Digestibility of Diets Including Canola Expeller and Wheat Millrun Fed with a Multi-Enzyme Blend to Weaned Pigs

by

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ABSTRACT

Pork production in Canada faces many hurdles to maintain economic and environmental sustainability, and feeding coproducts to nursery pigs opens doors to improve both. Feeding coproducts, however, is not without unique challenges when fed to nursery pigs in terms of nutrient digestibility. Fibre can be detrimental to nutrient digestibility and subsequent growth performance; however, supplemental dietary enzymes can mitigate some of the risks by increasing digestibility of the fibre and other nutrients. As a unique coproduct from pressing of canola seed for oil, canola expeller (CE) has more remaining oil compared to canola meal, which can add energy to weaned pig diets. In Chapter 2, a multi-enzyme blend was added to diets containing 250 g CE/kg and fed to ileal cannulated weaned pigs. Two different samples of CE were fed to determine the difference in processing facilities on nutritive value. Twelve cannulated weaned pigs [12-18 kg body weight (BW)] were fed in a double 6 (pig) × 3 (period) Youden square. The coefficient of apparent ileal and total tract digestibility (CAID and CATTD) was calculated and compared to feeding a basal control diet. Feeding CE decreased the CAID and CATTD of dry matter (DM), crude protein (CP) and gross energy (GE). Conversely, feeding CE increased the digestible energy (DE) and net energy (NE) value compared with the basal diet. Despite variances in proximate analyses of the CE samples (CP, fibre and crude fat), digestibility did not differ between the two CE sources. Multi-enzyme inclusion did not affect diets including CE but did increase the CATTD of DM and GE and NE value of the basal diet. Canola expeller inclusion can increase the energy content of diets and thus assist weaned pigs in meeting their energy requirements without added liquid canola oil. Wheat millrun (WM) is a valuable coproduct from flour milling that is usually fed to livestock, including older pigs. Its relative high fibre content precludes the inclusion in diets for young pigs to ensure optimal growth and efficiency during the critical young production stage. In Chapter 3, 75 g WM/kg was included in 4 experimental diets. The positive control (PC) had a higher energy value and lysine content than the negative control (NC), whereas the remaining 2 experimental diets were the NC containing either 0.2 or 0.4 g/kg of a commercial multi-enzyme blend, containing amylase, cellulase, glucanase, invertase, protease, xylanase, to determine a dose response. The experimental diets were fed to ileal cannulated weaned pigs (11-15 kg BW) in a double 4 × 5 Youden square. As expected, the CAID and lysine, and DE and NE values were greater for feeding the PC than NC diet. However, effects of enzyme dose on digestibility of the NC diet were not observed. In summary, adding a single or double dose of multi-enzyme did not increase CAID and CATTD of DM, CP, GE and AA compared to the PC. In conclusion, adding 250 g CE/kg to weaned pig diets creates an opportunity to maintain or increase the energy; however, enzyme inclusion did not specifically affect CE digestibility. Similarly, inclusion of two multi-enzyme doses did not affect digestibility of diets containing WM. The findings from this thesis provide evidence supporting dietary enzyme and coproduct inclusion in weaned pig diets; however, for both coproducts specifically, the optimal enzyme blend, activity and substrate requires further evaluation.

PREFACE

This MSc thesis is comprised of 4 chapters including a literature review, manuscripts 1 and 2 and a general discussion. Chapters were written according to the guidelines for Animal Feed Science and Technology. Animal use in Chapters 2 and 3 was approved and procedures reviewed by the University of Alberta Animal Care and Use Committee for Livestock.

Chapter 2 includes a manuscript with A. Janine Soderstrom, Lifang Wang, Rob Patterson, Eduardo Beltranena, Ruurd T. Zijlstra as authors, “Use of multi-enzyme blend to enhance nutrient digestibility of wheat-canola expeller diet in ileal-cannulated pigs”. A. Janine Soderstrom was responsible for investigation, processing, partial chemical analysis, and writing the original draft. Li Fang Wang performed statistical analysis, validation, data curation, manuscript review and editing. Rob Patterson assisted with conceptualization, enzyme acquisition, funding and manuscript reviewing and editing. Eduardo Beltranena was responsible for resource acquisition, methodology, manuscript review and editing. Ruurd T. Zijlstra was responsible for funding acquisition, project administration, conceptualization, manuscript review and editing. Chapter 2 was presented as a poster abstract and published in the 2023 Banff Pork Seminar proceedings.

Chapter 3 is a collaborative work by the above-mentioned authors with similar contributions and titled “Feeding two multi-enzyme blend doses to enhance nutrient digestibility of wheat grain-wheat millrun diets in ileal-cannulated weaned pigs”.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ANF	Anti-nutritional factor
AX	Arabinoxylan
βG	Beta glucan
BW	Body weight
CAID	Coefficient of apparent ileal digestibility
CATTD	Coefficient of total tract digestibility
CE	Canola expeller
CM	Canola meal
CLDN3	Claudin-3
CRP	C-reactive protein
DM	Dry matter
GE	Gross energy
G:F	Feed efficiency (ADG/ADFI)
GIT	Gastrointestinal tract
HCL	Hydrochloric acid
IL-8	Interleukin 8
LI	Large intestine
NDF	Neutral detergent fibre

NE	Net energy
NSP	Non-starch polysaccharide
MC	Multi-carbohydrase
OCLN	Occludin
SCFA	Short-chain fatty acids
SBM	Soybean meal
SI	Small intestine
SID	Standardized ileal digestibility
TGF-β1	Transforming growth factor
TNF-α	Tumour necrosis factor
WBC	White blood cell
WM	Wheat millrun

Chapter 1: Mode of action of feed enzymes and their inclusion in weaned pig nutrition: A review

1.1 Introduction

To address the dilemma of competition for resources between animal feed and human food, nutritionists formulate pig diets to include feedstuffs that humans cannot directly utilize such as coproducts from crop production systems, human food processing or bioindustry. Two coproducts fed to swine from the human food industry include canola meal (CM) and wheat millrun from the culinary oil and bread industries, respectively (Torres-Pitarch et al., 2019). Feeding coproducts to pigs reduces feed cost for the producer; however, some coproducts are high in dietary fibre. Including fibre-degrading enzymes in pig diets containing coproducts can moderately address the fibrous content and increase energy and nutrient digestibility (Torres-Pitarch et al., 2019). In addition, feeding coproducts with enzymes to increase their digestibility may lower the environmental footprint of both crop and livestock production by decreasing land use per unit of lean pork produced. Feeding coproducts aims to convert low quality vegetable by-products not utilized by humans to high quality animal protein, while maximizing cropland use for human food and bioindustries (Ali et al., 2017). Additionally, intensive animal operations must deal with accumulation of manure that causes environmental concerns. Feed enzymes increase the amount of nutrients available to the pig for absorption, thus resulting in fewer undigested nutrients excreted in manure and urine (Torres-Pitarch et al., 2019). Including coproducts and feed enzymes in pig diets at different stages of production, can therefore improve the economic, social, and environmental sustainability of pork production reducing waste streams along the production chain.

1.2 Plant-based limitations on nutrient digestibility

1.2.1. Fibre

Feeding plant-based diets dominates in commercial pig production not only because the lower cost of cereals, pulses and oilseed coproducts (Vila et al., 2018), but also the health risk of introducing disease (e.g., African Swine Fever, Porcine Epidemic Diarrhoea) into herds associated with feeding animal by-products (e.g., meat and bone meal, feather meal, tallow). Carbohydrates in feedstuffs are largely divided into starch and fibre. Fibre, or more broadly non-starch polysaccharides (NSP), have been studied extensively in both human and animal nutrition. In general, fibre consists of the largely insoluble, woody-type, polysaccharides in plant cell walls such as cellulose, and the soluble gummy-type, such as hemicellulose, β-glucan (βG) and arabinoxylans (AX), respectively (Bindelle et al., 2008; Gutierrez et al., 2014; Jha and Berrocoso, 2015; Lei et al., 2016). Although comprised of sugars, fibre is complex due to the glycosidic linkages creating polymers of hexoses and pentoses (Gutierrez et al., 2014). For example, AX consists of a linear backbone of β-(1-4)- xylopyranosyl with various sidechains (Figure 1.1; Lei et al., 2016). Ingredients contain different fibre types, for example, wheat grain contains high levels of AX, whereas oats contain βG (Yu et al., 2016). Content of AX ranges from 73 g/kg in wheat to 84 g/kg in barley, both having more than corn grain (45 g/kg; Patience and Petry, 2019).

Total dietary fibre is used as a broad description for all types of fibre (Petry and Patience, 2020). Resistant starch, defined as starch molecules that escape digestion in the foregut, is also considered to be dietary fibre, due to similar fermentation characteristics in the hindgut (Tan et al., 2021). More recently, the properties of fibre have been used to distinguish types of fibre. The physiochemical properties of fibre vary greatly between sources and thus alter the physiological response in the pig gastrointestinal tract (GIT), such as passage rate, nutrient absorption and satiety (Bindelle et al., 2008). Some of these physiochemical properties include fermentability, viscosity, and solubility.

Fermentability describes the degree of fermentation by hindgut microbiome producing volatile fatty acids and gases. Microbial fermentation in the colon breaks down fibre structure to produce short chain fatty acids (SCFA) including acetate, propionate, and butyrate and gases such as methane, hydrogen, and carbon dioxide. The degree of fibre fermentation is variable based on the physical structure of NSP (Bindelle et al., 2008). For example, cellulose, hemicellulose, and lignin, present in wheat bran and corn grain are less fermentable than pectic molecules present in soybean hulls. β-glucans and AX are highly fermentable fibre and produce large amounts of SCFA, altering energy metabolism in the animal, therefore affecting feed intake, faeces consistency, and weight gain.

Viscosity describes the physiochemical property of fibre when mixed with drinking water and gastrointestinal fluid. Associated with solubility, viscous polysaccharides create gels in the intestinal lumen. Although viscous digesta slows passage rate through the GIT, it also decreases digestion and absorption of fats and other nutrients by decreasing lipid emulsification and physical interaction with the intestinal brush border. Soluble fibre can increase endogenous losses by increasing mucous production and epithelial cell sloughing and proliferation (Jha and Berrocoso, 2015). Some AX and βG specifically, increase digesta viscosity and are poorly digested in the small intestine (SI). Digesta then passes to the large intestine (LI) as abundant substrate for bacterial fermentation, categorizing AX and βG as soluble and fermentable (Wu et al., 2018).

Although often categorized together, solubility and fermentability are not mutually exclusive. For example, soybean hulls and wheat bran are insoluble yet fermentable. Soluble fibres include pectins, gums, hemicelluloses and βG, whereas examples of insoluble fibres are cellulose and lignin (Jha and Berrocoso, 2015). More than half of the NSP in soybean meal are pectic substances, often cementing other polysaccharides and proteins within the cell matrix (Tahir et al., 2008). Soluble fibre typically slows passage rate, whereas insoluble fibre speeds up passage rate. Wheat bran often relieves sow constipation due to faecal bulking (Jha and Berrocoso, 2015).

Young pigs have a largely undeveloped hindgut, with limited ability to degrade fibre, that often reduces the digestibility of other nutrients such as starch, protein, and minerals. For example, when high fibre ingredients, ~45% insoluble fibre, were fed to weaned pigs, decreased apparent total tract digestibility (ATTD) of dry matter (DM) and gross energy (GE) resulted in reduced average daily feed intake (ADFI) and average daily growth (ADG; Yu et al., 2016). Total tract digestibility of fibre ranged from 0.4 to 0.6 and is markedly lower than the 0.8 or greater for starch, protein and fat (Bindelle et al., 2008). Fibre acts as a physical structure in plant cell walls, entrapping nutrients such as starch, protein, and minerals creating an encompassing matrix. This surrounding matrix prevents physical contact and digestion by limiting the surface area that digestive enzymes can act on their respective substrates (Tahir et al., 2008; Gutierrez et al., 2014; Wang et al., 2018, Zouaoui et al., 2018; Zhang et al., 2020). Growth performance parameters, such as ADFI and ADG are negatively correlated with dietary fibre inclusion, such that as little as 5% fibre can reduce growth (Yu et al., 2016; Wu et al., 2018). Dietary fibre increases the rate of endogenous losses, through friction and abrasion against the gut wall and sequential increased mucin production (Zouaoui et al., 2018).

Weaned pigs lack the endogenous enzymes to digest fibre, resulting in increased fermentation of undigested nutrients by hindgut microbiome (Bindelle et al., 2008; Jha and Berrocoso, 2015; Torres-Pitarch et al., 2019). The NSP such as βG, AX, and pectin are mostly fermented in the cecum and proximal colon, whereas insoluble NSP are more slowly degraded in the distal colon (Jha and Berrocoso, 2015). Microbes in the LI can cleave the complex structure of fibre into oligosaccharides and monosaccharides (Yu et al., 2016). Although less efficient than foregut enzymatic digestion of sugars, hindgut fermentation of sugars results in production of SCFA that can be used as energy source by the pig (Bindelle et al., 2008). These SCFA are passively absorbed by colonocytes and are either metabolized locally or mobilized to the liver. Butyrate specifically, is metabolized locally by colonic cells, thereby increasing growth and proliferation of colonic cells, resulting in improved gut health in monogastric species (Elia and Cummings, 2007; Regassa and Nyachoti, 2018). As the young pig has an immature gut microbiome, hindgut fermentation and therefore SCFA production and absorption is limited compared to growing pigs and sows. Fibre is the main energy source for the microbiome and thus it is an important contributor to microbiome development when fibre inclusion is increased in tolerable increments at a young age (Jha and Berrocoso, 2015).

1.2.2. Plant Protein

Protein content and quality are essential in monogastric diets to ensure appropriate health and growth. Protein and amino acids are essential components of organ and muscle tissues that are mainly supplied by dietary plant and animal ingredients. Proteins consist of three-dimensional chain structures and sequences of individual amino acids (AA), giving specific amino acid profiles, which determines their three-dimensional structure quality of the protein ingredient (Joye, 2019). The AA profile is of vital consideration when formulating pig diets (Son et al., 2019). Not all AA are considered equal, as essential amino acids, such as lysine, threonine, methionine and tryptophan are required in the diet (termed indispensable), as they cannot be synthesized endogenously (Templeman and Shoveller, 2022). Another method of describing protein quality is the ability for AA present in the diet to be digested and absorbed (Templeman and Shoveller, 2022). In terms of presence and bioavailability of the 10 essential AA, proteins of animal origin are considered nutritionally superior compared with plant-based proteins. Protein ingredients, however, are typically the most costly component of pig diets after energy. Animal-based protein ingredients are typically more costly than plant-based ingredients. Furthermore, in 2001, feeding of ruminant by-products was banned by the European Commission for food safety reasons, limiting animal protein ingredients in pig diets to fish, poultry, swine meals, whey, and plasma (Jezierny et al., 2010).

The increased protein and AA digestibility of animal protein meals as compared with plant protein meals has been well established. In vitro, chicken meat has a protein digestibility of 92% in contrast to 77.5% in barley (Carbonaro et al., 2012). Similarly, apparent ileal digestibility (AID) of crude protein was 80% for milk protein versus 51% for soybean meal (SBM) diets (Asche et al., 1989). In the stomach, protein digestion starts with denaturation by hydrochloric acid (HCl). Animal proteins, like whey, are highly soluble in acidic conditions and are rapidly absorbed in the duodenum (Berrazaga et al., 2019). In contrast, protein in SBM is largely digested and absorbed by the end of the ileum (Asche et al., 1989). The digestibility of proteins is affected by a variety of intrinsic and extrinsic factors. Intrinsic factors include AA profile, protein folding, and crosslinking. Extrinsic factors include anti-nutritional factors (ANF) and physical entrapment within cell structures (Joye, 2019). The physical structure of plant protein, such as folding and aggregation, limits the access of acids and digestive enzymes, resisting denaturation and hydrolysis. The protein structures of animal proteins consist of only α-helix, whereas plant proteins contain high content of β-sheet conformations (Berrazaga et al., 2019). β-sheet structures are more resistant to proteolysis in the GIT due to hydrophobic centres preserving the native structure limiting the interaction with digestive secretions. Specific AA, such as tyrosine, phenylalanine, tryptophan, threonine, valine and isoleucine, are predisposed to hydrophobicity and therefore reduced digestibility (Carbonaro et al., 2012). Protein digestibility varies between plant sources due to species, cultivar, agronomic practices, environment and processing (Cotten et al., 2016). Oilseed meals, such as CM and SBM are high in protein; however, the AA content and digestibility varies widely among oilseed meals (Cotten et al., 2016). In growing pigs, the AID of AA was greater in dehulled SBM than other plant protein sources (Son et al., 2019). In typical livestock diets, SBM is the preferred plant protein source because of its superior AA profile including abundance of lysine, threonine and tryptophan.

Like fibre, weaned pigs initially struggle to digest plant protein. The abrupt change from highly digestible sows' milk to dry feed, limits initial steps of protein digestion in partially acidic conditions. In suckling pigs, the conversion of lactose to lactic acid by *Lactobacillus* bacteria is the primary source of acidity. After weaning, limited HCl production results in a pH near 5, preventing full protein denaturation (Pearlin et al., 2020). Due to slow development or intestinal atrophy at weaning, the lack of pancreatic and intestinal peptidases decreases digestibility of proteins (Caine et al., 1997). The AA profile and bioavailability in animal ingredients like fishmeal, plasma, and poultry meal are similar to sows' milk, making these easily digestible by weaned pigs, and are thus included in weaned pig diets to increase feed intake (Asche et al., 1989; Deng et al., 2022; Wang et al., 2022). Although feeding animal-based proteins to pigs seem to outweigh the decreased digestibility of plant proteins, producers and nutritionists seek to transition pigs to feeding plant-based ingredients as early as possible to decrease feed cost.

1.2.3. Phytate

Phosphorus is an essential mineral for bone growth and multiple physiological functions and therefore must be supplied in sufficient quantities in the diet (Humer et al., 2015). Inorganic phosphorus is sourced from finite sources, often altering natural phosphorus cycles, and exacerbating the effect of livestock on phosphorus pollution (Abbasi et al., 2019). Plant-based ingredients do not provide sufficient phosphorus because of limited bioavailability, making phosphorus the third most costly nutrient in swine production (Humer et al., 2015). Cereals, oilseeds, and pulses contain phosphorus; however, its bioavailability varies greatly (Abbasi et al., 2019; Kumar et al., 2015; Sebastian et al., 1998). Phosphorus is mostly stored in plant seed in the indigestible form of phytate or phytic acid. Although abundant, phytate-bound phosphorus is poorly available to monogastric species, often needing to supplement dietary inorganic phosphorus to meet requirements. 60 - 80% of the phosphorus in monogastric diets is bound within phytate complexes (Sebastian et al., 1998; Lei and Stahl, 2000; Kumar et al., 2015). In commercial pig diets, 50 - 80% of dietary phosphorus is unabsorbed, and thus excreted via manure creating environmental concerns (Humer et al., 2015). Considered an ANF, phytate also binds, and consequently prevents the digestion of nutrients such as starch, protein, lipids, minerals and vitamins (Humer et al., 2015; Zouaoui et al., 2018).

Also known as myo-inositol hexaphosphate, phytate is a simple ringed carbohydrate, with 6 phosphate groups (Woyengo et al., 2009; Humer et al., 2015; Kumar et al., 2015; Zouaoui et al., 2018). Phytate chelates various cations (Figure 1.2) including AA, calcium, iron and zinc. Phytic acid content linearly decreased phosphorus, sodium, potassium, calcium, and magnesium digestibility when present in piglet diets (Woyengo et al., 2009). Phytate forms electrostatic connections to create complexes with proteins at various pH values, forming de novo within the GIT (Selle et al., 2000; Humer et al., 2015). Protein-phytate complexes have poor digestibility due to the inhibition of protease activity (Kumar et al., 2015; Humer et al., 2015; Zouaoui et al., 2018). Phytate also increases mucin secretion into the gut, increasing endogenous proteins losses and lowering the AID of AA (Selle et al., 2000; Zouaoui et al., 2018). In high- and low-phytate diets fed to growing pigs, high phytate reduced AID of CP and ATTD of DM, CP and GE compared with a low phytate diet (Yang et al., 2020). Phytate is hydrolysed by phytase, partially liberating the nutrients bound in the complex to differing extent and thus increasing nutrient utilization.

1.3 Mechanisms of enzyme activity

Being omnivorous, pigs have the inherent ability to digest some plant material by secreting a limited array of endogenous enzymes. These enzymes catalyse, thus accelerate, digestion of specific substrates in the diet. By targeting specific chemical bonds, enzymes break apart polymers into monomers. Endogenous enzymes are produced and secreted based on what is present in the diet; however, synthesis often reaches a biological limit, as seen in weaned pigs (Owsley et al., 1986). Low gastric and pancreatic enzyme production and activity occurs after weaning due to immaturity and change in composition and amount of feed ingested (Hedemann and Jensen, 2004). One method to exploit enzymes is to create and include exogenous enzymes in diets. Bacteria and fungi are the main sources of these exogenous enzymes, and since the 1990's, feed enzymes, such as phytase have been researched and applied to commercial livestock feed. Feeding these dietary enzymes helps increase gain-to-feed (G:F), thus lowering feed needs and production cost. The mode of action of enzymes outlined in the literature is summarized in Table 1.1 (Patience and Petry, 2019; Velázquez-De Lucio et al., 2021).

Feeding exogenous enzymes can increase the access of endogenous enzymes to substrates through degrading nutrient encapsulation and (or) causing a synergistic effect, seen more frequently when feeding low quality diets (Bedford, 2000; Musigwa et al., 2021). Feeding a variety of enzymes, for example, NSP-degrading enzymes with phytase, can target multiple substrates and further increase digestibility benefits (Bedford, 2000). Dosage and activity are important factors when formulating diets including feed enzymes. Enzyme manufacturers establish level and dose typically recommended based on purity, and thus activity of product, and the cost-benefit. Typically, curvilinear increases in digestibility up to a plateau occur with increased levels of dietary enzyme (Torres-Pitarch et al., 2019). Similarly, increased ADG was reported when grower pigs were fed higher dose of xylanase (Fang et al., 2007). A variety of factors can affect the activity of an enzyme. In general, enzymes are sensitive to environmental changes such as temperature, pH and moisture. Feed processing at high temperatures would typically damage the added enzyme reducing its activity. Thus, development of thermotolerant and encapsulated enzymes is currently being studied to protect feed enzymes from damage or early activation in the GIT (Bedford, 2000). Table 1.2 outlines common feed enzymes and their substrates in swine nutrition. Depending on their substrate, a wide variety of differing enzyme blends can be developed. Typical blends applied to feed could include xylanase, β-glucanase, cellulase and phytase (Zhang et al., 2020).

1.3.1. Carbohydrases

Fibre content negatively affects nutrient digestibility in weaned pigs, but it also provides prebiotic effects (not discussed here). As fibre is present in most plant-based ingredients, nutritionists can include NSP-degrading enzymes or carbohydrases in the diet to increase fibre digestibility to some extent (Zeng et al., 2018). In poultry, inclusion of 1% carbohydrase blend increased NSP digestion by 34% (Slominski and Campbell, 1990). The most common carbohydrases fed in the pork industry are xylanase and β-glucanase to degrade AX and βG, respectively (Torres-Pitarch et al., 2019). In general, carbohydrases degrade cell wall carbohydrate to varying extent to increase the digestibility of fibre itself but also increase the availability of entrapped nutrients (Yang et al., 2020). If carbohydrases partially degrade the insoluble fibre, the pigs' endogenous enzymes have increased opportunity to act in the foregut given the limited transit time (Velázquez-De Lucio et al., 2021). Several carbohydrases are commonly fed in pig nutrition today, with many more being developed. Each enzyme has unique mechanisms to work on specific substrates.

Xylanase. Arabinoxylan (AX) is a type of hemicellulose found in wheat and cereal grains and requires multiple enzymes to achieve complete degradation. The AX consists of β-1-4 linked xylopyranosyl backbone, with sidechains of arabinofuranose held with α-1-2 or α-1-3 glycosidic linkages (Figure 1.1). The AX often exists with various degrees of side chains including ferulic acid and O-acetyl (Lei et al., 2016). Xylanase, also known as endo-β-1,4-xylanase, targets the β-1-4 glycosidic bonds randomly, to release polysaccharides, oligosaccharides, and pentoses that can be further degraded (Petry and Patience, 2020). Another form of xylanase, β-1,-4 xylosidase acts on the main AX chain at the non-reducing end to produce xylose (Lei et al., 2016). L-arabinofuranosidases, feruloyl esterase, α-D-glucuronidases and acetyl esterase are all enzymes that target the side chains. This synergistic activity of various enzymes may reduce digesta viscosity in pigs fed corn-based diets (Patience and Petry, 2019)

Glucanase. β-glucans are soluble hemicellulose found in oats and barley grain. βG are polymers of glucose units connected by β-1-3 or β-1-4 glycosidic linkages. Endo-1-3(4)-β-glucanase targets βG randomly to release simple sugars. Four forms of glucanase are most common: β-1,3(4)-glucanase, β-1,3-1,4-glucanase, β-1,4-glucanase and β-1,3-glucanase (Patience and Petry, 2019).

Cellulase. Cellulose is an insoluble structural fibre consisting of β-D-glucopyranose. To completely depolymerize cellulose, 3 enzymes are required. Endoglucanase (β-1,4-glucanase), exoglucanase and β-glucosidase. These enzymes hydrolyse the glycosidic bonds in cellulose to produce fermentable sugars (Patience and Petry, 2019). Specifically, endoglucanases cleave internal glycoside bonds toward the amorphous regions of the fibre to produce long chain oligomers. Two variants of exoglucanase cleave these oligomers at both the non-reducing and reducing ends to produce short chain polysaccharides, followed by β-glucosidases producing glucose (Figure 1.3). Lignin greatly reduces cellulase activity, and often results in a reduced efficacy in cellulose hydrolysis (Juturu and Wu, 2014).

Mannanase. As a part of hemicellulose, mannans play a structural role in most legumes such as soybeans (Patience and Petry, 2019). They can be classified into linear mannan, glucomannan, galactomannan and galactoglucomannan. Galactomannans are linear chains of β-1-4 mannose with galactose side groups (Malgas et al., 2015). Mannanases, specifically β-mannanases, hydrolyse bonds to produce monomers of mannobiose and mannotriose. Similar to cellulose, various enzymes work together to fully degrade the mannans (Malgas et al., 2015; Torres-Pitarch et al., 2019).

1.3.2. Protease

Protein digestion begins with the disruption of the three-dimensional structure of feed proteins. Then the protein chains must be broken down into smaller peptides, dipeptides and individual AA to be absorbed across the intestinal wall and used by the animal. The GIT hydrolyses peptide bonds by acidic or enzymatic degradation via proteases. Two categories of proteases present in the gastrointestinal tract are: endoproteases and exoproteases. Endoproteases cleave internal peptide bonds to release larger peptides, whereas exoproteases cleave C or N-terminal peptide bonds releasing AA (Figure 1.4; Sharma et al., 2019). Exopeptidases can be further categorized based on how many AA are released at once, aminopeptidase vs. dipeptidase for example (Rawlings, 2013). Peptidases have different specificities to the amino acid sequence they hydrolyses; therefore, the different AA profiles in peptides present in the GIT have different susceptibilities to the peptidases present (Rawlings, 2013).

The pig has intrinsic gastric, pancreatic and brush border proteases and peptidases; however, young pigs struggle to synthesize adequate quantities (Caine et al., 1997; Tactacan et al., 2016). Including exogenous proteases in livestock diets has been used in the last 10 years to increase the digestibility and thus absorption of peptides and AA (Zhu et al., 2022). Protease supplementation disrupts the protein complex, increasing digestibility of all nutrients, and may thereby increase ADG (Torres-Pitarch et al., 2019). Results can be variable however, Caine et al. (1997) reported no increase in AID of CP in SBM diets with protease, whereas Yu et al. (2020) reported increased AA digestibility in SBM diets with protease. Factors such as source, specificity, dose and gastric degradation can limit the efficacy of enzymes. As proteins, exogenous enzymes themselves are digested or degraded in the stomach becoming inactivated or absent in the small intestine. Coating (encapsulation) can be added to delay enzyme digestion and degradation to increase the efficacy of the enzyme. Coated protease in the small intestine has a reported 85% retention rate compared with 35% for the uncoated protease (Yu et al., 2016). Improvements in feed enzyme technology may increase both efficacy of exogenous proteases, and their economic advantage to pork producers (Pan et al., 2016).

1.3.3. Phytase

Phytate is a complex molecule that binds not just phosphorus but a variety of nutrients. To access those nutrients, phytase is required to hydrolyse the bonds to release mostly inorganic phosphate from myo-inositol (Figure 1.5). Phytases are found naturally in the environment, produced by plants, fungi and bacteria (Kumar et al., 2015). Cereals including wheat, rye and barley contain negligible amounts of intrinsic phytase; therefore, exogenous phytase is included in livestock diets. Expressed as FTU, 1 FTU of phytase liberates 1 µmol of inorganic orthophosphate from 0.0051 mol sodium phytate (Selle et al., 2000). Liberating phytate-bound phosphorus from the phytate decreases the amount of dietary inorganic phosphorus needed to meet phosphorus requirements, thereby reducing excretion in faeces (Bedford, 2000). Degradation of the phytate molecule often increases the bioavailability of the entrapped nutrients, increasing the digestibility of those nutrients (Yang et al., 2020). Inclusion of 500 FTU of phytase increased digestibility of all indispensable AA in pig diets (Zouaoui et al., 2018).

1.3.4. Enzyme Blends

Each feed enzyme typically works on a specific substrate. Plant-based diets often have a wide array of substrates that weaned pigs struggle to digest. Adding multiple enzymes as a blend can have an additive or synergistic effect to break down the substrate more effectively (Torres-Pitarch et al., 2019). The NSP generally needs multiple enzymes for increased degradation as seen with AX, where xylanase action is complemented by arabinofuranosidase to liberate smaller sugars (Musigwa et al., 2021). Pectinase breaks apart the pectic polysaccharides (Tahir et al., 2008). Combining pectinase with other carbohydrases such as xylanase and β-glucanase can further degrade the cell wall and reduce digesta viscosity (Musigwa et al., 2021). In in vitro digestibility studies, the effect of single enzymes on CP and DM digestibility was insignificant; however, combination of cellulase, pectinase and hemicellulase increased CP and DM digestibility by 17.5% and 8.94 %, respectively (Tahir et al., 2008). In growing pigs, feeding the optimal carbohydrase mixture including cellulase, xylanase, β-glucanase, galactosidase, β-mannanase and pectinase to corn or wheat-based diets, increased ATTD of nutrients and diet DE value (Zhang et al., 2020).

Phytic acid commonly cross-links with AX to form an indigestible complex (Figure 1.6) limiting access of xylanase and phytase. The addition of phytase and xylanase together can increase solubility, and therefore digestion of the complex (Zouaoui et al., 2018). Additionally, the extent of significance can vary in animal experiments (Torres-Pitarch et al., 2018). Common enzyme blends seen in industry typically include phytase, β-glucanase, amylase, cellulase, pectinase, xylanase, and protease; however, the combination and activity are commonly customized based on the ingredient composition of the diet (Assadi et al., 2011).

1.4 Effect of feed enzyme inclusion on diet nutrient digestibility and growth performance in weaned pigs

1.4.1. Nutrient Digestibility

Swine nutritionists judge the nutritive value of feed ingredients to properly formulate diets to meet animal nutrient requirements. Nutritive values are created incorporating digestibility data obtained from ileal digesta as a measure of AID and faeces as a measure of

ATTD. Greater AID and ATTD indicate higher digestibility, thus leading to the assumption of greater G:F. Testing the digestibility of diets with exogenous enzyme compared to a diet without the enzyme can indicate the efficacy to aid digest a specific substrate.

Carbohydrases. The digestibility of diets containing multi-carbohydrases (MC) has been studied in swine nutrition previously. In growing pigs, feeding a MC blend containing galactanase, xylanase, mannanase, α-amylase and cellulase included in wheat bran diets, increased AID of DM, CP, NDF, GE, histidine, lysine, threonine and isoleucine. Additionally, feeding the enzyme blends increased the ATTD of DM and CP in wheat bran diets but did not affect diets without wheat (Zeng et al., 2018). Using the slaughter method, the effect of feeding a MC on AA digestibility was tested (Dadalt et al., 2016). The MC increased the AID of DM and ATTD of CP and DE and ME values. However, when looking at individual AA, feeding MC only increased the AID of histidine. Similarly, including MC in wheat bran diets fed to weaned pigs showed increased AID of CP, DM, and DE and ME values (Neto et al., 2020). Research on exogenous enzymes can be inconsistent; however, trials conducted by the same research group found no difference in AID or SID of AA when including a MC in yellow corn diets (Trindade Neto et al., 2020).

Protease. In cannulated growing pigs, the effects of feeding a coated protease on digestibility of SBM diets was evaluated (Pan et al., 2016). Protease increased AID of CP and essential AA such as arginine, isoleucine, and leucine. In addition, the coated protease increased both AID and ATTD of DM and GE. The authors concluded that protease could increase the utilization of protein in the diet. Similarly, coated protease inclusion to SBM diets fed to cannulated weaned pigs increased AID and standardized ileal digestibility (SID) of CP and total AA compared with no protease inclusion (Yu et al., 2016).

Phytase. Phytase has strong efficacy in releasing phosphorus from phytate, and due to the reduction of expensive inorganic phosphorus inclusion, most producers choose to add phytase. Feeding phytase to weaned pigs increased ATTD of DM and SID of histidine, arginine, leucine, lysine, valine, alanine and proline (Dadalt et al., 2016). Similarly, increasing dietary phytase linearly increased ATTD of phosphorus (Bento et al., 2012; Dersjant-Li et al. 2017), demonstrating the efficacy of phytase in weaned pig diets. Inconsistencies with phytase digestibility trials are commonly reported. While reporting an effect on phosphorus digestibility, Bento et al. (2012) reported no change in ATTD of DM, unlike Dadalt et al., (2016). Similarly, in four experiments testing the digestibility of 1) corn-SBM, 2) wheat-SBM, 3) wheat-SBM+ canola meal and 4) barley- pea- canola meal diets, feeding phytase did not affect AID of CP and AA for diets 1, 2 and 4 (Liao et al., 2005). However, they observed a tendency for increased AID of CP and AA and ATTD of CP and DE value for diet 3 indicating the importance of linking substrate and phytase efficacy.

When measuring digestibility, an assumption can be made that the greater utilization of feed would result in increased growth and superior G:F. However, often the increase in digestibility does not translate into increased growth or G:F, especially with carbohydrases like xylanase and β-glucanase are fed (Petry and Patience, 2020).

1.4.2. Growth Performance

Pig performance is the leading indicator for enzyme efficacy as it determines the payback to a producer or nutritionist incorporating enzymes in diet formulation. Enzymes, although added at small amounts can be costly. Phytase inclusion, for example, directly increases the amount of phosphorus released by plant ingredients, reducing the inclusion of inorganic phosphorus, in the form of mono- di-calcium phosphate. Studies showed a linear increase in ADG and G:F feeding up to 2,000 FTU/kg of phytase (Dersjant-Li et al., 2017). Effects of other exogenous enzymes are not as well established providing an opportunity for researching enzyme and substrate combinations or enzyme blends. For example, Duarte et al. (2019) reported an overall increase in ADG in weaned pigs fed xylanase, but ADG was further increased when xylanase and protease were fed together. Similarly, feeding enzyme blends increased ADG of weaned pigs by 17% in the first two weeks post-weaning (Wang et al., 2018). In a 42-day growth trial with pigs weaned at 21 days, feeding a carbohydrase blend was evaluated in four dietary treatments: 1) net energy (NE), 2) low energy (LE), 3) LE + 0.1% carbohydrase and 4) LE + 0.2% carbohydrase. Pigs fed diets NE and LE + 0.2 % had greater ADG, indicating the importance of enzyme dose on efficacy (Ao et al., 2020).

Not all research groups report a benefit of enzyme inclusion on growth performance traits. In a study with 210 weaned pigs 20 ± 2 days of age, carbohydrase blend inclusion reduced ADG compared with feeding control diets (Bloxham et al., 2018). In a trial with 492 weaned pigs (initial BW: 9.15 kg), feeding SBM and canola meal (CM) diets with a carbohydrase blend included, had no effect on ADFI, ADG or G:F (Pedersen et al., 2016). In both these experiments, enzyme increased ATTD of nutrients and GE, confirming previous statements of efficacy (Bloxham et al., 2018). A study looking at inclusion of xylanase and an enzyme blend of cellulase, β-glucanase and xylanase in high fibre diets fed to weaned pigs (6.43 ± 0.06 kg initial BW), revealed that including xylanase alone did not affect ADG, but enzyme blend inclusion did increase ADG (Li et al., 2018). Interestingly, they concluded that the increase in ADG was due to increased intestinal barrier and reduced immune activation, as there was no improvement in ADFI and G:F feeding any of the enzyme diets.

1.5 Effect of feed enzymes on gut health in weaned pigs

1.5.1. Immune Function

Newly weaned pigs often struggle with the stress of changing from liquid milk to a dry, plant ingredient-based diet, a new environment changing from nursing pens to weaner decks and establishing a new social order changing from littermates. Culmination of these stressors may lead to post-weaning lag and (or) post weaning diarrhoea even in the absence of disease challenge. Nutritionally, providing a highly digestible diet and introduction to creep feed before weaning can reduce at least one of these stressors. Plant-based diets are less costly but may exacerbate post-weaning lag in weaned pigs, therefore, researchers are interested in feeding enzymes to alleviate feed-related post-weaning lag and improve immune gut function in weaned pigs (Vila et al., 2018). In a trial with 75 weaned pigs (28 days of age), the effect of feeding proteases on immune response and inflammatory markers such as white blood cells (WBC), tumour necrosis factor (TNF-α), transforming growth factor (TGF-β1) and C-reactive protein (CRP) was tested (Lee et al., 2020). Feeding protease reduced the number of WBC, TNF-α and TGF-β1. Reduced inflammation can contribute to increased growth and decreased post-weaning mortality. Feeding carbohydrase may improve gut barrier integrity (Patience and Petry, 2019). Gut barrier integrity is paramount to both nutrient absorption and disease resistance by reducing bacteria translocation and thereby local inflammation. Including a blend of cellulase, β-glucanase, and xylanase increased small intestinal barrier integrity and reduced immune activation (Li et al., 2018). Tissue samples from the SI showed an increase in tight junction function, specifically colonic IL-17, occludin (OCLN), and claudin 3 (CLDN3) indicating increased gut barrier integrity. Blood samples also showed a decrease in inflammatory markers interleukin 8 (IL-8) and TNFα, indicating decreased overall immune activation (Li et al., 2019).

The literature is inconsistent on effects of feeding enzymes on immune function. For example, carbohydrase inclusion to low energy diets did not affect lymphocyte levels, indicating no effect on immune function (Ao et al., 2020). In contrast, the inclusion of an enzyme blend induced a pro-inflammatory response in growing pigs fed a high NSP diet, yet the mechanism still needs to be elucidated (Vila et al., 2018).

1.5.2. Microbiome

The composition of the gut microbiome and its change over time is a critical factor in gut health. Environmental conditions, maternal microbiome, and diet each play a critical role in establishing and developing the microbiome (Zhang et al., 2018). In weaned pigs, gut development and maturation are slow and therefore create a risk for pathogenic bacteria to colonize. Fibre acts as a prebiotic, by providing the commensal bacteria in the LI an energy source, and thereby cultivates the development of a healthy microbiome (Bindelle et al., 2008). Additionally, increased SCFA production in the hindgut lowers the pH, preventing the colonization of pathogenic Enterobacteriaceae and *Clostridia* (Tan et al., 2021). For example, feeding pigs wheat-based diets increased microbiota diversity and species richness, compared to corn-based diets (Zhang et al., 2018). Production of SCFA, particularly butyrate, by a mature microbiome also contributes energy to the pig, further contributing to gut health (Zhang et al., 2018). For weaned pig diets specifically increasing amounts of undigested protein and starch can be fermented in the LI. Fermentation by an immature microbiome can be detrimental to growth performance traits in weaned pigs, through toxic fermentation products or decreased appetite (Lee et al., 2020).

Feeding enzymes that increase nutrient digestibility reduces the amount of undigested substrates in the LI, preventing excessive fermentation. For example, the degradation of AX liberates oligosaccharides and monosaccharides. Monosaccharides can be absorbed by the epithelial cells in the foregut whereas oligosaccharides bypassed into the hindgut act as a prebiotic for the microbiome (Torres-Pitarch et al., 2019). The presence of xylose, which is a product of xylanase activity can stimulate growth of Bifidobacteria (Patience and Petry, 2019). Inclusion of a carbohydrases blend and its effects on composition of the microbiome has been studied previously. A decrease in ileal *Lactobacillus*, but a greater ileal and caecal *Enterobacteriaceae* population occurred compared with no enzyme inclusion (Li et al., 2019). Feeding the enzyme blend, however, decreased colonic *Lactobacillus* and total SCFA production likely due to increased ileal digestibility of both fibre and starch. In a trial with 96 grower pigs (initial BW 22.7 kg), feeding xylanase altered beta diversities in the caecal microbiota compared with the control. More specifically, xylanase inclusion reduced the influence of Bacteroidetes and promoted Firmicutes phylum. The authors suggested that breakdown of AX into monosaccharides reduced the availability of polysaccharides to Bacteroidetes. However, based on the observed taxonomy, feeding the xylanase treatments provided a greater number of hub bacterial taxa belonging to the Firmicutes phylum compared with the control group (Zhang et al., 2018).

1.6 Including feed enzymes to enhance flexibility in diet formulation

Availability and volatility in ingredient prices may influence producers to adopt niche feed ingredients. Feeding local coproducts provides an opportunity to lower feed cost, because of lower commodity price as well as transportation cost (Torres-Pitarch et al., 2019). In Canada, common coproducts include CM or canola expeller, wheat millrun or middlings from flour milling. These coproducts are high in NSP that can reduce digestibility of other nutrients. For example, wheat coproducts from flour milling can contain 8.0 - 49.2% NSP depending on processing method. Feeding these high fibre coproducts to older animals with a developed hindgut is well established; however, feeding these to weaned pigs is limited. Therefore, the inclusion of exogenous enzymes could improve utilization of these relatively high fibre feed ingredients in weaned pigs and provide pork producers opportunities to lower feed cost (Torres-Pitarch et al., 2018).

1.6.1. Canola Coproducts

Canola is a common oilseed grown in Canada to produce oil for culinary human food consumption. After the oil is pressed out and the leftover coproduct is washed with solvent, the remaining meal is relatively high in protein (36 to 38% CP as is), with a favourable AA profile (Jha and Berrocoso, 2015). The amount of hull lignin, cellulose and other NSP limit its inclusion in weaned pig diets (Pedersen et al., 2016). Researchers have explored the feeding of enzymes such as protease and carbohydrases to increase the nutritive value of canola meal (Lee et al., 2018). In vitro, an enzyme blend containing xylanase, glucanase, cellulase, mannanase, invertase, protease, amylase and pectinase was tested against a variety of canola coproducts including CM. The multi-enzyme inclusion tended to increase DM digestibility of all coproducts. Additionally, multi-enzyme inclusion reduced total gas production and total SCFA production of cold-pressed canola meal. Therefore, the multi enzyme improved the utilization of nutrients in canola coproducts in vitro (Lee et al., 2018). Using 96 weaned pigs (initial BW: 7.2 ± 1.2 kg), Zijlstra et al. (2004) tested the inclusion of 25% canola meal including a carbohydrase on diet digestibility and growth performance. They reported an increase in ADFI and ADG feeding diets with carbohydrase compared with control. However, they also reported that carbohydrase supplementation did not increase G:F or digestibility, indicating that the enzyme helped to stimulate feed intake of diets low in DE value.

1.6.2. Cereal coproducts

Various cereal coproducts are readily available in western Canada. For example, wheat milling for human bread and pasta consumption produces wheat millrun, middlings, and (or) shorts as coproducts that contain NSP, specifically AX (Zhang et al., 2018). In growing pigs, the inclusion of flour milling by-products decreased AID and ATTD of energy (Nortey et al., 2007, 2008). Due to high levels of AX, both trials examined feeding xylanase to increase nutrient digestibility. Xylanase increased AID of energy by 19% and ATTD of energy by 7% (Nortey et al., 2007). In the second study, feeding a combination of xylanase and phytase with wheat millrun increased calcium and phosphorus digestibility and retention (Nortey et al., 2008). For growth performance, the same study found that the inclusion of wheat millrun decreased ADFI, ADG and final BW. Evaluating dietary enzyme inclusion, xylanase increased G:F whereas phytase reduced ADFI (Nortey et al., 2008). However, carbohydrase inclusion to diets including 120-500 g wheat millrun/kg fed to weaned pigs did not affect pig performance or diet digestibility (l'Anson et al., 2014).

1.7 Conclusions

Both improving gut health and reducing feed cost is of great interest to pork producers and nutritionists for all stages of production. Among life stages, weaned pig diets are the costliest because of the inclusion of highly digestible, specialty ingredients. Lower cost, plant-based ingredients have lower digestibility because of greater fibre and phytate and lower protein content compared with feeding animal by-products. Fibre has major physical and physiological effects on the GIT including digestion, absorption, feed intake, growth and G:F in weaned pigs. Pigs cannot utilise indigestible fibrous components in feed that may dilute dietary nutrient content and cause nutrients to remain entrapped within the fibre matrix. Similarly, plant-proteins can have a poor amino acid profile, combined with poor digestibility in weaned pigs compared with animal proteins. Introducing plant-based proteins too abruptly after weaning can be detrimental to piglet feed intake, growth and health. Additionally, phytate is a common anti-nutritional factor in swine diets, preventing digestion and absorption of phosphorus, calcium, AA, minerals and vitamins. Exogenous feed enzyme inclusion can help increase the utilisation of lower cost plant ingredients by increasing digestibility and improving gut health. For example, including carbohydrase in diets high in fibre can improve the utilization of fibre and starch, while increasing the digestibility of other nutrients. Protease can increase the digestion and absorption of plant proteins in weaned pigs, while phytase can increase the digestibility of multiple nutrients tied to phytate rings. Furthermore, feed enzyme blends containing select enzymes to match the ingredient substrates in the diet can have additive or synergistic activity to maximize digestion and consequently increase nutrient absorption in weaned pigs. Feeding enzymes may increase nutrient digestibility and growth performance of pigs; however, the results can be inconsistent with many trials reporting limited or no benefit in digestibility, or more commonly pig performance. These conflicting results suggest that dosage, combination, and activity level of enzymes is extremely sensitive. Another important aspect of weaned pig management is gut health. Thus far, literature has reported a broad benefit to immune response and microbial populations including feed enzymes. Furthermore, feeding exogenous enzymes can increase the digestibility of common coproducts such as canola meal, and wheat millrun to provide alternatives and flexibility when sourcing ingredients for weaned pig diets. Increasing the digestibility and utilization of nutrients within the feed not only lowers feed cost, but also has an environmental benefit by increasing efficiency and reducing the amount of undigested nutrients excreted in manure, improving the sustainability of pork production (Péron and Partridge, 2009).

1.8 Knowledge Gaps

Feeding highly fibrous diets, including coproducts can be detrimental to weaned pig growth performance and health, ultimately reducing efficiency of pork production in western Canada. Additionally, sourcing common feed ingredients such as SBM or corn grain from other countries creates volatility in feed prices, increasing the price of dietary energy and protein. The efficacy of phytase in weaned pig diets is well established; thus, incentives exist to include enzymes for other substrates such as fibre, starch and plant protein. However, enzyme efficacy can be multi-factorial, so that the dose, activity level, substrate, environmental conditions can all affect the feasibility of including enzymes in weaned pig diets.

1.9 Thesis hypothesis and objectives

The null hypotheses of the present thesis were: (a) Inclusion of a multi-enzyme blend to diets containing 250 g canola expeller/kg would not improve the nutrient digestibility in weaned pigs, and that the nutritional value between canola expeller samples would not differ; (b) Inclusion of multi-enzyme blend to diets containing 75 g wheat millrun/kg would not improve the nutrient digestibility in weaned pigs, and that the dose of the enzyme blend would not increase nutrient digestibility.

The objectives of this thesis were therefore:

a) To measure and compare the apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of diets containing 250 g/kg of two canola expeller samples, with or without the inclusion of a multi-enzyme blend fed to ileal cannulated weaned pigs.

b) To measure and compare the apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of diets containing 75 g/kg wheat millrun with the inclusion of two doses of a multi-enzyme blend fed to ileal cannulated weaned pigs.

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Table 1.1 Proposed mode of action of exogenous enzyme included in swine diets

Mode of action	Reference
Hydrolyse bonds not possible by endogenous enzymes	Velázquez-De Lucio et al., 2021
Limits effects of antinutritional factors	Velázquez-De Lucio et al., 2021 Patience and Petry, 2019
Reduction of endogenous losses	Velázquez-De Lucio et al., 2021 Patience and Petry, 2019
Eliminates nutrient encapsulation	Velázquez-De Lucio et al., 2021 Patience and Petry, 2019 Musigwa et al., 2021
Improves gut health. (i.e., barrier function)	Patience and Petry, 2019
Stimulates endogenous enzyme production	Patience and Petry, 2019
Reduces losses to fermentation or excretion	Patience and Petry, 2019 Bedford, 2000
Releases beneficial end-products. (i.e., monosaccharides)	Patience and Petry, 2019

Table 1.2 Enzyme name and respective substrate

Enzyme	Substrate	Reference
Amylase	Starch	Bedford, 2000
Xylanase	Arabinoxylans	Torres-Pitarch et al., 2019
L-arabinofuranosidases	Arabinofuranose	Lei et al., 2016
Feruloyl esterase	Ferulic acid	Lei et al., 2016 Musigwa et al., 2021
α-D-glucuronidases	Glucuronic acid	Lei et al., 2016 Musigwa et al., 2021
Acetyl esterase	O-acetyl	Lei et al., 2016 Musigwa et al., 2021
β-glucanase	β-glucans	Patience and Petry, 2019
β-1,4-glucanase	Cellulose	Juturu and Wu, 2014 Patience and Petry, 2019
exoglucanase	Cellulose	Juturu and Wu, 2014 Patience and Petry, 2019
β-glucosidase	Cellulose	Juturu and Wu, 2014 Patience and Petry, 2019
β-mannanases	Galactomannans	Malgas et al., 2015
Pectinase	Pectins	Tahir et al., 2008
Proteases/ peptidases	Proteins and peptides	Rawlings, 2013 Sharma et al., 2019
Phytase	Phytate or phytic acid	Humer et al., 2015 Kumar et al., 2015 Velázquez-De Lucio et al., 2021

Figure 1.1 Schematic of arabinoxylan (AX) with respective side chains (Adapted from Correia et al., 2011)

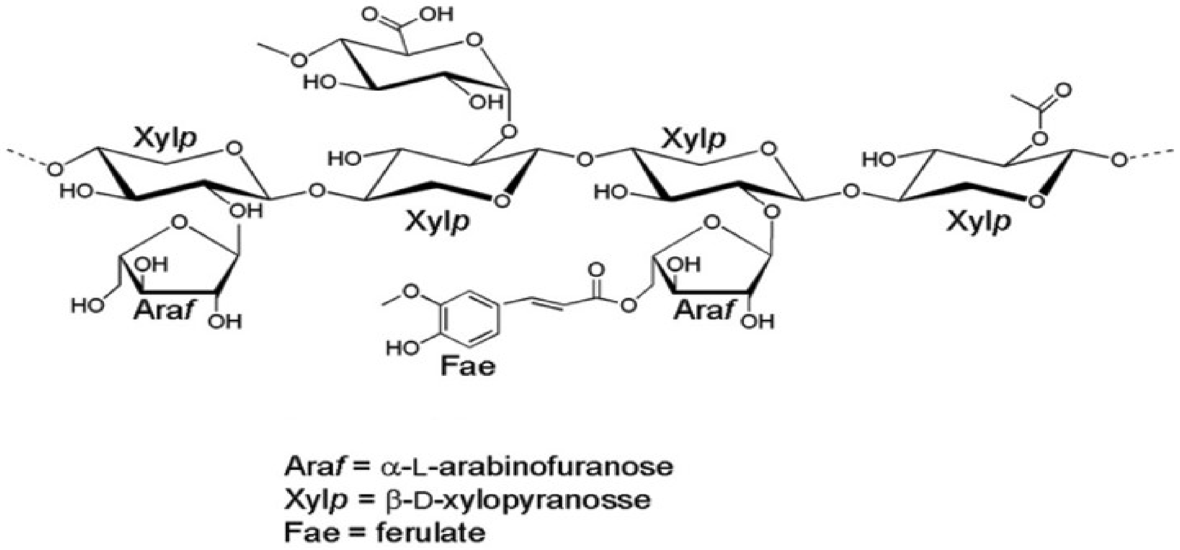


Figure 1.2 Molecular structure of phytic acid and chelated a) minerals or b) amino acids.

(Adapted from Humer et al., 2015)

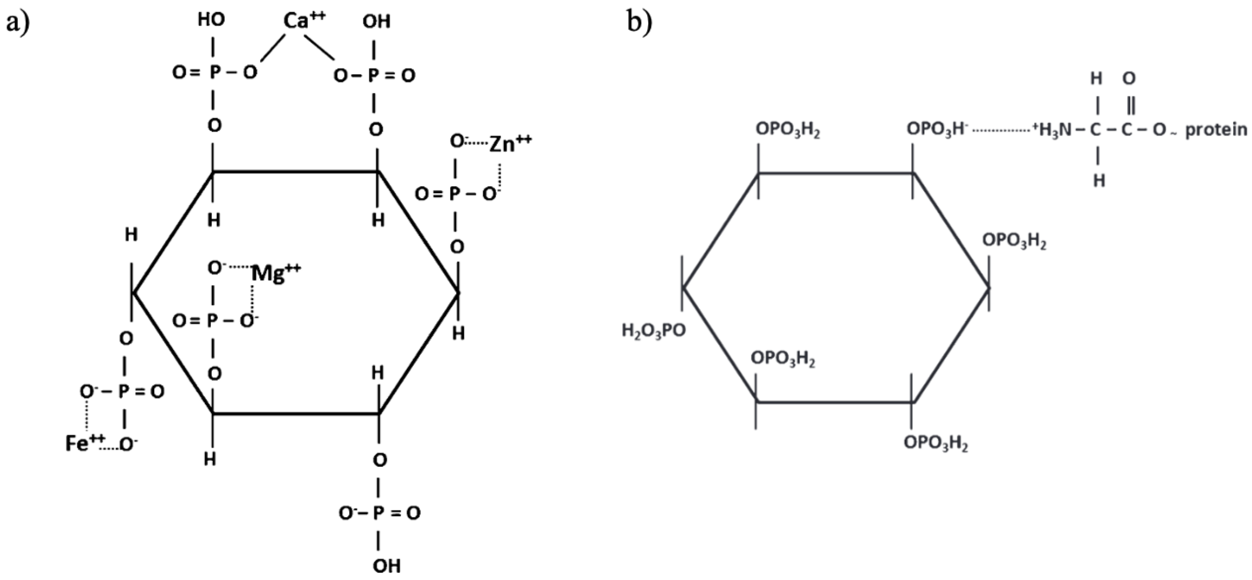


Figure 1.3 Schematic of carbohydrase degradation of fibre (Juturu and Wu, 2014)

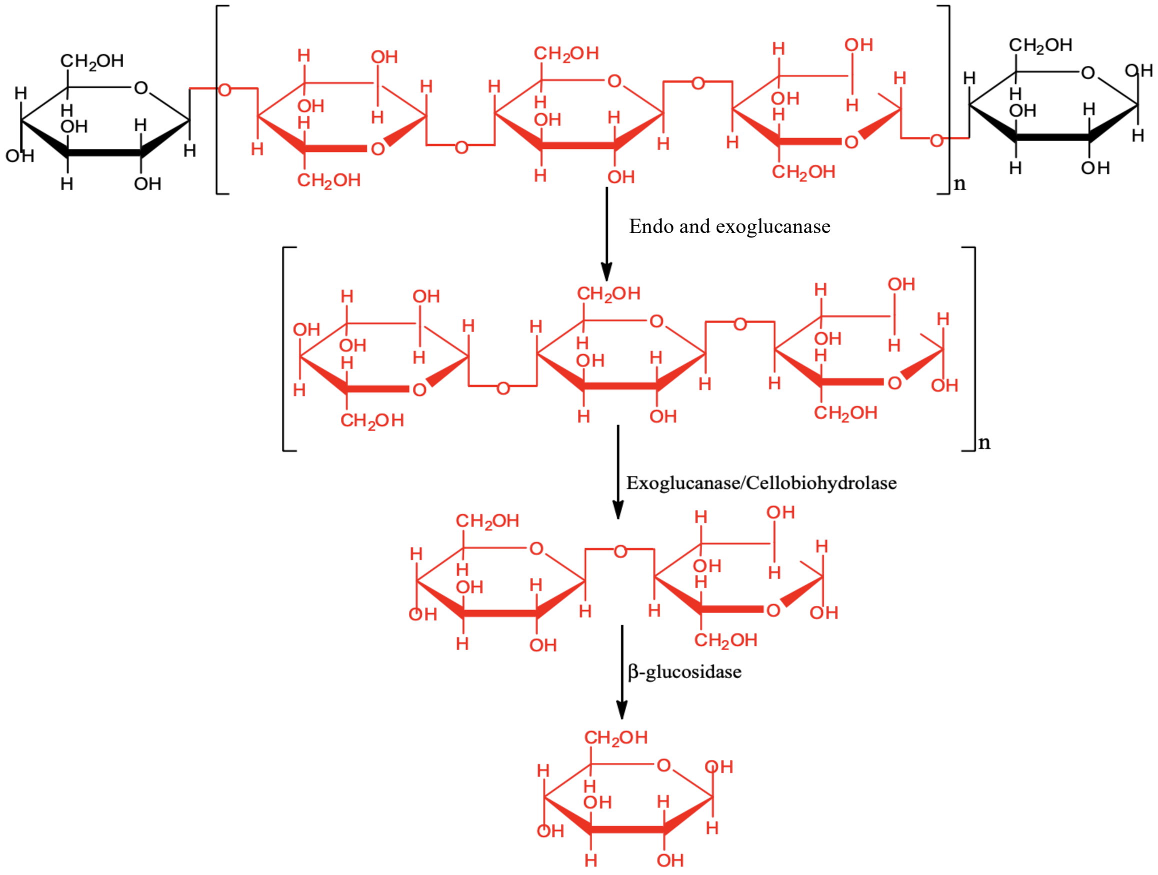


Figure 1.4 Amino acid sequence and cleavage sites of respective peptidases (Rawlings, 2013)

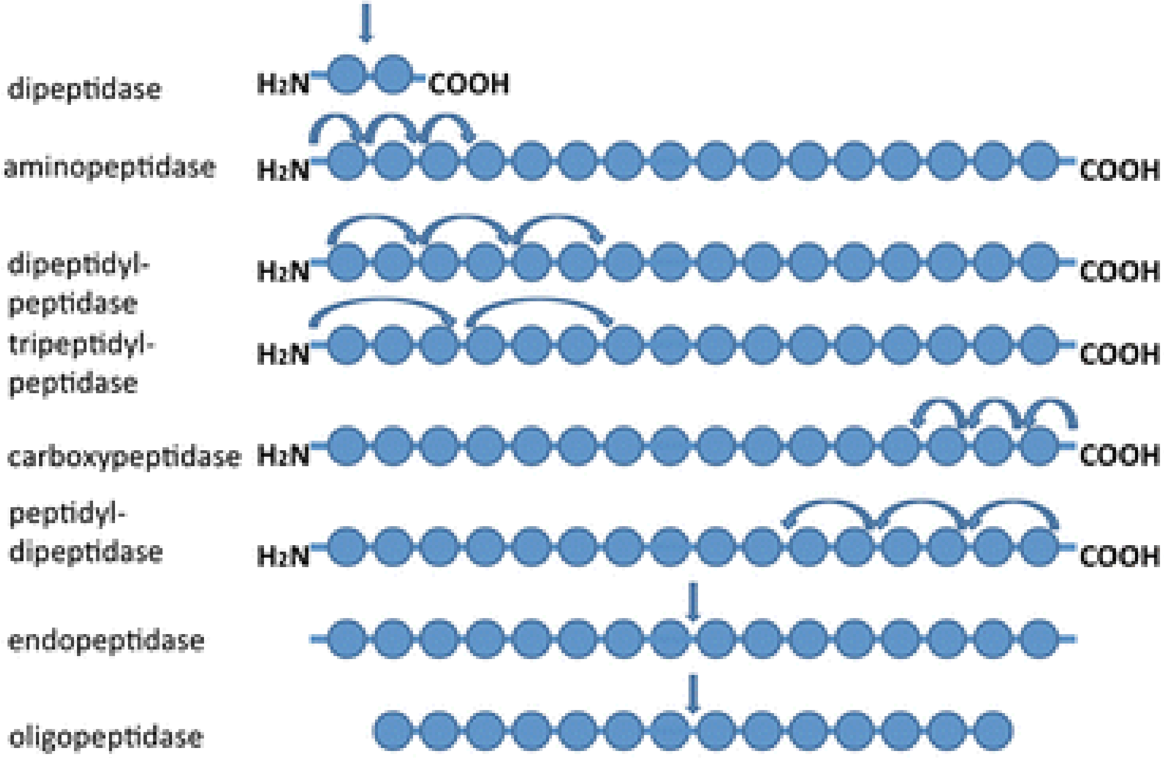


Figure 1.5 Phytate hydrolysis by phytase to produce inositol and inositol monophosphate (Kumar et al., 2015)

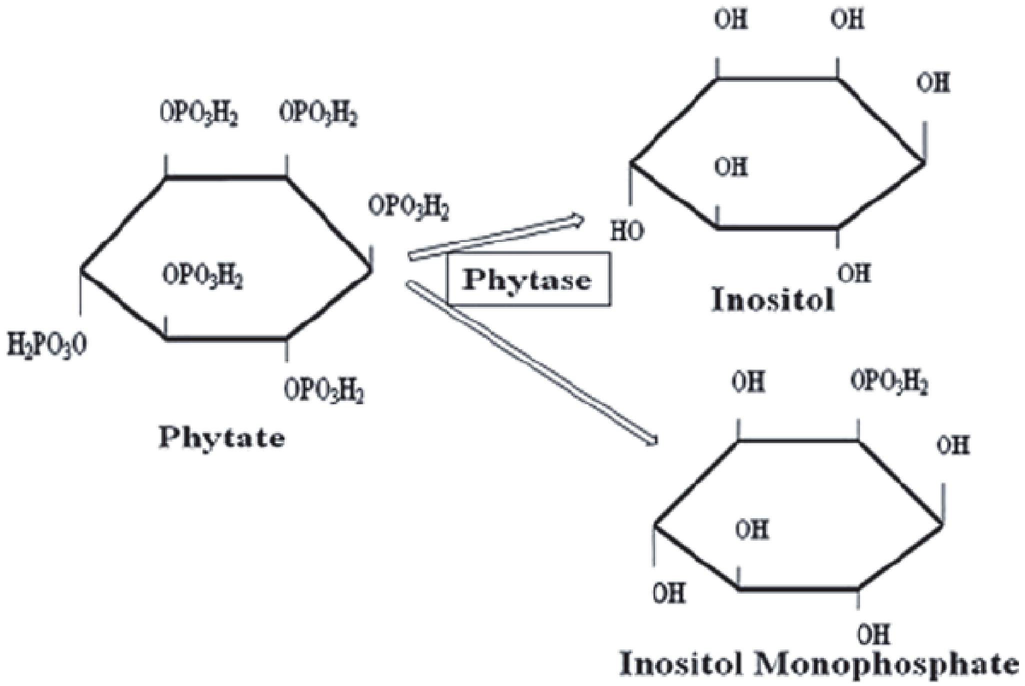
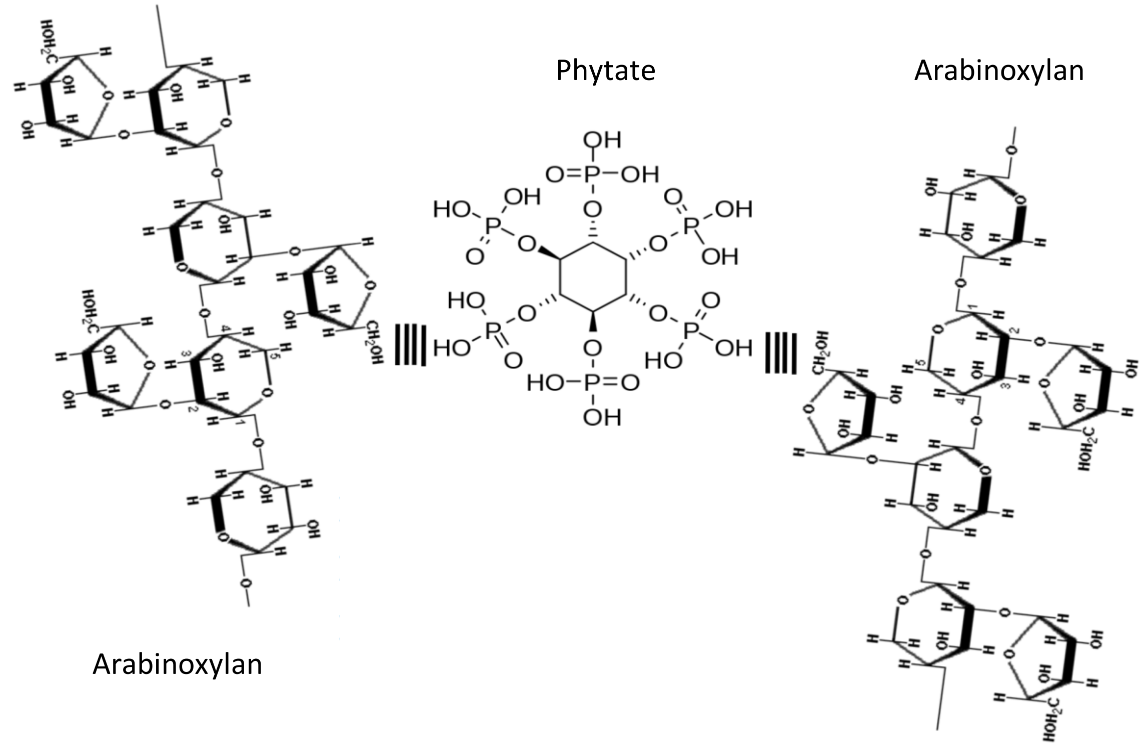


Figure 1.6 Phytate cross-linking arabinoxylan (AX)



Chapter 2: Feeding a multi-enzyme blend to enhance the nutrient digestibility of wheat-canola expeller diets in ileal-cannulated weaned pigs.

2.1 Introduction

Expansion of canola production in western Canada has coincided with increased tonnage of seed; however, not all canola grown meets human food oil grade. Excess food grade and lower grade canola seed can be diverted to feed markets (cake and oil feeding) and biodiesel production (Gaber et al., 2018). Facilities without solvent extraction produce canola coproducts such as ‘cold-pressed’ canola cake (around 200 g/kg remaining oil) or canola expeller (CE; up to 200 g/kg remaining oil) based on the processing equipment used (Seneviratne et al., 2010, 2011). Utilizing excess and non-food grade canola seed can increase sustainability of the crop and livestock sectors by feeding these coproducts to livestock (Canola Council of Canada, 2021). Locally produced, CE is a discounted feedstuff (Seneviratne et al., 2011; Zijlstra and Beltranena, 2013) and its feeding provides an opportunity to lower feed cost (Jha et al., 2013; Woyengo et al., 2014). Beyond soybean meal (SBM), canola coproducts are a good source of protein and amino acids (AA) in swine diets. Because of oil pressing without solvent extraction, CE contains more residual oil (100–200 g oil/kg) than canola meal (CM; 26 g crude fat/kg) and SBM (22 g crude fat/kg) increasing its net energy (NE) value. Apart from differences in lipid content between CE and CM, variation in available nutrients among CE samples may also occur from processing. For example, extruded canola seed can be subjected to seed conditioning and pressing friction temperature variation (95-130°C) that can lower the bioavailability of some AA such as lysine (Mustafa et al., 2000; Almeida et al., 2014). Other factors, e.g., cultivar, seed quality and equipment variables, can also influence CE digestibility (Leming and Lember, 2005).

Canola coproducts contain several anti-nutritional factors (ANF). Glucosinolates are low in modern canola cultivars, yet are concentrated in CE after pressing, and can reduce CE and CM digestibility (Seneviratne et al., 2011; Woyengo et al., 2014). Phytic acid impairs digestibility of phosphorus, minerals and AA (Wanasundara et al., 2016). Finally, the fibre content of CE is similar to CM, but greater than dehulled SBM, and can hinder digestibility of energy and other nutrients (Péron and Partridge, 2009; Smit et al., 2014). The CE contains 240 g NDF/kg and 160 g ADF/kg compared with 82 and 53 g/kg in dehulled SBM, respectively (Landero et al., 2012; NRC, 2012). Fibre content in canola coproducts primarily limits the inclusion rate in weaned pig diets as glucosinolate content is no longer an issue (< 10 µmol/kg; Adewole et al., 2016). Dietary inclusion of feed enzymes may increase digestibility of fibre in canola coproducts. Recently, supplementation of a multi-enzyme blend, including xylanase, glucanase, cellulase, mannanase, invertase, protease, pectinase, and amylase increased in vitro dry matter digestibility of canola coproducts (Lee et al., 2018). Degradation of fibre in CE may increase the efficacy of gastric and pancreatic enzymes to digest dietary nutrients and thereby allow young pigs to meet their high AA and NE requirements (Adeola and Cowieson, 2011; Woyengo et al., 2018). Finally, the ileal nutrient digestibility of CE in weaned pigs has not been described often. More reports are needed to convince pig producers of the benefits of feeding canola coproducts.

The null hypotheses of the present study were that diet nutrient and energy digestibility would not change with inclusion of CE, that the nutritional value would not differ between two CE samples, and that the addition of a multi-enzyme blend would not alter the nutrient digestibility of CE diets or CE individually. The objectives were to determine and compare the coefficient of apparent ileal digestibility (CAID) and coefficient of apparent total tract digestibility (CATTD) of energy and nutrients in a basal diet and two diets containing 250 g/kg of different CE samples with or without inclusion of a multi-enzyme blend fed to ileal-cannulated weaned pigs.

2.2 Materials and Methods

The animal experiment was conducted at the Swine Research and Technology Centre of the University of Alberta (Edmonton, AB, Canada) following animal use approval and review of procedures by the Animal Care and Use Committee for Livestock following guidelines established by the Canadian Council on Animal Care (CCAC, 2009).

2.2.1. Test materials and diet processing

A wheat-barley basal diet was sourced from Country Junction Feeds (Wetaskiwin, AB, Canada). The basal diet was formulated based on NE values from NRC (2012) and digestible amino acid values from INRA (2013). Two CE samples were sourced locally: canola expeller A from Milford Hutterite Colony (Raymond, AB, Canada) and canola expeller B from Prairie Home Hutterite Colony (Conrad, AB, Canada). Canola expeller diets included 750 g basal/kg plus 250 g CE-A or CE-B/kg, with or without a 0.5 g/kg of multi-enzyme blend. Per gram of multi-enzyme blend contained 900 U cellulase, 1,200 U xylanase, 250 U glucanase, 12,000 U amylase, 6,000 U protease, 700 U invertase and 2,400 U pectinase (Superzyme-Conc®, CBS Bio-Platforms; Calgary, AB, Canada). Diets included 5 g titanium dioxide (TiO_2)/kg as an indigestible marker for digestibility determination (Table 2.1). Diets were mixed (6 minutes) using a 300-kg horizontal paddle mixer (model 3061; Marion Process Solutions, Marion, IA, USA) and were fed as a mash. Particle size of the ingredients and diets were analysed using a mechanical sieve shaker (Model RX-29, W.S. Tyler, ON, Canada) following the method of the American Society of Agricultural and Biological Engineers (2008; Table 2.2).

2.2.2. Experimental design and management

This experiment was designed as a replicated 6 (pigs) × 3 (periods) Youden square to reach 6 observations per dietary treatment. In total, 12 Duroc × Large white/Landrace F1 barrows (12 to 18 kg body weight) were surgically cannulated at the distal ileum. Pigs recovered from cannulation surgery and had a gradual increase of feed allowance over 7 days. Introduction of the experimental diets was carried out by substituting 250, 500, 750 g/kg of the pre-surgical diet with the specific experimental diet over 3 days ending with 1,000 g/kg experimental diet by the beginning of the first diet acclimation period. Daily feed allowance was calculated at 2.8 (group 2) or 3.0 (group 1) × maintenance DE (110 kcal of DE per kg of $BW^{0.75}$; NRC 1998) divided into two meals offered at 08:00 and 15:00. Throughout the trial, pigs were housed in individual metabolic pens measuring 1.2 m length × 1.2 m width × 0.95 m height (1.8 m^2). The pens had walls made of polyvinyl plastic planking with 0.16 m^2 plexiglass windows on three sides. Free access to water was provided from a cup drinker placed 0.25 m above the floor beside the feeder. The stainless-steel feeder measured 0.35 m width × 0.4 m height with the trough 0.12 m above the pen floor. The climate-controlled room was maintained at 25 ± 2.5ºC for pigs < 15 kg and 22 ± 2.5ºC for pigs >15 kg. Lighting was provided daily from 07:00 to 19:00.

Each nine-day period started with 5 days of acclimation to the experimental diet, followed subsequently by 2-day collection of faeces and 2-day collection of digesta. Faeces were collected with plastic bags secured between leather and Velcro rings glued around the tail and anus for 48 h starting at 08:00 and monitored throughout both faecal collection days (van Kleef et al., 1994). Faeces from each pig were promptly pooled and frozen. Following completion of collection, faeces were thawed, homogenized and subsampled. Digesta was collected continuously from 08:00 to approximately 19:00 through the opened T-cannula via attached plastic collection bags containing 15 mL of 50 g/kg formic acid to prevent bacterial fermentation. Meals were provided at 08:00, 10:00, 13:00 and 15:00 during digesta collection to allow for continuous flow of digesta from the cannula. Bags containing digesta were promptly pooled and frozen. Following completion of collection, digesta was thawed, homogenized and subsampled. Once subsampled, faeces and digesta specimens remained frozen (–20°C) until lyophilisation.

2.2.3. Chemical analyses

Test ingredients, diets and lyophilized digesta and faeces were ground through a 1-mm screen using a centrifugal mill (Model ZM200, Retch GmbH, Haan, Germany). Test ingredients and diets were analysed for starch (assay kit STA-20; method 76-13; AACC, 2023) and using AOAC-methods for crude fat (method 920.39A), neutral detergent fibre (NDF) assayed without a heat-stable amylase and expressed inclusive of residual ash (Holst, 1973), acid detergent fibre (ADF) inclusive of residual ash (method 973.18) and ash (method 942.05) at the Agricultural Experiment Station Chemical Laboratories (ESCL), University of Missouri (Columbia, MO, USA; AOAC, 2006). Test ingredients were analysed for total dietary fibre (method 985.29), soluble and insoluble dietary fibre (method 991.43) and crude fibre (method 934.01), calcium (method 968.08), phosphorus (method 946.06), amino acids (AA; method 982.30E a-c) and chemically available lysine (method 975.44) at ESCL (AOAC, 2006). Digesta was analysed for moisture, crude protein (CP) and AA at ESCL. Diets, digesta and faeces were analysed for dry matter (DM; method 930.15; AOAC, 2006), CP by LECO (nitrogen × 6.25; method 990.03), gross energy (GE) using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany) and titanium dioxide (TiO_2) content at the University of Alberta (Myers et al., 2004).

2.2.4. Calculations

The CAID (AA) and CATTD of DM, CP and GE of the diets were calculated for each diet using the index method via the following calculation:

$$\text{CAID or CATTD} = 1 - [\left(\frac{(\text{Concentration of TiO2}_{\text{feed}}) \times (\text{Concentration of component}_{\text{digesta or feces}})}{(\text{Concentration of TiO2}_{\text{digesta or feces}}) \times (\text{Concentration of component}_{\text{feed}})}\right)]$$

The CAID and CATTD of DM, CP, AA and GE of the two CE ingredients were calculated using the difference method was used using the following calculation (Adeola, 2001):

$$\text{CAID or CATTD} = \left(\frac{(\text{CAID or CATTD}_{\text{diet}} \times \text{proportion}_{\text{diet}}) - (\text{CAID or CATTD}_{\text{Basal}} \times \text{proportion}_{\text{basal}})}{\text{proportion}_{\text{test ingredient}}}\right)$$

Diet and ingredient DE (MJ/kg) was calculated by the following formula:

$$\text{DE} = (\text{GE}_{\text{Diet or ingredient}} \times \text{GE CAID or CATTD}_{\text{diet or ingredient}})$$

Diet and ingredient NE (kcal/kg) were calculated using the equation of Noblet et al. (2004) as adopted by the NRC (2012), where nutrient values were expressed as g/kg DM, then converted to MJ/kg:

$$\text{NE} = (0.7 \times \text{DE}) + (1.61 \times \text{EE}) + (0.48 \times \text{Starch}) - (0.91 \times \text{CP}) - (0.87 \times \text{ADF})$$

2.2.5. Statistical analysis

Data were analysed using the GLIMMIX procedure of SAS as a replicated 2 × 3 (Diets) and 2 × 2 (Ingredients) factorial arrangement. Normality and homogeneity of variance for the residual of each variable and carry-over effect of diets fed in previous period were tested prior to ANOVA analysis. Pig was the experimental unit. Diet was the fixed effect, whereas square, pig (nested in square) and period (nested in square) were random terms. For multiple comparisons, *P* values were adjusted using Tukey-Kramer method. To test the hypothesis, $P < 0.05$ was considered significant.

2.3 Results

Pigs remained healthy throughout the experiment and maintained daily feed intake regardless of test diet offered. Diets including CE contained 13% less starch and 10% less ash than basal diets (Table 2.2). Diets including CE contained more CP, crude fat, ADF and NDF and 1.35 MJ/kg more GE than basal diets.

The CE-A contained 26 g/kg more crude fat than CE-B (Table 2.3). However, CE-B contained 12 g more CP/kg, 5 g more total dietary fibre (TDF)/kg and had 49 μm larger particle size than CE-A. For both CE samples, 98% of the TDF was insoluble.

For CATTD of DM and GE and diet NE values, an interaction between diet and enzyme supplementation was observed (P < 0.05; Table 2.4). Dietary inclusion of CE reduced ($P < 0.05$) diet CAID and CATTD of DM, GE and CP for both diets with and without supplemental enzyme. Dietary inclusion of CE increased ($P < 0.05$) diet ileal and total tract DE and NE values. Addition of multi-enzyme blend increased ($P < 0.05$) the CATTD of DM and GE of the basal diet by 0.012 and 0.015, respectively. Addition of multi-enzyme blend increased NE value of the basal diet by 0.34 MJ/kg.

Dietary inclusion of CE decreased ($P < 0.05$; Table 2.5) diet CAID of AA. For CAID of diet methionine, tryptophan and tyrosine, an interaction between diet and enzyme supplementation was observed ($P < 0.05$). Specifically, dietary enzyme inclusion reduced (*P* <

0.05) CAID of tryptophan for CE-A, reduced (*P* < 0.05) CAID of methionine for CE-B and increased (*P* < 0.05) CAID of tryptophan and tyrosine for CE-B.

The CAID of DM, GE and CP and the CATTD of DM and GE did not differ between the two CE samples (Table 2.6). Ingredient NE value was 0.87 MJ/kg lower (*P* < 0.05) for CE-B than CE-A. Dietary enzyme increased (*P* < 0.05) the CATTD of CP for CE-A only.

The CAID of most AA did not differ between CE-A and CE-B (Table 2.7). However, inclusion of dietary enzyme decreased (*P* < 0.05) CAID of methionine for CE-B only.

2.4 Discussion

2.4.1. Canola expeller inclusion

The CE may contain up to 200 g remaining oil/kg instead of the 30 g crude fat/kg in canola meal thus, inclusion in weaned pig diets can increase the energy content (Seneviratne et al., 2010; Grageola et al., 2013; Woyengo et al., 2016). The two CE samples obtained for the present study contained 66–93 g/kg more crude fat than previously reported for CE (Landero et al., 2012; Grageola et al., 2013), confirming that nutritional quality and energy value differs among CE samples. Both CE samples contained similar ADF to previously reported values (Grageola et al., 2013). In the present study, a highly digestible wheat and barley-based basal diet was fed as a control. Adding 250 g CE/kg decreased diet CAID and CATTD of DM, GE and CP and CAID of total AA likely because of increased dietary fibre content, as reported in previous studies (Landero et al., 2012; Grageola et al., 2013; Woyengo et al., 2016). The non-starch polysaccharides present in the CE diets reduced digestibility of other nutrients, particularly in weaned pigs because of an underdeveloped hindgut (Jha and Berrocoso, 2015). In previous nutrient digestibility studies feeding ileal-cannulated weaned pigs, dietary inclusion of

100 g CM/kg reduced CAID of CP and AA (Mariscal-Landín et al., 2008). As the first limiting AA, CAID of lysine is often considered more important than CP digestibility (Seneviratne et al., 2011). In the present study, CAID of lysine was reduced compared with the control, a reduction consistent with that reported by Mariscal-Landín et al. (2008) in weaned pigs and Seneviratne et al. (2010) in growing pigs. Pressing canola seed can create high temperature in the product through steam conditioning prior to flaking and friction within the barrel of the expeller press. However in the present study, 97% of the lysine was chemically available in both CE samples, indicating that Maillard reactions were biologically not relevant and heat damage during processing was minimal (Landero et al., 2012). In the present study, the remaining oil in the CE samples increased diet DE and NE, ileal DE and ileal NE values compared with feeding the control diet (Table 2.4). This approach contrasts similar studies that formulate consistent NE values for the experimental diets (Seneviratne et al., 2011; Heyer et al., 2021). Increasing the inclusion rate up to 250 g CE/kg in weaned pig diets may lower the required inclusion of energy-rich fats such as canola oil to maintain diet energy value and may thereby lower feed cost.

The nutritional value of CE may vary among small-scale domestic processing facilities in Canada because of seed quality and processing variables (Adewole et al., 2016). Regarding seed, cultivar, growing conditions, harvest and storage conditions and moisture are important factors. Regarding processing, press type, processing temperature and moisture conditions are important. Among 11 canola solvent extraction processing facilities in Western Canada, CM varied in nutrient content (Adewole et al., 2016). Specifically, CM ranged from 402–429 g/kg in CP and from 26.0–43.0 g/kg DM in ether extract (EE). Interestingly, without differences in NSP content, NDF and lignin content did differ between all the samples suggesting variation between nutritional value. In the present study, the available lysine content was consistent between the two CE samples, indicating similar temperature conditions during processing. These two CE samples differed mainly in crude fat, NDF and ADF content and particle size (Table 2.3). Using the difference method, it was established that the two CE samples did not differ in AID and ATTD of DM, GE and CP, likely due to their similar macronutrient content and physiochemical properties. However, CE-A had a greater NE value than to CE-B, likely because greater crude fat content (Paternostre et al., 2021). Fat in the diet may decrease passage rate and thereby increase nutrient digestion and absorption (van Avesaat et al., 2015). Although fibre decreased nutrient digestibility, the increased NE value from crude oil contained in CE was greater than the reduction in NE because of its fibre, as confirmed by Paternostre et al. (2021).

2.4.2. Enzyme inclusion

Enzyme inclusion in pig diets has been studied extensively to increase nutrient digestibility and subsequent growth performance in all stages of production (Adeola and Cowieson, 2011). Weaned pig diets provide the largest enzyme market in swine production because several substrates such as NSP, protein and phytate can reduce nutrient digestibility and exacerbate the post-weaning lag or increase incidence of diarrhoea due to an immature gut (Torres-Pitarch et al., 2017). With increased diet complexity and fibrous ingredients, diets can be supplemented with multi-enzyme blends to increase degradation of dietary substrates that hinder nutrient digestion (Diebold et al., 2004; de Lange et al., 2010; Velayudhan et al., 2019). Enzyme blends can have an additive or synergistic effect to increase digestibility in pig diets further than can be achieved feeding a single enzyme (Velayudhan et al., 2019). Dietary inclusion of single or multiple enzymes increased ileal digestibility of GE, DM and CP with xylanase and phospholipase in wheat-based diets (Diebold et al., 2004) and with cellulase, galactanase, mannanase and pectinase in corn-based diets fed to weaned pigs (Omogbeonigun et al., 2004). In the present study, inclusion of the multi-enzyme blend, increased ATTD of DM and GE for the wheat and barley-based basal diet. The enzyme blend included in the present experiment included cellulase, xylanase, glucanase, amylase, invertase and pectinase activity that was consistent with the substrates present in the basal diet, specifically arabinoxylans in wheat and β-glucans in barley. Interestingly, although protease was included in the enzyme blend, neither AID or AA or ATTD of CP were increased because of enzyme blend inclusion in any of the experimental diets. However, looking at the ingredients independently, enzyme inclusion increased the ATTD of CP solely for CE-A. In studies with a similar enzyme blend containing protease, AID or SID of CP or AA was not affected in growing pigs fed fibrous camelina cake (Woyengo et al., 2018). Particle size is another consideration for enzyme efficacy. Increased particle size (> 800 μm) typically leads to lower nutrient digestibility but may result in greater enzyme efficacy (Ngoc et al., 2011). In the present study, the particle size of the CE diets was consistent with controls, and industry standard (600 μm) for wheat-based mash diets, suggesting the particle size was not a factor for the lack of enzyme efficacy in the CE diets (Vukmirović et al., 2017; Table 2).

In the present study, feeding the enzyme blend did not affect nutrient digestibility of diets including either CE. Similarly, the enzyme blend did not increase the AID of DM, GE and CP and ATTD of DM and GE of the CE samples individually. The canola seed itself is small in size, measuring 1 mm in diameter compared with cereal grain (e.g., 2.75 mm for wheat) or pulse grains, e.g., 6 mm for field pea (Gatti et al., 2011; Manitoba Canola Growers, 2023; Khalid et al., 2022). Because of this small size, the highly ligneous hull (300 g/kg of CM) can severely impact enzyme efficacy and overall nutrient digestibility (Slominski and Campbell, 1990). As primarily insoluble fibre, the canola hull and therefore expeller, is difficult to be degraded enzymatically through decreased contact and increased passage rate (de Lange et al., 2010; Jha et al., 2019). Often, effects of multi-enzyme inclusion are therefore inconsistent in pig diets containing canola coproduct and other fibre rich ingredients. Similar to the present study, inclusion of a multienzyme blend did not affect energy digestibility in diets containing cassava meal fed to nursery pigs (Abella et al., 2020). Similarly, supplementation of xylanase and β-glucanase blend did not affect AID of DM, energy, ADF and NDF in weaned pigs fed 250 g CM /kg (Zijlstra et al., 2004). Unlike the present trial, multi-enzyme inclusion did increase digestibility of diets containing 60 g CM /kg (Omogbenigun et al., 2004) and did increase AID of DM, energy and CP of diets containing 200 g rice bran /kg (Trindade Neto et al., 2021) fed to weaned pigs.

2.4.3. Canola expeller and enzyme interaction

The multi-enzyme blend increased the CATTD of DM and GE for the basal diet only, indicating the importance of matching enzyme type to the correct substrate. In diets containing CE, the presence of lignin might have reduced the efficacy of cellulase, lowering the overall enzyme activity compared with feeding the wheat-based diet alone (Juturu and Wu, 2014). However, in an in vitro study, similar enzyme blends containing xylanase, glucanase, cellulase, mannanase, invertase, protease, amylase and pectinase added to similar substrates (cold-pressed canola cake) increased digestion of nutrients (Lee et al., 2018). Previous reports attest to the accuracy of using in vitro digestibility results to predict enzyme efficacy in swine (Fang et al., 2007b). In vivo digestibility is subject to more factors that can affect digestibility coefficients versus in vitro digestibility. Pig factors such as feed and water intake, passage rate, environmental temperature, sample collection and homogenization can interfere with the measured values for nutrient digestion, potentially explaining the reduced enzyme efficacy in CE observed in the present study. In growing pigs, feeding multi-enzymes increased ME value, SID of CP and AA and ATTD of ADF and NDF in diets containing double-low rapeseed expeller (Li et al., 2020). The dose of enzyme supplemented to the diet is critical for ensuring adequate substrate hydrolysis. Increasing enzyme dose has been associated with a linear increase and subsequent plateau in nutrient digestibility and pig performance (Zijlstra et al., 2004; Fang et al., 2007a; Torres-Pitarch et al., 2019). With more substrate for the supplemented enzymes present in the CE than control diets in the present trial, the enzyme dose might have been insufficient for proper substrate hydrolysis. Similar to the present trial, dietary inclusion of 250 g CM/kg reduced the ATTD of DM, GE and CP in weaned pigs; however, feeding a multi-carbohydrase enzyme increased the ATTD of DM, GE and CP compared with a control using SBM (Sanjayan et al., 2014). In the present trial, the enzyme increased the NE value of the basal diet by 0.34 MJ/kg. Despite this increase, diet NE, diet DE and ileal DE values were still higher in for CE diets regardless of enzyme inclusion. Our results indicate that the enzyme blend composition and dosage still need to be elucidated for feeding weaned pig diets containing CE.

2.5 Conclusion

Inclusion of 250 g CE /kg in weaned pig diets reduced the CAID and CATTD of GE, CP and AA. While the fibre in CE reduced nutrient digestibility, the extra energy that CE provided to the diet from remaining oil increased the NE, DE and ileal DE values of experimental diets. Slight variation in energy and CP digestibility between the two CE samples indicated that variation in nutritional value exists among CE sources and should be considered in diet formulations. Multi-enzyme blend inclusion increased nutrient digestibility solely in the basal diet, indicating a proper match between enzyme blend and substrate for the wheat and barley-based diets. However, the proper enzyme blend, composition, and dose still needs to be elucidated for diets including canola expeller.

2.6 References

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Table 2.1 Ingredient composition (g/kg diet, as fed) of experimental diets.

Ingredient	Without Enzyme			Multi-Enzyme		
	Basal	CE-A	CE-B	Basal	CE-A	CE-B
Wheat	615.90	461.89	461.89	615.28	461.58	461.58
Hulled barley	150.60	112.93	112.93	150.44	112.86	112.86
Canola expeller A[1]	–	250.00	–	–	250.00	–
Canola expeller B[2]	–	–	250.00	–	–	250.00
Menhaden fish meal	99.30	74.50	74.50	99.24	74.45	74.45
Soy protein concentrate HP300[3]	99.30	74.50	74.50	99.24	74.45	74.45
Limestone	10.90	8.20	8.20	10.92	8.19	8.19
Mono- di-calcium phosphate	7.95	5.96	5.96	7.94	5.96	5.96
Salt	5.96	4.47	4.47	5.95	4.47	4.47
Choline chloride 600 g/kg	0.99	0.75	0.75	0.99	0.74	0.74
Mineral premix[4]	1.88	0.58	0.58	1.88	0.58	0.58
Vitamin premix[5]	0.54	1.4	1.4	0.54	1.4	1.4
TiO_2	6.60	5.00	5.00	6.60	5.00	5.00
Multi enzyme blend[6]	–	–	–	0.50	0.50	0.50

[1] Milford Hutterite Colony, Raymond, AB, Canada.

[2] Prairie Home Hutterite Colony, Conrad, AB, Canada.

[3] HP300 (Hamlet Protein Inc., Findlay, OH, USA).

[4] Supplied per kilogram of basal diet: 532 mg Zn, 126 mg Cu, 527 mg Fe, 103 mg Mn, 1.0 mg I, 0.63 mg Se.

[5] Supplied per kg of basal diet: 12,000 IU vitamin A, 1,200 IU vitamin D, 111 IU vitamin E, 104 mg niacin, 28 mg pantothenic acid, 2.6 mg folacin, 15.8 mg riboflavin, 8.7 mg pyridoxine, 8.2 mg thiamine, 520 mg choline, 4.8 mg vitamin K, 0.76 mg biotin, 0.04 mg vitamin B_{12}.

[6] Contained (U/kg of diet): cellulase, 450; xylanase, 600; glucanase, 125; amylase, 6,000; protease, 3,000; invertase, 350; pectinase, 1,200 (Superzyme-Conc®; CBS Bio-Platforms, Calgary, AB, Canada).

Table 2.2 Analysed nutrient composition (g/kg, as fed) and gross energy (GE) value of experimental diets.

Nutrient	Without Enzyme			Multi-Enzyme		
	Basal	CE-A	CE-B	Basal	CE-A	CE-B
Moisture	109	109	104	110	105	104
Starch	255	225	220	307	222	198
Crude protein	229	233	241	216	240	241
Crude fat	9.44	58.6	53.3	11.2	56.7	54.2
Acid detergent fibre	43.0	56.0	62.8	32.9	62.1	61.3
Neutral detergent fibre	107	130	127	106	126	157
Ash	73.8	66.4	66.6	73.9	65.7	65.6
Indispensable amino acids						
Arginine	1.19	1.31	1.31	1.22	1.30	1.34
Histidine	0.49	0.56	0.57	0.51	0.56	0.57
Isoleucine	0.88	0.95	0.95	0.90	0.96	0.97
Leucine	1.50	1.62	1.63	1.53	1.64	1.66
Lysine	1.11	1.25	1.23	1.13	1.24	1.25
Methionine	0.39	0.43	0.43	0.39	0.46	0.44
Phenylalanine	0.99	1.03	1.04	1.02	1.06	1.05
Threonine	0.73	0.85	0.88	0.77	0.85	0.87
Tryptophan	0.29	0.28	0.25	0.27	0.23	0.29
Valine	0.98	1.13	1.13	1.02	1.11	1.14
Dispensable amino acids						

Alanine	0.99	1.05	1.07	1.02	1.05	1.08
Aspartic acid	1.69	1.74	1.74	1.71	1.76	1.77
Cysteine	0.34	0.46	0.46	0.37	0.47	0.47
Glutamic acid	4.56	4.59	4.70	4.67	4.64	4.70
Glycine	1.11	1.17	1.20	1.15	1.17	1.20
Proline	1.55	1.55	1.60	1.60	1.65	1.61
Serine	0.79	0.85	0.86	0.81	0.85	0.87
Tyrosine	0.65	0.67	0.62	0.65	0.72	0.68
Total amino acids	20.72	22.00	22.18	21.24	22.22	22.47
GE (MJ/kg)	16.1	17.5	17.4	16.0	17.5	17.4
Xylanase units/kg	112	76	76	814	623	1047
Particle size, µm[1]						
Mean	578	582	607	559	607	613
Standard deviation	2.13	2.17	2.12	2.30	2.11	2.09

[1] Particle size was measured in triplicate.

Table 2.3 Analysed nutrient composition (g/kg, as fed) of canola expeller samples.

Nutrient	Canola expeller A[1]	Canola expeller B[2]
Moisture	85.6	86.3
Starch	55.3	52.4
Crude protein (N × 6.25)	304	316
Total dietary fibre	280	285
Insoluble dietary fibre	274	281
Soluble dietary fibre	5.67	3.7
Neutral detergent fibre	188	194
Acid detergent fibre	139	156
Crude fat	196	172
Ash	51.6	52.7
Phosphorus	8.63	8.6
Calcium	5.92	5.4
Indispensable amino acids		
Arginine	17.6	19.1
Histidine	8.0	8.4
Isoleucine	12.0	13.5
Leucine	20.0	22.3
Lysine	17.3	18.2
Methionine	6.0	6.4
Phenylalanine	12.0	13.2
Threonine	12.0	13.1

Tryptophan	4.0	4.7
Valine	15.2	16.7
Dispensable amino acids		
Alanine	12.7	13.8
Aspartic acid	20	22.1
Cystine	8	8.8
Glutamic acid	49	53.5
Glycine	14.2	15.4
Proline	19	20.6
Serine	11	11.4
Tyrosine	9	9.4
Total amino acids	274.3	297.0
Chemically available lysine	16.7	17.6
GE (MJ/kg)	21.8	21.3
Particle size, µm[3]		
Mean	676	725
Standard deviation	1.83	1.89

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[1] Milford Hutterite Colony, Raymond, AB, Canada.

[2] Prairie Home Hutterite Colony, Conrad, AB, Canada.

[3] Standard deviations based on triplicates.

Table 2.4 Coefficient of apparent ileal digestibility (CAID) and coefficient of apparent total tract digestibility (CATTD) of dry matter, gross energy and crude protein, and the digestible energy (DE) and calculated net energy (NE) values of experimental diets (standardised to 900 g dry matter/kg)[1].

Variable	Without Enzyme			Multi-Enzyme			SEM[2]	*P*-value		
	Basal	CE-A	CE-B	Basal	CE-A	CE-B		Diet	Enzyme	Enzyme × Diet
CAID										
Dry matter[3]	0.737	0.697	0.695	0.747	0.699	0.710	0.010	< 0.001	0.133	0.678
Gross energy[3]	0.757	0.717	0.716	0.764	0.717	0.728	0.010	< 0.001	0.252	0.726
Crude protein[3]	0.827	0.782	0.779	0.815	0.779	0.790	0.008	< 0.001	0.752	0.156
CATTD										
Dry matter	0.827^{b}	0.802^{c}	0.809^{c}	0.839^{a}	0.811^{c}	0.806^{c}	0.003	< 0.001	0.005	0.019
Gross energy	0.828^{b}	0.795^{c}	0.804^{c}	0.843^{a}	0.803^{c}	0.801^{c}	0.005	< 0.001	0.014	0.045
Crude protein[3]	0.867	0.823	0.841	0.870	0.844	0.842	0.008	< 0.001	0.102	0.145
Energy values, MJ/kg										
Diet DE	13.4^{b}	14.0^{a}	14.1^{a}	13.7^{b}	14.1^{a}	14.0^{a}	0.001	< 0.001	0.036	0.037
Diet NE[4]	9.02^{d}	9.62^{ab}	9.60^{ab}	9.36^{c}	9.67^{a}	9.46^{bc}	0.001	< 0.001	0.014	< 0.001
Diet ileal DE[5]	12.3	12.6	12.5	12.4	12.6	12.7	0.002	0.024	0.316	0.772
Diet ileal NE[5]	8.11	8.66	8.53	8.45	8.61	8.57	0.001	< 0.001	0.097	0.054

[1] Least square means based on 6 pig observations per diet.

[2] SEM = standard error of the mean.

[3] Basal > CE-A and CE-B ($P < 0.05$); CE-A not different from CE-B.

[4] Diet NE values were calculated using Eq. 5 from Noblet et al. (1994) using measured diet DE value and analysed dietary crude fat, starch, crude protein and acid-detergent fibre content.

[5] Basal < CE-A and CE-B ($P < 0.05$); CE-A not different from CE-B.

[a-c] Within a row means without a common superscript differ ($P < 0.05$).

Table 2.5 Coefficient of apparent ileal digestibility of amino acids of experiments diets[1].

Variable	Without Enzyme			Multi-Enzyme			SEM[2]	*P*-value		
	Basal	CE-A	CE-B	Basal	CE-A	CE-B		Diet	Enzyme	Enzyme × Diet
Indispensable amino acids										
Arginine[3]	0.895	0.864	0.860	0.897	0.861	0.868	0.006	< 0.001	0.549	0.382
Histidine[3]	0.863	0.841	0.839	0.869	0.840	0.848	0.007	< 0.001	0.298	0.544
Isoleucine[3]	0.866	0.811	0.804	0.865	0.812	0.817	0.007	< 0.001	0.352	0.351
Leucine[3]	0.869	0.825	0.819	0.869	0.826	0.832	0.007	< 0.001	0.270	0.391
Lysine[3]	0.836	0.810	0.794	0.836	0.798	0.811	0.010	< 0.001	0.748	0.145
Methionine	0.888[a]	0.854[a]	0.851[a]	0.886[a]	0.863[a]	0.805[b]	0.012	< 0.001	0.066	0.009
Phenylalanine[3]	0.873	0.830	0.825	0.874	0.832	0.837	0.007	< 0.001	0.200	0.500
Threonine[3]	0.804	0.759	0.751	0.806	0.746	0.760	0.009	< 0.001	0.877	0.223
Tryptophan	0.874[a]	0.835[b]	0.801[c]	0.862[a]	0.791[c]	0.833[b]	0.009	< 0.001	0.121	< 0.001
Valine[3]	0.840	0.790	0.777	0.840	0.786	0.792	0.008	< 0.001	0.619	0.143
Dispensable amino acids										
Alanine[3]	0.825	0.796	0.786	0.825	0.788	0.799	0.009	< 0.001	0.767	0.309

Aspartic acid[3]	0.809	0.774	0.760	0.804	0.766	0.776	0.009	< 0.001	0.799	0.129
Cystine[5]	0.777	0.768	0.755	0.786	0.749	0.776	0.012	0.037	0.625	0.069
Glutamic acid[3]	0.904	0.877	0.875	0.913	0.875	0.885	0.006	< 0.001	0.122	0.346
Glycine[3]	0.778	0.744	0.743	0.787	0.719	0.755	0.017	< 0.001	0.924	0.232
Proline[3]	0.865	0.825	0.829	0.880	0.833	0.836	0.008	< 0.001	0.045	0.807
Serine[3]	0.828	0.786	0.777	0.826	0.776	0.785	0.008	< 0.001	0.734	0.266
Tyrosine[4]	0.880^{a}	0.830^{bc}	0.805^{c}	0.872^{a}	0.837^{b}	0.837^{b}	0.008	< 0.001	0.029	0.009
Total amino acids[3]	0.855	0.818	0.811	0.859	0.813	0.823	0.007	< 0.001	0.410	0.274

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[1] Least square means based on 6 pig observations per diet.

[2] SEM = standard error of the mean.

[3] Basal > CE-A and CE-B ($P < 0.05$); CE-A not different from CE-B.

[4] Basal > CE-A and CE-B ($P < 0.05$); CE-A greater than CE-B ($P < 0.05$).

[5] Basal > CE-A ($P < 0.05$); CE-A not different from CE-B.

[a-c] Within a row means without a common superscript differ ($P < 0.05$).

Table 2.6 Coefficient of apparent ileal (CAID) and coefficient of total tract digestibility (CATTD) of dry matter, gross energy, and crude protein, and digestible energy (DE) and calculated net energy (NE) values of canola expeller samples (standardised to 900 g dry matter/kg)[1].

Variable	Without Enzyme		Multi-Enzyme		SEM[2]	*P*-value		
	CE-A	CE-B	CE-A	CE-B		CE	Enzyme	Enzyme × CE
CAID[3]								
Dry matter	0.586	0.554	0.552	0.597	0.042	0.825	0.812	0.099
Gross energy	0.632	0.608	0.609	0.644	0.024	0.791	0.699	0.093
Crude protein	0.678	0.673	0.696	0.735	0.029	0.420	0.081	0.320
CATTD[3]								
Dry matter	0.731	0.748	0.732	0.710	0.017	0.883	0.124	0.097
Gross energy	0.724	0.736	0.721	0.699	0.018	0.766	0.104	0.147
Crude protein	0.719^{b}	0.771^{ab}	0.794^{a}	0.781^{ab}	0.022	0.355	0.007	0.024
Energy value MJ/kg								
DE	15.5	15.4	15.4	14.6	0.004	0.228	0.104	0.147
NE[4]	10.6	10.3	10.6	9.73	0.003	0.023	0.104	0.147

Ileal DE	13.5	12.8	13.0	13.5	0.005	0.739	0.707	0.095
Ileal NE	9.26	8.41	8.91	8.93	0.004	0.206	0.707	0.095

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[1] Least square means based on 6 pig observations per diet.

[2] SEM = standard error of the mean.

[3] Digestibility coefficients of dry matter, crude protein and gross energy in canola expeller were calculated using the difference method (Adeola, 2001), where the basal diet without enzyme was used as basal diet for the test ingredients CE-A and CE-B without enzyme, and the basal diet with enzyme was used as basal diet for the test ingredients of CE-A and CE-B with enzyme.

[4] Ingredient NE values were calculated using the calculated ingredient DE value and analysed ingredient crude fat, starch, crude protein, and acid-detergent fibre content using Eq. 5 from Noblet et al. (1994).

[a-c] Within a row means without a common superscript differ ($P < 0.05$).

Table 2.7 Coefficient of apparent ileal digestibility of amino acids of canola expeller samples[1,2].

Variable	Without Enzyme		Multi-Enzyme		SEM[3]	*P*-value		
	CE-A	CE-B	CE-A	CE-B		CE	Enzyme	Enzyme × CE
Indispensable amino acids								
Arginine	0.802	0.800	0.789	0.818	0.014	0.196	0.770	0.138
Histidine	0.801	0.801	0.782	0.810	0.017	0.269	0.672	0.255
Isoleucine	0.692	0.684	0.694	0.721	0.021	0.506	0.206	0.256
Leucine	0.727	0.722	0.727	0.755	0.018	0.398	0.218	0.225
Lysine	0.760	0.718	0.725	0.766	0.030	0.982	0.774	0.067
Methionine	0.789^{a}	0.785^{a}	0.819^{a}	0.655^{b}	0.038	0.008	0.086	0.010
Phenylalanine	0.726	0.722	0.727	0.751	0.018	0.456	0.251	0.298
Threonine	0.679	0.665	0.635	0.679	0.027	0.451	0.448	0.116
Tryptophan	0.753^{a}	0.667^{b}	0.650^{b}	0.782^{a}	0.028	0.259	0.770	< 0.001
Valine	0.695	0.673	0.665	0.706	0.021	0.542	0.910	0.052
Dispensable amino acids								
Alanine	0.556	0.565	0.463	0.599	0.060	0.113	0.489	0.162

Aspartic acid	0.688	0.651	0.668	0.711	0.027	0.889	0.313	0.058
Cysteine	0.704	0.703	0.631	0.717	0.041	0.168	0.321	0.159
Glutamic acid	0.458^{a}	0.456^{ab}	0.382^{b}	0.448^{ab}	0.025	0.093	0.032	0.076
Glycine	0.666	0.667	0.554	0.684	0.060	0.152	0.228	0.159
Proline	0.727	0.748	0.716	0.732	0.022	0.264	0.427	0.879
Serine	0.695	0.672	0.664	0.696	0.021	0.753	0.818	0.090
Tyrosine	0.721^{ab}	0.655^{b}	0.761^{a}	0.766^{a}	0.023	0.084	< 0.001	0.049
Total amino acids	0.734	0.714	0.717	0.751	0.021	0.631	0.512	0.090

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[1] Digestibility coefficients of dispensable and indispensable amino acids in canola expeller were calculated using the difference method (Adeola, 2001), where the basal diet without enzyme was used as basal diet for the test ingredients CE-A and CE-B without enzyme and the basal diet with enzyme was used as basal diet for the test ingredients CE-A and CE-B with enzyme.

[2] Least square means based on 6 pig observations per diet.

[3] SEM = standard error of the mean.

[a-b] Within a row means without a common superscript differ ($P < 0.05$)

Chapter 3: Feeding two multi-enzyme blend doses to enhance nutrient digestibility of wheat grain-wheat millrun diets in ileal-cannulated weaned pigs.

3.1 Introduction

Feed cost in animal production continues to increase because of multiple factors including uncertainty of Ukraine's grain production and distribution, increased global inflationary pressure and cost of food commodities as sequel to the COVID-19 pandemic, and localized drought conditions in North America. Due to competition with human consumption and the biofuel industry, dietary energy feedstuffs have become particularly expensive. Vegetable oil, for example, has doubled in price because of human culinary demand and increased demand for biodiesel (Zijlstra and Beltranena, 2022), resulting in nutritionists removing vegetable oil from most swine diets. Feeding coproducts from food industries such as flour milling, provides an opportunity to replace some cereal ingredients (energy) in swine feeds, thus lowering feed cost (Jha et al., 2012). Additionally, reduced transportation distance by sourcing locally produced coproducts reduces the carbon footprint of pork production due to decreased fuel consumption (Zijlstra and Beltranena, 2013, 2022). Processing wheat into flour leaves coproducts classified as wheat middlings, shorts, bran, and millrun (WM) based on increasing fibre content (Rosenfelder et al., 2013; Kpogo et al., 2021). Wheat millrun may also include offal and screenings after producing flour or receiving the wheat, resulting in variable nutritional content. Wheat millrun may contain 190 g non-starch polysaccharide (NSP)/kg DM compared with 108-130 g NSP/kg in wheat grain (Rosenfelder et al., 2013). Inclusion of high fibre cereal coproducts can reduce the digestibility of nutrients, which are more critical in diets intended for weaned pigs, as they have lower feed consumption and greater nutrient requirements compared with older counterparts (Garcia et al., 2015). For example, despite a greater gross energy (GE) value in WM than wheat grain, the digestible energy (DE) value of WM is lower because of the greater NSP content (Rosenfelder et al., 2013). In growing pigs, inclusion of 300 g WM/kg decreased apparent total tract digestibility coefficient (CATTD) of energy, protein and phosphorus resulting in reduced average daily weight gain (ADG; Kpogo et al., 2021). Feeding weaned pigs, inclusion of 200 g WM/kg reduced the CATTD of dry matter (DM) and energy, but did not affect ADG (Garcia et al., 2015).

In growing pigs, inclusion of dietary enzymes increases the nutrient digestibility of wheat millrun (Nortey et al., 2007, 2008). Thus, inclusion of a multi-enzyme blend may increase nutrient digestibility of high-fibre wheat coproducts in weaned pigs. Carbohydrase enzymes may break apart some of the complex fibre matrix to release trapped nutrients such as amino acids and minerals (Adeola and Cowieson, 2011). Previously, in an experiment feeding a diet containing wheat screenings and WM to weaned pigs, a multi-carbohydrase enzyme increased the apparent ileal digestibility (CAID) of DM, GE, crude protein (CP), starch and NSP (Omogbenigun et al., 2004). To mitigate lower feed intake, weaned pig diets are typically most costly because of greater energy and nutrient density, through the inclusion of more digestible feedstuffs targeted to aid development of the young digestive tract. Thus, an opportunity exists to lower feed cost by including wheat coproducts and exogenous enzymes to maximize diet energy and nutrient digestibility.

For this study, the null hypothesis was that inclusion of a multi-enzyme blend at two different doses would not increase the energy and nutrient digestibility of wheat millrun diets with reduced vs. adequate nutrient specifications intended for weaned pigs. Therefore, the objectives were to measure and compare the CAID of DM, GE, CP and AA and CATTD of DM, GE and CP in diets fed to ileal-cannulated weaned pigs: 1) between diets either adequate or deficient in dietary energy and lysine containing 75 g WM/kg, and 2) in energy and lysine deficient diets containing 75 g WM/kg with two (0.2 g/kg and 0.4 g/kg) inclusions of a multi-enzyme blend.

3.2 Materials and Methods

Animal use was approved, and experimental procedures were reviewed by the Animal Care and Use committee for Livestock of the University of Alberta based on guidelines established by the Canadian Council on Animal Care (CCAC, 2009). The animal experiment was conducted at the Swine Research and Technology Centre, University of Alberta (Edmonton, AB, Canada).

3.2.1. Experimental Design

To test the hypothesis, the study was designed as a double Youden square (4 pigs × 5 periods) feeding 4 experimental diets to reach 10 observations per dietary treatment. In total, 10 Duroc × Large white/Landrace F1 barrows were selected between 10 and 15 kg body weight. Pigs had a T-cannula surgically inserted at the distal ileum for the collection of digesta. Pigs recovered fully from cannulation surgery while fed a commercial starter diet increasing in allowance over the 7 days following surgery. Subsequently, feeding of the experimental diets was introduced by replacing 25, 50, 75 and 100% of the starter feed with the assigned experimental diet over 4 days. The latter day marked the start of the first acclimation period for feeding the experimental diet. Daily feed allowance feeding the experimental diets was calculated as 3.0 × maintenance DE (110 kcal of DE per kg of $BW^{0.75}$; NRC 1998) divided into two meals offered at approximately 08:00 and 15:00. Throughout the trial, pigs were housed in individual metabolic pens, measuring 1.2 m length × 1.2 m width × 0.95 m height (1.8 m^2). The pens had walls made of polyvinyl plastic planking with 0.16 m^2 plexiglass windows on three sides to allow pigs to view neighbouring pigs. Free access to water was provided with a cup drinker placed 0.25 m above the floor beside the feeder. The stainless-steel feeder measured 0.35 m width × 0.4 m height with the feed trough 0.12 m above the pen floor. The climate-controlled room was maintained at 25 ± 2.5ºC when pigs were less than 15 kg and 22 ± 2.5ºC when pigs exceeded 15 kg. Lighting was provided daily from 07:00 to 19:00.

Pigs consumed the assigned four experimental diets for five nine-day periods. The first five days was considered acclimation to the experimental diet, followed by four days of sample collections consisting of two days of faeces collection and subsequent two days of digesta collection. Faeces were collected via colostomy bags. A Velcro ring was spray-glued around the tail and anus and a plastic bag was snapped between a second bottom Velcro ring and a top leather ring (van Kleef et al., 1994). Faeces were collected continuously for 48 h starting at 0800 on the first day of faeces collection, replaced as needed, and ending at 0800 of the first day of digesta collection. Digesta was collected continuously from approximately 08:00 to 19:00 through the opened T-cannula via the attachment of plastic collection bags filled with 15 mL of 50 g/kg formic acid that were replaced when full. Meals were offered at 08:00, 10:00, 13:00 and 15:00 during digesta collection to attain a more continuous flow of digesta. Faeces or digesta for each pig were pooled by period and frozen to –20°C in a chest freezer. Following completion of each period, faeces or digesta were thawed, homogenized, and subsampled. Once subsampled, both faeces and digesta remained frozen until transported to the laboratory for freeze drying.

3.2.2. Test Materials and Diets

Wheat millrun was sourced from C.B. Constantini Ltd. (Vancouver, BC, Canada). Hard red spring wheat grain (CS Accelerate™, Canterra Seeds, Winnipeg, MB, Canada) was grown at a University of Alberta farm (St. Albert, AB, Canada) and ground through a 6/64 mm screen using a hammer mill (model Jacobson 5550-113-01, Carter Day International, Minneapolis, MN) at the University of Alberta Metabolic Unit. Diets (Table 3.1) were mixed using a horizontal paddle mixer (model SPC2748, Marion Mixers Inc., Marion, IA, USA) and fed as a mash. The diet formulation consisted of a positive control (PC), a negative control (NC), and two NC diets with inclusion of two doses of a multi-enzyme blend. The PC diet was formulated based on age-specific nutrient requirements (NRC, 2012) and included canola oil and L-lysine HCl to meet the energy and lysine requirements. The multi-enzyme blend consisted of 14,000 U/g amylase, 8,000 U/g xylanase, 5,000 U/g cellulase, 1,000 U/g glucanase, 2,600 U/g protease, 400 U/g invertase and was included in the NC diets at either 0.2 g/kg or 0.4 g/kg (Superzyme- W Conc™, CBS Bio-Platforms; Calgary, AB, Canada). All diets included 0.5 g titanium dioxide (TiO_2)/kg as indigestible marker and 500 FTU/kg of phytase. Particle size of the ingredients and diets was analysed using a mechanical sieve shaker (Model RX-29, W.S. Tyler, ON, Canada) following the method of the American Society of Agricultural and Biological Engineers (ASABE, 2008; Table 2).

3.2.3. Chemical analyses

Test ingredients, diets, and lyophilized faeces and digesta for each pig were ground through a 1-mm screen using a centrifugal mill (model ZM200, Retch GmbH, Haan, Germany). Test ingredients, diets, digesta and faeces were analysed for dry matter (DM; method 930.15), CP by LECO (nitrogen × 6.25; method 990.03), gross energy (GE) using an adiabatic bomb calorimeter (model 5003; Ika-Werke, Staufen, Germany) and TiO_2 (Myers et al., 2004) at the University of Alberta (AOAC, 2006). Test ingredients and diets were analysed for starch (assay kit STA-20; Sigma, St. Louis, MO, USA), and using AOAC (2006)-methods for crude fat (method 920.39A), neutral detergent fibre (NDF) assayed without a heat-stable amylase and expressed inclusive of residual ash (Holst, 1973), acid detergent fibre (ADF) inclusive of residual ash (method 973.18) and ash (method 942.05) at the Agricultural Experiment Station Chemical Laboratories (ESCL), University of Missouri (Columbia, Missouri, USA; AOAC, 2006). Test ingredients were analysed for soluble and insoluble dietary fibre (method 991.43) and crude fibre (method 934.01), calcium (method 968.08), phosphorus (method 946.06), amino acids (AA; method 982.30E a-c), and chemically available lysine (method 975.44) at ESCL (AOAC, 2006). Digesta was analysed for moisture (method 930.15), CP, and AA (method 982.30E a-c) at ESCL.

3.2.4. Calculations

The CAID of DM, GE, CP, and AA, and CATTD of DM, GE, and CP of diets were calculated using the index method (Adeola, 2001):

$$\text{CAID or CATTD} = 100 - [100\left(\frac{(\text{Concentration of TiO2}_{\text{feed}})\text{ x }(\text{Concentration of component}_{\text{digesta or feces}})}{(\text{Concentration of TiO2}_{digesta\ or\ feces})\text{x}(\text{Concentration of component}_{\text{feed}})}\right)]$$

Diet DE (kcal/kg) was calculated by the following formula:

$$\text{DE} = \frac{(\text{GE}_{\text{Diet}} * \text{GE CAID or CATTD}_{\text{diet}})}{100}$$

Diet NE (kcal/kg) was calculated using equation 5 of Noblet et al. (1994) as adopted by the NRC (2012), where nutrient values were expressed as g/kg DM:

$$\text{NE} = (0.7 * \text{ DE}) + (1.61 * \text{ EE}) + (0.48 * \text{ Starch}) - (0.91 * \text{ CP}) - (0.87 * \text{ ADF})$$

3.2.5. Statistical analyses

Digestibility data were analysed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). The model included diet fed as fixed effect, and square, period nested within square, and pig nested within square as random effects. Individual pig was the experimental unit. Normality and homogeneity of variance for the residual of each variable and carry-over effect of diets fed in previous period were tested prior to ANOVA analysis. For multiple comparisons, *P*-values were adjusted using Tukey-Kramer method. To test the hypothesis, $P < 0.05$ was considered significant, whereas $0.05 \leq P < 0.10$ was considered a trend.

3.3 Results

The PC diet contained 8% less starch than the NC diet and 11-14% less starch than the enzyme diets, respectively (Table 3.2). The PC diet contained 13% more crude fat and 10% more GE than the average of NC and enzyme diets. Diet crude protein content was consistent and ranged from 237-252 g/kg as fed. The PC contained 11.5% more lysine than the average of NC and enzyme diets. Xylanase activity was not detectable for both the PC and NC diets and was 1,179 and 1,385 U/kg for the 0.2 g/kg and 0.4 g/kg multi-enzyme doses, respectively. The millrun sample provided 370 g/kg total fibre, 99.7% of which was insoluble (Table 3.3). It also contained 172 g/kg of starch, 178 g/kg of protein and 7.2 g/kg of total lysine, 98% of which was chemically available.

The CAID and CATTD of DM, GE did not differ between the PC and NC diets (Table 3.4). Feeding the PC diet resulted in greater ($P < 0.001$) DE, NE, ileal NE and ileal DE values, and a trend ($P < 0.10$) for CAID CP than the NC diet. Including the dietary multi-enzyme at 0.2

or 0.4% had no effect on CAID, CATTD of DM, GE and CP, or energy values compared to the NC diet.

Feeding the PC diet resulted in greater ($P < 0.01$; Table 5) CAID of lysine compared with the NC diet and enzyme diets. The dietary inclusion of multi-enzyme at both doses had no effect on CAID of individual or total AA when compared to the NC diet.

3.4 Discussion

Due to increasing feed cost globally, replacement of traditional feedstuffs in pig diets with lower cost coproducts is expanding (Zijlstra and Beltranena, 2013). Most of the 3.4 million metric tonnes of wheat grain processed in Canadian flour mills yearly is grown in western Canada, making wheat coproducts abundant for Canadian livestock producers to feed (CNMA, 2022). Processing wheat grain into flour mostly removes the starchy endosperm, leaving the bran, germ, and aleurone layers to be classified based on fibre content into wheat bran, middlings, or shorts separately or one or more of these fractions can be pooled with screenings as WM (Nortey, 2007; Rosenfelder et al., 2013; Kpogo et al., 2021). Representing as much as 25% of the original kernel, WM can contain more CP and ash and greater GE value than the parent wheat (Rosenfelder et al., 2013). However, WM may also contain more than double the NSP than the parent wheat grain, specifically insoluble arabinoxylans (AX), which can have a CAID and CATTD as low as 0.10 and 0.46, respectively (Bach Knudsen and Canibe, 2000; Slominski et al., 2004; Garcia et al., 2015). Therefore, depending on what kernel fractions are included, WM can be a highly variable coproduct in terms of nutrient content.

In young pigs specifically, the digestibility of WM is not well established, because too high dietary NSP can exacerbate the post-weaning growth lag (l'Anson et al., 2013; Torres-

Pitarch et al., 2017; Park et al., 2020). Decreased CAID and CATTD of DM, nutrients, and energy with inclusion of WM in pig diets is well documented (Nortey et al., 2008; Garcia et al., 2015; Woyengo and Zijlstra, 2021). In poultry, multi-carbohydrase enzymes including xylanase, β-glucanase, and galactomanase increased the CAID of DM by 2%, of energy by 3%, and of CP by 4% in diets containing 80 wheat bran g/kg (Wickramasuriya et al., 2019). Supplementing diets with fibre-degrading enzymes, and thereby increasing the digestibility of wheat coproducts may develop into an opportunity to expand the feedstuff matrix for feed formulation for weaned pigs (Kpogo et al., 2021). In pigs, inclusion of xylanase alone has shown to increase the digestibility of WM because arabinoxylan makes up 56-60% of NSP that limits nutrient digestibility and is the main substrate for xylanase (Nortey et al., 2007; Rosenfelder et al., 2013). However, a variety of substrates are present in the fibre matrix of coproducts that could be hydrolysed by supplementation of multiple enzymes (Adeola and Cowieson, 2011; Aranda-Aguirre et al., 2021). In growing pigs, dry matter digestibility was increased when enzymes were combined compared with feeding mannanase or xylanase alone (Aranda-Aguirre et al., 2021). Multi-enzyme blends including multiple carbohydrases such as amylase, cellulase, glucanase, xylanase, invertase and pectinase can synergistically hydrolyse NSP present in WM to yield simple sugars (Rosenfelder et al., 2013; Garcia et al., 2015; Park et al., 2020; Kpogo et al., 2021). Absorption of these simple sugars, as well as freed AA and other nutrients can contribute to increased G:F and ADG (Aranda-Aguirre et al., 2021).

3.4.1. Reduced nutrient specifications

Reduced nutrient specification of the NC diet compared with the PC diet was designed to test the efficacy of two multi-enzyme inclusion levels in the experimental diets. If the NC + either enzyme level had similar nutrient digestibility to PC, a strong nutritional benefit would have existed to replace at least some of the canola oil added in diets containing wheat grain and WM. As expected, based on the results of Wickramasuriya et al. (2019), feeding the PC diet in the present study resulted in greater energy values and CAID of lysine than the NC diet. These results are consistent with diet nutrient specification, where the PC diet was formulated to include added canola oil and L-lysine HCL whereas the NC diet had only extra wheat grain. Although the NC diet contained more starch than the PC diet, the NC also contains more fibre, likely limiting the digestibility of other nutrients, especially in young pigs (Zijlstra et al., 2012; Jha et al., 2019; Sun et al., 2020). The AX and cellulose in wheat insoluble fibres entrap nutrients within a matrix, limiting access of enzymes to these nutrients in weaned pigs, thus more fibre in the diet can decrease nutrient digestibility (Jha and Berrocoso, 2015). An increase in dietary insoluble fibre can also increase passage rate throughout the GIT, thereby limiting the amount of contact time digestive enzymes have with their substrates to complete digestion, further limiting nutrient absorption (Owusu-Asiedu et al., 2006; Ratanpaul et al., 2019). The CAID of DM and CP and CATTD of DM and CP in the present trial did not differ between the PC and NC diet. In contrast, a study in weaned pigs reported a 3, 12 and 10% decrease in CATTD of DM, CP and energy, respectively, when 25 g poultry fat/kg was excluded in the NC diet but included in the PC diet (Tsai et al., 2017). Overall, the clear difference between the PC and NC showed the efficacy of the experimental design.

3.4.2. Enzyme supplementation

In the present study, significant effects of the two enzyme inclusion levels were not observed. In vitro studies of WM digestibility simulating pigs have shown similar results, where an enzyme blend of xylanase and β-glucanase did not increase digestibility or fermentability of WM (Woyengo et al., 2015). In contrast, in vitro studies with wheat-based diets with xylanase supplementation indicated a 36% increase in xylan solubilisation compared with the control (Pedersen et al., 2012). In vivo, like the present study, inclusion of a dietary multi-carbohydrase blend had no effect on CATTD and growth performance when feeding diets containing 150 or 300 g WM/kg to growing pigs (Kpogo et al., 2021). Our results were further mirrored in weaned pigs fed diets containing 500 g/kg of wheat millrun without an effect of supplemental xylanase on CAID, ADG and G:F (l'Anson et al., 2014). Contrarily, inclusion of xylanase or xylanase + arabinofuranosidase increased CAID and CATTD in growing pigs fed 300 g/kg wheat middlings (Moran et al., 2016) or 300 g/kg wheat bran, respectively (Sun et al., 2020). Coproduct inclusion is particularly limited in diets for weaned pig based on the risk of post-weaning growth lag such as decreased feed intake and weight gain. Therefore, the present study included only 75 g WM/kg to remain close to commercially relevant nursery diets. Increased inclusion of WM would have provided more substrate for the enzyme blend to hydrolyse and possibly increase digestibility. Increasing dietary content of AX from 69 g/kg (low AX) to 118 g/kg (high AX) coincided with a decrease in CATTD GE; however, enzyme inclusion increased CATTD of GE that indicated increased AX hydrolysation (Sun et al., 2020). Using extrapolated and average AX content for wheat grain and wheat bran from Kaprelyants et al., (2019), the present study experimental diets contained 60.7 g AX/kg diet, which is less than the lowest AX content reported by Sun et al. (2020). This comparison may indicate lack of available AX present for adequate multi-enzyme blend hydrolysis, and subsequent lack of enzyme effect on nutrient digestibility. However, in contrast to the present study, 4,375 U xylanase/kg increased apparent jejunal digestibility of energy from 0.360 to 0.595 and CATTD of energy from 0.819 to 0.857 in weaned pigs fed a diet containing 75 g WM/kg (Nortey, 2007). A study with an enzyme blend containing xylanase, β-glucanase and phytase in weaned pigs fed a diet containing canola meal, also reported no difference in digestibility with added enzyme (Lu et al., 2016). Comparison of results among multiple studies is difficult due to variety in dietary substrate composition and content, enzyme blend composition, dietary enzyme dose and enzyme source (Adeola and Cowieson, 2011; Pedersen et al., 2012; Ndou et al., 2015; Sun et al., 2020).

3.5 Conclusion

In conclusion, the positive control diet was formulated with canola oil and HCL-lysine, so the greater energy value and lysine content did indeed result in greater digestibility of energy and lysine than the negative control diet. However, including two multi-enzyme blend levels added to the negative control diet fed in the present study did not increase the energy values (DE, NE) or the CAID and CATTD of DM, GE, CP or AA for either enzyme dose.

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Table 3.1 Ingredient composition (g/kg diet, as fed) of experimental diets[1].

Ingredients	PC	NC	NC + 0.2	NC + 0.4
Wheat	603.1	640.1	640.1	640.1
Soybean meal	200.0	200.0	200.0	200.0
Wheat millrun	75.0	75.0	75.0	75.0
Fish meal	50.0	50.0	50.0	50.0
Canola oil	34.0	–	–	–
Limestone	8.50	8.50	8.50	8.50
Salt	5.00	5.00	5.00	5.00
Vitamin premix[2]	5.00	5.00	5.00	5.00
Trace mineral premix[3]	5.00	5.00	5.00	5.00
L-lysine HCl	4.00	1.00	1.00	1.00
Mono-/di-calcium phosphate	2.50	2.50	2.50	2.50
L-threonine	1.00	1.00	1.00	1.00
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00
DL-methionine	0.30	0.30	0.30	0.30
Carrier (flour)	0.50	0.50	0.30	0.10
Titanium dioxide	0.50	0.50	0.50	0.50
Superzyme-W-conc[5]	–	–	0.20	0.40
Phytase [4]	0.10	0.10	0.10	0.10

[1] PC, positive control; NC, negative control; NC + 0.2, negative control + 0.2 g multi-enzyme blend/kg; NC + 0.4, negative control + 0.4 g/kg multi-enzyme.

[2] Supplied per kilogram of diet: 7,500 IU of vitamin A, 750 IU of vitamin D, 50 IU of vitamin E, 4 mg menadione, 15 mg of pantothenic acid, 38 mg of niacin, 5 mg of riboflavin, 2.5 mg of folic acid, 2.5 mg of thiamine, 1.5 pyridoxine, 0.25 mg of biotin and 0.0015 mg of vitamin B_{12}.

[3] Supplied per kilogram of diet: 125 mg of Zn as ZnO 72%, 50 mg of Cu as $CuSO_4$, 75 mg of Fe as $FeSO_4$, 25 mg of Mn as MnO, 0.5 mg of I as $Ca\ (IO_3)_2$, and 0.6 mg of Se as Na_2SeO_3.

[4] Bio-phytase 5000™ contained: 500 FTU/kg of diet (CBS Bio-Platforms; Calgary, AB, Canada).

[5] Contained (U/kg for the 0.2 g/kg diet): glucanase, 200; xylanase, 1,600; cellulase, 1,000; amylase, 2,800; protease, 520; invertase, 80 (Superzyme-W-Conc ® CBS Bio-Platforms; Calgary, AB, Canada). The 0.4 g/kg diet contained double the dose.

Table 3.2 Analysed nutrient composition (g/kg as fed) and gross energy value of experimental diets[1].

Item	PC	NC	NC+0.2	NC+0.4
Moisture	103	108	109	109
Starch	332	361	375	383
Crude protein	240	252	244	237
Total dietary fibre	148	119	142	136
Insoluble fibre	146	118	141	135
Soluble fibre	1.88	0.98	1.34	0.98
Neutral detergent fibre	128	106	127	120
Acid detergent fibre	41.7	43.3	41.3	37.9
Crude fat	41.3	5.98	3.47	4.72
Ash	56.2	60.4	55.6	57.7
Phosphorus	5.10	5.68	5.57	5.91
Calcium	7.89	8.35	7.48	8.85
Gross energy (MJ/kg)	17.3	16.5	16.5	16.9
Indispensable amino acids				
Arginine	12.9	12.8	12.3	13.7
Histidine	5.74	5.89	5.52	5.88
Isoleucine	10.5	10.4	10.1	10.5
Leucine	16.9	17.1	16.3	17.2
Lysine	14.8	13.5	12.4	12.5
Methionine	4.04	3.92	3.65	4.10

Phenylalanine	11.0	11.2	10.8	11.2
Threonine	8.88	9.28	8.38	8.46
Tryptophan	2.24	2.41	2.32	2.67
Valine	11.5	11.4	11.1	11.7
Dispensable amino acids				
Alanine	9.96	10.4	9.80	10.3
Aspartic acid	19.7	20.0	18.8	19.8
Cystine	4.04	3.92	4.10	4.19
Glutamic acid	55.7	57.6	55.8	57.4
Glycine	10.8	11.3	10.7	11.3
Proline	17.0	18.3	17.6	18.3
Serine	9.42	10.2	9.27	9.36
Tyrosine	5.29	4.82	4.90	6.59
Total amino acids	233	236	225	238
Xylanase, U/kg[2]	ND	ND	1,179	1,385
Particle size, µm[3]				
Mean	578	559	582	607
Standard deviation	2.13	2.30	2.17	2.11

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[1] PC, positive control; NC, negative control; NC + 0.2, negative control + 0.2 g multi-enzyme blend/kg; NC + 0.4, negative control + 0.4 g/kg multi-enzyme.

[2] ND: Not detectable.

[3] Particle size was measured in triplicate.

Table 3.3 Analysed nutrient composition (g/kg, as fed) of wheat millrun.

Item	Millrun
Moisture	131
Insoluble dietary fibre	368
Soluble dietary fibre	< 1
Neutral detergent fibre	356
Acid detergent fibre	130
Crude protein (N × 6.25)	177
Starch	172
Crude fat	24.3
Ash	44.2
Phosphorus	8.02
Calcium	1.00
Indispensable amino acids	
Arginine	10.8
Histidine	4.50
Isoleucine	6.05
Leucine	10.4
Lysine	7.21
Available lysine	7.09
Methionine	2.33
Phenylalanine	6.75
Threonine	5.10

Tryptophan	1.30
Valine	8.30
Dispensable amino acids	
Alanine	7.96
Aspartic acid	10.8
Cystine	3.37
Glutamic acid	34.5
Glycine	8.91
Proline	10.6
Serine	5.79
Tyrosine	3.46
Total amino acids	151
Gross energy, MJ/kg	16.9
Particle size, µm	
Mean	622
Standard deviation[1]	2.06

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[1] Standard deviation based on 3 replicates.

Table 3.4

Coefficient of apparent ileal digestibility (CAID) and coefficient of apparent total tract digestibility (CATTD) of dry matter, gross energy and crude protein, and the digestible energy (DE) and calculated net energy (NE) values of experimental diets (the energy values standardised to 900 g dry matter/kg)[1].

Variable		Reduced nutrient specifications			SEM[2]	*P*-value
	PC	NC	NC + 0.2	NC + 0.4		
CAID						
Dry matter	0.735	0.721	0.738	0.736	0.010	0.363
Gross energy	0.764	0.741	0.753	0.753	0.010	0.159
Crude protein	0.840	0.823	0.819	0.819	0.009	0.097
CATTD						
Dry matter	0.864	0.861	0.865	0.865	0.004	0.554
Gross energy	0.861	0.853	0.858	0.859	0.004	0.212
Crude protein	0.900	0.895	0.901	0.903	0.007	0.684
Energy values, MJ/kg						
Diet DE	15.1^{a}	14.3^{b}	14.4^{b}	14.4^{b}	0.177	< 0.001
Diet NE[3]	10.7^{a}	9.90^{b}	9.95^{b}	9.96^{b}	0.124	< 0.001
Diet Ileal DE	13.4^{a}	12.4^{b}	12.6^{b}	12.6^{b}	0.427	< 0.001
Diet Ileal NE	9.45^{a}	8.59^{b}	8.73^{b}	8.73^{b}	0.299	< 0.001

[1] PC, positive control; NC, negative control; NC + 0.2, negative control + 0.2 g/kg multi-enzyme blend; NC + 0.4, negative control + 0.4 g/kg multi-enzyme.

[2] SEM = standard error of the mean. Least square means based on 10 pig observations per diet.

[3] Diet NE values were calculated using Eq. 5 in Noblet et al. (1994) using measured diet DE value and analysed dietary crude fat, starch, crude protein, and acid-detergent fibre content.

[a-b] Within a row, means without a common superscript differ ($P < 0.05$).

Table 3.5

Coefficient of apparent ileal digestibility of amino acids of experiments diets[1].

Variable	PC	Reduced nutrient specifications			SEM[2]	*P*-value
		NC	NC + 0.2	NC + 0.4		
Indispensable amino acids						
Arginine	0.896	0.886	0.883	0.884	0.006	0.091
Histidine	0.880	0.872	0.865	0.868	0.001	0.494
Isoleucine	0.879	0.864	0.861	0.864	0.007	0.073
Leucine	0.878	0.864	0.860	0.864	0.009	0.225
Lysine	0.875^{a}	0.842^{b}	0.827^{b}	0.831^{b}	0.010	0.001
Methionine	0.907^{a}	0.893^{ab}	0.886^{b}	0.889^{b}	0.007	0.032
Phenylalanine	0.886	0.874	0.872	0.875	0.008	0.296
Threonine	0.832	0.813	0.804	0.809	0.010	0.080
Tryptophan	0.833	0.833	0.835	0.836	0.010	0.991
Valine	0.857	0.843	0.838	0.842	0.010	0.222
Dispensable amino acids						
Alanine	0.825	0.809	0.800	0.805	0.012	0.182
Aspartic acid	0.832^{a}	0.805^{ab}	0.792^{b}	0.794^{b}	0.012	0.008
Cystine	0.818	0.813	0.811	0.805	0.011	0.705
Glutamic acid	0.924	0.918	0.921	0.920	0.005	0.620
Glycine	0.792	0.779	0.785	0.776	0.012	0.563
Proline	0.890	0.886	0.890	0.882	0.009	0.767

Serine	0.858	0.849	0.845	0.848	0.009	0.536
Tyrosine	0.863	0.861	0.850	0.852	0.010	0.479
Total amino acids	0.874	0.860	0.857	0.858	0.008	0.158

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[1] PC, positive control; NC, negative control; NC + 0.2, negative control + 0.2 g multi-enzyme blend/kg; NC + 0.4, negative control + 0.4 g/kg multi-enzyme.

[2] SEM = standard error of the mean. Least square means based on 10 pig observations per diet.

[a-b] Within a row, means without a common superscript differ ($P < 0.05$).

Chapter 4: General discussion

4.1 Discussion

Sustainable and profitable swine production is influenced by many factors, most notably feed ingredient prices. In livestock operations, feed cost represents the highest proportion of variable and total cost (de Lange et al., 2010; Woyengo et al., 2014). Weaned pig diets are costly due to inclusion of high-density energy and protein ingredients (de Lange et al., 2010; Sun et al., 2020). This production stage provides an opportunity to lower feed cost, through inclusion of less costly ingredients such as coproducts. Of the many benefits of feeding coproducts to feed livestock, reduced price and transportation cost provide a direct financial and benefit to producers (Beltranena and Zijlstra, 2022). Feeding livestock coproducts from existing food industries reduces the environmental impact of livestock production (Vonderohe et al., 2022). Land use changes for agriculture production is a leading cause of biodiversity loss and greenhouse gas release. Circularity of feeding animals' inedible 'by-products' from existing primary crop industries optimizes the amount of edible protein per land area produced (de Boer et al., 2018). Historically however, coproduct inclusion has been limited in nursery diets as fibre content can interfere with digestibility of other nutrients, impacting growth performance, thereby lowering efficiency (Bindelle et al., 2008; Vonderohe et al., 2022; Aranda-Aguirre et al., 2021)

4.2 Main findings and conclusions

The objectives of this thesis were to measure nutrient digestibility of common western Canadian coproducts fed either with or without enzyme blends to ileal-cannulated weaned pigs. Feeding canola coproducts to pigs in western Canada has been practiced in older animals, as they have more capacity to adequately digest fibre. As a valuable energy source, the remaining oil content in canola expeller (CE) can to some extent offset the negative effect of fibre reducing energy digestibility by increasing the gross energy values in pig diets (Bindelle et al., 2008; Zijlstra and Beltranena, 2013; Beltranena and Zijlstra, 2022). In recent years, biofuel production, weather patterns including drought, and world events has increased the cost of energy feedstuffs for producers to feed to swine herds. Demand for canola oil has increased local production and processing, leading to a correlated increase in canola coproducts, specifically CE (Canola Council of Canada, 2023). Expeller pressing canola produces cake with greater remaining crude fat content than solvent-extracted canola meal. There is increasing pressure for nutritionists and producers to feed novel feed ingredients to weaned pig diets to lower feed cost, while maintaining nutritional value. Thus, inclusion of dietary enzymes to increase digestibility of these ingredients is also practiced. In Chapter 2, feeding a multi-enzyme blend was tested against a wheat basal diet and CE included at 25%. Results from this trial showed the CE inclusion indeed decreased CAID and CATTD of all nutrients, yet enzyme inclusion did not have an effect on the digestibility of CE. Nonetheless, the enzyme did increase the NE value of the wheat-barley based basal diets, indicating that the enzyme was active and increased energy utilization in this study. Perhaps the enzyme blend composition or dose were insufficient in hydrolysing the substrates present in CE. Although the CAID and CATTD of most nutrients were decreased by CE, CE inclusion provided more DE and NE than feeding the basal diet alone, indicating the benefits of including CE when energy prices are high.

Wheat millrun (WM) is an abundant coproduct in western Canada, as a by-product of flour milling for the bakery industry. As a nutrient-rich ingredient offered at a discounted price, including wheat millrun in diets for weaned pigs is expanding (Slominski et al., 2004).

Consistent with other coproducts, the fibre content of WM can be detrimental to overall nursery diet nutrient digestibility. In chapter 3, the CAID and CATTD were measured in weaned pigs, feeding diets including wheat millrun and a multi-enzyme blend. The results of this study showed no effect of the enzyme on diet nutrient digestibility. A clear decrease in CAID lysine and energy values occurred between the positive and negative control, which was formulated with less energy and lysine, ensuring that the selected model can detect important differences in nutrient digestibility from enzyme inclusion. In the diets containing the enzyme, there was increased xylanase activity compared to the diets without enzyme, indicating overall enzyme activity. Thus, the reason why nutrient digestibility of diets containing WM was not increased remains inconclusive.

In conclusion, two common coproducts in western Canada, CE (Chapter 2) and WM (Chapter 3) studied here were not sufficiently hydrolysed by the included enzyme-blend, so that CAID or CATTD of DM, GE, CP and most AA of these two test feedstuffs was not increased.

4.3 Practical implications

Profitable pork production in Canada is vulnerable to market conditions, specifically market pork prices and production costs. As 66 to 75% of the total cost of production, feed arguably plays the greatest role affecting profitability (Alberta Pork, 2019). Feeding pigs lower cost coproducts from existing food, fuel or bioindustries without reducing performance would lower feed cost. For example, as common protein sources, CM is currently $570/ tonne compared with $875/ tonne for soybean meal (Alberta Pork, 2023). Wheat millrun is currently priced at $345/tonne compared with wheat grain at $426/ tonne.

Increasing canola seed production may also increase tonnage of lower quality seed available to small scale, local processors, thus increasing the abundance of CE available for feeding. The benefit of greater GE and NE value of CE compared with CM provides an opportunity to supplement energy to nursery diets at a lower cost. In chapter 2, increased energy values may benefit producers by increasing inclusion of a discounted feed ingredient while maintaining adequate nutritional value. Due to drought in western Canada, canola oil was $2,055/ tonne in 2022, compared with $974/ tonne in 2018 (Canola Council of Canada, 2023). Although there is a decrease in diet nutrient digestibility, feeding CE can reduce the cost of added liquid canola oil in weaned pigs' diets, thus lower feed costs.

In chapter 2, inclusion of the multi-enzyme blend increased the NE value of the control diet only but had no effect on nutrient digestibility of coproducts: CE (Chapter 2) or millrun (Chapter 3). There are inconsistent results on the benefit of feed enzymes in swine production, however, based on the scale of production in Canada where barns average 1,900 hogs, even a small effect of feed enzymes can offset the cost to producers (Statistics Canada, 2023). In nursery pig diets, primary benefits to carbohydrase supplementation are increased growth, G:F and decreased mortality wean to finish (Wang et al., 2018; Petry and Patience, 2020; Vangroenweghe et al., 2021). Taken together, increased feed efficiency and decreased mortality improve profitability at a rather expensive production stage (Table 4.1; 4,2; Zier-Rush et al., 2016). Shown in chapter 2, an increase in NE uplift from inclusion of the multi-enzyme blend in the basal diet, saved money directly related to the reduction of canola oil inclusion to increase dietary energy value (Table 4.3). An increase in digestibility would theoretically increase growth performance and efficiency during the energy-dependent phase of growth; however, this is not always the case (Aranda-Aguirre et al., 2021). Commercially, an improvement in performance or efficiency is essential for acceptance of novel feed ingredients and enzyme inclusion in weaned pig production. Efficacy of multi-enzyme supplementation is highly dependent on substrates present in the diet thus, the optimal blend, activity, and dose of multi-enzyme for both CE and WM still need to be elucidated.

4.4 Limitations

Overall, the experiments designed and executed in chapters 2 and 3 accurately tested the hypotheses. However, there were notable limitations during both experiments. For both experiments, intermittent and irregular COVID-19 restrictions impacted research activities for prolonged periods of time. For Chapter 2, acquisition and amount of sufficient CE samples was hindered due to prohibition of visitors on respective farms. Due to this constraint, the experimental design was adjusted from a 6 × 6 Latin square to a double 3 × 6 Youden square to preserve CE. Having had more CE samples and conducting a 6 × 6 Latin square, each experimental unit (pig) could have consumed each diet thereby strengthening the experimental design. For both trials, more cannulated pigs would have increased the power of the experimental design by increasing number of observations per dietary treatment. However, due to limited resources only 12 (Chapter 2) and 10 (Chapter 3) barrows could be cannulated. For Chapter 3, a resurgence of COVID-19 in 2022 prompted renewed in-person restrictions at the University of Alberta. This limited in-person research activities at SRTC delaying the start date of the trial from March 2022 to June 2022.

Ileal cannulation in weaned pigs is challenging due to rapid growth of the pigs. In part, the design of the T-cannulae wings used in chapter 3 allowed the cannula to fall out of six pigs as they quickly outgrew the cannula. Termination of these six pigs before the trial end date resulted in lost observations, consequently changing the experimental design from a double 4 × 4 Latin square to a 4 × 5 Youden square.

4.5 Future research

Both chapter 2 and 3 evaluated ileal and total tract digestibility of common coproducts and dietary inclusion of a multi-enzyme. Digestibility studies provide valuable insight to nutritionists for formulating diets and can imply better feed efficiency or growth in pigs. However, growth performance trials testing similar dietary treatments can further illustrate the commercial application of coproducts or multi-enzyme inclusion to weaned pig diets. Future research with CE inclusion should include performance traits in weaned pigs, specifically ADFI, ADG and G:F.

Multi-enzyme inclusion in pigs is not well established or accepted by swine nutritionists, due to contradictory published results (Dadalt et al., 2016; Zeng et al., 2018; Trindade Neto et al., 2020; Kpogo et al., 2021). Additionally, coproducts such as WM and CE are typically included at low levels in nursery diets. As the abundance of coproducts increases, future studies should be done to narrow down optimal multi-enzyme blend and dose for individual coproducts. In vitro studies can be used to determine the coproduct and corresponding enzyme blend (dose, activity and composition) prior to in vivo testing to enhance enzyme efficacy (Petry and Patience, 2020)

Lastly, post-weaning lag or diarrhoea impacts pig producers by loss of efficiency and increased mortality in the nursery. Imminent reduction or banning of antibiotics and zinc oxide in North American nursery pig diets provides an opportunity to improve gut health and immune function with alternative methods. Feeding multi-enzyme blends can improve gut barrier function, immune function, microbiome diversity and nutrient digestibility culminating in improved growth and welfare for nursery pigs (Li et al., 2018; Vila et al., 2018; Zhang et al., 2018; Patience and Petry, 2019; Li et al., 2019; Lee et al., 2020). Thus, further research should be conducted to explore the benefits of dietary enzyme inclusion to prevent post-weaning lag or diarrhoea.

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Table 4.1 Efficiency and feed cost scenario with a 1% improvement in feed efficiency due to multi-enzyme blend inclusion in diet.

	G:F	Cost ($) of feed per day in nursery[1,2]	Cost ($) per 28-day cycle	Difference in cost per cycle
Without enzymes	0.67	369	10,343	-
With CHO enzyme blend	0.68	366	10,237	106.65

[1] Based on $502/tonne of feed. Average cost of enzyme addition was $2.23/ tonne.

[2] Based on 1,000 head nursery.

Table 4.2 Forecasted savings from decreased mortality due to dietary multi-enzyme inclusion.

	Pig weight (kg)	Pork price ($/kg)	Revenue lost/pig	Deceased pigs[1]	Revenue lost/nursery
2% mortality	25	2	$50	20	$1,000
1.9% mortality[1]	25	2	$50	19	$950
			Saving from enzyme inclusion:		**$50**

[1] If enzyme scenario decreases mortality, saving one pig.

[2] Based on 1,000 pig nursery.

Table 4.3 Feed cost savings due to enzyme uplift of NE in Chapter 2.

	MJ/kg	Price /kg	Savings/tonne	Savings with enzyme price/tonne[2]
Canola oil	31.61	$2		
Uplift from enzyme[1]	0.34	$0.02	$21.51	$19.28

[1] Based on NE (MJ/kg) increase due to enzyme inclusion to a wheat barley basal diet in chapter 2

[2] Based on $2.23/tonne enzyme price.

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