptimal performance. Thus, it is crucial to understand the effects of anti-nutritional factors on animals to optimize the utilization of canola meal as shown in **Table 2.5**.

Table 2.5 Amount of anti-nutritional factors in canola meal

Types	Amount	Effect	References
Sinapine, %	0.6-1.8	Bitter taste; dark color; indigestibility; lower nutritional value; decrease feed intake	Bell (1993); Khajali and Slominski, 2012
Phytic acid, %	3.0-5.0	Bind minerals; decrease availability and digestibility; decrease digestion of starch and protein	Bell (1993); Khajali et al., (2012)
Glucosinolates, $\mu\text{mol/g}$	1.73-5.26	Reduce feed intake; impaired thyroid function; enlargement of internal organs like liver and kidney; decrease growth rate and cause hemorrhagic liver syndrome	Khajali et al., (2012)
Tannins, %	1.5-3.0	Reduce digestion of protein; interfere with digestive enzymes; influence growth performance	Bell (1993)

2.4.1 Phenolics

Canola meal and its by-products are rich in various bioactive compounds, including phenolic compounds, tannins, and erucic acid. High concentrations of phenolic compounds in canola seeds, as compared to other oilseeds, have been documented (Naczek et al., 1998). It was as early as 1959 that Bate-Smith and Ribereau-Gayon first identified tannins in rapeseed hulls. Containing 0.68–0.77% tannins (Naczek and Shahidi, 2004), canola meal holds high levels of insoluble tannins, accounting for 70–96% of the total tannins present in canola hulls. Significant is the fact that Khajali and Slominski (2012) determined the antinutritive effects of these tannins to be minimal, as their predominantly water-insoluble nature restricts their activity to the hull fraction.

Not only are tannins notable, but also the phenolic compounds of canola meal, with sinapine being the most abundant (Naczki and Shahidi, 2004). In soybean flour, by contrast, ferulic, syringic, and vanillic acids dominate among polyphenolics (Lee et al., 2008). Polyphenolic compounds, due to their chemical properties, can bind to nutrients such as vitamins, minerals, proteins, lipids, and carbohydrates, thereby reducing their bioavailability (Alu'datt et al., 2014). Yet, contrary to these adverse effects, phenolics and flavonoids exhibit beneficial properties—anti-inflammatory, antioxidant, and anticancer activities being notable among them (Nandasiri et al., 2022; Tileuberdi et al., 2022).

Erucic acid is a long-chain monounsaturated fatty acid (cis-13-docosenoic acid, C22:1) formerly found at high levels in traditional rapeseed oil (Nath et al., 2009). Diets rich in erucic acid were linked to myocardial lipidosis and cardiac steatosis in poultry and swine (Charlton et al., 1975). As a result, Canadian researchers developed canola cultivars with low erucic acid (< 2% in canola oil) and glucosinolate (< 30 $\mu\text{mol/g}$ in canola meal) levels (Chen et al., 2015). Consequently, only trace amounts of erucic acid remain in modern canola meal, making it negligible in animal nutrition.

2.4.2 Phytic acid

Phytic acid, a predominant antinutritional factor in canola meal, significantly impacts nutrient absorption and poses challenges to animal performance and environmental sustainability (Pallauf and Rimbach, 1997). Its dual role as a phosphorus storage compound and an inhibitor of nutrient utilization underscores the necessity of mitigation strategies, such as phytase supplementation, in animal feed formulations (Zhou and Erdman, 1995; Cheryan and Rackis, 1980). Known also as myo-inositol hexakisphosphate, phytic acid is both the primary storage form of phosphorus and

inositol in grains (Kerovuo et al., 2000; Wu et al., 2009). Binding proteins and key minerals—Ca, iron, zinc, manganese (Mg), and magnesium—phytic acid forms insoluble complexes that reduce bioavailability and prompt nutrient excretion (Cabahug et al., 1999; Cowieson and Roos, 2016). Not negligible is the fact that phytic acid content in canola meal ranges from 3.1% to 3.7% (McCurdy and March, 1992). Its inhibitory effect on several enzymes, including α -amylase, trypsin, tyrosinase, and pepsin, further diminishes nutrient digestibility, leading to impaired nutrient utilization in various livestock species, particularly monogastric animals (Deshpande and Cheryan, 1984; El-Batal and Abdel Karem, 2001).

In canola, over 60% of phosphorus exists as phytate or phytic acid, a form unavailable to monogastric animals due to their lack of phytase, the enzyme required to degrade phytate and release absorbable phosphorus (Bell, 1993; Kerovuo et al., 2000). Not only does undigested phytic acid result in significant phosphorus excretion into the environment, contributing to eutrophication but it also increases sodium loss in monogastric animals (Cowieson et al., 2004). Furthermore, imbalances in Ca and P ratios caused by poor phosphorus utilization led to deficiencies in structural development (Bell, 1993; Shapiro and Heaney, 2003).

2.4.3 Glucosinolates

Glucosinolates, another group of anti-nutritional factors present in canola, are classified into two primary categories: aliphatic and aromatic glucosinolates (Tripathi and Mishra, 2007). Compared to *Brassica napus* canola co-products, *Brassica juncea* canola co-products contain a higher concentration of aliphatic glucosinolates. The degradation products of aliphatic glucosinolates are known to be toxic, while no established toxicity has been found for aromatic glucosinolates (Tripathi and Mishra, 2007). Naturally, glucosinolates are in plant tissues near the enzyme

myrosinase, which, in the presence of moisture, hydrolyzes them into various toxic compounds such as aglucones, isothiocyanates, nitriles, and thiocyanates. These toxic byproducts can disrupt thyroid function and negatively impact growth performance (McCurdy and March, 1992). Bitter in taste, glucosinolates may contribute to the poor performance observed in monogastric animals by decreasing feed intake (Jia et al., 2012; Mejicanos et al., 2016). The liver and kidneys must detoxify glucosinolates, which increases metabolic activity in these organs, leading to hyperplasia, hypertrophy, and necrosis of cells (Tripathi and Mishra, 2007; Zhang et al., 2020). As a result, the ingestion of glucosinolates can cause liver damage, increased energy expenditure in the liver, reduced feed intake, slower growth, and higher mortality rates (McNeill et al., 2004). Although the concentrations of glucosinolates in canola meal have been reduced to low levels, evidence suggests that the conversion of rapeseed meal to canola meal has not fully eliminated the risk of liver toxicity (Bell, 1993; Tripathi and Mishra, 2007). Therefore, precautions should be taken when feeding canola meal to pigs, and liver toxicity should be monitored while determining optimal inclusion levels through controlled feeding trials and biochemical markers of liver function. The tolerance level of gestating sows to total glucosinolates has been reported to be up to 5.0 $\mu\text{mol/g}$ of diet dry matter, and dietary glucosinolates concentration should remain below 2.0 $\mu\text{mol/g}$ (Opalka et al., 2001; Schone et al., 2001). Notably, SCM contains lower glucosinolate levels than expeller- or cold-pressed canola meal, as some glucosinolates are lost during desolvetization (Newkirk and Classen, 2002). As such, SCM may be more suitable for swine diets.

2.4.4 Trypsin inhibitors

Trypsin, a proteolytic enzyme, is essential for protein digestion in swine, playing a pivotal role in breaking down dietary proteins into AAs. Produced by the pancreas in an inactive form known as

trypsinogen, it is activated once it enters the small intestine, where digestion occurs (Walsh et al., 1964). Trypsin inhibitors, also referred to as protease inhibitors, are enzymes that reduce the activity of trypsin and chymotrypsin, both crucial for protein breakdown. These inhibitors are thought to possess antimicrobial, anticancer, and anti-inflammatory properties (Kuhar et al., 2014). Broadly, they are classified into two categories: Kunitz trypsin inhibitors and chymotrypsin inhibitors. While Kunitz trypsin inhibitors are the dominant form in soybean meal, chymotrypsin inhibitors are more prevalent in grain legumes (Batista et al., 1996).

In the gastrointestinal tract, dietary trypsin inhibitors bind to trypsin and chymotrypsin, forming inactive complexes that reduce AA digestibility (Gilani et al., 2005). Consequently, growth rates and feed conversion efficiency are impaired. Furthermore, trypsin inhibitors stimulate the secretion of cholecystokinin, which in turn decreases voluntary feed intake by reducing AA digestibility (Ripken et al., 2015). When consumed, these inhibitors form irreversible complexes with the trypsin enzyme, causing a drop in trypsin levels within the intestine and hindering protein digestibility (Cabrera-Orozco, 2013). Moreover, the presence of trypsin inhibitors can lead to pancreatic hypertrophy or hyperplasia in monogastric animals (Embaby, 2010). Notably, trypsin inhibitors are heat-labile and can be destroyed during the heat processing of soybeans before oil extraction (Cabrera-Orozco, 2013). Most trypsin inhibitors are eliminated during the desolventizing-toasting stage of oil extraction, resulting in solvent-extracted SBM with reduced trypsin inhibitor activity (Cabrera-Orozco, 2013).

Trypsin inhibitors are well-recognized anti-nutritional factors in soybean, as they interfere with protein digestion by inhibiting digestive enzymes such as trypsin and chymotrypsin. However, thermal processing, such as heating, can effectively reduce trypsin inhibitor activity in soybean meal, excessive heat treatment can also cause AA, especially lysine, to bind with carbohydrates,

forming Maillard reaction products that reduce digestibility and contribute to AA degradation (Cromwell et al., 1999). In contrast, the presence of trypsin inhibitors in canola meal is low and has received relatively little attention compared to other anti-nutritional factors.

2.4.5 Fiber

Fiber is a diverse group of indigestible compounds, composed primarily of plant cell wall components such as cellulose, pectin, β -glucans, pentosans, heteroxylans, and xyloglucans, which cannot be hydrolyzed by endogenous enzymes of monogastric animals (Kumar et al., 2012). The fiber forms the cell wall structure of various grains and legumes, which are resistant to degradation by mammalian enzymes (Lattimer and Haub, 2010). Fiber has a lower nutritive level in monogastric animals who lack the digestive enzymes to degrade non-starch polysaccharides (Jha and Berrocoso, 2015). The dietary fiber can be categorized as soluble or insoluble fiber. Soluble fiber can increase digesta viscosity and prevent endogenous enzymes from interacting with feed substrates, lowering nutrient digestibility (Jha and Berrocoso, 2015). On the other hand, non-soluble fiber can hasten the digesta passage rate, resulting in less time for digestion and, thus, less nutrient utilization (Khajali and Slominski, 2012).

Soybean meal contains approximately 10% free sugars, 6% soluble NSP, 18–21% insoluble NSP, and less than 1% starch (Knudsen, 1997; Choct et al., 2010). Soybean contains NSP, namely: arabinans, arabinogalactans and acidic polysaccharides. Furthermore, canola meal contains approximately 34.5% dietary fiber, owing to the high level of seed hull remaining in the meal (Adewole et al., 2016). Non-starch polysaccharides, lignin, associated polyphenols, cell wall glycoproteins, and minerals bound to the cell wall are all structural components that contribute to the overall fiber content in canola meal (Simbaya, 1996; Jia et al., 2012). In canola meal,

approximately 91.0% of dietary fiber is water-insoluble, while approximately 9.0% is water-soluble (Simbaya, 1996; Khajali and Slominski, 2012; Wickramasuriya et al., 2015). The presence of these NSP in swine diets lead to poor growth performance. The presence of NSP in swine diets inhibits feed utilization and decreases nutrient bioavailability, which adversely affect animal performance (Högberg and Lindberg, 2004; Liu et al., 2016a). Indeed, it has been reported that canola meal has indigestible substrates such as NSP that do not only decrease overall digestibility of feed but also reduce utilization of other nutrients (Singh et al., 2017). As a result, the high amount of fiber in canola meal is one of the major factors limiting its use in the swine industry.

2.5 USE OF CANOLA MEAL IN SWINE DIETS

Because of the relatively low cost, higher availability, and balanced AA profile, canola meal is a better substitute for SBM in swine diets to reduce feed costs in pork production. However, the higher dietary fiber contents limit the use of canola meal in swine nutrition, even though meals from new canola cultivars are low in anti-nutritional factors such as glucosinolates, phytic acid, and phenolic components.

2.5.1 Dietary inclusion

Although canola meal has been extensively studied in pig feeding trials, the effects of different levels of dietary canola meal inclusion reported in the literature vary. In the study of Hong et al. (2021), the average daily feed intake (**ADFI**) of nursery pigs responded quadratically to increase in canola meal from 0% to 20% and then decreased when dietary canola meal was increased to 40% as shown in **Table 2.6**. The low feed intake with high canola meal inclusion could be attributed to the high fiber in canola meal and the high level of glucosinolates, which has been shown to impair

thyroid function by inhibiting thyroid hormone production and reducing swine feed intake due to its bitter taste (Tripathi and Mishra, 2007). In a review on canola meal, Schöne et al. (1997) suggested that growing pigs can tolerate less than 2 $\mu\text{mol/g}$ in the diet. In addition, sows have been shown to tolerate daily glucosinolates intakes of less than a maximum level of 5 $\mu\text{mol/g}$ in gestation diets without affecting reproductive performance. In contrast, the recommended glucosinolates content in lactation diets for sows is less than 2 $\mu\text{mol/g}$ (Quiniou et al., 2012). The levels of glucosinolates, on the other hand, are relatively low in new canola cultivars, with an overall mean of 3.57 $\mu\text{mol/g}$ glucosinolates (dry-matter basis) from a 7-year survey of 13 Canadian processing plants (Radfar et al., 2017). Thus, low glucosinolates content in current canola varieties may result in the increased utilization of canola meal.

Previous research in growing-finishing pigs has shown that pigs can tolerate up to 30% canola meal in their diets without negatively impacting pig growth performance (King et al., 2001; McDonnell et al., 2010). Landero et al. (2011) found that increasing the amount of canola meal in the diet by up to 20% in place of soybean meal did not affect the average daily gain (**ADG**), ADFI, or gain-to-feed ratio in weaned pigs. A study by Sanjayan et al. (2014) demonstrated that canola meal from *B. napus* and *B. juncea* could be included in up to 25% of weaned pig diets without affecting growth performance. Roth-Maier et al. (2004) noticed that growing pigs could tolerate up to 26% canola meal in diets without affecting growth performance. According to King et al. (2001), SCM can be included at levels of up to 250, 300, and 202 g/kg in diets of weaned pigs, grower/finisher pigs, and lactating sows, respectively, without affecting pig performance.

As for sows, Velayudhan et al. (2018) found that 30% dietary canola meal in sow diets had no adverse effects on sow feed intake or performance. Sows may have higher nutrient and energy utilization from dietary fiber than younger pigs because they have a larger digestive tract and better

ferment fiber (Jørgensen et al., 2007; Lindberg, 2014). Diets based on standardised ileal digestible (SID) AA content and net energy (NE) value have been shown to reduce the negative impact of high-fiber ingredient feeding in swine diets (Velayudhan and Nyachoti, 2017). More research is needed to evaluate the feasibility of using canola meal as a cost-effective and sustainable alternative protein source in swine diets, with a particular focus on its impact on sow reproductive performance, pig growth performance, and overall production efficiency.

2.5.2 Effects on growth performance

High-fiber diets were thought to reduce feed intake and, thus, energy intake in nursery pigs during the energy-dependent growth phase. According to Seneviratne et al. (2010) including 15% SCM in weaned pig diets did not affect body weight gain. In nursery pigs, similar results were obtained with a higher inclusion level of 20% SCM (Landro et al., 2011; Wang et al., 2017). According to Hong et al. (2021), including 30% or 40% canola meal in corn-soybean meal-based diets for nursery pigs had no adverse effects on growth performance. Another study in weaned pigs found that meals containing both *Brassica napus* black and *Brassica juncea* yellow could be included at a 25% level without affecting performance (Sanjayan et al., 2014). There were two proposed explanations for weaned pigs' unchanged performance at high canola meal inclusion. First, in the past, diets were primarily based on crude protein (CP) and DE rather than SID AA or NE. Diets based on SID AA content and NE value have been shown to reduce the negative impact of high-fiber ingredient feeding in swine diets. The second reason is that advanced canola meal processing, including improved desolventization techniques and selective breeding for low levels of glucosinolate, have enhanced its overall quality (Landro et al., 2011; Adewole et al., 2016). These

improvements make modern canola meal being a more viable alternative protein source in swine diets.

As for sows, feed intake, the body weight (**BW**) and backfat thickness change, weaning to estrus interval, and milk composition were not affected by up to 30% canola meal in lactation diets up to 30% canola meal in lactation diets (Velayudhan and Nyachoti, 2017). In a study by (Liu et al., 2018), including 23.3% dietary canola meal when all soybean meal was replaced in gestation and lactation diets increased the weaning to estrus interval but had no adverse effect on sow BW or backfat loss. According to the findings of this study, canola meal may replace all soybean meal in sow diets during gestation and lactation.

2.5.3 Effects on digestion and metabolism

Canola meal has lower and more inconsistent AA digestibility than soybean meal. The reason for the reduced nutrient digestibility is, at least in part, linked to processing parameters. It is well known that overheating canola seeds during processing can result in a content and digestibility losses (Newkirk et al., 2003b). In two studies (Landro et al., 2011; Landro et al., 2012), increasing the inclusion of SCM and ECM resulted in a reduction in apparent total tract digestibility (**ATTD**) of energy and nutrients, primarily attributed to dietary fiber content. According to Landro et al. (2011), weaned pigs fed canola meal at levels of 0, 50, 100, 150, and 200 g/kg in place of SBM had reduced ATTD of gross energy (**GE**), dry matter (**DM**), and CP linearly. Huang et al. (2005) concluded that the digestibility of AA in both canola meal and soybean meal varies with age.

In a sow study, the ATTD of DM, GE, CP, and P in sows fed 15% and 30% canola meal inclusion was lower than in soybean meal diets from d 115 of gestation to weaning (Velayudhan

and Nyachoti, 2017). However, feeding 30% dietary canola meal to sows from d 60 of gestation until weaning had no adverse effects on energy and nutrient digestibility (Velayudhan et al., 2018). The effects of canola meal on nutrient digestibility may also be related to adaptation time. Sows with a long adaptation period can efficiently utilize high dietary fiber, most likely due to an increase in hindgut fermentation due to increased gut microbial mass (Varel and Yen, 1997).

Table 2.6 The effect of solvent-extracted canola meal in sow diets

Levels	Period	Effects	References
20% SCM	Weaned pigs	No adverse effects on ADG or ADFI, but reduced nutrients digestibility	Landero et al. (2011)
20% SCM	Nursery pigs	Decreased ADFI and cecal butyric acid concentration	Hong et al. (2021)
25% SCM	Weaned pig	No adverse effects on growth performance	Sanjayan et al. (2014)
26% SCM	Growing pigs	No adverse effects on growth performance	Roth-Maier et al. (2004)
30%ECM	Growing pigs	Decreased ADFI and colon weight; increased the weight of the thyroid gland	Velayudhan et al., (2017)
30% SCM	Lactation	Decreased plasma urea nitrogen and nutrient digestibility; no adverse effects on milk composition	Velayudhan and Nyachoti (2017)
23.3% SCM	d 7 of gestation until end of lactation	No influence on feed intake or litter performance; increased wean to estrus interval	(Liu et al., 2018)

30% SCM	d 60	of	Reduced plasma urea nitrogen; no	Velayudhan et al.,
	gestation	until	adverse effects on ATTD of CP, GE,	(2018)
	weaning		Ca; Increased gut lactic acid bacteria	

2.5.4 Effects on microbiota

The high amount of dietary fiber may act as a substrate for gut microbiota in pigs and contribute to the maintenance of gut health. Some soluble fiber can be fermented in the small intestine's lower part, while most insoluble fiber is fermented in the hindgut (Molist et al., 2014). When challenged with *E. coli*, Hong et al. (2021) discovered that 20% dietary canola meal reduced cecal butyric acid concentration by 61% and tended to reduce total volatile fatty acid concentration in the colon digesta of weaned pigs. The tendency of dietary canola meal to reduce cecal volatile fatty acid concentration could also be attributed to canola meal's higher insoluble fiber content than soybean meal. Furthermore, dietary canola meal at 20% decreased the relative abundance of the Bacteroidetes phylum and tended to increase the relative abundance of the Firmicutes phylum in the study (Hong et al., 2021). The shift in microbial populations, particularly the increased Firmicutes-to-Bacteroidetes ratio, suggests that canola meal could impact fiber degradation and energy extraction in the gut. Weaned pigs fed diets of 30 and 40% canola meal had higher lactulose-to-mannitol ratios in the urine and lower ileal transepithelial electrical resistance (Hong et al., 2021). In addition, 30% canola meal in sow diets increased the level of gut lactic acid bacteria in sows (Velayudhan et al., 2018). Therefore, these findings suggest that canola meal can positively influence the gut microbiome by modulating microbial composition and fermentation patterns.

2.5.5 Long-term effects on sows

Most research has primarily focused on short-term outcomes, such as reproductive performance, feed intake, and nutrient digestibility (Liu et al., 2018; Velayudhan et al., 2018). However, the long-term effects of dietary canola meal inclusion over multiple reproductive cycles remain largely unexplored. Short-term studies suggest that high-fiber diets may initially impact sow metabolism and performance, but these effects may change as sows adapt to dietary modifications. For instance, diets with a high degree of bulkiness fed during the first two parities have been shown to increase litter weight and postnatal growth in the second parity (Matte et al., 1994). Similarly, a high-fermentation capacity fiber diet (29.3% ADF) has been reported to enhance lactation feed intake and improve piglet performance in the second reproductive cycle compared to a low-fermentation fiber diet (3.8% ADF) (Tan et al., 2018). These findings suggest that physiological responses to dietary fiber evolve over time, highlighting the need for long-term studies to determine optimal feeding strategies.

The long-term metabolic adaptations, interactions with gut microbiota, and the effects of anti-nutritional factors resulting from prolonged canola meal feeding remain unclear. These factors could either mitigate or exacerbate initial negative effects, making it critical to assess whether extended canola meal feeding influences reproductive longevity, litter quality, and overall herd productivity across multiple parities.

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

2.6.1 Enzyme supplementation

There are few studies that have been conducted to evaluate the effect of carbohydrase on digestibility and performance of pigs fed diets supplemented with canola meal. For instance, in a study with weaned pigs, Zijlstra et al. (2004) found that carbohydrase supplementation to a wheat- and canola meal-based diet improved average daily feed intake and average daily gain, though it did not enhance feed efficiency or ATTD of energy or nutrients. Zhang et al. (2014) reported that when exogenous multi-enzyme supplementation was used in piglets aged 35 to 65 days, the ATTD of DM, CP, and GE were higher compared to diets without enzyme supplementation. In contrast, Thacker (2001) observed no effect of multi-carbohydrase enzyme supplementation on the growth performance or ATTD of nutrients in growing pigs fed barley-based diets containing canola meal. The enhanced activities of amylase, lipase, and protease in the small intestine of pigs were enhanced with the inclusion of carbohydrase supplementation in the diets (Zhang et al., 2014). Improved endogenous enzyme activity may contribute to enhanced nutrient digestibility in pigs.

As previously mentioned, the fiber portion of canola meal is considered an anti-nutritional factor. However, it can be converted into substances that stimulate healthy microbial growth in the gastrointestinal tract using commercially available in-feed enzymes (Zijlstra et al., 2010). By supplementing diets containing canola meal with NSP-hydrolyzing enzymes, galacto-, gluco-, manno-, or xylo-oligomers with prebiotic effects can be produced. Not only do prebiotics selectively encourage the proliferation of beneficial microbial populations, but they also reduce the prevalence of enteric pathogens in the intestinal system (Niu et al., 2023). When treated with carbohydrase enzymes, canola meal reduces substrate availability for harmful microbial growth in

the ileum, thereby improving nutrient digestion and absorption (Thacker, 2001; Zijlstra et al., 2010). Furthermore, multi-carbohydrase enzymes acting on NSP in canola meal can reduce the amounts of water-insoluble NSP while increasing water-soluble NSP and their hydrolysis products, such as monosaccharides. Among these, galactose, glucose, and uronic acid are predominant (Kiarie et al., 2008). Similarly, an appropriate blend of carbohydrase enzymes targeting NSP has been shown to produce low-molecular-weight polysaccharides, simple sugars, and oligosaccharides, which serve as prebiotics, improving the gut environment for beneficial microbes (Theodoridou and Yu, 2013). Additionally, supplementation with exogenous fiber-degrading enzymes in canola meal improved *in vitro* dry matter digestibility and reduced total gas and volatile fatty acids production (Lee et al., 2018). These findings highlight the potential of enzyme supplementation to enhance the nutritional value of canola meal by improving fiber digestibility, promoting beneficial gut microbiota, and reducing anti-nutritional effects, ultimately supporting improved performance in swine diets

While carbohydrase enzymes target the NSP fraction of canola meal, another widely used exogenous enzyme in monogastric nutrition is phytase, which specifically hydrolyzes phytic acid to release digestible phosphorus and improve mineral utilization (Kong and Adeola, 2011; Newkirk and Classen, 1998). Supplementation with exogenous phytase in canola meal-based diets has been shown to significantly increase phosphorus digestibility and reduce anti-nutritional effects. For example, supplementation of phytase at 500 FTU/kg in diets containing around 300g/kg SCM meal for growing pigs significantly improved the apparent and standardized total tract digestibility of phosphorus and calcium (Adhikari et al., 2016). The basal endogenous losses of Ca were not affected when phytase was included in diets containing SCM fed to growing pigs (González-Vega et al., 2013). In addition, another study showed that in finishing pigs,

supplementation of phytase at 500 FTU/kg in a barley-canola meal (20% SCM) diets increased ATTD of P (Woyengo et al., 2008). Thus, these findings demonstrate that phytase supplementation is an effective strategy to enhance mineral utilization and mitigate the negative impacts of phytic acid in canola meal-based swine diets.

2.6.2 Fermentation

Microbial fermentation of canola meal involves the utilization of a complex microbial community and enzyme system to detoxify anti-nutritional factors, thereby enhancing the nutritional value of the meal. Glucosinolates, the primary anti-nutritional factor found in canola meal, are effectively broken down through fermentation. For example, Zhang et al. (2020) reported that by fermenting rapeseed meal with *Lactobacillus delbrueckii* and *Bacillus subtilis*, glucosinolate content was reduced from 64.56 $\mu\text{mol/g}$ to 3.47 $\mu\text{mol/g}$, achieving a remarkable degradation rate of 94.62%. Likewise, when canola meal was fermented with *Bacillus licheniformis*, yeast, and *Lactobacillus*, significant increases were observed in soluble protein, lactic acid, and total amino acid content, while glucosinolate and neutral detergent fiber levels were markedly reduced (Wang et al., 2019). The lactic acid produced not only contributes to a more acidic flavor but also facilitates protein denaturation, enhancing texture. Furthermore, fermentation of canola meal with *Aspergillus ficuum* has been shown to reduce phytic acid levels and increase protein content (Nair and Duvnjak, 1990). It has been emphasized that optimizing phosphate and glucose concentrations is crucial for maximizing phytase production and phytic acid reduction during fermentation with *A. ficuum* (Ebune et al., 1995). Additionally, *Lactobacillus salivarius* fermentation of canola meal led to a reduction in both glucosinolate and crude fiber content, while crude protein levels increased (Ahmed et al., 2014). Notably, isolated lactic acid bacteria from traditional fermented foods and

identified *L. salivarius* as the most efficient strain for canola meal fermentation was shown to reduce glucosinolate and crude fiber content from 22.0% to 13.6% and 12.0% to 10.1%, respectively (Ahmed et al., 2014). These findings demonstrate that microbial fermentation not only enhances nutrient availability but also promotes gut health and digestion, supporting its application in animal feed formulations.

2.7 LIVE YEAST PROBIOTICS IN SWINE DIETS

There are numerous natural alternatives to antibiotics that can enhance animal performance and overall health (Collier et al., 2011). Among these, live yeast and yeast cell wall products, derived from various yeast strains with probiotic properties, are widely recognized. These strains belong to genera such as *Saccharomyces boulardii*, *Saccharomyces cerevisiae*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Candida saitoana*, and *Candida pintolopesii* are widely recognized (Coenen et al., 2000; Bovill et al., 2001). The most extensively used yeast in livestock feed additives is *Saccharomyces cerevisiae* (*S. cerevisiae*) (Kumura et al., 2004). For centuries, *S. cerevisiae* has been integral to processes like brewing and bread-making. This strain is a rich source of digestible proteins, essential vitamins (e.g., vitamin B6, thiamine, biotin, riboflavin, nicotinic acid, and pantothenic acid), magnesium, and zinc (Haldar et al., 2011). The primary components of the *S. cerevisiae* cell wall include polysaccharides such as α -D-mannan, chitin, and β -D-glucan. Thus, more recently, yeast and its cell wall components have been adopted as dietary supplements in swine and poultry feed (Patterson et al., 2023; Sun et al., 2022; Zanello et al., 2013). These supplements are used primarily to improve performance and support animal health and overall well-being.

2.7.1 Mechanisms of action

S. cerevisiae contributes to enhanced gut health, improved nutrient utilization, and overall animal performance through multiple mechanisms (Elghandour et al., 2020). The cell wall components of *S. cerevisiae*, particularly beta-glucans and mannans, act as potent immunomodulators. These polysaccharides, which form a significant part of its cell wall, directly interact with immune cells and bind to bacteria, thereby preventing the attachment and colonization of pathogens in the gastrointestinal tract (Kogan et al., 2005; Zhou et al., 2023). Unlike other probiotics, yeast does not naturally adhere to the gastrointestinal tract in monogastric animals. Instead, it moves through the digestive system alive and active without colonizing the gut lining (Rodrigues et al., 2000). This unique transient behavior allows *S. cerevisiae* to function as an effective probiotic, positively influencing gut health and animal performance through multiple pathways. The key mechanisms by which *S. cerevisiae* exerts its benefits in livestock production (Christensen et al., 2022; Elghandour et al., 2020; Kiarie et al., 2011;) are included as follows:

1. Immunomodulation: Yeast and yeast cell wall products derived from *S. cerevisiae* can stimulate the functionality of immune cells such as macrophages and neutrophils, enhance their activity and promoting the release of pro-inflammatory cytokines like TNF- α and IL-6 during immune activation. The immune response is regulated by modulating cytokine production and promoting anti-inflammatory pathways, thereby preventing excessive inflammation. This balanced immunomodulatory effect strengthens the animal's defense against pathogens while supporting overall health and disease resistance in livestock.
2. Modulation of gut microbiota: Live yeast promotes the growth of beneficial gut bacteria, such as *Lactobacillus* and *Bifidobacterium*, while suppressing pathogenic microbes

through competitive exclusion. By stabilizing the gut microbiota, yeast contributes to a healthier gut environment and reduces the risk of gastrointestinal infections.

3. Enhancement of digestive enzyme activity: Yeast supplementation is associated with increased production of endogenous digestive enzymes, such as proteases and amylases. These enzymes improve the breakdown of dietary proteins, fats, and carbohydrates, enhancing nutrient availability and feed efficiency.

4. Metabolic and environmental effects: Yeast influences the gastrointestinal environment to optimize conditions for digestion and nutrient absorption. By removing oxygen from the gut, yeast creates anaerobic conditions that favor microbial fermentation and the proliferation of anaerobic bacteria. This process stabilizes gut pH, reduces methane and lactate production, and enhances microbial protein synthesis. Additionally, yeast alters volatile fatty acid profiles, increases feed intake, and improves overall feed digestibility, leading to enhanced animal productivity and health.

2.7.2 Effects on pig performance

In pigs, dietary inclusion of *S. cerevisiae* has been shown to stimulate immune response, modulate gut microbiota and increase nutrients digestibility as shown in **Table 2.7** (Ogbuewu et al., 2019). Supplementation with 2×10^{10} CFU/kg of *S. cerevisiae* in nursery pigs, average daily weight gain; serum IgA, IgG levels and fecal butyric acid as well as increased total volatile fatty acid concentrations were increased (Long et al., 2021). Furthermore, reduced the fecal score and pig mortality were shown in weaned pigs challenged with *Escherichia coli* K88+ receiving diets supplemented with 5×10^{10} CFU/kg *S. cerevisiae* (Trevisi et al., 2015). The benefits of yeast supplementation extend beyond immune modulation to include enhanced intestinal morphology

and growth performance. Shen et al. (2009) reported that pigs fed yeast-based products demonstrated similar growth performance, nutrient digestibility, and intestinal morphology compared to those receiving antibiotic growth promoters, suggesting yeast as a potential alternative to antibiotics. Supplementation with live yeast, finely ground yeast, or yeast fermentation products has been associated with increased villus height and villus-to-crypt ratios, as well as longer villi and deeper crypts in the ileum of piglets (Domeneghini et al., 2004; Bontempo et al., 2006). Yeast supplementation may also stimulate gut cell proliferation and increase glycoconjugate concentrations in mucin, enhancing resistance to pathogenic invasion (Bontempo et al., 2006). Yeast and yeast-based products also improve performance in swine. Increased average daily feed intake and decreased *Escherichia coli* adhering to the mucosa have been reported in weaned piglets challenged with *Escherichia coli* supplemented with *S. cerevisiae* fermentation products yeast products (Kiarie et al., 2011).

In sows, yeast supplementation during gestation has been linked to enhanced maternal protein utilization, evidenced by reduced plasma urea nitrogen levels, and improved progeny performance, including increased weaning weights and higher average daily gain (Shen et al., 2009). Although no significant effects on body weight or feed intake were reported in gestating sows, the total number of piglets born and born alive was greater in sows receiving yeast supplements (Elghandour et al., 2020). Besides, yeast supplementation during gestation has demonstrated transgenerational benefits. For example, Shen et al. (2009) found that supplementation of *S. cerevisiae* to gestating sows altered hematological parameters, including increased neutrophil counts, in their offspring. In addition, sows fed with 5×10^{10} CFU/kg *S. cerevisiae* from late gestation until weaning had a reduced farrowing duration and backfat losses during lactation and increased feed intake of sows and feed conversion ratio of creep feed in

suckling pigs (Sun et al., 2022). It also has been shown that 5×10^{10} CFU/kg *S. cerevisiae* in gestating and lactating sow diets increased IgG level in colostrum and decreased piglet diarrhea incidence (Zanello et al., 2013). These findings highlight the potential of *S. cerevisiae* as a valuable feed additive to improve immune function, growth performance, and reproductive outcomes in swine production systems.

Table 2.7 Impact of supplementation of swine diets with *Saccharomyces cerevisiae*

Yeast does	Period	Impacts	References
<i>S. cerevisiae</i> , 2×10^{10} CFU/kg	Nursery pigs	Increased average daily weight gain; serum IgA, IgG and fecal butyric acid; increased total volatile fatty acid concentrations	(Long et al., 2021)
<i>S. cerevisiae</i> , 5×10^{10} CFU/kg	weaned challenged ETEC	pigs with Reduced the fecal score; reduced ETEC excretion; reduced pigs' mortality	(Trevisi et al., 2015)
<i>S. cerevisiae</i> , 2×10^{10} CFU/kg	Sows from late gestation until weaning	Shortened farrowing duration; increased feed intake of sows; decreased backfat losses during lactation; improved feed conversion ratio of suckling pigs;	(Sun et al., 2022)

<i>S. cerevisiae</i> , 5×10^{10} CFU/kg	Sows during gestation lactation	late and in	Increase of IgG level in colostrum; decreased diarrhea incidence	(Zanello et al., 2013)
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2.8 SUMMARY

Canola meal is commonly used in swine diets due to the low price and balanced AA profile. Nevertheless, the high fiber content compared to soybean meal has restricted its use. The effects of different levels of canola meal inclusion on swine performance and gut microbiota are inconsistent due to the influence of variety, different stages of pig development. Therefore, more studies are necessary to investigate the effects of canola meal to optimize the canola meal inclusion levels in swine diets without causing adverse effects. In addition, recent studies about the live yeast effect on sows showed beneficial effects on nutrient digestibility and reproductive performance. However, there was no synergistic effects of canola meal and live yeast probiotics in sows.

Previous studies evaluated that inclusion of up to 300 g/kg solvent-extracted canola meal in late-gestating and lactating sow diets did not impair reproductive performance, but reduced nutrient digestibility, particularly during gestation period. While enzyme supplementation has been explored as a potential strategy to mitigate this issue, there remains limited evidence on effective approaches to enhance nutrient utilization. Additionally, no studies have evaluated the usage of expeller-extracted canola meal in sow diets, despite its distinct nutritional and physical properties. Furthermore, previous studies were conducted from late-gestating sows over a single reproductive cycle, leaving it unclear whether sows may adapt to high-fiber diets over time. Therefore, it is necessary to investigate both the potential of different processing methods (solvent vs. expeller) and the impact of long-term dietary inclusion of canola meal on nutrient digestibility and overall sow performance.

CHAPTER 3

HYPOTHESES AND OBJECTIVES

The studies tested the following hypotheses:

- 1) Up to 300 g/kg canola meal inclusion could be used as a primary protein source in late-gestating and lactating sow diets without adversely affecting sow reproductive performance and the growth performance of suckling piglets.
- 2) Probiotic supplementation is expected to benefit sow performance and nutrient utilization regardless of basal diet type and may mitigate any potential reductions in performance when high levels of canola meal are included.
- 3) Solvent-extracted canola meal and expeller-pressed canola meal differ in nutrient profile, which may lead to distinct effects on sow performance and nutrients digestibility.
- 4) Continuous feeding of canola meal over two reproductive cycles may support nutrient digestibility comparable to soybean meal

The overall objective was to explore the nutritional strategies for optimizing canola meal utilization in sow diets.

The Specific objectives were:

- 1) To study the effects of high inclusion of solvent-extracted canola meal in gestation and lactation diets with or without probiotics on sows and piglet performance.
- 2) To investigate the energy and nutrient utilization in sows fed high canola meal diets with different processing methods (solvent vs. expeller) and the effects of probiotics on nutrient utilization and gut microbiome.
- 3) To study the high inclusion of canola meal in sow diets on sow and litter performance over two reproductive cycles.

CHAPTER 4**MANUSCRIPT I****HIGH CANOLA MEAL INCLUSION IN GESTATION AND LACTATION SOW DIETS
WITH SACCHAROMYCES CEREVISIAE PRODUCT ON REPRODUCTIVE
PERFORMANCE< MILK COMPOSITION AND NUTRIENT DIGESTIBILITY OF
SOW AND LITTER PERFORMANCE**

Xiaoxiao Zhang*, Debora Muratori Holanda*, Anna Rogiewicz*, Elijah G. Kiarie†, Chengbo Yang*, and Charles Martin Nyachoti*¹

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

†Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Xiaoxiao Zhang: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing- original draft

Debora Muratori Holanda: Conceptualization, investigation, supervision, methodology, writing - review & edition

Anna Rogiewicz: Formal analysis, writing - review & edition

Elijah G. Kiarie: Methodology, writing - review & edition

Chengbo Yang: Writing - review & edition

Charles Martin Nyachoti: Project administration, resources, supervision, writing - review & edition

4.1 ABSTRACT

The objective of this study was to assess how sow and litter performance and nutrient utilization were affected by dietary probiotic supplementation in gestation and lactation diets that contained high levels of canola meal. Seventy-five sows were allotted to one of three treatment diets, starting on d 80 of gestation. The experimental diets included a control diet (**CTRL**) composed of corn and soybean meal, or a modified CTRL diet where soybean meal was substituted with 300 g/kg of canola meal, provided either with (**CCM-P**) or without (**CCM**) *Saccharomyces cerevisiae* product supplementation. On d 80 and d 111 of pregnancy, as well as on d 1 and d 21 following farrowing, the sow body weight (**BW**) and backfat thickness were recorded. Piglet weights were measured on d 1 and d 19 after birth. Milk and blood samples from sows were collected on d 1 and d 19 post-farrowing to measure nutrient composition. Additionally, fecal samples were gathered on d 110 of gestation and d 19 of lactation to analyze apparent total tract digestibility (**ATTD**) with titanium dioxide as an indicator. Data were analyzed through the PROC MIXED procedure in SAS 9.4, following a randomized complete block design. Results indicated that the inclusion of CCM in sow diets had no significant effect on sow or litter growth performance, as well as plasma urea N levels. However, in contrast to gestating sows fed the CTRL diet, those fed the CCM diet had lower ($P < 0.05$) ATTD of gross energy, dry matter, and crude protein. In contrast, the CCM-P diet led to increased ($P < 0.05$) ATTD of phosphorus and tended to increase ($P = 0.08$) ATTD of calcium relative to the CCM group. Furthermore, lactating sows fed CCM diets demonstrated higher ($P < 0.05$) ATTD of neutral detergent fiber (**NDF**) compared to the CTRL group. Milk fat content was significantly greater ($P < 0.05$) in sows consuming CCM diets than those fed the CTRL diet. In conclusion, incorporating 300 g/kg canola meal into sow diets during late gestation and lactation maintained similar reproductive and litter performance compared to the control diet

but negatively impacted nutrient digestibility in late gestating sows. Supplementing the canola meal diet with *S. cerevisiae* product improved phosphorus digestibility and milk fat content, suggesting that probiotics may mitigate some negative effects of canola meal in sow nutrition.

Keywords: Canola meal, digestibility, milk, piglets, probiotic, sows

4.2 INTRODUCTION

Feed represents the most significant cost in swine production, making it critical to identify cost-effective alternative protein sources (Boumans et al., 2022). Canola meal, a secondary product derived from oil extraction of canola seeds, is a valuable alternative to soybean meal due to its availability, affordability, favorable crude protein (**CP**) content and well-balanced amino acid (**AA**) composition (Khajali and Slominski, 2012; Adewole et al., 2016). However, the inclusion level of canola meal in swine diets is limited due to anti-nutritional factors, such as glucosinolates, which negatively affect nutrient digestibility and animal performance. Over time, plant breeding and processing advancements have significantly reduced glucosinolates content in canola meal, improving its safety and applicability in animal nutrition (Canola Council of Canada, 2024). Consequently, the adoption of low-glucosinolate canola meal in swine diets has increased over time.

However, the total dietary fiber content in canola meal is approximately 33.80%, which is notably higher than that of soybean meal (21.80 %), can negatively impact nutrient digestibility by physically interfering with enzyme activity and nutrient absorption (Agyekum and Nyachoti, 2017). Therefore, when formulating swine diets with canola meal, it is essential to consider standardized ileal digestible (**SID**), AA contents and net energy (**NE**) values. It has been showed that adult pigs, particularly gestating sows, exhibit enhanced energy utilization from the dietary fiber abundant in canola meal, compared to younger pigs (Noblet et al., 2013). For gestating sows, high-fiber diets help manage feed intake, preventing excessive fat accumulation that can lead to insulin resistance, metabolic imbalances, and impaired lactation (Jo and Kim, 2023). Dietary fiber in gestation diets without modifying daily net energy intake has been shown to enhance voluntary feed intake during lactation, however, this increase may not be sufficient to fulfill the nutrient

requirements for milk production or to maintain ideal body condition in highly prolific sows (Jo and Kim, 2023). Several studies have explored the use of canola meal in sow diets on sow reproductive performance and their litter performance. For example, King et al. (2001) reported no adverse effects on feed intake, body weight, or piglet growth performance when 200 g of canola meal was included per kg of diet for lactating sows. Likewise, Velayudhan & Nyachoti (2017) reported no notable negative impacts on sow or piglet performance when canola meal was included at 300 g/kg in diets for lactating sows, although digestibility of energy and nutrients was slightly reduced. These findings showed that canola meal could be a feasible alternative to soybean meal in swine diets. However, strategies to mitigate its potential limitations, such as reduced nutrients digestibility, need to be further investigated.

Studies have indicated that supplementation with *Saccharomyces cerevisiae* (*S. cerevisiae*) enhances gut health, nutrient utilization, and animal performance through mechanisms such as immunomodulation, modulation of gut microbiota, enhancement of digestive enzyme activity, and optimization of the gastrointestinal environment (Rauch and Lynch, 2010; Shen et al., 2011). In gestating sows, the addition of yeast has been associated with improved maternal protein utilization, as indicated by lower plasma urea nitrogen levels. It has also been linked to enhanced litter performance, including greater weaning weights and increased average daily gain (Shen et al., 2009). One previous study showed that sows fed with 5×10^{10} CFU/kg *S. cerevisiae* from late gestation until weaning had a reduced farrowing duration and backfat losses during lactation and increased feed intake of sows and feed conversion ratio of creep feed in suckling pigs (Sun et al., 2022). These findings underscore the potential of *S. cerevisiae* as a beneficial feed additive for sows. To the best of our knowledge, however, no in vivo studies have clearly examined how canola meal and *S. cerevisiae* product interact to affect sow reproductive function, highlighting a crucial

area for further study. Accordingly, this study aimed to evaluate the impact of high canola meal inclusion, with or without probiotic supplementation, in late-gestating and lactating sows on reproductive performance and nutrient digestibility, as well as suckling piglet growth.

4.3 MATERIALS AND METHODS

4.3.1 Animals and experimental design

The experimental procedures received approval from the University of Manitoba Animal Care Committee (AC11687), and all sows and piglets were managed in accordance with the Canadian Council on Animal Care (CCAC, 2009) guidelines.

This study was conducted at the Glenlea Research Station, University of Manitoba (Winnipeg, MB, Canada). A total of seventy-five gestating sows (TN70, Topigs Norsvin, Oak Bluff, MB, Canada) were included on d80 of gestation, averaging a parity of 2.7 ± 0.33 and an initial backfat thickness of 15.2 ± 0.58 mm. A randomized full block design was used to allocate sows at random to one of three dietary groups. The trial was carried out across three consecutive farrowing groups, with farrowing occurring every three weeks. Each treatment group included 25 sows per farrowing group.

From d 80 until d 111 of pregnancy, sows were kept in group housing in gestation stalls individual pens. On d 111 of pregnancy, sows were washed and transferred to the farrowing room, where each crate (2.10×0.69 m) was equipped with an individual feeder and water dispenser to ensure unrestricted access to feed and water for both sows and piglets. Each pen was equipped with a heat lamp and a mat to provide warmth for piglets. In the farrowing room, the temperature was gradually reduced from 38°C to 31°C by the time of weaning. To balance litter sizes, cross-

fostering was carried out within treatment groups within 48 hours after birth, ensuring each sow nursed approximately 12–13 piglets. At around 21 days of age, piglets were weaned from the sow.

4.3.2 Experimental diets

For both lactating and gestating sows, the experimental diets were prepared using standardized ileal digestible (**SID**) AA and net energy (**NE**) values (**Table 4.1**). The nutritional needs for gestating sows as stated by the NRC (2012) were met or exceeded in the formulation of all diets. The lactation diets were formulated for piglets who were predicted to gain 230 g of weight per day and sows that had an average post-farrowing body weight of 210 kg. The SID AA values for soybean meal and canola meal were sourced from previous studies (González-Vega and Stein, 2012; Velayudhan et al., 2019). The study included three dietary treatments: 1) **CTRL**, corn-soybean meal-based diet; 2) **CCM**, corn- 300 g/kg canola meal-based diet; 3) **CCM-P**, CCM diet supplemented with *S. cerevisiae* product (Actisaf® Sc 47, *Saccharomyces cerevisiae*: CNCM I-4407, 10¹⁰ CFU/g, Phileo Lesaffre Animal Care, France). Gestation diets include 250 mg/kg of *S. cerevisiae* product, while lactation diets contain 500 mg/kg of *S. cerevisiae* product based on the manufacturer's recommendation. Additionally, titanium dioxide was incorporated at 3.0 g/kg as an indigestible marker.

4.3.3 Sampling and measurements

Sows were provided with 3.0 kg/day of the gestation diet from d 80 until d 111 of gestation, followed by 3.0 kg/day of the lactation diet from d 111 until farrowing. After farrowing, sows were offered feed three times daily at 0700, 1100, and 1500 h. The amount of lactation feed was gradually increased by approximately 0.5 kg/day until d 6, after which feed was made available ad

libitum until weaning on d 21. Throughout both gestation and lactation, the amount of feed supplied, and feed leftover were weighed daily. The average daily feed intake (**ADFI**) was calculated as the total feed provided minus any feed refusals.

On d 80 and d 111 of pregnancy, body weight (**BW**) and backfat thickness were measured, along with additional assessments on the first day of lactation (farrowing) and at weaning (d 21 of lactation). An a-mode ultrasonic device (Renco Lean-Meater series 12, Renco Corporation, Minneapolis, MN, USA) was used to measure backfat thickness. Measurements were taken at the 10th rib, 6 cm lateral to the midline. Values from both sides were averaged to obtain a single backfat measurement, following the method described by Wang et al. (2008) to measure changes in sow body condition. Estrus detection was performed after weaning to assess the interval from weaning to estrus.

Litter size, including the total number of piglets born, born alive, mummified, and weaned per sow, was recorded. Litters were weighed on the first day of lactation (d 1). To account for age differences due to varying farrowing dates, all piglets were weighed again on d 19 post-farrowing to determine litter weight gain.

4.3.4 Blood collection and analysis

On d 1 and d 19 post-farrowing, blood samples were obtained from sows by performing venipuncture on the jugular vein and collected into 10 mL sodium heparinized vacutainer tubes (BD Vacutainer®, Franklin Lakes, NJ, USA) with sampling conducted 2 h after the morning feeding. Following collection, blood samples were centrifuged at 3,000×g for 15 minutes at 4°C. Before being subjected to additional examination, the collected plasma was placed in plastic screw-cap vials and kept at -80°C. Plasma was transported to Manitoba Veterinary Diagnostic

Services for measurement of plasma urea N concentrations utilizing a VITROS 250 Chemistry System (Ortho-Clinical Diagnostics Inc., Raritan, Rochester, NY).

4.3.5 Colostrum and milk collection and analysis

On the day of farrowing, 50 mL of colostrum was collected, and an additional 50 mL of milk was obtained on d 19 post-farrowing. Sows were injected with 1 mL oxytocin (Rafter 8 Products Inc., Calgary, AB, Canada) before sample collection to facilitate milk release. Fresh colostrum and milk samples were manually collected from all functional teats, thoroughly mixed, and sent to Horizon Lab Ltd. (Winnipeg, MB, Canada) for analysis of crude fat, crude protein, and lactose composition using Fourier transform infrared spectroscopy with the CombiFoss 6000 system (Foss Electric, Denmark).

4.3.6 Fecal collection and digestibility analysis

On d 110 of pregnancy and d 19 post-farrowing, fecal samples were obtained from all sows through grab sampling via rectal palpation. Prior to additional analysis, the obtained samples were kept at -20°C. Fecal samples were ground into a fine powder and dried at 55°C in a forced-air oven before to chemical examination.

Both diet and fecal samples were ground to a fine consistency for dry matter (**DM**), gross energy (**GE**), CP, Ca, P and neutral detergent fiber (**NDF**) composition analysis. The content of acid detergent fiber (**ADF**), crude fat and glucosinolates content in diets were also determined. Dry matter was analyzed following AOAC (method 934.01; 2006), while GE was quantified using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL, 2005) with benzoic acid as a calibration standard. Crude protein was estimated based on nitrogen content multiplied by

6.25, whereas nitrogen content was determined through the combustion method (method 990.03; AOAC, 2006) using a LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI). Calcium and phosphorus concentrations were assessed after ashing and processed according to AOAC (2006; method 985.01), followed by analysis with a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Crude fat concentrations were analyzed in diets following AOAC (method 920.39; 2006). Neutral detergent fiber and ADF were measured using the Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY), following the method described by Van Soest et al. (1991). Titanium dioxide content was examined based on the method of Lomer et al. (2000) and analyzed using an inductively coupled plasma spectrometer (Vista-MPX; Varian Canada Inc., Mississauga, ON, CA). Glucosinolate levels in the diets were evaluated according to the procedures outlined by Slominski and Campbell (1987).

4.3.7 Calculations and statistical analysis

The equation was used to calculate the apparent total tract digestibility (**ATTD**) of nutrients:

$$\text{ATTD (\%)} = 100 - \{[(\text{Nd/Nf}) \times (\text{Tf/Td})] \times 100\}$$

In the equation, Nd represents the concentration of energy or nutrients in fecal samples, Nf denotes the energy or nutrient levels in feed, Tf corresponds to the titanium dioxide concentration in feed, and Td refers to the titanium dioxide concentration in fecal samples.

The MIXED procedure in SAS (SAS Inst. Inc., Cary, NC, USA) was used to analyze the data, applying a randomized complete block design. In the model, treatment was considered a fixed effect, while farrowing group and block were included as random effects. To account for variability due to seasonal and management factors, farrowing group was set as a random variable. Each sow or litter served as the experimental unit. Initial backfat thickness on d 80 of pregnancy was

incorporated as a covariate for BW and backfat thickness analysis. Covariates were retained in the model only if their effects were significant ($P < 0.10$); otherwise, they were excluded.

To assess normality and variance homogeneity, residuals were examined using diagnostic plots generated from the model. The Shapiro-Wilk test and Levene's test were applied to evaluate residual normality and homogeneity, respectively. Outliers were excluded if standardized residuals exceeded ± 3 standard deviations. Least square means were analyzed, and differences were considered significant at $P < 0.05$, while trends were identified for values between 0.05 and 0.10.

Table 4.1 Ingredient composition and analyzed nutrient content of experimental diets (as-fed basis).

Item	Diet ¹			
	Gestation ²		Lactation ³	
	CTRL	CCM	CTRL	CCM
Ingredient, g/kg				
Corn	720.2	625.0	707.8	618.8
Canola meal	-	300.0	-	300.0
Soybean meal	220.0	-	225.0	-
Vegetable oils	23.0	44.0	25.5	45.0
Limestone	13.0	12.5	11.6	11.5
Monocalcium phosphate	9.8	3.0	15.0	7.8
Salt	4.0	4.0	4.7	4.7
Vitamin-mineral premix ⁴	10.0	10.0	10.0	10.0
L- Lysine	-	1.5	0.4	1.8
L-Tryptophan	-	-	-	0.1
L-Valine	-	-	-	0.3
Calculated composition				
Gross energy, kcal/kg	4,025	4,215	3,985	4,170
Net energy, kcal/kg	2,604	2,603	2,568	2,568
Crude protein, %	17.00	17.03	16.60	16.60
Calcium, %	0.74	0.73	0.76	0.76
Total phosphorus, %	0.55	0.56	0.63	0.65
STTD P, %	0.30	0.30	0.39	0.39
Analyzed composition				
Gross energy, kcal/kg	4,073	4,225	4,064	4,244
Crude protein, %	17.20	17.70	17.50	17.40

Crude fat, %	5.54	7.77	5.85	7.63
Acid detergent fiber, %	2.95	7.00	2.58	6.45
Neutral detergent fiber, %	8.07	12.98	6.42	12.58
Calcium, %	0.64	0.66	0.66	0.63
Total phosphorus, %	0.55	0.58	0.64	0.64
Total glucosinolates ($\mu\text{mol/g}$)	0.05	0.64	0.07	0.75

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg canola meal-based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *Saccharomyces cerevisiae* product in lactation diets.

²As-fed basis. All gestation diets were formulated to contain 2.60 Mcal/kg of NE with 0.80%, 0.28%, 0.56%, and 0.17% standardized ileal digestible Lys, Met, Thr, and Trp, respectively, and 0.73% and 0.30% Ca and standardized total tract digestible (STTD) P, respectively. Values were calculated based on values for standardized ileal digestibility of AA in corn and crystalline AA published by NRC (2012), and standardized ileal digestibility values for AA in soybean meal and canola meal were from González-Vega & Stein, (2012) and Velayudhan et al. (2019).

³All lactation diets were formulated to contain 2.56 Mcal/kg of NE with 0.84%, 0.26%, 0.55%, and 0.17% standardized ileal digestible Lys, Met, Thr, and Trp, respectively, and 0.76% and 0.39% Ca and STTD P, respectively.

⁴Supplied the following per kg of finished gestation diets: vitamin A, 4400 IU; vitamin D, 880 IU; vitamin E, 48 IU; vitamin K, 0.6 mg; choline, 1.37 g; pantothenic acid, 13 mg; riboflavin, 4.13 mg; folic acid, 1.43 mg; niacin, 11 mg; thiamin, 1.1 mg; vitamin B6, 1.1 mg; biotin, 0.21 mg; vitamin B12, 16 μg , Cu, 11 mg as copper sulfate; Zn, 110 mg as zinc oxide; Fe, 88 mg as ferrous sulfate; Mn, 27.5 mg as manganese sulfate; I, 0.15 mg as potassium iodate; Se, 0.16 mg as sodium selenite. Supplied the following per kg of finished lactation diets: vitamin A, 2200 IU; vitamin D, 880 IU; vitamin E, 48 IU; vitamin K, 0.6 mg; choline, 1.37 g; pantothenic acid, 13 mg; riboflavin, 4.13 mg; folic acid, 1.43 mg; niacin, 11 mg; thiamin, 1.1 mg; vitamin B6, 1.1 mg; biotin, 0.21 mg; vitamin B12, 16 μg , Cu, 22 mg as copper sulfate; Zn, 110 mg as zinc oxide; Fe, 88 mg as ferrous sulfate; Mn, 27.5 mg as manganese sulfate; I, 0.15 mg as potassium iodate; Se, 0.16 mg as sodium selenite.

4.4 RESULTS

4.4.1 Sow performance

No dietary effect ($P > 0.10$) was found on sow BW or backfat thickness during the entire experimental period (**Table 4.2**). However, sows fed the CCM diet had a tendency ($P = 0.09$) for lower BW gain from d 80 to d 111 of pregnancy compared to those fed the CTRL diet. No difference ($P > 0.10$) in ADFI in lactation period was found among sows fed the different experimental diets. Dietary treatment had no effect ($P > 0.10$) on the wean to estrus interval of sows.

4.4.2 Litter performance

As shown in **Table 4.3**, there were no effects ($P > 0.10$) of sow dietary treatment on number of total piglets born, piglets born live, born dead and mummies. No difference ($P > 0.10$) on the number of piglets after cross-fostering, as intended, or on d19 post-farrowing. Sow dietary treatments had no effect ($P > 0.10$) on the survival rate from farrowing to weaning. In addition, the average body weight of piglets on farrowing and d19 post-farrowing were not influenced ($P > 0.10$) by dietary treatment.

4.4.3 Milk composition and plasma urea N

The nutrient composition of sow colostrum and milk, including crude fat, crude protein, lactose, and oligosaccharides, is presented in **Table 4.4**. No significant differences ($P > 0.10$) were observed among treatments for these components. There was no difference ($P > 0.10$) on milk crude protein and lactose contents among sows fed different diets. However, sows fed the CCM-

P diet had a significantly higher ($P < 0.05$) crude fat value than sows fed the CTRL and CCM diet on d 19 post-farrowing.

Sows fed the CCM diet showed a tendency ($P = 0.09$) for lower plasma urea N levels on farrowing day compared to those fed the CTRL diet, but the addition of probiotics to the CCM-P diet did not affect ($P > 0.10$) the plasma urea N level relative to the CTRL diet as shown in **Table 4.5**. No difference ($P > 0.10$) was found in plasma urea N levels of sows fed the CTRL, CCM or CCM-P diets on d 19 post-farrowing.

4.4.4 Apparent total digestibility of energy and nutrients

In **Table 4.6**, the energy and nutrients digestibility in gestating sows were shown. Sows receiving CCM and CCM-P diets exhibited lower ($P < 0.05$) ATTD values for DM, GE, and CP compared to those fed the CTRL diet. Gestating sows in the CCM-P group tended to have higher Ca digestibility than those in the CCM group ($P = 0.08$), while no difference ($P > 0.10$) was observed between the CCM and CTRL groups. While no significant differences ($P > 0.10$) were observed in the ATTD of P between sows fed the CTRL and CCM diets, the probiotic supplementation in the CCM-P diet significantly improved ($P < 0.05$) the ATTD of P relative to the CCM diet. Nevertheless, the ATTD of Ca and P in sows fed the CCM-P diet was comparable ($P > 0.10$) to that in the CTRL group.

Table 4.7 showed that the ATTD coefficients for energy and nutrients in sows fed different dietary treatments during the lactation period. No dietary treatment effects ($P > 0.10$) were observed on the ATTD of GE, CP, Ca or P. However, the addition of probiotic to the CCM-P diet tended to decrease ($P = 0.07$) the ATTD of DM in comparison to sows fed the CTRL diet, while no difference was found between the CCM and CTRL groups. Additionally, sows consuming

canola meal-based diets (CCM and CCM-P) showed a significantly higher ($P < 0.05$) ATTD of NDF than those on the CTRL diet.

Table 4.2 Effect of dietary canola meal inclusion and probiotic in sow diets on sow performance during late gestation and lactation

Item	Diet ¹			SEM	<i>P</i> -value
	CTRL	CCM	CCM-P		
Parity	2.83	2.96	2.60	0.325	0.731
Sow body weight, kg					
d 80 of gestation	267.3	264.7	262.4	8.55	0.924
d 111 of gestation	301.9	297.7	294.6	8.61	0.717
d 1 post-farrowing	280.2	280.8	276.4	4.09	0.698
d 21 post-farrowing	249.9	250.0	247.5	6.89	0.944
Gestation gain ²	30.26	24.38	26.19	1.96	0.085
Lactation loss ³	30.18	26.77	29.35	5.00	0.535
Sow backfat thickness, mm					
d 80 of gestation ⁴	15.18	14.99	15.35	0.409	0.818
d 111 of gestation	15.53	15.84	15.84	0.542	0.826
d 21 post-farrowing	13.31	14.42	13.31	0.636	0.183
Gestation gain	0.32	0.71	0.59	0.542	0.830
Lactation loss ⁵	1.70	1.40	2.29	0.514	0.458
Gestation ADFI, kg/d	2.98	2.96	2.96	0.012	0.166
Lactation ADFI, kg/d	5.99	5.72	5.65	0.291	0.162
Wean to estrus interval, d	4.22	4.13	4.28	0.223	0.880

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg -based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *Saccharomyces cerevisiae* product in lactation diets.

²Gestation bodyweight or backfat thickness gain was calculated as the difference between sow bodyweight at d 80 and d 111 of gestation.

³Lactation bodyweight gain was calculated as the difference between sow body weight at d 1 and d 21 of post-farrowing.

⁴Sow initial backfat thickness at d 80 of pregnancy was used as a covariate in the statistical model for BW and backfat thickness data analysis.

⁵Lactation backfat thickness loss was calculated as the difference between d 111 of gestation and d 21 of post-farrowing.

Table 4.3. Effect of dietary canola meal inclusion and probiotic in sow diets on reproductive performance at farrowing and litter performance during the suckling period

Item	Diet ¹			SEM	<i>P</i> -value
	CTRL	CCM	CCM-P		
Litter size ²					
Total born	16.69	15.24	16.53	0.817	0.236
Born alive	15.53	14.09	15.32	0.629	0.140
Stillborn	1.08	0.96	1.40	0.309	0.573
Mummified	0.17	0.16	0.20	0.086	0.938
After cross-fostering	14.34	13.43	13.90	0.554	0.115
Weaned	12.82	12.19	12.42	0.300	0.193
Piglet survival pre-weaning, ³ %	90.11	88.85	89.27	2.203	0.891
Litter weight, kg					
d 1	20.65	18.92	19.64	1.543	0.136
d 19	76.33	72.21	72.93	3.779	0.331
Daily weight gain, ⁴ kg/d	2.93	2.80	2.81	0.140	0.521

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg -based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *Saccharomyces cerevisiae* product in lactation diets.

²Litter size, the number of piglets per litter

³Piglet survival pre-weaning (%) = (the number of weaned piglets /the number of piglets after cross fostering) × 100.

⁴Daily weight gain = (litter weight on d 19 – litter weight on d 1)/19.

Table 4.4. Effect of dietary canola meal inclusion and probiotic on colostrum and milk composition on d 19 post-farrowing

Item	Diet ¹			SEM	P-value
	CTRL	CCM	CCM-P		
Colostrum composition, g/kg					
Crude fat	7.32	8.08	7.40	0.446	0.422
Crude protein	6.56	6.41	6.89	0.399	0.683
Lactose	5.60	5.59	5.59	0.095	0.988
Milk composition, g/kg					
Crude fat	7.43 ^b	7.48 ^b	8.24 ^a	0.309	0.014
Crude protein	4.61	4.61	4.79	0.084	0.213
Lactose	7.18	7.12	7.11	0.068	0.509

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

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg - based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *Saccharomyces cerevisiae* product in lactation diets.

Table 4.5. Effect of dietary canola meal inclusion and probiotic on plasma urea nitrogen of sows at farrowing and on d 19 post-farrowing

Item	Diet ¹			SEM	<i>P</i> -value
	CTRL	CCM	CCM-P		
Plasma urea N, mmol/L					
Farrowing day	3.83	3.34	3.68	0.164	0.089 ²
d 19 post-farrowing	4.58	4.27	4.44	0.143	0.274

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg - based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *Saccharomyces cerevisiae* product in lactation diets.

²The tendency ($P = 0.09$) for lower plasma urea N at farrowing day was observed only between CCM and CTRL.

Table 4.6. Effect of dietary canola meal inclusion and probiotic on apparent total tract digestibility (ATTD) coefficients of nutrients and energy in gestation sows on d 110 of gestation

Item	Diet ¹			SEM	P-value
	CTRL	CCM	CCM-P		
Dry matter, %	85.87 ^a	82.60 ^b	83.13 ^b	0.63	< 0.001
Gross energy, %	85.83 ^a	83.34 ^b	83.40 ^b	0.68	< 0.001
Crude protein, %	86.47 ^a	81.51 ^b	82.59 ^b	0.65	< 0.001
Calcium ² , %	34.63	31.47	37.75	1.89	0.085
Phosphorus, %	31.96 ^a	27.23 ^b	33.52 ^a	2.04	0.011
NDF ³ , %	52.35	52.19	52.56	2.35	0.991

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

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg canola meal-based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *cerevisiae* product in lactation diets.

²A tendency for higher ($P = 0.08$) calcium digestibility was observed in the CCM-P group compared with the CCM group, and no difference was observed between CCM-P and CTRL groups.

³NDF, neutral detergent fiber.

Table 4.7. Effect of dietary canola meal inclusion and probiotic on apparent total tract digestibility (ATTD) coefficients of nutrients and energy in lactation sows on d 19 post-farrowing

Item	Diet ¹			SEM	<i>P</i> -value
	CTRL	CCM	CCM-P		
Dry matter ² , %	83.57	82.57	82.19	0.57	0.073
Gross energy, %	83.86	83.69	82.83	0.46	0.209
Crude protein, %	84.46	83.47	83.28	0.49	0.141
Calcium, %	30.14	30.56	30.97	1.90	0.923
Phosphorus, %	34.48	32.30	36.75	1.49	0.113
NDF ³ , %	43.27 ^a	54.72 ^b	54.21 ^b	2.24	<0.001

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

¹Experimental diets consisted of CTRL, corn-soybean meal-based diet; 2) CCM, corn-300 g/kg canola meal-based diet; 3) CCM-P, CCM diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47). 250 mg/kg of *Saccharomyces cerevisiae* product in gestation diets, 500 mg/kg of *cerevisiae* product in lactation diets.

²The tendency ($P = 0.07$) for lower dry matter digestibility was observed only between CCM-P and CTRL; no difference was shown between CCM and CTRL.

³NDF, neutral detergent fiber.

4.5 DISCUSSION

This study investigated the effects of including 300 g/kg of canola meal in sow diets during late gestation and lactation, with or without probiotic supplementation. Overall, the inclusion of canola meal did not compromise sow reproductive outcomes, litter performance, milk composition, and nitrogen metabolism, except for some reductions in energy and protein digestibility during late gestation. While digestibility of some nutrients was reduced, particularly during late gestation, probiotic supplementation partially alleviated this effect by improving phosphorus digestibility. These findings suggest that canola meal is a potential substitute to replace soybean meal as a protein source in sow nutrition when combined with dietary strategies to optimize nutrient digestibility.

Previous studies (Baidoo et al., 1986; McIntosh et al., 1986) have reported that the inclusion of canola meal in swine diets impaired growth performance, likely due to diet formulations based primarily on CP content and digestible energy (**DE**) values. The DE system often overestimates the energy values of diets high in protein or fiber (Velayudhan et al., 2015). In contrast, the NE system offers a more precise assessment of energy utilization compared to other energy evaluation methods, making it more effective to use canola meal in animal diets without negatively influencing animal performance (Velayudhan et al., 2015; Agyekum and Nyachoti, 2017). It has been suggested that canola meal may be included at levels of up to 200g/kg in diets for lactating sows without negatively affecting reproductive performance or litter growth performance when formulated diets based on NE system (King et al., 2001). The results of sow reproductive performance and litter growth performance in the current study align with more recent research on lactating sows (Velayudhan and Nyachoti, 2017), wherein it was observed that inclusion of up to 300 g/kg canola meal in lactation diet did not change body weight during

lactation or weaning to estrus interval when diets were formulated according to SID AA and NE content.

In the current study, sows fed with 300 g/kg canola meal had a similar ADFI during the entire experimental period compared to sows fed with soybean meal diets. This may be partially explained by the higher crude fat levels in CSCM-based diets than CTRL diets (7.77% vs. 5.54% during gestation; 7.63% vs. 5.85% during lactation), which could have improved feed palatability. Dietary fat enhances aroma, texture, and mouthfeel, all of which are known to stimulate voluntary feed intake in pigs (Varona et al., 2021). The improved palatability may have helped mitigate negative effects of high dietary fiber on feed intake. Additionally, dietary fiber has a higher heat increment due to its fermentation in the hindgut and associated increase in metabolic heat, while dietary fat has the lowest heat increment among macronutrients (Schoenherr et al., 1989). Therefore, the higher fat content in the CSCM-based diets may have helped reduce the additional heat production typically associated with fiber-rich formulations, thereby supporting more efficient energy utilization and maintaining feed intake. However, in previous studies, an increased level of canola meal in the diet of weaned or growing pigs led to a decrease in ADFI, closely related to the metabolic effects of glucosinolates, which disrupt iodine metabolism, affect thyroid gland function, and thereby influence animal performance (Mullan et al., 2000; Parr et al., 2015). As demonstrated by Bell (1993) and Mawson et al. (1994), growing pigs may tolerate up to 2.5 $\mu\text{mol/g}$ of total glucosinolates in their diets, while sows can take up to 4.0 $\mu\text{mol/g}$ without experiencing any negative effects on their reproductive performance. However, in the current study, the glucosinolate concentration in canola meal was 2.4 $\mu\text{mol/g}$ and the glucosinolate in gestation and lactation diets were 0.6 $\mu\text{mol/g}$ and 0.7 $\mu\text{mol/g}$, respectively, therefore, the low glucosinolates may explain why sows fed diets containing high levels of canola meal did not

experience a decrease in ADFI in this study. Similarly, previous studies demonstrated that diets with glucosinolate concentrations below 2.0 $\mu\text{mol/g}$ do not negatively impact daily feed intake in lactating sows (Schöne et al., 2001; Quiniou et al., 2012). The unaffected ADFI in sows fed canola meal in the current study may also be attributed to increased gut capacity following an adaptation period. Previous research has shown that diets rich in non-starch polysaccharides (NSP) or pectin led to the development of larger stomachs and colons in growing pigs, with both volume and empty weight increasing (Jørgensen et al., 1996b). Several studies have observed an increase in feed intake during lactation when high-fiber diets were provided during gestation, likely due to the gastrointestinal tract becoming adapted to the larger volumes of feed associated with high fiber intake (Agyekum et al., 2019; Shang et al., 2019). However, the differences in results may be attributed to variations in dietary fiber types and the length of the adaptation period. Thus, future studies should further explore the long-term effects of high-fiber canola meal diets during both gestation and lactation on gut capacity and feed intake in sows and digestive system adaptation.

In addition, the present experiment showed that litter performance, including litter size at farrowing and weaning, as well as piglet average daily gain, was not affected by dietary treatment. These results are consistent with the findings of Velayudhan and Nyachoti (2017), who similarly reported no adverse effects when 300 g/kg of canola meal was included in sow diets. These results support the hypothesis that canola meal can be used in sow diets to fully replace soybean meal without adverse effects on sow and suckling piglet performance. The lack of effect on litter size at birth may be due to the limited influence of dietary changes during late gestation on the number of piglets born. Although there was a numerical difference (approximately 1.5 piglets) in the number of piglets born alive between the CTRL and CCM groups, this difference was not statistically significant. To further explore whether this variation was influenced by total litter size,

we included total born as a covariate in the statistical model. After adjustment, the least square means (\pm SEM) for piglets born alive were CTRL: 14.61 ± 0.22 , CCM: 14.61 ± 0.22 , and CCM-P: 14.91 ± 0.22 . The treatment effect remained non-significant ($P > 0.10$), while total born as a covariate was highly significant ($P < 0.001$). These results suggest that the variation in the number of piglets born alive was primarily driven by natural variation in total litter size, rather than by a direct effect of dietary treatment. However, litter size at birth is largely determined by ovulation rate, early embryonic survival, and uterine capacity, which are established during early gestation (Bennett and Leymaster, 1989). Additionally, litter size at weaning primarily depends on neonatal survival, which is largely influenced by the piglet's energy reserves from hepatic and muscular glycogen, as well as intake of colostrum and transitional milk in the first few days after birth (Theil et al., 2014). Piglet average daily gain is largely related to both the quantity and quality of milk provided by the sow, although other factors such as litter size, piglet health, and environmental conditions also contribute to growth (King et al., 1997). In the present study, the absence of dietary effects on litter size at weaning and litter weight gain likely reflects the fact that milk composition was not adversely affected by the inclusion of 300 g/kg of canola meal in the maternal diet. The precise formulation of diets to meet SID amino acid and NE requirements likely ensured adequate lactational nutrient supply, which helped maintain piglet growth and survival despite the higher fiber content and inclusion of 300 g/kg canola meal in the CCM diet. Furthermore, the litter or piglet weights at birth and weaning were not significantly affected by the supplementation of *S. cerevisiae* product in this study. This is in line with other research (Jang et al., 2013; Le Floc'h et al., 2022) that demonstrated that live yeast supplementation in sow diets had no effect on growth performance of their litters. However, yeast culture supplementation during pregnancy and lactation has been shown in some studies to increase litter growth performance (Kim et al., 2008;

Liu et al., 2023; Christensen et al., 2024). The tested probiotic in the present study is a live yeast (*S. cerevisiae*), in contrast to yeast culture, which consists of yeast metabolites and cereal grain fermentation byproducts that may provide additional nutrients to enhance litter weight gain. It has also been reported that live yeast increased litter weight gain and average weaned piglets per litter in sows receiving live yeast (Domingos et al., 2021). Furthermore, variations in yeast strain, dosage, and duration of supplementation may influence the degree and consistency of live yeast effects on sow reproductive outcomes (Patterson et al., 2023).

Milk composition is a crucial factor for the growth and development of suckling piglets (Farmer, 2013). The composition and yield of colostrum and milk in sows are influenced by a number of factors, such as the environment, diets, breed, and health state (Amatucci et al., 2022). The current study found no significant difference in milk composition between sows fed canola meal diets and those in the control group, consistent with findings from Velayudhan and Nyachoti (2017). The underlying mechanism is likely due to the diets being formulated with equivalent NE and SID AA contents. Additionally, sow milk production tended to remain stable even when dietary protein and energy levels are slightly deficient (De Bettio et al., 2016). During late gestation, sows allocate energy and AA to fetal and placental growth, fluids and membrane as well as for mammary gland development (Langendijk et al., 2023). In lactation, most of the absorbed nutrients and energy are directed toward milk production and further mammary gland development (Bauman and Bruce Currie, 1980; Hurley et al., 2000). When sows do not receive sufficient nutrients, particularly protein and energy, they break down body tissue protein to maintain milk production (Dourmad et al., 2008). The absence of major differences between the CTRL and CCM groups in milk composition suggests that sows fed the high-fiber canola meal diet maintained adequate nutrient intake during lactation to support milk production without the need for

substantial body weight or backfat mobilization, as reflected in the unchanged sow performance indicators.

In the present study, dietary supplementation with *S. cerevisiae* product during lactation increased milk fat concentration at weaning in sows fed canola meal-based diets. Similar findings have been reported with yeast culture, a fermentation-derived product of *S. cerevisiae*, which, when supplemented at 500 or 800 mg/kg from day 30 of gestation, increased colostrum fat and lactose concentrations (Ma et al., 2023). However, not all studies have reported positive effects of yeast supplementation on milk composition. For instance, Jang et al. (2013) observed no improvements in milk composition, including fat, lactose and protein, when live yeast was provided in lactation. One possible explanation for these divergent findings is the substantial difference in yeast dosage: while Jang et al. used live yeast at 10^7 CFU/g, the present study applied a higher dosage of 10^{10} CFU/g at 500 mg/kg during lactation. In the present study, one possible explanation for the increase in milk fat concentration is the enhanced nutrient digestibility observed with live yeast supplementation. By promoting beneficial gut microbiota and stabilizing the gastrointestinal environment, live yeast may improve the breakdown and absorption of dietary fiber, thereby increasing the energy supply required for milk fat synthesis (Kritas et al., 2006). In addition, sows fed canola meal-based diets supplemented with probiotics produced more milk fat compared to those in the CTRL group. While this may be partially attributed to the probiotic effects, another contributing factor could be the higher crude fat content in the CCM-P diets during lactation. Review papers by Pettigrew (1981) and Rosero et al. (2016) summarized that fat-rich diets increase milk fat output in sows by stimulating milk fat synthesis, with additional benefits for piglet growth. However, in this study, neither the higher crude fat levels in the CCM and CCM-P diets nor the inclusion of *S. cerevisiae* product improved the growth performance of suckling

piglets. The limited impact of dietary fat on early piglet growth may be explained by the fact that protein and water retention are the primary drivers during this stage (Noblet and Etienne, 1987). It has been shown that dietary fat intake and milk fat output are not major determinants of piglet growth, as evidenced by Neal et al. (1999), who found no significant difference when fat levels were increased from 3% to 9%. Instead, the quantity of milk consumed may play a more critical role during the suckling phase than the actual composition of the milk (Quesnel et al., 2012). This aligns with previous findings showing that increased milk production in sows and greater milk intake by piglets are strongly correlated with piglet daily weight gain (Strathe et al., 2017).

Urea is the primary nitrogenous waste product formed during the breakdown of dietary protein that is either not utilized by the body or results from tissue protein turnover (Weiner et al., 2015). In this study, during gestation, sows fed the CCM diet tended to have lower plasma urea N levels and reduced ATTD of CP. This effect may be attributed to the higher fiber content and the slightly lower AA digestibility compared to soybean meal, which can hinder protein digestion and absorption (Newkirk and Classen, 2002). The decreased digestibility of CP could also result in less nitrogen being absorbed, thereby contributing to reduced plasma urea N levels (Eggum, 1970). However, during lactation, neither the ATTD of CP nor plasma urea N levels were significantly affected by the canola meal diet, indicating that all diets were balanced for AA to the same degree. This stability could be due to the increased protein demand during lactation, which enhances nitrogen utilization efficiency (Kim et al., 2013). Additionally, improved nitrogen recycling and potential adaptations of the gut microbiota to the higher fiber content of the canola meal diet may contribute to these outcomes.

Research on the effects of canola meal on nutrient digestibility shows mixed results, with some indicating reduced energy and nutrient absorption (Velayudhan and Nyachoti, 2017), while

others found no negative effects when substituting soybean meal with canola meal (Agyekum et al., 2014; Sanjayan et al., 2014). These discrepancies may arise from variations in factors such as fiber composition, processing methods, and the physiological state of the pigs (Noblet and Shi, 1993; McDougall et al., 1996). Therefore, understanding the impact of canola meal on nutrient digestibility, especially in sow diets, is essential. In the present study, inclusion of 300 g/kg of canola meal during gestation resulted in reduced ATTD of DM, GE and CP. This reduction may be attributed to several interacting factors, particularly the higher crude fat and dietary fiber contents in the canola meal-based diet compared to the control diet. Several studies have demonstrated that dietary fat can enhance nutrient digestibility. For instance, Jørgensen et al. (1996a) reported improved ATTD of CP when pigs were fed diets containing 4%, 8%, and 16% rapeseed oil. Furthermore, pigs fed oil-supplemented diets showed higher ATTD of fat compared to those fed basal diets (Jørgensen et al., 2000). However, Jørgensen and Fernández (2000) observed that at higher fat inclusion levels, fat digestibility becomes independent of dietary fat content. While the true ileal and total tract digestibility of fat may remain constant at higher inclusion levels (Kil et al., 2010), increased fiber intake can lead to greater bile secretion and mucosal turnover, thereby increasing endogenous fat excretion and reducing ATTD values. This may help explain why, in the current study, the crude fat level of 7.7% did not result in improved energy or protein digestibility. In addition, the increased dietary fiber content in this study may be the cause of the declines in energy and crude protein digestibility values of late gestating sows fed diets containing 300 g/kg of canola meal. Although the increased fiber did not negatively affect growth indicators in this trial, the reduction in nutrient digestibility coefficients aligned with previous findings, where lactating sows fed diets containing 15-30% canola meal from d 115 of gestation until weaning exhibited similar outcomes (Velayudhan and Nyachoti, 2017). Likewise,

inclusion 200 g/kg canola meal to replace soybean meal in weaned pig diets decreased the ATTD coefficients of DM, GE and CP (Wang et al., 2017). The reduced ATTD of CP suggested that canola meal supplied less digestible AA, likely due to the presence of hulls in canola meal, which contain 150 g/kg CP but are challenging to digest (Khajali and Slominski, 2012).

In the current study, lactating sows fed diets containing 300 g/kg canola meal showed no differences in nutrients and energy digestibility, which is consistent with findings by Velayudhan et al., (2018), where high-fiber canola meal diets did not affect energy and nutrient digestibility coefficients. This lack of impact could be attributed to the sow ability to adapt over time, allowing them to more efficiently utilize the high dietary fiber (Jørgensen et al., 1996b). However, lactating sows fed canola meal-containing diets demonstrated increased ATTD of NDF compared to those fed the CTRL diet in the present study. This may be partially explained by the higher NDF levels in the canola meal diets. Additionally, progressive adaptation to the canola meal diet over time may have contributed to improved fiber utilization during the later stages of the current study. Despite using similar diet formulations with 300 g/kg of canola meal during lactation, Velayudhan et al. (2017) did not observe any improvement in NDF digestibility, which may be due to differences in the duration of dietary exposure. Given the potential for ingredient effects to accumulate across reproductive cycles, future research could explore how long-term inclusion of canola meal from early gestation over multiple reproductive cycles affects sow nutrient metabolism and lifetime productivity.

Supplementation of canola meal-based diets with *S. cerevisiae* product improved the ATTD of P during gestation. This improvement is likely linked to the enzymatic activity stimulated by the yeast, particularly phytase, which can hydrolyze phytate complexes in plant-based ingredients and enhance the release of bound minerals (Kaur et al., 2007). Previous studies have

also reported similar effects of yeast products on phosphorus availability. For instance, inclusion of live yeast in sow diets during gestation and lactation improved phosphorus digestibility in nursery pigs (Lu et al., 2019). Fermentation of dietary fiber has been suggested to increase intestinal phosphorus availability in pigs (Metzler and Mosenthin, 2008), and improved P digestibility has been observed in broilers and growing pigs supplemented with yeast culture (Gao et al., 2008; Kim et al., 2014). However, no improvement in Ca and P digestibility was observed during lactation in the present study. This differential response may be attributed to physiological differences between gestation and lactation. During lactation, sows consume significantly more feed, which may accelerate gastrointestinal transit time and reduce the contact time required for effective enzymatic hydrolysis of phytate complexes (De Bettio et al., 2016). As a result, the addition of *S. cerevisiae* product may enhance the nutritional value of canola meal by mitigating its negative impact on phosphorus digestibility during gestation, thereby improving its feasibility as a sustainable alternative to soybean meal in sow diets.

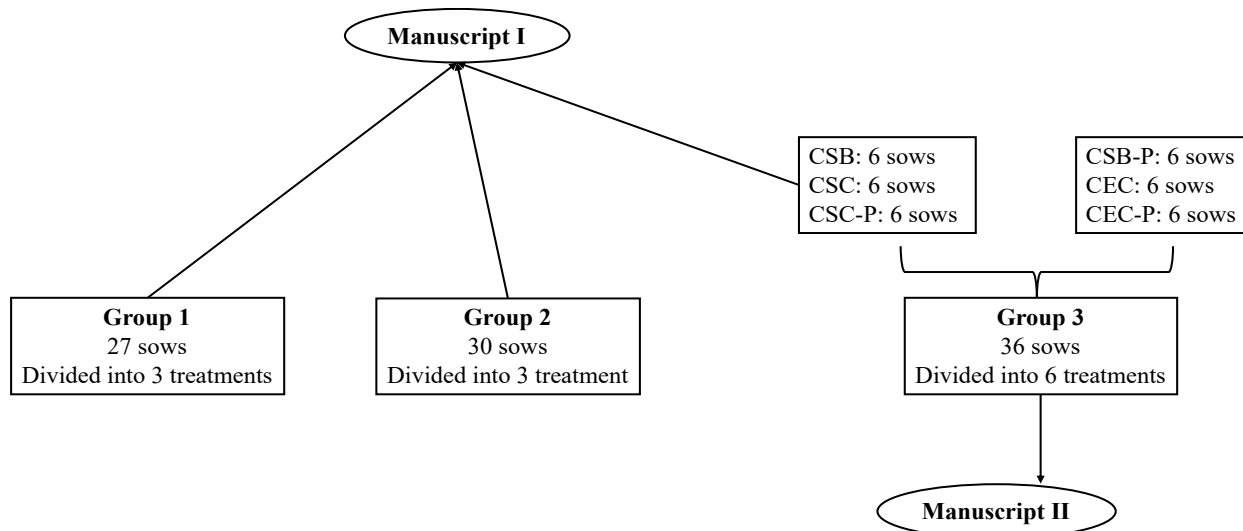
4.6 CONCLUSION

In conclusion, canola meal at 300 g/kg can effectively replace soybean meal in gestating and lactating sow diets. The inclusion of canola meal negatively influenced the apparent total tract digestibility of energy and nutrients during gestation but without compromising sow or litter performance. The supplementation of *Saccharomyces cerevisiae* product alleviated the negative impact of canola meal on phosphorus digestibility in gestating sows and increased milk fat content at weaning. While these findings highlight the potential of canola meal as a sustainable protein source, the reduction in energy and protein digestibility was only observed during gestation, but not during lactation, suggesting that sows may gradually adapt to canola meal with repeated use. Therefore, further studies are needed to determine whether long-term feeding across multiple cycles can mitigate the initial negative effects observed during gestation.

TRANSITION STATEMENT

Following the manuscript I, which evaluated the effects of including 300 g/kg solvent-extracted canola meal supplemented with live yeast on sow reproductive performance and litter outcomes, Manuscript II was designed to further explore this feeding strategy by comparing canola meal sourced from two different extraction methods—solvent and expeller—both in combination with *S. cerevisiae* product. This allowed for an assessment of whether the method of oil extraction influences the effectiveness of probiotic supplementation in sow diets.

In addition, several sows were shared between Manuscript I and II, as the treatments assigned to the third group in the barn were consistent across both studies, as illustrated in the figure below. This strategy was designed to optimize animal use and reduce experimental costs.



CHAPTER 5**MANUSCRIPT II****EFFECTS OF HIGH SOLVENT- AND EXPELLER- EXTRACTED CANOLA
MEAL INCLUSION IN LATE-GESTATION AND LACTATION SOW DIETS WITH
SACCHAROMYCES CEREVISIAE PRODUCT ON SOW REPRODUCTIVE
PERFORMANCE, NUTRIENT DIGESTIBILITY, MICROBIOME AND LITTER
PERFORMANCE**

Xiaoxiao Zhang*, Debora Muratori Holanda*, Daniel Flores Orozco*, Hooman Derakhshani*,
Elijah G. Kiarie*[†], Anna Rogiewicz*, Chengbo Yang*, and Charles Martin Nyachoti*¹

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

[†]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Xiaoxiao Zhang: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing- original draft

Debora Muratori Holanda: Conceptualization, investigation, supervision, methodology, writing - review & edition

Daniel Flores Orozco: Formal analysis, methodology, visualization

Hooman Derakhshani: Formal analysis, writing - review & edition

Anna Rogiewicz: Formal analysis, writing - review & edition

Elijah G. Kiarie: Writing - review & edition

Chengbo Yang: Writing - review & edition

Charles Martin Nyachoti: Project administration, resources, supervision, writing - review & edition

5.1 ABSTRACT

The experiment was conducted to determine the effects of dietary solvent-extracted canola meal (**SCM**) and expeller-extracted canola meal (**ECM**) inclusion in late-gestation and lactation diets with probiotic supplementation on sow and litter performance and microbiome. Thirty-six sows were randomly allotted 1 of 6 diets for a 3×2 factorial arrangement on d 80 of pregnancy. Diets consisted of a corn and soybean meal control (**CSB**) or the CSB diet with soybean meal replaced by either 300 g/kg SCM (**CSC**) or 300 g/kg ECM (**CEC**), provided with or without *Saccharomyces cerevisiae* product (Actisaf® Sc 47) supplementation. Sow body weight (**BW**) and backfat (**BF**) thickness were determined on d 80 and d 111 of pregnancy and on d 1 and d 21 post-farrowing. Piglets were weighed on d 1 and d 19 post-farrowing. Milk and blood samples from sows were collected on d 1 and d 19 post-farrowing to determine milk composition and plasma urea nitrogen. Fecal samples from sows were collected on d 110 of gestation and d 19 of lactation for apparent total digestibility (**ATTD**) determination using titanium dioxide as an external marker. Fecal samples from sows and piglets were collected for microbiome analysis on d 19 of lactation. Data were analyzed using the PROC MIXED procedure of SAS 9.4 for a completely randomized design. Sows fed ECM-containing diets had reduced ($P < 0.05$) BW on d 1 and d 21 post-farrowing compared to SBM-fed sows, whereas SCM-containing diets did not alter sow performance. Dietary treatments and *S. cerevisiae* product supplementation tended to interact on gestational feed intake ($P = 0.07$), but no differences were observed during lactation. Litter performance, including litter size, weight and survival rate, was unaffected. Probiotic supplementation increased ($P < 0.05$) milk fat content on d 19 post-farrowing. In gestating sows, CEC diets reduced ($P < 0.05$) CP digestibility, while *S. cerevisiae* product supplementation improved ($P < 0.05$) the ATTD of gross energy (**GE**), crude protein (**CP**) and Ca. In lactating sows, no differences in ATTD of nutrients

were observed, but probiotics improved ($P < 0.05$) CP digestibility in ECM-containing diets, mitigating its negative effects. Thus, 300 g/kg SCM can be included in sow diets as the main protein-source substitutes for SBM, but a 300 g/kg ECM inclusion may require supplementation with *S. cerevisiae* product to minimize adverse effects on sows.

Keywords: Canola meal, sows, *S. cerevisiae*, piglets, milk, digestibility

5.2 INTRODUCTION

Soybean meal is a widely used protein source in swine diets due to its high nutritional value and digestibility (Babatunde et al., 2021). However, the need to diversify protein sources in swine feeding programs has driven interest in alternative ingredients, such as canola meal, a co-product of oil extraction which offers a cost-effective and sustainable protein source for pigs (Khajali and Slominski, 2012; Mejicanos et al., 2016). Two types of canola meal are commonly produced: solvent-extracted canola meal (**SCM**) and expeller-extracted canola meal (**ECM**). Canola oil is commonly extracted using solvent extraction methods, resulting in a meal with less than 5% residual oil (Bell 1993). Expeller extraction methods can also be used to obtain canola oil but are less efficient and result in a meal with a higher residual oil (8.0 to 15%) (Landro et al., 2012). The higher oil content in ECM provides additional energy, but its increased fiber and glucosinolate levels may limit nutrient digestibility and animal performance (Landro et al., 2012; Toghyani et al., 2015). Studies in growing pigs have shown that ECM inclusion reduces average daily feed intake (**ADFI**) and body weight (**BW**) at 30% inclusion level (Velayudhan et al., 2017). Despite these challenges, ECM's fiber content may enhance microbial fermentation and gut health, as evidenced by Inglis et al. (2021), who reported altered microbiota composition and increased short-chain fatty acid production in broilers fed ECM. Fiber-rich diets like canola meal also have physiological effects on sows that extend beyond nutrient digestibility (Jha and Berrocso, 2015). They can increase gastrointestinal bulk, reduce stereotypic behaviors, and support reproductive performance by promoting gut health and microbial activity (Danielsen and Vestergaard, 2001; Tokach et al., 2019). Our previous study has shown that SCM inclusion, particularly with probiotics, enhances ADFI and nutrient digestibility in sows, demonstrating its potential as a viable alternative to soybean meal. However, the effects of ECM on sows, particularly during gestation

and lactation, remain underexplored, necessitating further research to optimize its inclusion in sow diets.

Probiotics, such as *Saccharomyces cerevisiae* (*S. cerevisiae*), are well-established for their ability to improve nutrient utilization in fibrous diets. Probiotic supplementation has been shown to enhance gut microbial activity, enzymatic breakdown of fiber, and nutrient absorption, improving digestibility and positively influencing milk composition (Shen et al., 2011; Ogbuewu et al., 2019). Moreover, *S. cerevisiae* has been associated with improved growth performance, milk production, and reproductive outcomes in pigs (Shen et al., 2017; Elghandour et al., 2020). Despite these promising benefits, the combined effects of canola meal and *S. cerevisiae* supplementation on sow performance, milk composition, and gut microbiota have not been studied. Therefore, the objective of this study was to determine the effects of a high dietary SCM and ECM with or without *S. cerevisiae* product on sow performance, nutrient utilization, and gut microbiota. By addressing these gaps, this research provides insights into the potential of canola meal and probiotics as sustainable strategies for improving sow productivity and supporting swine production systems.

5.3 MATERIALS AND METHODS

5.3.1 *Animals and experimental design*

The experimental protocols were reviewed and approved by the University of Manitoba Animal Care Committee (AC11687) and the sows and piglets were handled according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

The experiment was conducted at Glenlea Research Station, University of Manitoba (Winnipeg, Canada). A total of 36 gestating sows on d 80 of pregnancy with an average parity of 2.8 ± 0.88 and an average initial back fat thickness of 15.50 ± 1.10 mm were used and randomly assigned to one of the 6 treatments according to a 3×2 factorial arrangement.

The sow housing system and management procedures, including individual gestation and farrowing housing, environmental control, piglet care, and cross-fostering, were the same as those described in Section 4.3.1 of Chapter 4.

5.3.2 *Experimental diets*

Experimental diets (gestation and lactation, **Table 5.1**) were formulated based on standardized ileal digestible (**SID**) AA and net energy (**NE**) contents. The nutrients of all experimental diets met or exceeded NRC (2012) nutrient requirement recommendations for gestating sows whose average parity were over 2, the average post-farrowing BW were 210 kg, and the expected mean daily weight gain of piglets were 230 g. The SID AA values of SBM, SCM and ECM were obtained from other studies (González-Vega and Stein, 2012; Velayudhan et al., 2019).

Thirty-six sows were randomly allotted 1 of 6 diets for a 3×2 factorial arrangement on d 80 of pregnancy. Diets consisted of a corn and soybean meal control (**CSB**) or the CSB diet with soybean meal replaced by either 300 g/kg SCM (**CSC**) or 300 g/kg ECM (**CEC**), provided with

or without *Saccharomyces cerevisiae* product (Actisaf® Sc 47, *Saccharomyces cerevisiae*: CNCM I-4407, 10^{10} CFU/g, Phileo Lesaffre Animal Care, France).

The six experimental diets consisted of 1) **CSB**, corn-soybean meal-based diet; 2) **CSB-P**, CSB diet supplemented with *S. cerevisiae* product; 3) **CSC**, corn-300g/kg solvent-extracted canola meal-based diet; 4) **CSC-P**, CSC diet supplemented with *S. cerevisiae* product; 5) **CEC**, corn-300g/kg expeller-extracted canola meal-based diet; 6) **CEC-P**, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contain 500 mg/kg of *S. cerevisiae* product. Titanium dioxide was included at 3 g/kg feed as an indigestible marker.

The feeding strategy for sows during gestation and lactation, including the amount of feed provided, the timing of feed increases, and the transition between gestation and lactation diets, was consistent with the protocol described in Section 4.3.2. Additionally, average daily feed intake (**ADFI**) was recorded using the same procedures as outlined in Section 4.3.2.

5.3.3 Sampling and measurements

Sampling procedures and measurements for this experiment followed the same protocol as detailed in Section 4.3.3 of Chapter 4. Please refer to that section for a complete description.

5.3.4 Blood collection and analysis

Blood collection and analysis for this experiment followed the same protocol as detailed in Section 4.3.4 of Chapter 4. Please refer to that section for a complete description.

5.3.5 Colostrum and milk collection and analysis

Colostrum and milk collection and analysis for this experiment followed the same protocol as detailed in Section 4.3.5 of Chapter 4. Please refer to that section for a complete description.

5.3.6 Fecal collection and chemical analysis

Fecal collection and chemical analysis for this experiment followed the same protocol as detailed in Section 4.3.6 of Chapter 4. Please refer to that section for a complete description.

5.3.7 DNA extraction and microbiome analysis

On d 19 post-farrowing, fecal samples were collected from each sow and one randomly selected piglet per litter for microbiome analysis. Fecal samples were collected directly from the rectum and immediately stored at -80°C until further processing. For analysis, 200 mg of each sample was thawed on ice, and bacterial DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). Microbial cells were lysed by heating at 95°C, and the extraction process followed the manufacturer's instructions. DNA concentration and quality were assessed using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. The V4 hypervariable region of the 16S rRNA gene was amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Libraries were prepared using the NEBNext Ultra II FS DNA Library Prep Kit (NEB, USA) according to the manufacturer's instructions. Sequencing was performed on an Illumina NovaSeq platform at Genome Quebec (Montreal, Quebec, Canada) to generate 150 bp paired end reads. Raw reads were processed using Trimmomatic (v0.39) to remove

adapters, low-quality bases (Phred < 15), and short reads (< 30 bp). Sequence quality was assessed using FastQC (v0.12). Taxonomy classification was performed using Kraken2 (v2.1.3) with the NCBI 16S rRNA database, and relative abundances were refined using Bracken (v3.0). The dominant phyla (> 1% relative abundance) were visualized as stacked bar plots using ggplot2 (R package). Microbial community differences were assessed using differential heat trees (metacoder, R package), with log₂ fold changes (log₂ FC) encoded by color and node sizes scaled to taxonomic abundance. Alpha diversity (Shannon, Chao1, and Simpson indices) and beta diversity (Bray-Curtis dissimilarity, PCoA) were calculated using the vegan R package.

5.3.9 Statistical analysis

The apparent total tract digestibility (**ATTD**) of nutrients and energy was determined using the same equation as described in Section 4.3.7 of Chapter 4.

Data were analyzed using the MIXED procedure of SAS (v9.4, SAS Institute Inc., Cary, NC, USA) for a factorial design with a completely randomized design. The individual sow or litter was considered the experimental unit. The statistical model included diet, probiotic, and their interaction as fixed effects. For sow BW and backfat thickness analysis, initial BW and backfat thickness on d 80 of pregnancy were included as covariates and retained in the model only if their effects were significant ($P \leq 0.10$); otherwise, they were removed. Residual normality and homogeneity of variance were assessed using the Shapiro-Wilk test and Levene's test, respectively. Least square means were compared using the Tukey test and differences were considered significant when $P < 0.05$, and trends were noted when $0.05 < P < 0.10$.

For microbiome 16S rRNA analysis, alpha diversity indices (Shannon, Chao1, and Simpson) were analyzed using ANOVA or Kruskal-Wallis tests in R (v4.2.0). Beta diversity

differences were tested using PERMANOVA (Adonis test, vegan R package). Differential abundance analysis was conducted using DESeq2, with false discovery rate (FDR) correction ($q < 0.05$).

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

Item	Diet ¹					
	Gestation ²			Lactation ³		
	CSB	CSC	CEC	CSB	CSC	CEC
Ingredient, %						
Corn	72.02	62.50	65.30	70.78	61.88	64.19
Solvent-extracted canola meal	-	30.00	-	-	30.00	-
Expeller-extracted canola meal	-	-	30.00	-	-	30.00
Soybean meal	22.00	-	-	22.50	-	-
Vegetable oils	2.30	4.40	1.50	2.55	4.50	1.70
Limestone	1.30	1.25	1.25	1.16	1.15	0.99
Monocalcium phosphate	0.98	0.30	0.25	1.50	0.78	1.13
Salt	0.40	0.40	0.40	0.47	0.47	0.47
Vitamin-mineral Premix ⁴	1.00	1.00	1.00	1.00	1.00	1.00
L- Lysine	-	0.15	0.30	0.04	0.18	0.35
L-Tryptophan	-	-	-	-	0.01	0.04
L-Valine	-	-	-	-	0.03	0.06
Calculated composition						
Net energy, kcal/kg	2,604	2,603	2,604	2,568	2,568	2,568
Crude protein, %	17.00	17.03	16.73	16.60	16.60	16.30
Calcium, %	0.74	0.73	0.72	0.76	0.76	0.77
Total phosphorus, %	0.55	0.56	0.58	0.65	0.65	0.75
SID Lys	0.80	0.80	0.80	0.84	0.84	0.84
SID Met	0.28	0.28	0.28	0.26	0.26	0.26
SID Thr	0.56	0.56	0.56	0.55	0.55	0.55

SID Trp	0.17	0.17	0.17	0.17	0.17	0.17
STTD P, %	0.30	0.30	0.29	0.39	0.39	0.39
Analyzed composition						
Gross energy, kcal/kg	4,073	4,225	4,135	4,064	4,244	4,224
Crude protein, %	17.20	17.70	17.50	17.50	17.40	17.60
Neutral detergent fiber, %	8.07	12.98	13.77	6.42	13.85	13.67
Calcium, %	0.64	0.66	0.64	0.66	0.63	0.67
Total phosphorus, %	0.53	0.56	0.54	0.63	0.68	0.64
Total glucosinolates ($\mu\text{mol/g}$)	0.05	0.64	1.53	0.07	0.75	1.67

¹Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

²As-fed basis. All gestation diets were formulated to contain 2.60 Mcal/kg of NE with 0.80%, 0.28%, 0.56%, and 0.17% standardized ileal digestible Lys, Met, Thr, and Trp, respectively, and 0.73% and 0.30% Ca and standardized total tract digestible (STTD) P, respectively.

³All lactation diets were formulated to contain 2.56 Mcal/kg of NE with 0.84%, 0.26%, 0.55%, and 0.17% standardized ileal digestible Lys, Met, Thr, and Trp, respectively, and 0.76% and 0.39% Ca and STTD P, respectively.

⁴Supplied the following per kg of finished gestation diets: vitamin A, 4400 IU; vitamin D, 880 IU; vitamin E, 48 IU; vitamin K, 0.6 mg; choline, 1.37 g; pantothenic acid, 13 mg; riboflavin, 4.13 mg; folic acid, 1.43 mg; niacin, 11 mg; thiamin, 1.1 mg; vitamin B6, 1.1 mg; biotin, 0.21 mg; vitamin B12, 16 μg , Cu, 11 mg as copper sulfate; Zn, 110 mg as zinc oxide; Fe, 88 mg as ferrous sulfate; Mn, 27.5 mg as manganese sulfate; I, 0.15 mg as potassium iodate; Se, 0.16 mg as sodium selenite. Supplied the following per kg of finished lactation diets: vitamin A, 2200 IU; vitamin D, 880 IU; vitamin E, 48 IU; vitamin K, 0.6 mg; choline, 1.37 g; pantothenic acid, 13 mg; riboflavin, 4.13 mg; folic acid, 1.43 mg; niacin, 11 mg; thiamin, 1.1 mg; vitamin B6, 1.1 mg; biotin, 0.21 mg; vitamin B12, 16 μg , Cu, 22 mg as copper sulfate; Zn, 110 mg as zinc oxide; Fe, 88 mg as ferrous sulfate; Mn, 27.5 mg as manganese sulfate; I, 0.15 mg as potassium iodate; Se, 0.16 mg as sodium selenite.

⁵Amino acids are indicated as standardized ileal digestible AA. Values were calculated based on values for standardized ileal digestibility of AA in corn and crystalline AA published by NRC (2012), and standardized ileal digestibility values for AA in soybean meal and solvent- and expeller-extracted canola meal were from González-Vega & Stein, (2012) and Velayudhan et al. (2019).

5.4 RESULTS

5.4.1 Sow performance

On d 1 and d 21 post-farrowing, sows fed ECM-containing diets had a decreased BW compared to sows fed SCM-containing diets ($P < 0.05$; **Table 5.2**). There was no significant difference in BW between sows fed the SBM-containing diet and those fed SCM-containing diets ($P > 0.10$). Dietary treatment had no effect ($P > 0.10$) on sow backfat thickness or changes in BW and backfat thickness throughout the experimental period. No dietary or probiotic effects ($P > 0.10$) were observed on ADFI during gestation or lactation. In addition, probiotic supplementation had no effect ($P > 0.10$) on sow growth performance and wean to estrus interval, and no interaction effects were detected between dietary ingredients and probiotics.

5.4.2 Litter performance

As shown in **Table 5.3**, there were no dietary effects ($P > 0.10$) on number of total piglets born, piglets born live, born dead and on d 19 post-farrowing. There was no dietary effect or probiotic effect ($P > 0.10$) on body weight of litter, ADG and the survival rate from farrowing to weaning. No interaction effect ($P > 0.10$) between ingredients and probiotics was found on litter performance.

5.4.3 Milk composition and plasma urea N

The composition of sow colostrum and milk is shown in **Fig 5.1**. No differences ($P > 0.10$) were observed in colostrum fat, protein and lactose oligosaccharides content among treatments. There was no significant difference ($P > 0.10$) on milk crude protein and lactose oligosaccharides

contents in sows fed different diets on d 19 post-farrowing. Probiotic supplementation in sow diets (CSB, CSC and CEC) increased the levels of milk fat on d 19 post-farrowing ($P < 0.05$).

No differences ($P > 0.10$) were found in PUN levels of sows fed the CSB, CSC, CEC diets with or without probiotic supplementation on d 1 and d 19 post-farrowing as shown in **Fig 5.2**.

5.4.4 Apparent total digestibility of energy and nutrients

The coefficients of ATTD of energy and nutrients in late-gestating sows are presented in **Table 5.4**. Sows fed diets containing SCM and ECM decreased ($P < 0.05$) ATTD of DM, GE and CP than sows fed SBM-containing diets, however the *S. cerevisiae* product supplementation in late-gestating sow diets increased ($P < 0.05$) ATTD of DM, GE and CP across all diets. The ATTD of Ca was significantly affected by dietary ingredient type ($P < 0.05$) and probiotic supplementation ($P < 0.05$), with a significant interaction between these factors ($P < 0.05$). In diets without probiotics, sows fed the CSB diet had higher calcium ATTD ($P < 0.05$) than those fed the CSC and CEC diets. Similarly, in probiotic-supplemented diets, Ca ATTD was higher ($P < 0.05$) in sows fed the CSB-P diet compared to those fed the CSC-P diet but did not differ from those fed the CEC-P diet. The ATTD of P was not influenced ($P > 0.10$) by replacing SBM with SCM or ECM, but *S. cerevisiae* product supplementation increased ($P < 0.05$) ATTD of P in late-gestating sows. In addition, Sows fed diets containing SCM and ECM had a higher ($P < 0.05$) ATTD of NDF than sows fed SBM-containing diets, and *S. cerevisiae* product supplementation increased ($P < 0.05$) ATTD of NDF in late-gestating sows.

In **Table 5.5**, the coefficients of ATTD of energy and nutrients in lactating sows are presented. Sows fed ECM-containing diets had a lower ($P < 0.05$) ATTD of DM than those fed SBM-containing diets, and replacing SBM with SCM tended ($P = 0.09$) to decrease the ATTD of

DM in lactating sows. Similarly, the ATTD of GE was lower ($P < 0.05$) in sows fed ECM-containing diets compared to those fed SBM-containing diets, while replacing SBM with SCM showed a tendency ($P = 0.08$) to decrease the ATTD of GE. In addition, sows fed SCM- and ECM-containing diets had a lower ($P < 0.05$) ATTD of CP than those fed SBM-containing diets. There was no significant probiotic effect ($P > 0.10$) on the ATTD of energy and nutrients. However, probiotic supplementation in SCM- and ECM-containing diets mitigated the reduction in ATTD of DM, GE, and CP compared to sows fed SBM-containing diets.

5.4.5 Microbiome analysis

Alpha diversity (**Fig 5.3**), measured using the Chao1, Shannon, and Simpson indices, was used to evaluate microbial richness and diversity across different dietary treatments and probiotic supplementation. For species richness (Chao1 index), probiotic supplementation significantly increased ($P < 0.05$) microbial richness in sows fed CEC diets. However, no significant differences ($P > 0.10$) in species richness were observed among piglets across dietary treatments (CSB, CSC, or CEC). For microbial diversity (Shannon index), probiotic supplementation significantly increased ($P < 0.05$) Shannon diversity in piglets ($P = 0.01$), while no significant effect ($P > 0.10$) was observed in sows. Similarly, for community evenness (Simpson index), probiotic supplementation increased ($P < 0.05$) Simpson diversity in piglets, though this effect was not statistically significant ($P > 0.10$) in sows. However, no significant probiotic effects ($P > 0.10$) on Shannon or Simpson diversity were detected in sows. There were no significant differences ($P > 0.10$) in alpha diversity (Chao1, Shannon, or Simpson indices) among dietary treatments (CSB, CSC, CEC) in either sows or piglets.

Beta diversity assessed using PCoA based on Bray-Curtis dissimilarity, showed no significant ($P > 0.10$) clustering based on dietary treatment (CSB, CSC, or CEC) or probiotic supplementation at either the genus level or phylum level (**Fig 5.4**).

The community abundances of samples from at the phylum level are presented in **Fig 5.5**, with *Bacillota* and *Bacteroidota* being the two most abundant phyla in all treatment groups. Sows fed the CEC diet had the highest *Bacillota* abundance ($64 \pm 16.4\%$), while CSC-fed sows had the highest *Bacteroidota* abundance ($29.5 \pm 11.3\%$). However, probiotic supplementation did not significantly alter the relative abundance of *Bacillota* and *Bacteroidota* across dietary treatments. At the genus level, *Bacteroides* was most abundant in CSC-fed sows ($26.7 \pm 11.8\%$), while *Limosilactobacillus* was highest in CEC-fed sows with probiotic supplementation ($9.6 \pm 10.3\%$). *Lactobacillus* abundance was relatively higher in probiotic-fed sows under the CSC diet ($10.4 \pm 11.8\%$). In addition, probiotic supplementation increased the abundance of *Faecalibacterium* ($2-7\%$) compared with sows in non-probiotic groups.

As for piglets, at the phylum level, *Bacillota* and *Bacteroidota* were the dominant phyla across all dietary treatments. Piglets from sows fed CSB diets had the highest *Bacillota* abundance ($66.5 \pm 10.2\%$), whereas *Bacteroidota* was highest in piglets from CEC-fed sows ($46.1 \pm 29.4\%$). Piglets from CSC-fed sows exhibited a more balanced distribution of *Bacillota* ($44.7 \pm 19.7\%$) and *Bacteroidota* ($26.5 \pm 14.8\%$). At the genus level, *Bacteroides* was most abundant in piglets from CEC-fed sows ($30.2 \pm 24.8\%$), whereas *Escherichia* was highest in piglets from CSC-fed sows ($9.5 \pm 7.7\%$). In addition, the heat tree analysis (**Fig 5.6**) showed increased *Lactobacillus* and *Faecalibacterium* in probiotic-fed piglets. *Bacteroides* abundance decreased in probiotic-supplemented piglets fed CEC diets.

Table 5.2 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation in sow diets on sow performance

Item	Diet ¹						SEM	P-value		
	No-probiotic			Probiotic				Ingr ²	Pro ²	Ingr x Pro
	CSB	CSC	CEC	CSB-P	CSC-P	CEC-P				
Sow bodyweight, kg										
d 80 of gestation	272.2	255.8	248.0	258.5	258.5	259.2	14.41	0.686	0.996	0.661
d 111 of gestation	286.2	283.4	279.9	279.5	285.5	281.4	3.33	0.477	0.668	0.315
d 1 post-farrowing	271.8	273.4	262.7	266.5	277.8	266.7	4.58	0.047	0.746	0.489
d 21 post-farrowing	255.7	257.8	242.6	249.2	262.4	243.4	4.72	0.003	0.929	0.456
Gestation gain ³	26.8	25.3	22.5	21.2	27.2	23.0	3.42	0.544	0.678	0.477
Lactation loss ⁴	17.4	16.5	22.3	18.2	17.4	24.2	4.03	0.348	0.563	0.902
Sow backfat thickness, mm										
d 80 of gestation	15.20	15.17	15.17	15.50	16.17	15.80	1.100	0.961	0.493	0.953
d 111 of gestation	16.89	15.93	17.27	16.67	17.13	17.11	0.938	0.755	0.716	0.659
d 21 post-farrowing	14.00	14.50	13.50	13.83	15.00	13.40	1.058	0.440	0.926	0.937

Gestation gain	1.60	0.67	2.00	1.17	2.40	1.40	1.023	0.957	0.792	0.489
Lactation loss ⁵	2.80	1.33	3.67	2.83	1.60	3.80	1.040	0.133	0.872	0.994
Gestation ADFI, kg/d	2.98	2.93	2.85	2.93	2.94	2.96	0.038	0.456	0.463	0.109
Lactation ADFI, kg/d	6.88	6.05	6.26	6.13	6.16	6.25	0.401	0.566	0.489	0.479
Wean to estrus interval, d	5.00	4.67	4.50	4.00	4.00	4.75	0.321	0.779	0.151	0.249

¹Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

²Ingr, ingredient effect; Pro, probiotic (*S. cerevisiae* product) effect.

³Gestation bodyweight or backfat thickness gain was calculated as the difference between sow bodyweight at d 80 and d 111 of gestation. Sow initial BW at d 80 of pregnancy was used as a covariate in the statistical model for BW data analysis. Sow initial BF thickness BW at d 80 of pregnancy was used as a covariate in the statistical model for backfat thickness data analysis.

⁴Lactation bodyweight gain was calculated as the difference between sow body weight at d 1 and d 21 of post-farrowing.

⁵Lactation backfat thickness loss was calculated as the difference between d 111 of gestation and d 21 of post-farrowing.

Table 5.3 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation in sow diets on litter performance

Item	Diet ¹						SEM	P-value		
	No-probiotic			Probiotic				Ingr ²	Pro ²	Ingr x Pro
	CSB	CSC	CEC	CSB-P	CSC-P	CEC-P				
Litter size ³										
Born total	18.20	14.00	18.17	14.33	14.00	16.00	1.623	0.113	0.108	0.410
Born alive	14.00	14.75	14.75	12.83	12.80	13.40	1.311	0.839	0.111	0.933
Born dead	0.33	0.67	1.67	1.33	1.33	1.00	0.467	0.622	0.431	0.241
After cross-fostering ⁴	14.00	13.60	14.33	13.33	13.60	14.20	0.530	0.437	0.567	0.823
Weaning	11.80	12.40	12.50	11.83	11.80	13.40	0.652	0.246	0.846	0.565
Piglet survival to weaning, ⁵ %	83.94	91.66	86.69	88.76	86.90	94.82	3.210	0.425	0.336	0.174
Piglet body weight, kg										
d 1 post-farrowing	21.36	20.40	22.86	20.50	20.24	22.36	1.651	0.349	0.701	0.977
d 19 post-farrowing	71.41	77.26	76.13	77.26	76.28	86.94	4.671	0.114	0.375	0.379
Pig ADG, ⁶ kg/d	0.234	0.258	0.239	0.265	0.258	0.255	0.015	0.906	0.142	0.748

¹Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

²Ingr, ingredient effect; Pro, probiotic (*S. cerevisiae* product) effect.

³Litter size, the number of piglets per litter

⁴Cross-fostering, piglets were cross-fostered within the dietary treatment to equalize litter sizes within 24 h of birth. The sizes and bodyweight of per litter were recorded.

⁵Piglet survivability (%) = (the number of weaned piglets /the number of piglets born alive after cross fostering) × 100.

⁶Pig ADG = (average pig weight on d 19 – average live birth weight)/days of lactation.

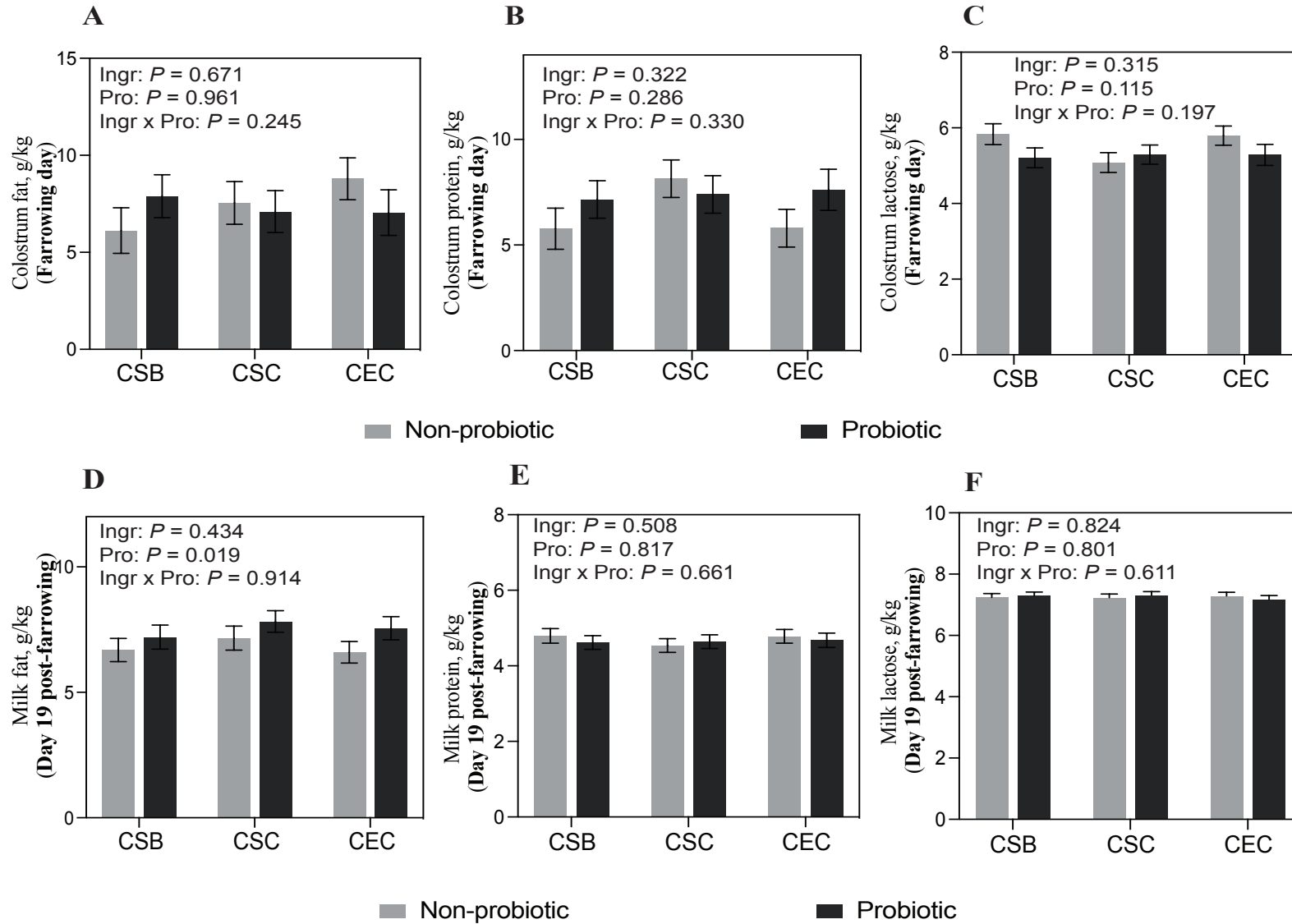


Fig 5.1 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on colostrum and milk composition on d 19 of lactation. Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

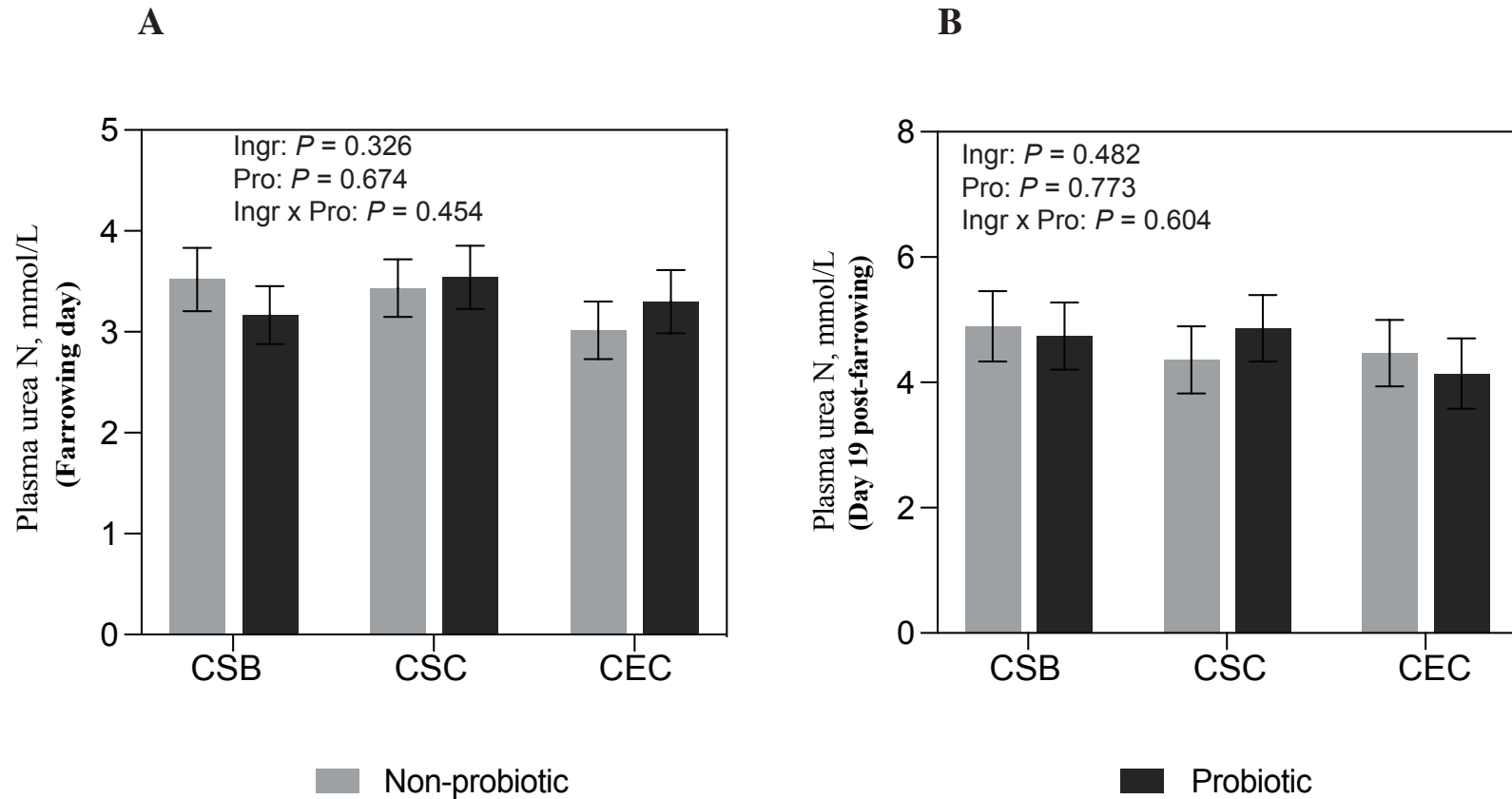


Fig 5.2 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on plasma urea N on d 1 and d 19 of lactation. (A) plasma urea N on farrowing day; (B) plasma urea N on d 19 post-farrowing. Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC

diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

Table 5.4 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on apparent total tract digestibility (ATTD) of nutrients and energy in in gestating sows.

Item, %	Diet ¹						SEM	P-value		
	No-probiotic			Probiotic				Ingr ²	Pro ²	Ingr x Pro
	CSB	CSC	CEC	CSB-P	CSC-P	CEC-P				
Dry matter	85.26	83.56	83.04	88.38	85.14	85.35	0.681	0.001	<0.001	0.550
Gross energy	85.59	82.46	83.82	88.50	86.05	86.18	0.645	<0.001	<0.001	0.584
Crude protein	85.12	83.04	83.46	88.02	85.07	85.14	0.807	0.004	0.001	0.708
Calcium	31.20 ^b	36.40 ^b	26.40 ^b	50.05 ^a	36.75 ^b	39.62 ^{ab}	2.863	0.048	<0.001	0.011
Phosphorus	35.03	30.60	31.93	43.78	36.99	41.51	2.803	0.166	0.001	0.842
NDF	49.07	57.36	57.80	53.91	61.14	62.13	2.619	0.001	0.023	0.983

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

¹Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

² Ingr, ingredient effect; Pro, probiotic (*S. cerevisiae* product) effect.

Table 5.5 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on apparent total tract digestibility (ATTD) of nutrients and energy in lactating sows

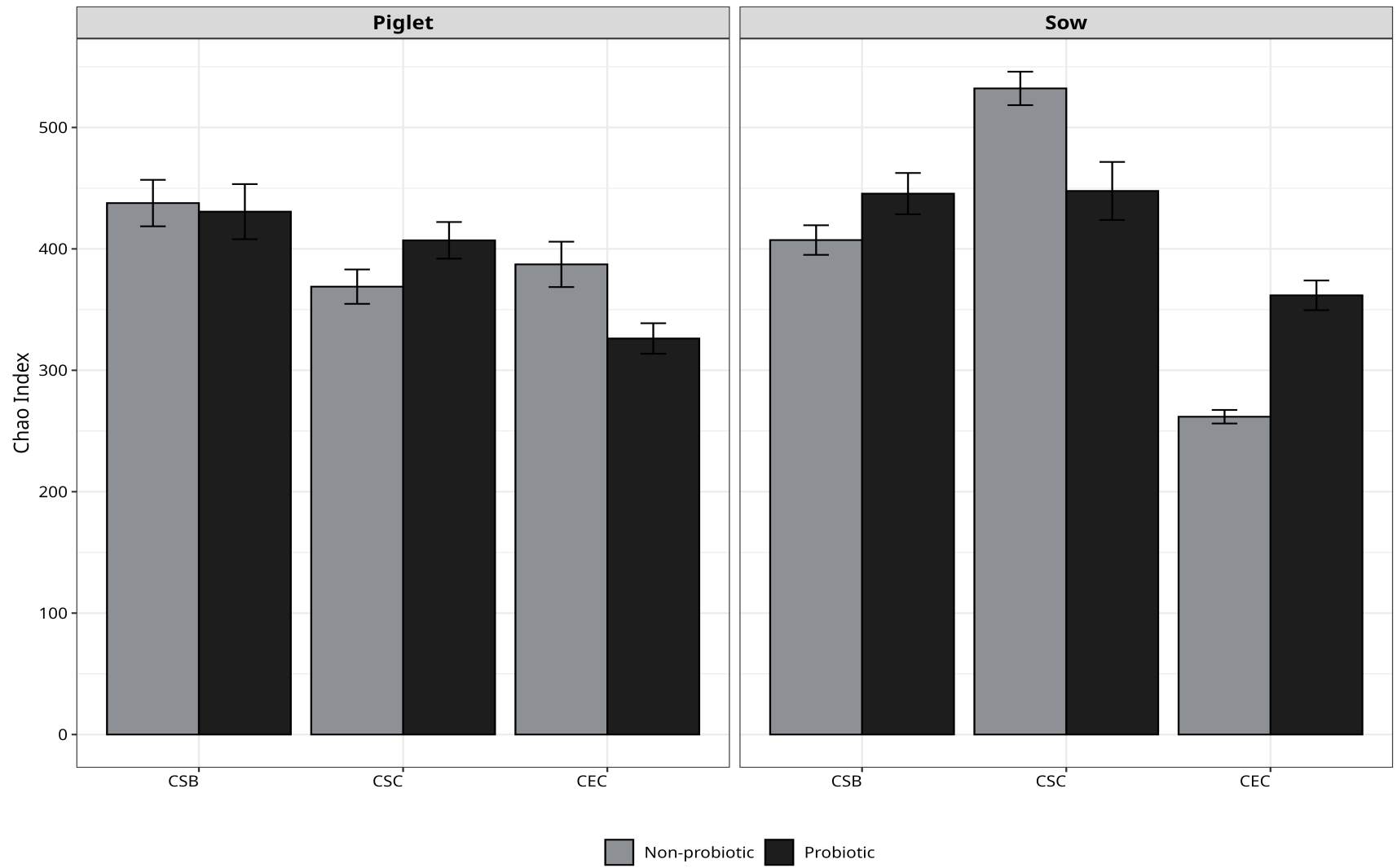
Item, %	Diet ¹						SEM	P-value		
	No-probiotic			Probiotic				Ingr ²	Pro ²	Ingr x Pro
	CSB	CSC	CEC	CSB-P	CSC-P	CEC-P				
Dry matter	83.88	81.09	78.95	84.12	81.07	81.73	1.446	0.031	0.377	0.535
Gross energy	84.63	81.55	79.61	84.59	81.80	82.54	1.276	0.029	0.337	0.476
Crude protein	86.67	81.57	79.93	85.47	82.89	83.24	1.370	0.004	0.288	0.244
Calcium	27.06	27.64	22.00	29.86	27.10	32.44	3.744	0.942	0.191	0.359
Phosphorus	34.64	30.77	27.43	33.48	33.53	38.96	3.617	0.876	0.162	0.247
NDF	45.64	53.05	52.36	50.66	53.31	52.88	1.362	0.569	0.647	0.874

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

¹Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

² Ingr, ingredient effect; Pro, probiotic (*S. cerevisiae* product) effect.

A



B

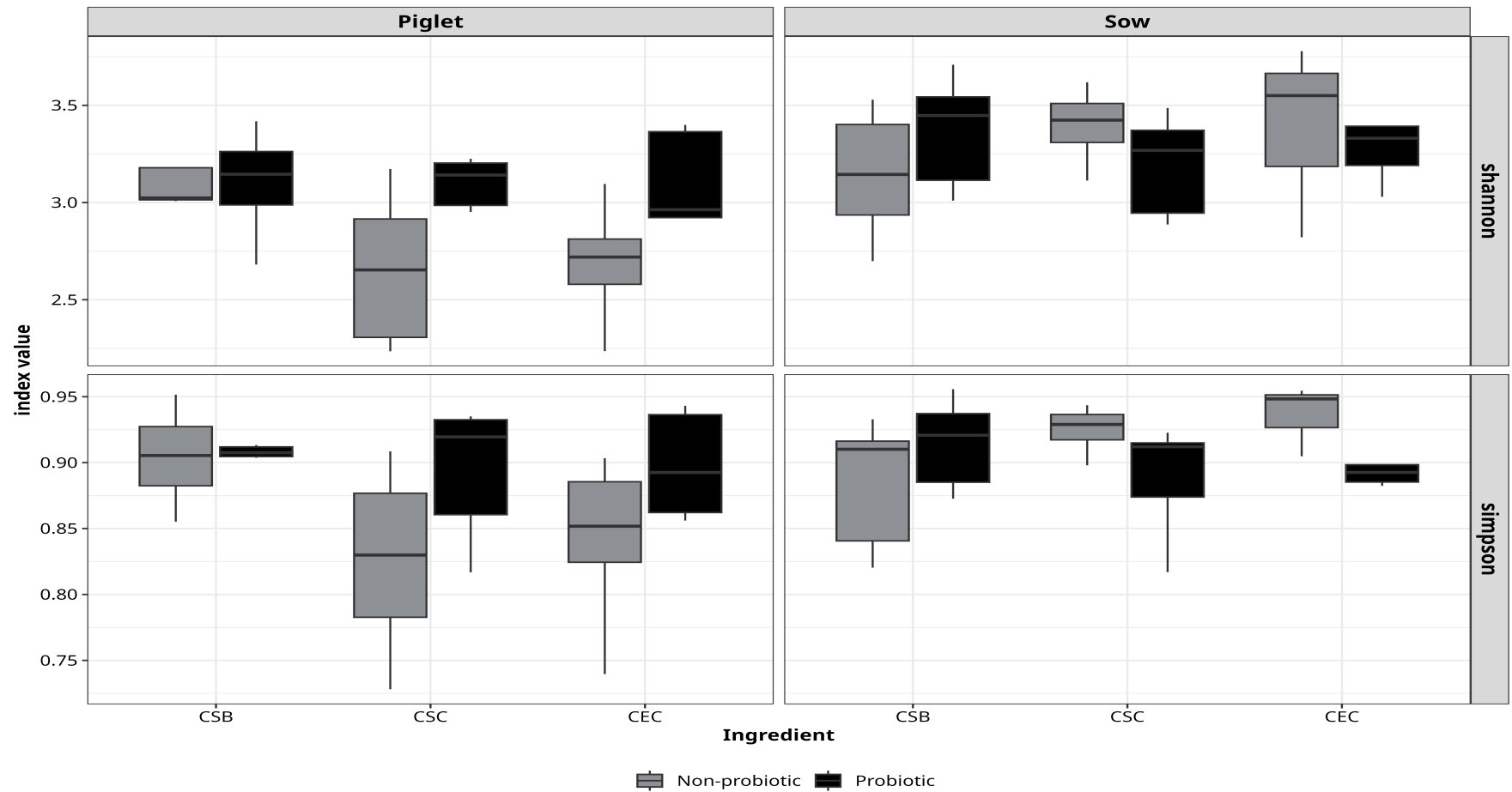
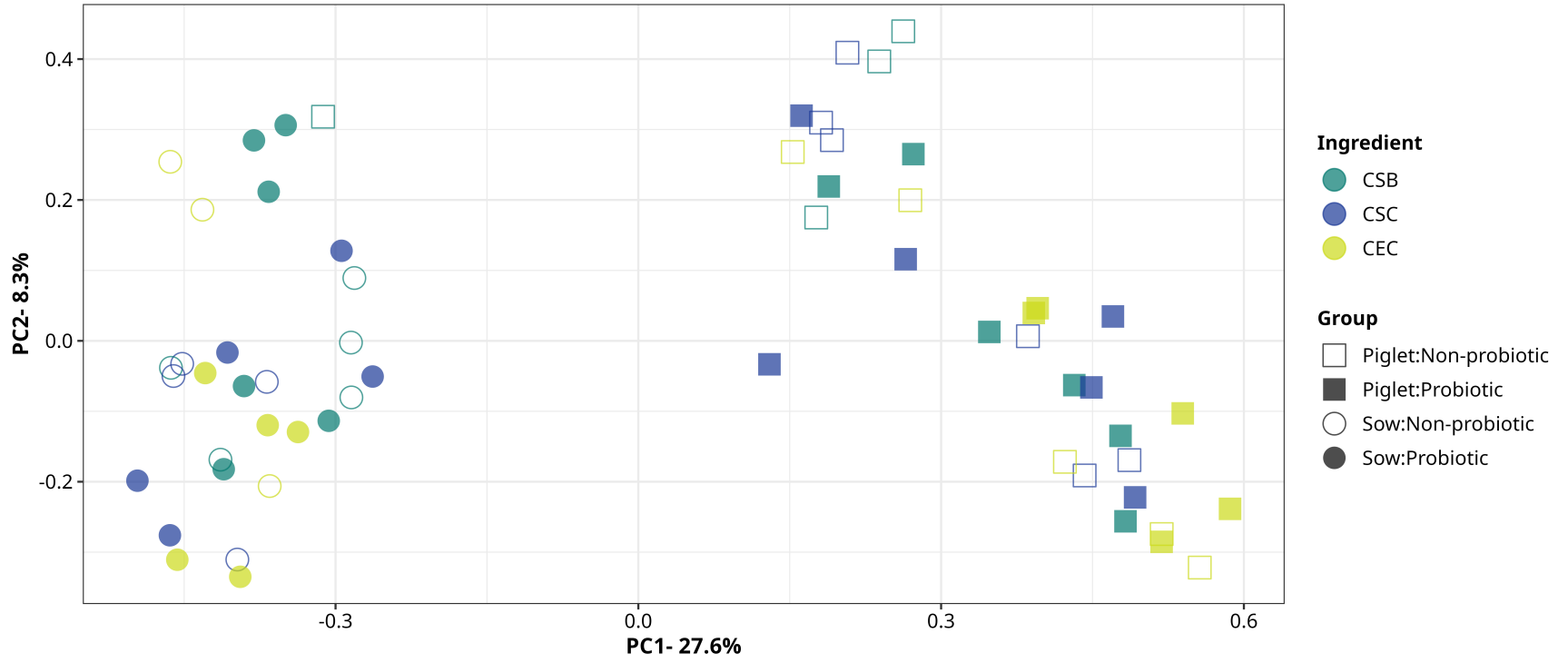


Fig 5.3 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on alpha diversity indices of microbiota in the feces of sows and piglets. Chao index (A), Shannon and

simposon (**B**). Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

A

PCoA of microbial abundances at the genus level



B

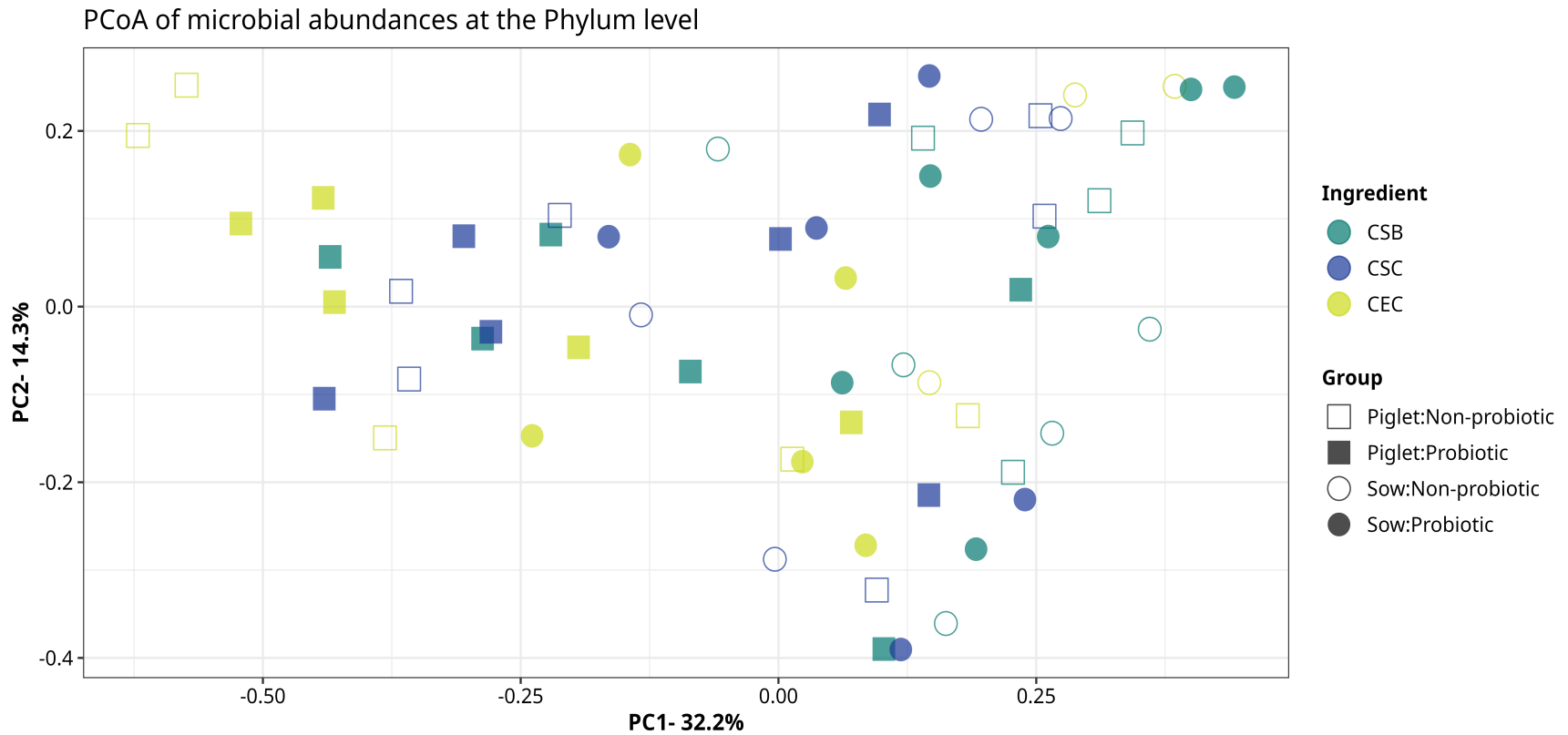
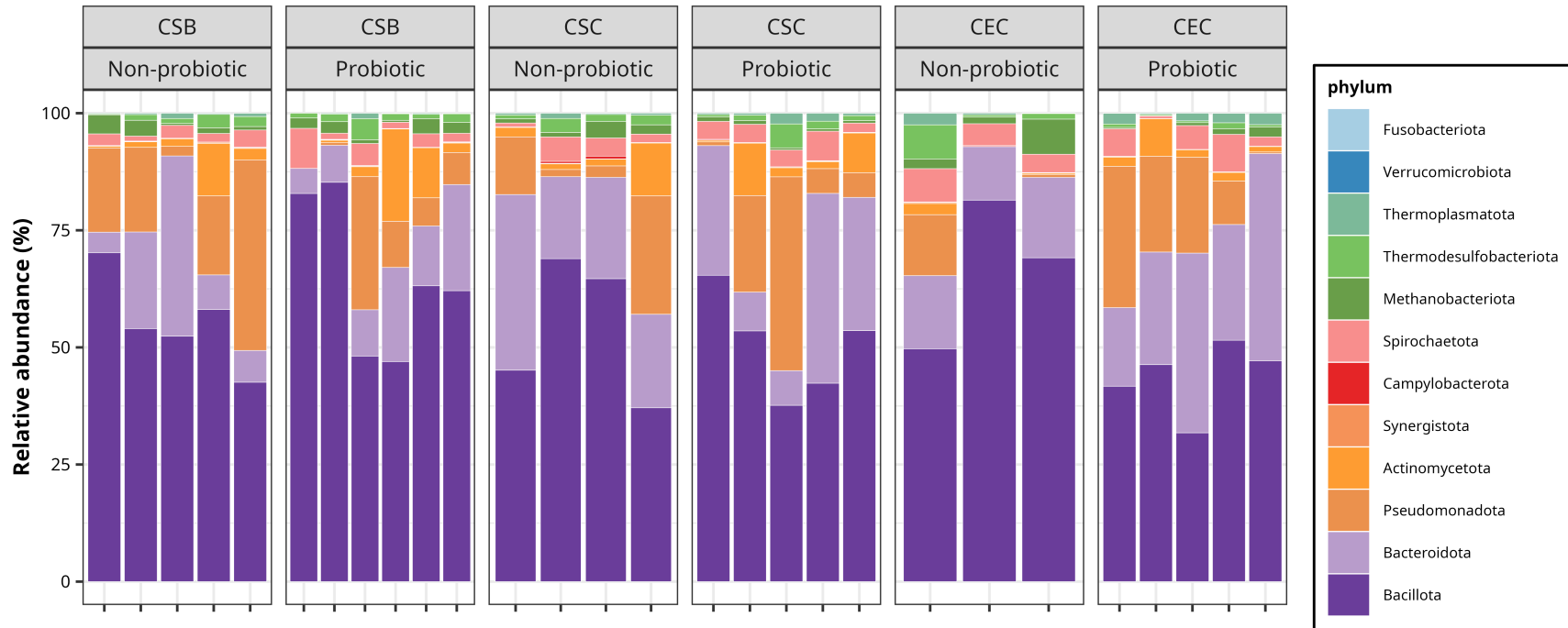


Fig 5.4 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on beta diversity indices of microbiota in the feces of sows and piglets. PCoA of microbial abundance at the genus level (A), PCoA of microbial abundance at the phylum level (B). Experimental diets consisted of 1) CSB, corn-soybean meal-

based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

A

Microbial Communities at the Phylum level in Sow



B

Microbial Communities at the Phylum level in Piglets

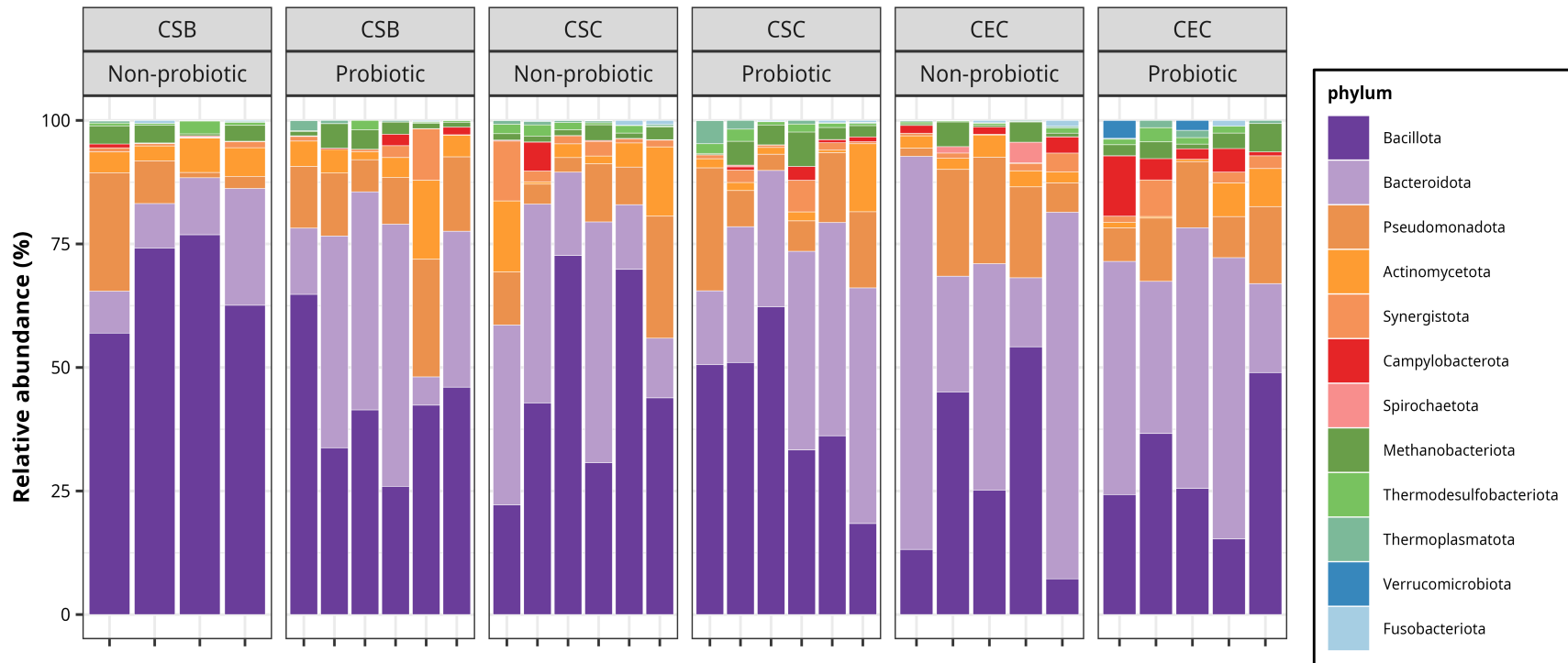


Fig 5.5 Effect of dietary solvent-extracted canola meal and expeller-extracted-canola meal inclusion with *Saccharomyces cerevisiae* product supplementation on microbial communities at phylum level in the feces of sows and piglets. In sows (A), in piglets (B). Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented

with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

Fig 5.6 Microbial composition analysis in sows and piglets in response to dietary solvent-extracted canola meal and expeller-extracted canola meal inclusion with *Saccharomyces cerevisiae* product supplementation. **(A)** Microbial network analysis in sows. **(B)** Microbial network analysis in piglets, showing taxonomic classification and log-fold change (\log_2 FC) of differentially abundant operational taxonomic units (OTUs) based on dietary treatments. Experimental diets consisted of 1) CSB, corn-soybean meal-based diet; 2) CSB-P, CSB diet supplemented with *Saccharomyces cerevisiae* product (Actisaf® Sc 47); 3) CSC, corn-300g/kg solvent-extracted canola meal-based diet; 4) CSC-P, CSC diet supplemented with *S. cerevisiae* product; 5) CEC, corn-300g/kg expeller-extracted canola meal-based diet; 6) CEC-P, CEC diet supplemented with *S. cerevisiae* product. In CSB-P, CSC-P and CEC-P diets, 250 mg/kg of *S. cerevisiae* product was included in gestation diets, while lactation diets contained 500 mg/kg of *S. cerevisiae* product.

5.5 DISCUSSION

The incorporation of canola meal, both SCM and ECM, with or without *S. cerevisiae* product, addresses significant gaps in understanding how alternative protein sources and probiotics interact to affect sow performance and nutrient utilization. Despite ECM's potential advantages, such as its higher residual oil content and cost-effectiveness, its application in sow diets remains underexplored (Seneviratne et al., 2010; Woyengo et al., 2010). This is largely due to concerns about the high fiber and glucosinolate content in canola meal, which can impair energy and nutrient digestibility (Velayudhan et al., 2017). The supplementation of *S. cerevisiae* product may be able to alleviate some of these challenges by enhancing gut microbial fermentation, enzymatic activity, and nutrient absorption (Jiang et al., 2015; Ogbuewu et al., 2019). These improvements not only boost digestibility but may also positively impact sow reproductive performance (Shen et al., 2011; Chen et al., 2020). Thus, the purpose of this study was to investigate these interactions by evaluating the effects of high dietary inclusion of canola meal with probiotics on reproductive performance, nutrient digestibility, milk composition, piglet growth and gut microbiota, contributing to the sustainability and efficiency of sow production systems. In this study, sows fed ECM-containing diets showed reduced BW on both d 1 and d 21 post-farrowing compared to those fed CSM-containing diets. Similarly, ADG of grower-finisher pigs fed the ECM-containing diet was reduced due to the increased dietary glucosinolates content (Seneviratne et al., 2010). It has been reported that weaned pigs had a low nutrient digestibility and energy retention due to the utilization of ECM, and that up to 200-240 g/kg ECM can replace soybean meal in nursery pig diets (Landerio et al., 2012; Le et al., 2014). The high fiber content in ECM can encapsulate nutrients, reducing their availability for enzymatic digestion and thereby limiting energy extraction (Mejicanos et al., 2016; Omotosho et al., 2024).

Furthermore, dietary treatment and *S. cerevisiae* product supplementation did not influence the ADFI of gestating and lactating sows. This finding aligns with our previous study, which demonstrated that *S. cerevisiae* product supplementation had no significant effect on ADFI in sows fed diets containing SCM. It has been shown that diet palatability can be reduced when glucosinolate levels exceed 3 $\mu\text{mol/g}$ in the diet (Bell, 1993). In the current study, the glucosinolate levels were less than 2 $\mu\text{mol/g}$ in both SCM- and ECM-containing diets, which could be the reason why the ingredient did not change the ADFI of sows. However, it has been reported a positive effect of *S. cerevisiae* product supplementation on ADFI. For instance, Li et al. (2006) observed in weaned pigs, supplementation with *S. cerevisiae* at a concentration of 2.6×10^7 CFU/g has been shown to increase ADFI, which may be attributed to its potential role in improving palatability. The variability in responses across studies may be influenced by differences in yeast concentration, strain specificity, and dietary composition. Additionally, the lack of differences in BF or BW changes suggests that sows were able to maintain body condition, likely through physiological adaptations to high-fiber diets, such as enhanced microbial fermentation in the hindgut and increased volatile fatty acid production, can compensate for energy deficits (Noblet et al., 2013). Additionally, high-fiber diets are known to improve satiety and reduce excessive fat accumulation, as documented in other studies (Ewan et al., 1996; Holt et al., 2006).

The ATTD data in this study provide insights into how dietary treatments influence nutrient utilization in sows. In late-gestating sows, both SCM- and ECM-containing diets led to reduced ATTD of DM, GE, Ca and CP compared to SBM-fed sows, likely due to the higher fiber content in canola meal, which creates physical barriers that limit enzymatic access to nutrients and reduces energy and protein digestibility (Bell, 1993; Khajali and Slominski, 2012). A notable finding is that late-gestating sows fed SCM and ECM diets exhibited higher ATTD of NDF than those fed

SBM-containing diets, which may be due to the adaptation of gut microbiota to fiber-rich diets, leading to improved fermentation and fiber utilization (Patience et al., 2020). Additionally, probiotic supplementation further increased NDF digestibility, reinforcing the role of *S. cerevisiae* product in enhancing fiber fermentation and microbial activity in the hindgut. Supplementation with *S. cerevisiae* product significantly improved the ATTD of DM, GE, and CP across all diets, suggesting that the probiotic enhances nutrient breakdown and absorption, potentially through improved gut microbiota balance and enzyme secretion (Labussière et al., 2022). Interestingly, the ATTD of P was not affected by dietary ingredient type but was significantly improved by *S. cerevisiae* product supplementation. Live yeast supplementation has been shown to enhance fiber degradation and energy utilization, which may also increase intestinal phosphorus availability and digestibility through microbial fermentation processes (Metzler and Mosenthin, 2008). Similar improvements in phosphorus digestibility with yeast culture have been reported in broilers (Gao et al., 2008) and growing pigs (Kim et al., 2014). The increased P digestibility could be due to microbial phytase activity from the probiotic, which enhances P availability by hydrolyzing phytate complexes in canola meal (Shen et al., 2022).

In lactating sows, sows fed ECM-containing diets had a lower ATTD of DM, GE and CP than sow fed SBM-containing diets. This discrepancy likely reflects the impact of ECM's high fiber content on nutrient utilization as increased fiber intake has been shown to elevate endogenous nitrogen losses, including mucins and digestive enzymes under high-fiber conditions (Souffrant, 2001; Barber et al., 2020). Furthermore, the slower digestion and altered digesta transit time caused by the fiber in ECM can reduce protein digestion and absorption in the small intestine (Freire et al., 2000; Low et al., 2020). These findings are consistent with studies by Landero et al. (2012), who observed that higher ECM inclusion in pig diets reduced CP digestibility despite containing

similar SID AA profiles, likely due to nitrogen losses in the hindgut caused by microbial fermentation, which cannot be utilized by the host. Additionally, the reduced digestibility of CP and GE in ECM-fed sows may help explain the observed decrease in BW during gestation, as lower energy and nutrient absorption can result in energy deficits, requiring sows to mobilize body reserves to meet their metabolic needs. Interestingly, supplementation with *S. cerevisiae* product significantly improved the ATTD of energy and nutrients in sows fed canola meal diets, indicating that the probiotic enhanced microbial activity and nutrient fermentation in the gastrointestinal tract, which facilitated fiber degradation and nutrient release (Shen et al., 2011). Probiotic supplementation likely offset some of the adverse effects of high fiber by increasing microbial enzyme production and fostering a more favorable gut environment for beneficial bacteria (Shen et al., 2011).

It has been shown that dietary glucosinolate levels ranging from 0.45 to 0.78 $\mu\text{mol/g}$ during growth and lactation, and from 0.16 to 0.32 $\mu\text{mol/g}$ during pregnancy, did not produce any deleterious effects on sows and piglets (Tripathi and Mishra, 2007). However, a dietary glucosinolate level of 1 $\mu\text{mol/g}$ has been associated with thyroid hypertrophy. In the current study, SCM-containing diets had glucosinolate levels of less than 0.75 $\mu\text{mol/g}$, which are below the tolerance thresholds. This indicates that the observed performance and digestibility outcomes for sows fed with SCM-containing diets are unlikely to have been directly influenced by glucosinolate toxicity. However, ECM-containing diets had glucosinolate levels exceeding 1.5 $\mu\text{mol/g}$, which may explain the lower ATTD of CP observed in sows fed ECM without probiotics in the current study. Glucosinolates can negatively influence nutrient absorption by disrupting thyroid function, leading to altered metabolism and impaired protein utilization. Additionally, the breakdown products of glucosinolates can increase gut irritation and mucosal damage, further reducing the

efficiency of nutrient absorption (McCurdy and March, 1992). The interaction between ECM and *S. cerevisiae* product on CP digestibility indicated that *S. cerevisiae* product supplementation plays a key role in mitigating the negative effects of ECM on nutrient utilization. The inclusion of *S. cerevisiae* product likely enhanced microbial proteolytic activity and fermentation in the gut, improving CP digestibility (Chaves-López et al., 2011). This aligns with findings by Ogbuewu et al. (2019), who demonstrated that probiotics enhance protein digestibility by supporting microbial activity and creating a more favorable gut environment, particularly in diets with high fiber content. The ATTD of GE and Ca was also improved in sows fed diets with yeast culture supplementation (Ma et al., 2023). Besides, the absence of differences in plasma urea N levels across treatments suggests stable protein metabolism, even in sows fed ECM-containing diets with reduced CP digestibility. The lack of significant differences in the digestibility of GE, Ca, P, and NDF among dietary treatments in the present study may reflect the natural adaptation of sows to fibrous diets during lactation. As reported by Noblet et al. (2013), sows exhibit greater energy efficiency and fermentation capacity over time when fed high-fiber diets, which can offset initial reductions in nutrient digestibility seen during gestation. Landero et al. (2012) similarly observed that pigs fed ECM over an extended period adapted to its fiber content, resulting in improved digestibility despite initial challenges. These findings suggest that while sows can adapt to high-fiber diets over time, *S. cerevisiae* product can accelerate this process and enhance the utilization of protein in fibrous diets, which is important during lactation, when nutrient requirements are high, and maintaining efficient nutrient utilization is critical for milk production and litter performance.

The consistent litter performance observed in this study, despite differences in the nutrient digestibility in gestating sows, suggests that factors beyond late gestation dietary treatments had a stronger influence on reproductive and litter outcomes. Litter size, including the number of piglets

born alive or dead, is determined earlier in gestation, largely influenced by factors such as ovulation rate, fertilization success, and embryonic development (Macdonald et al., 1963; Pope and First, 1985; Johnson et al., 1999). By d 80 of gestation, these processes are largely complete, which likely explains why dietary treatments introduced from d 80 of pregnancy had no effect on litter size. Moreover, piglet average daily gain and survival rates are primarily driven by the quantity and quality of milk provided by the sow (Alexopoulos et al., 2018). In this study, the consistent CP and lactose content in milk across treatments ensured an adequate nutrient supply to meet the requirements of the litter and support piglet growth. The significant improvement in milk fat content in sows fed *S. cerevisiae* product-supplemented diets indicates that *S. cerevisiae* product likely enhanced energy transfer to the milk, potentially by increasing gut microbial activity and volatile fatty acids production, such as acetate and butyrate are precursors for milk fat synthesis, which is consistent with other studies (Shen et al., 2017; Song et al., 2017; Ma et al., 2023). Despite the improvement in milk fat content, no differences in piglet ADG were observed, which could be attributed not only to milk composition but also to milk yield, which was not measured in this study, as piglet ADG is influenced by both milk composition and the overall volume of milk produced (King et al., 1997; Miao et al., 2019). Overall, the ability of sows to maintain litter growth and survival despite reduced energy and protein digestibility may reflect adaptive mechanisms such as enhanced nutrient redistribution and metabolic prioritization for reproduction and lactation.

Microbiome analysis revealed no significant effects of dietary treatments (CSB, CSC, or CEC) on alpha diversity (Chao1, Shannon, and Simpson indices) in sows, suggesting that replacing SBM with SCM or ECM did not alter overall microbial richness and community evenness. However, probiotic supplementation influenced microbial composition, with a significant increase in Shannon and Simpson diversity in piglets, indicating a more diverse and potentially resilient

microbial ecosystem. One possible explanation for the lack of significant changes in sow microbial diversity is that the gut microbiota tends to be more resilient and stable due to long-term microbial colonization, dietary adaptation, and a well-established microbial ecosystem in adult animals (Mai et al., 2004; Tannock et al., 2004). Evidence suggests that probiotics can be transmitted from sows to piglets through direct contact with maternal feces, influencing early microbial establishment (Jadamus et al., 2001). Higher microbial diversity in piglets is generally considered beneficial, as it can contribute to improved gut health, metabolic capacity, and immune system development (Baker et al., 2013; Lan and Kim, 2020).

A notable finding was that sows fed ECM diets exhibited the highest *Bacillota* abundance, whereas those fed SCM diets had the highest *Bacteroidota* abundance. This difference in microbial composition may explain the observed discrepancies in digestibility between SCM and ECM diets. *Bacteroidota* is commonly associated with fiber fermentation, as members of this phylum produce enzymes that degrade complex plant polysaccharides (Comstock and Coyne, 2003). Higher *Bacteroidota* abundance in SCM-fed sows may have contributed to improved NDF digestibility, as members of this phylum are known for their fiber-degrading capabilities (Xu, et al., 2003). In contrast, ECM-fed sows had a higher proportion of Firmicutes, which may have reduced fiber breakdown efficiency due to differences in microbial enzymatic activity compared to SCM-fed sows (Simpson and Campbell, 2015). This microbial difference may also explain why SCM-containing diets supported similar energy and nutrient digestibility in lactation period, while ECM-fed sows had lower ATTD of GE, DM, and CP than those fed SBM-containing diets. In addition, probiotic supplementation did not significantly alter the relative abundance of Firmicutes or *Bacteroidota* in sows but selectively increased *Limosilactobacillus* in CEC-fed sows. The increase

in *Limosilactobacillus*, a beneficial lactic acid bacterium, suggests a potential role in modulating gut pH and enhancing nutrient absorption (Mack et al., 1999; Jiang et al., 2022).

In the current study, despite these microbial shifts, beta diversity analysis showed no significant clustering based on dietary treatments or probiotic supplementation, suggesting that while individual bacterial taxa were affected, the overall gut microbial community structure remained relatively stable. This stability may explain why probiotic supplementation did not fully mitigate the reductions in nutrient digestibility observed in SCM- and ECM-fed sows, as the microbial shifts may not have been sufficient to compensate for the lower digestibility of canola meal components.

5.6 CONCLUSION

This study demonstrated that high dietary inclusion of SCM and ECM, with or without *S. cerevisiae* product, influenced nutrient digestibility and BW in sows without negatively affecting milk composition or litter performance. Both SCM and ECM diets reduced the ATTD of GE, while ECM diets also decreased CP digestibility and led to lower BW in gestating sows. Despite these effects, consistent milk quality and sufficient nutrient provision supported piglet growth and survival across treatments. Probiotic supplementation improved CP digestibility and milk fat content in both SCM and ECM diets, mitigating some limitations associated with high-fiber diets. These findings support the use of SCM and ECM as cost-effective protein sources in sow diets, particularly when supplemented with *S. cerevisiae* product, for sustainable swine production. However, further research is needed to evaluate the effects of different inclusion levels of ECM on sow metabolism and to determine the tolerance of pigs to varying glucosinolate concentrations across trials. Additionally, future studies should explore the long-term effects of these dietary strategies on sow reproductive performance and milk yield to optimize the use of canola meal in sow feeding systems.

TRANSITION STATEMENT

Results from manuscript I and II indicated that while the inclusion of 300 g/kg solvent-extracted canola meal did not impair reproductive performance but negatively affected nutrient digestibility, particularly during the gestation period. Therefore, manuscript III was designed to investigate whether prolonged feeding of solvent-extracted canola meal over two consecutive reproductive cycles would intensify these negative effects and further impact sow productivity and nutrient utilization.

CHAPTER 6

MANUSCRIPT III

HIGH CANOLA MEAL INCLUSION IN GESTATION AND LACTATION DIETS ON SOW REPRODUCTIVE AND PIGLET GROWTH PERFORMANCE, MILK COMPOSITION, SERUM METABOLITES PROFILE AND NUTRIENT DIGESTIBILITY OVER TWO REPRODUCTIVE CYCLES

Xiaoxiao Zhang*, Anna Rogiewicz*, Elijah Kiarie*[†], Chengbo Yang*, and Charles Martin Nyachoti*¹

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

[†]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Xiaoxiao Zhang: Conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing- original draft

Anna Rogiewicz: Resources, writing - review & edition

Elijah G. Kiarie: Writing - review & edition

Chengbo Yang: Writing - review & edition

Charles Martin Nyachoti: Project administration, resources, supervision, writing - review & edition

6.1 ABSTRACT

The experiment was conducted to determine the long-term effects of canola meal inclusion as the main protein source in sow diets on sow and litter performance. Sixty-eight sows from two batches were randomly allotted one of two diets on d 80 of pregnancy. The control diet (**CON**) consisted of corn, barley and soybean meal, and a treatment diet of corn, barley and canola meal (**CSCM**). The body weight and backfat thickness of sows were measured on d 1, d 35, d 80, and d 111 of pregnancy, and on d 1 and d 21 post-farrowing. Piglets were weighed on d 1 and d 19 post-farrowing. Milk and blood samples from sows were collected on d 1 and d 19 post-farrowing to determine milk composition and serum metabolites. Faecal samples from sows were collected on d 110 of gestation and d 19 of lactation to determine apparent total digestibility (**ATTD**) determination using titanium dioxide as an indigestible marker. Data were analysed using the PROC MIXED procedure of SAS 9.4 for a randomized complete block design with repeated measures, where individual sow was considered the experimental unit and measured across two consecutive reproductive cycles. Over the two reproductive cycles, results showed that sow BW and backfat thickness were unaffected by diet but varied between cycles, with greater BW and backfat thickness observed in the second cycle ($P < 0.05$). Sows fed the CSCM diet exhibited lower ($P < 0.05$) BW gain during late gestation in the first cycle but not in the second cycle. Litter performance, including weight and size, was unaffected by dietary treatment, though litter weight at birth was higher ($P < 0.05$) in the second cycle. The composition of colostrum and milk composition was generally unaffected by diet or cycle, except for colostrum fat, which showed a significant interaction ($P < 0.05$) between diet and cycle. Serum metabolites revealed tendencies ($P < 0.10$) for increased creatinine and gamma-glutamyl transferase (**GGT**) with the CSCM diet, while reproductive cycle effects were observed for GGT, cholesterol, calcium, protein, and

albumin levels. Apparent total tract digestibility of nutrients was not significantly influenced by diet or reproductive cycle, though the CSCM diet tended to reduce ($P = 0.06$) the ATTD of crude protein. In conclusion, feeding gestation and lactation diets containing canola meal to sows over two reproductive cycles resulted in similar sow reproductive and litter performance compared to the control.

Keywords: Alternative protein source, feed efficiency, metabolic adaptation, lactation performance, sow nutrition

6.2 INTRODUCTION

Sow nutrition is a critical determinant of productivity, affecting reproductive efficiency, litter performance, and overall herd health (Campos et al., 2012; Kim et al., 2013). During gestation and lactation, sows have heightened nutritional requirements to support fetal growth, colostrum and milk synthesis, and postpartum recovery (Aherne and Kirkwood, 1985; Close and Cole, 1986; Pettigrew and Yang, 1997). Protein content in sow diets is an essential dietary component, as it directly influences reproductive outcomes and the growth of piglets (Easter and Kim, 1998). Soybean meal has traditionally been the primary protein source in swine diets due to its high protein concentration and excellent digestibility. However, the increasing feed costs and rising global demand for soybean meal have increasingly strained profitability in the pork industry (Boumans et al., 2022). These challenges have intensified the search for alternative protein sources to reduce dependence on soybean meal. The second most common protein source in animal diets is canola meal, which is a byproduct of extracting canola seed oil (Bell, 1993). This is especially true in Canada, where canola production has greatly increased. Compared to soybean meal, canola meal has lower lower crude protein (CP), gross energy, and lysine levels but it contains higher concentrations of methionine and cysteine (Bell, 1993; Adewole et al., 2016).

Although these benefits, the high fibre content and anti-nutritional components of canola meal, like phytic acid and glucosinolates, have prevented it from being widely used in swine diets as a full substitute for soybean meal (Khajali and Slominski, 2012; Radfar et al., 2017). However, sows can better ferment dietary fiber more effectively than younger pigs due to their larger digestive tracts and advanced microbial populations (Jørgensen et al., 2007; Serena et al., 2008). Furthermore, high-fibre diets have demonstrated additional benefits for sows. High-fibre diets during gestation may provide bulk to the diet without adding excess energy, which could help

control weight gain while promoting satiety (Ewan et al., 1996; Holt et al., 2006). Additionally, fibre during the transition period may alleviate constipation, reducing farrowing duration and stillbirth rates, and can positively influence reproductive hormones, ultimately improving litter size and piglet weight (Holt, et al., 2006). Research on canola meal utilization in sow diets has yielded mixed results. For example, diets with 300 g/kg canola meal inclusion from d 60 of gestation until weaning supported satisfactory sow and litter performance (Velayudhan et al., 2018), whereas inclusion from d 7 of gestation increased the wean-to-estrus interval without affecting litter outcomes (Liu et al., 2018). In contrast, our previous study demonstrated that 300 g/kg canola meal inclusion from d 80 of gestation negatively affected nutrient digestibility in gestating sows.

Additionally, few studies have examined the long-term effects of canola meal inclusion on sow and litter performance, despite evidence suggesting that extended adaptation periods are needed for sows to fully benefit from high-fiber diets. Previous findings also indicate that high-fiber diets may enhance reproductive performance, with improvements in litter size and weight occurring after two or three reproductive cycles, though initial reductions in performance have been noted during the first reproductive cycle (Li et al., 2021). Thus, this study hypothesizes that an extended adaptation period to dietary canola meal supplementation can achieve comparable or improved reproductive performance without negatively affecting nutrient digestibility or serum metabolites compared to soybean meal-based diets. The objective was to evaluate the effects of canola meal on sow and piglet performance across two consecutive reproductive cycles, offering insights into its viability as a sustainable and economically viable alternative to soybean meal in swine production.

6.3 MATERIALS AND METHODS

6.3.1 Animals and experimental design

The experimental protocols were reviewed and approved by the University of Manitoba Animal Care Committee (AC11687) and the sows and piglets were handled according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

The experiment was carried out at Glenlea Research Station, University of Manitoba (Winnipeg, MB, Canada). A total of 68 gestating sows at d 80 of pregnancy with an average parity of 1.11 ± 0.05 and an average initial back fat thickness of 16.1 ± 0.35 mm were used and randomly assigned to one of the 2 dietary treatments. Sows were fed their designated dietary treatments starting from d 80 of pregnancy and continued through farrowing, lactation, weaning, and rebreeding, until the completion of a second reproductive cycle ending at the second weaning. Throughout the whole experimental period, sows were kept on their assigned dietary treatments unless they were removed because of structural or reproductive issues or unsatisfactory litter production. Sows were housed in groups during gestation until d 111 of pregnancy and had continuous access to their electronic sow feeder. Sows were washed and then transported to the farrowing room on d 111 of pregnancy. The sow housing system and management procedures, including individual gestation and farrowing housing, environmental control, piglet care, and cross-fostering, were the same as those described in Section 4.3.1 of Chapter 4.

6.3.2 Experimental diets

The present study was an experiment study with a randomized complete block design with repeated measures. Sixty-eight sows from two batches were randomly allotted one of two diets on d 80 of pregnancy. The study involved two diets: a control diet (**CON**) consisting of corn, barley

and soybean meal, and a treatment diet (**CSCM**) consisting of corn, barley and canola meal as shown in **Table 6.1**. Experimental diets for gestating and lactating sows were formulated based on standardized ileal digestible (**SID**) amino acid (**AA**) contents and net energy (**NE**) values, with **SID AA** values for soybean meal and canola meal obtained from other studies (González-Vega and Stein, 2012; Velayudhan, et al., 2019). All experimental diets were formulated to meet or exceed NRC (2012) nutrient requirements, based on an expected post-farrowing body weight of 210 kg for gilts and an anticipated mean daily weight gain of 270 g for piglets.

The feeding strategy for sows during gestation and lactation, including the amount of feed provided, the timing of feed increases, and the transition between gestation and lactation diets, was consistent with the protocol described in Section 4.3.2. Additionally, average daily feed intake (**ADFI**) was recorded using the same procedures as outlined in Section 4.3.2.

6.3.3 Sampling and measurements

Sow bodyweight (**BW**) and backfat thickness measurements were consistent with the protocol described in Section 4.3.3 of Chapter 4. Sow **BW** and backfat thickness were measured on d 35, d 80 and d 111 of gestation, farrowing day and weaning day. Litter size and litter weight measurements for this experiment followed the same protocol as detailed in Section 4.3.3 of Chapter 4. Please refer to that section for a complete description.

6.3.4 Blood collection and serum metabolites profile analysis

Blood samples (5 mL) were collected from all sows via jugular venipuncture into 10 mL serum separator tubes (SST; BD Vacutainer, Franklin Lakes, NJ, USA) prior to the morning meal on d 1 and d 19 post-farrowing. Samples were allowed to clot at room temperature, then centrifuged at

3,000 × g for 15 min at 4°C. The serum was pipetted into plastic screw-cap vials and stored at –80°C for further analysis. Serum samples were submitted to the Veterinary Diagnostic Services of Manitoba Agriculture (Winnipeg, MB, Canada) for biochemical analysis using a VITROS 250 Chemistry System (Ortho-Clinical Diagnostics Inc., Raritan, Rochester, NY). The blood parameters analysed included renal function markers, which consisted of urea and creatinine, and hepatic function markers included gamma-glutamyl transferase (**GGT**). Muscle function markers were represented by creatine kinase. The serum protein profile included total protein, albumin, globulin, and the albumin-to-globulin (**A/G**) ratio. The mineral profile included calcium (**Ca**) and phosphorus (**P**). Biochemical analyses were performed using an automated clinical chemistry analyser (e.g., Roche Cobas C501, Roche Diagnostics, Indianapolis, IN) following standardized laboratory protocols and internal quality control procedures.

6.3.5 Colostrum and milk collection and analysis

Colostrum and milk collection and analysis for this experiment followed the same protocol as detailed in Section 4.3.5 of Chapter 4. Please refer to that section for a complete description.

6.3.6 Fecal collection and chemical analysis

Fecal samples and chemical analysis for this experiment followed the same protocol as detailed in Section 4.3.6 of Chapter 4. Please refer to that section for a complete description.

6.3.7 Calculation and statistical analysis

The apparent total tract digestibility (**ATTD**) of nutrients and energy was determined using the same equation as described in Section 4.3.7 of Chapter 4.

All data were analysed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC USA). The model included diet, reproductive cycle, and their interaction as fixed effects. Farrowing group was included as a random effect (block) to account for variability introduced by seasonal or management factors. Additionally, the nesting structure of the experiment was modelled by including farrowing group nested within diet and cycle (diet*cycle*group) as a random effect, allowing for proper consideration of dependencies arising from the grouping of animals within specific farrowing batches. Individual sows were treated as a random effect nested within group and diet (ID (group*diet)) to account for sow-level variability within each group-diet combination.

Initial BW on d 80 of gestation was used as a covariate for BW data analysis, while initial backfat thickness served as a covariate for backfat thickness analysis. Covariates were included in the model only if their effects were significant ($P < 0.10$); otherwise, they were removed. Residuals were evaluated for normality and homogeneity of variance using diagnostic plots generated from the model. The normality and homogeneity of residuals were assessed using the Shapiro-Wilk test and Levene's test, respectively. Outliers were removed if standardized residuals exceeded ± 3 standard deviations. Least square means for diet, cycle, and their interaction were compared using Tukey's adjustment for multiple comparisons. Results are presented as least square means with standard errors, with statistical significance set at $P < 0.05$ and tendencies noted for $0.05 \leq P < 0.10$.

Table 6.1 Ingredient composition and analyzed nutrient content of experimental diets¹ (as-fed basis).

Ingredient, %	Gestation ²		Lactation ³	
	CON	CSCM	CON	CSCM
Corn	45.78	59.16	40.15	51.19
Barley	30.00	13.50	28.00	8.00
Canola meal (solvent-extracted)	-	14.50	-	24.50
Soybean meal	8.00	-	14.50	-
Wheat	10.00	7.00	9.00	8.00
Vegetable oils	2.70	2.70	3.36	4.00
Limestone	1.15	1.25	1.25	1.17
Monocalcium phosphate	0.82	0.56	1.25	1.17
Salt	0.40	0.40	0.50	0.50
Vitamin-mineral Premix ⁴	1.00	1.00	1.00	1.00
L- Lysine	0.08	0.07	0.32	0.32
DL-Methionine	0.04	-	0.07	-
L-Threonine	0.03	-	0.11	0.06
L-Tryptophan	-	0.01	0.01	0.03
L-Valine	-	-	0.14	0.11
Calculated composition				
Metabolize energy, kcal/kg	3,307	3,321	3,322	3,346
Net energy, kcal/kg	2,549	2,549	2,535	2,535
Crude protein, %	12.50	12.90	15.20	15.90
Calcium, %	0.61	0.61	0.80	0.80
Total phosphorus, %	0.49	0.51	0.67	0.70
SID Lysine	0.52	0.52	0.87	0.87

SID Methionine	0.24	0.24	0.28	0.28
SID Threonine	0.40	0.40	0.55	0.55
SID Tryptophan	0.13	0.13	0.17	0.17
STTD P, %	0.29	0.29	0.45	0.45
Analyzed composition				
Gross energy, kcal/kg	4,069	4,104	4,064	4,163
Crude protein, %	12.20	12.85	15.20	16.10
Neutral detergent fiber, %	8.71	12.20	9.51	12.29
Calcium, %	0.58	0.56	0.76	0.75
Total phosphorus, %	0.47	0.48	0.62	0.64

¹Experimental diets consisted of 1) CON, corn-barley and soybean meal control diet; 2) CSCM, corn-barley and canola meal treatment diet

²All gestation diets were formulated to contain 2.55 Mcal/kg of NE with 0.52%, 0.24%, 0.40%, and 0.13% standardized ileal digestible Lys, Met, Thr, and Trp, respectively, and 0.73% and 0.29% Ca and standardized total tract digestible (STTD) P, respectively.

³All lactation diets were formulated to contain 2.53 Mcal/kg of NE with 0.87%, 0.28%, 0.55%, and 0.17% standardized ileal digestible Lys, Met, Thr, and Trp, respectively, and 0.80% and 0.45% Ca and STTD P, respectively.

⁴Supplied the following per kg of finished gestation diets: vitamin A, 4400 IU; vitamin D, 880 IU; vitamin E, 48 IU; vitamin K, 0.6 mg; choline, 1.37 g; pantothenic acid, 13 mg; riboflavin, 4.13 mg; folic acid, 1.43 mg; niacin, 11 mg; thiamin, 1.1 mg; vitamin B6, 1.1 mg; biotin, 0.21 mg; vitamin B12, 16 µg, Cu, 11 mg as copper sulfate; Zn, 110 mg as zinc oxide; Fe, 88 mg as ferrous sulfate; Mn, 27.5 mg as manganese sulfate; I, 0.15 mg as potassium iodate; Se, 0.16 mg as sodium selenite. Supplied the following per kg of finished lactation diets: vitamin A, 2200 IU; vitamin D, 880 IU; vitamin E, 48 IU; vitamin K, 0.6 mg; choline, 1.37 g; pantothenic acid, 13 mg; riboflavin, 4.13 mg; folic acid, 1.43 mg; niacin, 11 mg; thiamin, 1.1 mg; vitamin B6, 1.1 mg; biotin, 0.21 mg; vitamin B12, 16 µg, Cu, 22 mg as copper sulfate; Zn, 110 mg as zinc oxide; Fe, 88 mg as ferrous sulfate; Mn, 27.5 mg as manganese sulfate; I, 0.15 mg as potassium iodate; Se, 0.16 mg as sodium selenite.

⁵Amino acids are indicated as standardized ileal digestible AA. Values were calculated based on values for standardized ileal digestibility of AA in corn and crystalline AA published by NRC (2012), and standardized ileal digestibility values for AA in soybean meal and canola meal were from González-Vega & Stein, (2012) and Velayudhan et al. (2019).

6.4 RESULTS

6.4.1 Sow performance

Over two reproductive cycles, dietary treatments had no significant effect ($P > 0.10$) on sow BW or backfat thickness on d 80 and d 111 of pregnancy, as well as on d 1 and d 21 post-farrowing as shown in **Table 6.2**. However, sows fed the CSCM diet exhibited a lower ($P < 0.05$) BW gain from d 80 to d 111 of gestation compared to sows on the CON diet. A significant cycle effect ($P < 0.05$) was observed, with increased BW gain in gestating sows during the second reproductive cycle. Furthermore, there was a significant interaction ($P < 0.05$) between dietary treatments and reproductive cycle. In the first reproductive cycle, sows fed the CSCM diet showed lower ($P < 0.05$) BW gain during gestation compared to those on the CON diet, while no differences ($P > 0.10$) were detected in the second cycle. Sows in the second reproductive cycle exhibited higher ($P < 0.05$) BW and backfat thickness on d 80 and d 111 of pregnancy, at farrowing, and on d 19 post-farrowing compared to sows in the first cycle. However, gestating sows in the second cycle had a lower ($P < 0.05$) backfat thickness gain from d 80 to d 111 of pregnancy than those in the first cycle. Additionally, there was a significant interaction ($P < 0.05$) between diet and cycle on sow BW at farrowing. Regarding average daily feed intake during both the gestation and lactation periods, no differences ($P > 0.10$) were observed between dietary treatments or reproductive cycles. No dietary treatment or reproductive cycle effect ($P > 0.10$) on the wean to estrus interval of sows was found.

6.4.2 Litter performance

As shown in **Table 6.3**, dietary inclusion of canola meal had no effect ($P > 0.10$) on litter weight throughout the trial. However, litter weight at birth and after cross-fostering was significantly

higher ($P < 0.05$) during the second reproductive cycle compared to the first cycle. Neither dietary treatments nor reproductive cycles influenced ($P > 0.10$) litter size throughout the experimental period. Similarly, no dietary effects ($P > 0.10$) were observed on the total number of piglets born, piglets born alive, piglets born dead, or piglets on d 19 post-farrowing. Regarding the average daily gain of piglets and piglet survivability from birth to d 19 post-farrowing, no significant differences ($P > 0.10$) were detected between dietary treatments or reproductive cycles during either the gestation or lactation periods.

6.4.3 Colostrum and milk composition

The composition of sow colostrum and milk is presented in **Table 6.4**. Dietary treatments had no effect ($P > 0.10$) on colostrum fat, protein, or lactose oligosaccharides throughout the experiment. However, a significant interaction ($P < 0.05$) between reproductive cycle and diet was observed for colostrum fat. In the first cycle, colostrum fat was higher ($P < 0.05$) in sows fed the CSCM diet compared to those fed the CON diet, whereas no difference ($P > 0.10$) was observed in the second cycle. Dietary inclusion of canola meal in sow diets over the two reproductive cycles did not influence ($P > 0.10$) milk fat, protein, or lactose oligosaccharides on d 19 post-farrowing. Additionally, there was no significant effect ($P > 0.10$) of reproductive cycle or interaction between diet and cycle on the nutrient composition of milk.

6.4.4 Serum metabolites

Serum biomarkers of renal, hepatic, and muscle function, as shown in **Fig 6.1**, included urea, creatinine, creatine kinase, and GGT on d 1 and d 19 of lactation. Sows in the second reproductive cycle exhibited significantly lower ($P < 0.05$) serum GGT concentrations compared to those in the

first reproductive cycle on farrowing day. On the farrowing day, sows fed the CSCM diet tended to have higher ($P = 0.09$ and 0.07 , respectively) serum creatinine and GGT concentrations compared to those fed the CON diet ($P = 0.08$). On d 19 of lactation, serum urea concentrations were lower ($P < 0.05$) in sows from the second reproductive cycle than in those from the first reproductive cycle. Furthermore, serum creatine kinase concentrations were higher ($P < 0.05$) in sows from the second reproductive cycle than in those from the first reproductive cycle. No significant dietary effects ($P > 0.10$) were observed on serum urea or creatine kinase concentrations.

Fig 6.2 shows the serum concentrations of total protein, albumin, globulin, and the A/G ratio on d 1 and d 19 of lactation. On d 1 of lactation, serum albumin and total protein concentrations were significantly lower ($P < 0.05$) in sows from the second reproductive cycle compared to those in the first cycle. Conversely, on d19 of lactation, serum globulin concentrations were higher ($P < 0.05$) in sows from the second reproductive cycle than in those from the first cycle, whereas the A/G ratio was significantly lower in the second cycle ($P < 0.05$). As shown in **Fig 6.3**, serum mineral concentrations, including Ca and P, were measured on d 1 and d 19 of lactation. On d 19 of lactation, sows fed the CSCM diet tended to have lower serum calcium concentrations than those fed the CON diet ($P = 0.07$). No reproductive cycle effect ($P > 0.10$) was observed on serum Ca or P levels at either time point.

6.4.5 Apparent total digestibility of energy and nutrients

The ATTD coefficients of energy and nutrients in late-gestating sows are presented in **Table 6.5**. Sows fed the CSCM diet showed a tendency ($P = 0.06$) for reduced ATTD of CP compared to sows fed the CON diet. Sows fed the canola meal-containing diets tended ($P = 0.05$) to exhibit a decreased ATTD of gross energy. Additionally, an interactive effect ($P < 0.05$) between diet and

reproductive cycle was observed. Specifically, in the first reproductive cycle, sows fed canola meal-containing diets had a lower ($P < 0.05$) ATTD of gross energy compared to those fed the CON diet. However, in the second cycle, no differences ($P > 0.10$) in ATTD of gross energy were observed between the dietary treatments. Across the two reproductive cycles, neither diet nor reproductive cycle significantly influenced ($P > 0.10$) the ATTD coefficients of DM, Ca, P or NDF in late-gestating sows. Furthermore, no interaction effects ($P > 0.10$) between diet and reproductive cycle were observed for the ATTD of energy or any of the measured nutrients during gestation. The ATTD of energy and nutrition coefficients for nursing sows are given in **Table 6.6**. No significant dietary effects ($P > 0.10$) were detected on the ATTD of DM, gross energy, CP, Ca, P or NDF in lactating sows. Additionally, there were no significant differences ($P > 0.10$) in the ATTD of these components between the first and second reproductive cycles. Likewise, no interaction effects ($P > 0.10$) between dietary treatment and reproductive cycle were observed for energy or nutrient digestibility during lactation.

Table 6.2 Effect of dietary canola meal in inclusion on sow performance over two reproductive cycles

Item	First cycle		Second cycle		SEM	<i>P</i> -value		
	CON ¹	CSCM	CON	CSCM		Diet	Cycle	Diet× Cycle
No. of sows	34	34	26	30	-	-	-	-
Sow bodyweight, kg								
d 35 of gestation	-	-	256.61	252.41	2.843	0.277	-	-
d 80 of gestation	245.68	246.06	285.17	290.22	6.951	0.702	0.007	0.740
d 111 of gestation	277.32	271.38	305.28	310.35	7.118	0.946	0.010	0.419
Gestation gain ²	31.23 ^a	25.17 ^b	20.29 ^b	20.70 ^b	1.477	0.037	<0.001	0.017
Farrowing	261.07	251.47	290.36	291.49	9.721	0.221	< 0.001	0.006
Weaning	234.92	227.33	263.59	260.28	12.897	0.461	0.017	0.754
Lactation change ³	27.07	24.93	28.50	30.72	4.576	0.992	0.462	0.649
Sow backfat thickness, mm								
d 35 of gestation	-	-	16.25	15.60	0.446	0.281	-	-
d 80 of gestation	16.15	16.10	17.92	18.41	0.327	0.454	<0.001	0.369
d 111 of gestation	17.45	17.57	18.25	18.95	0.426	0.364	0.001	0.379

Gestation gain	1.32	1.49	0.32	0.57	0.377	0.549	0.005	0.903
Farrowing	16.42	16.55	17.21	18.68	0.517	0.184	0.037	0.208
Weaning	14.99	14.14	15.02	15.46	0.748	0.808	0.263	0.275
Lactation change	1.46	2.47	2.26	3.17	0.758	0.290	0.387	0.946
Average daily feed intake, kg/day								
Gestation	2.93	2.94	2.81	2.87	0.059	0.604	0.147	0.702
Lactation	6.57	6.40	6.52	6.14	0.205	0.420	0.105	0.537
Wean to estrus interval	4.34	4.49	4.50	4.57	0.143	0.401	0.422	0.751

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

¹Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet

²Gestation bodyweight or backfat thickness gain was calculated as the difference between sow bodyweight at d 80 and d 111 of gestation.

³Lactation bodyweight gain was calculated as the difference between sow body weight at d 1 and d 21 of post-farrowing.

⁴Lactation backfat thickness loss was calculated as the difference between d 111 of gestation and d 21 of post-farrowing.

⁵Sow initial BW at d 80 of pregnancy was used as a covariate in the statistical model for BW data analysis.

⁶Sow initial BF thickness BW at d 80 of pregnancy was used as a covariate in the statistical model for backfat thickness data analysis.

Dash (-) indicates that the measurement was not applicable for this time point or treatment.

Table 6.3 Effect of dietary canola meal inclusion on litter performance over two reproductive cycles

Item	First cycle		Second cycle		SEM	<i>P</i> -value		
	CON ¹	CSCM	CON	CSCM		Diet	Cycle	Diet× Cycle
No. of litters	34	34	24	27		-	-	-
Litter size ²								
born total	16.09	16.07	16.72	17.05	1.071	0.852	0.368	0.830
born alive	14.60	14.73	15.40	15.61	0.795	0.785	0.247	0.956
born dead	1.38	1.11	0.91	1.14	0.269	0.946	0.376	0.313
after cross-fostering ³	13.96	13.83	13.90	14.12	0.604	0.867	0.662	0.502
d 19	13.02	13.48	13.32	13.34	0.556	0.491	0.794	0.500
Litter weight, kg								
Born alive	21.73	22.88	24.67	24.56	1.256	0.524	<0.001	0.306
After cross-fostering ³	22.61	22.59	23.98	23.83	1.225	0.881	0.007	0.891
d 19	79.74	81.79	79.74	84.09	4.384	0.722	0.056	0.504
Gain	58.03	59.95	61.27	61.26	3.556	0.682	0.311	0.637
Piglet ADG ⁴ , g/day	236.07	232.71	241.88	241.97	6.433	0.806	0.125	0.723
Piglet survivability ⁵ , %	94.03	95.52	96.31	94.65	1.188	0.577	0.932	0.113

¹Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet

²Litter size, the number of piglets per litter

³Cross-fostering, piglets were cross-fostered within the dietary treatment to equalize litter sizes within 24 h of birth. The sizes and bodyweight of per litter were recorded.

⁴Pig ADG = (average pig weight on d 19 – average live birth weight)/days of lactation.

⁵Piglet survivability (%) = (the number of weaned piglets /the number of piglets born alive after cross fostering) × 100.

Dash (-) indicates that the measurement was not applicable for this time point or treatment.

Table 6.4 Effect of dietary canola meal inclusion on colostrum and milk composition in lactating sows on d 1 and d 19 post-farrowing over two reproductive cycles

Item	First cycle		Second cycle		SEM	<i>P</i> -value		
	CON ¹	CSCM	CON	CSCM		Diet	Cycle	Diet× Cycle
Fat, %								
d 1	7.21 ^b	9.45 ^a	7.20 ^b	6.70 ^b	0.916	0.148	0.140	0.019
d 19	7.48	7.66	7.92	8.30	0.504	0.612	0.327	0.848
Crude protein, %								
d 1	8.61	7.21	8.44	9.59	0.749	0.856	0.103	0.062
d 19	4.62	4.60	4.43	4.55	0.106	0.621	0.237	0.484
Lactose oligosaccharide, %								
d 1	4.90	4.99	5.14	4.69	0.281	0.332	0.912	0.126
d 19	7.04	6.96	6.88	6.97	0.065	0.877	0.297	0.251

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

¹Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet

Table 6.5 Effect of dietary canola meal inclusion on apparent total tract digestibility (ATTD) of nutrients and energy in gestating sows

Item	First cycle		Second cycle		SEM	<i>P</i> -value		
	CON ¹	CSCM	CON	CSCM		Diet	Cycle	Diet× Cycle
Dry matter, %	83.41	80.45	82.34	82.23	0.861	0.155	0.691	0.177
Gross energy, %	84.05 ^a	81.63 ^b	83.43 ^{ab}	84.26 ^a	0.800	0.121	0.053	0.003
Crude protein, %	82.51	78.50	81.30	80.56	0.915	0.055	0.657	0.137
Calcium, %	36.33	35.12	31.69	32.89	5.401	0.999	0.561	0.833
Phosphorus, %	41.84	37.58	34.92	34.16	4.098	0.556	0.269	0.676
NDF ² , %	39.02	42.09	38.58	48.72	4.000	0.155	0.459	0.403

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

¹Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet

²NDF, neutral detergent fiber.

Table 6.6 Effect of dietary canola meal inclusion on apparent total tract digestibility (ATTD) of nutrients and energy in lactating sows ATTD over two reproductive cycles

Item	First cycle		Second cycle		SEM	<i>P</i> -value		
	CON ¹	CSCM	CON	CSCM		Diet	Cycle	Diet× Cycle
Dry matter, %	81.10	78.81	80.70	80.32	2.386	0.388	0.704	0.521
Gross energy, %	82.64	79.97	82.64	82.37	2.132	0.296	0.382	0.383
Crude protein, %	82.69	79.25	81.01	80.68	1.958	0.238	0.929	0.317
Calcium, %	32.13	34.68	36.29	37.85	5.290	0.349	0.101	0.817
Phosphorus, %	43.95	42.22	41.49	41.96	5.712	0.877	0.740	0.786
NDF ² , %	40.00	41.06	49.84	47.48	7.783	0.901	0.189	0.745

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

¹Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet

²NDF, neutral detergent fiber

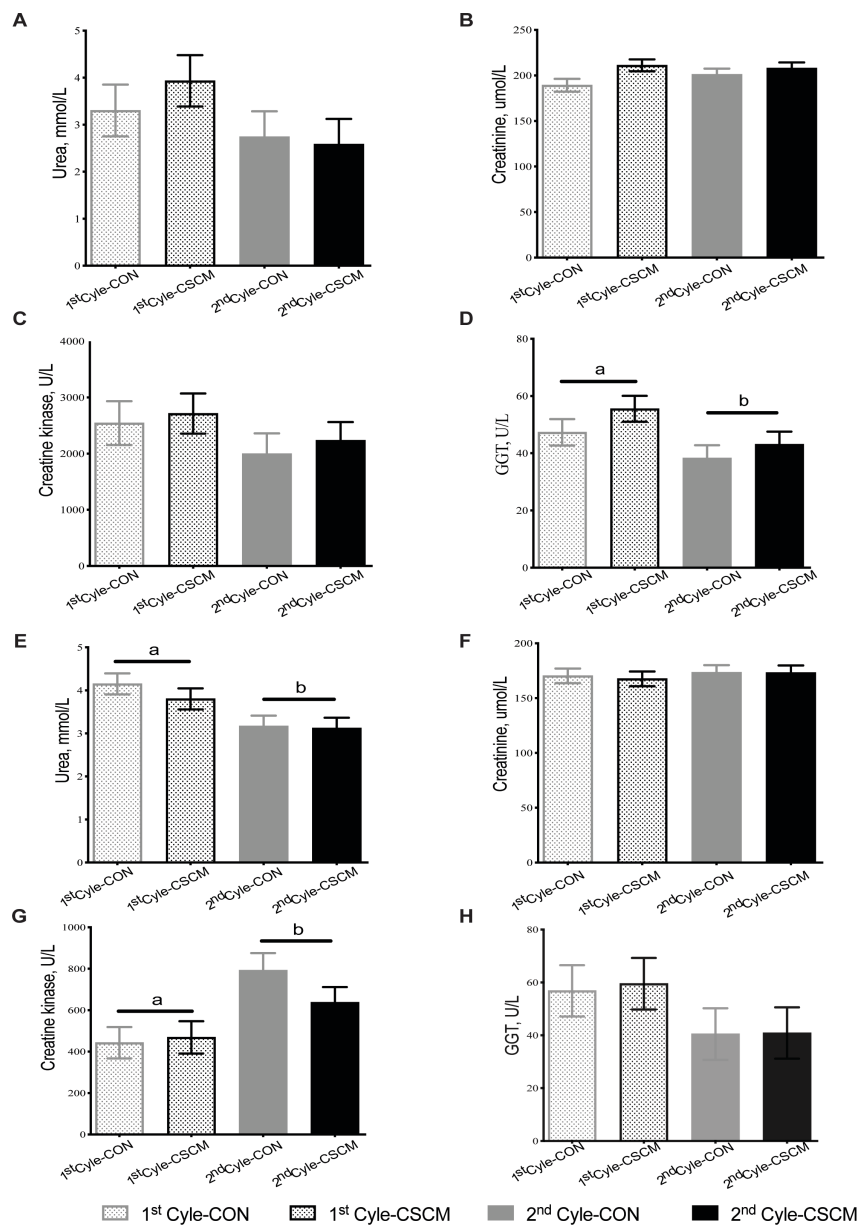


Fig 6.1. Effect of dietary canola meal inclusion on sow serum biomarkers of renal, hepatic, and muscle function on d 1 and d 19 of lactation over two reproductive cycles. Serum urea (A) (mmol/L), creatinine (B) ($\mu\text{mol/L}$), creatine kinase (C) (U/L), and gamma-glutamyl transferase (GGT) (D) (U/L) were measured on d 1 of lactation. Similarly, urea (E), creatinine (F), creatine kinase (G), and GGT (H) were assessed on d 19 of lactation. Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet. Means with different superscripts (a, b) differ significantly ($P < 0.05$).

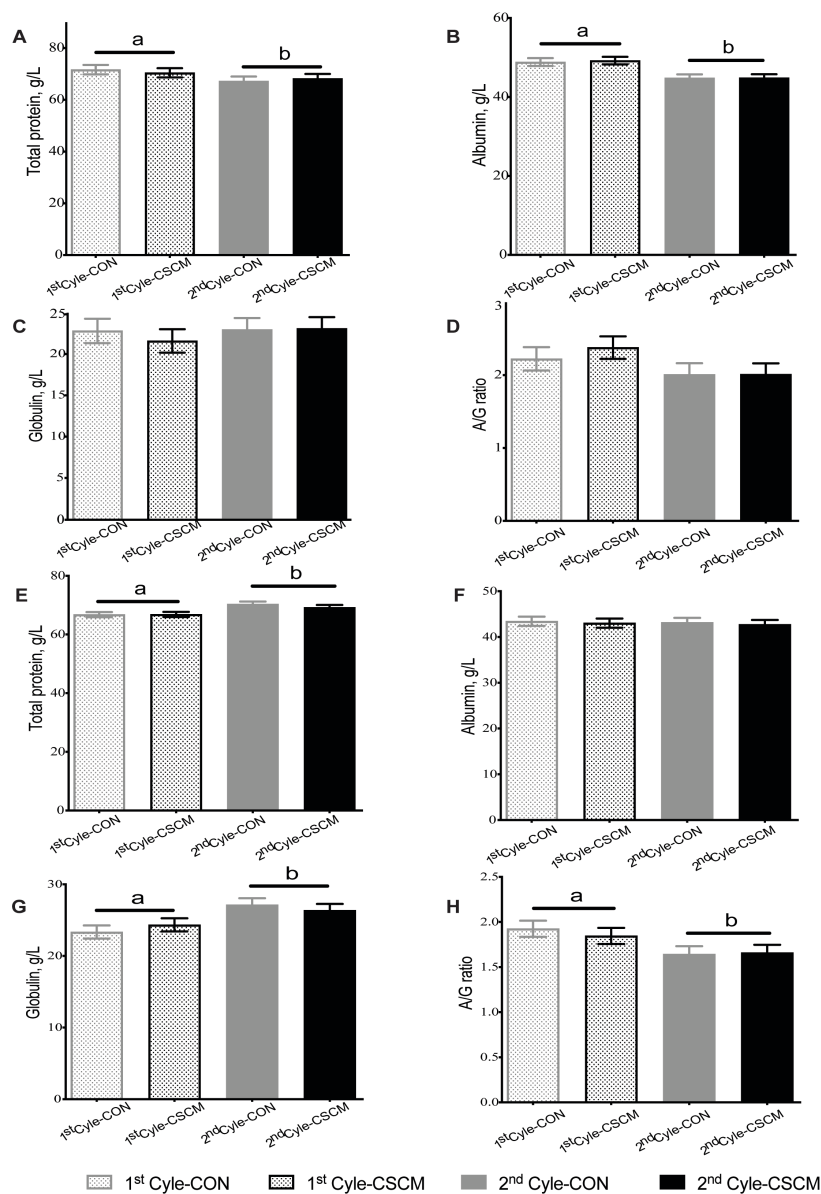


Fig 6.2. Effect of dietary canola meal inclusion on sow serum concentrations of total protein, albumin, globulin, and the albumin-to-globulin (A/G) ratio on d 1 and d 19 of lactation over two reproductive cycles. Total protein (A) (g/L), albumin (B) (g/L), globulin (C) (g/L), and albumin to globulin ratio (A/G ratio) (D) were measured on d 1 of lactation. Similarly, total protein (E), albumin (F), globulin (G), and A/G ratio (H) were assessed on d 19 of lactation. Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet. Means with different superscripts (a, b) differ significantly ($P < 0.05$).

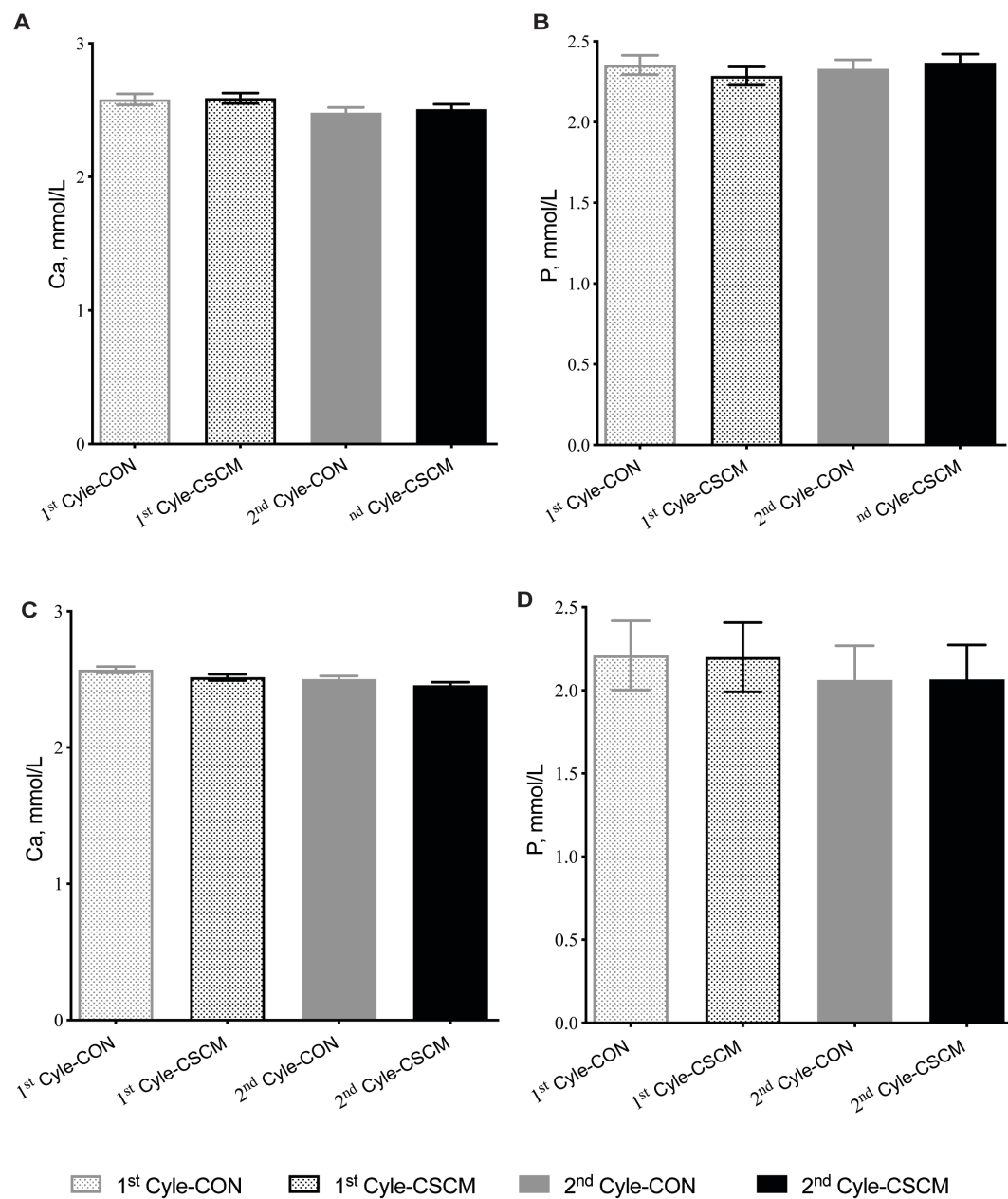


Fig 6.3. Effect of dietary canola meal inclusion on sow serum mineral concentrations, including Ca and P on d 1 and d 19 of lactation over two reproductive cycles. Calcium (Ca) (A) (mmol/L) and phosphorus (P) (B) (mmol/L) were measured on d 1 of lactation. Similarly, Ca (C) and P (D) were assessed on d 19 of lactation. Experimental diets consisted of CON, corn-barley and soybean meal control diet; CSCM, corn-barley and canola meal treatment diet. Means with different superscripts (a, b) differ significantly ($P < 0.05$).

6.5 DISCUSSION

The findings from this study are significant in addressing key challenges faced by the pork industry, where the rising feed costs are a major concern, especially for sows, which require large amounts of feed (Kim et al., 2019). Canola meal offers potential economic and nutritional benefits, including higher mineral content, a more balanced AA profile, and higher fiber content than soybean meal (Khajali and Slominski, 2012; Radfar et al., 2017). While concerns about anti-nutritional factors such as glucosinolates, phytic acid, and high fiber content have limited its inclusion, advancements in canola breeding have reduced glucosinolate levels, making canola meal a more viable option (Aider and Barbana, 2011; Adewole et al., 2016). Previous studies have shown that 20-30% dietary canola meal inclusion in swine diets had no influence on sow reproductive performance, which supports the evidence for using canola meal to replace soybean meal in swine diets (Landerio et al., 2011; Liu et al., 2016; Mejicanos et al., 2016). Additionally, the benefits of high-fiber diets in sow feeding, such as improved satiety, reduced aggressive behavior, and positive effects on reproductive outcomes, further supports its inclusion, particularly since sows have a greater ability to ferment fiber compared to young pigs (Zhuo et al., 2020; Schwennen et al., 2022; Jo and Kim, 2023). Evaluating canola meal over multiple reproductive cycles is necessary because the full adaptation of sows to high-fiber diets may require more than one reproductive cycle. Studies have shown that high-fiber diets can improve reproductive outcomes over time, with litter size and weight potentially increasing after two or three cycles (Ewan et al., 1996; Zhuo et al., 2020). Furthermore, our previous research has shown that 300 g/kg canola meal inclusion can negatively affect the digestibility of energy and nutrients in sows, particularly during gestation, despite demonstrating the feasibility of canola meal in sow diets. This decreased digestibility may be due to insufficient long-term feeding and adaptation to canola

meal, which could explain the negative effects observed in short-term studies. Therefore, this study was necessary to explore how long-term adaptation to canola meal supplementation over two reproductive cycles may mitigate these negative effects and improve overall nutrient digestibility, providing insights into optimizing its use as a sustainable, cost-effective alternative to soybean meal in sow diets.

Results of the present study are consistent with findings from previous research showing that inclusion of up to 30% canola meal in sow diets had no effects on overall sow BW or BF thickness (Velayudhan et al., 2018). However, in our study, sows fed the CSCM diet showed reduced BW gain in the first reproductive cycle, which aligns with an earlier study that observed lower BW gains in sows fed high-fiber diets during gestation, likely due to reduced energy intake caused by the high fiber inclusion (Guillemet et al., 2007). Studies have shown that high-fiber diets can decrease BW gain in gestating sows even if average daily feed intake was not influenced (Holt et al., 2006). Lower BW gain of sows fed the canola meal-containing diet may be due to the slower adaptation of sows to increased fiber levels as this study was started from d 80 of pregnancy. However, in the second reproductive cycle, no significant differences in BW gain were observed, which suggests that sows may adapt to the high inclusion of canola meal diet over time. This is supported by other research, where sows fed high-fiber diets over two or three reproductive cycles showed improved reproductive outcomes, including average daily gain of lactating sows in the second cycle (Tan et al., 2018). Furthermore, the increased BW and BF thickness observed in sows during the second reproductive cycle could reflect the natural physiological changes that occur as sows adjust to the diet, prioritizing body condition for the next reproductive phase (Guillemet et al., 2007; Tan et al., 2018).

Sow lactation feed intake is an important driver of improved piglet BW at weaning (Craig et al., 2017). The lack of differences in average daily feed intake between dietary treatments and reproductive cycles is consistent with previous studies, which have suggested that sows adapt their feeding behavior over time to meet energy needs, even if dietary composition varies (Quiniou et al., 2012). However, a high-fiber diet may enhance voluntary feed intake in sows during lactation, potentially serving as a compensatory mechanism for reduced nutrient intake and lower body weight gain during gestation (Holzgraefe et al., 1986). In the current study, lactation feed intake was not affected by feeding the canola meal-containing diet. The possible reason might be that fiber supplementation during gestation mainly improved sow feed intake during the first week of lactation (Guillemet et al., 2007; Quesnel et al., 2009). Overall, these results suggest that the inclusion of canola meal, a high-fiber ingredient, in sow diets may not adversely affect sow performance when fed over multiple reproductive cycles, with the sow's adaptation to the fiber content likely contributing to improved nutrient utilization in subsequent cycles.

Results of the current study showed that dietary inclusion of canola meal had no adverse effects on litter performance, with consistent litter weight, litter size, and piglet growth observed across reproductive cycles. The lack of influence on litter performance aligns with our previous results from the same project and is consistent with findings by Velayudhan et al. (2018). Similarly, Liu et al. (2018) found that replacing 50–100% of soybean meal with canola meal in sow diets did not impair litter size, piglet weight, or survivability, highlighting that nutritionally balanced canola meal diets can serve as an effective alternative to soybean meal. This may be attributed to improved canola meal processing techniques in recent years, which have significantly reduced antinutritional factors such as glucosinolates. On the contrast, earlier research has shown that fiber supplementation during gestation can enhance litter weight and the number of piglets born alive

over successive reproductive cycles (Veum et al., 2009; Li et al., 2021). The improved reproductive performance of sows fed high-fiber diets in later parities has been associated with enhanced placental development and alterations in plasma reproductive hormones, including progesterone, estrogen, and luteinizing hormone, which are pivotal in regulating female reproduction (Wang et al., 2016). For example, the results of Vallet et al. (2010) indicated a positive relationship between litter size and plasma estrogen on d 110 of gestation. In addition, the variations in fiber types and reproductive cycles across studies may contribute to the inconsistent results regarding litter performance (Renteria-Flores et al., 2008; Quesnel et al., 2009). The stable growth performance of sows fed canola meal-containing diets in this study suggests that canola meal inclusion did not negatively impact maternal energy reserves or nutrient intake during lactation—key factors influencing litter outcomes such as piglet growth and survivability. Additionally, the observed decrease in gestational BW and BF gain may explain the improved reproductive performance in the second reproductive cycle, consistent with findings by Wientjes et al. (2013).

Colostrum and milk composition of sows contributed to the growth and survivability of piglet particularly in the early stages of life when colostrum provides essential immunity and nutrients (Farmer and Quesnel, 2009; Declerck et al., 2015, 2016). In the present study, dietary canola meal supplementation in sows during two reproductive cycles did not change colostrum and milk composition, which was consistent with previous results reported by Velayudhan et al. (2018). However, in the first cycle, the canola meal-containing diets increased colostrum fat, which is inconsistent with our previous results, where even a 30% inclusion of canola meal in gestation diets did not influence colostrum fat levels compared to sows fed soybean meal-containing diet, while the inclusion level in this study was 14.50%. Colostrum is produced during a short, energy-

intensive period around farrowing, and the ability of sows to produce milk relies on their ability to mobilize body reserves and efficiently use dietary nutrients (Devillers et al., 2007; Foisnet et al., 2010). Canola meal, with its higher fiber, may alter metabolic processes by providing acetate through hindgut fermentation and unsaturated fatty acids from residual oil, both of which are key precursors for fat synthesis, potentially boosting colostrum fat levels as the sow prioritizes this early-stage output (Shang et al., 2019; Wealleans et al., 2021). During the early stages of the first reproductive cycle, sows fed canola meal diets might experience a metabolic response to the changed diets such as dietary fiber and anti-nutritional factors, which can influence energy metabolism and lipid mobilization. This initial response might explain the higher colostrum fat levels. In contrast, milk production at weaning reflects a prolonged lactational phase where nutrient demands are sustained and rely more heavily on dietary intake rather than body reserves (Krogh et al., 2016; Tokach et al., 2019). Over time, sows appeared to adapt, normalizing colostrum fat content by the second cycle. This adaptive response may reflect improved nutrient utilization and metabolic efficiency with prolonged dietary exposure. Overall, the absence of differences in milk composition during lactation, coupled with consistent litter performance, supports the hypothesis that high canola meal inclusion can meet the nutritional demands of lactation when properly formulated.

The observed changes in the serum metabolites and digestibility results of sows provide insight into the physiological effects of dietary inclusion of canola meal over two reproductive cycles on metabolic adjustments. The tendency for reduced ATTD of CP in late-gestating sows fed the CSCM diet likely reflects the impact of high fiber content (Newkirk and Classen, 2002). This reduction may have contributed to the observed changes on d 1 post-farrowing in serum metabolites, such as the tendency for increased creatinine, a byproduct of muscle metabolism due

to the slightly lower energy availability associated with the high-fiber content of canola meal, prompting greater reliance on protein catabolism for energy (Bufarah et al., 2017). The increased creatine has been shown to be related with the endogenous protein catabolism to compensate for lower dietary protein availability (Carlotti et al., 2008; Rempel et al., 2018). Serum GGT, an enzyme involved in glutathione metabolism and a critical component of the body's antioxidant defense system, also serves as an indicator of liver function, with its activity increasing in response to cellular damage that causes hepatic enzyme leakage (Hanigan and Pitot, 1985; Hanigan, 2014). The slightly increased GGT level in sows fed the canola meal-containing diet on farrowing day may indicate an upregulation of glutathione recycling in response to oxidative stress or might reflect mild metabolic stress or an adaptive response to anti-nutritional factors in canola meal, which can affect nutrient metabolism and liver activity. However, in this study, the serum GGT level or creatinine were not influenced in sows after two reproductive cycles of adaptation, indicating a possible adaptive mechanism over time.

Despite the tendency for reduced CP digestibility, no significant dietary effects were observed on the ATTD of other nutrients during gestation or lactation. This suggests that the overall energy and nutrient availability in the canola meal diet were adequate to meet the metabolic demands of sows. However, the slight decrease in serum calcium levels in sows fed the CSCM diet on d 19 post-farrowing might be linked to the high phytic acid content in canola meal, which can bind minerals and reduce their bioavailability, particularly during lactation when calcium demands for milk production are high (Bell, 1993). Reproductive cycle effects on both blood profiles highlight the physiological adaptations of sows over time. Similarly, the reduced serum urea levels in the second cycle suggest enhanced nitrogen utilization, likely reflecting better efficiency in amino acid metabolism as the sows adjusted to the high-fiber, canola meal-containing

diet (Eggum, 1970; Orok and Bowland, 1975; Brendemuhl et al., 1987; Pan et al., 2013). However, the increased serum total protein and albumin levels in the second cycle, alongside the lower albumin to globulin ratio, indicate improved protein metabolism and immune modulation in the second cycle, potentially reflecting better adaptation to dietary fiber and improved nutrient utilization over time (Malt, 1965; Verheyen et al., 2007). These metabolic adaptations are consistent with the lack of significant changes in digestibility across reproductive cycles, suggesting that sows efficiently utilized nutrients after initial adaptation. Overall, these findings suggest that the inclusion of canola meal in sow diets leads to slight changes in metabolic markers, likely due to its unique composition, including high fiber, and anti-nutritional factors. However, the absence of severe effects and the adaptation observed across cycles make canola meal be a sustainable protein source when carefully formulated in sow diets. Further studies are needed to explore the long-term metabolic and hormonal responses associated with high canola meal inclusion in sow diets, particularly in relation to high fiber intake, to optimize inclusion rates and maximize sow and litter performance.

6.6 CONCLUSION

This study demonstrated that dietary canola meal can be used as a primary protein source in sow diets over two reproductive cycles without compromising sow or litter performance. While initial metabolic adjustments, including a tendency for decreased serum gamma-glutamyl transferase and calcium levels, were observed during the first cycle, these effects diminished in the second cycle, indicating successful physiological adaptation. Additionally, the slight reduction in crude protein digestibility in gestating sows but also highlights the consistent digestibility of energy and fibre across treatments and cycles, suggesting that canola meal diets can provide adequate nutritional support for sows. These findings highlight the potential of canola meal as a sustainable alternative to soybean meal, with long-term benefits when diets are properly formulated based on the digestible energy and amino acids values. Future research should explore the metabolic mechanisms underlying these adaptations to further enhance the efficacy of canola meal in sow diets.

CHAPTER 7

GENERAL DISCUSSION

Canola meal has been considered a potential alternative protein source to soybean meal in sow diets due to its favorable amino acid profile, particularly higher methionine and cysteine levels (Radfar et al., 2017; Gołębiewska et al., 2022). However, limitations such as lower lysine content, high fiber, and anti-nutritional factors have historically restricted its use in monogastric diets. Advances in plant breeding have significantly reduced glucosinolate levels in modern canola cultivars, making them safer for livestock consumption. Previous studies have shown that 300 g/kg solvent-canola meal inclusion can be safely included in late-gestating and lactating sows without compromising sow reproductive performance but negatively affected nutrient digestibility (Velayudhan and Nyachoti, 2017). Expeller-pressed canola meal (ECM) differs from SCM in oil content, fiber composition, and residual glucosinolate levels, which may alter its nutritional value and physiological effects in sows. However, no study has shown the utilization of ECM in sow studies.

To address this, the overall objective of this research was to evaluate the nutritional strategies for optimizing utilization for canola meal in sow diets. Live yeast supplementation has been shown to improve gut health by promoting beneficial microbiota, increasing nutrient digestibility, and mitigating the adverse effects of dietary fiber. These synergistic effects suggest that combining canola meal with probiotics may improve its nutritional value, support reproductive and lactational performance, and enhance the sustainability of swine production systems.

Across all three studies, litter performance and piglet growth performance did not differ between sows fed soybean meal (SBM)-containing diets and those fed SCM-containing diets,

regardless of probiotic supplementation. This lack of difference is likely due to the comparable standardized ileal digestible (SID) amino acid and net energy (NE) contents of the experimental diets, which ensured that nutrient supply met the physiological requirements of the sows during both gestation and lactation (Noblet and Perez, 1993). These results are consistent with previous findings that high dietary inclusion of canola meal can effectively replace SBM without compromising reproductive or litter performance in sows (Velayudhan et al., 2018). The consistency of these outcomes across multiple reproductive cycles and experimental conditions strengthens the evidence that canola meal can be used as the sole protein source in sow diets without negative effects on maternal or offspring performance. However, in manuscript II, sows fed ECM-containing diets showed reduced body weight on both d1 and d21 post-farrowing compared to those fed SBM-containing diets. This reduction in BW may be explained by the higher fiber content and greater dietary glucosinolate concentrations in ECM relative to SBM and SCM, which can reduce dietary energy density and feed intake (Guillemet et al., 2007; Quesnel et al., 2009).

Regarding milk composition, the inclusion of either SCM or ECM in place of SBM had minimal effects on most milk components across the three studies. This indicates that replacing SBM with canola meal did not compromise the synthesis of key nutrients in sow milk. However, in Fig 7.1, the comparison of milk fat content on d19 of lactation between SBM- and SCM-containing diets showed a consistent pattern across manuscript I and manuscript II, where probiotic supplementation was associated with higher milk fat levels. This positive effect was observed in both studies, suggesting a reproducible response of milk fat yield to probiotic inclusion, regardless of study-specific conditions. The positive effect of probiotics on milk fat content may be linked to their ability to modulate gut microbiota composition and fermentation activity, thereby enhancing

nutrient absorption and energy utilization. In Study II, microbiota analysis revealed that probiotic supplementation increased the relative abundance of fiber-fermenting and short-chain fatty acid (SCFA)-producing bacteria. SCFAs, particularly acetate and butyrate, are important precursors for de novo fatty acid synthesis in the mammary gland (Palmquist et al., 1993; Shi et al., 2020). Additionally, improved gut barrier function and reduced systemic inflammation associated with probiotic feeding could have supported better nutrient partitioning toward milk production rather than immune defense, further contributing to increased milk fat yield.

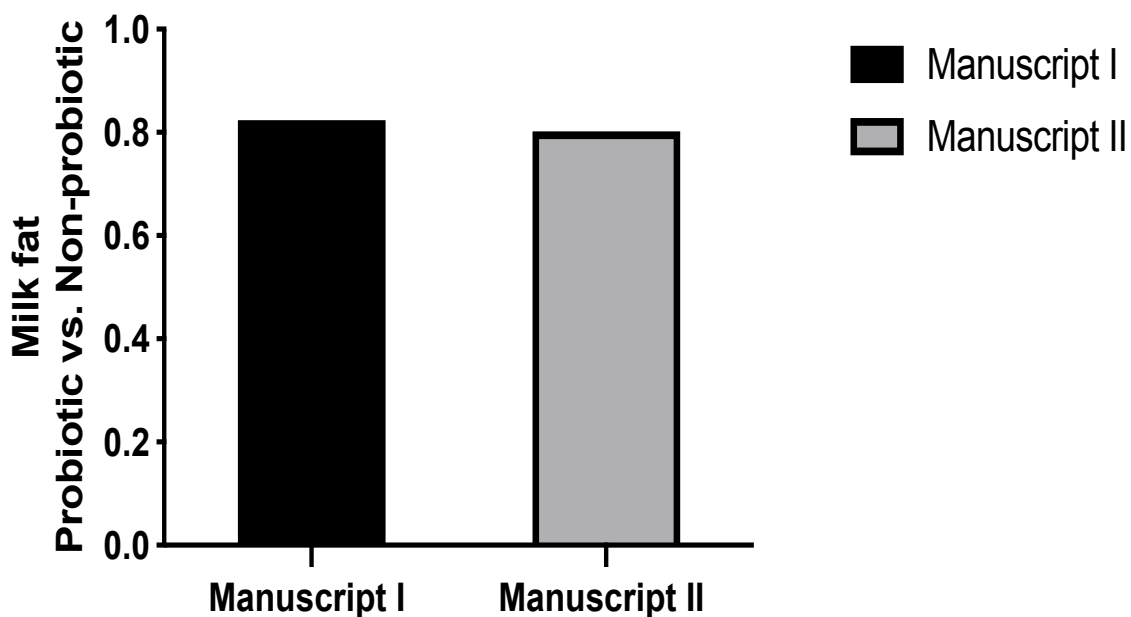


Fig 7.1 Differences in milk fat content (g/kg) on d19 post-farrowing between probiotic-supplemented and non-supplemented sows in manuscript I and manuscript II. Values represent the mean difference (SBM – SCM) calculated from each study.

To provide an integrated perspective, Fig 7.2-7.5 summarizes the effects of SCM inclusion and probiotic supplementation on nutrient digestibility across the three studies. In Fig 7.2, across the three studies, the apparent total tract digestibility (ATTD) of energy and nutrients in gestating

sows showed a generally consistent pattern when comparing SBM- and SCM-containing diets. In Manuscripts I and II, sows fed SCM-containing diets had lower digestibility of DM, GE, CP, Ca and P compared with those fed SBM-containing diets. This reduction is likely attributable to the higher fiber content and the presence of antinutritional factors in SCM, which can reduce nutrient availability. The reduced ATTD of crude protein suggests that the less digestible amino acids, likely due to the hulls containing 150 g/kg CP, combined with the lower fermentability of canola meal fiber compared to soybean meal, may contribute to microbial protein production, potentially lowering the ATTD of crude protein (Khajali and Slominski, 2012). In contrast, in Manuscript III, the differences in nutrient digestibility between the two diet types were minimal. One possible explanation is that in this study, sows were fed the experimental diets from late gestation through two consecutive parities, allowing more time for physiological adaptation to the high-fiber, CM-based diet, thereby narrowing the digestibility gap. In addition, in both Manuscripts II and III, the ATTD of NDF was higher in sows fed SCM-containing diets than in SBM-containing diets. This may be because the additional dietary fiber from SCM stimulates hindgut fermentation, promoting the growth of fiber-degrading bacteria and increasing cellulolytic enzyme activity (Jha and Berrocso, 2015; Lindberg, 2014). Over time, this microbial adaptation can enhance the breakdown of structural carbohydrates, leading to improved fiber digestibility in sows fed canola meal-based diets.

In manuscript II, the reduced ATTD of GE and CP in sows fed ECM-containing diets may be explained by the nitrogen losses in the hindgut caused by microbial fermentation and protein synthesis, which cannot be utilized by the host. Additionally, the reduced digestibility of CP and GE in ECM-fed sows may help explain the observed decrease in BW during gestation, as lower nutrient absorption can result in energy deficits, requiring sows to mobilize body reserves to meet

their metabolic needs. Even though sows are known to tolerate up to 4.0 $\mu\text{mol/g}$ of total glucosinolates in SCM-containing diets, while lactating sows can tolerate up to 2.8 $\mu\text{mol/g}$ in SCM without impairing reproductive performance (Bell, 1993; Velayudhan and Nyachoti, 2017), there is no study showing the maximum levels of ECM included in sow diets without influencing reproductive performance. Thus, further research is needed to evaluate the effects of different inclusion levels of ECM on sow metabolism and to determine the tolerance of pigs to varying glucosinolate concentrations across trials.

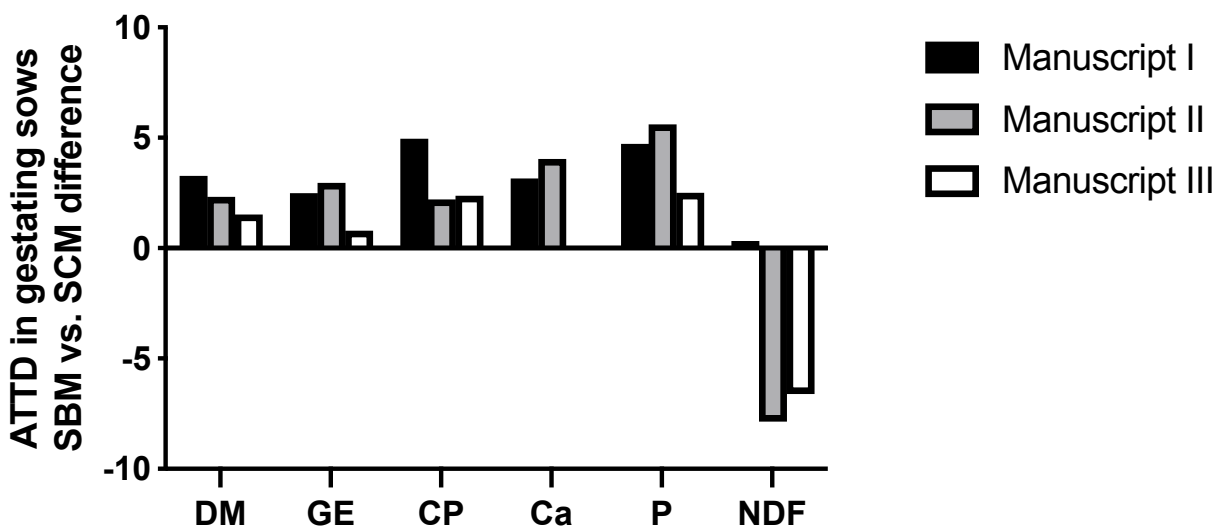


Fig 7.2 Differences in apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), crude protein (CP), calcium (Ca), phosphorus (P), and neutral detergent fiber (NDF) between sows fed soybean meal (SBM) and canola meal (SCM) diets during gestation in manuscript I, manuscript II and manuscript III. Values represent the mean difference (SBM – SCM) calculated from each study.

In Fig 7.3, probiotic supplementation increased the ATTD of nutrients in both Manuscripts I and II, with a particularly notable improvement in Ca and P digestibility during gestation. Moreover, the reductions in nutrient and energy digestibility typically observed with SCM diets were partially mitigated when probiotics were included, suggesting that the additive helped counteract some of the negative effects associated with SCM. The potential mechanisms underlying this improvement could be that the supplementation of *S. cerevisiae* product may alleviate some of these challenges by enhancing gut microbial fermentation, enzymatic activity, and nutrient absorption (Jiang et al., 2015; Ogbuewu et al., 2019). Probiotic supplementation likely offset some of the adverse effects of high fiber by increasing microbial enzyme production such as phytase and fostering a more favorable gut environment (Nakamura et al., 2000). Additionally, probiotics may stabilize intestinal pH and reduce oxidative stress in the gut environment, supporting more efficient nutrient absorption and utilization (Shen et al., 2011).

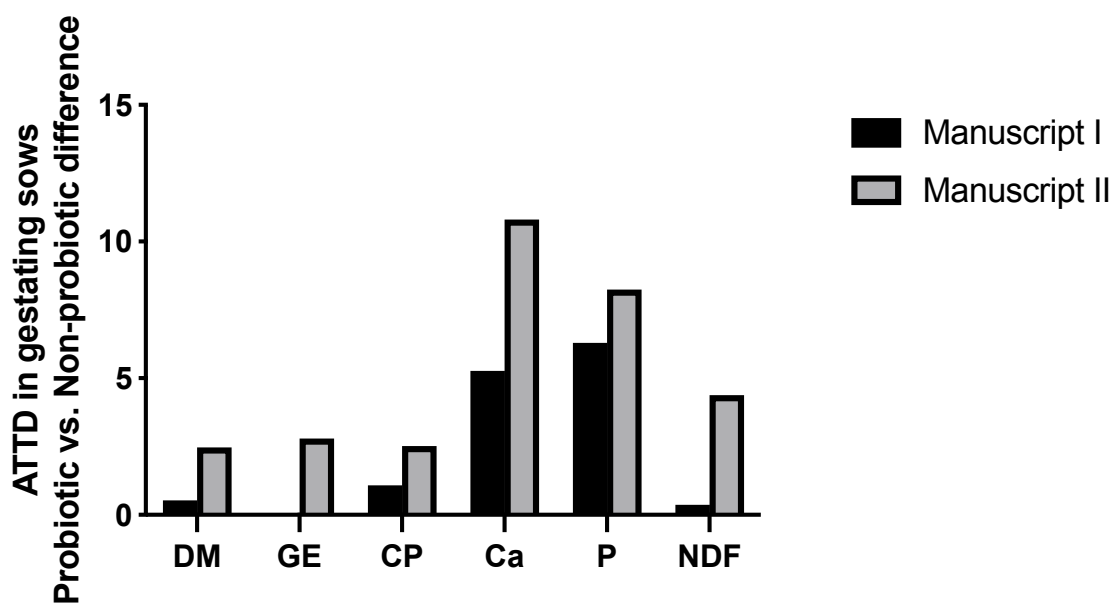


Fig 7.3 Differences in apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), crude protein (CP), calcium (Ca), phosphorus (P), and neutral detergent fiber (NDF) between

probiotic-supplemented and non-supplemented sows during gestation in manuscript I and manuscript II. Values represent the mean difference (Probiotic – Non-probiotic) calculated from each study.

In Fig. 7.4, the differences in nutrient digestibility between sows fed SCM- and SBM-containing diets during lactation were smaller than those observed in gestation through the three studies. The lack of impact on nutrients and energy digestibility of lactating sows could be attributed to the sows' ability to adapt over time, allowing them to more efficiently utilize the high dietary fiber, which may be due to increased hindgut fermentation as a result of greater microbial mass in the gut (Varel, 1987). Thus, the long-term feeding of canola meal inclusion from early gestation to lactation is needed to better understand the mechanisms that influence nutrient digestibility in sows. In manuscript III, the objective was to determine the long-term effects of dietary canola meal inclusion as the main protein source in gestation and lactation diets on sow and litter performance. Manuscript I and manuscript II demonstrated that including canola meal at 300 g/kg in gestation and lactation diets is safe; however, this inclusion level provided more nutrients than required, particularly for gestating sows, potentially masking differences in performance. Thus, to better assess the impact of nutrient limitations, manuscript III was designed with canola meal inclusion levels that aligned more closely with sow requirements, allowing any negative effects to be more easily detected. The unchanged serum GGT level or creatinine were not influenced in sows after two reproductive cycles in manuscript III, indicating a possible adaptive mechanism over time. Also, the increased serum total protein and albumin levels in the second cycle, alongside the lower A/G ratio, indicate improved protein metabolism and immune modulation in the second cycle, potentially reflecting better adaptation to dietary fiber and improved nutrient utilization over time (Malt, 1965; Verheyen et al., 2007). These metabolic

adaptations are consistent with the lack of significant changes in digestibility across reproductive cycles, suggesting that sows efficiently utilize nutrients after initial adaptation.

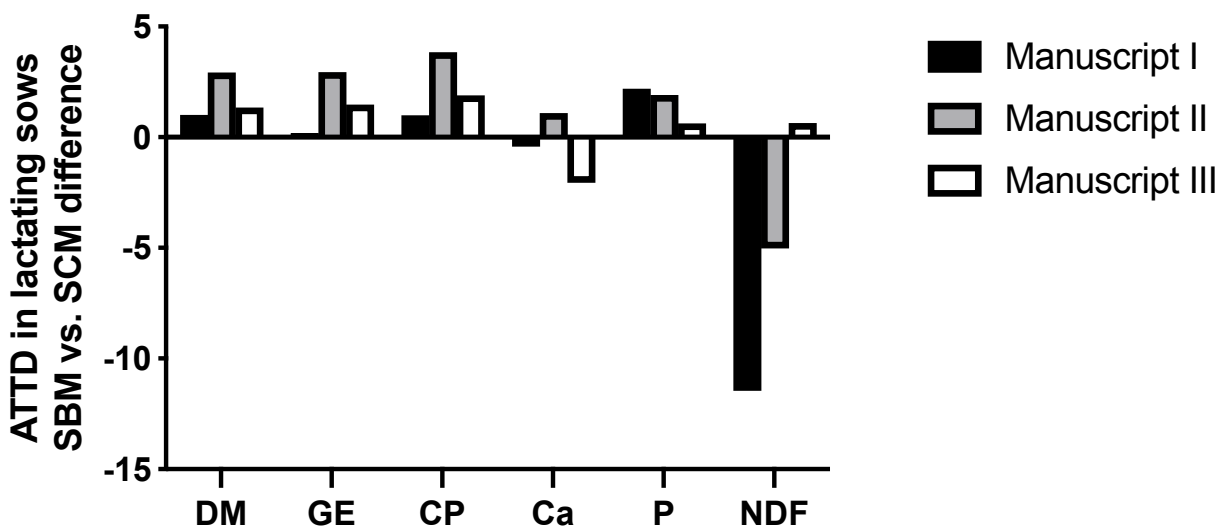


Fig 7.4 Differences in milk fat content (g/kg) on d19 post-farrowing between sows fed soybean meal (SBM) and canola meal (SCM) diets during lactation in manuscript I, manuscript II and manuscript III. Values represent the mean difference (SBM – SCM) calculated from each study. Positive values indicate higher milk fat in sows fed SBM diets.

The beneficial effects of probiotics on the ATTD of most nutrients were limited, but an improvement in the ATTD of P was still observed as shown in Fig 7.5. The reduced difference during lactation suggests that sows adapt to dietary treatments over time, narrowing the digestibility gap between diets. Overall, these findings suggest that the inclusion of canola meal in sow diets leads to slight changes in metabolic markers, likely due to its unique composition, including high fiber, and anti-nutritional factors. However, the absence of severe effects and the adaptation observed across cycles make canola meal be a sustainable protein source, with long-term benefits with proper dietary formulation.

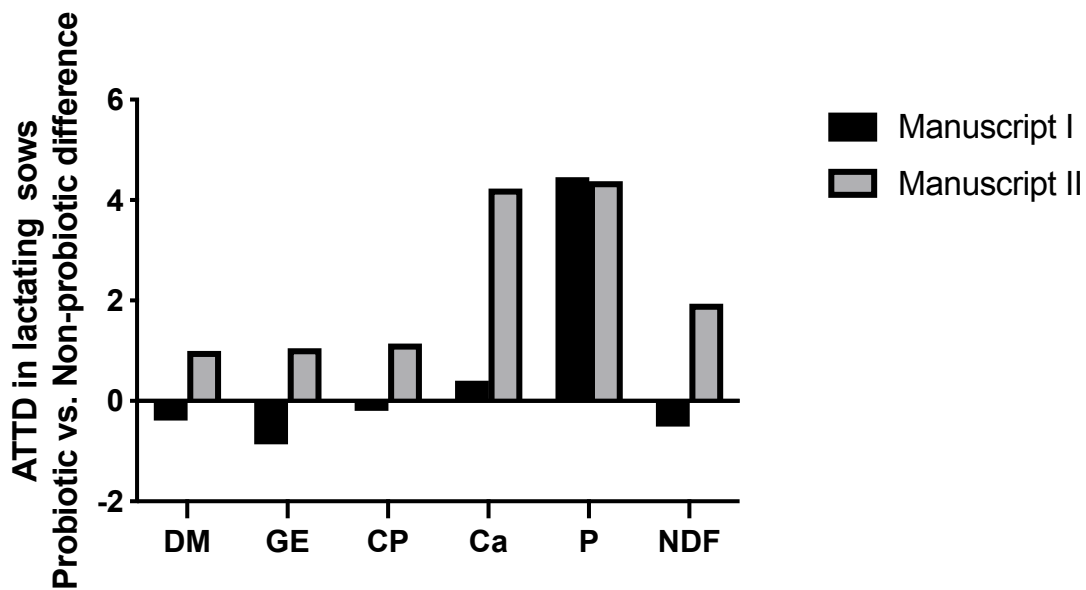


Fig 7.5 Differences in apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), crude protein (CP), calcium (Ca), phosphorus (P), and neutral detergent fiber (NDF) between probiotic-supplemented and non-supplemented sows during lactation in manuscript I, manuscript II and manuscript III. Values represent the mean difference (Probiotic – Non-probiotic) calculated from each study.

CHAPTER 8

CONCLUSIONS AND FUTURE STUDIES

8.1 CONCLUSION

This research investigated the utilization of high dietary inclusion of canola meal in gestating and lactating sows, considering its effects on sow and piglet performance, nutrient utilization, and gut microbiota modulation through probiotic supplementation. Based on the findings from three studies, the following conclusions can be drawn:

1. High inclusion of canola meal in sow diets

Replacing soybean meal with 300 g/kg solvent-extracted canola meal in late-gestating and lactating sow diets did not influence sow reproductive performance and suckling piglet growth, supporting the feasibility of using canola meal as a primary protein source in lactating sows.

2. Energy and nutrient digestibility

High levels of solvent- or expeller-extracted canola meal in late-gestating sow diets led to reduced energy and nutrient digestibility, likely due to the increased fiber content and insufficient adaptation period. However, with longer adaptation, sows were able to utilize the diets effectively, demonstrating the potential of canola meal inclusion when dietary adaptation is considered.

3. Probiotic supplementation effects

The addition of *S. cerevisiae* product to high-canola meal diets improved nutrient digestibility and increased milk fat content on d 19 post-farrowing, highlighting its potential to mitigate some negative effects of high-fiber diets. The *S. cerevisiae* product inclusion also enhanced the digestibility of gross energy and crude protein, supporting its beneficial role in sow nutrition.

4. Long-term feeding effects across parities

Over two reproductive cycles, feeding solvent-extracted canola meal resulted in reduced body weight gain from d 80 to d 111 of gestation in the first reproductive cycle. However, in the second reproductive cycle, colostrum composition and overall sow performance remained stable, suggesting that any initial challenges may be mitigated over time.

5. Metabolic and serum biochemical changes

Sows fed canola meal-containing diets showed alterations in serum metabolites, with increased serum creatinine and gamma-glutamyl transferase levels and reduced serum calcium concentrations, indicating potential metabolic adjustments. These findings suggest that long-term canola meal feeding may require nutritional adjustments to ensure metabolic balance in sows.

6. Colostrum and milk composition

The first reproductive cycle showed an increase in colostrum fat content in sows fed solvent-extracted canola meal, whereas no differences were observed in the second

reproductive cycle, indicating that dietary adaptation or metabolic adjustments may influence colostrum composition over time.

7. Differences between solvent- and expeller- extracted canola meal

While solvent-extracted canola meal inclusion had a slight negative effect on apparent total tract digestibility of nutrients in lactating sows, expeller-extracted canola meal resulted in a more pronounced reduction in nutrient digestibility. This suggests that the optimal inclusion level of expeller-extracted canola meal should be further studied to determine its suitability in lactating sow diets.

8. Digestibility adaptations over time

In the first reproductive cycle, sows fed canola meal-containing diets had lower apparent total tract digestibility of gross energy, but in the second reproductive cycle, no significant differences in the apparent total tract digestibility gross energy were observed between dietary treatments. This indicates that sows may require an extended adaptation period to efficiently utilize high fibre ingredient in their diets over successive reproductive cycles.

9. Implications for practical feeding strategies

These findings collectively support the potential use of canola meal in sow diets, when appropriate feed formulation strategies are applied and nutrient levels are properly balanced. Gradual adaptation, probiotic supplementation, and metabolic monitoring are essential to maximize its benefits and minimize potential adverse effects on nutrient utilization and metabolic health.

8.2 FUTURE STUDIES

1. Evaluate the long-term effects of using expeller-extracted canola meal as the primary protein source on sow reproductive performance and nutrient digestibility across consecutive reproductive cycles.
2. Mechanistic investigations to understand how long-term canola meal feeding affects gut morphology, microbiota composition, and nutrient transporter expression.
3. To repeat the study in a commercial facility with a larger number of sows to evaluate performance outcomes under practical production conditions and to investigate the economic feasibility of replacing soybean meal with canola meal in real-world feeding programs.

CHAPTER 9

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