Evaluating the Feeding Value of Western Canadian Expeller-pressed Soybean Meal Fed to Growing Pigs

by

Shiven Patel

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I. ABSTRACT

The objectives of this study were to evaluate phosphorous (P) digestibility and energy contents of expeller-pressed soybean meal (SBE) derived from Manitoba grown soybeans as a potential source for swine. The study aimed to compare the Manitoba derived SBE with Ontario derived SBE, yielding the results that can be used to showcase the comparative soybean quality in swine nutrition, enhancing the reputation of western Canadian grown soybean in foreign markets and concurrently reducing the dependence on imports. Four SBEs were used in the experiment, with three derived from soybeans grown in Manitoba (MB1, MB2, and MB3) whereas the fourth was derived from soybeans grown in Ontario. Experiment 1 was conducted to determine the apparent total tract digestibility (ATTD) of calcium (Ca) and P and standardized total tract digestibility (STTD) of P in SBE and to examine the effects of microbial phytase supplementation on ATTD and STTD of P in SBE. The STTD values of P with and without phytase supplementation averaged 69.9% and 79.7%, respectively. The ATTD and STTD of P were not different among the SBE types. Phytase supplementation increases the P digestibility in SBE irrespective of source. Experiment 2 was conducted to determine the digestible energy (DE), metabolizable energy (ME) and net energy (NE) content in SBE derived from Manitoba and Ontario regions. Manitoba and Ontario derived SBEs were not different in terms of DE, ME and NE contents when fed to growing pigs. The average contents of DE, ME and NE in studied SBE was 4280, 4056, and 2986 kcal/kg DM, respectively. Manitoba derived and Ontario derived SBE were not different in terms of P digestibility and energy content. Thus, average values of STTD of P, and NE values can be efficiently utilized in swine diet formulations. In conclusion, western Canadian grown soybeans hold promise as a viable protein ingredient for swine nutrition, however, further studies are necessary to understand its full potential.

II. DEDICATION

This thesis is dedicated to my pillars of support: my parents, Mayaben Patel and Surendrabhai Patel,

brother, Chirag Patel and sister-in-law Bhavini Patel

and

beloved nephew Nishal Patel

III. ACKNOWLEDGEMENTS

This thesis is the result of the endeavours encompassing the support and encouragement of many people. I shall ever, remain thankfully indebted to all those learned souls, who directly or indirectly motivated me to achieve my goal and enlightened me with the touch of their knowledge and constant encouragement. I feel this is an extremely significant and joyous opportunity bestowed upon me by the Almighty God, to think about and thank all those persons, who dedicated their valuable time for my tomorrow.

First and foremost, I would like to express my gratitude to my advisor, Dr. Martin Nyachoti, for providing me with the opportunity to work on this project and for his invaluable guidance through numerous meetings, discussions, and feedback sessions. His swift actions have enabled me to engage in laboratory work and make progress in my writing endeavours. I am also very grateful to the members of my thesis committee, Dr. James House, and Dr Chengbo Yang, for their valuable insights, constructive feedback, and academic contributions that have greatly enhanced the quality of this work.

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IV. FOREWORD

This thesis was prepared following a manuscript format and is composed of two separate journal manuscripts. Manuscript-1 abstract was accepted to ASAS-2024 Midwest Meeting as a Poster.

1. S. S. Patel, J. Lee, N. Mohamed, C Yang, J. House, R. Patterson, and C. M. Nyachoti. Determination of standardized total tract phosphorus digestibility in expeller-pressed Canadian prairie grown soybean meal fed to growing pigs. American Society of Animal Science 2024 Midwest Meeting. March 10-13, 2024.

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V. ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fibre
ANFs	Anti-nutritional factors
ATP	Adenosine triphosphate
ATTD	Apparent total track digestibility
BW	Body weight
C	Carbon
Ca	Calcium
CF	Crude fiber
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
CS	Comparative slaughter
d	Day
DCP	Digestible crude protein
DE	Digestible energy
dEE	Digestible ether extract
DESBM	Dry extruded expelled soybean meal
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
EU	European union
Exp	Experiment
FHP	Fasting heat production
FU	Feed unit
FSBM	Fermented soybean meal
g	Gram
GE	Gross energy
H	Hydrogen
h	Hour
HI	Heat increment
HP	Heat production
IC	Indirect calorimetry
J	Joule
K	Potassium
kcal	Kilocalorie
kg	Kilogram
kJ	Kilojoules
ME	Metabolizable energy

N	Nitrogen
Na	Sodium
NDF	Neutral detergent fiber
NE	Net energy
NSP	Non-starch polysaccharides
O_2	Oxygen
P	Phosphorus
RE	Retained energy
RQ	Respiratory quotient
SB	Soybean
SBE	Expeller-pressed soybean meal
SBM	Soybean meal
SD	Standard deviation
SEM	Standard error of the mean
ST	Starch
STTD	Standardized total track digestibility

1. INTRODUCTION

Soybean, also known as soja bean or soya bean (Glycine max), is called the "miracle crop" due to its high oil content (18%) and high-quality protein (about 40%) (Modgil et al. 2021). Approximately 85% of soybeans are processed into soybean meal (SBM) and oil, and from that 98 % of SBM is used as a protein source in animal feed (Pope et al. 2023). As soybean contains higher amount of crude protein (CP), it serves as a primary source of essential and non-essential amino acids (AA) in monogastric animals (Willis 2003; Stein et al. 2008). There are two types of soybean processing techniques: Solvent extracted SBM and Expeller-pressed SBM (SBE) (Aydeniz et al. 2014). The latter techniques yield higher oil content resulting into lower CP. Nutrient composition of SBM is affected by processing conditions and composition of soybeans. Soybean composition is affected by type of variety, geographical location, and environmental factors (de Coca-Sinova et al. 2008; Rotundo et al. 2016; Cober et al. 2023). Most of the research in past decades focused on improving soybean yield without taking into account its nutritional composition (Mahmoud et al. 2006; de Borja Reis et al. 2020), which resulted into variable nutritional content. Moreover in Canada, quality factors like oil or protein content are not considered when registering new soybean varieties (Barthet and Puvirajah 2022).

In 2022-23, Canadian soybean supplies reached 7.3 Mt, driven by higher production and stable imports, compared to 6.2 Mt in 2021-22 (AAFC 2023). Manitoba contributes 22% of Canada's soybean production (Statistics Canada 2023). Despite this significant production, a report from Manitoba Agriculture (2021) revealed that the province imported 250 thousand tonnes of SBM, incurring a cost of $164 million USD. In terms of exports, Manitoba exported 1.2 million tonnes of soybean for the same year. However, when it comes to the pricing, Western Canadian soybean are discounted because of lower CP levels compared to Ontario soybean (MPSG 2022).

Ingredient composition varies from region to region even within the same variety. For instance, Ontario and Manitoba has major differences in temperature and precipitation which results in variability in growing conditions and managemental practices. Animal nutritionists encounter the challenge of integrating new ingredient varieties into diet formulations, as the standard nutritional values may lead to under- or over-supply of nutrients relative to the specific requirements of the animal. Therefore, to efficiently incorporate Manitoba-grown SBE in swine diet formulation, it is important to determine its nutritional value. Determination of nutritive value of locally available ingredient will allow cost-effective diet formulation for pigs.

Chemical and nutrient composition of an ingredient can be determined through proximate analysis in a laboratory. However, those measurements are not adequate to determine bioavailability of nutrient for an animal (D’Alfonso 2002). Nutrient bioavailability varies across animal species as each animal has distinct digestive system. Digestibility values for phosphorous, crude protein, and energy for an ingredient are the key factors in cost-effective swine diet formulation. Nutritional value of Manitoba-grown SBE for swine is yet to be determine. In the current study, three types of soybean seeds were selected based on seed protein content, two from Manitoba having high (40.1 % DM) and low CP (31.0 % DM) content, and one from Ontario (44.6 % DM). Using expeller-press method, Manitoba soybean seeds were processed into three types of SBE and one Ontario derived SBE (ON) as a control. Manitoba derived SBE labelled as Manitoba commercial -1 (MB1), Manitoba commercial -2 (MB2), and Manitoba commercial -3 (MB3).

2. LITERATURE REVIEW

2.1. SWINE INDUSTRY

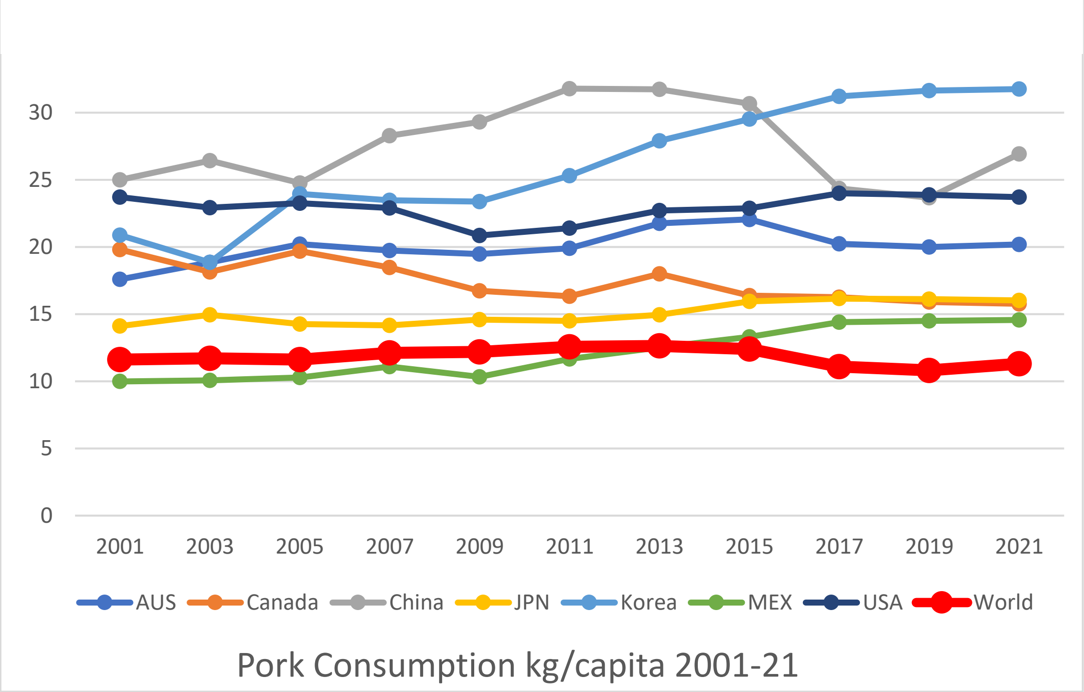
With 7,000 pig farms and an annual pig production exceeding 25.5 million, Canada's pork industry constitutes a substantial sector, yielding $4.1 billion in direct farm gate sales. Ranking fourth in Canadian farm cash receipts, it sustains 31,000 on-farm jobs and influences over 103,000 direct, indirect, and induced positions nationwide. The holistic economic impact, inclusive of farms, inputs, processing, and pork exports, impressively totals $23.8 billion (CPC 2023).

In Manitoba, the hog industry ranks as the third most significant contributor to farm cash receipts, amounting to $1.1 billion. Manitoba ranked number one in Canada with over 8.2 million hog and pig sales in 2020 (Canada 2022). Integral to Canada's economic stability, it ensures abundant, locally sourced, affordable, high-quality protein for Canadians and a global market, all while upholding stringent standards for animal welfare and environmental stewardship.

An increase in global pork production is expected due to higher production in China (USDA 2023). United States department of agriculture (USDA) foreign agricultural service (FAS) report indicates that worldwide pork consumption is set to rise to 127 million metric tons within the next decade, constituting approximately one-third of the overall growth in meat consumption. While per capita pork consumption (Fig 3.1) is anticipated to see a minor uptick globally in this forecasted timeframe, a decline is projected in most developed countries due to their already high levels of per capita consumption across all meat types. Increasing worldwide pork demand presents growth opportunities for the swine industry but escalates sustainability challenges in terms of ingredient supply, including on-farm greenhouse gas emissions, feed-related indirect emissions, land use changes, and heightened risks of pollutants in soil, water, and air.

In 2021, Pork was the world's 119th most traded product, with a total trade of $36.9B (OEC 2023). Worldwide pork export is primarily controlled by major exporting nations (Figure 2.2) i.e., North America (USA and Canada), European Union (Netherland, Denmark, Germany, Spain etc), and Brazil. The leading ten exporters collectively accounted for 91% of the global pork exports (measured in carcass weight equivalent). In the year 2020, 41% of the aggregate export originating from Canada was exclusively directed to China, followed by USA, 21% (CanadaPork 2020). In terms of imports, China has become the foremost global importer (Fig 2.3), accounting for 29% of worldwide imports from 2019 to 2021 due to substantial supply disruptions in recent years. In 2018, China's African swine fever (ASF) outbreaks, as reported by Han et al. (2022), inflicted significant harm on the nation's pig and sow populations, resulting in severe shortages of pork supply.

Figure 2.1 Global pork consumption, kg per capita, 2001-2021 (OECD data)



These trade patterns in the global pork industry are significantly shaped by the price and accessibility of animal feed, with a particular emphasis on maize and soybeans, predominantly originating from North and South America. Europe and China are prominent animal feed importers, particularly China, which imports more soybeans (over 100 million metric tons) than it produces (Yu and Jensen 2022). Ending such imports would jeopardize China's pork self-sufficiency goal, leading to increased pork imports and potentially reshaping global swine production and soybean trade patterns (Yu and Cao 2015). A considerable number of countries across the world lack self-sufficiency in protein supply, and they heavily depend on imports of SBM. For instance, similar to China, the EU produces only 42% of its total protein demand (Hendriks et al. 2019).

Swine are monogastric animals that requires diets lower in fibre and higher in energy unlike ruminants. They have a relatively simple digestive system that is optimised for the digestion of carbohydrates and proteins such as cereal grains. Swine diets comprises of six basic nutrients (Water, Carbohydrates, Protein, Fats, Vitamins and Minerals). Each of these nutrients plays direct or indirect role in overall growth, reproduction, and maintenance. Cereal grains such as corn, wheat, barley, and their by-products serve as a primary source of energy and represent the most significant component of the overall feed expenditure (Myer and Brendemuhl 2013). Apart from cereal grains, supplemental fats and oils are commonly added not only to increase energy density but also to improve palatability and to reduce dust.

After energy, protein ranks as the second most significant cost component in swine diets, playing a vital role in growth and muscle development. Selecting a swine diet protein source involves evaluating amino acids content, digestibility, energy, anti-nutritional factors, nutrient consistency, sourcing reliability, costs, and production goals for a well-rounded decision-making. In conclusion, availability of plant based protein considering the production and trade patterns around the globe plays crucial role in pig production efficiency and cost.

Figure 2.3 Global pork exports by various nations from 2019–2021 (OECD data)

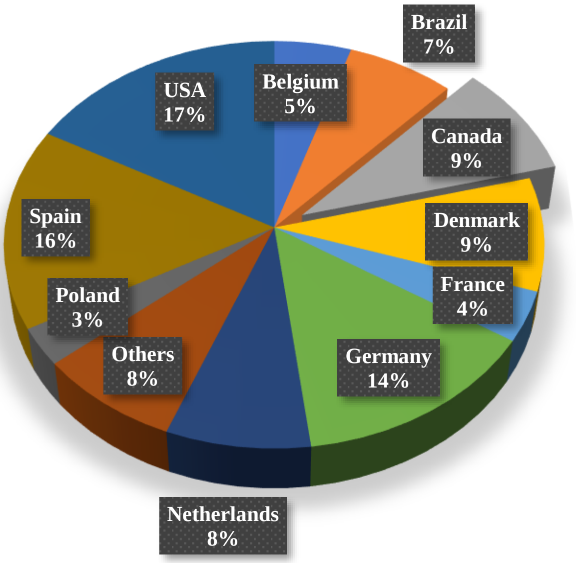
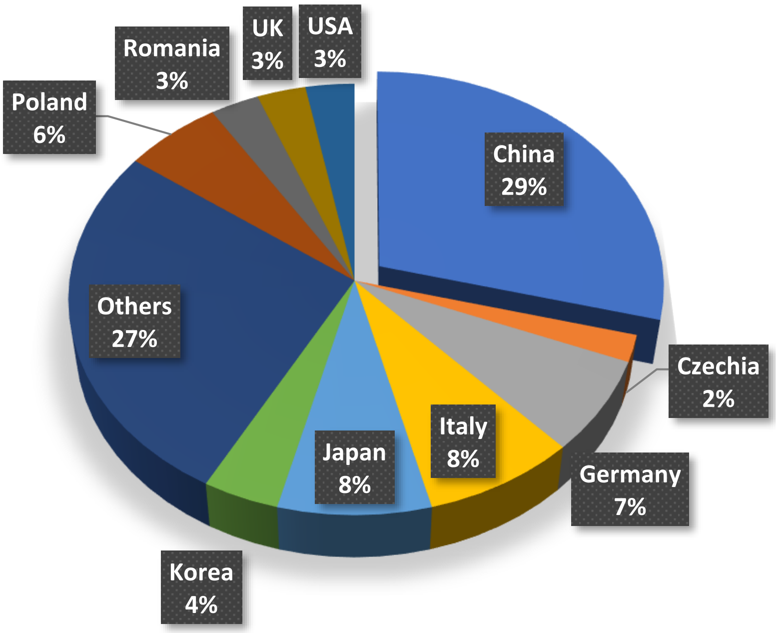


Figure 2.2 Global pork imports by various nations from 2019-2021 (OECD data)



2.2. THE SOYBEAN

Soybean, also known as soja bean or soya bean (*Glycine max*), is called the "miracle crop" due to its high oil content (18%), high-quality protein (about 40%), soil fertility improvement, high yield, and profitability. Soybean cultivation, with its origins in China, dates back 4000-5000 years, and it became a significant commercial crop in the United States in the nineteenth century (Tanwar and Goyal 2021). USA produced 50% of soybean till 1980s. However, Brazil a tropical food giant leads the soybean production (Table 2.1) followed by USA and Argentina (Pagano and Miransari 2016).

The global demand for soybeans is driving land use changes and deforestation in South America, increasing the carbon footprint of feed production (Świątkiewicz et al. 2021). Despite its negative environmental impact, soybeans are the top choice for protein feeds due to their quality and availability in monogastric animals, as its co-product SBM is the major protein source in diets (Tallentire et al. 2018). Approximately 85% of soybeans are processed into SBM and oil, and from that 98% of SBM is used as a protein source in animal feed and rest in soy proteins and flour (Modgil et al. 2021).

In Canada, soybeans have been grown on a limited basis in specific areas of southwestern Ontario, particularly near Windsor, since the 1920s. However, substantial expansion in the number of farms engaged in soybean production began during the mid-1970s. Between 1971 and 2021, soybean yields increased by 59% (Statistics Canada 2023). According to Soy Canada, Canada ranks as the world's seventh-largest soybean producer, with an annual output of 6.54 million metric tonnes. Most of the Canada's soybean harvest (Fig 2.4) is concentrated in Ontario (58%), Manitoba (22%) and Quebec (18%) (Soy Canada 2023).

Table 2.1 Soybean production, million tonnes, 2018-2022 (OECD data)

Country	2018	2019	2020	2021	2022
World	366	340	363	373	377
Brazil	120.7	126.3	132.9	134.3	136.5
USA	120.5	96.6	112.5	115.3	116.2
Argentina	55.3	49.0	48.0	50.9	51.1
China	15.9	18.1	19.6	19.9	20.3
India	13.2	11.2	12.6	12.8	12.9
Paraguay	8.7	10.5	10.0	10.2	10.4
Canada	7.4	6.1	6.3	7.2	7.3
Russia	4.3	4.3	4.2	4.2	4.3
Ukraine	4.4	3.7	2.8	3.1	3.1

Figure 2.4 Soybean production regions in Canada (Barthet and Puvirajah 2022)

Table 2.2 Oil and protein content (13% moisture) of 2023 Canadian oilseed-type soybeans on as-is basis (Canadian Grain Commission 2023)

	Oil (%)	Protein (%)
Eastern	21.8	39.0
Western	22.3	37.1

The expansion of soybean cultivation has nearly doubled in the past decade, mainly driven by the commercialization of short-season cultivars (Pinar 2023). Manitoba experiences a shorter growing season, spanning 65 to 135 frost-free days, while Ontario has an extended growing season of 90 to 190 days for soybean cultivation (Ort et al. 2022). Soybean plants require a certain duration to allocate energy and resources for protein synthesis, and this process is constrained by the limited time available during the shorter growing seasons in northern regions. Excessive early-season moisture or late-season drought, high temperatures during seed-fill, and cool temperatures, limiting nitrogen fixation can all affect soybean protein levels. These influences vary depending on regional soil types and their water retention capabilities (MPSG 2019).

Rotundo et al. (2016) reported a gradient of low-to-high protein levels in the United States from 2006 to 2013, extending from the northwest to the southeast not only due to geographical variation but also due to year to year weather variation. A study carried out in Brazil aimed to identify the environmental conditions (high and low altitude) that are conducive to optimal grain, protein, and oil yields in 28 different soybean cultivars. The results of the study indicated that high-altitude regions with lower temperatures can lead to a significant increase of 6.15% in protein content in the soybeans (Capelin et al. 2022). Possible reason for the less protein in Manitoba grown soybeans is attributed to lower mean temperatures and lower precipitation in northern regions (Cober and Morrison 2019). Genetic variations, which usually influence long-term trends, interact with environmental conditions to create distinctions between eastern and western Canadian soybeans. In Canada, breeding efforts primarily prioritize maximizing yield and enhancing agronomic factors. Quality factors like oil or protein content are not considered when registering new soybean varieties (Barthet and Puvirajah 2022).

Farmer’s net return was increased by 55% in Winnipeg, soybeans, soy oil and soymeal futures prices were up by 52%, 26% and 54%, respectively in 2021 compared to 2020, indicating the importance of meal component (Ferris 2021). In order to enhance the competitive positioning of soybeans in the global market and establish a positive image for Western Canadian soybeans, it is essential to determine nutritional content and bioavailability of locally produced soybeans for swine diets.

2.3. SOYBEAN MEAL

Soybean meal is a vital component of animal feed and plays a significant role in the global livestock industry. It is a byproduct of soybean oil production, obtained by extracting oil from soybean seeds (Liu and Proctor 1997; Modgil et al. 2021). SBM is highly palatable and easily digestible high-quality protein source that contains all nine essential amino acids that are essential for animal growth and development (Karr-Lilienthal et al. 2004). In swine diets, SBM is typically the primary source of protein, used in all phases of production, from gestation and lactation to nursery, grower, and finisher pigs (Manu and Baidoo 2020).

Soybean meal has a high crude protein content of 44-50%, depending on the processing method (Moheimani et al. 2018). While SBM is relatively deficient in B complex vitamins (Banaszkiewicz 2011), it remains a essential ingredient in the formulation of feed for monogastric animals. Additionally, SBM is a good source of fibre, which helps to maintain gut health and promote digestion.

Traditionally recognized for their protein content, yet the substantial carbohydrate component, which can constitute up to 40% of the meal (NRC 2012; Henchion et al. 2017), has often been overlooked. This carbohydrate fraction in SBM primarily comprises non-starch polysaccharides (NSP) and free sugars, at less than 1% (Choct 2015; Ruiz et al. 2020). These carbohydrates require specific enzymes for proper digestion, an enzymatic capacity largely absent in monogastric animals. Proteins, lipids, starch, vitamins, and minerals, forms fibrous complexes with NSPs leading to reduced absorption and digestion in the intestinal lumen (Wu 2017; Moore 2018). Pigs have physiological advantage over poultry in terms of lower digestive tract (Choct et al. 2010). Increased capacity and longer transit time favours NSP fermentation leading to higher metabolizable energy (ME) value in swine compared to poultry (Coon et al. 1990). By employing appropriate strategies, the negative effects of NSP and oligosaccharides in SBM can be mitigated and the nutritional value of this important feed ingredient can be maximized (Karr-Lilienthal et al. 2005; Faber et al. 2012).

Soybean lipids are a valuable component of animal diets, offering a range of benefits for animal health and performance. They are a concentrated source of energy containing essential fatty acids, and other nutrients (Nahashon and Kilonzo-Nthenge 2011). The addition of soybean lipids to animal diets can improve feed intake, weight gain, and overall performance (Leszczynski et al. 1992). Soybean oil can be used to improve the pelleting process in feed manufacturing and to bind feed ingredients together (Wood 1987). Soybean oil can also be used to coat feed particles, which can help to reduce dustiness and improve the palatability of feed (Hutjens 1998).

Soybean processing is an important step that can affect the quality of SBM. Achieving accurate heat processing is crucial for SBM, as overprocessing results into increase amount of Maillard reaction products leading to reduced digestibility of amino acids (Parsons 2000). Several reports analysed the effect of overprocessing or under-processing of soybean that affects SBM quality (Araba and Dale 1990; Lee et al. 1991; Parsons et al. 1992; Caprita et al. 2010; Dunmire et al. 2019).

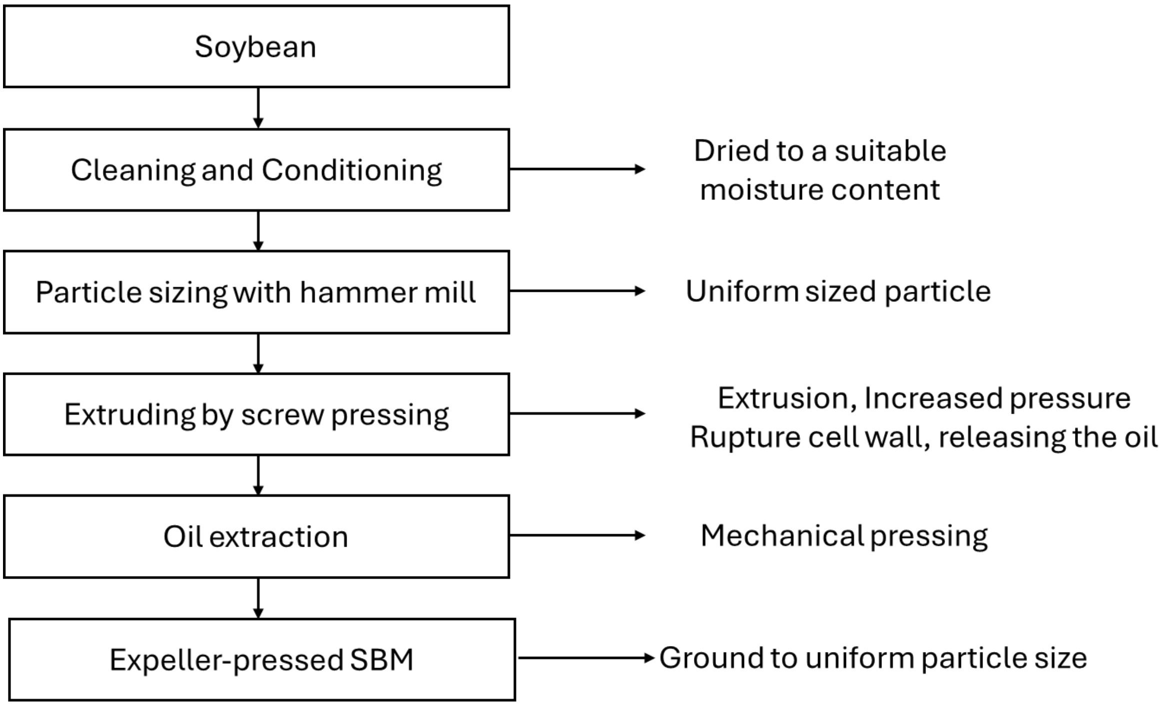
2.4. PROCESSING OF SOYBEAN

Soybean holds significant global value primarily due to the nutritional effectiveness of SBM as a food and feed component (Shea et al. 2020; Gupta and Manjaya 2022). There are two methods for oil extraction, which are mechanical pressing and solvent extraction (Aydeniz et al. 2014).

In the solvent extraction process, soybean seeds undergo a series of preparatory steps, including drying, cleaning, cracking, dehulling, conditioning, and flaking. Conditioning, performed at approximately 74°C, softens the soybeans, while flaking expedites the oil-extraction process (Liu and Proctor 1997). Hexane serves as the solvent for defatting the flakes, resulting in a meal with less than 1% oil and approximately 30-35% residual hexane, needing de-solventization at elevated temperatures (e.g., 105-115°C) (Paraíso et al. 2008). After the oil extraction process, what remains is referred to as defatted SBM, valued as an exceptional protein source for animal feed (Stein et al. 2008).

Extrusion is not widely used in the swine industry due to high costs. The production of SBE through mechanical pressing requires the preparation of soybeans through cleaning, conditioning, and drying. Following this, the soybeans are passed through a hammer mill for particle size reduction. Subsequently, they enter an extruder where a combination of feeding and compression takes place. Inside the extruder, extrusion occurs, cooking and expanding raw materials, rupturing the cell walls of the soybeans, releasing oil, and deactivating trypsin inhibitors, a key process in SBE production (Liu and Proctor 1997; Toomer et al. 2023). Even though the meal resulting from this process has a higher oil content, it lacks the hexane residues found in solvent-extracted meal that can influence the protein structure of SBE (Day and Swanson 2013; Mozafarpour et al. 2019).

Figure 2.5 Processing of soybeans to make expeller-pressed soybean meal



2.5. ANTI-NUTRITIONAL FACTORS IN SOYBEAN MEAL

Antinutritional factors (ANFs) are naturally occurring plant compounds that can interfere with the absorption of essential nutrients (Thangaraj 2019). Soybean meal contains ANFs such as phytate and glycoproteins as lectin and trypsin inhibitors (Pettersson and Pontoppidan 2013), which can reduce the nutritional value of SBM for humans and animals (Fuller 2004; Vagadia et al. 2017).

Anti-nutritional factors in SBM can be categorized into two main groups: thermolabile and thermostable (Liener 1994; Gu et al. 2010; Yasothai 2016; Wu 2017). Thermolabile ANFs include anti-vitamins, lectin as glycoprotein, and protease-inhibitors. Thermostable ANFs include saponins, tannins, flatulence factors, and phytate. Heat treatment can inactivate thermolabile ANFs in SBM, but the efficiency of inactivation depends on the duration, temperature, and moisture content of the samples (Vagadia et al. 2017).

Soybean meal contains approximately 2% trypsin inhibitors, which reduce the activities of proteolytic enzymes involved in protein digestion (Liener 1994; Vagadia et al. 2017). SBM contains two types of trypsin inhibitors: the soybean Kunitz trypsin inhibitor and the Bowman-Birk inhibitor. These inhibitors play a crucial role in regulating protein digestion and nutrient availability. Roasting is the most common method for inactivating trypsin inhibitors, but it is only 85% effective (Barać and Stanojević 2005).

Extrusion is another effective method for reducing protease inhibitors in soybeans. Other approaches include radiation (gamma and infrared), pressure drop (controlled) and ultrasound (Petres et al. 1990). Pre-treating soybeans with exogenous enzymes can also decrease protease inhibitors. A meta-analysis by Cowieson and Roos (2013) found that exogenous proteases can increase ileal amino acid digestibility by 2.7 to 5.4%. The study concluded that proteases were most effective when amino acid digestibility was low, with their efficacy doubling for every 10% decrease in ileal amino acid digestibility.

Soybean meal contains approximately 4% lectins, glycoproteins can negatively impact monogastric growth performance. In the small intestine, absorption of nutrients were reduced when lectin bind to the epithelial cells, reducing nutrient absorption and decreasing the immune response in animals, promoting colonization by coliforms (McDonald et al. 2002), affecting amino acid absorption and damaging the intestinal membrane (Liener 1994; Vagadia et al. 2017; He et al. 2018) . Lectin is responsible for growth retardation effect by at least 50% from raw SBM. Heat treatment is effective in destroying hemagglutinins, and adding moisture during the process enhances their inactivation compared to dry heat (Liener 1994). Therefore, proper processing of SBM is essential before consumption to eliminate lectins.

Tanin is polyphenolic in nature and under particular pH condition it forms tannin-protein complex (Siebert et al. 1996), causes intestinal mucosa damage, disorder in vit B12, glucose and iron absorption (Chung et al. 1998). As tannin is found mostly in soybean hulls, SBM prepared by de hulling process contains lower levels of tannins (Liener 1994).

Flatulence factors in human and animal nutrition are low molecular weight oligosaccharides (LMOs), such as raffinose and stachyose. These compounds are resistant to digestion in the small intestine and are fermented by the gut microbiota, producing gases like CO_2, H_2, and methane (Liener 1994; Choct et al. 2010). Notionally, in animal gut, α-galactose-containing LMOs, like stachyose and raffinose, can reduce amino acid digestibility through an osmotic effect (Choct et al. 2010). A study by Li et al. (2017) developed a prediction model to estimate amino acid digestibility in pigs based on stachyose and raffinose content, with high R^2 values for some amino acids. This confirms that stachyose and raffinose reduce amino acid digestibility.

Phytate, a cyclic compound with six phosphate groups, is a major concern in soybeans, where more than 50% of element P is bound to phytate (Liener 1994). Phytate constitute almost 1-1.5 g phytic acid/100g of dry matter of soybean and its products (Yasothai 2016). Phytate also chelates calcium, magnesium, zinc, and iron, making these minerals less absorbable and reducing the effectiveness of endogenous enzymes (Joye 2019; Thangaraj 2019; Samtiya et al. 2020; Wang and Guo 2021). Moreover, it likely hinders the effective utilization of various dietary nutrients such as proteins, starches, and lipids (Humer et al. 2015). Selle et al. (2000) reported reduced bioavailability of carbohydrate as the phytate tends to decrease the enzymatic activity of amylase, trypsin and pepsin. Various technologies have been utilized to improve the phytate degradation such as thermal treatment, mechanical processing, soaking/germination and fermentation (Humer et al. 2015). Exogenous enzymes, specifically phytases is targeted method to minimize the negative effects of phytate on the feeding value of feedstuffs (Humer et al. 2015). Mucosal phytase in stomach and intestine in pigs have negligible enzyme activity

(Schlemmer et al. 2001). Due to the limited capacity of endogenous mucosal phytase in monogastric animals to effectively hydrolyze phytate-bound phosphorus, the common strategy for enhancing mineral and nutrient absorption is the addition of exogenous microbial phytase to diets. Reports have demonstrated that phytase addition improves growth performance in pigs, but it can disrupt the balance of P, Ca, and dietary electrolytes (Selle and Ravindran 2007). On the other hand, phytic acid also exhibits anti-carcinogenic properties, inhibiting cancer cell proliferation (Kumar et al. 2010). This dual role of phytic acid highlights its importance in plant biology and human health. Furthermore, plant breeders select soybean genotypes with improved yield and seed viability while minimizing phytic acid content (Spear and Fehr 2007).

Understanding and addressing the impact of ANFs in soybeans is essential for optimizing the nutritional value of soy-based products and ensuring their positive contribution to human and animal health. Soybean variety if grown in different region, their chemical and nutritional composition of seed and co-products changes. Bioavailability of nutrients for animal is the key factor in determining the nutritive value of feedstuff. Digestibility of nutrients determined by measuring the disappearance of nutrients from the gastrointestinal tract. Over supplementation of nutrients leads to increased cost and over supplied nutrient excreted in manure causing environmental pollution especially in terms of Nitrogen and Phosphorous (Kornegay 1996). On the other hand, under supplying the nutrient could hampered the growth of animal and animal fails to manifest its true genetic potential. Phosphorous, energy and protein contents in diets are the main components that drives the feed cost. Therefore, current study evaluates Manitoba-grown SBE for their chemical composition and feeding value in terms of P and energy contents.

2.6. PHOSPHOROUS NUTRITION IN SWINE

Phosphorous is essential for animal health, impacting processes like reproduction, energy metabolism, and structural development. To meet these needs, inorganic P is commonly added to animal diets (Kebreab et al. 2013). However, overfeeding P can lead to inefficiency in P utilization, causing increased P excretion. Consequently, in commercially fed pigs, approximately 50% of the ingested P is excreted when diets lacking phytase supplementation are used (Zhang 1999). Furthermore, over-supplementing P can be costly and deplete non-renewable phosphate resources (Liu et al. 2019).

As previously mentioned, phytic acid is one of the ANFs that hinders the absorption of trace elements and affects the activity of gut enzymes. New varieties of oilseed grains have been created to reduce phytate concentration and enhancing P digestibility of an ingredient. For instance, low-phytate corn exhibited a 15% higher P digestibility compared to regular corn (Sands et al. 2001), and low-phytate SBM displayed a 26% higher P bioavailability in contrast to traditional SBM (Sands et al. 2003). Phytase is naturally present in various plant species. However, there is considerable variation in plant phytase activity among different plant species, leading to varying degrees of gastrointestinal phytate hydrolysis in monogastric animals. Their effectiveness is typically only about 40% when compared to phytase enzymes from microbial sources. For example, wheat's endogenous phytase exhibits 9% activity in the stomach, reducing to 2% in the small intestine (Schlemmer et al. 2001).

Majority of P is processed in the small intestine as large intestine has no significant role to play. Because of that, available P levels were found to be similar between entire digestive tract and ileum (She et al. 2017b). Thus, ATTD of P can be calculated by calculating retention of P by pig. Researchers uses either total collection method or index method to determine the P intake and P output in feces (Adhikari et al., 2016). Housing conditions, inclusion levels, the selection of feed ingredients, and the choice of markers can impact the calculation of digestibility. This leads to variations among authors when assessing P digestibility (Jang et al. 2014; Prawirodigdo et al. 2021; Zhai et al. 2022). The endogenous loss of P in pigs is influenced by their total dry matter (DM) intake, which results in inconsistent and non-reproducible findings. The NRC (2012) established the STTD of P for various feed ingredients by utilizing basal endogenous P losses of 190 mg/kg of DM intake to adjust the ATTD of P.

The improved digestibility of P in SBM during the early 2000s, compared to earlier varieties, can be attributed in part to alterations in the soybean itself, as suggested by Goebel and Stein (2011). Therefore, it is essential to periodically update P digestibility values for modern cereal grains. Table 2.4 shows list of authors conducted trials to test ATTD and STTD of P in SBM.

Sotak-Peper et al. (2016) procured SBM samples from 22 crushing plants in US and separated them based on growing areas into 4 zones: northern, eastern, western and Illinois. The study concluded that ATTD, STTD and content of P were not different among SBM samples from the different regions. Moreover, the study also reported phytase inclusion in diets increase ATTD of Ca and P, and STTD of P. She et al. (2018) evaluate corn, canola meal and SBM diets fed to growing pigs and found ATTD and STTD values to be 33.12% and 37.76%, 50.19% and 56.62%, 34.93% and 39.45%, respectively. Moreover, the study also found that the mixture of corn and SBM, corn and CM, and corn, SBM, and CM were not different in terms of STTD of P.

Almeida et al. (2017) studied the dose-dependent effects of microbial phytase (0-2,000 FTU/kg) on P digestibility in SBM. Their findings showed that phytase efficacy varied based on ingredient inclusion, which is important to consider while formulating complete diets for pigs. Using an exponential model, the estimated phytase dose needed for highest STTD of P was 160 FTU/kg for 55.5% in SBM. Similarly, She et al. (2017a) studied the graded level of microbial phytase in 4 sources of Canola meal (CM) and SBM and found that STTD of P in SBM was not different at 597, 1333 and 2133 FTU/kg phytase levels. Rojas and Stein (2012) explored the effect of fermentation and phytase inclusion on SBM. The study concluded that phytase inclusion increases the ATTD and STTD of P in conventional SBM but there was no difference between conventional SBM and fermented SBM when phytase was added in the diets. Goebel and Stein (2011) studied the effect of enzyme treatment on SBM by formulating 2 enzyme-treated SBM (HP-310; enzyme mixture devoid of phytase and HP-340; treated with enzyme mixture that includes phytase) and conventional SBM. They found that ATTD of P was improved in HP-340.

Akinmusire and Adeola (2009) aimed to determine the true digestibility of P in CM and SBM with 3 inclusion levels (13.2%, 26.4%, and 39.6%) while evaluating the impact of microbial phytase (0, 1000 FTU/kg) on total P digestibility. For SBM at 26.4% and 39.6% inclusion level the ATTD of P with 1000 FTU/kg, was doubled compared to diets devoid of phytase. Ajakaiye et al. (2003) also determine true digestive utilization of P with different SBM inclusion leading to 4 levels of P (0.098, 0.196, 0.293, and 0.391% on a DM). The STTD of P from SBM was calculated to be 51.3%. Dilger and Adeola (2006) research on conventional and low-phytate (LPA) SBM using regression method to estimate true P digestibility and endogenous P loss and found total track digestibility to be 45.15% and 62.11%, respectively. Similarly, Hill et al. (2009) studied additive effects of LPA corn, LPA SBM and phytase resulted into decrease in P excretion by 42% compared to conventional corn and SBM diets.

Phosphorous digestibility for SBM is extensively researched in swine nutrition. It reflects the variation exists among soybean varieties and how the digestibility values were influenced by ingredient inclusion rate, phytate content in soybeans and phytase levels in diets.

Eutrophication, driven by P pollution, is a major global concern. In 2003, farmers in Manitoba for the first time began incorporating phytase into swine diet formulations. By 2010, a remarkable 95% of the finisher feed in the region contained phytase (Sheppard 2019). The main concern related to P in Manitoba pertains to the eutrophication of rivers, lakes, and, notably, Lake Winnipeg, as highlighted by the Manitoba Phosphorus Expert Committee (2006). Establishing standardized values for P digestibility for SBE derived from Manitoba-grown soybeans enables nutritionists to create diets that fulfil the P requirements of pigs while concurrently reducing P excretion.

Table 2.3 Calcium and phosphorus concentrations and apparent and standardized total tract digestibility of phosphorus in soybeans, soybean meal (SBM), and other soybean products (as-fed basis), NRC (2012)

Product	Full-fat soybeans	Dehulled SBM	Non-dehulled SBM	Enzyme treated SBM	Fermented SBM	Soy protein concentrate	Soy protein isolate
Total P, %	0.53	0.71	0.64	0.75	0.80	0.82	0.75
ATTD P, %	39	39	39	60	60	39	39
STTD P, %	48	48	48	66	66	48	48
Phytate bound P, %	0.33	0.38	0.36	-	-	-	-
Phytate bound P, % of total P	62.3	53.5	56.3	-	-	-	-
Non-phytate P, %	0.2	0.33	0.28	-	-	-	-
Non-phytate bound P, % of total P	37.7	46.5	43.8	-	-	-	-
Total Ca, %	0.31	0.33	0.35	0.31	0.3	0.32	0.17
ATTD Ca, %	-	62.9	-	60.9	50.7	-	-

Table 2.4 Published ATTD and STTD of P in soybean meal fed to growing pigs.

Ingredient	Phytase level	ATTD	STTD	Reference
SBM		50.90	56.80	(She et al. 2015)
SBM		37.20	44.80	(Kong et al. 2021)
SBM		50.19	56.62	(She et al. 2018)
Corn-SBM		42.07	45.81	
SBM		41.10	44.50	(Dilger and Adeola 2006)
Low phytate SBM		58.10	62.60	
SBM	0	59.54	65.67	(She et al. 2017a)
	597	76.94	82.37	
	1333	83.77	89.90	
	2133	84.14	89.90	
SBM inclusion levels				
13	0	34.33	40.85	(Akinmusire and Adeola 2009)
26	0	36.33		
40	0	38.63		
13	1000	68.01	70.77	
26	1000	70.42		
40	1000	71.19		
Low phytic acid corn + normal SBM	0	38.40	-	(Hill et al. 2009)
	500	46.80		
Low phytic acid corn + LPA SBM	0	48.20		
	500	60.10		

SBM HP 310	0	59.80	-	(Goebel and Stein 2011)
	500	77.70		
SBM HP 340	0	83.80		
	500	87.70		
Conventional SBM	0	65.50		
	500	79.50		
Fermented SBM	0	60.90	65.50	(Rojas and Stein 2012)
	800	67.50	71.90	
Conventional SBM	0	41.60	46.10	
	800	66.20	66.20	
Zone 1: Northern (Michigan, Minnesota, and South Dakota)	0	50.61	56.96	(Sotak-Peper et al. 2016)
	500	65.89	72.23	
Zone 2: Eastern (Georgia, Illinois, Indiana, and Ohio)	0	51.42	57.56	
	500	64.61	70.81	
Zone 3: Western (Iowa, Missouri, and Nebraska)	0	49.69	55.74	
	500	63.58	69.88	
SBM	0	-	36.70	(Almeida et al. 2017)
	125		51.00	
	250		64.80	
	500		54.30	
	1000		59.90	
	2000		55.50	

2.7. ENERGY SYSTEM IN SWINE

Feed accounts for nearly 70% of the production cost in swine and nearly 35% of the cost accounts for supplying energy to the animal (Noblet et al. 2022). Therefore, it is of high importance to determine with precision the energy needs of the animal to maintain the highest efficiency in costs and growth performance.

Gross energy (GE), which indicates the energy released when burning feed, does not account for digestibility and is not commonly utilized by nutritionists in feed formulation. Instead, digestible energy is calculated by comparing the energy content in the consumed feed with that in feces, offering a more accurate measure (Velayudhan 2013). However, metabolizable energy (ME) offers advantages over digestible energy (DE) as it accounts for energy losses in urine.

The first systematic efforts to estimate metabolizable energy were made by (Atwater 1891). The ME of the feed can be calculated subtracting urinary energy in the form of N from the DE. For the trial, urine is separately collected minimum for 3 days in digestibility crates. Energy content can be derived using bomb calorimeter. However, the procedure is time consuming with low accuracy, and therefore a prediction equation was proposed for growing pigs from urinary N content: Urinary energy (J) = 0.19 + 0.031* urinary N (Noblet and Van Milgen 2004; Noblet et al. 2022).

Net energy (NE) system is a more recent approach for calculating dietary energy, aiming to determine the NE consumed by animals after deducting heat increment (HI). Metabolizable energy (ME) content (k) also known as utilization of ME for NE is an important variable depicting the efficiency of ME utilization by different nutrients. For CP, k is 60%, while for fat it is 90%, resulting in increased HI (per unit of energy) with metabolic utilization of CP compared to fat (Noblet et al. 1999). Nevertheless, nutritionists continue to formulate diets using the ME system. Reasons for this includes insufficient research supporting the advanced

NE system, as measurements of NE is complex and requires specific equipment and expertise. However, A simple NE system using a validated prediction equation can be applied using DE or ME values of feeds, eliminating the need for complex NE measurements (Noblet et al. 2022).

The investigation on SBM revealed several factors affecting energy content (Table 2.6). Goebel and Stein (2011) studied enzymatic treatment of SBM, which altered its composition but did not significantly affect DE and ME values. Similarly, Rojas and Stein (2012) explored the use of fermented soybean meal (FSBM) as an alternative to conventional SBM in animal diets, found that FSBM could be a suitable substitute without impacting energy contents. Rodriguez et al. (2020) reported differences between SBE and SBM, with SBE having lower CP and amino acids but higher acid hydrolyzed ether extract (AEE), resulting in increased concentrations of DE, ME, and NE in SBE compared to SBM. Baker and Stein (2009) used high protein content, low oligo saccharides and conventional type extruded expelled SBM and reports no difference in DE and ME between low oligosaccharides SBM and conventional SBM. Velayudhan (2013) compared IC and prediction equation methods to calculate NE content of DESBM using 3 dietary formulation designs and reported least disparity of NE values between two methods with constant corn: SBM formulations.

Lopez et al. (2020) conducted a comparative analysis of SBM from different countries, emphasizing the impact of regional disparities on energy values. The study reports Less variability exist between SBM from Argentina, Brazil and USA. For United States, Sotak-Peper et al. (2016) highlighted regional differences and suggested that existing feed composition tables might underestimate SBM energy values, affecting diet formulation. The study also reported NE content of SBM using prediction equations.

Table 2.5 Concentration of energy (kcal/kg) in soybeans, soybean meal (SBM), and other soybean products (as-fed basis) (NRC 2012)

Product	Full-fat soybeans	Dehulled SBM	Non-dehulled SBM	Extruded-expelled SBM	Enzyme-treated SBM	Fermented SBM	Soy protein concentrate	Soy protein isolate
Gross energy	5227	4256	4257	4692	4451	4533	4605	5386
Digestible energy	4193	3619	3681	3876	3914	3975	4260	4150
Metabolizable energy	3938	3294	3382	3573	3536	3607	3817	3573
Net energy	2874	2087	2148	2344	-	-	2376	2187

Table 2.6 Published energy content of soybean expeller or soybean meal fed to pigs

		DE	ME	NE	NE*	Author
Dry extruded-expelled SBM (DESBM)		3384	3324	2652	2624	(Velayudhan et al. 2015)
Conventional extruded expelled SBM		3827	3620			(Baker and Stein 2009)
Conventional Solvent extracted SBM		3845	3672			
SBE		4306	4124		2736	(Rodriguez et al. 2020)
SBM		3749	3515		2194	
SBE		4591	4099	3189		(Koo et al. 2021)
Heat treated SBE		4222	3692	3234		
SBE		4497	4301			(Cristobal. et al. 2023)
SBE		4352	4105			(Woodworth et al. 2001)
SBM		4572	4339			(Berrocoso et al. 2015)
SBM		4020	3756			(Park et al. 2021)
SBM from different countries	Argentina	4139^{a}	3903^{a}			(Lopez et al. 2020)
	Brazil	4018^{ab}	3795^{ab}			
	China	3989^{b}	3769^{ab}			
	India	3812^{c}	3566^{c}			
	USA	4001^{b}	3750^{b}			
Fermented SBM		4040	3627			(Espinosa et al. 2020)
SBM		4131	3900			

Short-time enzyme-treated SBM		4333	3926			(Goebel and Stein 2011)
Normal-time enzyme-treated SBM		4316	3914			
SBM		4347	3980			
SBM		4306	3896			(Liu et al. 2016)
SBM autoclaved at different temperature						
Autoclaving 110℃	15	3991	3704			(Oliveira et al. 2020)
	30	4039	3704			
Autoclaving 150 ℃	3	3776	3441			
	6	3752	3346			
	9	3561	3202			
	12	3346	2868			
	15	2987	2557			
	18	3035	2557			
SBM derived from different zones in USA						
Zone 1: Northern		4343^{a}	4096^{ab}		2534^{a}	(Sotak-Peper et al. 2015)
Zone 2: Eastern		4319^{a}	4117^{a}		2497^{ab}	
Zone 3: Western		4136^{b}	3926^{b}		2391^{b}	
Zone 4: Illinois		4247^{ab}	4038^{ab}		2448^{ab}	
SBE		3876	3573		2344	(NRC 2012)

[a,b, c] Means not sharing a common superscript are significantly different.

NE*: Net energy values predicted by equations (Noblet et al. 1994)

Zone 1: Northern (Michigan, Minnesota, and South Dakota), Zone 2: Eastern (Georgia, Indiana, and Ohio), Zone 3: Western (Iowa, Missouri, and Nebraska), Zone 4: Illinois

Overall, based on the literature NE content in SBM is variable and it is affected by the region where soybeans were grown, the type of processing conditions used and the use of enzyme. Therefore, it is necessary to evaluate the GE, ME and NE content of Manitoba-grown SBE to efficiently formulate the swine diets.

2.8. THESIS HYPOTHESIS AND OBJECTIVES

Ingredient evaluation should be done to define the feeding value of an ingredient. Current study aims to determine the P digestibility and energy contents of Manitoba-grown SBE. Hypothesis of the study was SBE from Manitoba has similar energy content, ATTD and STTD of P as compared to the Ontario SBE, when fed to growing pigs.

The overall objectives of studies were:

1. To determine ATTD of P and Ca, and STTD of P in western-prairie derived SBEs fed to growing pigs.
2. To determine DE, ME and NE content of western-prairie derived SBEs fed to growing pigs.

3. MANUSCRIPT -1: DETERMINATION OF STANDARDIZED TOTAL TRACT PHOSPHORUS DIGESTIBILITY IN CANADIAN PRAIRIE GROWN EXPELLER-PRESSED SOYBEAN MEAL FED TO GROWING PIGS.

S. S. Patel[1], J. Lee[1], N. Mohamed[2], C Yang[1], J. House[2], R. Patterson[3], and C. M. Nyachoti[1]

[1]Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2; [2]Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; [3]CBS Bioplatforms, Inc., Calgary, Alberta, Canada T2C 0J7

Contribution of authors:

SP: Development of protocol, Designed, implement and conducted the animal trial, laboratory analyses, data collection, and prepare first draft of manuscript; JL: participated in experimental design, data analysis and reviewed manuscript; NM: Participated in acquisition of research fund, laboratory analyses, and reviewed manuscript; CY: Acquisition of research fund, conceptualization of research project and reviewed the paper; JH: Acquisition of research fund, conceptualization of research project and reviewed the paper; RP: Reviewed development of animal protocol and reviewed manuscript; CMN oversaw the research project and had primary responsibility for the final content; and all authors: read and approved the final manuscript.

3.1. ABSTRACT

The objectives of this study were 1) to determine the apparent total tract digestibility (ATTD) of calcium (Ca) and phosphorous (P) and the standardized total tract digestibility (STTD) of P in soybean expeller (SBE) and 2) to examine the effects of microbial phytase supplementation on ATTD and STTD of P in SBE. A total of 48 growing barrows (15.5 ± 0.7 kg, mean ± SD) were assigned to 1 of 8 dietary treatments (4 with 500 phytase units/kg and 4 without phytase) in a completely randomized design to give 6 replicates per treatment. SBE was the only source of P in the diets. Four SBE were used in the experiment, with three derived from soybeans grown in Manitoba (MB1, MB2, and MB3) whereas the fourth was derived from soybeans grown in Ontario. The experiment was conducted over 3 consecutive 12 d periods; within each period the initial 7 d were for adaptation followed by 5 d for total fecal collection using the marker to marker procedure. Data were analyzed using the MIXED procedure of SAS. Fixed effects were the type of SBE and phytase whereas period was included as a random effect in the model. Dry matter (DM), Ca and P levels were analyzed in diets and feces. The ATTD and STTD of P were not affected by the source of SBE. Phytase supplementation significantly increased ($P < 0.001$) P digestibility in all SBE types. The ATTD and STTD values of P with phytase supplementation averaged 72.8% and 79.7%, respectively. There was no interaction effect between type of SBE and phytase supplementation. The ATTD of Ca ($P < 0.001$) in MB2 was significantly lower (55.7%) than in the other SBE whose values averaged 71.6%. Ca digestibility was not affected by phytase supplementation. In conclusion, the ATTD and STTD of P were not different among the SBE types and averaged 63.0% and 69.9%, respectively. Phytase supplementation increased the P digestibility in SBE irrespective of source.

Key words: digestibility, phosphorous, phytase, pig, soybean expeller

3.2. INTRODUCTION

Understanding the nutritional value of soybean meal (SBM) in swine nutrition remains essential for optimizing swine health and production. Soybean meal, renowned for its rich protein content and balanced amino acid profile, serves as a cornerstone in swine diets, offering crucial nutrients for growth and feed efficiency (Pluske 2012). The variations in structural component of soybean affect how phytate and other component interact, and the structural variation is influenced by environmental conditions, geographical location, season, and variety. (Medic et al. 2014; Gaudin et al. 2015). Recognizing and examining the intricacies of soybean composition hold significant implications for advancing swine nutrition and production strategies.

Phosphorus (P) plays a crucial role in the nutrition of pigs, influencing various physiological processes, including bone formation and energy metabolism. However, the majority of P in plant-based feed ingredients is bound to phytate (phytic acid), a complex molecule that restricts its bioavailability to monogastric animals, such as pigs (Selle and Ravindran 2008). The phytate-P complex constitutes about two-thirds of P in plant-based meals like cereals, grains, and legumes. It is present at a concentration of approximately 1 to 1.5 g per 100 g of dry matter (DM) in soybean and its products (Yasothai 2016), playing a significant role in reducing the bioavailability of essential minerals like P, Ca, Zn, Mg, Fe, and K (Modgil et al. 2021). Additionally, phytate is a potent chelating agent that negatively affects the bioavailability of proteins by forming complex structures with various nutrients including amino acids, and carbohydrates by inhibiting gastric enzymatic activity (Selle et al. 2006).

The limitations caused by phytate in P absorption can have substantial repercussions for pig diets, notably reduced growth performance and elevating nutrient excretion (Metzler and Mosenthin 2008). To address this challenge, pig diets often grapple with reduced P absorption, which subsequently affects the overall efficiency of the animals' growth and increases nutrient waste. To mitigate this issue, the incorporation of microbial phytase enzymes in pig diets has been a strategic response, effectively enhancing the digestibility of P, thereby improving nutrient utilization within the animals' digestive systems (Poulsen et al. 2010; Rojas and Stein 2012). The determination of P digestibility is critical for formulating nutritionally balanced and cost-effective diets for pigs. Values for ATTD and STTD of P in different types of SBM were reported for pigs (Dilger and Adeola 2006; Goebel and Stein 2011; Rojas and Stein 2012; Sotak-Peper et al. 2016; She et al. 2017a; She et al. 2018; Lee and Nyachoti 2021). However, to our knowledge, there are no published reports on the the ATTD and STTD of P for Manitoba derived soybean meal expeller (SBE). The first objective of this study was to test the hypothesis that soybeans from either eastern or western Canadian regions possess similar concentrations of phytate, P, and Ca, potentially resulting in comparable ATTD and STTD of P in SBE. The second objective is to investigate whether the addition of microbial phytase enhances the ATTD and STTD of P in SBE, irrespective of the region in which the soybeans were grown.

3.3. MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC 2009) (F18-038/1/2/3, AC11419).

3.3.1. ANIMALS AND HOUSING

Forty-eight 6-week-old growing barrows [Tempo × TN70; Topigs Norsvin, Winnipeg, MB, Canada] with an average initial body weight (BW) of 15.5 ± 0.7 kg (mean ± standard deviation (SD)) were obtained from the University of Manitoba's Glenlea Research Unit. Pigs were individually housed in metabolic crates (118 cm × 146 cm) in a temperature controlled room (22°C ± 2°C). Metabolic crates were equipped with a feeder and nipple drinker, a fully slatted floor, a screen floor for total feces collection and a urine tray for the total urine collection.

3.3.2. EXPERIMENTAL DIETS

All nutrients including vitamins and minerals except P were provided to meet or exceed requirements for growing pigs (15.5 ± 0.7 kg, mean ± SD) (NRC 2012). Three samples of Manitoba SBE based on their CP content were obtained from a nearby soybean crushing plant (Delmar Commodities, Winkler, MB, Canada) and labelled as Manitoba commercial (MB1), Manitoba commercial 2 (MB2) and Manitoba commercial 3 (MB3). Additionally, one sample of SBE was acquired from Ontario (ON) (Grand Valley Fortifiers, Cambridge, ON, Canada) as a control. Eight diets were formulated using the 4 types of SBE (Table 3.1). Four diets lacked microbial phytase supplementation, while the other four contained 500 microbial phytase (CBS Bioplatforms, Inc., Calgary, Alberta, Canada) units (FTU/kg). The daily amount of feed fed to pigs was calculated at approximately 2.8 times the maintenance energy requirement (i.e., 197 kcal ME/kg $BW^{0.60}$; NRC, 2012) based on their BW on d 1 and d 10. Pigs were fed once daily at 0900 h and had free access to drinking water throughout the study.

Table 3.1 Diet formulation and analyzed composition.

	MB1		MB2		MB3		ON	
Diets	A	B	C	D	E	F	G	H
Phytase	0	500	0	500	0	500	0	500
Cornstarch	39.27	39.27	39.27	39.27	39.27	39.27	39.27	39.27
Sucrose	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Soybean expeller	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Vegetable oils	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66
Salt	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Vitamin-mineral premix[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys-HCl	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
DL-Met	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
L-Thr	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Analyzed Composition of Diets								
DM, %	92.88	92.89	92.68	92.55	93.14	93.12	93.04	93.32
GE kcal/kg, DM	4592	4578	4620	4613	4595	4615	4638	4604
P, % DM	0.28	0.27	0.28	0.27	0.28	0.27	0.25	0.26
Ca, % DM	0.77	0.79	0.74	0.74	0.73	0.75	0.83	0.81
Phytase activity[a]	213	638	106	618	280	560	242	599

[a] Phytase activity analysed using ISO 30024:2009 method.

[b] Supplied the following per kg of complete diet: vitamin A, 1500 IU; vitamin D, 400 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B_6, 2.5 mg; biotin, 70 µg; vitamin B_{12}, 20 mg; Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg.

Where, MB1= Manitoba commercial 1, MB2= Manitoba commercial 2, MB3= Manitoba commercial 3 and ON= Ontario

3.3.3. EXPERIMENTAL DESIGN

The experiment was conducted in a completely randomized design. The study was carried out in 3 consecutive periods using 48 pigs to get 6 replicates per treatment. For each period a total of 16 growing pigs were obtained at 4 weeks of age. Each period consisted of 15 d; the first 10 d were for adaptation, and the last 5 d were for sample collection. Fecal samples were collected according to the marker-to-marker procedure (Adeola 2001). On d 10 and 15 (0800 h), pigs received 100 g of their assigned experimental diet with 5 g of ferric oxide (Sigma-Aldrich, St. Louis, MO), and the remaining portion of feed was provided after pigs finished consuming the feed. Metabolic crates were washed after feeding the assigned diets in the morning on day 8, and fecal collection was started when the marker appeared in feces. Also, the collection of feces was terminated when the marker appeared in feces after day 15. Fecal samples were collected once daily in the morning and immediately stored at −20 °C until further analysis.

3.3.4. CHEMICAL ANALYSES

The fecal samples were dried in a forced air oven at 60°C and pooled per pig. Diet, ingredient, and fecal samples were ground using a coffee grinder (Smart Grind; Applica Consumer Products Inc., Miami Lakes, FL, USA) before analysis. All analytical procedures were conducted in duplicates. DM was determined in ingredients, diets and fecal samples following AOAC guidelines (method 934.01; 2006). For DM assessment, 1 g of the sample was carefully weighed in a pre-weighed silica dish and subjected to overnight drying at 104°C in an oven. The following day, the sample was taken out, allowed to cool in a desiccator, and re-weighed. Gross energy content in the experimental diets was assessed using an adiabatic oxygen bomb calorimeter (Model 6300; Parr Instrument Co., Moline, Illinois), with benzoic acid serving as the calibrating standard. Additionally, both ingredients and diets were analyzed for CP following AOAC protocols (2006) utilizing an N analyser.

Total P and Ca analyses in ingredients, feed, and feces followed the outlined procedures (method 985.01; AOAC, 2007) and were examined using an inductively coupled plasma (ICP) spectroscopy (Varian Inc., Palo Alto, CA). For Ca and P analyses, approximately 1 g of the sample was measured and placed in a labelled tube without a screw cap. The samples were ashed overnight in a furnace at 600°C. Following ashing, the tubes were allowed to cool before acid digestion. A mixture of 10 mL of 5N HCl/HNO_3 (1% v/v) was added to the tubes, capped with screws, and digested for 1 h in a sonication bath preheated to 70°C. After cooling the samples from the sonication bath, all digested sample (1 mL for feces and 10 ml for ingredients and diets) was pipetted and diluted with deionized water using a 100 ml volumetric flask. Subsequently, the samples were filtered into 20 ml scintillation vials. Then the samples were sent for ICP analysis. Phytic acid in each ingredient was determined using the Megazyme Phytic acid kit (K-PHYT; Cedarlane, Burlington, ON, Canada). The concentrations of phytate-bound P in test ingredients were calculated as previously described by Tran et al. (2004).

Table 3.2 Analysed composition of soybean meal expeller (DM basis)

	MB1	MB2	MB3	ON
CP %	41.0	40.7	41.8	45.3
Ca %	0.27	0.27	0.26	0.35
P %	0.71	0.63	0.65	0.71
Phytate P[1]	0.29	0.25	0.28	0.28
Non-Phytate P[2]	0.42	0.38	0.37	0.43

[1] Phytate-bound phosphorus was determined as 28.2% of phytate (Tran and Sauvant 2004)

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

Where, MB1= Manitoba commercial 1, MB2= Manitoba commercial 2, MB3= Manitoba commercial 3 and ON= Ontario

3.3.5. CALCULATIONS AND STATISTICAL ANALYSIS

The ATTD of Ca and P in experimental diets were calculated as outlined by Petersen and Stein (2006) using the following equation:

$$ATTD = ([P_i - P_f]/P_i) \times 100,$$

Where, ATTD represents the apparent total tract digestibility values in percentages; P_i / Ca_i represents the total P / Ca intake (g) from d 11 to d 15 of the experimental period, and P_f represents the total fecal P / Ca output (g) that originated from the feed fed from d 11 to d 15. The ATTD of P was corrected for basal endogenous losses of P to derive STTD of P according to the equation as follows:

$$\text{STTD of P} = [Pi - (Po - \text{basal endogenous losses of P})] / Pi$$

A basal endogenous P loss was assumed to be 190 mg/kg DM intake (NRC, 2012).

Data were analyzed using the MIXED procedure of SAS. Diet was treated as the fixed variable and period was added as random effect. The individual pig was considered the experimental unit. The model contained main effects of the diet, phytase supplementation and their interaction. Least squares mean for each treatment were calculated using the LSMeans procedure in SAS, and if significant differences were detected, means were separated using the PDIFF option with the Tukey adjustment. Results were considered significant at $P \leq 0.05$ and considered a trend at $0.05 \leq P \leq 0.10$.

3.4. RESULTS

All pigs remained visibly healthy and readily consumed their daily ration throughout the experimental period. The average body weight on d 10 of the experiment was at 18.2 ± 0.14 kg (mean ± SEM). The detailed compositional analysis of the various SBE types evaluated in this study is shown in Table 3.3.

Diets that contained phytase were formulated to contain 500 FTU/kg, and the analysed value were 638, 618, 560 and 599 FTU for MB1, MB2, MB3 and ON, respectively. The DM content for the diets based on Manitoba and Ontario SBE averaged 92.95% and 93.18%, respectively, and the content was similar among diets. ATTD and STTD of P demonstrated no difference irrespective of the SBE source. However, the inclusion of phytase led to a significant increase in digestibility values in all SBE types. In the absence of supplemental phytase, STTD values for MB1, MB2, MB3, and ON diets were 58.06%, 56.20%, 63.80% and 62.67%, respectively. Conversely, with phytase supplementation, P digestibility substantially improved, resulting in values of 75.40%, 80.06%, 80.40% and 82.35% for MB1, MB2, MB3, and ON based SBE diets, respectively. Notably, the variation in SBM types did not affect both feed and P intake. However, the addition of phytase corresponded to increased ($P < 0.05$) feed consumption and consequently elevated trend ($P < 0.10$) for P intake. As P absorption increased ($P < 0.01$) due to dietary phytase supplemented diets, a notable reduction in P excretion was observed ($P < 0.01$). There was no interaction between type of SBE and phytase supplementation ($P > 0.10$).

Table 3.3 Apparent (ATTD) and standardized (STTD) total tract digestibility of P in Canadian SBE based diets supplemented without and with 500 U/kg of phytase.

	MB1		MB2		MB3		ON		SEM	SBM	Enzyme	SBM* Enzyme
	0	500	0	500	0	500	0	500				
DMI g/d	720	721	716	726	712	735	700	734	4.061	0.954	0.045	0.481
P input g/d	1.97	1.98	1.98	2.00	1.99	2.02	1.93	2.02	0.011	0.651	0.071	0.548
P output g/d	0.93	0.62	1.00	0.54	0.86	0.54	0.85	0.50	0.031	0.085	<0.001	0.330
Absorbed P g/d	1.04	1.37	0.98	1.46	1.14	1.49	1.08	1.53	0.035	0.120	<0.001	0.435
P ATTD %	51.16	68.94	49.29	73.16	56.90	73.49	55.76	75.45	1.627	0.055	<0.001	0.446
P STTD[a] %	58.06	75.84	56.20	80.06	63.80	80.40	62.67	82.35	1.627	0.055	<0.001	0.447
Ca input/d	5.92	6.59	5.81	5.81	5.54	5.92	6.43	6.37	0.060	<0.001	0.002	0.003
Ca output/d	1.82	1.81	2.66	2.49	1.61	1.49	1.93	1.82	0.083	<0.001	0.484	0.986
Ca ATTD %	69.30	72.65	54.28	57.14	71.04	74.93	70.08	71.65	1.378	<0.001	0.409	0.873

[a] Values for the basal endogenous loss of P were assumed at 190 mg/kg dry matter intake (NRC, 2012) to calculate STTD of P by correcting the ATTD values.

3.5. DISCUSSION

Among the three types of Manitoba SBE, average P content was 0.66% (DM) as compared to Ontario SBE 0.71% (DM). For Manitoba SBE average total P content was similar to values reported by Sotak-Peper et al. (2016), Rojas and Stein (2012), Goebel and Stein (2011) and NRC (2012). The results from a meta-analysis study about the total P content of SBM from different countries were in agreement with the values reported for the USA (0.67%) and Argentina (0.675%), while higher than those for India (0.57%) and Brazil (0.62%) (Ibáñez et al. 2020). Phytate bound P values were 0.27% which was lower than the values reported by Sotak-Peper et al. (2016); 0.42% and NRC (2012); 0.36%. Decrease in the phytate bound P compared to reported values by other authors might be due to genetic improvement of soybean variety over the time.

Variations in mineral levels within the soil can affect the mineral content in soybeans and subsequently in SBM (Sotak-Peper et al. 2016). Furthermore, discrepancies in the analytical methods employed can lead to differing mineral measurements in feed components. Nonetheless, most of the mineral values observed in the SBE utilized in this study aligned with the values documented by the NRC (2012).

Phosphorus digestibility in pigs depends on several factors such as dietary Ca, P, and phytate contents, animal factors such as breed, age and sex and feeding regime (Rosenfelder-Kuon et al. 2020). Results for ATTD and STTD of P in diets without phytase supplementation was similar to the results reported by Rojas and Stein (2012) but P digestibility was higher in the phytase containing diets in current study. In the current study, the ATTD and STTD were higher than the values reported by Akinmusire and Adeola (2009); Almeida and Stein (2010); Rojas and Stein (2012). Phytase inclusion in SBE diets increased P digestibility by 9 to 30% in published reports (Akinmusire and Adeola 2009; NRC 2012; Sotak-Peper et al. 2016; She et al. 2017a). In the current experiment, the increase in ATTD and STTD of P was 19% when microbial phytase was added in SBE diets, which is lower than values reported by Akinmusire and Adeola (2009); 30%, Almeida and Stein (2010); 26%, Rojas and Stein (2012); 25%, but higher than Goebel and Stein (2011); 14%, Sotak-Peper et al. (2016); 12%, She et al. (2017a); 16%, Almeida et al. (2017); 17%. The ATTD and STTD of P increased in SBE diets as phytase supplementation hydrolysed the phytate which lower the inositol phosphates, increasing the availability of P simultaneously decreasing the antinutritional effect of phytate (Zeller *et al.,* 2015; Rosenfelder-Kuon *et al.,* 2020). Moreover, differences in P digestibility arise from factors such as diet quality, inclusion of mineral P, composition of feed, and feeding practices, whether ad libitum or restrictive. Important aspects such as feed processing techniques like pelleting and feed acidification, as well as critical nutrients like zinc (Zn) and copper (Cu), significantly impact phytase efficacy, highlighting the complexities influencing P utilization (Blavi et al. 2017; Rosenfelder-Kuon et al. 2020).

The ATTD of Ca in MB2 was significantly lower (55.71%) than other SBE diets (~ 71.60%). The ATTD of Ca was not affected by phytase inclusion in SBE diets. However, most of the literature reports that the ATTD of Ca was increased by 6% to 11% due to the addition of microbial phytase (González-Vega et al. 2015; Sotak-Peper et al. 2016). Calcium from limestone (Calcium Carbonate) tends to bind with phytase, thereby reducing the solubility or digestibility of Ca (González-Vega et al. 2015), this could be a possible explanation for the lack of phytase effect on ATTD of Ca. Ideal Ca: P ratio is 2:1 for complete diets (NRC 2012). However, for the current study with 2.4:1 Ca: P ratio, SBE diets contains ~6.8 g/kg and ~2.9 g/kg of P and Ca, respectively. The Ca: P ratio was higher in the diets as SBE was the only source of P, while Ca was supplied to meet the NRC requirements.

In conclusion, Ca, P and phytate concentrations in prairie derived SBEs were not different than the SBE derived from Ontario. The ATTD and STTD of P for the SBE diets utilized in the present study showed that P digestibility among MB1, MB2, MB3 and ON were not different.

3.6. CONCLUSION

Phytase supplementation significantly increased ($P < 0.001$) P digestibility in all SBE types. The ATTD and STTD values of P with phytase supplementation averaged 72.8% and 79.7%, respectively. There was no interaction effect between type of SBE and phytase supplementation. This suggests that Manitoba-derived SBE can be incorporated at the same proportion as Ontario SBE.

4. MANUSCRIPT -2: DETERMINATION OF DIGESTIBLE, METABOLIZABLE AND NET ENERGY CONTENT IN CANADIAN PRAIRIE GROWN EXPELLER-PRESSED SOYBEAN MEAL FED TO GROWING PIGS.

S. S. Patel[1], A. Olivo Espinal[1], N. Mohamed[2], C Yang[1], J. House[2], and C. M. Nyachoti[1]

[1]*Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2;* [2]*Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada.*

Contribution of authors:

SP: Development of protocol, designed, implement and conducted the animal trial, laboratory analyses, data collection, and prepare first draft of manuscript; AE: Implementation of trial and data collection, laboratory analysis and reviewed manuscript; NM: Participated in acquisition of research fund, laboratory analyses, and reviewed manuscript; CY: Acquisition of research fund, conceptualization of research project and reviewed the paper; JH: Acquisition of research fund, conceptualization of research project and reviewed the paper; CMN oversaw the research project and had primary responsibility for the final content; and all authors: read and approved the final manuscript.

4.1. ABSTRACT

The objective of this study was to determine the digestible energy (DE), metabolizable energy (ME) and net energy (NE) contents in soybean expellers (SBE) derived from Manitoba and Ontario grown soybeans. A total of 30 growing barrows (16.4 ± 0.8 kg, mean ± SD) were assigned to 1 of 5 dietary treatments in a completely randomized design to give 6 replicates per treatment. Experimental diets were: a corn-based basal diet and four diets containing 42% of one of the four SBE at the expense of corn. Three of SBE were derived from Manitoba- grown soybeans whereas the fourth originated from Ontario-grown soybeans. Manitoba derived SBE were Manitoba commercial-1 (MB1), Manitoba commercial-2 (MB2), and Manitoba commercial-3 (MB3). The experiment was conducted over 3 consecutive 30-day periods; each consisting of an initial 10 d adaptation period followed by 5 d of total fecal and urine collection (marker to marker procedure) to calculate DE and ME. On d 16, 3 pigs were transferred to indirect calorimetry (IC) chambers for 36 hours to measure total heat production (HP) for 24 hours and fasting heat production (FHP) for 12 hours. Urine was collected for 24 hours and 12 hours separately to measure urinary nitrogen loss. Data were analyzed using the MIXED procedure of SAS. Fixed effects were the type of SBE whereas period was included as a random effect in the model. Dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), gross energy (GE), starch, ash, crude protein (CP) and ether extract (EE) contents were analyzed for ingredients and diets. There were no differences in DE, ME and NE contents among the four SBE. The average contents of DE, ME, and NE in the evaluated SBE were 4280, 4056, and 2986 kcal/kg DM, respectively. In conclusion, regardless of source, SBE contained similar energy values for growing pigs.

Key words: digestible energy, metabolizable energy, net energy, pig, soybean expeller

4.2. INTRODUCTION

Animals obtain energy from their food through cellular respiration, involving metabolic reactions in cells. This energy transformation converts nutrients into adenosine triphosphate (ATP), the primary energy unit in tissues (Brafield and Llewellyn 1982; Van Milgen and Noblet 2003). Essentially, energy is harnessed through digestive processes and can only be measured during its conversion (Kreiber 1985). Energy is crucial component in swine nutrition as it accounts for more than 70% of feed cost (Noblet and Van Milgen 2004). Energy contents in ingredients or diets can be expressed as calories (cal), joule (J), kilojoule (kJ), kilocalories (kcal) of gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy (NE) (NRC 2012).

Current DE and ME system in swine nutrition poses economic challenges due to overestimated energy values for protein-rich ingredients (Noblet et al. 1994). For any ingredient or diets, contents of carbohydrates and fats are the main source of energy. For growing pigs energy lost as heat increment is 0.75, 1.0, 1.2 and 2.0 kcal per g of starch, digestible ether extract, digestible fiber and digestible crude protein (DCP), respectively (Noblet et al. 1994). Energy requirements for growth or maintenance are independent of diet formulation when compared based on NE values (Noblet 2007). Therefore, there is ongoing interest in swine industry to derived NE values for different ingredients.

Soybean meal (SBM) is a vital source of energy and amino acids for swine (Patience 2013). As a well-known protein rich ingredient, SBM is one of the most commonly used ingredients in swine diets. Factors like soil conditions, weather fluctuations, and storage duration can influence the nutrient content of soybeans indirectly affecting the end product SBM or soybean expeller (SBE) (García-Rebollar et al. 2016).

Early maturing soybean varieties has led to an increase in soybean production around Canada, especially in Manitoba and Ontario (Soy Canada 2023). Understanding the digestibility characteristics of Manitoba-grown soybean meal is essential for formulating diets that meet the energy requirements of swine and identifying potential limitations or factors affecting energy utilization, leading to more accurate recommendations for swine producers.

To our knowledge, currently there is limited data demonstrating the DE, ME, and NE in Canadian prairie derived SBE. The objective of the study was to assess the DE, ME, and NE of SBE produced from soybeans cultivated in Manitoba. This assessment was conducted in comparison to SBE from Ontario. NE values were also predicted using equations (Noblet et al. 1994).

4.3. MATERIALS AND METHODS

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

4.3.1. ANIMALS AND HOUSING

Thirty growing barrows [Tempo × TN70; Topigs Norsvin, Winnipeg, MB, Canada] aged 6 week old, with an average initial body weight (BW) of 16.4 ± 0.8 kg (mean ± SD) were obtained from the University of Manitoba Glenlea Research Unit for use in the current study. Pigs were individually housed in metabolic crates (118 cm × 146 cm) in a temperature controlled room (22°C ± 2°C). Metabolism crates were equipped with a feeder and nipple drinker, a fully slatted floor, a screen floor for total feces collection and a urine tray for the total urine collection.

4.3.2. EXPERIMENTAL DIETS

Vitamins and minerals were provided to meet or exceed requirements for growing pigs (NRC 2012). Five dietary formulations were corn-based basal diet and four diets containing 42% of one of the four SBE at the expense of corn. Three types of Manitoba derived SBE, extracted through expeller pressing and labelled as Manitoba commercial (MB1), Manitoba commercial 2 (MB2) and Manitoba commercial 3 (MB3), obtained from Delmar Commodities, located in Winkler, MB, Canada. Additionally, one SBE from Ontario, labelled as Ontario SBE, was acquired from a crushing plant situated in Cambridge, Ontario, operated by Grand Valley Fortifiers, Cambridge, ON, Canada. Ontario SBE was chosen to act as control to compare the digestibility values to Manitoba derived SBEs. Feed allowance was calculated at approximately 2.8 times the maintenance energy requirement (i.e., 197 kcal ME/kg $BW^{0.60}$; NRC, 2012) based on their BW on d 1, and d 10. Pigs were fed once daily in the morning (07:00 AM) and had free access to drinking water at all times.

Table 4.1 Diet formulation and analysed composition of experimental diets (DM basis)

Item	ON	MB1	MB2	MB3	Basal
Ingredients, % in diets					
Corn	51.12	51.12	51.12	51.12	91.18
Soybean expeller	42.00	42.00	42.00	42.00	0.00
Vegetable oils	2.47	2.47	2.47	2.47	4.41
Limestone	1.28	1.28	1.28	1.28	1.28
Monocalcium phosphate	1.63	1.63	1.63	1.63	1.63
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00	1.00
Analyzed composition of experimental diets					
Dry Matter, %	91.34	91.84	91.89	91.61	90.24
Starch, %	5.49	4.94	5.07	4.73	3.02
Ether extract, %	32.55	29.58	31.80	34.29	54.37
Crude protein, %	25.18	23.41	22.53	21.94	9.63
Acid detergent fibre, %	3.57	3.39	4.36	3.52	1.07
Neutral detergent fibre, %	9.98	12.21	12.70	10.85	9.11
Ash, %	7.89	8.22	7.94	7.53	7.31
Gross energy, kcal/kg	4670	4631	4660	4661	4743

[1] Supplied the following per kg of complete diet: vitamin A, 1500 IU; vitamin D, 400 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B_6, 2.5 mg; biotin, 70 µg; vitamin B_{12}, 20 mg; Cu, 10 mg; Zn, 110 mg; Fe, 120 mg; Mn, 10 mg; I, 0.4 mg; Se, 0.3 mg.

Where, ON= Ontario, MB1= Manitoba commercial 1, MB2= Manitoba commercial 2, MB3= Manitoba commercial 3.

4.3.3. EXPERIMENTAL DESIGN AND PROCEDURES

The research was carried out in 3 consecutive periods, with 10 pigs per period, using consistent experimental conditions, and procedures. The pigs were randomly assigned to one of 5 experimental diets, resulting in 6 replicates per diet. The pigs were provided with one of the experimental diets throughout the study, 10 d of adaptation and 5 d for the total collection of feces and urine to estimate DE and ME (Woyengo et al. 2010). Fecal samples were collected according to the marker-to-marker procedure (Adeola, 2001). On d 11 and d 16, morning meal diets contained 0.5% Ferric oxide as an indigestible marker to determine the initiation and termination of fecal collection. From d 11 to d 16, fecal samples were collected once every morning and preserved at -20°C. The collection of urine began on the morning of 11 d and concluded on the morning of d 16. Urine was collected in containers containing 10 mL of 6N HCl to minimize nitrogen (N) losses. A portion constituting 10% of the total urine weight was filtered through glass wool and subsequently stored at -20°C.

On the d 17, three pigs were placed in calorimetric chambers (1.22 × 0.61 × 0.91 m; Columbus Instruments, OH, USA) for 36 h. This period included 24 h for measuring heat production (HP) and an additional 12 h for measuring fasting heat production (FHP) based on oxygen consumption, carbon dioxide production, and N excretion. At the time of this study, there were 3 calorimetric chambers available. The pigs were transferred into the calorimetric chambers within 1 h of consuming their daily feed ration. They had access to fresh water throughout their time in the chambers. Urine produced during the 24 h and 12 h periods was collected separately, weighed, sampled, and then stored at -20°C to analyse for N content. The temperature inside the chambers was carefully maintained at 22 ± 1°C, and precautions were taken to limit the movement of personnel in the chamber room to ensure that the pigs were not distressed during the measurements of both HP and FHP.

4.3.4. CHEMICAL ANALYSES

Fecal samples were dried in an oven at 60°C for 5 d, pooled and finely ground (1 mm) before chemical analysis. Urine samples from metabolism crates and calorimetry chambers were thawed and separately pooled for each pig. Ingredient, diets and fecal dry matter (DM) content were determined in accordance with AOAC (1990; method 925.09) by drying 1 g of the sample at 102°C overnight. The GE content of ingredients, diets, feces, and urines were assessed using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL), which had been calibrated with benzoic acid as a standard. Nitrogen content in ingredients, diets, feces, and urine was determined through the combustion method (method 990.03; AOAC, 1990) using the LECO N analyser (model CNS-2000; LECO Corp., St. Joseph, MI), and CP was calculated as N multiplied by 6.25. Crude fat in diet and ingredient samples was determined after hexane extraction (method 920.39; AOAC, 1990) using an extraction apparatus. Dietary starch content was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland). The ash content was determined in accordance with AOAC (1990; method 942.05). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents in the diets were assessed following the procedure outlined by Goering and Van Soest (1970).

To determine the GE of urine, 0.5 g of cellulose were dried at 100°C for 24 h, and 2 ml of a urine sample were added to it and the weight of the resulting mixture was them recorded. Subsequently, this mixture of urine and cellulose, along with a sample of pure cellulose, were dried in an oven at 50°C for 24 h and weighed to estimate the DM content of the urine. The GE of dried urine-cellulose mixture and the pure cellulose were determined using an adiabatic bomb calorimeter. The GE of the urine samples was then calculated using the difference method (Fleischer et al. 1981).

4.3.5. CALCULATIONS

The dietary DE, ME and NE contents were computed following the guidelines specified in the (NRC 2012).

Information regarding O_2 and CO_2 levels was accumulated for first 24 h and final 12 h of the fasting period. As fasting period was only for 12 h the final value from FHP was adjusted for 24 h. The HP from the pigs during the collection period was determined using the equation provided by Brouwer (1965),

$$HP = 3.87 \times O_2 + 1.20 \text{ x } CO_2 - 1.43 \times \text{Urine N}$$

Where HP = heat production (kcal), O_2 = oxygen consumption (L), CO_2 = carbon dioxide production (L) and urinary nitrogen in grams (g).

FHP during fasting was determined similarly to HP. Heat increment was computed by subtracting FHP from HP, and NE concentration was calculated as per NRC (2012).

$$NE_{kcal/kg} = ME - (HP - FHP)/\text{feed intake}$$

Where, ME is in kcal/kg, HP and FHP are in kcal, and feed intake is in kg as fed or DM.

Net energy was also calculated according to the following equations (Noblet et al. 1994):

Eq.1: $NE = 0.843 \times DE - 463$

Eq.2: $NE = 0.700 \times DE + 1.61 \times EE + 0.48 \times ST - 0.91 \times CP - 0.87 \times ADF$

Eq.3: $NE = 0.870 \times ME - 442$

Eq.4: $NE = 0.726 \times ME + 1.33 \times EE + 0.39 \times ST - 0.62 \times CP - 0.83 \times ADF$

Where, NE = net energy (kcal/kg DM), ME = metabolizable energy (kcal/kg DM), DE = digestible energy (kcal/kg DM), CP = crude protein (% DM), EE = ether extract (% DM), ST = starch (% DM), CF = crude fibre (% DM), NDF = neutral detergent fibre (% DM) and ADF = acid detergent fibre (% DM)

Net energy values were calculated using prediction equations. Values obtained from equations were averaged to determine NE values. The energy content of SBE was determined using the difference method (Woyengo et al. 2010). by subtracting the NE contribution of the basal diet from the NE of the diets that contained 42% of SBE.

The NE of test SBE diet was calculated as follow:

NE_{SBE} (kcal/kg DM) = $NE_{Basal\ diet} - [(NE_{Basal\ diet} - NE_{SBE})/0.42]$

4.3.6. STATISTICAL ANALYSES

Data were analysed using the mixed procedure of SAS (SAS inst. Inc., Cary, NC). The statistical model included factors related to diet as a fixed effect and period as a random effect. Each individual pig was treated as the experimental unit. Least-square means were calculated and separated with Tukey’s adjustment. Significance was considered at a probability level of $P < 0.05$.

4.4. RESULTS

Pigs remained in good health and consistently consumed their daily feed allowance throughout the experimental period. Table 4.1 and 4.2 contains analyzed composition of diets and ingredients, respectively. In terms of ingredient composition, the CP content was higher in ON SBE (45.3%) compared to Manitoba-derived SBE (41.0%, 40.7%, and 41.8% for MB1 MB2, and MB3, respectively). Conversely, fat content was lower in ON SBE (7.82%) compared to Manitoba-derived SBE (9.52%, 9.13%, and 9.30% for MB1, MB2, and MB3, respectively).

Details of energy balance including total and fasting heat production values are presented in Table 3.2. For the diets containing SBE, DM intake, GE intake, GE output, GE in urine, DE, ME, energy, and protein utilization values were similar. Energy contents of diets were 3981, 4073, 4075, 4061, and 4216 kcal/kg DM for DE, 3850, 3937, 3969, 3932 and 4180 kcal/kg DM for ME, 3873, 3953, 3871, 3912 and 4130 kcal/kg DM for NE, for diets ON, MB1, MB2, MB3 and basal, respectively. Predicted NE values were 2847, 2917, 2931, 2912 and 3081 kcal/kg for ON, MB1, MB2, MB3 and basal diets.

Calculated energy content of the SBE ingredients were 4101, 4384, 4228, and 4308 kcal/kg DM for DE, and 3896, 4135, 4143, and 4077 kcal/kg DM for ME, for Ontario, MB1, MB2, and MB3, respectively. For predicted NE, the values were 2843, 3073, 3048, and 3008 kcal/kg DM for ON, MB1, MB2, and MB3, respectively. The DE and predicted NE of ON SBE differed from MB1 but not different than MB2 and MB3 SBE. Metabolizable energy for SBE were not different. Since the energy contents did not vary significantly among the Manitoba-derived SBEs, an average value of 4340 kcal/kg DM for DE, 4118 kcal/kg DM for ME, and 3043 kcal/kg DM for predicted NE can be utilized as representative values for Manitoba SBEs.

Table 4.2 Analysed composition of soybean meal expeller (Dry matter basis)

Soybean Expeller	ON	MB1	MB2	MB3
Dry matter, %	93.1	95.5	95.2	95.3
Starch, %	1.93	1.26	1.30	1.48
Ether extract, %	7.82	9.52	9.13	9.30
Crude protein, %	45.3	41.0	40.7	41.8
Acid detergent fibre, %	16.49	13.02	15.88	17.93
Neutral detergent fibre, %	13.62	17.51	24.01	20.89
Ash, %	5.90	5.78	5.60	5.83
Gross Energy, kcal/kg	5001	5035	5019	5037

Where, ON= Ontario, MB1= Manitoba commercial 1, MB2= Manitoba commercial 2, MB3= Manitoba commercial 3

Table 4.3 Energy balance of diets fed to growing pigs

	Ontario	MB1	MB2	MB3	Basal	SEM	*P*-value
DMI, g/d	833[a]	837[a]	849[a]	838[a]	747[b]	9.33	<0.01
GE Intake, kcal/d	3889[a]	3877[a]	3957[a]	3904[a]	3545[b]	40.14	<0.01
GE output, kcal/d	573[a]	493[a]	494[a]	504[a]	408[b]	15.14	<0.01
GE in Urine, kcal/d	107	109	109	108	80	4.40	0.142
ATTD of GE, %	85.33[a]	87.27[b]	87.45[b]	87.12[b]	88.87[b]	0.06	<0.01
DE, kcal/kg DM	3981[a]	4073[a]	4075[a]	4061[a]	4215[b]	18.01	<0.01
ME, kcal/kg DM	3850[a]	3937[a]	3969[a]	3932[a]	4180[b]	24.03	<0.01
HP, kcal/kg DM	972	977	969	1010	704	39.49	-
FHP, kcal	899	837	749	829	528	39.71	-
NE[2], kcal/kg DM	3873	3953	3871	3912	4130	33.62	0.063
NE[3], kcal/kg DM	2847[a]	2917[a]	2931[a]	2912[a]	3081[b]	12.77	<0.01
Net protein utilization, %	82.51[a]	85.47[a]	85.11[a]	84.80[a]	77.82[b]	0.85	<0.01
Energy utilization							
ME / DE	96.73[a]	96.68[a]	97.40[a]	96.84[a]	98.94[b]	0.18	<0.01
NE[2] / ME	100.60	100.38	97.57	99.50	98.81	0.71	-
NE[3] / ME	73.95	74.11	73.86	74.06	73.69	0.23	0.082

[a,b, c] Means not sharing a common superscript are significantly different.

[1] Heat production = [3.87 × O_2 + 1.20 × CO_2 – 1.43 × urinary N]/DMI

[2] Fasting heat production = [3.87 × O_2 + 1.20 × CO_2 – 1.43 × urinary N]

[3] Net Energy value predicted by equations (Noblet *et al.*, 1994)

Where, MB1= Manitoba commercial 1, MB2= Manitoba commercial 2, MB3= Manitoba commercial 3, DM = Dry Matter, DE= Digestible Energy, ME= Metabolizable Energy, NE= Net Energy, HP= Total Heat Production, FHP= Fasting Heat Production

Table 4.4 Energy content of soybean expellers fed to growing pigs

Ingredients (DM basis)	Ontario	MB1	MB2	MB3	SEM	*P*-value
DE [1]	4101^{a}	4384^{b}	4328^{ab}	4308^{ab}	41.87	0.051
ME [1]	3869	4135	4143	4077	44.59	0.054
NE [2]	3516	3709	3512	3611	74.99	0.823
NE [3]	2813^{a}	3073^{b}	3048^{ab}	3008^{ab}	33.60	0.049
Ingredients (As-fed basis)						
DE [1]	3909	4174	4133	4012		
ME [1]	3688	3937	3956	3796		
NE [2]	3351	3531	3354	3362		
NE [3]	2681	2925	2911	2800		
Energy Utilization						
ME / DE	94.35	94.31	95.71	94.61	0.36	0.328
NE [3]/ ME	74.75	75.29	74.57	74.99	0.14	0.301
NE [3]/ DE	69.74	70.36	70.87	70.30	0.17	0.161

[a,b, c] Means not sharing a common superscript are significantly different

[1] DE and ME values for ingredients were determined by indirect (difference) method (Adeola, 2001)

[2] NE of SBE was calculated using the difference method (Woyengo et al., 2010)

[3] Net Energy values predicted by equations (Noblet et al., 1994)

Where, MB1= Manitoba commercial 1, MB2= Manitoba commercial 2, MB3= Manitoba commercial 3, DM = Dry Matter, DE= Digestible Energy, ME= Metabolizable Energy, NE= Net Energy

4.1. DISCUSSION

The nutritional composition of feed ingredients may vary between seasons and among different suppliers. Hence, it is vital to regularly evaluate and determine the nutrient content of each ingredient. Nutritional content of SBE can differ based on both the origin of the seeds and the extraction method employed (Karr-Lilienthal et al. 2006; Opapeju et al. 2006).

Net energy values from the IC method were different than predicted values due to a technical issue with the IC machine, resulting in faulty measurements of O_2 consumption and CO_2 production. These led to unreliable NE values as calculated NE to ME ratio was almost 100% (Table 4.3). According to the literature, calculated NE/ME ratio ranges from 70 to 80% for complete diets. Hence, NE values derived from IC method are not discussed further in this thesis.

A basal diet containing only corn was used in the experiment to determine DE and ME values using the difference method. The apparent total track digestibility (ATTD) of GE in the corn-basal diet was similar to reported literature (Rojas and Stein 2013; Sotak-Peper et al. 2015; Lopez et al. 2020; Rodriguez et al. 2020). The average fat content in SBE from Manitoba was about 9% on DM basis, while SBE from ON had a fat content of 7.82%, which were comparable to values reported by Velayudhan et al. (2015) and Powell et al. (2011) for expeller-extruded SBM. In contrast, values reported by other authors (Baker and Stein 2009; Rodriguez et al. 2020; Koo et al. 2021) were lower, which might be due to different soybean processing conditions and soybean itself. The NDF content of studied SBEs were higher than previously reported values (Baker and Stein 2009; NRC 2012), as hulls were included in the current soybean processing that can influence the fibre level (Sakkas et al. 2019).

Analysed CP level was 41.2% DM for Manitoba derived SBE, which was lower than published literature (Woodworth et al. 2001; Karr-Lilienthal et al. 2006; Opapeju et al. 2006; Baker and

Stein 2009; Powell et al. 2011; Sakkas et al. 2019; Rodriguez et al. 2020; Koo et al. 2021; Cristobal. et al. 2023). Tested SBEs had a higher content of fat, the difference in CP content can be attributed to that. Protein-rich ingredients are often overestimated in terms of their energy values when expressed on a DE and ME basis because starch and fat have higher energy efficiency than CP (Van Milgen et al. 2001). Due to the greater energy output and reduced metabolic heat production associated with fat, it is considered more efficient than starch or proteins (Just 1982).

The GE and ME of SBE for Manitoba-derived SBE were 4340 kcal/kg DM and 4118 kcal/kg DM, respectively. These values were comparable to values reported by Woodworth et al. (2001) and Rodriguez et al. (2020) where DE was determined to be 4352 kcal/kg, 4306 kcal/kg, and ME 4105 kcal/kg, 4124 kcal/kg, respectively. Cristobal. et al. (2023) with SBE and Koo et al. (2021) with extruded-expelled SBM reported 4497 kcal/kg and 4591 kcal/kg for DE, 4301 kcal/kg and 4099 kcal/kg for ME, respectively. In contrast, Baker and Stein (2009) and Velayudhan et al. (2015) with extruded-expelled SBM reported 3827 kcal/kg and 3384 kcal/kg for DE, 3620 kcal/kg and 3384 kcal/kg for ME, respectively. The variation in results could be explained by variations in the nutritional content of SBE, including differences in fat levels, the presence or absence of hulls, and the processing methods used.

The NE values derived using prediction equations were different among ON and MB1 SBE types. Velayudhan et al. (2015) determined NE values for extruded-expelled SBM and found that NE values obtained using IC method was higher than predicted NE values. Predicted NE values for SBE were 2624 kcal/kg (Velayudhan et al. 2015) and 2736 kcal/kg (Rodriguez et al. 2020) on DM basis, which was lower than the average value determined in current study (2986 kcal/kg). The DE and ME values which were higher in current study were considered in the prediction equations, and as result this may have led to the higher NE values as compared to values reported by both authors. Researchers from University of Illinois compiled soybean meal studies with pigs conducted from 2009 to 2014 and reports average values of 4413, 3979, and 2592 kcal/kg DM for DE, ME and predicted NE, respectively. Notably the DE values for SBM were higher and NE value was lower by 350-400 kcal/kg DM. The difference might be due to the higher CP content in SBM in the region, which negatively correlated with NE values in the prediction equation and in the current study SBE contains higher crude fat percentage than solvent extracted SBM. Moreover, it's worth noting that the study carried out at the University of Illinois used single equation (Equation 2) from Noblet et al. (1994), which doesn't account for ME values.

4.2. CONCLUSION

Manitoba and Ontario derived SBEs were not different in terms of DE, ME and NE values except MB1. The average content of DE, ME and NE in SBE was 4280, 4056, and 2986 kcal/kg DM, respectively. Results of this research indicates SBE from Ontario and Manitoba has similar energy content and the derived NE value can be used in swine diet formulation.

5. GENERAL DISCUSSION

United States Department of Agriculture (USDA) Foreign Agricultural Service (FAS) report indicates that worldwide pork consumption is set to rise to 127 million metric tons within the next decade, constituting approximately one-third of the overall growth in meat consumption. Soybean meal or SBE serves as a major protein supplying ingredient in swine diets and there is increasing demand of soybean and its products globally. The recent growth in soybean cultivation in Manitoba, particularly due to the development of short-season cultivars, has notably increased soybean production. Manitoba ranks second in oil-seed type soybean production, with an output of 1.3 million metric tonnes. Despite this significant production, a report from Manitoba Agriculture (2021) revealed that the province imported 250 thousand tonnes of SBM, incurring a cost of $164 million USD. The importation of Soybean Meal involves extended transportation, which leads to increased resource utilization and the release of environmentally impactful gases, contributing to ecological concerns. Utilizing locally sourced SBE can significantly contribute to reginal economic growth with reduced greenhouse gas emissions. Moreover, prompting the use of local soybeans can lead to reduced dependence on highly volatile SBM international markets. Domestic production of soybean meal presents a potential mitigation strategy for the challenges associated with global trade tensions. This approach ensures a consistent supply of feed ingredients for the pig sector, concurrently fostering economic autonomy and enhancing regional food security.

Chemical and nutritional composition of soybean is affected by type of variety, geographical location, and environmental factors (de Coca-Sinova et al. 2008; Rotundo et al. 2016; Cober et al. 2023), thus it is critical to evaluate the nutritional composition of locally produced SBE for swine. This information is crucial for the efficient use of a local feed resource, representing a significant step forward in Manitoba's agricultural and swine nutrition practices.

Ingredient evaluation requires in vivo study of nutrient digestibility and animal performance trials to determine the nutritive value of a feed ingredient. In this thesis, two experiments were conducted to determine the nutritive value of Manitoba-grown SBE for swine. In manuscript I, the ATTD and STTD of P was evaluated for Manitoba-grown SBE and Ontario-grown SBE. Effect of phytase was also evaluated on the SBE diets. The results from this experiment showed that ATTD and STTD of P was not different among the SBE diets, similarly the findings of Sotak-Peper et al. (2016), who reported similar P digestibility across different U.S. regions, our study likewise finds no significant regional variation in P digestibility within Canadian soybean varieties. Microbial phytase inclusion in diets improve P utilization in swine. The reported STTD of P in SBM in literature is 40% to 60% and the digestibility was increased to 65% to 80% by addition of microbial phytase (Akinmusire and Adeola 2009; Goebel and Stein 2011; Rojas and Stein 2012; Sotak-Peper et al. 2016). Current study reports 19% increase in P digestibility as phytase was added in diets and ATTD and STTD values for SBE types averaged 63.0% and 69.9%, respectively.

In Manuscript II, the DE, ME and NE content for SBE was determined for three Manitoba-grown SBE and one Ontario-grown SBE. For ingredient composition, the CP content was higher in ON SBE compared to Manitoba-derived SBE and conversely, fat content was lower in ON SBE compared to Manitoba-derived SBE. Content of CP and fat in diets can influence the heat increment in pigs indirectly affecting the NE value. Despite that, the current study found no significant difference in DE, ME and NE contents among the four SBE. The overall average contents of DE, ME and NE in the evaluated SBE were 4280, 4056, and 2986 kcal/kg DM, respectively.

It is essential to acknowledge the limitation encountered in the present study. This acknowledgment serves as a foundation for planning future trials and mitigating potential hurdles when conducting similar research with other ingredients. First, the composite samples of soybeans were selected based on their CP content for Manitoba derived soybeans. Moreover, two composites soybean varieties were processed to make three types of SBE with low, medium, and high CP contents. However, end results of SBE did not show the variable protein levels as expected. Secondly, the processing conditions provided was more generalized and therefore could not allow any speculations to know how this may have influenced composition of SBE. Having important information about processing conditions could have helped better understand if the processing conditions were ideal or not. Thirdly, nutritive value of ingredient can be improved by reducing the particle size or including enzyme mixture that could increase the digestibility of nutrients.

Given soybeans' importance in swine diets, future research is necessary to investigate its amino acid digestibility. The analysis of amino acid profile allows for the determination of limiting amino acid concentrations in Manitoba grown SBE, facilitating an assessment of its suitability for swine diets compared to Ontario grown SBE. Results from such trials will be critical in guiding the selection of the optimal Manitoba soybean variety for swine diet formulations. Moreover, conducting growth performance trials is crucial to evaluate the impact of different SBE types on swine growth performance.

Based on the findings of this thesis, it can be concluded that for Manitoba derived SBE and Ontario derived SBE, P digestibility and energy contents were not different. The values derived can be used in swine diet formulations to improve feed efficiency.

6. GENERAL CONCLUSION

Based on the results obtained in the thesis, it can be concluded that:

1. The ATTD and STTD of P for Manitoba-grown SBE and Ontario-grown SBE were not different and averaged 63.0% and 69.9%, respectively.
2. Phytase inclusion significantly increased P digestibility in SBE diets.
3. Phytase inclusion in SBE diets has no effect on Ca digestibility.
4. The DE, ME and NE content of Manitoba-grown SBE and Ontario-grown SBE were not different and averaged 4280, 4056, and 2986 kcal/kg DM, respectively.

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