THE EFFECT OF FEEDING CANOLA MEAL ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE IN PIGS

By

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE

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ABSTRACT

Three experiments were conducted to determine the nutritive value of black *Brassica napus* (BBN), yellow *Brassica juncea* (YBJ) and yellow *Brassica napus* (YBN) in pig diets. In experiment 1, effect of multi-carbohydrase supplementation on SID of AA in BBN, YBJ and YBN was evaluated in growing pigs. The YBJ had similar SID of AA to BBN and enzyme did not affect SID of most of AA. The second and third experiments investigated the effect of high dietary inclusion of BBN and YBJ on weaned pig performance and nutrient digestibility with enzyme supplementation. The studies showed that weaned pigs can be fed diets containing up to 250 g/kg of either BBN or YBJ. Enzyme supplementation improved the nutrient digestibility. In conclusion, BBN and YBJ had similar digestible nutrient contents and there were no detrimental effects detected when pigs were fed up to 250 g/kg of BBN and YBJ in weaned pigs.

DEDICATION

This thesis is dedicated to :-

My husband Mr. Sanjayan Satchithanantham and our daughter Shanika Sanjayan

My parents Subramaniam Ananthapavan and Yogeswary Ananthapavan

My brother Nishanthan Ananthapavan

ACKNOWLEDGEMENT

I would like to express my sincere thanks and heartfelt gratitude to my supervisor Dr. C. M. Nyachoti for providing me an opportunity to work on this project and to for his continuous guidance throughout the programme. My sincere thanks to Drs. B. Slominski, W. K. Kim and Genyi Li for accepting to be on my advisory committee and their valuable suggestions and contributions during our meetings. Special thanks to Dr. G. Crow for statistical advice.

I acknowledge Canola Council of Canada for funding this project and University of Manitoba for the travel award. I am thankful to Drs. J. Heo, E. Kiarie and J. S. Sands for their guidance and taking time to clarify my questions. My appreciation also goes to technical and administrative staffs of Department of Animal Science, especially Atanas Karamano, Lisa Rigaux, Robert Stuski, Darwin Ramos, Akin Akinwumi, Carol Schlamb, Kathy Graham, Cathy Plouffe, Mei Ding, Anna Rogiewicz, Karen Carrette, Harry Muc, Prakash Sharma, Margaret Ann Baker and Janice Haines. I wish to thank my fellow graduate students for their support and assistance.

My sincere appreciation goes to my husband and daughter for their support and love which made my study enjoyable and less stressful. Many thanks to my parents and brother for their care and guidance. I could not go this far without your love and support.

FOREWORD

This thesis was written in manuscript format and it is composed of three manuscripts. Part of manuscript I was presented at the ASAS–ADSA Midwest meeting in Des Moines, Iowa (March, 2012). Manuscript III was presented at Western Nutritional Conference in Winnipeg, Manitoba (September, 2012). Part of first manuscript and other two manuscripts were presented at the Canola Cluster meeting in Winnipeg, Manitoba (September, 2012).

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LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
ATTD	Apparent total tract digestibility
BBN	Black-seeded *Brassica napus*
BW	Body weight
CM	Canola meal
CP	Crude protein
Cys	Cysteine
DE	Digestible energy
DM	Dry matter
EPCM	Expeller pressed canola meal
Exp	Experiment
g	Gram
G:F	Gain to feed ratio
Glu	Glutamic acid
Gly	Glysine

h	Hour
His	Histidine
Iso	Isoleucine
kcal	Kilocalorie
kg	Kilogram
Leu	Leucine
Lys	Lysine
Met	Methionine
ME	Metabolizable energy
N	Nitrogen
NDF	Neutral detergent fibre
NE	Net energy
NSP	Non-starch polysaccharide
Phe	Phenylalanine
P	Phosphorus
Pro	Proline
Ser	Serine
SD	Standard deviation
SEM	Standard error of the mean
SECM	Solvent extracted canola meal
SID	Standardized ileal digestibility
Thr	Threonine
Val	Valine

YBJ	Yellow-seeded *Brassica juncea*
YBN	Yellow-seeded *Brassica napus*

1.0 General introduction

Feed cost represents almost 70% of the total production cost in a typical swine operation (Payne and Zijlstra, 2007). Canola meal (CM), a co-product of the canola oil crushing industry, is a possible cost effective protein substitute for protein sources in pig diets. It contains high levels of protein and well balanced amino acids (Bell, 1993).

Canola is an offspring of rapeseed, which was bred to have low levels of erucic acid and glucosinolates. Canola (*Brassica napus*) is one of the most important oil seed crops in Canada. Canola seed is crushed to yield approximately 42% oil which is used for human consumption and 58% meal which is used for livestock feeding (Unger, 1990). Canola meal is the second most important protein ingredient after soybean meal. It contains approximately 36-39% crude protein (CP) compared to 44-48% in soybean meal (Bell, 1993; Simbaya, 1996; Canola Council of Canada, 2009). Although CM contains high levels of protein and amino acids, anti-nutritional factors such as glucosinolates and fibre limit its utilization in swine diet.

In the past inclusion of CM up to 5% in the nursery diets was considered as safe because beyond this level, it led to poor performance (Thacker, 1990). When CM replaced 0, 25, 50 and 100% of soybean meal in young pig diet, ADG and ADFI were linearly reduced (Baidoo et al., 1987; McIntosh et al., 1986). The reduced performance might have been due to old formulation method which was not based on net energy (NE) and SID of amino acids and higher glucosinolate content (Payne and Zijlstra, 2007; Landreo et al., 2011). Bell et al. (1988) described that canola meal based diets formulated based on total amino acids are deficient in digestible amino acids which affect performance in pigs. In addition to that, several studies demonstrated that pigs fed diets containing CM had similar performance to soybean meal if the diet was formulated according to digestible amino acids (Mullan et al., 2000; Siljander-Rasi et al., 1996).

In order to minimize the anti-nutritional factors, canola breeders have developed new varieties of YBJ and YBN (Bell et al., 1998; Simbaya et al., 1995; Slominski, 1997). *Brassica juncea* contains slightly more protein, but lower ADF and NDF than BBN (Newkirk et al., 1997). In 1998, Bell and his collogues found that B. *juncea* had similar feeding value to *B. napus* in pigs in terms of apparent total tract digestibility of energy and protein. Yellow seeded *B. napus* is another new canola cultivar which was introduced in order to reduce fibre and increase the protein content (Simbaya et al., 1995; Slominski, 1997).

Enzyme supplementation has been recognized as a method to improve nutrient digestibility and consequently to allow for higher inclusion level of ingredients that contain anti-nutritional factors. Furthermore, in an in-vitro study Meng and Slominski (2005a) reported that combination of multiple carbohydrase enzymes improve the digestibility of NSP by a maximum of 36% in CM. Therefore, multi-carbohydrase supplementation may enhance the nutrient digestibility thereby improving the performance of pigs.

There are few studies conducted in order to determine the nutritive value of *B. napus* and *B. juncea* in pigs (Bell et al., 1998; Montoya and Leterme, 2009). However, there is limited information about standardized ileal amino acids digestibility of new cultivars of CM compared to conventional cultivars, maximum inclusion level of current varieties of CM in weaned pigs and effect of enzyme supplementation on nutrient utilization.

Therefore the objectives of this thesis were:

1. To determine the standardized ileal AA digestibility of canola cultivars [i.e., BBN, YBJ and YBN (YN01-429)] fed to ileal cannulated grower pigs.
2. To determine the effect of supplemental enzymes on SID of AA and apparent total tract digestibility of DM , CP and energy.
3. To evaluate the effect of increasing inclusion of BBN and YBJ for weaned pigs in terms of performance and ATTD of nutrients.

2. 0 Literature review

2.1 Introduction

Canola is an offspring of rapeseed which belongs to the cabbage family or Brassicas. The genus Brassica also contains plants such as cabbage, radish, kale, mustard and cauliflower (Bell, 1984). Rapeseed oil contain around 25-45% erucic acid in oil and 110-150 μmoles/g of aliphatic glucosinolate in the meal (Bell, 1993). Rapeseed was cultivated 3,000 years ago in India and 2,000 years ago in China and Japan. *Brassica campestris* or *Brassica rapa* was introduced to Canada in 1936 from Poland (Bell, 1984). After a few years *Brassica napus* was introduced from Argentina to Canada. Because of their origins *B. rapa* referred to as polish type and *B. napus* referred to as Argentine type. Glucosinolates in rapeseed meal was reduced by various plant breeding techniques as they are unpalatable and toxic to most animals.

Mawson et al. (1994) described that hydrolyzed products of glucosinolates by myrosinase enzyme have goitrogenic effects which has the ability to interfere the iodine metabolism and thereby affect the thyroid function and animal performance. In 1974 a “double low” cultivar was registered in Canada which has low level of both erucic acid and glucosinolate. In 1979 all double low cultivars produced in Canada were named as Canola (Bell, 1984). Canola has become the most popular crop in Canada with an annual production of approximately 9 million tonns (Canola Council of Canada, 2009). During crushing, canola seed yield 42% of oil, which is used as vegetable oil for human consumption and 58% meal, which is used as a protein source in animal feed (Unger, 1990). Canola meal has less than 2% of total fatty acid in the form of erucic acid in the oil portion and less than 30µ moles/g of glucosinolates in the meal portion (Bell, 1993).

2.2 Canola cultivars

2.2.1 Black-seeded *Brassica napus*

Black seeded *B. napus,* is an offspring of rapeseed, is an important oil seed crop in western Canada. *Brassica napus* is commonly known as canola or rapeseed. In the past it (rapeseed) was not used for animal and human consumption due to the present of toxic substances such as glucosinolates and erucic acid. However, the recent varieties of *B. napus* (canola) were produced by plant breeding techniques in order to minimize the erucic acid (< 2%) in the oil and glucosinolates (30 µmol/g) in the meal portion (Bell, 1993).

2.2.2 Yellow-seeded *Brassica juncea*

Brassica juncea is a species of mustard family. It is grown as a spice crop in North America and as an oil seed crop in Asia. It is grown in western Canada in order to produce condiment mustard. *Brassica juncea* has many advantages over *Brassica napus* including greater tolerance to heat and drought, resistance to blackleg disease and vigorous seedling growth (Woods et al, 1991; Newkirk, et al., 1997; Burton et al, 2003). Compared to *B. napus*, *B. juncea* suffers less to western Canadian weather condition. It was also found that *B. juncea* mature earlier compared to *B. napus*. These characteristics resulted in high yield of oil and low chlorophyll (Rakow and Raney, 1993). Low erucic acid and glucosinolates containing canola quality *B. juncea* (CQJ) has been developed recently (Potts *et al.*, 1999). It contains high amount of protein and oil compared to CM due to its thinner yellow seed coat (Burton et al, 2003).

2.2.3 Yellow-seeded *Brassica napus*

Yellow seeded *B. napus* is a newly introduced canola cultivar in order to reduce the fibre and to increase the protein and oil content (Simbaya et al., 1995; Slominski, 1997). Bell (1993) suggested yellow seeded canola has higher energy digestibility compared to black seed canola. It was also suggested that yellow-seeded canola has low fibre but high in protein and oil. A recent study described that bigger seed size, thinner hull and lower lignin and polyphenol contents of the hull are the major contributing factors for the lower fibre content in YBN (Khajali and Slominski, 2012). It was also described that because of thinner seed coat and larger embryo, YBN have high amount oil. Rashid and Rakow (1999) indicated that YBN line YN01-429 is the advanced canola quality arrived from complex intra specific and inter specific crosses.

2.3 Composition of canola meal

2.3.1 Nutritional composition

Canola meal contains protein, carbohydrate, lipid and fibre. It contains high levels of protein and well balanced amino acid (Bell, 1993). In Table 1. nutritional composition of CM and SBM are shown.

Crude protein contents of CM vary depending on the cultivars from which the meal were produced (Clandinin et al., 1981). For instance, the meal from cultivars of *B. juncea* contains a slightly high amount of crude protein (45.9%) compared to BBN (44.6%) on a dry matter basis (Newkirk et al., 1997) (Table 2). Canola meal is a good source of sulphur amino acids; methionine and cysteine (Newkirk et al., 2003a). It contains 2% methionine as a percent of total protein, but soybean meal has 1.5%. However, CM has lower amount of lysine compared to soybean meal. Canola meal contains 10% lower available lysine compared to soybean meal (Sauer et al., 1982). For this reason, these two meals are most of the time included together in a diet in order to complement each other (Khajali and Slominski, 2012).

One of the main factors that limit the nutritive value of CM is its low digestibility of energy which is a reflection of its high crude fibre content (Thacker, 1990). Compared to soybean, canola contains high amount of oil (40 – 45% on a dry matter basis) due to the presence of gum (Dale, 1996). Carbohydrate is the other major component present in CM. Simple sugars, sucrose, oligosaccharides, starch and non-starch polysaccharides are the major components of carbohydrates, which represents one-third of the CM (Khajali and Slominski, 2012). Compared to BBN, meal derived from YBN contains high amount of CP and sucrose but lower in glucosinolates and fibre content (Khajali and Slominski, 2012). Interestingly *Brassica juncea* contains intermediate levels of CP, sucrose, fibre and glucosinolates to BBN and YBN (Table 2).

Canola meal is a richer source of most of the minerals (Bell, 1993). Compare to soybean meal, CM has relatively high amount of Ca, P, S, Mg, Mn and Se, but K and Cu contents are lower (Bell et al., 1999). However, the presence of phytic acid and high fibre in the meal reduces the availability of most of the minerals. Although the availability of most of the minerals are low in CM, it has high amounts of available calcium, iron, manganese, phosphorus and selenium compared to soybean meal (Clandinin et al., 1986).

Compared to soybean meal, CM is a richer source of Vitamin B such as choline, biotin, niacin, riboflavin, thiamine and folic acid. However, pantothenic acid content is lower in CM (Clandinin et al., 1986).

Table 1. Chemical composition of canola meal compared to soybean meal

Components	Canola meal	Soybean meal
Dry matter, %	90.0	90.0
Crude protein, %	36.5	45.6
Ether extract, %	3.6	1.3
Gross energy, MJ/kg	18.6	20.1
Carbohydrates, %		
Starch	2.5	0.7
Sucrose	6.0	6.2
Sugar	7.7	6.9
Oligosaccharide	2.5	5.3
Fibre, %		
Crude fibre	11.6	5.4
Non-starch polysaccharide	18.0	17.8
Neutral detergent fibre	26.0	12.0
Acid detergent fibre	18.2	7.5
Total dietary fibre	31.7	21.8
Amino acids, %		
Arginine	2.04	3.23
Lysine	2.00	2.86
Threonine	1.57	1.74
Methionine	0.74	0.65
Cysteine	0.85	0.67
Tryptophan	0.48	0.64
Minerals, %		
Calcium	0.7	0.3
Phosphorus	1.2	0.7
Magnesium	0.6	0.3
Sodium	0.08	0.01
Potassium	1.29	2.0
Vitamins, mg/kg		
Biotin	1.0	0.3
Folic acid	2.3	1.3
Niacin	169.5	29.0
Pantothenic acid	9.5	16.0
Riboflavin	3.7	2.9
Thiamine	5.2	4.5

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

Table 2. Chemical composition of canola varieties (% of dry matter)

Components	Black *B.napus*	Yellow *B. juncea*	Yellow *B. napus*
CP	43.8	47.4	49.8
Fat	1.8	1.7	1.6
Ash	7.3	7.2	7.0
Starch	0.4	0.3	0.4
Sucrose	8.8	9.2	10.2
NSP	20.2	20.0	18.7
ADF	20.06	12.68	-
NDF	25.72	21.07	-
TDF	30.10	25.8	24.1
Glucosinolates (μmol/g)	30.7	18.8	20.0

Adapted from, Newkrik, 1997; Slominski et al., 1999; Khajali and Slominski, 2012.

2.3.2 Anti-nutritional components

The main anti-nutritional components of CM include fibre, glucosinolates, phytate and phenolic components such as tannins and sinnapine.

2.3.2.1 Fibre

Canola meal contains high amount of fibre compared to soybean meal which is 3 times higher than soybean meal (Bell, 1993). Its smaller seed size (2 mm diameter) and greater oil content are the possible reasons for the high percentage of fibre in the meal portion (Dale, 1996). High protein soy and 44% soy with hulls added back, contain around 4% and 7.5% fibre respectively whereas CM has more than 10% crude fibre (Dale, 1996). Canola meal contains cellulose (4-6%), non-cellulosic polysaccharide (13-16%), lignin and polyphenols (5-8%) and proteins and minerals associated with fibre fraction as the major fibre components (Slominski and Campbell, 1990). Previous studies demonstrated that yellow-seeded meal has low amount of fibre compared to black-seeded meal. For instance, ADF and NDF contents of *B. juncea* (12.7% and 21.1%) are lower compared to those (20.1% and 25.7%) of *B. napus* (Newkirk et al., 1997) (Table 2).

Fibre represents one third of the meal. It is because canola seed has high amount of hull compared to the size of seed. Hulls represent 16% of the seed weight and about 30% of the meal weight. Hulls contain high amount of non-starch polysaccharide (NSP) and lignin (Liang, 2009). Fibre mainly contains NSP, lignin associated with poly phenols, glycoprotein and minerals associated with fibre (Simbaya, 1996). Non starch polysaccharide components of CM are shown in Table 3. Slominski and Campbell (1990) suggested that 87% of glucose molecules are derived from cellulose.

Pectic polysacchardies are present in CM as a non-cellulosic polysaccharide, which is indicated by the presence of uronic acid (Slominski and Campbell, 1990). Arabinose, xylose, galactose and rhamnose are the main components of galacturonic acid. Part of the arabinose and galactose were derived from arabinan and / or arabinogalactan. Presence of xylose indicates the presence of xylan and xyloglucans. Xyloglucans contain xylose, glucose, galactose and fucose (Slominski and Campbell, 1990). Cellulose, arabinose, arabinogalactan and pectins are the major NSP components in CM (Slominski and Campbell, 1990; Meng, 2005; Kiarie, 2008). In a recent study, Meng and Slominski (2005b) found that CM contained 174.5 mg/g total NSP of which 14.3 mg/g was water soluble.

Table 3. Non-starch polysaccharides components of canola meal (mg/g)

Components	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*
Rhamnaose	1.4	1.8	1.4
Fucose	2.0	1.8	1.9
Arabinose	37.3	45.3	40.0
Xylose	15.8	14.9	13.8
Mannose	3.7	3.8	3.5
Galactose	15.0	18.4	14.8
Glucose	52.3	66.3	48.0
Uronic acids	51.5	59.2	45.1

Simbaya, 1996

2.3.2.2 Glucosinolates

Glucosinolate is the other major anti-nutritional factor present in CM. Rapeseed meal contained 110-150 μmol/g of glucosinolates (Bell, 1993). However, through plant breeding techniques new canola varieties have been developed with low level of (< 30 μmol/g) glucosinolates. Canola meal contains two types of glucosinolates (Table 4); aliphatic (85%) and indolyl (15%) (Newkirik et al., 2003). Gluconapin, glucobrassicanapin, progoitrin and napoleiferin are the major aliphatic glucosinolates present in CM of which progoitrin is the major factor which is responsible for the anti-nutritional effect (Fenwick and Curtis, 1980; Simbaya, 1996). Intact glucosinolates do not cause any harmful effects to animals. However, the break down products of glucosinolates either by enzyme myrosinase or by non-enzymatic factors such as heat, low pH, anatomical and physiological structure of the gastrointestinal tract, digesta transit time and microbial activity cause harmful effects to animals (Bell, 1993). Depending on the nature of glucosinolates, reaction condition and concentration, the break down products- thiocyanate, isothiocyanate, oxazolidinethione (goitrin) and nitriles may be formed and impair not only feed intake (due to their bitter taste) and growth performance but also affect thyroid function there by inhibit thyroid hormone production and impair liver and kidney function (Bell, 1993; Campbell and Schone, 1998; Mullan et al., 2000).

Previous studies showed that growing pigs can tolerate a maximum of 2.0-2.5 μmol/g of glucosinolates in the diet (Bell, 1993; Schone et al., 1997; Roth-Maier et al., 2004).

Table 4. Glucosinolates content of canola meal (μ mols/g dry matter)

Component	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*
Total glucosinolate	11.4	21.7	11.4
Allyl (Sinigrin)	-	1.2	-
3-Butenyl (Gluconapin)	2.3	16.1	1.8
4-Pentenyl (Glucobrassicanapin)	0.5	1.2	0.5
2-OH-3 butenyl (Progoitrin)	4.6	1.8	4.6
2-OH-3-pentenyl (Napoleiferin)	0.1	0.1	0.2
3-Indolylmethyl (Glucobrassicin)	0.3	-	0.5
4-OH-indolylmethyl (Hydroxyglucobrassicin)	3.5	1.3	3.8

Simbaya, 1996

2.3.2.3 Phytate

Phytate (myo-inositol hexakisdihydrogen phosphate or IP_6) is the another considerable anti-nutritional factor which is found in most plant seeds as the primary storage form of phospharus (Adeola and Cowieson, 2011). Phytate has the capacity to bind with protein and minerals such as Ca, Fe, Zn, Mn and Mg and form insoluble complex which is not available to the animal (Cabahug et al., 1999). Canola meal contains considerably high amount of phytate-bound phosphorus in proportion to total phosphorus and which ranges from 36% to over 70% (Khajali and Slominski, 2012). Due to this reason bioavailability of phosphorus had been estimated to be around 30 to 50% of the total phosphorus in CM (Enami, 2011).

2.3.2.4 Tannins

Another anti-nutritional factor in canola meal is tannins, which are complex polyphenolic compound. They can be subdivided into hydrolysable and condensed portion which is mostly found in seed coat (Yapar and Clandinin, 1972; Theander et al., 1977). Khajali and Slominski (2012) reported that tannins have the potential to bind with protein and proteolytic enzymes in gastrointestinal tract, thereby reducing the protein digestibility .

2.3.2.5 Sinapine

Sinapine is the choline ester of sinapic acid (Butler et al., 1982). It is bitter in taste which may limit the feed intake. It has been found that sinnapine can cause fishy taint in eggs due to the presence of Trimethylamine in the yolk (Butler et al., 1982).

2.4 Canola meal processing

Canola meal is a by product of canola oil crushing industry. There are three main methods used to extract the canola oil and meal. These are solvent extraction (where oil is extracted from the meal by physical expeller extraction followed by solvent washing), expeller pressed (where oil is physically extracted using heat) and cold pressed (where oil is physically extracted without heat treatment) (Mailer, 2004).

The solvent extraction method is the most common and efficient method of extracting the oil from the seed. This results in a meal that has less than 5% residual oil (Spragg and Mailer, 2007). Canola oil also can be extracted using expeller pressed method where the oil and meal is physically extracted with added heat, but this method is less efficient and result in a meal with higher residual oil content (8-15%) (Spragg and Mailer, 2007). In the process of solvent extraction, CM is subjected to higher moisture (15-18%) and moderate temperature (95 -115℃), whereas in the expeller extraction method, the meal is subjected to less than 12% moisture and higher temperature up to 160℃ (Canola Council of Canada, 2009). The steps involved in solvent extraction process are discussed briefly below.

2.4.1 Seed cleaning

The first step in canola processing is cleaning the seeds from dockage materials such as leaves, sticks, stones and cereal grains (Beach, 1983). Canola seed is then graded based on the seed damage, maximum moisture content and chlorophyll level.

2.4.2 Seed flaking

The cleaned seed is first flaked by roller mills set for narrow clearance of 0.2 - 0.3 mm to physically rupture the seed coat without damaging the quality of the oil (Pickard, 1993). The seeds must be preconditioned to flaking by heating at 30-40˚C to prevent shattering (Unger, 1990).

2.4.3 Cooking

The flakes are cooked by passing them through a series of steam heated drums or stack type cooker at 80-105˚C for 15-20 minutes to complete the cell breakdown and to reduce the viscosity of the oil. The cooking cycle also deactivates the myrosinase enzyme and thus prevents the breakdown of glucosinolates in the meal to undesirable products which would affect the quality of oil and meal (Mailer, 2004).

2.4.4 Pressing

After the cooking, flaked canola is pressed in a series of screw presses or expellers which consist of rotating screw shaft within a cylindrical barrel. The rotating shaft presses the cake against the adjustable choke which removes the oil while avoiding excessive pressure and temperature (Mailer, 2004).

2.4.5 Solvent Extraction

In this step pressed cake is deposited in the extractor and solvent hexane heated to 50-60˚C is passed in opposite direction to the movement of press cake. The residual oil content is removed in a continuous counter current extraction method. The meal at this stage is referred as "marc" (Unger, 1990).

2.4.6 Desolventizing and Toasting

The solvent extracted meal is placed into the desolventizer-Toaster and solvent is removed by the use of steam which provides heat to vaporize the hexane. During this process the meal is heated to 95-115˚C and moisture content increased to 12-18%. The desolventized meal is then toasted on heated metal plates. The final products contain 10% moisture and less than 15 oil content (Mailer, 2004). In the processing plant some of the canola oil refining products including gums and soap stocks may be added into the meal to increase the energy value and meal quality.

2.5 Digestibility of amino acids in pigs

The amino acids contained in practical swine diet are not fully biologically available to animals. This is because most of the proteins in the feed ingredients are not fully digested and the amino acids are not fully absorbed and metabolically available to the animal (NRC, 1998). Therefore, it is important to formulate the diets based on digestible amino acids rather than total amino acids in order to provide adequate, but not excessive amino acids which will reduce the nitrogen excretion and improve the performance of the animals.

Amino acid digestibility is the most important factor which determines the amino acids utilization of pigs from the feedstuffs (Fan, 1994). Amino acid digestibility is defined as the difference between the amount of amino acids ingested by the animal and the amount that is excreted in the feces or in ileal fluid divided by the amount that is ingested (Sauer and Ozimek, 1986). Digestibility assays are divided into two main categories: fecal and ileal digestibility (Hoehler et al., 2006). Compared to fecal analysis method, ileal method is a more appropriate method since there is bacterial metabolism of amino acids in the large intestine and cecum, which leads to an over- or under- estimation of the amino acids digestibility (Sauer and Ozimek, 1996). Some studies showed that there was a net synthesis of lysine and methionine and disappearance of cysteine, threonine and tryptophan in the large intestine (Low, 1980; Tanksley and Knabe, 1982; Sauer and Ozimek, 1986). In addition, it was also reported that amino acids will not be absorbed from the cecum and large intestine (Just et al., 1981).

2.5.1 Apparent, standardized and true ileal digestibility

Ileal digestibility of amino acids are expressed as apparent (AID), standardized (SID) and true (TID) ileal digestibility depending on the proportion of the ileal endogenous amino acid out flow included in the calculation (Stein et al., 2007a). In order to avoid the bacterial manipulation in the large intestine, analysis of ileal content is preferred . There are many different approaches, including Simple T-cannulation, Post valvular T-cecum cannulation, Ileao-rectal anastomosis and slaughter methods, that have been used to collect digesta from the ileum of the pigs (Gabert et al., 2001). Apparent ileal digestibility (AID) is calculated by subtracting the total amount of specific nutrient at the ileal out flow from the total amount of that ingested (Stein et al., 2007a). However, according to the word "apparent", ileal out flow contains both non-digested amino acids and the amino acids of endogenous (IAA_{end}) origin such as digestive enzymes, mucoproteins, desquamated cells, serum albumin, peptides, free amino acids, amines and urea (Nyachoti et al., 1997; Bacte-Moughan and Schuttert, 1991).

Endogenous amino acids are the protein substances that are endogenously synthesized and secreted into the intestinal lumen of the pig and neither digested nor absorbed prior to the distal ileum. Endogenous losses are divided into two portions: basal endogenous losses and specific endogenous losses (Nyachoti et al., 1997; Stein et al., 2007a, b). The basal losses (non specific loss) are not influenced by the diet that is being fed. However, it is related to the total dry matter intake (Stein et al., 2007b) . The basal losses are measured by N-free diet, regression method and the peptide alimentation techniques (Stein et al.,2007b). The specific losses are influenced by the specific characteristics of the feed ingredients such as type and amount of fibre and other anti-nutritional factors (Stein and Nyachoti, 2003). There is no any direct procedures to measure the specific endogenous losses. However, the homoarginine and isotope dilution techniques are used to estimate the total endogenous losses which include both basal and specific endogenous losses (Stein et al., 2007b).

Stein et al. (2005) mentioned that AID values of individual feed ingredients are not additive in mixed diet. This is a major concern in feed formulation, because additivity of individual ingredients is important for the prediction of growth performance in pigs. In order to partly overcome this problem, it has been suggested that AID values had to be corrected for basal endogenous losses (Jansman et al., 2002). Such value is called as standardized ileal digestibility (SID) and that is calculated similar to AID but basal IAA_{end} are subtracted from ileal out flow. Determination of SID is important for a perfect feed formulation as SID values are additive in mixed diets which overcome the disadvantages of apparent and true ileal digestibility (Stein et al., 2007). True ileal digestibility (TID) values are also calculated similar to AID but total IAA_{end} are subtracted from ileal out flow. True ileal digestibility values are rarely available for feed ingredients because it is difficult to accurately measure the total IAA_{end}.

2.6 Factors affecting digestibility

2.6.1 Animal factor

Amino acid digestibility is affected by age of the animal. The ability to digest protein and amino acids increase with the age of the animal (Wilson and Leibholz, 1981). The reason for the low digestibility of proteins of vegetable origin in young pigs is that the activity of some of the protein digesting enzymes is low in early life. Moughan (1993) suggested that the activity of the enzyme that digest vegetable protein is low in the young pigs compared to older pigs. He also mentioned that trypsin activity of the young pig (3 week old) is only 10% of that of the adult pigs.

Stein et al. (1999a) demonstrated that gestation sows with restricted feeding and the grower pigs with ad-libitum feeding had similar AID values of AA. However, Stein et al. (2001) demonstrated that gestation sows have higher SID values compared to grower pigs due to higher endogenous loss in gestation sows which was because of lower feed intake (Stein et al., 1999b). It was suggested that if the body weight of the pig is higher than 60 kg, the AA digestibility is not influenced by age, body weight and physiological condition. However, this is not the case for pigs weighing less than 60 kg; AA digestibility changes with the age or maturity (Stein and Nyachoti, 2003). They also suggested that protein and AA digestibility do not change with the genetic line of the pigs.

2.6.2 Level of feed intake

It has been reported that AID of amino acids increase with the level of feed intake from the level that is calculated to provide the maintenance energy requirement up to the level that is twice the amount of maintenance energy requirement (Rayadurg and Stein, 2003). It was suggested that the reason for the lower AID in pigs that had lower feed intake, was increased basal endogenous amino acids loss compared to the pigs that had higher feed intake (Stein et al., 1999b; Rayadurg and Stein, 2003). However, SID linearly decrease with increased feed intake as the values of SID are calculated by correcting AID values with the basal IAA_{end} (Rayadurg and Stein, 2003).

2.6.3 Chemical composition and physical characteristics of diet

It has been demonstrated that dietary factors such as protein concentration, dietary fibre, fat content and the anti-nutritional factors influence the digestibility of amino acids. Htoo et al. (2007) found that decreasing the CP content of the diet tended to reduce the AID of protein and most of the AA in young pigs. It is also interesting to note that protein which is derived from animal origin is more digestible than that of plant origin (Gabert et al., 2001). Solubility of the protein is another factor which influences its digestibility. Diets containing low solubility protein sources such as fish meal, soy protein concentrate, meat meal, sunflower meal etc, and high solubility protein sources such as casein were different in AID of AA (Jansman et al., 1995).

Dietary fibre is the other important component that might affect the digestibility of nutrients. The studies showed that the level of dietary fibre had an negative effect on the digestibility of AA (Mosenthin et al., 1994; Schulze et al., 1994). On the other hand there are also reports of no effect of dietary fibre on amino acid digestibility

(Sauer et al., 1991; Li et al., 1994). The reason for the inconsistent results might be due to the different sources of fibre. However, previous studies showed that, soluble fibre such as pectin, guar gum and rye binds with water and increase the digesta viscosity thereby reducing the digestibility of nutrients (Renteria-Flores et al., 2008). On the other hand insoluble fibre such as cellulose, hemicellulose and lignin increase the digesta passage rate (Anderson, 1985), thereby reducing the digestion and absorption of nutrients (Mroz et al., 1986; Bedford and Schulze, 1998). In general, inclusion of fiber in pig diet not only increases the endogenous AA loss but also adsorb with peptides and AA, thereby withholding them from absorption (Sauer and Ozimek, 1986).

Anti-nutritional factors of feed ingredients also have a negative effect on nutrient digestibility. Stein (2003) described that anti-nutritional factors increase the specific endogenous loss of AA thereby reducing the AID and SID values. Several studies also noted reduced digestibility of N and AA in pigs containing pea protein supplemented with trypsin inhibitor (le Guen et al., 1995), soybean lectins (Schulze et al., 1995). Furthermore, differences in AA digestibility may also result from the tannin content of the diet. Tannin has the potential to bind either with AA of the diet and form a complex which is resistant to proteolytic enzyme activity or directly bind with the protein molecule of the enzyme, thereby reducing the digestibility of AA (Sauer and Ozimek, 1986). Stein and Nyachoti (2003) reported that dietary fibre and anti-nutritional factors reduce the AID by increasing the specific endogenous losses.

The fat content of the diet also has been shown to improve the AID values, if the diet contained high amount of fat (i.e. 10-15%) (Li and Sauer, 1994). Stein (2003) described that the diet which contained high level of fat have increased retention time in the small intestine, thus give more time for protein digestion. Further investigation on the effect of dietary source and level of fat, it was observed that both source and level of fat has an effect on AID of AA in pigs (Albin et al., 2001). For instance AID of lysine and threonine were increased in young pigs with the increased inclusion of canola oil (Li and Sauer, 1994). It was also observed that apparent fecal digestibility was increased with increased soybean oil in grower pigs (Jorgensen and Fernandez, 2000).

2.6.4 Feed processing

Amino acid digestibility is also affected by the processing method. (Sauer et al., 1977) found that finely ground wheat had higher AA digestibility than cracked wheat. This was because grinding decreases the particle size, thereby improving the digestibility by increasing the exposure to digestive enzyme. Heat treatment during processing can also influence the digestibility by inactivating the trypsin inhibitors present in soy protein, thereby improving the digestibility (Gabert et al., 2001). However, over-heating has a negative effect on protein digestibility.

2.7 Factors affecting nutritive value of canola meal

Canola meal quality is affected by several factors. Enami (2011) suggested that the level of glucosinolates and their break down products, heat treatment during processing and the residual oil content in the meal are the three main factors affecting the quality of CM.

2.7.1 Available energy

Available energy is an important factor in swine diet formulation. Energy requirement of animals vary depending on the age, species and breed.

Glucosinolates are the main factor which limits the available ME value in CM and they also affect the digestive function (Bell, 1991). Metabolizable energy value of the meal improves if the glucosinolate content is reduced via plant breeding (Bell, 1993). Other factors such as fibre content, protein and oil also influence the ME content of CM. These factors depend on the variety and quality of the seed and the processing method. Fibre content of the meal varies according to the size of the seed, ratio between hull and embryo, colour and composition of the hull. Majority of the fibre is present in hull of the seed, which represents 16% of the seed and 30% of the meal weight. Compared to dark hulls, hulls of the yellow-seeded *Brassica* have higher energy digestibility (Bell, 1993). Oil content of CM also affects the ME which is influenced by efficiency of oil extraction during processing and amount of gum (phosphatidyl compounds) which has around 50% of the canola oil. March and Soong (1978) found that added gum increased the energy value of the meal by 150 kcal/kg. It was also suggested that CM contains low level of starch (2.5%) and sucrose (7.7%), which might also contribute to the lower energy content in the meal (Slominski and Campbell, 1991; Newkirik et al., 2003).

2.7.2 Protein and amino acids

Protein quality of CM is affected by the temperature during processing. Temperature during processing should be the minimum required to inactivate the myrosinase enzyme. Excessive heat causes Maillard or browning reactions and reduces AA availability, especially for lysine (Bell, 1993). Dale (1996) mentioned that excessive heat will reduce the availability and digestibility of lysine present in the meal. Since lysine is the first limiting AA in swine diets, excessive heat affects usage of CM in swine diet. Newkirk (2003) mentioned that quality of the CM won't be affected before the desolventization toasting phase of processing. Desolventization / toasting change the colour of the meal from yellow to brown and reduce the lysine availability of the meal (Newkirk et al., 2003). Fiber is another factor which might reduce the digestibility of AA. Newkirk et al. (2003) indicated that the hull is negatively correlated with protein digestibility, suggesting that protein in the hull is not digested by the enzymes.

Carnovale et al. (1988) found that phytate is another factor which negatively affects the digestibility of protein. It was mentioned that phytic acid has the potential to bind with protein bodies and reduce its solubility and availability for enzymatic activity thereby reduce the digestion and absorption (Lenis and Jongbloed, 1999).

Plant genotype also has an impact on nutrient content of the meal. For instance, YBN has less fibre and more protein compared to BBN (Slominski et al. 1999). It was also found that lysine content of YBJ is lower compared to BBN (Newkirk, 2002).

2.7.3 Minerals

Mineral contents of meal vary among crushing plants in western Canada. Genetic and environmental factors mainly influence in variation of mineral contents. Bell et al. (1999) mentioned that location has a big influence in the mineral content of the meal. Environmental factors such as temperature and precipitation and season of the year in which crops were grown affect the nutrient availability and pH of the soil which influence the absorption of some minerals from the soil (Bell et al., 1999).

2.8 The use of canola meal in swine feed

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

2.8.1 Starter pigs

It appears that majority of the studies on CM use in starter pig diets were mainly focused on growth performance. In the past, it was suggested that complete (McKinnon and Bow-land, 1977) or partial (Castell, 1977) replacement of soybean meal with CM had negative effects on pig performance. It was also documented that increasing inclusion of CM linearly reduced ADG and ADFI in weaned pigs (McIntoshi et al., 1986; Baidoo et al., 1987). In a preference trial, weaned pigs were offered a choice between a soybean based control diet and CM at 5-20% inclusion level (Baidoo et al., 1986). They found that pigs preferred to eat a SBM based control diet more than any of the diet containing CM. There was also a significant reduction in the amount of feed consumed when CM inclusion level increased from 5 to 20%. The possible reason for the low intake of a diet containing CM in starter pigs may be the influence of glucosinolate breakdown products on thyroid function and the reduced palatability due to the presence of glucosinolates and their break down products (McKinnon and Bow-land, 1977).

However, recent findings are contrary to the results of past research. For instance, a recent study reported that either solvent-extracted canola meal (SECM) or expeller-pressed canola meal (EPCM) at 150 g/kg inclusion level combined with crude glycerol can partially replace SBM and wheat in weaned pig diets (Seneviratne et al., 2011). Another study by Landero et al. (2011) who also fed 0, 50, 100, 150 and 200 g SECM / kg , in replacement for soybean meal to weaned and found no significant differences in growth performance but there were linear reductions in ATTD of DM, energy and CP and quadratic reductions in the digestible energy (DE) content of the diet. Landero et al. (2012) also conducted another experiment to determine the effect of feeding increasing levels of EPCM up to 200 g/ kg diet to weaned pigs and found no significant differences in growth performance although there were linear reductions in apparent total tract digestibility of DM, energy and CP. There were two possible explanations proposed for the better performance of weaned pigs at high CM inclusion. Firstly, in the past diet was formulated mainly on CP and DE and not based on SID AA or NE. Zijlstra and Payne (2007) suggested that formulating diets with by-products as alternative feedstuffs would minimise the risk associated with reductions in growth performance if the NE and SID AA systems were used. The second reason is that recent cultivars of CM have comparatively low amounts of glucosinolates compared to old cultivars (Landreo et al., 2011).

2.8.2 Grower - finisher pigs

Previous studies reported that CM can be used to replace only up to 50% of the supplemental protein from SBM in grower pigs (McKinnon and Bow-land, 1977). However, replacement of 75% or complete replacement of SBM by CM significantly reduced the growth rate (Baidoo and Aherne, 1987). Sauer et al. (1982) described that lower DE and lysine contents in CM compared to soybean meal and the effect of glucosinolates on feed intake and metabolic process might be the possible reasons for the low performance in grower pigs. Thacker (1990) suggested that good performance could be achieved in grower pigs, if CM supplies only one half of the supplementary protein in the diet. In a review on CM, Schone et al. (1997) suggested that growing pigs can tolerate a maximum level of 2 μmol/ g of glucosinolates in the diet. But the total glucosinolate content of Canadian CM is around 7.2 μmol/g (Newkirk et al., 2003). This might lead to include the maximum level of 33% CM in growing pig diet.

There are few studies conducted in order to determine digestibility of nutrients in CM. For instance, Bell et al. (1998) reported that BBN and YBJ had similar digestible protein and energy in finisher pigs. Indeed, a recent experiment using toasted and non toasted BBN, YBJ and YBN in grower pigs suggested that DE and NE content of YBN is higher than that of BBN and YBJ (Montoya and Leterme, 2009). The SID of AA of SECM and EPCM in grower pigs has been reported by a few studies (Woyengo et al., 2010; Seneviratne et al., 2010). In the afore mentioned studies, EPCM had greater digestible AA and energy compared to SECM (Woyengo et al., 2010).

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

2.9 Exogenous enzymes in swine diets

Exogenous enzymes are widely used in swine diets in order to increase the nutritive value of the feed ingredients. Feed enzymes are mainly dominated by carbohydrases and phytase (Adeola and Cowieson, 2011). Mode of action of enzyme is described by a couple of possible mechanisms such as breakdown of anti-nutritional factors present in feed ingredients, elimination of nutrient encapsulation effect thus increasing availability, breakdown of specific chemical bonds in raw materials that are otherwise not cleaved by endogenous enzymes, thus releasing more nutrients, and complementation of the enzymes produced by young animals (Simon, 1998; Bedford and Schulze, 1998).

Swine diets mainly contain plant materials such as cereals and vegetable proteins which cannot be fully digested and utilized by pigs because many cereals and their by products contain high amount of NSP such as cellulose, xylose, arabinose β-glucans glactonic acid, gums, pectins and mucillage (McDonald et al., 1995). Some of these compounds are present as a part of the cell wall, thus shielding substrates from coming into contact with digestive enzymes and limit the digestibility of nutrients (Bedford, 2000; Adeola and Cowieson, 2011). Furthermore soluble NSP such as β-glucans and arabinaoxylans increase the digesta viscosity and reduce digestibility and nutrient intake (Bedford and Schulze, 1998). The adverse effects can be overcome by supplementation of exogenous enzymes. Feed enzymes should have the ability to break down plant cell wall materials and lower the intestinal viscosity (Ahmed et al., 2007).

The most common fibre degrading enzymes used in pig and poultry diets are β-glucanase, xylanase, and cellulase (Xu, 2008). Several studies indicated that young pigs fed wheat and hull-less barley with NSP degrading enzyme had improved energy and protein digestibility. Whereas growing finishing pigs had poor response to NSP degrading enzyme supplemented to wheat- based diet (Xu, 2008). Enzymes are widely used to remove the anti-nutritional factors and toxins, increase the existing nutrients and NSP digestibility and supplement host enzymes (Bonneau and Laarveld, 1999). This minimizes both nutrient wastage and manure production. Enzyme usage is specific to certain feed ingredients and growth stage of pigs. Certain feed ingredients also require specific enzymes depending on the proportion of NSP present in them. For instance barley is rich in β-glucan, whereas wheat and rye are rich in arabinoxylans. In this condition glucanase can be used to improve the digestibility of viscous cereals such as barley and reduce their viscosity in the gut (Marquardt et al., 1994). Xylanases are use to increase the digestibility of viscous polymers in wheat

(Bedford and Morgan, 1996). Bedford et al. (1998) reported that enzymes had higher response to poor quality ingredients compared to higher quality ingredients.

In general the use of exogenous enzymes to improve nutrient digestibility has yielded inconsistent results. The studies show that carbohydrase supplementation improved the nitrogen (Yin et al. 2000; Reilley et al., 2010), dry matter (Li et al., 1996; Olukosi et al., 2007a), energy (Yin et al., 2000; Diebold et al., 2004) and AAs digestibility (Li et al., 1996; Baidoo et al., 1998; Barrera et al., 2004) in pigs. On the other hand there are also reports of no effect of carbohydrase enzyme on crude protein (Nitrayova et al., 2009), dry matter (Woyengo et al., 2008) and energy digestibility (Olukosi et al., 2007a).

There are few studies that have been conducted to evaluate the effect of NSP-degrading enzymes on digestibility and performance of pigs fed diets supplemented with CM. For instance, Thacker, (2001) fed barley-based diets containing CM and supplemented with muti-carbohydrase enzymes to growing pigs and found that enzyme had no effect on growth performance and ATTD of nutrients. In a study with weaned pigs, Zijlstra et al. (2004) found that carbohydrase supplementation to wheat and CM based diet improved the ADFI and ADG, but did not improve the feed efficiency and ATTD of nutrients. They postulated that carbohydrase enzymes reduced the digesta viscosity thereby increasing the passage rate which led to an increase in ADFI. Furthermore, in an in-vitro study Meng and Slominski (2005b) reported that combination of multi-carbohydrase enzymes improve the digestibility of NSP by a maximum of 36% in CM.

2.10 Conclusion

There is increasing interest in utilizing CM in swine diet as it is a cost effective protein source. This literature review provides information about the nutritive value of CM and recent techniques (i.e., development of new canola cultivar and supplementation of feed enzymes) which have been used to improve the nutritive value of CM. Determination of SID of AA of new cultivars of canola is very important in order to formulate the diet efficiently thereby helping to achieve predictable growth performance in pigs. Furthermore, enzyme supplementation to cereal based diets has yielded inconsistent results.

Therefore based on the available literature, it was hypothesized that YBJ and YBN would be higher in SID of AA and have better growth performance in pigs compared to BBN as they have lower fibre and glucosinolates contents in the meal. The second hypothesis was that multi-carbohydrase supplementation would improve the digestibility and performance in pigs. The purpose of this research was to evaluate the effect of multi-carbohydrase in a diet containing CM on nutrient digestibility and growth performance in pigs.

3.0 Manuscript 1

Standardized ileal digestibility of amino acids in canola meal containing diets fed to growing pigs in response to enzyme supplementation

3.1 Abstract

The study was conducted to investigate the effect of multi-carbohydrase supplementation on apparent ileal (AID) and standardized ileal digestibility (SID) of black-seeded *Brassica napus* (BBN), yellow-seeded *Brassica juncea* (YBJ) and yellow-seeded *Brassica napus* (YBN) in growing pigs. A total of 18 barrows with an initial body weight of 20.9 ± 1.6 kg (mean ± SD) fitted with a T-cannula at the distal ileum were fed one of six experimental diets in a replicated completely randomized design in a factorial arrangements with the factors being i) 3 types of canola meal (i.e., BBN, YBJ and YBN) and ii) without and with added enzyme (i.e., multi-carbohydrase). The diets were cornstarch-based with either BBN or YBJ or YBN as the sole source of protein and amino acids (AA) and were formulated to contain approximately 140 g/kg crude protein. Titanium dioxide (3 g/kg) was included in the diets as an indigestible marker. Each experimental period lasted for 9 days. Pigs were acclimatized to their respective diets for 7 days followed by 12 hours of continuous digesta collection on days 8 and 9. Daily feed allowance was based on the body weight of the pig at the beginning of each period and was calculated to supply 2.6 times the maintenance energy requirements for energy and offered in two equal portions at 0800 and 1600 hours as a dry mash. Pigs had free access to water at all times. The AID and SID of CP and AA in BBN and YBJ were greater ($P < 0.05$) compared with YBN. Enzyme had no effect ($P > 0.10$) on the AID and SID of His, Ile,

Leu, Lys, Thr, Val, Ala and Ser among treatments. Interestingly, supplemental enzyme decreased ($P < 0.05$) the AID of DM in BBN and YBN, AID of N and energy in YBN and SID of Arg, Met, Cys, Glu and Tyr in BBN, YBJ and YBN. In conclusion, the average AID and SID of indispensable AA, were (73.4 and 80.8%), (73.2 and 81.1%) and (68.5 and 76.1%) in BBN, YBJ and YBN without added multi-carbohydrase, respectively.

3.2 Introduction

Feed accounts for more than 60% of the total production cost in a typical swine operation, and protein is the second most contributing factor to the total cost of feed in pigs. Canola meal (CM) (i.e.,black-seeded *Brassica napus*) is recognized for its high level of protein and well-balanced AA profile (Bell, 1993). However, high fibre content and anti-nutritional factors such as glucosinolates in the rapeseed are problematic to its use in pig diets (Bell, 1993). In this regard, rapeseed breeders have developed new varieties of rapeseed known as canola to overcome those anti-nutritional factors [i.e, yellow-seeded *Brassica juncea* and yellow-seeded *Brassica napus*] (Simbaya et al., 1995; Slominski, 1997; Bell et al. 1998). Newkirk et al. (1997) reported YBJ contained greater CP, but lower ADF and NDF contents compared to BBN. In addition, Bell et al. (1998) reported that YBJ and BBN had similar apparent total tract digestibility of energy and CP in finisher pigs.

There has been an increasing interest in utilizing feed enzyme in pig diets in order to improve nutrient digestibility and consequently to allow for higher inclusion level of ingredients that contain anti-nutritional factors. Furthermore, in an in-vitro study

Meng and Slominski (2005b) reported that combination of multi-carbohydrase enzymes improve the digestibility of non-starch polysaccharide (NSP) by a maximum of 36% in CM. However, digestibility and nutrient values (e.g. CP, energy and AA) of new cultivars of CM compared to conventional cultivars in response to enzyme supplementation has not been investigated extensively.

It was hypothesized that i) feeding diets containing YBJ and YBN would have higher SID of AA compared to BBN and ii) multi-carbohydrase supplementation will improve nutritional value of those CM varieties. Therefore, the objective of the study was to determine the AID and SID of CP and AA of BBN, YBJ and YBN in response to multi-carbohydrase supplementation using ileal cannulated growing pigs.

3.3 Materials and Methods

All procedures described in this experiment were approved by University of Manitoba Animal Care Committee and pigs were handled and cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

3.3.1 Animals and housing

A total of 18 barrows (Yorkshire-Landrace × Duroc) with an average body weight of 20.9 ± 1.6 kg (mean ± SD) were obtained from Glenlea Swine Research unit, University of Manitoba. They were housed in individual pens within an environmentally controlled room with the average temperature of 21 ±2˚C. During the 5-day acclimation period they were fed a commercial grower pig diet and had free access to water at all times. After that pigs were surgically fitted with a simple T-cannula at the distal ileum as described by Nyachoti et al. (2002). The study was started after a 14-day post-surgical recovery period. During the recovery period they were fed with the same commercial grower pig diet.

3.3.2 Diets

Canola meal derived from BBN and YBJ were obtained from a local crushing plant at Bunge Canada (Altona, Manitoba, Canada) and YBN obtained from POS pilot plant in Saskatoon, Canada using conventional solvent extraction process. Experimental diets included a cornstarch-based diet containing BBN, YBJ or YBN and fed without and with multi-carbohydrase enzyme addition (Superzyme OM; Canadian Bio-Systems Inc., Calgary, Alberta, Canada). Enzyme supplement supplied 1,100 U of pectinase, 50 U of cellulase, 1,000 U of xylanase, 600 U of glucanase, 400 U of mannanase, 50 U of galactanase and 2,500 U of amylase per kilogram of diet. Canola meals were used as a sole source of protein and diets were formulated to contain 14% crude protein. Titanium dioxide (3 g/kg) was used as an indigestible marker.

3.3.3 Experimental design

The experiment was conducted according to a replicated completely randomized design in a factorial arrangements with the factors being i) 3 varieties of canola meal (i.e., BBN, YBJ and YBN) and ii) without and with added enzyme (i.e., multi-carbohydrase) to give six observations per treatment. Each period lasted for 9 days. Pigs were fed twice daily (0830 and 1530) at 2.6 times maintenance energy requirement (NRC, 1998). Pigs were adapted to experimental diets and environmental conditions for 7 d followed by 12 hours (0800 to 2000) of continuous ileal digesta collection on days 8 and 9 (Nyachoti et al., 2002). Digesta were collected into sample bags containing 10 ml of formic acid to minimize bacterial activity and stored immediately at -20˚C until required for further analysis.

Table 5. Ingredients and calculated composition of basal diets

Items	*Black B. napus*	*Yellow B. juncea*	*Yellow B. napus*
Ingredients, %			
Canola meal	37.20	35.60	39.50
Corn starch	59.80	61.40	57.50
Limestone	0.60	0.60	0.60
Monocalcium phosphate	0.80	0.80	0.80
Iodized salt	0.30	0.30	0.30
Vitamin-mineral premix[1]	1.00	1.00	1.00
Titanium dioxide	0.30	0.30	0.30
Calculated nutrient composition			
Dry matter, %	92.7	92.9	93.3
Crude protein, %	14.03	14.02	14.02
Metabolizable energy (MJ/kg)	15.1	15.2	15.1
Digestible lys, g /Mcal	1.6	1.6	1.7
Calcium, %	0.67	0.66	0.68
Total Phosphorus, %	0.57	0.57	0.60
Available Phosphorus, %	0.24	0.24	0.25
Neutral detergent fiber, %	9.02	5.67	10.01
Acid detergent fiber, %	6.40	6.12	6.79

[1]Supplied per kg of diet- vitamin A: 2000 IU; vitamin D3:200 IU; vitamin E: 40 IU; vitamin K: 2 mg; riboflavin: 7 mg; niacin: 21 mg; vitamin B12: 20 mg; biotin: 70 mg; Cu: 10 mg as CuO; I: 0.40 mg as $Ca(IO_3)_2$; Fe 120 mg as $FeSO_4 .H_2O$; Mn: 10 mg as MnO; Se: 0.30 mg as Na_2SeO_3; Zn: 110 mg as ZnO.

3.3.4 Sample preparation and chemical analysis

Digesta samples were thawed and pooled for each pig in each period. They were homogenized in a blender (Waring Commercial, Torringtonn, CT, USA), sub-sampled and freeze dried. The dried digesta and diet samples were ground in Thomas wiley mill model 4 (Labwrench, Midland, ON, Canada) and mixed prior to chemical analysis.

Ileal digesta samples and diet samples were analysed for DM, N, gross energy, amino acids and titanium. Canola meal samples were analysed for DM, N, fat, total dietary fibre, NSP, sucrose, glucosinolate and AA. Dry matter was determined according to the method of AOAC (1990; method 925.09). Nitrogen content was determined by the combustion method (method 990.03; AOAC 1990) using a combustion analyzer (model CNC-2000; Leco Corporation, St. Joseph, MI, USA). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA) which had been calibrated using benzoic acid as a standard. The amino acid contents in the sample were analysed by acid hydrolysis according to the method of AOAC (1984; method 982.30) as modified by Mills et al. (1989). For amino acids analysis except for methionine and cysteine, 100 mg of samples were digested in 4 mL of 6 M HCl for 24 h at 110°C. Digested mixture was neutralized with 4 mL of 6.25 M NaOH and allowed to cool to room temperature. The mixture was then made up to 50 mL volume with sodium citrate buffer solution (pH 2.2) and analyzed using an AA analyzer (Sykam, Eresing, Germany). Samples for cysteine and methionine analysis were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not analysed. Concentration of titanium was determined according to

Lomer et al. (2000). Fat content of CM samples was determined using VELP Organic Solvent Extraction Apparatus in which hexane was used to extract the fat (method 920.39; AOAC, 1990). ADF and NDF content were analyzed according to the method of Goering and Van Soest (1970). Sucrose content of the samples was determined by gas liquid chromatography according to Slominski et al. (1994). Dietary fibre was determined by combination of NDF and detergent soluble-NSP and calculated as the sum of both NDF and detergent soluble-NSP (Slominski et al., 1994). Non-starch polysaccharides were analyzed using gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The procedure for neutral sugars was performed as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). Uronic acids were determined using the procedure described by Scott (1979). Glucosinolate content of the meal was analysed according to the procedure described by Thies (1977) with some modifications (Slominski and Campbell, l987).

3.3.5 Calculations and statistical analysis

Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) were calculated using the following equations.

$AID = 100 - [(Nd/Nf) \times (Tif/Tid)]$

where

Nd = nutrient concentration in ileal digesta (% DM)

Nf = nutrient concentration in feed (% DM)

Tif = TiO_2 concentration in feed (% DM)

Tid = TiO_2 concentration in ileal digesta (% DM)

SID = AID + (EAL/AAf)

Where

EAL = non specific endogenous loss of amino acids at the distal ileum (mg/kg DMI)

AAf = dietary content of the amino acid (% DMI)

EAL is measured at the distal ileum after feeding low casein protein diet to grower pigs and calculated according to the following equation.

EAL = AAd × (Mf/Md)

Where AAd is concentration of that amino acid in ileal digesta (mg/kg DMI), Mf is marker concentration in feed (mg/kg DM) and Md is marker concentration in ileal digesta (mg/kg DM).

The non-specific (basal) endogenous ileal N and AA losses (mg/kg DMI) (Table 6) used to calculate the SID values derived from averaging five independent studies conducted in our laboratory (Opapeju et al., 2006; Kiarie and Nyachoti, 2007; Lan et al., 2008; Woyengo et al., 2010; Yang et al., 2010). The average body weight of pigs used in those studies ranged from 20.2 to 82.0 kg.

Data were analysed as a 3 × 2 factorial arrangement in completely randomized design using the GLM procedure of SAS (SAS software release 9.1, SAS Inst., Inc., Cary, NC) with the main effects being 3 varieties of CM and added multi-carbohydrase or no added multi-carbohydrase. Least-squares analysis and 2-factor linear model with all interactions were used to evaluate the response. Statistical significance was accepted at $P < 0.05$. Duncan's multiple range test was used to separate treatment means when a significant treatment effect was observed.

Table 6. Endogenous ileal amino acid and nitrogen losses (g/kg DMI)

Items	Average ENL (g/kg DMI)
Nitrogen	3.083
Arg	0.765
His	0.335
Iso	0.438
Leu	0.574
Lys	0.449
Met	0.126
Phe	0.315
Thr	0.713
Val	0.559
Ala	0.668
Asp	0.991
Cys	0.289
Glu	1.501
Gly	1.997
Pro	6.987
Ser	0.871
Tyr	0.241

Values are an average of results reported by Opapeju et al., 2006; Kiarie and Nyachoti, 2007; Lan et al., 2008; Woyengo et al., 2010; Yang et al., 2010

3.4 Results

Nutrient composition of canola cultivars are presented in Table 7. The samples of BBN, YBJ and YBN contained 37.2, 37.7 and 35.5% CP respectively on as fed basis.

Pigs remained healthy and readily consumed their daily feed allowance. The effects of dietary treatments on AID and SID measurements are presented in Table 8 and 9. Yellow seeded *B. juncea* had similar ($P > 0.05$) AID values for DM, N and energy compared to BBN but had higher values ($P < 0.05$) than YBN. There were no differences ($P > 0.05$) in AID and SID of dispensable and indispensable AAs between BBN and YBJ. The AID and SID contents of Lys, Met, Thr, Val, Ala, Asp, Cys, Gly and Ser were greater ($P < 0.05$) for BBN and YBJ than for YBN.

Multi-carbohydrase supplementation did not improve ($P > 0.05$) AID and SID of His, Ile, Leu, Lys, Thr, Val, Ala and Ser regardless of varieties of CM. Although multi-carbohydrase supplementation decreased ($P < 0.05$) AID of DM in BBN and YBN and AID of N and energy in YBN, no effect ($P > 0.05$) in AID of DM, N and energy in YBJ was observed. However, enzyme supplementation decreased ($P < 0.05$) AID and SID values of Arg, Met, Cys, Glu and Tyr, compared to pigs fed diets without enzyme supplementation regardless of variety of CM. There was a trend for the enzyme to reduce ($P < 0.10$) the AID and SID of Phe, Asp, Gly and Pro in all three variety of CM. Interaction was not observed ($P > 0.05$) between CM varieties and enzyme supplementation for AID and SID of AA.

Table 7. Analysed chemical composition of canola meal (as fed basis, %)

Items	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*
Dry matter	89.7	90.3	92.2
Crude protein	37.2	37.7	35.5
Fat	2.6	2.6	2.0
Total dietary fiber	30.3	25.0	34.0
Total NSP	18.4	18.9	21.7
Glycoprotein	2.6	2.1	6.9
Glucosinolates (μ moles/g)	8.5	15.1	4.7
Indispensable AA			
Arg	2.13	2.36	1.80
His	0.97	0.96	0.89
Ile	1.23	1.20	1.08
Leu	2.64	2.64	2.38
Lys	2.03	1.92	1.93
Met	0.76	0.72	0.69
Phe	1.44	1.40	1.30
Thr	1.64	1.63	1.54
Val	1.63	1.52	1.46
Dispensable AA			
Ala	1.74	1.73	1.62
Asp	2.75	2.94	2.43
Cys	0.86	0.73	0.76
Glu	6.93	6.66	5.97
Gly	1.86	1.85	1.65
Pro	2.45	2.23	2.16
Ser	1.68	1.69	1.57
Tyr	0.97	0.96	0.89

Table 8. Apparent ileal digestibility (%) of dry matter, nitrogen, energy and amino acids in black-seeded *B. napus*, yellow-seeded *B. juncea* and yellow-seeded *B. napus* fed to growing pigs

Items	Black *B. napus* (+ Enzyme)	Yellow *B. juncea* (+ Enzyme)	Yellow *B. napus* (+ Enzyme)	Black *B. napus* (- Enzyme)	Yellow *B. juncea* (- Enzyme)	Yellow *B. napus* (- Enzyme)	Pooled SEM[1]	P-value Diet	P-value Enzyme	P-value Diet × Enzyme
Dry matter	68.8^{b}	70.2^{ab}	63.5^{c}	72.4^{a}	73.1^{a}	68.2^{b}	1.08	0.001	0.001	0.714
Nitrogen	60.9^{ab}	59.5^{bc}	52.0^{d}	64.4^{a}	62.2^{ab}	56.5^{c}	1.38	0.001	0.003	0.796
Energy	71.8^{bc}	73.4^{abc}	67.0^{d}	75.0^{ab}	76.6^{a}	71.4^{c}	1.10	0.001	0.001	0.815
Indispensable AA										
Arg	77.3^{b}	79.2^{ab}	78.7^{ab}	81.0^{ab}	83.2^{a}	80.6^{ab}	1.42	0.329	0.010	0.722
His	77.1	76.1	75.9	78.4	79.0	76.5	1.40	0.480	0.181	0.682
Ile	67.7^{ab}	69.5^{ab}	69.2^{ab}	71.6^{a}	72.0^{a}	65.1^{b}	1.62	0.096	0.568	0.045
Leu	72.4	72.2	71.1	74.7	75.1	70.8	1.53	0.154	0.201	0.548
Lys	70.5^{ab}	66.8^{bc}	65.0^{c}	72.9^{a}	69.9^{abc}	65.3^{bc}	1.71	0.002	0.180	0.704
Met	77.7^{bc}	77.2^{bc}	71.3^{d}	79.9^{ab}	82.8^{a}	75.2^{cd}	1.42	0.001	0.002	0.513
Phe	61.4^{ab}	57.1^{b}	59.2^{ab}	65.4^{a}	62.5^{ab}	60.6^{ab}	2.21	0.193	0.054	0.648
Thr	64.1^{ab}	60.0^{b}	59.1^{b}	66.7^{a}	63.8^{ab}	59.1^{b}	1.67	0.003	0.122	0.523
Val	65.8^{ab}	67.4^{ab}	66.7^{ab}	69.6^{a}	70.5^{a}	63.7^{b}	1.66	0.084	0.339	0.096
Dispensable AA										
Ala	74.2^{a}	73.0^{a}	64.1^{b}	72.2^{a}	71.9^{a}	59.3^{b}	2.53	0.001	0.218	0.744
Asp	66.2^{ab}	65.1^{abc}	60.0^{c}	68.5^{a}	69.8^{a}	61.2^{bc}	1.84	0.001	0.076	0.620
Cys	68.6^{ab}	65.0^{bc}	54.5^{d}	71.5^{a}	70.8^{ab}	59.8^{cd}	2.06	0.001	0.010	0.760
Glu	78.8	76.7	78.2	81.1	80.6	79.9	1.48	0.671	0.038	0.736
Gly	67.6^{a}	64.7^{ab}	51.4^{b}	64.1^{ab}	61.2^{ab}	38.4^{c}	4.48	0.001	0.078	0.477
Pro	52.4	57.0	57.5	64.4	59.7	77.1	7.06	0.387	0.078	0.538
Ser	67.3^{a}	62.0^{bc}	59.6^{c}	69.3^{a}	66.8^{ab}	59.6^{c}	1.71	0.001	0.113	0.385
Tyr	67.9	67.6	67.9	71.9	71.6	68.1	1.54	0.413	0.040	0.384

[1]- Standard error of mean

Table 9. Standardized ileal digestibility (%) of nitrogen and amino acids in black-seeded *B. napus*, yellow-seeded *B. juncea* and yellow-seeded *B.napus* fed to growing pigs

Items	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*	Pooled SEM[1]	P-value		
	+ Enzyme			- Enzyme				Diet	Enzyme	Diet × Enzyme
Nitrogen	76.1^{ab}	76.3^{ab}	67.9^{c}	79.8^{a}	79.2^{a}	73.3^{b}	1.38	0.001	0.001	0.665
Indispensable AA										
Arg	86.3^{b}	88.3^{ab}	88.0^{ab}	90.3^{ab}	92.4^{a}	89.9^{ab}	1.42	0.356	0.008	0.686
His	85.1^{ab}	84.8^{ab}	84.0^{b}	87.1^{ab}	88.7^{a}	85.0^{ab}	1.40	0.267	0.054	0.593
Ile	75.3^{ab}	77.0^{ab}	76.3^{ab}	79.7^{a}	80.2^{a}	73.6^{b}	1.62	0.082	0.224	0.080
Leu	77.6	77.8	76.3	80.3	81.1	76.5	1.53	0.117	0.103	0.573
Lys	76.1^{abc}	73.5^{bcd}	70.5^{d}	78.9^{a}	77.1^{ab}	71.3^{cd}	1.71	0.002	0.090	0.701
Met	81.9^{bc}	81.7^{bc}	75.6^{d}	84.2^{ab}	87.0^{a}	79.7^{cd}	1.42	0.001	0.002	0.565
Phe	66.4^{ab}	62.7^{b}	64.3^{ab}	70.8^{a}	68.4^{ab}	66.1^{ab}	2.21	0.253	0.036	0.680
Thr	73.7^{abc}	71.1^{bc}	68.8^{c}	77.1^{a}	75.2^{ab}	69.8^{c}	1.67	0.003	0.044	0.622
Val	74.3^{bc}	76.1^{abc}	74.3^{bc}	78.5^{ab}	79.8^{a}	72.6^{c}	1.66	0.034	0.135	0.163
Dispensable AA										
Ala	79.6^{a}	78.9^{ab}	71.0^{bc}	78.2^{ab}	78.3^{ab}	66.8^{c}	2.53	0.001	0.315	0.752
Asp	74.7^{ab}	74.2^{ab}	69.0^{b}	77.8^{a}	79.3^{a}	71.0^{b}	1.84	0.001	0.032	0.701
Cys	76.8^{a}	74.8^{ab}	63.2^{c}	79.8^{a}	80.1^{a}	68.9^{bc}	2.06	0.001	0.009	0.780
Glu	85.4	84.3	84.7	88.3	88.7	86.9	1.48	0.788	0.013	0.750
Gly	78.8^{a}	77.4^{a}	67.7^{ab}	76.5^{a}	74.6^{a}	56.1^{b}	4.48	0.002	0.140	0.516
Ser	77.6^{ab}	74.5^{bc}	71.1^{c}	80.7^{a}	79.4^{ab}	72.1^{c}	1.71	0.001	0.044	0.534
Tyr	74.4^{ab}	74.6^{ab}	73.9^{b}	78.7^{ab}	78.9^{a}	74.8^{ab}	1.54	0.231	0.018	0.447

[1]- Standard error of mean

[a, b, c, d] within a row, means without a common superscript differ ($P < 0.05$).

3.5 Discussion

The present study was conducted to test the hypothesis of that BBN, YBJ and YBN have similar SID of AA when fed to growing pigs and that addition of multi-carbohydrase would improve the degradation of NSP, thereby enhancing the digestibility of nutrients in those three CM varieties.

The contents of CP, total dietary fibre and AA in the BBN, YBJ and YBN samples used in the present study were different from the reported values (Trindade Neto et al., 2012). These differences could be due to differences in cultivars and processing condition (Bell et al., 1991). We expected to have similar amounts of AA in those three canola cultivars in our study, as there were not much difference in their CP content. However, this was not the case because YBN had lower content of most of the AA.

Black-seeded *B. napus* and YBJ had similar AID of AA but greater than the YBN; these data are comparable to data published elsewhere (Trindade Neto et al., 2012). Black-seeded *B. napus* and YBJ had similar AID values of CP (64.2 % and 62.2%), which were similar to previously published values for CM (Fan and Sauer, 1995; Woyengo et al., 2010; Trindade Neto et al., 2012). Standardized ileal digestibility values of Lys and Thr in BBN (78.9 and 77.1%, respectively) and YBJ (77.1 and 75.2%) from the current study were higher than those reported for CM (AmiPig, 2000). This might be because the old cultivars of CM contained higher fiber compared to current cultivars and fibre might reduce the digestibility and availability of nutrients. It is also known that dietary fiber not only reduces the digestibility of nutrients, but also increases endogenous protein losses (Noblet and Perez, 1993;

Schulze et al., 1994). In addition, Mosenthin et al. (1994) suggested that soluble fiber increase the endogenous AA losses and thereby reduce the digestibility of ileal AA in growing pigs. Another possible contributing factor could be due to anti-nutritional factor such as glucosinolates. Earlier cultivars (Newkirk et al., 2003; Seneviratne et al., 2010) had higher amount of glucosinolates compared to the cultivars used in our studies. In this regard, Stein (1986) suggested that dietary anti-nutritional factors (i.e., glucosinolates) reduce the SID values by increasing the specific endogenous losses. Furthermore, Stein and Nyachoti (2003) reported that dietary fiber and anti-nutritional factors are the major contributors to the specific endogenous losses.

The average SID values for indispensable AA in BBN (80.8 ± 5.7%) and YBJ (81.1 ± 7.4%) were similar to SID value of 79.0% in solvent extracted canola meal (SECM) but lower than the SID values of 84.9% for EECM (Woyengo et al. 2010). Since our meal was derived from conventional prepress solvent extraction method, SID values were lower compared to EECM reported by Woyengo et al. (2010) which can be due to formation of Maillard reaction products during the desolventization and toasting in SECM, that is not the case in EECM (Newkirk et al., 2003; Woyengo et al., 2010). Although our SID values for BBN, YBJ and YBN were in agreement with the values reported by Trindade Neto et al. (2012), current values were slightly lower (80.8, 81.1 and 76.1% vs. 82.8, 86.7 and 79.4%). This response could be due to the fact that the pigs used in the current study were lighter than the pigs used in the study by Trindade Neto et al. (2012) (20.9 vs. 30.9 kg, respectively). It has been reported that amino acid digestibility increase with the age of the animals as young pigs have lower enzyme activity to digest protein (Wilson and Leibholz, 1981; Moughan, 1993; Caine et al., 1997).

In an *in-vitro* study, Meng et al. (2005) demonstrated that multi-carbohydrases were effective in degradation of cell wall polysaccharide of CM. However, an earlier study demonstrated that multi-carbohydrase (i.e., Superzyme OM) did not improved NSP digestibility of CM in layers (Jia et al., 2008). The use of multi-carbohydrase (i.e., Superzyme OM) to improve the nutritive value of CM was less effective for pigs in our study at the level used. However, it is not clear from the current study, why the multi-carbohydrase enzyme reduced the AID of DM, energy, N and some of the AA compared with the control diet. It might be because the enzyme hydrolyzes the NSP in CM and produce high amount of soluble NSP, which might reduce the digestion and absorption of most nutrients by increasing the viscosity in the intestine. This notion could be supported by a study done by Bedford and Classen (1992) who suggested that hydrolysis of insoluble NSP is limited by the dose of enzyme. Zijlstra et al. (2004) explained that optimum inclusion level of enzyme is important in order to get better response. For instance they suggested that at higher dose, the enzyme might release excessive amount of nutrients thus cause nutrient imbalance, thereby it might indirectly inhibit digestibility. In addition, it was suggested that low level of enzyme in the diet might lead to increased amount of higher molecular weight soluble NSP which could increase the viscosity of the digesta (Pettersson and Aman, 1989; Bedford et al., 1991). When the viscosity increases in the intestine, it might reduce the rates of diffusion of solutes (Bedford, 1996) and compromise the ability of the gut to physically mix the contents (Edwards et al., 1988). This may explain the results of current study showing lower AID of some of the nutrients in enzyme supplemented diets.

It is documented that there is negative correlation between protein digestibility and total dietary fiber content of CM (Newkirk et al., 1997). In the present study, total dietary fiber content of BBN and YBJ were comparable (30.2 and 24.0%, respectively), this could explain similar SID of CP and AA in those two varieties.

In the present study, YBN had lower DM, N, energy and some of the AA (Lys, Met, Thr, Val, Ala, Asp, Cys, Gly and Ser) digestibility values compared to BBN and YBJ. This is ascribable to over toasting of the meal during processing. The optimal temperature for canola processing is around 107 - 108°C. However, an excessive heat during processing might lead to formation of Maillard reaction products and also negatively would affect the availability of AA, especially Lys (Clandinin et al., 1959). High glycoprotein content of the meal derived from YBN was the evidence of over toasting of the meal and formation of Millard reaction products. It had been also noticed that pigs fed diet containing YBN had lower digestibility of Lys which reflects the fact of high processing temperature. Lower digestibility values in YBN were also ascribable to lower fat content (2.02%) compared to that of BBN (2.60%) and YBJ (2.56%). Stein (2003) documented that diets containing high fat might have increased retention time in the small intestine, thus give more time for digestion. It is also interesting to note that YBN had higher dietary fibre (34.0%) compared to BBN (30.29%) and YBJ (24.97%), which could be another possible reason for the lower digestibility in YBN.

In conclusion, yellow-seeded *B. juncea* had comparable AID values of DM and energy and AID and SID of nitrogen and AA compared to BBN**.** Yellow-seeded *B. juncea* and BBN had higher AID and SID values of AA compared to YBN. Multi-carbohydrase enzyme did not affect the AID and SID of most of the AA.

4.0 Manuscript 2

Effect of canola meal inclusion level in a wheat-based diet on growth performance of weaned pigs

4.1 Abstract

A study was conducted to determine the effect of high dietary inclusion of canola meals (CM) from black-seeded conventional *Brassica napus* (BBN) and yellow-seeded *Brassica juncea* (YBJ) on weaned pig performance. A total of 168 weaned pigs with an initial body weight (BW) of 7.61 ± 0.76 kg (mean ± SEM) were randomly assigned to 42 pens and fed one of seven experimental diets in a completely randomized block design, with 6 replicates pens (n=6) per treatment. Experimental diets consisted of a wheat-soybean meal-based control diet without any CM and six other diets containing 50, 100 and 150 g/kg of CM derived from either BBN or YBJ. Pigs were fed phase I weaner diet (5-10 kg of BW) for the first 14 days followed by phase II weaner diet (10-20 kg of BW) for another 14 days. The experimental diets were formulated to meet nutrient requirement and were formulated to contain 2.4 and 2.3 Mcal/kg NE and similar standardized ileal digestible lysine (5.1 and 5.0 g/Mcal NE) contents in phase 1 and 2, respectively. Pigs were offered the experimental diets on an *ad libitum* basis and had free access to drinking water at all times. Body weight and feed intake were monitored weekly for 4 weeks after weaning, which allowed calculation of ADG, ADFI and gain to feed (G:F) ratio. No differences ($P > 0.05$) were found in ADG, ADFI and G:F for the entire experimental period. Increasing inclusion levels of either BBN or YBJ up to 150 g/kg did not hinder the ADG, ADFI and G:F of weaned pigs throughout the study compared with those fed the wheat-

soybean meal- based control diet. Thus, the results indicate that weaned pig diets can be formulated to contain up to 150 g/kg of either BBN or YBJ in place of soybean meal, without compromising growth performance for 28 days after weaning if the diet is formulated based on NE and SID of AA.

4.2 Introduction

There is an increasing interest in the use of CM in swine diets as an alternative and less expensive source of high quality protein and amino acids (AA) in substitution of expensive protein feedstuffs (e.g., soybean meal). Canola meal contains high amount of protein (i.e., around 36-41%) and balanced AA profile (Bell, 1993).

In the past decades, the nursery pig diet with added CM up to 50 g/kg was considered as safe because feeding nursery pig diets containing more than 50 g CM /kg caused detrimental effects on growth performance (Baidoo et al., 1987; Thacker 1990). Furthermore, McIntoshi et al. (1986) demonstrated that feeding each percent increase of CM in diets to pigs reduced ADG and ADFI by 2 g and 4 g, respectively. In this regard, canola breeders have developed new varieties of canola meal (i.e, yellow *Brassica juncea* and yellow *Brassica napus)* to overcome those anti- nutritional factors (Simbaya et al., 1995; Slominski, 1997; Bell et al., 1998). As a consequence, recent studies have demonstrated that feeding solvent-extracted and expeller-pressed-canola meal to the upper limit of 200 g/kg by partially removing soybean meal as a source of energy, protein and AA on growth performance in weaned pigs had no negative effects on growth performance when the diets were formulated to contain similar level of the net energy (NE) and SID AA content (Landero et al., 2011, 2012). Based on a report by Newkirk et al. (1997), YBJ contained greater CP, but lower

ADF and NDF contents compared to BBN. Bell et al. (1998) reported that YBJ and BBN had similar apparent total tract digestibility of energy and CP.

However, the upper inclusion limit without any detrimental effects on growth performance of current major varieties of CM (i.e., BBN and YBJ) in weaned pig diets remains to be established. The purpose of this experiment, therefore, was to evaluate the upper inclusion limit of the current major varieties of CM for weaned pigs in terms of growth performance immediately after weaning. The hypothesis tested in the study was that feeding a diet with higher inclusion level of the CM will not adversely affect growth performance of pigs when diets were formulated to contain equal contents of NE and SID AA compared with pigs fed a wheat-soybean meal based control diet without any CM.

4.3 Materials and methods

The experimental protocol used in the present study was reviewed and approved by the Animal Care Committee of the University of Manitoba. Animals were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

4.3.1 Animals and housing

A total of 168 pigs (Yorkshire-Landrace × Duroc) with an average initial BW of 7.62 ± 0.76 kg (mean ± SEM) and weaned at 21 ± 1 days were used. Pigs were acquired from the Glenlea Swine Research Unit, University of Manitoba. Based on their initial BW and sex, they were randomly blocked and allocated to one of 7 experimental treatments. Four pigs housed in a pen, each with plastic covered expanded metal floor (space allowance of 0.45 m^2 per pig). Each pen was equipped with a nipple bowl drinker and a metal feed trough. The ambient temperature was maintained at 29 ± 1ºC for the initial week and then gradually decreased by 1ºC every week. Pigs were offered the experimental diets on an *ad libitum* basis for 4 weeks, and had free access to drinking water at all times. Body weight and feed intake were monitored weekly for 4 weeks after weaning, which allowed calculation of ADG, ADFI and gain to feed (G:F) ratio.

4.3.2 Diets

Canola meal derived from BBN and YBJ were obtained from a local crushing plant at Bunge Canada (Altona, Manitoba, Canada) using conventional solvent extraction process. . Experimental diets consisted of a wheat-soybean meal-based control diet without any CM and six other diets containing 50, 100 and 150 g/kg of CM derived from either BBN or YBJ. All diet were formulated to contain 2.4 and 2.3 Mcal/kg NE and similar amounts of standardised ileal digestible Lys (5.1 and 5.0 g/Mcal NE) in phase 1 and 2, respectively and were formulated to meet NRC (1998) requirement for weaned pigs. Canola meals from BBN and YBJ were used in substitution of soybean meal. All diets were fed in mash form. Composition and nutrient contents of the experimental diets are presented in Table 10 and 11. Pigs were fed with a first phase weaner diet (5-10 kg of BW) for the first 14 days followed by the second phase weaner diet (10-20 kg of BW) for another 14 days.

Table 10. Composition and analyzed nutrient contents of phase I[1] diets (%, as-fed basis)

Items	Control	Yellow *B. juncea*			Black *B. napus*		
		50	100	150	50	100	150
Ingredients, %							
Barley	-	10.30	5.75	-	-	-	-
Corn	19.70	5.00	-		10.25	1.99	5.00
Wheat	33.00	27.02	35.65	39.05	33.10	38.10	36.30
Millrun	5.00	5.00	5.00	7.57	5.00	7.00	5.00
Canola meal	-	5.00	10.00	15.00	5.00	10.00	15.00
SBM, 44 % CP	20.00	15.00	10.00	5.00	15.00	10.00	5.00
Menhaden fish meal	8.00	8.00	8.00	8.00	8.00	8.00	9.00
Dried whey	11.00	20.00	20.00	20.00	20.00	20.00	20.00
Vegetable oil	1.20	2.51	3.36	3.59	1.51	2.88	3.03
Limestone	0.40	0.60	0.60	0.44	0.60	0.47	0.30
Monocalcium phosphate	0.40	0.40	0.40	0.10	0.40	0.40	0.20
Vitamin-Mineral premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine-HCl	0.11	1.00	0.16	0.20	0.10	0.14	0.15
DL-Methionine	0.03	0.05	0.05	-	0.02	-	-
Threonine	0.03	0.02	0.03	0.04	0.02	0.02	0.01
Tryptophan	-	-	-	0.01	-	-	0.01
Calculated nutrient composition							
Crude protein, %	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Net energy, kcal/kg	2,390	2,390	2,390	2,390	2,390	2,390	2,390
Calcium, %	0.83	0.94	0.93	0.80	0.94	0.88	0.81
Available Phosphorus, %	0.52	0.58	0.60	0.56	0.58	0.61	0.59
SID Lys, g/Mcal NE	5.1	5.1	5.1	5.1	5.1	5.1	5.1
Analysed nutrient composition							
Crude protein, %	23.3	22.8	23.2	23.8	22.9	22.4	23.0
NDF, %	9.33	9.72	9.73	10.52	9.45	10.37	11.97
ADF, %	2.90	3.27	3.33	3.84	3.42	4.17	4.96
Gross energy, kcal/kg	4,110	4,112	4,135	4,182	4,115	4,120	4,159

[1]Phase I, days 1-14

[2]Supplied per kg of diet- vitamin A: 8250 IU; vitamin D3:825 IU; vitamin E: 40 IU; vitamin K: 4 mg; niacin: 22.5 mg; vitamin B12: 0.25 mg; choline chloride: 500 mg; biotin: 0.20 mg; folic acid:2 mg; Cu: 25 mg as CuO; I: 0.4 mg as $Ca(IO_3)_2$; Fe 100 mg as $FeSO_4 .H_2O$; Mn: 50 mg as MnO; Se: 0.3 mg as Na_2SeO_3; Zn: 150 mg as Zno.

Table 11. Composition and analyzed nutrient contents of phase II [1]diets (%, as-fed basis)

Items	Control	Yellow *B. juncea*			Black *B. napus*		
		50	100	150	50	100	150
Ingredients, %							
Barley	14.00	13.00	13.00	7.22	13.00	11.20	7.70
Corn	10.30	10.00	9.20	10.10	9.00	7.00	5.50
Wheat	37.00	37.40	37.30	41.00	38.60	41.40	45.26
Millrun	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Canola meal	-	5.00	10.00	15.00	5.00	10.00	15.00
SBM, 44 % CP	20.00	15.00	10.00	5.00	15.00	10.00	5.00
Menhaden fish meal	3.50	4.00	4.50	5.00	4.00	4.50	5.00
Vegetable oil	2.15	2.72	3.37	3.88	2.67	3.33	4.00
Limestone	0.90	0.90	0.90	0.90	0.80	0.90	0.86
Monocalcium phosphate	0.71	0.53	0.30	0.47	0.55	0.31	0.30
Vitamin-Mineral premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine-HCl	0.27	0.30	0.33	0.36	0.29	0.31	0.34
DL-Methionine	0.10	0.05	0.03	0.02	0.04		
Threonine	0.07	0.10	0.07	0.05	0.05	0.05	0.04
Calculated nutrient composition							
Crude protein, %	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Net energy, kcal/kg	2,300	2,300	2,300	2,300	2,300	2,300	2,300
Calcium, %	0.76	0.74	0.71	0.75	0.70	0.71	0.70
Available Phosphorus, %	0.42	0.40	0.38	0.44	0.41	0.38	0.40
SID Lys, g/Mcal NE	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Analysed nutrient composition							
Crude protein, %	21.8	21.1	21.6	20.7	22.0	21.6	21.2
NDF, %	13.48	12.82	12.34	13.65	12.42	14.11	15.31
ADF, %	4.37	4.63	4.09	4.48	4.60	5.70	6.30
Gross energy, kcal/kg	4,159	4,182	4,254	4,278	4,158	4,230	4,302

[1]phase II, days15-28

[2]Supplied per kg of diet- vitamin A: 8250 IU; vitamin D3:825 IU; vitamin E: 40 IU; vitamin K: 4 mg; niacin: 22.5 mg; vitamin B12: 0.25 mg; choline chloride: 500 mg; biotin: 0.20 mg; folic acid:2 mg; Cu: 25 mg as CuO; I: 0.4 mg as Ca $(IO_3)_2$; Fe 100 mg as $FeSO_4 .H_2O$; Mn: 50 mg as MnO; Se: 0.3 mg as Na_2SeO_3; Zn: 150 mg as Zno.

4.3.3 Experimental design

The experiment was conducted as a completely randomised block design with 42 pens in two rooms to give 6 replicates per treatment. Room was considered as a blocking factor.

4.3.5 Sample preparation and chemical analyses

Sample preparation and chemical analysis for CM and diet samples were similar to those described in manuscript I.

4.3.4 Statistical analysis

Data were analysed as a repeated measure using MIXED procedure of SAS (SAS software release 9.1, SAS Inst., Inc., Cary, NC). The pen was considered as the experimental unit for all measures, and the model included block as a random factor, and treatments and gender as sources of variation. Since no gender effect was detected ($P>0.10$), data were pooled and analysed for treatments effects. Initial BW was used as a covariate to analyse the growth performance data. Orthogonal polynomial contrasts were used to test for linear or quadratic effects of CM inclusion. Statistical significance was accepted at $P<0.05$.

4.4 Results

Nutrient composition of canola cultivars are presented in Table 12. The samples of BBN and YBJ contained 37.2 and 37.7% CP and 30.3 and 25.0% total dietary fibre as fed basis.

All pigs remained healthy and no signs of disease were observed during the study. All measured growth performance indices (i.e., ADF, ADFI and G:F) are presented in Table 13. Neither increasing dietary inclusion nor varieties of CM affected (P>0.10) ADG, ADFI and feed efficiency compared with pigs fed control diet without any CM for 4 weeks immediately after weaning. However, increasing inclusion of BBN tended to linearly increase (P=0.055) ADG and quadratically (P=0.094) ADFI compared with pigs fed control diet from days 22 to 28.

Table 12. Analysed chemical composition of black- seeded *B. napus* and yellow-seeded *B. juncea* (%, as fed- basis)

Item	Black *B. napus*	Yellow *B. juncea*
Dry matter	89.7	90.3
Crude protein	37.2	37.7
Fat	2.6	2.6
Total dietary fibre	30.3	24.9
Total NSP[1]	18.4	18.9
Sucrose	5.0	6.2
Glucosinolate (µmol/g)	8.5	15.1
Indispensable AA		
Arg	2.13	2.36
His	0.97	0.96
Ile	1.23	1.20
Leu	2.64	2.64
Lys	2.03	1.92
Met	0.76	0.72
Phe	1.44	1.40
Thr	1.64	1.63
Val	1.63	1.52
Dispensable AA		
Ala	1.74	1.73
Asp	2.75	2.94
Cys	0.86	0.73
Glu	6.93	6.65
Gly	1.86	1.85
Pro	2.44	2.22
Ser	1.67	1.69
Tyr	0.97	0.95

[1]Non-starch polysaccharide

Table 13. Growth performance of weaned pigs fed diets with increasing inclusion levels of black- seeded *B. napus* and yellow-seeded *B. juncea* in place of soybean meal[1]

Item	Control	Yellow *B. juncea*			Black *B. napus*			SEM	P-value	Control vs *B. juncea*		Control vs *B. napus*	
		50	100	150	50	100	150			Lin	Quad	Lin	Quad
ADG (g/day)													
Day 0-7	284	329	325	324	328	288	312	21.8	0.597	0.273	0.331	0.273	0.331
Day 8-14	469	421	445	417	467	459	411	27.7	0.581	0.285	0.715	0.143	0.409
Day 15-21	504	493	517	491	493	510	516	31.3	0.991	0.908	0.822	0.686	0.753
Day 22-28	556	541	563	589	602	615	619	32.2	0.508	0.511	0.619	0.055	0.358
Day 0-28	452	440	444	454	472	468	453	16.5	0.794	0.896	0.491	0.979	0.288
ADFI (g/day)													
Day 0-7	382	405	370	391	449	398	424	28.2	0.505	0.818	0.952	0.223	0.563
Day 8-14	711	692	649	668	681	689	631	34.6	0.707	0.264	0.574	0.113	0.667
Day 15-21	870	874	913	872	844	926	891	26.8	0.386	0.688	0.388	0.264	0.863
Day 22-28	1001	1015	1026	1069	1135	1093	1070	45.8	0.390	0.376	0.795	0.421	0.094
Day 0-28	741	746	740	750	778	777	754	21.7	0.777	0.856	0.915	0.686	0.165
G:F (g/g)													
Day 0-7	0.75	0.83	0.88	0.84	0.74	0.73	0.77	0.064	0.507	0.268	0.305	0.820	0.706
Day 8-14	0.66	0.62	0.69	0.62	0.69	0.67	0.67	0.046	0.876	0.813	0.796	0.967	0.710
Day 15-21	0.58	0.56	0.57	0.56	0.59	0.55	0.58	0.029	0.975	0.657	0.833	0.765	0.734
Day 22-28	0.56	0.54	0.54	0.55	0.53	0.56	0.58	0.025	0.819	0.881	0.579	0.310	0.304
Day 0-28	0.60	0.59	0.59	0.60	0.60	0.59	0.60	0.014	0.954	0.910	0.194	0.714	0.941

[1] Least square means based on 6 pens of 4 pigs per diet

Abbreviations are; Lin = Linear response, Quad = quadratic response ADG = average daily gain, ADFI = average daily feed intake, G:F = gain to feed ratio SEM = pooled standard error of mean

4.5 Discussion

Data from the current study supported our hypothesis that pigs fed diets with higher inclusion levels of CM (up to 150 canola g/kg) from BBN and YBJ would not have any signs of loss in production in the immediate post-weaning period compared with those fed a control diet without any CM. This is in agreement with recent findings, indicating that solvent-extracted canola meal and expeller-pressed canola meal could be included up to 200 g/kg in substitution of soymeal meal in weaner pig diets formulated to equal NE and SID AA without hindering growth performance (Seneviratne et al., 2011; Landero et al., 2011, 2012). Zijlstra and Payne (2007) suggested that formulating diets with by-products as alternative feedstuffs would minimise the risk associated with reductions in growth performance indices if the NE and SID AA systems were used. In contrast, diets containing more than 5.5% of CM resulted in a significant reduction in ADG in growing pigs from 20 to 60 kg (Baidoo and Aherne, 1987) when the diets were formulated based on DE system and total AA contents. Bell et al. (1988) suggested that CM-based diets formulated on the basis of total amino acids had poor performance in pigs as the diet were deficient in digestible amino acid content. However, Seneviratne et al. (2010) reported that feeding diets containing expeller pressed canola meal (150-225 g/kg) had reduced ADFI and ADG in growing-finishing pigs (20 to 100 kg), although diets were formulated based on NE and SID AA. It can be explained by the fact that the CM they used had high amount of glucosinolate (23.2 μmol/g) compared to current varieties. It is known that energy loss from urine and combustible gases are not taken into account when diets were formulated based on a DE system. Consequently, the contribution of protein and fibre to available energy to animals can be overestimated by the DE system (Noblet et al., 1994).

Glucosinolates are the other considerable factors which limit the utilization of CM in pig diets. Feeding diets containing high glucosinolates contents to pigs may adversely affect feed intake due to poor palatability (Bell, 1993). In the past, rapeseed had higher glucosinolates contents which ranged from 110 to 150 μmol/g (Bell, 1993). Thacker and Kirkwood (1990) reported that glucosinolate breakdown products inhibit synthesis of thyroid hormone, which plays an important role in body metabolism, thereby glucosinolates reduce the utilization of nutrients that resulted in poor growth performance. Mullan et al. (2000) reported that inclusion of 150 g/kg of CM (glucosinolate content above 10 μmol/g) compromised feed intake in growing pigs. However, diets containing up to 250 g/kg of low glucosinolate canola meal (< 5μmol/g) did not adversely affect growth performance in weaned pigs (King et al., 2001). Glucosinloates contents of BBN and YBJ in the current study were 8.49 and 15.12 μmol/g, respectively. To best of our knowledge, there is currently no data available on the maximum tolerance glucosinolates contents in weaner pig diets. However, it has been suggested that the maximum tolerance for glucosinolates contents were between 2.0 to 2.5 μmol/g in growing pig diets (Bell, 1993; Schone et al., 1997; Roth-Maier et al., 2004).

Although canola meal contains 3 times the amount of fibre in soybean meal (Bell, 1993), we could not find any difference in growth performance in piglets among treatments. This might be explained by the similar NDF content of soybean based control (13.4%) diet and diets containing 150 g/kg of both BBN (15.3%) and YBJ

(13.7%). The results of the present study were similar to those of recent studies by Landero et al. (2011, 2012) showing that inclusion of 0, 50, 100, 150 and 200 g/kg of solvent-extracted canola meal and expeller-extracted canola meal in replacement of soybean meal in weaned pig diets had no significant differences in growth performance. Pigs in the present study had excellent performance. The ADG for day 0-28 was 453 g/kg for diets containing YBJ and BBN at the 150g/kg inclusion level, whereas ADG was 509 g/kg in the study by Landreo et al. (2011) at 150g/kg inclusion of solvent extracted canola meal. It could be due to the fact that the pigs used in the current study were lighter than the pigs used the study by Landreo et al. (2011) (7.6 vs 8.1 kg, respectively).

In conclusion, BBN and YBJ can be included to serve as an alternative protein and energy source up to 150g/kg in weaned pig diets without compromising growth performance if the diet is formulated based on NE and SID AA.

5.0 Manuscript 3

Effect of high canola meal inclusion level and enzyme supplementation on nutrient utilization and growth performance of weaned pigs

5.1 Abstract

The study was conducted to determine the effects of high dietary inclusion level of canola meals (CM) [i.e., black-seeded *Brassica napus* (BBN) and yellow-seeded *Brassica juncea* (YBJ)] on growth performance and apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP) and gross energy (GE) without or with added multi-carbohydrase. A total of 162 weaned pigs with an initial BW of 7.26 ± 0.70 kg were randomly assigned to the 54 pens with 3 pigs per pen based on the BW and gender and fed one of nine experimental diets in a completely randomized block design, with 6 replicates per treatment. A wheat-soybean meal-based control diet and eight diets containing 200 and 250 g/kg of either BBN or YBJ without or with added multi-carbohydrase were formulated to contain comparable net energy (i.e., 2322 to 2398 and 2218 to 2299 kcal/kg) and standardized ileal digestible lysine (i.e., 5.2 to 5.3 and 4.5 to 4.6 g/Mcal NE) contents in phase 1 and 2, respectively. Titanium dioxide (3 g/kg) was included in the diets as an indigestible maker. Pigs were offered the experimental diets on an *ad libitum* basis for 4 weeks and had free access to drinking water at all times. Average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were monitored weekly for 4 weeks after weaning. On days 22 and 23, fresh fecal samples were collected to measure the ATTD of DM, CP and GE. Feeding the diets containing the higher level of BBN and YBJ (i.e., 250 g/kg) did not negatively affect (P>0.05) ADG, ADFI and G:F compared with pigs fed the control diet. The multi-carbohydrase had no effect (P>0.05) on growth performance but

improved ($P<0.05$) the ATTD of DM, CP and GE compared to pigs fed without multi-carbohydrase supplementation regardless of variety of CM. The diets containing 250 g/kg of CM had lower ($P<0.05$) ATTD of nutrients (i.e., CP, energy and DM) regardless of multi-carbohydrase supplementation or variety of CM. An interaction effect was observed ($P<0.05$) between CM type and inclusion level on ATTD of DM in which increasing inclusion level of both BBN and YBJ decreased ATTD of DM. In conclusion, weaned pigs can be fed diets containing BBN or YBN up to 250 g/kg with no detrimental effects on growth performance when diets are formulated based on similar net energy and standardized ileal digestibility (SID) of amino acid (AA). Furthermore, adding a multi-carbohydrase to mixed grain-based diets with high levels of BBN and YBN improved CP, DM and GE digestibility but had no effect on growth performance.

5.2 Introduction

Despite CM being a cost effective substitution for other plant protein ingredients such as soybean meal, the presence of anti-nutritional factors such as glucosinolates and a relatively high level of fibre have restricted high inclusion levels in diets of weaner pigs (Bell, 1993). One of the anti-nutritional factors in CM is non-starch polysaccharides (NSP) which occur in relatively high levels. Canola meal contains on average 2.5% α-galacto-oligosaccharides and 17.4 to 18.0% NSP of which 1.5% is soluble (Bell, 1993; Meng and Slominski, 2005). Cellulose and arabinose are the major NSP components in CM (Slominski and Campbell, 1990). Greater NSP content in a pig diet is known to reduce nutrient digestibility, increase endogenous protein loss, and hence reduce pig performance. In this regard, canola breeders have developed new varieties of CM to overcome those anti-nutritional factors (Simbaya et al., 1995; Slominski, 1997; Bell et al. 1998). As a consequence, CM has recently been reconsidered as a possible cost effective protein substitute for soybean meal in pig diets. Landero et al. (2011) found that increasing inclusion of canola meal up to 20% in weaned pig diets did not negatively affect the growth performance but found liner reductions in total tract digestibility of crude protein and energy.

Furthermore, there is a growing interest in the use of feed enzymes in pig diet in order to minimize the effect of anti-nutritional factors present in the feed ingredients. Thus, exogenous enzymes to degrade NSP might improve the utilization and digestion of nutrients.

Therefore, the hypotheses tested in the present study were that i) feeding CM (i.e., BBN and YBJ) up to 250 g/kg diet would not have negative effects on growth performance and nutrient digestibility and ii) multi-carbohydrase would improve digestibility of CP, energy and DM in those CM varieties, and subsequently aid to improve the growth performance. The objectives of the study were to i) evaluate the maximum level of current major varieties of CM in terms of total tract digestibility and growth performance, ii) examine the effect of enzyme supplementation on the total tract digestibility of CP, energy and DM and growth performance and iii) investigate any interactions between different types of CM and enzyme supplementation.

5.3 Materials and methods

The experimental protocol used in the present study was reviewed and approved by the Animal Care Committee of the University of Manitoba. Animals were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

5.3.1 Animals

A total of 162 pigs (Yorkshire-Landrace × Duroc) with an average initial BW of 7.26 ± 0.70 kg (mean ± SEM) and weaned at 21 ± 1 days were used. Pigs were acquired from the Glenlea Swine Research Unit, University of Manitoba. They were weighed and randomly assigned to the 54 pens with 3 pigs per pen based on the BW and gender. Each pen was equipped with a nipple drinker and a metal feed trough. The ambient temperature was maintained at 29 ± 1ºC for the initial week and then gradually decreased by 1ºC every week. Pigs were offered the experimental diets on an *ad libitum* basis for 4 weeks, and had free access to drinking water at all times. Body weight and feed intake were monitored weekly for 4 weeks after weaning, which allowed calculation of ADG, ADFI and gain to feed (G:F) ratio.

Freshly voided feces were collected from pen floor from 0800-1600 h by grab sampling on days 22 and 23 of the experiment. Samples were pooled by pen and frozen at - 20°C until required for further analyses.

5.3.2 Diets

Canola meal derived from BBN and YBJ were obtained from a local crushing plant at Bunge Canada (Altona, Manitoba, Canada) using conventional solvent extraction process. Experimental diets included wheat-soybean meal based control diet and eight diets containing two types of CM (i.e., BBN and YBJ) at 200 or 250 g/kg inclusion level and without or with multi-carbohydrase supplementation. The enzyme preparation supplied 1,400 U of pectinase, 1,600 U of cellulase, 1,600 U of xylanase, 1,200 U of glucanase, 300 U of mannanase, 40 U of galactanase, 1,000 U of invertase, 2,000 U of protease and 10,000 U of amylase per kilogram of diet. All diet contained similar amounts of standardised ileal digestible Lys (i.e., 5.2 to 5.3 and 4.5 to 4.6 g/Mcal NE) and comparable net energy (i.e., 2322 to 2398 and 2218 to 2299 kcal/kg) in phase 1 and 2, respectively. Diets were formulated to meet NRC (1998) requirement for weaned pigs. Canola meals from BBN and YBJ were used in place of soybean meal and all diets were fed in mash form. Composition and nutrient contents of phase I and II diets are presented in Tables 14 and 15. Pigs were fed with a first phase weaner diet (5-10 kg of BW) for the first 14 days followed by the second phase weaner diet (10-20 kg of BW) for another 14 days.

Table 14. Composition and analyzed nutrient contents of phase I diets (%, as-fed basis)

Items	Control	Yellow *B. juncea*		Black *B. napus*	
		20%	25%	20%	25%
Ingredient, %					
Corn	12.72	27.01	25.13	25.02	24.79
Wheat	41.57	19.85	19.44	21.40	19.03
Canola meal	-	20.0	25.00	20.00	25.00
Soybean meal, 44% CP	21.00	8.00	5.00	8.45	5.80
Dried whey	15.00	15.0	15.00	15.00	15.00
Vegetable oil	0.95	2.04	2.89	2.01	2.80
Limestone	0.59	0.46	0.48	0.44	0.46
Mono calcium phosphate	0.10	0.11	0.16	0.10	0.12
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00	1.00
Lysine-HCl	0.15	0.22	0.25	0.18	0.19
DL-Methionine	0.03	-	-	-	-
Threonine	0.03	0.02	0.02	0.01	0.01
Tryptophan	-	0.02	0.03	0.02	0.02
Isoleucine	-	0.04	0.06	0.02	0.04
Valine	-	0.03	0.04	-	-
Calculated nutrient composition					
Crude protein, %	22.00	22.00	22.00	22.00	22.00
Net energy, kcal/kg	2,397	2,337	2,325	2,333	2,322
Calcium, %	0.81	0.80	0.80	0.80	0.80
Available Phosphorus, %	0.45	0.45	0.45	0.45	0.45
SID Lys, g/ Mcal NE	5.22	5.26	5.25	5.28	5.26
Analysed nutrient composition					
Crude protein, %	23.9	23.7	23.4	23.4	23.6
NDF, %	8.67	10.70	11.45	11.56	13.30
ADF, %	2.65	4.24	4.78	6.20	7.00
Gross energy, kcal/kg	4,008	4,088	4,143	4,099	4,166

[1]Supplied per kg of diet- vitamin A: 8250 IU; vitamin D3:825 IU; vitamin E: 40 IU; vitamin K: 4 mg; niacin: 22.5 mg; vitamin B12: 0.25 mg; choline chloride: 500 mg; biotin: 0.20 mg; folic acid:2 mg; Cu: 25 mg as CuO; I: 0.4 mg as $Ca(IO_3)_2$; Fe 100 mg as $FeSO_4 .H_2O$; Mn: 50 mg as MnO; Se: 0.3 mg as Na_2SeO_3; Zn: 150 mg as Zno.

Table 15. Composition and analyzed nutrient contents of phase II diets (%, as-fed basis)

Items	Control	Yellow *B. juncea*		Black *B. napus*	
		20%	25%	20%	25%
Ingredient, %					
Corn	20.03	31.50	30.62	30.07	30.16
Wheat	48.75	32.00	30.26	32.36	30.05
Canola meal	-	20.00	25.00	20.00	25.00
Soybean meal, 44% CP	26.73	11.80	8.02	12.71	9.12
Vegetable oil	0.95	1.20	2.62	1.50	2.35
Limestone	0.95	0.90	0.86	0.88	0.85
Mono calcium phosphate	1.03	0.82	0.78	0.84	0.79
Vitamin-mineral premix[1]	1.00	1.00	1.00	1.00	1.00
Lysine-HCl	0.18	0.27	0.30	0.22	0.24
DL-Methionine	0.04	0.01	-	-	-
Threonine	0.04	0.04	0.04	0.02	0.02
Tryptophan	-	0.02	0.03	0.02	0.03
Isoleucine	-	0.10	0.11	0.08	0.09
valine	-	0.04	0.06	-	-
Titanium dioxide	0.30	0.30	0.30	0.30	0.30
Calculated nutrient composition					
Crude protein, %	20.00	20.00	20.00	20.00	20.00
Net energy, kcal/kg	2,299	2,218	2,230	2,223	2,220
Calcium, %	0.713	0.719	0.713	0.719	0.715
Available Phosphorus, %	0.338	0.321	0.322	0.323	0.321
SID Lys, g/ Mcal NE	4.48	4.58	4.57	4.56	4.58
Analysed nutrient composition					
Crude protein, %	21.6	20.6	21.1	20.3	20.7
NDF, %	9.26	10.98	12.33	12.43	14.13
ADF, %	3.55	4.43	5.44	6.60	7.22
Gross energy, kcal/kg	3,901	3,951	4,073	4,000	4,047

[1]Supplied per kg of diet- vitamin A: 8250 IU; vitamin D3:825 IU; vitamin E: 40 IU; vitamin K: 4 mg; niacin: 22.5 mg; vitamin B12: 0.25 mg; choline chloride: 500 mg; biotin: 0.20 mg; folic acid:2 mg; Cu: 25 mg as CuO; I: 0.4 mg as $Ca(IO_3)_2$; Fe 100 mg as $FeSO_4 .H_2O$; Mn: 50 mg as MnO; Se: 0.3 mg as Na_2SeO_3; Zn: 150 mg as Zno.

5.3.3 Experimental design

The experiment was conducted as a completely randomized block design with 54 pens in two rooms to give 6 replicates per treatment. Room was considered as a blocking factor. Nine diets containing 2 types of CM (BBN or YBJ), 2 levels of inclusion (200 or 250g/kg) and 2 levels of enzyme (without or with multi-carbohydrase) and ninth wheat-SBM-based control diet were randomly assigned to 6 replicates.

5.3.4 Sample preparation and chemical analysis

Sample preparation and chemical analysis for CM and diet samples were similar to those described in manuscript I.

Fecal samples were dried in an oven at 60 ºC for three days, pooled and ground in a coffee grinder (Applica Consumer Products Inc., Miami Lakes, FL) and mixed prior to analysis. Fecal samples were analysed for DM, N, TiO_2 and gross energy. Dry matter was determined according to the method of AOAC (1990; method 925.09). Nitrogen content was determined by the combustion (method 990.03; AOAC 1990) using a combustion analyzer (Model CNC-2000; Leco Corporation, St. Joseph, MI, USA). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Concentration of TiO_2 was determined according to Lomer et al. (2000).

5.3.5 Calculations and Statistical analysis

Apparent total tract digestibility (ATTD) was calculated using the following equations.

$$ATTD = 100 - [(Nf/Nd) \times (Tid/Tif)]$$

where

Nf = nutrient concentration in feces (% DM)

Nd = nutrient concentration in diet (% DM)

Tid = TiO_2 concentration in diet (% DM)

Tif = TiO_2 concentration in feces (% DM)

Digestible energy (DE) was calculated using the following equation

$$DE = D_{GE} \times \text{Gross energy} /100$$

Where D_{GE} is total tract digestibility of gross energy (%)

Growth performance and digestibility data were analysed as a repeated measure using MIXED procedure of SAS (SAS software release 9.1, SAS Inst., Inc., Cary, NC). Pen was considered as the experimental unit. Diet was considered as fixed effect and block was considered as a random effect. The eight dietary treatments which contained either BBN or YBJ with and without enzyme treatment, were compared as a 2 × 2 × 2 factorial arrangement and were together compared to control diet, using orthogonal contrasts. Treatment differences were considered as significant at $P < 0.05$.

5.4 Results

Average daily gain, ADFI and G:F are shown in Table 16. Regardless of variety and inclusion level, CM (i.e., BBN and YBJ) did not negatively affect the ADG, ADFI and G:F for 4 weeks after weaning. Multi-carbohydrase supplementation had no effect ($P>0.05$) on ADG, ADFI and feed efficiency.

Apparent total tract digestibility of CP, DM, energy and DE are presented in Table 17. Pigs fed diets containing YBJ had higher ($P<0.05$) ATTD of CP, DM and energy compared to pigs fed diets containing BBN. Apparent total tract digestibility of nutrients of diet containing 200g/kg YBJ were similar ($P>0.05$) to the soybean meal-wheat based control diet. Increasing dietary inclusion of both BBN and YBJ decreased ($P < 0.05$) the ATTD of CP, energy and DM.

Multi-carbohydrase supplementation improved ($P<0.05$) the ATTD of DM, CP and GE compared to pigs fed diets without enzyme supplementation regardless of variety of CM. There was an interaction ($P<0.05$) effect of CM type and inclusion level on ATTD of DM digestibility and DE content.

Table 16. Effect of high canola meal inclusion level and enzyme supplementation on growth performance of weaned pigs[1]

variable	Control	Yellow *B. juncea* 20% -	Yellow *B. juncea* 20% +	Yellow *B. juncea* 25% -	Yellow *B. juncea* 25% +	Black *B. napus* 20% -	Black *B. napus* 20% +	Black *B. napus* 25% -	Black *B. napus* 25% +	SEM	P-value * Con vs. rest	P-value * CM	P-value * Level	P-value * Enz
ADG (g/day)														
Day 0-7	235	212	200	212	218	207	210	189	192	19.9	0.165	0.431	0.732	0.982
Day 8-14	396	408	400	365	421	416	418	406	420	27.4	0.719	0.407	0.709	0.436
Day 15-21	458	455	470	441	497	479	485	461	473	22.0	0.600	0.579	0.794	0.161
Day 22-28	464	459	500	482	508	481	494	495	513	28.8	0.336	0.639	0.445	0.202
Day 0-28	400	386	384	373	407	389	401	388	393	18.9	0.991	0.852	0.683	0.402
ADFI (g/day)														
Day 0-7	312	285	262	283	291	282	279	257	248	20.6	0.086	0.365	0.635	0.642
Day 8-14	604	607	570	553	591	595	609	587	615	34.8	0.730	0.393	0.721	0.666
Day 15-21	767	744	760	744	810	779	770	786	753	33.2	0.944	0.684	0.734	0.611
Day 22-28	788	803	826	855	843	811	847	859	835	47.7	0.354	0.854	0.440	0.872
Day 0-28	617	610	604	606	634	617	626	622	613	21.3	0.968	0.693	0.767	0.720
G:F (g/g)														
Day 0-7	0.75	0.74	0.77	0.75	0.77	0.74	0.75	0.74	0.77	0.06	0.883	0.932	0.853	0.609
Day 8-14	0.66	0.67	0.71	0.66	0.71	0.70	0.68	0.70	0.68	0.03	0.390	0.818	0.865	0.540
Day 15-21	0.60	0.62	0.62	0.59	0.62	0.62	0.63	0.59	0.63	0.04	0.684	0.884	0.697	0.456
Day 22-28	0.60	0.58	0.62	0.57	0.61	0.61	0.61	0.58	0.61	0.04	0.969	0.776	0.759	0.377
Day 0-28	0.63	0.63	0.64	0.62	0.64	0.63	0.65	0.62	0.64	0.02	0.957	0.630	0.893	0.553

[1] Least square means based on 6 pens of 3 pigs each per diet.

Abbreviations are; ADG = average daily gain, ADFI = average daily feed intake, G:F = feed efficiency, SEM = pooled standard error of mean, con= control diet, CM=canola meal, (+ /-)= with or without multi-carbohydrase.

* There were no interaction effects.

Table 17. Effect of high canola meal inclusion level and enzyme supplementation on apparent total tract digestibility (ATTD) of nutrients[1]

Variable	Control	Yellow *B. juncea*				Black *B. napus*				SEM[2]	P-value					
		20%		25%		20%		25%			Con vs. rest	CM[3]	Level	Enz	Diet × Level	Diet Level × Enz
		-	+	-	+	-	+	-	+							
ATTD																
CP	82.4[a]	81.6[a]	81.0[ab]	76.6[cd]	79.3[bc]	77.6[cd]	78.7[bc]	73.8[e]	75.7[de]	0.98	0.001	0.001	0.001	0.004	0.210	0.210
Energy	81.5[a]	81.6[ab]	80.6[ab]	77.5[cd]	78.9[bc]	77.5[cd]	78.0[cd]	72.9[e]	76.1[d]	0.84	0.001	0.001	0.001	0.001	0.060	0.089
DM	81.8[a]	81.0[ab]	79.9[ab]	76.1[de]	78.1[c]	76.6[cde]	77.7[cd]	71.4[f]	75.2[e]	0.69	0.001	0.001	0.001	0.001	0.042	0.177
DE, MJ/kg	14.9[a]	14.5[bc]	14.9[a]	14.7[ab]	14.8[ab]	14.4[bc]	14.5[bc]	13.7[d]	14.3[c]	0.16	0.001	0.001	0.001	0.001	0.004	0.007

[1]Least square means based on 6 pens of 3 pigs per pen.

[a, b, c, d, e] within a row, means without a common superscript differ ($P < 0.05$).

[2]SEM = pooled standard error of mean.

[3]CM = canola meal.

5.5 Discussion

Data from the current study supported our first hypothesis that piglets fed diets containing higher inclusion level of both BBN and YBJ up to 250 g/kg diet would not negatively affect growth performance, although there were lower ATTD of nutrients at higher inclusion level (i.e., 250 g/kg).

To date very limited information is available regarding the dietary inclusion of CM on growth performance and nutrient digestibility of weaned pigs (Landreo et al., 2011, 2012). In the present study, ADG, ADFI and G:F of weaned pigs fed diets containing up to 250 g/kg of either BBN or YBJ did not significantly differ from those fed the soybean meal-wheat based control diet, suggesting that CM can be included up to 250g/kg inclusion in weaned pig diets. The findings in the current study are in agreement with the recent report demonstrating that inclusion of solvent extracted canola meal up to 200 g/kg did not negatively affect the growth performance in weaned pigs (Landreo et al., 2012). However, these results contradict with the earlier research indicating that increasing inclusion of CM linearly reduce the ADG and ADFI in weaned pigs (McIntoshi et al., 1986; Baidoo et al., 1987). These inconsistent results can be explained by higher glucosinolate content in previous cultivars of CM and by the use of old feed formulation system in terms of energy and AA supply (i.e., DE and CP basis) (Zijlstra and Payne, 2007; Landreo et al., 2012).

Numerous performance trials had been conducted to improve the growth performance of piglets with dietary multi-carbohydrase enzyme supplementation. However, inconsistencies had seen among studies that have determined the effect of multi-carbohydrase supplementation. For instance, some of the previous studies indicated that carbohydrase enzymes improve the ADG in weaned pigs (Bedford et al. 1992; Baidoo et al., 1998; Omogbenigun et al., 2004; Zijlstra et al., 2004). On the other hand, there were no effects of carbohydrase enzyme supplementation on growth performance in weaned pigs (Mavromichalis, et al., 2000; Olukosi et al., 2007b). Results of the current study were in accordance with the latter studies.

In the present study, ATTD of DM, CP and energy decreased while increased inclusion of both BBN and YBJ, which is in contrast to our hypothesis that increasing inclusion of both cultivars would not alter the ATTD of any nutrients in weaned pigs. This observation could be due to its high fibre content. Increasing inclusion of both BBN and YBJ from 200 g/kg to 250 g/kg increased NDF from 12.43% to 14.14% in BBN and from 10.98% to 12.33% in YBJ, which resulted in approximately a 4 to 5% reduction in ATTD of N, DM and energy. This is in agreement with published data, suggesting that increased dietary fibre in piglet diets decreases the ATTD of nutrients (Landero et al., 2011, 2012).

It is also of importance to note that, even though YBJ had higher amount of total dietary fibre content (24.97%) compared to soybean meal (17 to 20.7%) (Grieshop et al., 2003), inclusion YBJ at 200 g/kg resulted in similar digestible energy, DM and CP compared to the values obtained with feeding control diet. Interestingly, YBJ had higher ATTD of nutrients compared to BBN at 200 g/kg and 250 g/kg inclusion level. This might be explained by the total dietary fibre content which might affect the digestibility (Stein, 1986). Because YBJ (24.9%) had lower total dietary fibre compared to BBN (30.3%), the ATTD of nutrients were higher in YBJ.

Compared to 200 g/kg inclusion level, at 250 g/kg inclusion, enzyme had a much more pronounced positive effect on ATTD of DM, N and energy in both BBN and YBJ containing diets. Interestingly, current observations showed that piglets received 250 g/kg of either BBN or YBJ with enzyme supplementation had 2 to 3% higher ATTD of nutrients compared to the piglets which received the non-enzyme supplemented diets at the same inclusion level of CM. This results is in agreement with the previous study showing that multi-carbohydrase enzyme was more effective in diets with 30% DDGS than those that had 15% (Emiola et al., 2009). This observation is probably due to increased dietary fibre content in diets which contained CM at 250 g/kg inclusion level compared to 200 g/kg inclusion level (10.9 vs. 12.3% NDF in YBJ and 12.4 vs. 14.1% NDF in BBN at 200 and 250 g inclusion level per kg diet, respectively), which may have provided more substrate for the enzyme. Canola meal contains cellulose (i.e., insoluble), pectins and arabinoxlans (i.e., soluble) as the major NSP (Slominski and Campbell, 1990). Lower digestibility of nutrients are associated with high fibre diets which is mainly related to soluble fibre which is known to increase digesta viscosity thus reducing the interaction between digestive enzymes and dietary substrates (Bedford and Schulze, 1998; Zijlstra et al., 2004). In an in vitro study, a multi-carbohydrase enzyme blend was shown to increase fibre degradation and improve protein solubility (Slominski and Campbell, 1990; Alloui et al., 1994). The disruption of the cell wall polysaccharide, thereby eliminating nutrient encapsulating effects and reduction of digesta viscosity were most likely the contributing factors to the improved nutrient digestion with multi-carbohydrase supplementation in this study.

Multi-carbohydrase did not improve the feed efficiency at 250 g/kg inclusion of both BBN and YBJ, although it improved the energy and protein digestibility. These results are somewhat surprising given the fact that enzyme enhances the nutritive value of diet and thereby improving the growth performance. However, it was not the case in our study; this discrepancy might be explained by higher variability in feed efficiency than in nutrient digestibility.

In conclusion, BBN and YBJ can be included as protein and energy sources in weaned pig diets up to 250 g/kg without compromising growth performance. Multi-carbohydrase supplementation improved CP, energy and DM digestibility in weaned pig diets containing BBN and YBJ up to 250 g/kg inclusion level. Furthermore, results indicated that addition of multi-carbohydrase to mixed grain-based diets with high levels of BBN and YBJ improved CP, DM and energy digestibility in weaned pigs but had no effect on piglet performance.

6.0 General Discussion

Canola meal is a co-product of canola crushing industry and can be used as a cost effective protein and energy source in pig diets. However, high fibre content and anti-nutritional factors such as glucosinolates in the CM are problematic to its use in pig diets (Bell, 1993). Canola breeders have developed new cultivars of canola with lower glucosinolate and reduced fibre contents (i.e, *Brassica juncea* yellow and *Brassica napus* yellow) in order to minimize effects of anti-nutritional factors (Simbaya et al., 1995; Slominski, 1997; Bell et al., 1998). A limited number of studies have been conducted to determine the nutritive value of BBN and YBJ in pigs (Bell et al., 1998; Montoya and Leterme, 2009). However, these studies were mainly focused on protein and energy digestibility. Determination of SID of AA of feed ingredients in pig is very important in order to minimize the N output from the operation and for the effective feed formulation in the swine industry (Hoehler et al., 2006). It is also known that ileal AA digestibility is a good indicator of bioavailability of AA (NRC, 1998). Furthermore, the use of feed enzyme may also help to minimize the effect of some of the anti-nutritional factors present in pig feed ingredients. The studies show that multi-carbohydrase supplementation improved the AA digestibility in barley-CM based diets in pigs (Baidoo et al., 1998). However, the influence of enzyme supplementation on SID of AA in BBN and YBJ in pigs has not been determined yet.

Therefore, the purpose of manuscript I was to determine the AID and SID of CP and AA of BBN, YBJ and YBN in response to multi-carbohydrase supplementation in growing pigs. Our results indicated that pigs fed diets with BBN and YBJ had similar

AID and SID of amino acids but higher than YBN. The lower digestibility of YBN is ascribable to over toasting of the meal at processing plant. Lower digestibility of Lys YBN was evident for over toasting as Lys is the most susceptible AA to heat treatment. Higher glycoprotein content of YBN also ascribable to over toasting of the meal during processing. Furthermore, multi-carbohydrase supplementation did not have a beneficial effect on the digestibility of AA. It could be explained by the inclusion level of enzyme, which might be lower or higher than the optimum dose level. Zijlstra et al., (2004) explained that optimum inclusion level of enzyme is important in order to get better response. These findings highlight that BBN and YBJ had similar ileal digestible AA. It was expected that BBN and YBJ would have similar growth performance indices of weaned pigs, since both cultivars had similar SID amino acids.

In manuscript II, the effect of high dietary inclusion of BBN and YBJ were evaluated in weaned pigs in terms of growth performance. We used the SID values of amino acids of BBN and YBJ from our previous experiment and NE values from published prediction equations (Noblet et al., 1994) to formulate the diets for this experiment. Because NE values and SID of AA are the two important concepts in the effective formulation in order to get efficient and predictable growth performance (Payne and Zijlstra, 2007). We did not detect any differences in growth performance of piglets among diets. This suggested that both BBN and YBJ can be included up to 150 g/kg in weaned pig diets without negatively affecting ADG, ADFI and feed efficiency. This is in agreement with recent findings, indicating that solvent-extracted canola meal and expeller-pressed canola meal could be included up to 200 g/kg in substitution of soymeal meal of weaned pig diets formulated to equivalent NE and SID AA without hindering growth performance (Seneviratne et al., 2011; Landero et al., 2011, 2012). Zijlstra and Payne (2007) suggested that formulating diets with by-products as alternative feedstuffs would minimise the risk associated with reductions in growth performance indices by using the NE and SID AA system.

Therefore, it was decided to conducted another experiment in order to investigate further the higher inclusion level of CM in weaned pig diets. The objective of the manuscript III, was to determine the effect of high dietary inclusion level of BBN and YBJ in terms of growth performance and ATTD of DM, CP and GE. Furthermore, the effectiveness of the multi-catbohydrase on growth performance and nutrient digestibility also was evaluated in this study. Growth performance results demonstrated that both BBN and YBJ can be included in weaned pig diets up to 250g/kg without compromising performance. Increasing both BBN and YBJ resulted in 4 to 5% reduction in ATTD of CP, DM and energy. These observations appear to be attributable to its high fibre content. Increasing inclusion of both BBN and YBJ from 200 g/kg to 250 g/kg increased NDF from 12.43% to 14.14% and 10.98% to 12.33% , respectively (Manuscript III). Piglets fed multi-carbohydrase supplemented diet had higher ATTD of CP, energy and DM. However, exogenous enzyme effect was significant at 250 g/kg inclusion level in both BBN and YBJ. This observation is probably due to increased dietary fibre content in diets which contained 250 g/kg inclusion level compared to 200 g/kg inclusion level. However, these studies (manuscript II and III) indicate that both BBN and YBJ can be included up to 250g/kg in weaned pig diets without compromising the growth performance, when the diets were formulated based on SID AA values obtained for grower pigs in manuscript I.

7.0 Summary and conclusion

Yellow-seeded *B. juncea* and BBN had similar SID of of Lys (77.1 vs. 78.9%), Met (87.0 vs. 84.2%), Thr (75.2 vs. 77.1%), Ile (80.2 vs. 79.7%) and Val (79.8 vs. 78.5%). The average AID and SID of indispensable AA, were (73.4 and 80.8%), (73.2 and 81.1%) and (68.5 and 76.1%) in BBN, YBJ and YBN without added multi-carbohydrase, respectively. Canola meal (both BBN and YBJ) can be included into weaned pig diets up to 250 g/kg inclusion level without compromising the growth performance, when the diet is formulated based on similar NE and SID Lys/ kcal NE. Furthermore, enzyme supplementation to mixed grain-based diets with high levels of BBN and YBJ improved CP, DM and energy digestibility in weaned pigs but this improvements were not translated to improved piglet performance.

Recommendation for future research:

1. Determine the optimum dose of enzyme supplementation in order to improve SID of AA in BBN, YBJ and YBN.
2. Super dosing effect of multi-carbohydrase on SID of amino acid in canola meal.
3. Evaluate the nutritive value of canola meal in sows.
4. Examine the effect of new cultivars of canola meal inclusion on carcass quality and carcass characteristics.

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