MEANS TO IMPROVE THE UTILIZATION OF CANOLA MEAL BY BROILER CHICKENS: NEW LOW-FIBER CANOLA AND THE USE OF EXOGENOUS ENZYMES

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By

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DEDICATION

This writing is dedicated to my:

Dad, who implanted the seeds of science, hard work and persistence in me,

Mom, who taught me patience and resilience,

Sisters, who their support, kindness and care never left my side,

Late brother, who taught me to value life, science and art,

Husband, who brought joy, peace and happiness back to my life,

Son, who filled my journey with moments of joy and wonder,

And all the teachers and friends who helped me to find and respect my path.

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ABSTRACT

Means of improving the quality of CM for poultry, including breeding for low-fiber canola and the use of exogenous enzymes, have been proposed. The objectives of the current study were: (1) To evaluate the chemical and nutritive composition of new yellow-seeded *B. napus* and *B. juncea* canola, (2) To investigate the effect of canola type and enzyme supplementation on AME_n and SID of amino acids, and growth performance of broiler chickens, (3) To explore the new carbohydrase enzymes for their ability to depolymerize NSP of CM *in vitro*, and (4) To evaluate the effect of new enzyme combinations in vivo with broiler chickens. In comparison with the conventional meal, yellow-seeded *B. napus* and *B. juncea* contained more crude protein, more sucrose, and less dietary fiber. The AME_n and SID amino acid values for yellow-seeded *B. napus*, *B. juncea* canola, and the conventional black-seeded *B. napus* were 1865, 2092 and 1902 kcal/kg DM, and 82.5, 83.2, and 81.8%, respectively. Enzyme addition resulted in a more pronounced effect on the AME_n content of *B. juncea* meal. When birds were fed diets containing 15% CM, BWG averaged 2.32, 2.30, 2.19, and 2.31 kg for the SBM-based Control, black and yellow *B. napus*, and *B. juncea* meals, respectively. A lower ($P<0.05$) BWG was observed in birds fed a diet containing 30% of *B. juncea* meal. In another experiment, enzyme supplementation improved FCR in chicks fed *B. juncea* meal. In the *in vitro* enzyme incubation studies, enzyme preparations galactanase/pectinase and cellulase/xylanase showed NSP degradation of more than 40%, and were subsequently used in the broiler chicken experiments. A lower BWG and higher intestinal viscosity were observed in birds fed the enzyme-supplemented diet containing 30% of *B. juncea* meal. High digesta viscosity could be

attributed to the release of water-soluble NSP due to the low dietary enzyme concentration and/or unfavorable gut conditions. No differences in growth performance were observed in broiler chickens fed diets containing 15% of CM without and with enzyme preparations. Digestibility of NSP was higher in birds fed the enzyme-supplemented *B. napus* canola diet, although this effect was not translated into any visible improvement in growth performance.

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LIST OF ABBREVIATIONS

AA - Amino acid

ADF - Acid detergent fiber

AIAAD - Apparent ileal amino acids digestibility

AID - Apparent ileal digestibility

AME_n – Nitrogen corrected apparent metabolizable energy

BWG - Body weight gain

CM - Canola meal

DEB - Dietary electrolyte balance

DF - Dietary fiber

DM - Dry matter

FCR - Feed conversion ratio

FI - Feed intake

GLS - Glucosinolates

IEAA - ileal endogenous amino acids

ME - Metabolizable energy

NDF - Neutral detergent fiber

NDICP - Neutral detergent insoluble crude protein

NSP - Non-starch polysaccharides

P - Phosphorus

SBM - Soybean meal

SCFA - Short chain fatty acids

SID - Standardized ileal digestibility

TDF - Total dietary fiber

TMA - Trimethylamine

TME_n - Nitrogen corrected true metabolizable energy

1. GENERAL INTRODUCTION

Canola meal (CM), a co-product of the canola oil industry, with annual production of 4 million tons in Canada, is a valuable protein and well balanced amino acid supplement for poultry diets (Newkirk, 2009; CCC, 2015). According to the Canola Council of Canada (CCC, 2015), by definition, canola oil shall contain less than 2% erucic acid while the meal shall contain less than 30 μmol/g of aliphatic glucosinolates. The canola that is produced today is of superior quality to the original rapeseed which contained 24-45% erucic acid in the oil and 110-150 μmol/g aliphatic glucosinolates in the meal (Bell 1984; 1993). However, due to the presence of several anti-nutritional factors, including non-starch polysaccharides (NSP), glucosinolates, tannins and phytic acid, CM inclusion rates in monogastric diets are still limited (Slominski and Campbell, 1990; Bell, 1993; Khajali and Slominski, 2012). Currently, the recommended inclusion levels of CM in broiler chicken diets are limited to 10% in the starter and 20% in the grower phases (CCC, 2015).

Fiber components of CM include NSP, which mainly originate from the hull fraction of the seed and account for 18-20% of the meal (Bell and Shires, 1982; Slominski and Campbell, 1990; Meng and Slominski, 2005). Pectic polysaccharides are the major NSP polymers of CM and include rhamnogalacturonans and xylogalactouronan backbones with associated side chains of arabinose, galactose and xylose polymers. Other polysaccharides such as arabinans, arabinogalactans, galactans, xyloglucans, galactomannans and mannans are also present (Siddiqui and Wood, 1977; Slominski and Campbell, 1990; Meng and Slominski, 2005; Putsjens et al., 2013).

The NSP are not degraded by poultry endogenous enzymes, and thus increase gut viscosity and digesta passage rate and consequently reduces nutrient utilization (Annison, 1991; Jensen et al., 1995; Bedford and Morgan, 1996; Pustjens et al., 2012). A few studies demonstrated the inverse relationship between fiber content, including NSP, and metabolizable energy content (Downey and Bell, 1990; Ahmad et al., 2007; Mushtaq et al., 2007) and protein digestibility (Bell, 1993; Slominski, 1997).

Various approaches, including breeding for low-fiber canola or the use of exogenous enzymes, have been considered in an attempt to improve the nutrient value of CM for monogastric animals (Khajali and Slominski, 2012). In earlier germplasm characterization studies, some positive quality characteristics (i.e., low fiber, increased oil) have been demonstrated for yellow-seeded canola (Slominski et al., 1994; Simbaya et al., 1995; Slominski, 1997). More recently, a superior quality yellow-seeded canola has been developed at the Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada (Somers et al., 2011). In addition, canola quality *B. juncea* canola with agronomic advantages such as heat, drought and disease resistance, early maturity and high yield of oil has been developed at the Agriculture and Agri-Food Canada Research Centre in Saskatoon, Canada (Cheng *et al*., 2011). In comparison with the conventional canola meal, yellow seed coat in *B. juncea* would result in lower fiber content of the meal (Simbaya *et al*., 1995; Slominski 1997; Slominski *et al*., 2012; Zhou *et al*., 2013). However, and despite the positive characteristics, earlier studies demonstrated poor growth performance and low energy and amino acid digestibility in birds fed *B. juncea* meal. This could be due to the anti-nutritional properties of glucosinolates, with the different glucosinolate profile of *B. juncea* meal compared to that

of conventional *B. napus* canola (Jia et al., 2012). In all of the earlier studies, the processing and the production of CM from new yellow-seeded types was conducted on either the laboratory or the small pilot plant scale. In the current study, however, for the first time the seeds from new yellow-seeded *B. napus* and *B. juncea* canola were crushed in the commercial Bunge processing plant in 2 consecutive years 2010 and 2011. The resulting meals were evaluated and the outcomes of such studies have been presented in Chapters 3 and 4 of this thesis.

Another approach to improve the nutritive value of CM is the use of carbohydrase enzymes to target the cell wall NSP, reduce their encapsulating effect and, as a result, improve nutrient utilization by poultry (Campbell and Bedford, 1992; Meng *et al*., 2005; Khajali and Slominski, 2012). However, due to the complex nature and structure of NSP in different canola species, targeting such structures is challenging since a very diversified combination of enzymes is needed to maximize the enzyme efficacy (Slominski, 2011). Earlier studies from this laboratory demonstrated a significant depolymerization of cell wall polysaccharides of CM in vitro when carbohydrase enzymes were used in concert (Meng et al., 2005). However the effect of enzyme supplementation in vivo was not consistent. A significant improvement in starch, protein and NSP digestibility as well as growth performance was observed when birds were fed diets supplemented with enzyme (Meng et al., 2005). In some other studies, however, enzyme cocktails improved NSP digestibility but growth performance was not affected (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000, 2001; Mushtaq et al., 2007). Earlier studies from this laboratory demonstrated a significant effect of enzyme supplementation in chickens fed diets containing *B. juncea* meal, which

was attributed to different NSP structures and potentially higher pectic polysaccharide contents of this meal (Jia *et al*., 2012). Similar enzyme combination was used in the current study and its effect on different canola meals was documented in Chapters 3 and 4. However, in Chapter 5, a new carbohydrase enzyme with a very high affinity towards CM non-starch polysaccharides was introduced and further explored in vitro and in vivo.

Therefore, the overall objective of this research was to explore the potential for improved utilization of CM by broiler chickens. To achieve this objective, various experiments were conducted to address the following specific objectives:

1) To evaluate the chemical and nutritive composition of meals derived from newly developed yellow-seeded *B. napus* and *B. juncea* canola.
2) To investigate the effect of canola type and enzyme supplementation on metabolizable energy and standardized ileal amino acid contents, and growth performance of broiler chickens fed diets containing high levels of *B. napus* and *B. juncea* meals.
3) To explore the new carbohydrase enzymes and their combinations for the ability to depolymerize the specific cell wall polysaccharides of *B. napus* and *B. juncea* meals in vitro.
4) To evaluate the effect of new enzyme combinations on NSP and energy utilization, and growth performance of broiler chickens.

2. LITERATURE REVIEW

2.1 History of Canola Meal

Canola is one of the most important crops in Canada with 8 million hectares in Western Canada producing over 15 million tonnes of seed. Canola seeds are processed to produce oil (approximately 44%) and canola meal as a co-product, which serves as a protein source for livestock diets (CCC, 2015).

Canola originally known as rapeseed, a *Brassica* genus of cruciferae or cabbage family, was first planted in India over 3000 years ago with the oil used for cooking, medicinal effects and illumination (Åppleqvist and Olhson, 1972). The first rapeseed variety of *Brassica rapa* was brought from Poland and planted on a small farm in Saskatchewan in 1936 to diversify its crop production. Later, in 1942, *Brassica napus* seeds were imported from Argentina and these two rapeseed species have been grown in Canada for many years (Bell, 1982; 1984; 1993).

Rapeseed oil was originally used as a steam engine lubricant. After World War 2, with the introduction of diesel engines and ban of rapeseed oil for human consumption due to high erucic acid contents, the production of rapeseed was reduced. High levels of erucic acid (24-45%) in the seed were found in one study to reduce growth rate and to promote heart disease in rats, and was one of the factors that limited the usage of rapeseed oil in human diets. After many years of scientific research, scientist Dr. Baldur Stefansson of the University of Manitoba and Dr. Keith Downey of the Agriculture Canada Research Station in Saskatoon found *B. napus* seeds with lower erucic acid content and developed and registered the first low-erucic acid variety “Oro” in 1968 (Bell, 1993).

Another limitation of rapeseed was its high content of glucosinolates with some goitrogenic properties. Glucosinolates (GLS) cause thyroid dysfunction and consequently reduce animal performance (Mawson et al., 1994). Toasting meal up to 110°C deactivates myrosinase enzyme, which is responsible for hydrolysing glucosinolates to toxic end-products. The first low-erucic acid and low-glucosinolate variety of *B. napus* Tower was developed from Polish variety Bronowski in 1974. In 1977, the first canola quality *B. rapa* variety Candle was released (Bell 1984; 1993).

In 1979, rapeseed varieties of *B. rapa* (or *campestris*) and *B. napus* with less than 2% of erucic acid and less than 30 μmol/g of aliphatic glucosinolates were officially recognized as canola (Canadian oil) in North America by Western Canadian Oilseed Crushers' Association. The new varieties of canola are significantly superior to the original rapeseed which contained 25-45% of erucic acid and 110-150 μmole/g of aliphatic glucosinolates in the meal (Bell 1984; 1993). According to the Canola Council of Canada, Canola is officially defined as: "Seeds of the genus *Brassica* (*Brassica napus*, *Brassica rapa* or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile, and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid". To use the name Canola, rapeseed varieties have to meet the above standards.

2.2 Chemical Composition of Canola Meal

Canola meal contains an average value of 36% protein (12% moisture basis). According to the survey conducted from 2000 to 2014 by the Canadian Grain

Commission, protein content of CM varied between 37 to 42% (Barthet, 2014; CCC, 2015). This variation is due to growing conditions specially weather and soil characteristics (CCC, 2015). Canola meal is rich in sulphur amino acids such as methionine and cysteine but is low in lysine compared to soybean meal (SBM). Therefore, both meals can complement each other when used in livestock diets (Bell, 1984; CCC, 2015). Less metabolizable energy in CM (2000 kcal/kg) in comparison with SBM (2491 kcal/kg) is partially due to the high fiber content of this meal (NRC, 1994). Ether extract of CM averages 3.5% and varies due to processing condition. In Canada, and when using the pre-press solvent extraction process, ether extract of CM is usually 1-2% higher than that of other countries due to the amount of gums and soapstocks added back to the meal following oil refining (CCC, 2015). Canola meal is a good source of most vitamins and minerals especially sulphur, selenium, phosphorus and B vitamins (Bell et al., 1999; CCC, 2015).

2.2.1 Metabolizable Energy of Canola Meal for Poultry

Metabolizable energy content of pre-press solvent extracted CM for poultry averages ~2000 kcal/kg which is 287 kcal/kg (as-fed basis) lower than that of 44% SBM (Hijikuro and Takemasa 1985; NRC 1994; Mandal et al., 2005; Simbaya et al., 1996). Similarly, Newkirk et al. (1997) reported the average values of 2031 kcal/kg for six different samples of CM. According to Bell (1993) metabolizable energy (ME) contents of CM for broilers and laying hens range from 1771 to 2605 (AME_n) and 2055 to 2273 kcal/kg (TME_n) respectively. The ME values reported for laying hens are 1929 (Li et al., 1989) and 2249 kcal/kg (Deng, 1993).

There are many factors that contribute to low ME content of CM for poultry and

thus limit its inclusion in poultry rations. Sucrose content (5-6%, as-fed basis) and that of starch (2%) and simple sugars (0.5%) of CM would contribute around 320 kcal/kg to the ME content of CM (Slominski and Campbell, 1991). Oil extraction techniques affect ME values of the meal. Two common techniques have been used for canola oil extraction. The most common process combines the pre-pressing of the seeds followed by solvent extraction, which produces a final meal with less than 1% of residual oil. Another process is the expeller extraction technique, which is not as effective in extracting the oil, and would produce CM with 8 to 15% of residual oil (Spragg and Mailer, 2007), which, in turn, would contain more ME (Toghyani et al., 2014). Woyengo et al. (2010) observed higher ME values for expeller pressed meal compared to its solvent extracted counterpart. It is worth mentioning that in Canada gums and soapstocks produced during solvent extraction and oil refining are added back to CM (CCC, 2015). Gums mainly consist of glycolipids and phospholipids that increase CM ether extract content by 1-2% (McCuaig and Bell, 1981; CCC, 2015) and consequently ME values by approximately 150 kcal/kg (March and Soong, 1978). Another important factor is the temperature applied during expelling and meal de-solventization processes with meals produced by medium seed conditioning temperature showing higher ME in comparison with the ones produced by low or high temperature (Spragg and Mailer, 2007; Toghyani et al., 2014).

In an experiment conducted by Downey and Bell (1990), a negative correlation between fiber content and energy digestibility of CM was observed. Lessire et al. (1986) used de-hulled CM with 54% lower crude fiber content and observed the increase in ME values by 15 to 20%. Fiber dilutes nutrients and increases digesta passage rate, leading to reduced nutrient digestibility and energy availability (Bell, 1993). In many studies, the

effects and importance of other anti-nutritional factors of CM such as glucosinolates and tannins on ME values have been studied. Mutzar and Slinger (1980), Hijikuro and Takemasa (1985), Lessire et al. (1986) and Mandal et al. (2005) observed a negative relationship between the glucosinolate content and AME values.

2.2.2 Protein and Amino Acid Composition and Digestibility

Canola meal is a valuable source of protein with a minimum protein content of 36% (12% moisture basis) and well-balanced amino acid (AA) profile, with sulfur-containing amino acids methionine and cysteine predominating (Bell, 1984; CCC, 2015).

According to a survey conducted by the Canadian Grain Commission, the protein content of CM produced between 2000 and 2014 varied from 37 to 42% (12% oil-free, DM basis) (Barthet, 2014). In a recent study, protein content of CM from 11 crushing plants, collected between 2011 and 2014 was determined and was between 40.2 to 42.6% with the average value of 41.7% DM (Adewole et al., 2016). In earlier studies, protein content of CM was slightly higher with the average values of 43.9% (Newkirk et al., 1997) and 41.9% (Bell and Keith, 1991), ranging from 38.0 to 43.5%. Other studies reported crude protein (CP) values of 38.5% DM (Seneviratne et al., 2010), 41.4% DM (Woyengo et al., 2010), 37.8% DM (Woyengo et al., 2011) and between 36.2 to 40% (as-is basis) (Finlayson, 1974; Goh et al., 1980). These variations might be due to geographical, environmental, variety and processing conditions (Bell et al., 1991). This was confirmed by the recent study by Adewole et al. (2016) showing significant differences in CP and AA contents of CM produced in Canada between 2011 and 2014.

Canola meal is a well-balanced source of AA with high sulfur AA methionine and cysteine and less lysine content in comparion with SBM (Khajali and Slominski, 2012).

One of the important limitations of CM in terms of AA content is its arginine level (Khajali and Slominski, 2012), which is significantly lower than that of SBM. High inclusion levels of CM may reduce dietary arginine level below birds' requirement (Izadinia et al., 2010). Unavailability of synthetic arginine and lack of birds' ability to produce arginine make poultry more vulnerable to dietary arginine deficiency (Khajali and Wideman, 2010). Arginine is a precursor of nitric oxide, a potential vasodilator, and its deficiency may cause pulmonary hypertension, ascites and other vascular disorders, especially in environments with low oxygen pressure such is high altitude and cold conditions (Collier and Vallance, 1989; Shaul, 2002; Izadinia et al., 2010).

Availability of amino acids of CM varies due to processing conditions (NRC, 1994). To optimize CM usage in poultry and to minimize nitrogen excretion into the environment, diets have to be formulated based on the digestible AA contents for different types of poultry (Kong and Adeola, 2013). There are many assays to determine AA digestibility in poultry. In most of the studies, digestibility values are determined by excreta collection using adult roosters or precision feeding technique (Parsons, 1991; NRC, 1994; Rhone-Poulenc, 1995). Other studies, however, have questioned the crop-intubation of the birds due to its abnormal feeding pattern. Other criticism arises from the fact that the use of values produced from mature birds might not match the digestive capacity of the young chicken (Ravindran et al., 1999; Lemme et al., 2004). In this context, ileal digestibility technique is more reliable in comparison with the total tract digestibility measurements, which are affected by hindgut fermentation (Ravindran and Bryden, 1999; Lemme et al., 2004). Other studies compared the site of ileal digesta sampling in poultry. According to some studies, estimating digestibility at the proximal

ileal level disregards AA disappearance in the central or distal ileum and consequently might under-estimate the digestibility values. This explains higher AA digestibilities in the rooster assay in comparison with the apparent ileal digestibility (AID) assay with broiler chickens (Kluth et al., 2005; Rezvani et al., 2008). When poultry diets are formulated based on the standardized ileal AA digestibility (SID) values, diets are closer to birds' requirements, which subsequently reduces an excess of nitrogen excretion (Adedokun et al., 2007). Moreover, SID is more additive in diets consisted of different feed ingredients in comparison to AID (Angkanaporn et al., 1996; Stein et al., 2005). Amino acid endogenous losses used in the SID assay is another source of variation in the AA digestibility measurements. This has to be taken into consideration when calculating SIAAD using historical and/or book values for endogenous AA losses (Kim et al., 2012).

Different studies found AA digestibility to be age-dependent with higher AA digestibility values observed for older birds (Parsons et al., 1982; Wallis and Balnave; 1984; Ten Doeschate et al. 1993). Increased fiber digestibility in older birds in comparison with younger birds seem to be responsible for higher AA digestibilities (Fan et al., 1996). According to Kong and Adeola (2013), the AA digestibility values of CM were higher in broilers at 28 and 42 days of age in comparison with 14 d-old birds. Similarly, Huang et al. (2005) observed higher AA digestibility values for 42 vs 14 d-old broiler chickens with the most pronounced effect observed for arginine and threonine. Ingredient characteristics such as physical structure, anti-nutritional factors, processing technique as well as intestinal development and endogenous losses are among important factors affecting digestibility values (Huang et al., 2005). Endogenous losses, as an example, decreased in broilers from 5 to 15 d of age, which might be due to the

development of intestinal mucosa (Adedokun et al. 2007; Kong and Adeola, 2013).

According to NRC (1994), true lysine digestibility of CM is 10% lower than that of SBM with a similar pattern observed for other amino acids. High concentration of methionine and high digestibility of methionine both in layers and broilers has been observed in several studies. Ileal digestibility values of AA in laying hens (Rezvani et al., 2008) and broiler chickens (Rodehutscord et al., 2004; Kluth and Rodehutscord, 2006) have been reported to average 80%. In another study, ileal AA digestibility of 14 different varieties of Australian CM for broilers varied between 68 to 83% (Bryden et al., 2009). Similarly, pre-ceacal lysine digestibility of CM from Western Canada was between 66 and 86%, and 87 and 92% in toasted versus non-toasted samples, respectively (Newkirk et al., 2003).

According to Barbour and Sim (1991), Zuprizal Labrier et al. (1991) and Slominski et al. (1999), true AA digestibilities of CM were from 79 to 98% with the mean value of 91%, from 73.1 to 96.7% with the mean value of 80.1%, and between 83.2 and 85.9% with the mean value of 84.2%, respectively. Although there is high variation in AA digestibilities, a similar pattern was observed for individual AA with methionine and arginine showing the highest and valine and threonine the lowest digestibility values (Rezvani et al., 2012).

2.2.3 Ether extract

Amounts and composition of ether extract in CM depend on the processing conditions. Pre-press solvent extracted CM has higher ether extract contents, mostly due to the gums and soapstocks that are added back to the meal after oil refining. Therefore, Canadian CM would contain 3.5% of ether extract in comparison with 1-2% observed for

meals produced in other countries (Slominski et al., 1999; Slominski et al., 2012; CCC, 2015). Ether extract content of samples from 11 different crushing plants in Canada was found to be between 1.4 and 4.3% (90% DM-basis) with the mean value of 3.3% (Rogiewicz et al., 2012). In the most recent study, the ether extract varied from 2.7 to 4.3% with the mean value of 3.5% (Adewole et al., 2016). These differences could be due to the amount of gums and soapstocks added back to the meal in each crushing plant (Newkirk, 2009).

Gums and soapstocks are co-products of oil refining and mainly consist of phospholipids, glucolipids, triglycerides, free fatty acids, sterols and fat-soluble vitamins (Bell, 1984; CCC, 2015). Studies showed that quantities as high is 6% had no adverse effect on growth of poultry (Robblee et al., 1978; CCC, 2015) but rather would increase the ME content and reduce dustiness of the meal (CCC, 2015).

2.2.4 Carbohydrates

Canola meal carbohydrates include: 1) simple sugars glucose and fructose, 2) soluble di-saccharide sucrose and galacto-oligosaccharides raffinose and stachyose, and 3) unavailable and structural polysaccharides which fall under the non-starch polysaccharide or fiber categories and will be discussed in the next chapter of the thesis.

Canola meal contains 8-10% of simple sugars and di- and oligosaccharides (DM basis), which may vary due to growing, processing conditions, and method of analysis (Rao and Clandinin, 1972; Bach Knudsen, 1994; Slominski et al, 1994). In a recent survey of 11 crushing plants, the mean sucrose content was between 5.7 and 6.4% with the mean value of 6.1%, and oligosaccharides raffinose and stachyose between 2.6 and 3.2% with the mean value of 2.9% (Adewole et al., 2016).

Despite sucrose, which is highly digestible due to the presence of sucrase in the gut, oligosaccharides cannot be digested in the small intestine and are fermented by the lower gut microbiota, with the production of short chain fatty acids (SCFA) which would contribute to the energy content of CM (Rackis, 1975; Leske et al., 1993)

2.2.5 Minerals

Mineral content of CM has been investigated in numerous studies (Bell, 1984; Bell and Keith, 1991; Bell et al., 1999; Sauvant et al., 2002; Broderick et al., 2015; Slominski et al., 2015) demonstrating that CM is a good source of minerals especially phosphorus and selenium. In comparison with SBM, CM contains higher mineral content and would be considered a better source of minerals even though the availability of those minerals is lower in CM. Lower availability of minerals in CM is mostly due to higher levels of fiber and phytic acid and their binding and encapsulating effects (Nwokolo et al., 1976, Nwokolo and Bragg, 1977; Clandinin et al., 1986; Ward et al., 1991). Such negative effects could be overcome by exogenous enzymes such as phytase and carbohydrases (Ward et al., 1991; Slominski, 2012).

Environmental conditions, soil and fertilizer application are among factors that affect the mineral content of CM (Bell and Keith, 1991). According to some studies, however, the higher mineral content of CM could also be due to various admixtures such as sand, dust and foreign materials in the processing plant rather than the mineral content *per se* (Unger, 1990; Slominski et al., 1999).

Higher sulphur content of CM in comparison with SBM (1.14 vs 0.44%) has been a concern especially when high inclusion levels of CM are used (Summers, 1995; Ahmed et al., 2007).

2.2.6 Vitamins

When compared to SBM, CM is higher in most B vitamins such as folic acid, choline, biotin, niacin and riboflavin with the exception of pantothenic acid (Bell, 1984; Clandinin et al., 1986; Clandinin et al., 1989; NRC, 2012)

2.3 Anti-nutritional factors of CM

2.3.1 Dietary fiber

The term dietary fiber (DF) refers to edible components of plant cell walls, that are not digestible in the small intestine, but are fermented in the large intestine by gut microbiota (Van Der Kamp, 2004). Non-starch polysaccharides (NSP), lignin and polyphenols, and structural proteins and Maillard reaction products are all part of DF. The DF, specifically NSP, exist in all plant materials in different amounts and structure (McNab and Boorman, 2002; Van Der Kamp, 2004). In CM, NSP are composed of mostly pectic polysaccharides (Seddiqui and Wood, 1972; Voragen et al., 2001). Total tract digestibility of NSP in poultry is minimal and ranges from 2 to 8% (Slominski and Campbell, 1990; Meng and Slominski, 2005; Meng et al., 2006).

In poultry, DF is considered an important factor in diet formulation. It encapsulates nutrients and consequently prevents endogenous enzymes from accessing the nutrients. It also increases gut viscosity, which minimizes the efficacy of endogenous enzymes, preventing them from reaching the nutrients, and consequently resulting in increased passage rate of digesta and reduced digestibilities of protein, energy and minerals (Antoniou et al., 1981; White et al., 1981; Annison, 1991; Jensen et al., 1995). It also causes physiological changes in the digestive tract such as thickening of intestinal mucus membranes, which may reduce the absorption capacity of the gut (Choct, 2002).

Dietary fiber undergoes microbial fermentation in the large intestine in relatively small amounts. This process produces short chain fatty acids (SCFA) that, in poultry, have minimal contribution to the energy value of DF (Bell and Shires, 1982; Slominski and Campbell, 1990; McNab and Boorman, 2002). Other authors indicated that the effect of fiber is mostly due to the nutrient dilution rather than the anti-nutritive effect per se (Slominski et al., 1999).

Canola meal contains approximately 30% DF, originating mainly from the hull fraction of the seed (Bell and Shires, 1982; Bell, 1993; Naczk et al., 1994; Spragg and Mailer, 2007). Canola meal contains 18% NSP constituent sugars with the high concentration of uronic acid, glucose, and arabinose followed by galactose, xylose and mannose residues (Slominski and Campbell, 1990; Bell, 1993). The major pectic polysaccharides of CM consist of rhamnogalacuronan, homogalacturonan, and xylogalactouronan with associated side chains of xylose, galactose and arabinose (Slominski and Campbell, 1990, Meng and Slominski, 2005). Other polysaccharides such as arabinans, xylans, galactan, arabinogalactans, galactomannans and mannans are also present (Siddiqui and Wood, 1972; Slominski and Campbell, 1990; Meng and Slominski, 2005; Putsjens et al., 2013). A negative relationship between energy digestibility and fiber content of CM has been documented (Downey and Bell, 1990).

2.3.2 Glucosinolates

Glucosinolates (GLS) is a term used for a wide variety of secondary sulphur-contaning plant metabolites, which are common for cruciferous plants. All GLS contain a sulphonamide moiety and a β-D-thioglucose group with different side chains mostly derived from amino acids (Tripathi and Mishra, 2007). Based on different side-chain

structure, over 120 different GLS have been identified (Chen and Andersson, 2001). Glucosinolates of CM consist of two types: dominant aliphatic GLS and indole (indolyl) GLS (Slominski and Campbell, 1987), including gluconapin (3-butenyl), gluco-brassicanapin (4-pentenyl), progoitrin (2-hydroxy-3-butenyl), gluconapoleiferin (2-hydroxy-4-pentenyl), glucobrassicin (3-indolylmethyl), and 4-hydroxyglucobrassicin (4-hydroxy-3-indolyl-methyl), with the later being the predominant indole GLS in canola seeds (Bell, 1984; Slominski and Campbell, 1987; Shahidi and Gabon, 2007). Glucosinolates are expressed on the molecular (μmol/g) rather than weight (mg/g) basis. This is justified by the fact that their molecular weights differ due to different side chains (CCC, 2015).

The toxic effects of GLS derive from the breakdown products, which are produced by myrosinase (thioglucosidase glucohydrolase) enzyme. Glucosinolates are present in vacuoles and are separated from myrosinase which is located in the myrosin cells. In the presence of moisture and following the seed rupture and tissue damage, glucosinolates come into contact with myrosinase, which results in their hydrolysis to unstable aglucens (Bones et al., 1991; Morra and Kirkegaard, 2002) later re-arranging to isothiocyanates, goitrin, nitriles, and thiocyanates. As recently reviewed (Khajali and Slominski, 2012), toxic effects of GLS depend on the content, extent of hydrolysis and composition of the hydrolysis products. These products have bitter taste with anti-nutritional and goitrogenic effects, which negatively affect thyroid hormones synthesis, appetite, feed intake and growth performance (Slominski and Rakowska, 1985; McCurdy, 1990; Tripathi and Mishra, 2007; Mailer et al., 2008; Woyengo et al., 2011). Woyengo et al. (2011) observed an increase in the liver size and hepatic metabolic

activity in the birds fed expeller-extracted CM with potentially active myrosinase, which further changed nutrient metabolism in the liver and consequently reduced feed intake (FI). Other studies also reported increases in the activity of hepatic enzymes, resulting in liver hypertrophy (Slominski and Campbell, 1991; Roland et al., 1996; Vang et al., 2001; Newkirk and Classen, 2002; Tanii et al., 2008). It is of interest to note that young animals are more sensitive to the anti-nutritive effects of GLS (Tripathi and Mishra, 2007).

Myrosinase activity is affected by factors such as temperature, pH, pressure and presence of ascorbic acid (Ludikhuyze et al., 2000). Even though myrosinase is inactivated by the heat applied in the processing plant, thermal decomposition of GLS might produce similar toxic end-products (Campbell and Slominski, 1990). Microbiota of the lower gut especially in the caeca, showed some effect on degradation of GLS, especially the aliphatic ones (Slominski et al., 1987, 1988; Campbell and Slominski, 1989).

Glucosinolates occur in plants as defense mechanism and environmental conditions such as water stress, plant genetic and physiological state, health and nutrition affect the concentration of GLS in cruciferous plans including *Brassica napus* (Mailer and Cornish, 1987; Stowe 1998; Ciska et al., 2000; Dekker et al., 2000). Breeding programs have reduced the GLS content leading to the production of canola with less than 30 μmol/g of aliphatic glucosinolates in the oil-free meal (CCC, 2015). These levels could be further reduced during seed processing due to heat treatment, which inactivates myrosinase and decomposes GLS. The extent of myrosinase inactivation depends on the temperature, time and moisture applied during meal desolventization (Campbell and Slominski, 1990; Unger, 1990; Jensen et al., 1995). According to Newkirk and Classen

(2002), solvent extracted CM is less bitter in comparison with its expeller-pressed counterpart, which indicates the effect of desolventization on the loss of GLS hydrolysis products. These results were later confirmed by Glencross et al. (2004) and Spragg and Mailer (2007) who observed higher GLS content in expeller-pressed meal. Toasting of CM would reduce the GLS content further (Campbell and Slominski, 1990).

The GLS content of the current double-zero rapeseed meal or CM, has been reported to be as low as 18 μmol/g (fat-free basis; Brand et al, 2007), 10 μmol/g (CCC, 2009; Labalette et al., 2011), 4.3 μmol/g DM (Mikulski et al., 2012), 3.9 μmol/g (90% dry matter basis; Rogiewicz et al., 2012) and 4.2 μmol/g DM (Slominski, 2015). In the study by Mailer et al. (2008), total GLS content in eight cultivars grown in 9 different sites over two years was estimated. Based on the site, season and cultivar, the average total GLS content was 18 μmol/g (oil- and moisture-free basis).

Earlier studies with laying hens fed CM with high levels of GLS, showed high mortality due to hemorrhagic liver syndrome and liver hypertrophy resulting in reduced egg production (Ibrahim and Hill, 1980; Martland et al., 1984). These adverse effects limited the inclusion rate of CM to 10% (Mawson et al., 1994). In broilers, feeding high GLS CM increased mortality and reduced FI and growth performance (McNeill et al., 2004). In studies with high GLS CM high incidence of leg problems in broilers was observed due to decreased FI and interference of sulphur ion with calcium absorption (Summers et al., 1992). In several studies GLS tolerance levels in broiler chickens have been determined. Broilers were found to tolerate the GLS levels as high as 11.6 (Leeson et al., 1987) and 8.0 μmol/g (Tripathi and Mishra, 2007). As reviewed by Mawson et al. (1994), GLS levels lower than 4 μmol/g had no effect on growth performance of broilers.

However, when GLS content increased to 10 μmol/g, growth performance was severely depressed. Dietary inclusion level of CM containing less than 4 μmol/g GLS could be increased to more than the current level of 20% for broilers (Khajali and Slominski, 2012). In laying hens, however, 1.5 μmol/g GLS would be considered as no effect level due to the hemorrhagic liver syndrome. Therefore, the inclusion level of CM in laying hens could be increased to 15 - 20% of the diet (Campbell and Slominski, 1991; Khajali and Slominski, 2012).

2.3.3 Sinapine

Canola meal contains approximately 1% of sinapine, a choline ester of sinapic acid, which occurs naturally in *cruciferous* plants. Sinapine has bitter taste and reduces the palatability of CM (Mailer et al., 2008). Under normal circumstances, sinapine is hydrolyzed to trimethylamine (TMA) by the gastrointestinal microorganisms and is converted to the odorless nitrous oxide in the liver and kidney by the microsomal enzyme TMA-oxidase (Hill, 1979). However, in some strains of brown-egg laying hens (i.e. Rhode Island Red) with the lower production of this enzyme, TMA accumulates in the blood and consequently is deposited in the yolk with the production of fishy taint in the egg (Mueller et al., 1978; Curtis et al., 1978; Goh, et al., 1979; Butler et al., 1982). Breeding to correct for the genetic defect of some breeds of laying hens is underway (Honkatukia et al., 2005; Newkirk, 2009). Mailer et al. (2008) observed some variation in the sinapine content of different CM germplasms and concluded that the selection programs could further reduce the sinapine content of CM. No negative effect of sinapine in white leghorns and broiler chickens has been demonstrated (Qiao and Classen, 2003; Khajali and Slominski, 2012).

2.3.4 Tannins

Tannins are complex polyphenolic structures with the molecular weight of 500 to 3000 Da. Canola meal contains 1.5 to 3% tannins with higher levels observed in brown-seeded canola in comparison with its yellow-seeded counterpart (Khajali and Slominski, 2012; CCC, 2015). Tannins are divided into hydrolysable and condensed or insoluble tannins (Yapar and Clandinin, 1972) with condensed tannins predominating in CM (Theander et al., 1977). Condensed tannins are located within the hulls of CM and their quantity is related to the seed coat color (Stringam et al., 1974; Theander et al., 1977; Slominski and Campbell, 1990). Tannin content in canola hulls range between 1.5 to 6.2 per 100 grams of oil free hulls (Naczk et al., 2000; Newkirk, 2009; Khattab et al., 2010). Tannins of CM contain cyanidin, pelargonidin and leucocyanidin as basic units (Schofield et al., 2001). Yellow canola seeds have no proanthocyanidins in the hulls, which makes the hulls translucent and thus reflecting the yellow color of the oil in the seed embryo (Rashid and Rakow, 1999; Slominski et al., 1999; Slominski et al., 2012).

Anti-nutritional properties of tannins depend on the origin, chemical structure and bioavailability (He et al., 2006; Seeram, 2006; Mansoori et al., 2015). In CM, tannins cause bitter taste and dark color. Additionally, they form complexes with amino acids, minerals, proteins and enzymes in the gastrointestinal tract and consequently reduce AA and mineral digestibility and negatively affect endogenous AA loss, protein utilization and growth performance of chickens (Naczk and Shahidi, 1991; Mansoori and Acamovic, 2007, 2009; Khajali and Slominski, 2012; Gopinger et al., 2014). According to Mansoori and Acamovic (2007), poor digestibility of AA in the presence of tannins is related to the increased endogenous AA losses. In addition, tannins disturb the intestinal mucosal

glucose transporters and reduce glucose and simple sugar uptake (Kottra and Daniel, 2007; Mansoori et al., 2007). In a study by Mansoori (2009), 500 mg tannic acid per bird altered D-xylose uptake and transport and reduced D-xylose concentration in plasma of 26-day-old broiler chickens by 54%. Similarly cranberry condensed tannins as low as 24 mg/kg of the diet affected dry matter and nitrogen digestibility in broilers (Cross et al., 2011). Despite all the above studies, which observed the negative effects of tannins in poultry nutrition, tannins of CM are mostly water-insoluble and are present in the cells of CM hulls, which makes their anti-nutritional effect minimal (Naczk et al., 2000; Khajali and Slominski, 2012). Despite the higher tannin content of CM in comparison with SBM, the inclusion level of CM in poultry diets is much lower than that of SBM, which makes the total concentration of bioactive components of tannins negligible and would have a minimal effect on nutrient absorption in the small intestine (Mansoori et al., 2015).

2.3.5 Phytic acid

Phytic acid [myo-inositol (1, 2, 3, 4, 5, 6 - hexakis dihydrogen phosphate)] (i.e., myo-inositol with the six phosphate groups attached), is the main storage form of phosphorus (P) in almost all mature grains and seeds. Each phosphate group is charged and consequently tends to bind cations, and forms insoluble complexes with minerals such as calcium, iron, zinc, manganese, copper and magnesium which renders them unavailable for monogastric animals (Nwokolo and Bragg 1977; Rimbach et al., 1995; Cabahug et al., 1999; Khajali and Slominski, 2012). Phytate also increases endogenous losses of minerals, especially sodium which affects sodium partitioning in the gut. It also reduces Na^+K^+-ATPase activity, which influences the function of Na^+K^+-pump in the duodenum and the Na-dependent transport of nutrients such as glucose and peptides (Liu

et al., 2008; Khajali and Slominski, 2012). Endogenous excretion of other nutrients such as AA, iron, sulphur and sialic acid are also increased in the presence of phytic acid (Cowieson et al., 2004, 2008; 2009; Cowieson and Ravindran, 2007). Phytate may also reduce the activity of few endogenous digestive enzymes such as pepsin and trypsin (Pallauf and Rimbach, 1997). According to Mansoori (2010), the intestine capacity of intestine for sugar absorption could also be reduced in the presence of phytic acid.

Canola meal contains a relatively high proportion of phytic acid, which ranges from 36 to over 70% of the total P content (Summers et al., 1983; Broz and Ward, 2007; Khajali and Slominski, 2012). According to Bell (1993), of 1.22% of total P present in CM, 0.53% would account for phytate P. Canola meal has twice the level of phytate in comparison with SBM (3 vs 1.5% DM basis) (De Boland et al., 1975; Zhou et al., 1990).

Endogenous phytase enzymes has different activity based on the species and age of animals (March, 1991) and their moderate effect on phytate P utilization in poultry has been observed (Maenz and Classen, 1998). According to Mushtaq et al, (2007), phytate had a minimal effect on growth perfomance of mature birds, most likely due to the increase in the endogenous enzyme activity with age. One way to overcome any anti-nutritional properties of phytate is to use supplemental microbial phytase, which would increase availability of phosphorus as well as other minerals and AA (Ravindran et al., 1999). There have been many studies reported on the effect of phytase on phytate phosphorus release, which is beyond the scope of this dissertation.

2.3.6 Dietary electrolyte balance

Dietary electrolyte balance (DEB) is usually defined as dietary cation to anion ratio and is calculated as follows: level of Na plus the level of K versus level of Cl DEB=

(Na + K) – Cl and expressed as mEq/kg of the diet (Mongin, 1981). Optimal DEB is 250 meq/kg for achieving the best performance and livability in poultry (Oviedo-Rondón et al., 2001; Murakami et al., 2003). According to Johnson and Karunajeewa (1985) and Olanrewaju et al, (2007) 180 to 300 and 174 to 241 mEq/kg of DEB are acceptable, respectively, without causing any negative effect. Any shift in DEB may negatively affect FI, growth performance and litter quality in birds (Farahat et al., 2013). In a study by Mushtaq et al. (2007), growth performance of broilers was improved when fed CM based diets with the optimum DEB.

Canola meal contains lower levels of potassium and sodium and higher levels of sulphur, which consequently increase dietary anions (Cl and S), decrease dietary cations (K), lowers dietary cation to anion ratio or DEB and shifts the balance toward acidic side (Johnson and Karunajeewa, 1985; Bell and Keith, 1991; Ruiz-López et al., 1993; Khajali and Slominski, 2012). Higher sulphur levels in CM, in comparison with SBM (1.14 vs 0.44%) may negatively affect growth performance of broilers and interfere with calcium absorption, causing leg problems (Summers et al., 1989; Leeson and Summers, 2001; Khajali and Slominski, 2012).

Low levels of sodium and potassium and high levels of sulphur in CM could be alleviated by adding NaCl, potassium bicarbonate and extra calcium to the CM-based diets resulting in improved growth performance of broilers (March, 1984; Summers, 1995; Newkirk, 2009; Khajali et al., 2011; Khajali and Slominski, 2012). Increasing dietary calcium in broilers partially corrects the acid:base imbalance caused by high sulphur content of CM (Summers, 2005). According to Austic and Keshavarz (1988), the improvement in laying hen egg shell quality in CM-based diets supplemented with

calcium is due to the altered acid:base balance rather than the calcium level per se.

2.4 Improvements to canola meal quality

Over the years, various means have been used to improve the quality of CM for poultry. These include seed dehulling, optimization of processing conditions, breeding for low-fiber canola or the use of exogenous enzymes. In this section, only literature data related to the nutritive value of low-fiber canola and the effect of enzymes have been reviewed.

2.4.1 Genetic selection for yellow-seeded canola

Evaluation of meals derived from new cultivars of *Brassica* in this laboratory showed some positive characteristics related to the yellow-seeded canola (Slominski et al., 1994; Simbaya et al., 1995; Slominski, 1997). Yellow-seeded *B. napus* canola has been developed from interspecific crosses between yellow-seeded *B. juncea*, *B. carinata* and the conventional black-seeded *B. napus* (Rashid and Rakow, 1997; Slominski, 1997).

Higher sucrose and protein contents and lower fiber and glucosinolate contents are among characteristics observed in yellow-seeded canola in comparison with the conventional brown-seeded type. Due to those characteristics, higher ME values are expected (Bell and Shires, 1982; Jiang et al., 1999; Slominski et al., 1999; Slominski et al., 2012). In fact, yellow-seeded canola showed higher TME_n in comparison with its conventional black-seeded counterpart (Slominski et al., 1999). In a study by Bell and Shires (1982), higher NSP digestibility values were observed in yellow-seeded canola in comparison with its brown-seeded counterpart. In recent studies from this laboratory, meals derived from yellow-seeded *B. napus* canola showed superior quality characteristic with more protein and sucrose, less dietary fiber and improved apparent ileal amino acid

digestibility and ME content (Slominski et al., 2012; Jia et al., 2012). It has been demonstrated that quantitative improvements to yellow-seeded *B. napus* may not necessary translate into qualitative changes and improvements in growth performance of broilers (Jia et al., 2012).

Canola hulls are the main source of fiber, and the lower fiber component in yellow-seeded canola is due to the higher seed size and the lower hull weight (15.5 vs 12.0%) in the total seed mass. This is mostly because of thinner hulls and lower polyphenol content in this meal (Stringam et al., 1974; Theander et al., 1977; Slominski et al., 1994; Simbaya et al., 1995; Jiang et al., 1999; Slominski et al., 2012). A higher proportion of cotyledons in yellow-seeded canola, on the other hand, would increase the protein content. A strong negative correlation between the fiber and protein and oil contents has been observed (Stringam et al., 1974; Simbaya et al., 1995; Rashid and Rakow, 1999).

B. juncea canola is a yellow-seeded species with a large pure yellow seed coat. *B. juncea* suffers less from heat and drought stress and matures earlier than *B. napus*. Such characteristics are the basis for high yield of oil and low chlorophyll content of the *B. juncea* seed. In Canada, *B. juncea* is mainly produced in Manitoba, Saskatchewan and Alberta (Jia et al., 2012; Khajali and Slominski, 2012). *B. juncea* contains viscous polysaccharides in the seed coat and thus higher water-soluble fiber content than that of the conventional *B. napus* canola by 1.7%. A different NSP profile with somewhat higher uronic acid content in this meal is an indication of higher pectic polysaccharide content of *B. juncea* meal (Slominski et al., 2012). The GLS composition of *B. juncea* meal is also different with aliphatic 3-butenyl (gluconapin) predominating (Newkirk et al., 1997;

Slominski et al., 2012). Higher water-soluble dietary fiber content of *B. juncea* with potentially viscous properties may have a negative effect on energy and protein digestibility in poultry. In comparison with the conventional *B. napus* canola, this meal would also contain more protein, sucrose and less fiber (Simbaya et al., 1995; Newkirk et al., 1997) leading to potentially higher feeding value *B. juncea* meal (Newkirk et al., 1997).

2.4.2 Exogenous enzymes

Application of exogenous enzymes is among approaches undertaken to improve the nutritive value of CM for poultry. Enzyme preparations break down the cell wall polysaccharides into smaller polymers and therefore eliminate their viscous properties and minimize the encapsulating effect of the cell walls. They also increase the water-soluble NSP content, which contribute to hindgut fermentation and enhanced energy utilization (Theander et al., 1989; Campbell and Bedford, 1992; Graham and Pettersson, 1992; Choct 2002; Slominski et al., 1993; Meng et al. 2005; Gao et al., 2007; Slominski, 2011). Enzymes solubilize the water-insoluble cell wall polysaccharides throughout a slow process of surface peeling which would minimize their efficacy due to the short residence time of the digesta in the gastrointestinal tract (Hotten, 1991; Simbaya et al., 1996). Therefore, it is important that the optimal combination of enzymes is used to target the NSP structures of feed ingredients effectively (Mandel et al., 2005).

Different carbohydrase preparations have been demonstrated to be effective in the cell wall NSP degradation of CM *in vitro* through rapid degradation of water-soluble NSP followed by a slow hydrolysis of water-insoluble NSP (Slominski and Campbell, 1990; Simbaya et al., 1996; Meng et al., 2005). The same enzyme preparation increased

digestibility of protein, starch and NSP as well as growth performance of broiler chickens fed wheat, SBM, CM and peas based diet (Meng et al., 2005). When high levels of CM were used in poultry diets, enzyme supplementation increased NSP and protein digestibility but didn't have a visible and significant effect on growth performance of broiler chickens (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000; Mandel et al., 2005; Mushtaq et al, 2006; Mushtaq et al., 2007). The lack of enzyme effect on growth performance and ME content could be explained by the limited contribution of NSP hydrolysis products to the energy utilization throughout the fermentation process in poultry (Moran, 1982; Khajali and Slominski, 2012)**.** The inconsistent results from using enzymes in CM-based diets could be due to the inclusion level of CM, canola variety, processing and level and type of enzyme used (Chegeni *et al.,* 2011). In a study by Slominski et al. (2011), the effect of multi-carbohydrase enzyme application on AME content was more pronounced in *B. juncea* canola. It seemed that the enzyme preparation had higher affinity towards the viscous properties of NSP present in this species.

2.5 Research Hypothesis

The hypotheses of the current research were that CM meal utilization by poultry could be improved by using the meals from the low-fiber, yellow-seeded varieties, and that specific carbohydrase supplements could be identified and used to depolymerize the cell wall structure of CM, thereby improving NSP digestibility and growth performance of broiler chickens.

3. MANUSCRIPT I

Chemical composition and nutritive value of canola-quality *Brassica juncea* for poultry and the effect of enzyme supplementation[1]

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3.1 Abstract

Canola breeding programs undertaken to improve meal quality has led to the development of canola quality (i.e., low-glucosinolate, low-erucic acid) form of *B. juncea*, a mustard species known for its pure yellow seed coat. Under Western Canadian conditions, *B. juncea* suffers less from heat and drought stress and matures earlier than *B. napus*. Such characteristics are the basis for high yields of oil and low chlorophyll content in the seed. The objective of the current study was to evaluate the chemical and nutritive composition of meals derived from pre-press solvent extracted seeds of the conventional black-seeded *B. napus* canola and the canola-quality yellow-seeded *B. juncea*. In comparison with *B. napus* canola, meal derived from yellow-seeded *B. juncea* contained, on dry matter (DM) basis, similar amount of protein (417 vs. 415 g/kg) and fat (28 vs. 29 g/kg), more sucrose (69 vs. 56 g/kg), more starch (34 vs. 10 g/kg) and less dietary fibre (277 vs. 338 g/kg). Lower fibre content of *B. juncea* canola was reflected in lower content of lignin with associated polyphenols (40 vs. 104 g/kg). The nutritive value of canola meals was investigated with broiler chickens fed maize/soybean meal-based diets containing 300 g/kg of meals from 4 to 18 d of age. A lower ($P<0.05$) body weight gain (BWG) was observed in birds fed the *B. juncea* diet when compared with those fed the conventional black-seeded *B. napus* canola (479 vs. 515 g/bird). No difference in feed conversion ratio (FCR) was observed (1.44 vs. 1.42). Meal apparent metabolisable energy (AME_n) values for *B. juncea* and *B. napus* were determined with broiler chickens (from 14 to 19 d of age) and were 7.9 and 7.8 MJ/kg DM, respectively. Enzyme (multi-carbohydrase) addition resulted in the AME_n value of 8.3 MJ/kg DM for *B. napus* meal, with a more pronounced effect ($P<0.05$) observed for *B. juncea* canola (from 7.9 to 9.3

MJ/kg DM). In the follow-up study, growth performance of broiler chickens fed 15% of canola meals without and with enzyme supplementation was determined in the starter (1-21 d of age) and grower (22-35 d of age) phases of the experiment. Compared to the soybean meal (SBM) Control, canola meals decreased BWG of broiler chickens in the starter phase and the entire trial. Enzyme supplementation improved FCR in the chicks fed *B. juncea* meal ($P<0.05$). The enzyme effect was more pronounced in the young birds.

Keywords: *B. juncea* canola, nutritive value, broiler chicken, enzyme supplementation

Abbreviations: CM, canola meal; DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre; TDF, total dietary fibre, NSP, non-starch polysaccharides; NDICP, neutral detergent insoluble crude protein; BWG, body weight gain; FCR, feed conversion ratio; AME_n, apparent metabolisable energy; SBM, soybean meal.

3.2 Introduction

Canola meal (CM), a co-product of canola oil industry, is a suitable alternative protein source with well-balanced amino acids, especially methionine, for poultry diets (Canola Council of Canada, 2009). However, the presence of anti-nutritional factors such as glucosinolates, phytic acid or some fractions of fibre may restrict a full replacement of soybean meal by CM (Khajali and Slominski, 2012).

By definition, CM should contain less than 30 µmol/g of aliphatic glucosinolates in the meal (Canola Council of Canada, 2015). However, over the years the level of glucosinolates has been reduced significantly by canola breeding and on average it is now 4.6 µmol/g (Adewole *et al*., 2016). *Brassica juncea* mustard, a canola-quality low-glucosinolate and low-erucic acid type has been developed at the Agriculture and Agri-

Food Canada Research Centre in Saskatoon, Canada (Cheng *et al*., 2011). *B. juncea* has agronomic advantage, such as the heat, drought and disease resistance, and matures earlier than the conventional *B. napus* canola. This results in high yields of oil and low chlorophyll content in the seed. *B. juncea* is known for its thinner and pure yellow seed coat. Thinner seed coat would result in the lower hull fibre and consequently lower total fibre content of the meal compared with the conventional *B. napus* canola (Stringam *et al*., 1974; Simbaya *et al*., 1995; Slominski 1997; Beltranena and Zijlstra., 2011; Slominski *et al*., 2012; Zhou *et al*., 2013). The fibre content, including non-starch polysaccharides (NSP) of CM, is believed to be inversely related to energy digestibility (Downy and Bell, 1990) and protein contents (Bell, 1993; Slominski, 1997). Energy-diluting effect of canola fibre has a negative effect on feed intake and body weight gain (Zhou *et al*., 2013). On average, CM contains 180 g/kg of NSP of which very little (i.e., 1.4%) is water-soluble (Slominski and Campbell, 1990; Meng and Slominski, 2005). The major NSP components found in CM are arabinans, arabinogalactans, xyloglucans, galactomannans, and pectic polysaccharides, including rhamnogalacturonans with associated side chains consisting of arabinose, galactose and xylose residues (Siddiqui and Wood, 1977; Slominski and Campbell, 1990).

One way to improve CM utilization is to use carbohydrase enzymes to target NSP of the cell walls (Meng *et al*., 2005; Khajali and Slominski, 2012). Carbohydrase enzymes would facilitate the hydrolysis and/or solubilization of cell wall polysaccharides, and thus would reduce or eliminate the encapsulating effect of cell walls and enhance availability of protein and other nutrients (Theander *et al*., 1989; Campbell and Bedford, 1992; Chesson, 1993). This nutrient encapsulation concept, however, would more apply when

the canola seeds rather than the meal are fed to monogastric animals (Meng *et al*., 2006). This is due to the fact that in the commercial meal any nutrients, including protein, fat, or carbohydrates are freely available for digestion by endogenous enzymes of monogastric animals due to the effect of seed crushing, and thus cell rupture, during the canola pre-press solvent extraction process.

In some earlier studies, multi-carbohydrase supplementation was beneficial and resulted in depolymerization of CM NSP both *in vitro* and *in vivo*, and an improvement ($P<0.05$) in nutrients digestibilities and growth performance was noted (Meng *et al*., 2005). In some other studies, however, enzyme supplementation had no effect on growth performance of broiler chickens (Kocher *et al*., 2000, 2001; Meng and Slominski, 2005; Mushtaq *et al*., 2007). This is understandable since the release of enzyme hydrolysis products would only result in increased lower gut fermentation and the production of short chain fatty acids. This process may not be as effective as that resulting from improved fat, protein, and other nutrient utilization when the partly crushed or expelled canola seed are supplemented with the multi-carbohydrase enzymes. Earlier research from this laboratory demonstrated a significant effect of enzyme supplementation of *B. juncea*-containing diets, with potentially high pectic polysaccharide contents of this meal being responsible for an improvement with enzyme supplementation (Jia *et al*., 2012).

Regardless of enzyme supplementation, however, poor response from *B. juncea* meal in terms of growth performance of broiler chickens and available energy and digestible amino acid contents were observed in earlier studies from this laboratory (Jia *et al*., 2012). In addition, feeding *B. juncea* meal, caused more changes in the intestinal function in turkeys, including lower hydration and higher viscosity of the small intestinal contents

and increased bacterial enzyme activities in the ceaca compared with the conventional *B. napus* canola (Jia *et al*., 2012). Another factor responsible for growth depression and low nutrient utilization could be related to the antinutritive properties of glucosinolates, with *B. juncea* meal showing a distinct difference in the glucosinolate profile when compared with the conventional *B. napus* canola.

The objective of this study was to further evaluate the chemical composition and nutritive value of *B. juncea* canola in comparison with the conventional black-seeded *B. napus*. The effect of *B. juncea* meal, without and with enzyme supplementation, on metabolisable energy and gut viscosity of broiler chickens was investigated. The *B. juncea* meal was produced by the commercial crushing plant using the pre-press solvent extraction process. The same plant and process were used to produce the meal from the conventional black-seeded *B. napus* canola. Two growth performance experiments with broiler chickens fed diets containing high levels of CM without or with enzyme supplementation were conducted.

3.3 Materials and Methods

3.3.1 Plant material

Meal samples from the conventional black-seeded *B. napus* canola, and canola-type yellow-seeded *B. juncea* were obtained from Bunge Canola Processing Plant, Altona, Canada, following crushing of the respective seeds using the conventional pre-press solvent extraction process.

3.3.2 Analytical procedures

In preparation for chemical analyses, samples were ground to pass through a 1 mm sieve. The meals were subjected to crude protein (N × 6.25) analysis using combustion method (AOAC 968.06) and a nitrogen analyzer model TruSpecN (Leco Corp., St. Joseph, MI, USA). Standard AOAC (2005) procedures were used for dry matter (934.01), ether extract (2003.06), total phosphorus (965.17), and ash (942.05) determination. Phytate phosphorus was determined using the procedure described by Haug and Lantzsch (1983).

Samples for amino acid (AA) analysis were prepared according to the AOAC procedures 994.12 and then were determined using an amino acid analyzer (S4300, Sykam GmbH, Eresing, Germany).

Starch was analyzed using the Megazyme Total Starch Kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Carbohydrates glucose, fructose, sucrose, raffinose, and stachyose were determined by gas−liquid chromatography using 3% OV-7 column and Varian 430 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada) as described by Slominski *et al.* (1994). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using an Ankom fibre analyzer (Ankom Technology, Macedon, NY, USA) and AOAC procedures 989.03 and 2002.04, respectively. Non-starch polysaccharides were determined by gas−liquid chromatography (component neutral sugars) using SP-2340 column and Varian CP-3380 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada) and by colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) and the procedure described by Englyst and Cummings (1984, 1988) with modifications (Slominski and Campbell, 1990). The content of NSP was measured in the meals and the

NDF residues. Neutral detergent soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue, and total dietary fibre was determined by summation of NDF and NDF-soluble NSP. The contents of crude protein (N × 6.25) and ash in the NDF residue were also measured. The value for lignin with associated polyphenols was calculated by difference [NDF − (NSP + protein + ash)] (Slominski *et al*., 1994). Water-soluble NSP was determined using the procedure described by Slominski *et al*. (1993).

Glucosinolate analysis was performed as described by Slominski and Campbell (1987). Briefly, 300 mg of canola samples was weighed into 15 mL centrifuge tubes. Two milliliters of methanol, 1.0 mL of benzyl glucosinolate (internal standards, 0.5 mM), and 0.1 mL of lead−barium acetate were added to the tubes, extracted for 3 h at room temperature, and then centrifuged (*g* × 1690). Two milliliters of supernatant was transferred to a DEAE-Sephadex column, which was washed successively with 1 mL of each of 67% methanol, water, and pyridine acetate. Purified sulfatase solution was then added to the column, and the contents were incubated at room temperature overnight. The resulting desulfated glucosinolates were eluted with 4 mL of 60% methanol and dried. The dry residue was trimethylsilylated by adding 0.2 mL of a mixture of anhydrous acetone/N,O-bis (trimethylsilyl) acetamide (BSA)/ trimethylchlorosilane (TMCS)/1-methylimidazole (2:1:0.1:0.05 v/v) and incubated for 30 min at room temperature. The trimethylsilyl derivatives of desulfoglucosinolates were separated by gas−liquid chromatography using a glass column packed with 2% OV-7.

Myrosinase activity was determined by difference between total sample glucosinolate content and the glucosinolates remaining following incubation of the sample with distilled water (autolysis) at 40°C for a defined period of time. Glucosinolate

analysis was conducted as described above. One unit of myrosinase activity was defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmol of glucosinolate per 1 min (Niu *et al*., 2015).

3.3.3 Broiler chicken growth performances Experiment 1

A short-term broiler chicken study was conducted to evaluate the effect of meals derived from pre-press solvent extracted seeds of the conventional black-seeded *B. napus* canola and canola-type (low-glucosinolate) yellow-seeded *B. juncea* mustard. One-day-old male Ross 308 broiler chickens were purchased from a local hatchery and held in electrically heated battery brooders (James Mfg. Co., Monut Joy, PA) for 3 day pre-experimental period to ensure complete yolk sac absorption and were fed commercial chick starter crumbles (210 g/kg crude protein). On day 3, birds were fasted for 4 hours, individually weighted, and randomly distributed among treatments. There were five birds per cage and nine replicated cages per treatment. Birds were provided with continuous light, had free access to water, and were fed maize/soybean meal diets containing 300 g/kg of CM. The diets were formulated to provide 12.4 MJ/kg of ME and 220 g/kg of protein (Table 3.1). Body weight (BW) and feed intake were recorded at the end of experiment on day 19 with cage as the experimental unit. Feed conversion ratio (FCR) was calculated. On day 19, 5 birds were randomly selected from each treatment and killed by cervical dislocation. Fresh digesta (1.5 g) from jejunum was collected and centrifuged at 9961×g for 10 minutes and viscosity of the supernatant solution (0.5 mL) was measured at 40°C using Brookfield digital viscometer (DV-II+LV model; Brookfield Engineering Laboratories Inc., Stoughton, MA).

TABLE 3.1. Composition of experimental diets used in the growth performance Experiment 1

Item	Conventional *B. napus* meal (Control)	*B. juncea* meal
Ingredient (g/kg)		
Maize	463.0	480.0
Soybean meal	146.0	131.0
Conventional *B. napus* meal	300.0	-
B. juncea meal	-	300.0
Canola oil	50.0	47.0
Cacium carbonate	12.0	12.0
Dicalcium phosphate	11.5	11.5
DL- Methionine	0.5	0.4
Mineral premix[1]	5.0	5.0
Vitamin premix[2]	10.0	10.0
Titanium oxide	3.0	3.0
Total	1000.0	1000.0
Calculated composition (g/kg unless specified)		
Metabolisable energy (MJ/kg)	12.4	12.4
Crude protein	221.0	221.0
Calcium	10.2	10.2
Non-phytate P	4.1	4.0
Methionine	5.1	5.1
Methionine + cysteine	9.7	9.7
Lysine	11.7	11.6
Threonine	8.9	8.9
Analyzed composition (g/kg)		
Crude Protein	208.0	208.0

[1]Provided per kilogram of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride)

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg panthotenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg antioxidant, 11 mg virginiamycin, 99 mg monensin sodium

3.3.4 Apparent metabolizable energy (AME_n) assay with broiler chickens (Experiment 2)

A 2 × 2 factorial arrangement of treatments was used to evaluate the effect of CM and enzyme supplementation on metabolisable energy (AME_n) content of meals from conventional *B. napus* and *B. juncea* canola. A basal diet composed of practical feed

ingredients was used and was formulated to provide 13.4 MJ/kg ME and 230 g/kg protein (Table 3.2). The four experimental diets were composed of 70% of basal diet and 30% of test ingredients without or with enzyme supplementation. Each diet contained 3 g/kg of TiO_2 as an internal marker. The enzyme (multi-carbohydrase) supplement Superzyme OM (Canadian Bio-Systems Inc., Calgary, Alberta, Canada) was used and supplied 1,700 U of cellulase, 1,100 U of pectinase, 1,200 U of xylanase, 360 U of glucanase, 240 U of mannanase, 30 U of galactanase, 1,500 U of amylase, 120 U of protease per kg of diet.

One-day old male Ross-308 broiler chickens were purchased from a local commercial hatchery. The management procedures and housing conditions were the same as those described in the growth performance experiment above. A control group was fed the basal diet for the entire trial and was included to calculate the AME_n values of test ingredients by difference. There were 5 birds per cage, 6 replicate cages per treatment. Birds in experimental groups were fed the basal diet from day 3 to 14, and then the diets containing test ingredients were fed from day 15 to 19 (acclimatization period). On day 19, excreta samples from each cage were collected over a 3 h period, immediately frozen at −20 °C, freeze-dried, and finely ground. Excreta samples from the same cage were pooled to yield five replicates per treatment. Duplicate samples of diets and excreta were analyzed for titanium oxide (Lomer *et al*., 2000), nitrogen (nitrogen analyzer, model TruSpecN, Leco Corp., St. Joseph, MI, USA) and gross energy (Parr 6300 calorimerter, Parr Instrument Co., Moline, IL, USA). Nitrogen retention and AMEn values of test ingredients were calculated as described by Leeson and Summers (2001).

TABLE 3.2 Composition of a basal diet used in the AME_n assay Experiment 2

Item	g/kg
Ingredient	
Maize	350.0
Wheat	135.0
Soybean meal	316.0
Dried porcine plasma	50.0
Wheat middlings	40.0
Canola oil	64.0
Cacium carbonate	17.8
Dicalcium phosphate	10.4
DL- Methionine	1.1
Mineral premix[1]	5.0
Vitamin premix[2]	10.0
Total	1000.0
Calculated composition (g/kg unless specified)	
Metabolisable energy (MJ/kg)	13.4
Crude protein	230.0
Calcium	11.0
Non-phytate P	4.5
Methionine	5.0
Methionine + cysteine	8.3
Lysine	14.2
Threonine	9.0
Analyzed composition (g/kg)	
Crude protein	216.0

[1]Provided per kilogram of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride)

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg panthotenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg antioxidant, 11 mg virginiamycin, 99 mg monensin sodium

3.3.5 True metabolizable energy (TME_n) assay with adult roosters

Nitrogen-corrected true metabolizable energy content of canola meals was determined at the University of Illinois using the assay procedure described by Parsons (1985). Briefly, 30 g of each canola meal samples (*B. napus* black, and *B. juncea* yellow)

were precision-fed to 2 groups of 5 individually caged cecectomized Single Comb White Leghorn roosters after 24 h of feed withdrawal. Excreta were then collected during the next 48 h. The excreta samples were frozen, freeze-dried, weighed to determine total output and ground to pass through 1-mm sieve. Feed and excreta were analyzed for nitrogen (990.03, AOAC) and for gross energy using an adiabatic bomb calorimeter. TME_n was calculated as described by Parsons *et al*. (1992). Endogenous corrections for energy were made using fasted for 48 hours roosters to determine the endogenous excretion of energy and nitrogen.

3.3.6 Broiler chicken growth performance Experiment 3

A 3 × 2 factorial arrangement of treatments was conducted to investigate the effects of CM and enzyme supplementation on growth performance of broiler chickens. One-day-old male Ross-308 broiler chickens were obtained from a local commercial hatchery. All birds were randomly assigned to 5 cages of 60 birds each per treatment. The experimental diets were fed from day one. Diets containing wheat/maize/SBM/distiller's dried grains with solubles (DDGS) and either 50 g/kg of CM (Control) or 150 g/kg of CM provided 210 g/kg protein, 13.0 MJ/kg ME in the starter phase (1-21 d of age) and 190 g/kg protein, 13.0 MJ/kg ME in the grower phase (22-35 d of age) of the experiment (Table 3.3). Birds had free access to water and feed and were provided with continuous light. Body weight and feed intake were recorded at the end of each phase. Feed conversion ratio values were also calculated.

TABLE 3.3. Composition of experimental diets used in the broiler chicken growth performance Experiment 3

	Starter phase (1-21 d of age)			Grower phase (22-35 d of age)		
	Control	Conventional *B. napus* meal	*B. juncea* meal	Control	Conventional *B. napus* meal	*B. juncea* meal
Ingredient (g/kg)						
Wheat	320.0	300.0	293.0	341.0	325.0	302.0
Maize	256.0	239.0	254.0	304.0	290.0	314.0
Soybean meal	200.0	126.0	121.0	141.0	65.0	63.0
Conventional *B. napus* meal	50.0	150.0	-	50.0	150.0	-
B. juncea meal	-	-	150.0	-	-	150.0
Fish meal	50.0	50.0	50.0	50.0	50.0	50.0
DDGS	50.0	50.0	50.0	50.0	50.0	50.0
Canola oil	41.0	53.0	5.1	31.0	40.0	40.0
Calcium carbonate	11.2	10.5	10.5	10.5	9.5	9.5
Dicalcium phosphate	6.0	5.4	5.4	3.5	2.6	2.7
DL-Methionine	0.8	0.6	0.5	1.0	0.9	0.9
L-Lysine	-	-	-	2.0	1.0	2.0
Threonine	-	-	-	1.0	1.0	0.9
Mineral premix[1]	5.0	5.0	5	5.0	5.0	5.0
Vitamin premix[2]	10.0	10.0	10.0	10.0	10.0	10.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated composition (g/kg unless specified)						
Metabolisable energy (MJ/kg)	13.0	13.0	13.0	13.0	13.0	13.0
Crude protein	210.0	210.0	210.0	191.0	190.0	191.0
Calcium	10.0	10.1	10.0	9.1	8.9	8.9
Non-phytate P	4.3	4.3	4.3	3.8	3.7	3.7
Methionine	5.1	5.0	5.0	5.0	5.1	5.1
Methionine + cysteine	8.5	8.9	8.8	8.1	8.6	8.6
Lysine	11.1	11.0	11.0	11.1	10.1	11.0
Threonine	7.8	8.0	8.0	7.9	8.0	8.0
Analyzed composition (g/kg)						
Crude protein	217.0	220.0	217.0	203.0	199.0	197.0

[1]Provided per kilogram of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride)

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg panthotenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg

3.3.7 Animal care

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care with the animal protocols approved by the Animal Care and Use Committee of the University of Manitoba and University of Illinois (TME_n assay).

3.3.8 Statistical Analysis

The data were tested by the GLM procedure of the SAS program. Means were separated by Tukey's honestly significant difference. All statements of significance are based on $P \leq 0.05$. Results were presented as means and standard errors of the means**.**

3.4 Results and discussion

3.4.1 Chemical composition of canola meals

Table 3.4 shows the chemical composition of meals derived from canola-quality *B. juncea* mustard and the conventional *B. napus* canola. There was no difference between crude protein content of two meals. The values of 419 and 417 g/kg were close to the crude protein content of CM reported earlier (Bell and Keith, 1991; Canola Council of Canada, 2015). The ether extract content in both CM were similar, demonstrating no difference in the level of gums and other oil refining byproducts being added back to the

meal following oil extraction (Canola Council of Canada, 2015). The values were slightly lower than the value of 38 g/kg (as fed basis) or 35 g/kg (DM basis) reported earlier for conventional CM by NRC (1994) and Adewole *et al*. (2016), respectively, but higher than the value of 18 g/kg demonstrated earlier by Slominski *et al*. (2012) where no oil refining by-products were added back to the meals.

There was no difference in simple sugar and oligosaccharide contents between the conventional canola and *B. juncea* meal, with the quantities in agreement with the earlier reports from this laboratory (Slominski *et al*., 1994; 1999; 2012). Sucrose content was higher in the meal from *B. juncea* canola in comparison to its black-seeded counterpart (69 vs 56 g/kg DM) with similar values to those reported earlier (Slominski *et al.,* 1994). High sucrose content of *B. juncea* canola is an interesting phenomenon and it has been observed in other species, including yellow-seeded *B. napus* canola. In this context, a positive correlation between the percentage of yellow seeds in the sample and the concentration of sucrose in yellow-seeded canola has been reported (Slominski *et al*., 1994; 1999; 2012).

Acid and neutral detergent fibre values were lower in *B. juncea* canola (118 and 176 g/kg DM) in comparison with the conventional meal (217 and 270 g/kg DM). This is in agreement with the earlier studies demonstrating much lower NDF values for yellow-seeded canola (Stringam *et al*., 1974; Slominski and Campbell, 1990; Slominski *et al*., 1994) and reflects the thinner seed coat in yellow-seeded canola.

Total dietary fibre was lower in *B. juncea* "yellow" primarily due to the lower content of lignin with associated polyphenols. These findings are in agreement with earlier studies from this laboratory demonstrating similar results in yellow seeded canola

meals (Slominski and Campbell, 1990; Slominski *et al*., 1994; 1999; 2012). There was no difference in the NSP and water-soluble NSP content between the meals, which is in agreement with the earlier study (Slominski *et al*., 2012). However these authors demonstrated different NSP profile especially higher uronic acid contents in *B. juncea* meal, which is an indication of higher pectic polysaccharide contents in this meal.

Glycoproteins represent the structural protein of the cell walls and potentially the Maillard reaction products formed during the desolventization and toasting step of processing which results in the neutral detergent insoluble protein formation (Van Soest, 1994). Therefore, high glycoprotein content is an indication of Maillard reaction with the Maillard reaction products contributing to the dietary fibre content and thus being poorly digested by poultry (Jia *et al*., 2012). The lower lignin and polyphenol content of *B. juncea* mustard in this study is in agreement with earlier reports which demonstrated that yellow-seeded canola species are lower in lignin and polyphenol contents with polyphenols rather than lignin contributing to this fraction of canola (Theander *et al*., 1977; Slominski *et al*., 1994; Simbaya *et al*., 1995). The lower polyphenol content of yellow-seeded canola is a result of reduction or elimination of hull proanthocyanidins during the canola breeding programs, which results in the yellow seed coat color and an increase in embryo's oil content (Rashid and Rakow, 1999; Slominski *et al*., 1999; 2012). Although the amounts of phenolic compounds, including condensed tannins, are higher in canola seeds compared to soybean seeds, a great proportion of condensed tannins are located in the cells of hull fraction and are in the water-insoluble form (Naczk *et al*., 2000; Jia *et al*., 2012; Khajali and Slominski, 2012). However, lower inclusion levels of CM than that of SBM decreases the concentration of polyphenolic compounds in the diet

to the negligible levels so their direct anti-nutritional effect is minimal (Mansoori *et al*., 2015). However, the high polyphenol contents of conventional black-seeded rapeseed/canola would result in nutrient (i.e., protein and energy) dilution and has to be taken into consideration (Slominski *et al*., 2012).

As demonstrated in Table 3.5, *B. juncea* meal had higher glucosinolate content than *B. napus* "black", although these values are still within the definition of canola meal (i.e., less than 30 µmole/g of aliphatic glucosinolates). Newkirk *et al*. (1997) also found higher glucosinolate content in *B. juncea* in comparison with *B. napus* and *B. rapa* canola. Slominski *et al*. (2012) on the other hand, found lower glucosinolate content in *B. juncea* than the conventional meal. Adewole *et al*. (2016) observed different glucosinolate contents in meals from various crushing plants across Canada, which indicates the effect of processing on the glucosinolate content of CM and further explains the difference in glucosinolate content of the current *B. juncea* meal in comparison with the meal used in the earlier studies (Slominski *et al*., 2012). These authors, however, found different glucosinolate profile of *B. juncea* with gluconapin (3-butenyl) predominating, which is in agreement with this study. Beltranena and Zijlstra (2011) also found higher content (11 vs. 5 µmol/g) and different composition of glucosinolates in *B. juncea*. The implication of such difference on the nutritive value of the meal is not clear. There was no myrosinase activity detected in both the conventional and *B. juncea* meals, which indicates that sufficient heat-treatment conditions were used in the pre-press solvent extraction process for this enzyme inactivation.

TABLE 3.4. Chemical composition of conventional *B. napus* canola and canola quality *B. juncea* meals used in the study (g/kg, dry matter basis)[1]

Component	Conventional meal	*B. juncea* meal
Crude protein	415.0	417.0
Ether extract	29.0	28.0
Carbohydrates		
Simple sugars[2]	1.0	1.0
Sucrose	56.0	69.0
Oligosaccharides[3]	15.0	14.0
Starch	30.0	34.0
Fibre fractions		
Acid detergent fibre (ADF)	217.0	118.0
Neutral detergent fibre (NDF)	270.0	176.0
Total dietary fibre (TDF)	338.0	277.0
Non-starch polysaccharides (NSP)	205.0	210.0
NSP component sugars		
Arabinose	43.8	45.5
Xylose	19.0	20.4
Mannose	4.2	3.6
Galactose	16.1	16.6
Glucose	66.5	66.7
Uronic acid	55.0	54.8
Water-soluble NSP	28.0	24.0
Water-insoluble NSP	182.0	184.0
Lignin and polyphenols	104.0	44.0
Glycoprotein (NDICP[4])	29.0	23.0
Ash	83.0	79.0
Total phosphorus (P)	11.3	11.6
Phytate P	7.1	7.3
Non-phytate P	4.2	4.3
Glucosinolates (µmol/g)	9.5	16.7
Myrosinase activity (U/g)	0.0	0.0

[1]Samples were analysed in duplicates.
[2]Includes glucose and fructose
[3]Includes raffinose and stachyose
[4]Neutral detergent insoluble crude protein

TABLE 3.5. Glucosinolate content of conventional *B. napus* canola and canola quality *B. juncea* (μmol/g, dry matter basis)[1]

Glucosinolate	Conventional *B. napus* meal	*B. juncea* meal
Sinigrin (2-propenyl)	0.0	0.2
Gluconapin (3-butenyl)	2.0	13.5
Glucobrassicanapin (4-pentenyl)	0.2	0.5
Progoitrin ((2R)-2-hydroxy-3-butenyl)	3.8	1.1
Epi-progoitrin ((2S)-2-hydroxy-3-butenyl)	0.2	0.0
Gluconapoleiferin (2-hydroxy-4-pentenyl)	0.3	0.4
Glucoalyssin (5-methylsulphonylpentyl)	0.4	0.0
Glucobrassicin (3-indolylmethyl)	0.4	0.1
Hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl)	2.0	0.8
Neoglucobrassicin (1-methoxy-3-indolylmethyl)	0.2	0.1
Total	9.5	16.5

[1]Samples were analysed in duplicates.

Table 3.6 shows amino acid composition of *B. juncea* meal in comparison with the conventional *B. napus* meal. With the exception of arginine, *B. juncea* showed similar or higher amino acid levels than the conventional *B. napus* meal. Higher lysine content of *B. juncea* could be considered a desirable characteristic of this meal. This result is contrary to earlier studies, which demonstrated lower lysine and methionine content in different samples of *B. juncea* (Slominski *et al*., 1999; 2012). In general, the current *B. juncea* meal had higher ether extract and glucosinolate contents in comparison with the meal used earlier (Slominski *et al*., 2012). Both meals had similar water-soluble NSP contents and showed similar NSP component sugar profiles.

3.4.2 Broiler chicken growth performance Experiment 1

Growth performance of broiler chickens fed diets containing 300 g/kg of CM is shown in Table 3.7. Birds fed a *B. juncea* diet had lower ($P<0.05$) feed intake and body weight gain than those fed the conventional meal. However, FCR was not different between the

TABLE 3.6. Amino acid contents of conventional *B. napus* canola and canola-quality *B. juncea* (dry matter basis).

Amino acid	g/kg DM		g/16 g N	
	Conventional meal	*B. juncea* meal	Conventional meal	*B. juncea* meal
Indispensable amino acids				
Arginine	26.2	23.8	6.31	5.70
Histidine	10.9	10.9	2.63	2.60
Isoleaucine	13.3	13.8	3.20	3.30
Leucine	29.2	29.5	7.05	7.06
Lysine	21.3	22.7	5.14	5.44
Methionine	8.0	8.5	1.93	2.03
Phenylalanine	15.6	16.1	3.75	3.85
Valine	16.8	18.2	4.06	4.37
Dispensable amino acids				
Alanine	19.1	19.4	4.61	4.64
Aspartic acid	32.5	30.6	7.83	7.34
Cystine	8.0	9.6	1.94	2.29
Glutamic acid	73.7	77.3	17.76	18.53
Glycine	20.5	20.8	4.94	4.98
Proline	24.7	27.3	5.94	6.54
Serine	18.7	18.7	4.52	4.48
Tyrosine	1.06	10.8	2.55	2.60
Total	367.5	376.2	-	-

[1]Samples were analysed in duplicates.

treatments. This is contrary to the earlier study from this laboratory showing similar BWG but higher FCR in birds fed 300 g/kg of *B. juncea* meal (Jia *et al*., 2012). The gut viscosity values were low and didn't show any difference between the treatments. In agreement with this study, Kocher *et al*. (2000) also demonstrated very low gut viscosity throughout intestinal tract of birds fed CM. Gut viscosity tends to increase for the diets high in water-soluble NSP resulting in poor growth performance. However, the viscosity values observed in the current study would be considered to have minimal effect on growth performance.

Earlier studies have demonstrated the negative effect of glucosinolates of canola meal on the voluntary feed intake of broiler chickens even at the low levels (Mushtaq *et*

al., 2007; Min *et al*., 2011), which in turn, could affect feed intake and growth performance of the chickens (Tripathi and Mishra, 2007). Glucosinolates may reduce bird appetite and adversely affect liver function and metabolic activities. This will increase the expenditure of energy and other nutrients such as amino acids, minerals and vitamins in visceral organs for maintenance at the expense of growth and consequently would decrease body weight gain of broiler chickens (Mailer *et al*., 2008; Woyengo *et al*., 2011). Higher levels of CM have been used in earlier studies without having any negative effect on growth performance of broilers. Ahmad *et al*. (2007) used up to 200 g/kg and Naseem *et al*. (2006), SariCiqek and Serdar (2006) and Min *et al*. (2011) up to 250 g/kg of CM without any adverse effect on growth performance. However, the dietary glucosinolate level is also important. The glucosinolate profile of *B. juncea* mustard could be responsible for the poor performance of birds (Slominski *et al*, 1999; Jia *et al*., 2012). In addition, the negative effect of glucosinolates could be more pronounced in younger birds (Ahmad *et al*., 2007). As birds used in the current study were younger than 18 days old, they were expected to have less tolerance to glucosinolates, especially the aliphatic ones present in *B. juncea* meal.

TABLE 3.7. Effect of canola type on growth performance of broiler chickens (5-19 d of age), Experiment 1[1]

Item	Feed intake (g/bird)	Body weight gain (g/bird)	FCR (g feed/g gain)	Viscosity (mpa.s)
B. napus meal	732[a]	515[a]	1.42	1.40
B. juncea meal	689[b]	479[b]	1.44	1.55
SEM	8.8	4.8	0.021	0.062

[1]Each diet was fed to nine replicate cages of 5 birds each.
[ab]Means within columns with no common letters differ significantly (P<0.05).

3.4.3 Apparent (AME_n) and true (TME_n) metabolizable energy contents (Experiment 2)

Table 3.8 shows the apparent metabolisable energy values of conventional and *B. juncea* meals and the effect of enzyme supplementation. There was no difference in AME_n content between the meals. Inclusion of enzyme in both meals increased the metabolisable energy values for conventional meal by 7.7 and for *B. juncea* by 12.6% ($P<0.05$). Contrary to this study, Newkirk *et al*. (1997) demonstrated higher energy utilization in *B. juncea* meal. On the other hand, Jia *et al*. (2012) observed lower metabolisable energy values for *B. juncea* meal than *B. napus* species. Apparent metabolisable energy values in this study are in agreement with the mean value of 8.5 MJ/kg reported in earlier studies (NRC, 1994; Newkirk *et al*., 1997; Mandal *et al*., 2005). Ether extract, sucrose, fibre and glucosinolate contents are some of the factors that are responsible for the difference in AME_n values of canola meals (Mandal *et al*., 2005; Jia *et al*., 2012). Contrary to earlier studies (Hijikuro and Takemasa, 1985; Lessire *et al*., 1986) the glucosinolate content of *B. juncea* meal does not seem to have negative effect on AME_n values. This could be explained by the short experimental period of the AME_n assay, which could minimize the negative effect of glucosinolates. Although both meals contained similar oil contents, the lower fibre and higher sucrose contents of *B. juncea* meal didn't seem to improve the AME_n values.

Similarly to earlier study in our laboratory (Jia *et al*., 2012), enzyme addition increased energy values of *B. juncea* meal ($P<0.05$). According to Jia *et al*. (2012) the enzyme cocktail seems to have superior effect on *B. juncea* most likely due to a different NSP profile and/or water solubility. However, it was not the case in the current experiment. Earlier studies in our laboratory showed an increase ($P<0.05$) in NSP

digestibility but only a trend in AME_n improvement supplemented in birds fed CM with enzyme (Meng and Slominski, 2005). Somewhat similar results were observed by Simbaya *et al*. (1996), Kocher *et al*. (2000), and Mushtaq *et al*. (2007) when using less diversified enzyme cocktails.

As demonstrated in Table 3.8, true metabolizable energy value of the canola meals, was significantly higher in *B. juncea* meal than the conventional *B. napus* meal. This is in agreement with earlier study demonstrating higher TME_n value for the yellow-seeded canola in comparison with its black-seeded counterpart (Slominski *et al*., 1999), which could be justified by the difference in processing condition and carbohydrate and oil contents of the meal. In the current study, however, there was no difference in the oil content of the two meals although *B. juncea* meal contained more sucrose and starch than the conventional *B. napus* meal.

3.4.4 Broiler chicken growth performance Experiment 3

Growth performance of broiler chickens fed diets containing 150 g/kg of CM and the effect of enzyme supplementation is shown in Table 3.9. Diets containing CM decreased feed intake in the starter phase of the experiment although this effect was significant ($P<0.05$) only in the chicks fed *B. juncea* meal. Same pattern was observed in the grower phase when the conventional meal showed the reduction in feed intake compared to the Control. For the entire trial, chicks fed canola meals had lower feed intake ($P<0.05$) than that of the Control. Statistical analysis showed no enzyme effect on feed intake in the starter and grower phases as well as for the entire trial.

Body weight gain decreased ($P<0.05$) for the diets containing canola meals, however, *B. juncea* meal showed the lowest BWG in the starter phase. In the grower

TABLE 3.8. Effect of canola type and enzyme supplementation on apparent (AME_n)[1] and true (TME_n)[2] metabolisable energy content (MJ/kg, dry matter basis), Experiment 2

		AME_n	TME_n
Meal			
	Conventional meal	7.85[b]	9.15[b]
	B. juncea meal	8.60[a]	9.86[a]
	SEM	0.228	0.187
Enzyme[3]			
	-	7.82[b]	-
	+	8.62[a]	-
	SEM	0.263	-
Meal × Enzyme			
	Conventional meal	7.75[b]	-
	Conventional meal + enzyme	7.95[b]	-
	B. juncea meal	7.87[b]	-
	B. juncea meal + enzyme	9.30[a]	-
	SEM	0.373	-
P value			
Meal		0.00	0.03
Enzyme		0.02	-
Meal × Enzyme		0.07	-

[1]Each diet was fed to six replicate cages of 5 birds each.

[2]Each diet was fed to 5 individually caged cecectomized roosters.

[3]Enzyme supplement provided 1,700 U of cellulase, 1,100 U of pectinase, 1,200 U of xylanase, 360 U of glucanase, 240 U of mannanase, 30 U of galactanase, 1,500 U of amylase, 120 U of protease per kg of diet

[ab]Means within columns with no common letters differ significantly (P<0.05)

TABLE 3.9. The effect of canola type and enzyme supplementation on growth performance of broiler chickens (1-35 d of age), Experiment 3[1]

Effect	Feed intake (g/bird)			Body weight gain (g/bird)			Feed conversion ratio (g feed/g gain)		
	1-21 d	22-35 d	1-35 d	1-21 d	22-35 d	1-35 d	1-21 d	22-35 d	1-35 d
Meal									
Control	1220^{a}	2102^{a}	3321^{a}	846^{a}	1248^{a}	2093^{a}	1.44^{c}	1.68^{c}	1.59^{c}
Conventional meal	1157^{ab}	1965^{b}	3121^{b}	749^{b}	1090^{b}	1839^{b}	1.54^{b}	1.80^{b}	1.70^{b}
B. juncea meal	1116^{b}	2000^{ab}	3107^{b}	681^{c}	1045^{b}	1725^{c}	1.64^{a}	1.91^{a}	1.80^{a}
SEM	20.7	33.8	44.8	6.8	13.5	17.7	0.02	0.03	0.02
Enzyme									
-	1168	2009	3176	741^{b}	1114	1853^{b}	1.58^{a}	1.81	1.72^{a}
+	1161	2036	3191	776^{a}	1142	1917^{a}	1.49^{b}	1.79	1.67^{b}
SEM	16.6	26.6	36.5	5.5	10.6	14.5	0.02	0.02	0.02
Meal × Enzyme									
Control	1210^{a}	2064^{ab}	3272	818^{b}	1224^{a}	2038^{b}	1.48^{bc}	1.69^{b}	1.60^{c}
Control + enzyme	1230^{a}	2140^{a}	3370	875^{a}	1272^{a}	2147^{a}	1.40^{c}	1.68^{b}	1.57^{c}
Conventional meal	1145^{ab}	2012^{ab}	3155	735^{c}	1087^{b}	1822^{c}	1.55^{bc}	1.85^{ab}	1.73^{ab}
Conventional meal + enzyme	1170^{ab}	1917^{b}	3087	762^{c}	1092^{b}	1855^{c}	1.53^{bc}	1.76^{ab}	1.66^{bc}
B. juncea meal	1150^{ab}	1950^{ab}	3100	670^{d}	1030^{b}	1700^{d}	1.72^{a}	1.89^{a}	1.83^{a}
B. juncea meal + enzyme	1082^{b}	2050^{ab}	3115	692^{d}	1060^{b}	1750^{cd}	1.56^{b}	1.93^{a}	1.78^{ab}
SEM	27.6	47.8	63.3	9.1	19.0	25.1	0.03	0.04	0.03
Effects and their significance									
Meal	0.006	0.03	0.004	0.001	0.001	0.001	0.001	0.001	0.001
Enzyme	0.65	0.46	0.72	0.001	0.08	0.001	0.001	0.59	0.04
Meal × Enzyme	0.21	0.11	0.42	0.17	0.51	0.27	0.17	0.27	0.86

[1]Each diet was fed to six replicate cages of 60 birds.

[2]Enzyme supplement provided 1,700 U of cellulase, 1,100 U of pectinase, 1,200 U of xylanase, 360 U of glucanase, 240 U of mannanase, 30 U of galactanase, 1,500 U of amylase, 120 U of protease per kg of diet

[ab]Means within columns with no common letters differ significantly (P<0.05).

phase, BWG for canola-containing diets were lower (P<0.05) than the Control but no difference was observed between canola diets. Overall, *B. juncea* canola had the lowest BWG and both canola meals had lower BWG than the Control. Enzyme had significant effect (P<0.05) on BWG in the starter phase and the entire experiment.

Feed conversion ratio increased (P<0.05) for the diets containing canola meals in all phases, however *B. juncea* meal increased FCR even further in comparison with the conventional meal. Enzyme supplementation improved (P<0.05) the FCR in the starter phase and the entire trial. Effect of enzyme on FCR was less pronounced in the older birds (22-35 d of age).

Earlier studies have shown that several anti-nutritional factors of canola meal such as fibre, tannins and glucosinolates may be responsible for lower body weight gain in broiler chickens. Dietary fibre of CM has been implicated to reduce nutrient digestibility and absorption of protein and energy for the growth (Kocher *et al*., 2000; Meng *et al*., 2005; Landero *et al*., 2012). Tannins with bitter flavour may decrease feed intake as well as palatability of canola meal (Mansoori and Acamovic, 2007; Khajali *et al*., 2011). They may also reduce digestibility of protein by forming complexes in the gastrointestinal tract (Khajali and Slominski, 2012; Gopinger *et al*., 2014). Glucosinolates on the other hand, increase liver metabolic activities and energy consumption for maintenance in the expense of growth (Woyengo *et al*., 2011). Moreover, they cause bitter taste and negatively affect appetite and feed intake (Mushtaq *et al*., 2007; Mailer *et al.,* 2008; Min *et al*., 2011). Young birds have less tolerance to glucosinolates. According to Ahmad *et al*., (2007) only birds older than 21 days can tolerate 300 g/kg of canola meal with no effect on the performance. Canola meals, which are low in glucosinolates,

have been used up to 350 g/kg without adverse effect in broilers (Kocher *et al*. 2001). However, all the meals used in the current study were much lower in glucosinolate content. In this context, quantities as low as 4 μmol of glucosinolates per gram of the diet has been indicated as "no effect" level in broiler chicken diets (Khajali and Slominski, 2012).

In agreement with the present study, earlier research showed a lower BWG in chickens fed diets containing *B. juncea* meal than those containing *B. napus* or *B. rapa* meals (Slominski *et al*., 1999; Jia *et al*., 2012) and similarly to the current study the only factor that was different in *B. juncea* meal was its higher glucosinolate content which could be responsible for the poor growth performance. Moreover, different glucosinolate profile in *B. juncea* has to be taken into consideration as a factor responsible for growth depression (Jia *et al*., 2012).

Previous research has shown the positive effect of multi-carbohyrase enzyme in depolymerizing CM cell wall polysaccharides *in vitro* (Slominski and Campbell, 1990; Simbaya *et al*., 1996; Meng *et al*., 2005). However, when the same enzyme was applied *in vivo* the results were less promising due to the short transit time of digesta in the gastrointestinal tract (Shires *et al*., 1987; Simbaya *et al*., 1996). Jamroz *et al*. (2004) demonstrated an improvement in FCR of broilers fed the enzyme-supplemented diet. Kocher *et al*. (2001) demonstrated no effect of enzyme addition on growth performance of broilers fed high dietary levels of canola meal. Similarly, Simbaya *et al*. (1996), Meng and Slominski (2005) and Mushtaq *et al*. (2007) observed no effect of multi-carbohydrase enzyme in birds fed CM-based diets.

In the current study, however, the multi-carbohydrase supplementation significantly improved BWG and FCR for the starter phase and the entire trial. Earlier studies have confirmed the importance of using ingredient-specific combination of enzymes or "enzyme cocktail", which target the NSP structure of the feed ingredient of the choice (Chesson, 1993; Classen *et al*., 1996; Simbaya *et al*., 1996; Mandal *et al*., 2005; Meng *et al*., 2005). In agreement with the current study, earlier studies in our laboratory demonstrated a greater response of *B. juncea* meal than the other meals to the multi-carbohydase supplementation (Jia et al., 2012). In this context, water-soluble NSP, which are known to be more susceptible to enzyme addition (Danicke *et al*., 1999), were found to be similar in *B. juncea* and *B. napus* meals used in the earlier (Jia *et al*., 2012) and current study.

3.5 Conclusions

In comparison with the conventional meal, meal derived from *B. juncea* mustard contained similar amounts of protein (417 vs 415 g/kg DM), ether extract (28 vs 29 g/kg) and metabolisable energy (7.9 vs 7.8 MJ/kg), less dietary fibre (277 vs 338 g/kg DM) and more sucrose (69 vs 56 g/kg DM) and glucosinolates (16.7 vs 9.5 μmol/g). The higher glucosinolate content and their different profile in *B. juncea* meal could cause a reduction in body weight gain of broilers, especially in the starter phase. Multi-carbohydrase enzyme addition increased the BWG and FCR in younger birds fed CM.

3.6 Acknowledgements

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4. MANUSCRIPT II

Chemical composition and nutritive value of new low-fiber *Brassica napus* and *Brassica juncea* canola meal for broiler chickens

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4.1 ABSTRACT: Breeding attempts to increase the oil in the seed and to reduce the fiber content in the meal have led to the development of yellow-seeded *B. napus* canola and canola-quality *B. juncea*. The objective of the current study was to evaluate the chemical and nutritive composition of meals derived from yellow-seeded *B. napus* and *B. juncea* canola.

Apparent metabolizable energy (AME_n) and standardized ileal amino acid digestibility (SID) of yellow-seeded *B. napus*, *B. juncea*, and the conventional CM were determined with broiler chickens of 14 to 19 d of age (AME_n assay), or 14 to 21 d of age (SID assay) using 6 pens of 6 birds each per treatment. The nutritive value of canola meals was further validated in a growth performance study using 7 pens of 50 broiler chickens per treatment. Birds were fed wheat/corn/soybean meal-based diets containing 15% of canola meals in the starter (1-10 d), grower (11-24 d), and finisher (25-36 d) phases of the experiment. In comparison with the conventional meal, yellow-seeded *B. napus* and *B. juncea* contained (DM basis) more crude protein (43.4 and 47.2 vs. 41.1%), more sucrose (10.1 and 8.0 vs. 6.6%), and less total dietary fiber (29.8 and 28.9 vs. 35.0%), respectively. The highest content of all essential amino acids (except cysteine) was observed in *B. juncea* meal. The AME_n and SIAAD values for yellow-seeded *B. napus*, *B. juncea* canola, and the conventional black-seeded *B. napus* were 1865, 2092 and 1902 kcal/kg DM, and 82.5, 83.2, and 81.8%, respectively. In the growth performance study, BWG averaged 2.32, 2.30, 2.19, and 2.31 kg for the Control, black and yellow *B. napus*, and *B. juncea* meals, respectively, and no significant difference in FCR between the control and diets containing canola meals were observed indicating that all types of canola meal could be used effectively to replace SBM in broiler chicken rations

providing that the diets are formulated based on digestible amino acids and available energy contents.

Key words: Low-fiber canola, chemical composition, nutritive value, broiler chicken

4.2 INTRODUCTION

Canola is an important oilseed crop with 3 million tons of canola oil and 4 million tons of canola meal (CM) being produced annually in Canada (CCC, 2015). As a co-product of oil extraction, CM is widely used in livestock diets as a source of protein, with well balanced amino acids.

When compared to soybean meal (SBM), CM inclusion rates in poultry diets are limited due to the presence of several anti-nutritive factors, including glucosinolates, tannins, phytic acid, and certain fiber components. Fiber components of CM mainly originate from the hull fraction of canola seed (Bell and Shires, 1982) and consist of cellulose (4-6%), non-cellulosic polysaccharides (13-16%), lignin and polyphenols (8%), glycoproteins (3.5%) and minerals (1%) associated with the cell walls (Slominski and Campbell, 1990; Slominski et al., 1994). Fiber components are poorly utilized by poultry and are inversely related to metabolizable energy and digestible protein content of CM (Slominski, 1997). Based on the NRC specification (NRC, 1994), the metabolizable energy content of CM is 2000 kcal/kg, and is approximately 230 kcal/kg lower than that of SBM (Khajali and Slominski, 2012).

Selection for low-fiber, yellow-seeded canola, seed de-hulling, and the use of exogenous enzymes have been among approaches undertaken to improve the nutritive value of CM for monogastric animals (Khajali and Slominski, 2012). Some earlier studies

have demonstrated positive characteristics of yellow-seeded samples of canola in comparison with their black-seeded counterparts (Slominski et al., 1994; Simbaya et al., 1995; Slominski, 1997). Recently, further improvements to the agronomics (i.e., increased yield), and quality characteristics (i.e., true yellow color, low fiber, increased oil) of yellow-seeded *B. napus* canola have been achieved at the Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada. Such improvements led to the development of yellow-seeded *B. napus* canola with superior quality characteristics in comparison with the conventional black-seeded type (Somers et al., 2011). In addition, canola-quality *B. juncea* mustard has been developed. Under Western Canadian conditions, *B. juncea* suffers less from the heat and drought stress and matures earlier than *B. napus*. Such characteristics are the basis for high yields of oil and low chlorophyll content in the seed (Cheng et al., 2011).

The use of enzymes is among approaches to improve the nutritive value of CM. High fiber content of CM makes the cell wall degrading enzymes an effective choice in reducing nutrient encapsulating effects of cell walls to improve nutrient availability by poultry (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000, 2001; Meng et al., 2005; Meng and Slominski, 2005).

Based on the Canola Council of Canada recommendations (2015), CM could be included up to 10% in the starter and 20% in the grower diets for broiler chickens. However, Min et al. (2011) used 25% of CM in broiler diets while Kocher et al. (2000) replaced SBM with up to 100% of CM without any negative effect on growth performance. In another study, however, Peyvastegan et al. (2012) demonstrated that

20% of CM in a diet negatively affected growth and feed conversion ratio in broiler chickens.

The objective of this study was to evaluate the chemical composition and nutritive value of pre-press solvent extracted meals derived from black- and yellow-seeded *B. napus* canola and canola-quality yellow-seeded *B. juncea*. Apparent (AME_n) and true (TME_n) metabolizable energy contents and standardized ileal amino acid digestibility (SIAAD) of the meals were determined. The effect of a multi-carbohydrase supplement on the AME_n contents of different CM was also investigated. A growth performance study was conducted using diets containing 15% of different canola meals and fed to 1-35 d old broiler chickens. Diets were formulated based on the determined AME_n and SIAAD values.

4.3 MATERIALS AND METHODS

4.3.1 Plant Material

Large quantities of seeds of black-seeded *B. napus* canola and canola type yellow-seeded *B. juncea*, were produced and crushed at Bunge Canola Processing Plant, Altona, MB, Canada, using the conventional pre-press solvent extraction process. Seeds of yellow-seeded *B. napus* canola were processed at the POS Pilot Plant in Saskatoon, SK, Canada, using the same pre-press solvent extraction.

4.3.2 Analytical Procedures

For chemical analyses, CM samples were ground to pass through a 1 mm sieve. The meals were subjected to crude protein (N×6.25) analysis using a combustion method

(AOAC 968.06) and a nitrogen analyzer model TruSpecN (Leco Corp., St. Joseph, MI, USA). Standard AOAC (2005) procedures were used for dry matter (934.01), ether extract (2003.06), ash (942.05), and total phosphorus (965.17) determination. Phytate phosphorus was determined using the procedure described by Haug and Lantzsch (1983).

Samples for amino acid (AA) analysis were prepared according to the AOAC procedure 994.12 and were determined using an amino acid analyzer S4300 (Sykam GmbH, Eresing, Germany). Starch was analyzed using the Megazyme Total Starch Kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Carbohydrates glucose, fructose, sucrose, raffinose, and stachyose were determined by gas−liquid chromatography using a 3% OV-7 column and a Varian 430 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada) as described by Slominski et al. (1994). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined using an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA) and AOAC procedures 989.03 and 2002.04, respectively. Non-starch polysaccharides (NSP) were determined by gas−liquid chromatography (component neutral sugars) using SP-2340 column and Varian CP-3380 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada) and by colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) and the procedure described by Englyst and Cummings (1984, 1988) with modifications (Slominski and Campbell, 1990). The content of NSP was measured in the meals and in the NDF residues. Total dietary fiber (TDF) was calculated as the sum of NDF and detergent-soluble NSP. Detergent soluble NSP was calculated as total NSP minus NSP present in the NDF residue. The contents of crude protein (N×6.25) and ash in the NDF residues were also measured. The value for lignin

with associated polyphenols was calculated by difference [NDF − (NSP + protein + ash)] (Slominski et al., 1994).

Glucosinolate analysis was performed as described by Slominski and Campbell (1987). Briefly, 300 mg of canola samples was weighed into 15 mL centrifuge tubes. Two milliliters of methanol, 1.0 mL of benzyl glucosinolate (internal standards, 0.5 μM), and 0.1 mL of lead−barium acetate were added to the tubes, extracted for 3 h at room temperature, and then centrifuged ($g \times 1690$). Two milliliters of supernatant were transferred to a DEAE-Sephadex column, which was washed successively with 1 mL of each of 67% methanol, water, and pyridine acetate. Purified sulfatase solution was then added to the column, and the contents were incubated overnight at room temperature. The resulting desulfated glucosinolates were eluted with 4 mL of 60% methanol and dried. The dry residue was trimethylsilylated by adding 0.2 mL of a mixture of anhydrous acetone/N,O-bis (trimethylsilyl) acetamide (BSA)/trimethyl-chlorosilane (TMCS)/1-methylimidazole (2:1:0.1:0.05 v/v) and incubated for 30 min at room temperature. The trimethylsilyl derivatives of desulfoglucosinolates were separated by gas-liquid chromatography using a glass column packed with 2% OV-7.

Myrosinase activity was determined by difference between total sample glucosinolate content and the glucosinolates remaining following incubation of the sample with distilled water (autolysis) at 40°C for a defined period of time. Glucosinolate analysis was conducted as described above. One unit of myrosinase activity was defined as the amount of enzyme that catalyzes the hydrolysis of 1 μmol of glucosinolate per 1 min (Niu et al., 2015).

4.3.3 Apparent Metabolizable Energy (AME_n) Assay with Broiler Chickens

A 3 × 2 factorial arrangement of treatments was used to evaluate the effect of CM without and with enzyme supplementation on metabolizable energy (AME_n) content for broiler chickens. The six experimental diets were composed of 70% of basal diet and 30% of 3 types of CM as test ingredients without or with enzyme supplementation. The enzyme (multi-carbohydrase) supplement Superzyme OM (Canadian Bio-Systems Inc., Calgary, AB, Canada) was used and supplied 1,700 U of cellulase, 1,100 U of pectinase, 1,200 U of xylanase, 360 U of glucanase, 240 U of mannanase, 30 U of galactanase, 1,500 U of amylase, 120 U of protease per kg of diet. All diets contained 0.3% chromium oxide (Cr_2O_3) as an indigestible marker.

One-day old male Ross-308 broiler chickens were purchased from a local commercial hatchery. Birds were fasted for 4 hours, individually weighted, and randomly distributed among treatments. There were 6 birds per pen, 6 replicate pens per treatment. Birds were provided with continuous light, had free access to water, and were fed wheat/corn/soybean meal basal diet formulated to provide 3100 kcal/kg ME and 22% protein (Table 4.1). A control group was fed the basal diet for the entire trial and was included to calculate the AME_n values of test ingredients. Birds in experimental groups were fed the basal diet from day 1 to 14, and then diets containing test ingredients from day 15 to 19 (acclimatization period). On day 19, excreta samples from each pen were collected over a 3 h period, immediately frozen at −20 °C, freeze-dried, and finely ground. Excreta samples from the same pen were pooled to yield six replicates per treatment. Duplicate samples of diets and excreta were analyzed for chromium oxide using the AOAC procedure 985.01, nitrogen (nitrogen analyzer, model TruSpecN, Leco

Corp., St. Joseph, MI, USA) and gross energy (Parr 6300 calorimeter, Parr Instrument Co., Moline, IL, USA). Nitrogen retention and AME_n values of test ingredients were calculated as described by Leeson and Summers (2001).

TABLE 4.1. Composition and calculated analysis of a basal diet used in the AME_n assay

Item	%
Ingredient	
Wheat	44.0
Corn	31.0
Soybean meal	13.5
Dried porcine plasma	5.0
Canola oil	2.0
Calcium carbonate	1.65
Dicalcium phosphate	1.35
DL-Methionine	0.16
L-Lysine	0.10
Threonine	0.06
Mineral premix[1]	0.5
Vitamin premix[2]	1.0
Total	100.0
Calculated analysis (% unless specified)	
Metabolizable energy (kcal/kg)	3100
Crude protein	22.0
Calcium	0.99
Non-phytate P	0.45
Methionine	0.50
Methionine + cysteine	0.83
Lysine	1.09
Threonine	0.80
Analyzed composition (%)	
Crude protein	22.0

[1]Provided per kilogram of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride).

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg pantothenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg endox, 11 mg virginamycin, 99 mg monensin sodium.

4.3.4 True Metabolizable Energy (TME_n) Assay with Adult Roosters

A 2 × 2 factorial arrangement of treatments was used to evaluate the effect of *B. napus* black and *B. juncea* CM without and with enzyme supplementation on true metabolizable energy (TME_n) content. The enzyme (multi-carbohydrase) supplement Superzyme OM (Canadian Bio-Systems Inc., Calgary, AB, Canada) was used and supplied 1,700 U of cellulase, 1,100 U of pectinase, 1,200 U of xylanase, 360 U of glucanase, 240 U of mannanase, 30 U of galactanase, 1,500 U of amylase, 120 U of protease per kg of meal.

Nitrogen-corrected true metabolizable energy contents of CM were determined at the University of Illinois using the assay procedure described by Parsons (1985). Briefly, 30 g of each CM, with and without enzyme addition, were precision-fed to 4 groups of 5 individually caged Single Comb White Leghorn roosters after 24 h of feed withdrawal. Excreta were then collected during the next 48 h. The excreta samples were frozen, freeze-dried, weighed to determine total output and ground to pass through 1-mm sieve. Feed and excreta were analyzed for nitrogen (AOAC, 990.03) and for gross energy using an adiabatic bomb calorimeter (Parr 6300 calorimeter, Parr Instrument Co., Moline, IL, USA). The TME_n values were calculated as described by Parsons et al. (1992). Endogenous corrections for energy were made using fasted for 48 hours roosters to determine the endogenous excretion of energy and nitrogen.

4.3.5 Standardized Ileal Amino Acid Digestibility of Canola Meals

One-day old male Ross-308 broiler chicks were purchased from a local commercial hatchery and randomly distributed among 3 treatments with 6 birds per pen

and 6 replicate pens per treatment. Birds were provided with continuous light, had free access to water and were fed corn/wheat/ soybean meal basal diet, formulated to provide 3050 kcal/kg ME and 23% crude protein from day 1 to 14 (Table 4.2). From day 14 chicks were fed experimental diets with the test ingredient serving as the sole source of protein. Test diets were formulated to contain 2700-2800 kcal/kg ME and 22% crude protein. Each diet contained 0.3% of chromium oxide (Cr_2O_3) as an indigestble marker (Table 4.3). On day 21 all birds were euthanized in a CO_2 chamber, the contents of ileum (portion of the small intestine from Meckel's diverticulum to approximately 2 cm proximal to the ileo-ceacal junction) were collected, freeze-dried, ground and analyzed for amino acids as described in the Analytical Procedures Section. Digesta and excreta samples were analyzed for chromium after the samples were ashed at 600°C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian, Palo Alto, CA) according to the method of AOAC (2005, method 985.01). Apparent ileal amino acids digestibility (AIAAD) values were calculated and then standardized by using the ileal endogenous amino acids (IEAA) flow from birds fed the nitrogen-free diet.

The standardized ileal amino acids digestibility (SID) was calculated using the following equation (Adedokun et al, 2008):

SID (%) = AID (%) + [(IEAA flow / amino acids in the diet) x 100]

4.3.6 Growth Performance Study with Broiler Chickens

One-day-old male Ross-308 broiler chickens were used to investigate the effects of CM on growth performance of broiler chickens in 3 different phases. Birds were

obtained from a local commercial hatchery. All birds were randomly assigned to 7 pens of 50 birds each per treatment. Four different experimental diets were fed from day one. Diet composition is presented in Table 4.4. Diets were formulated based on Ross 308 breeder recommendations and digestible amino acid contents and provided 22% protein, 3000 kcal/kg ME in the starter phase (d 1-10 of age), 21% protein, 3050 kcal/kg ME in the grower phase (d 11-24 of age) and 19% protein, 3100 kcal/kg ME in the finisher phase (d 25-35 of age). Birds had free access to water and feed, and were provided with continuous light. Body weight and feed intake were recorded at the end of each phase. Feed conversion ratio values were also calculated.

TABLE 4.2. Composition and calculated analysis of a basal diet used in the standardized ileal amino acid digestibility assay

Item	%
Ingredient	
Wheat	13.0
Corn	45.4
Soybean meal	31.6
Meat and bone meal	2.0
Canola oil	3.4
Cacium carbonate	1.2
Dicalcium phosphate	1.25
DL- Methionine	0.14
L-Lysine	0.20
Threonine	0.03
Mineral premix[1]	0.5
Vitamin premix[2]	1.0
Chromium oxide	0.3
Total	100.0
Calculated analysis (% unless specified)	
Metabolizable energy (kcal/kg)	3054
Crude protein	23.0
Calcium	1.00
Non-phytate P	0.45
Methionine	0.50
Methionine + cysteine	0.83
Lysine	1.30
Threonine	0.85

[1]Provided per kilogram of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride).

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg pantothenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg endox, 11 mg virginamycin, 99 mg monensin sodium.

TABLE 4.3. Composition and calculated analysis of experimental diets used in the standardized ileal amino acid digestibility assay

Item	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow
Ingredient (%)			
Sucrose	30.3	36.1	35.6
B. napus, conventional black	59.7	-	-
B. juncea, yellow	-	-	54.5
B. napus, yellow	-	53.7	-
Canola oil	6.0	6.0	6.0
Cacium carbonate	0.90	1.20	0.93
Dicalcium phosphate	1.25	1.20	1.12
Mineral premix[1]	0.5	0.5	0.5
Vitamin premix[2]	1.0	1.0	1.0
Chromium oxide	0.3	0.3	0.3
Total	100.0	100.0	100.0
Calculated analysis (% unless specified)			
Metabolizable energy (kcal/kg)	2719	2823	2820
Crude protein	22.0	22.0	22.0
Calcium	1.00	1.00	1.00
Non-phytate P	0.46	0.45	0.45
Methionine	0.41	0.34	0.36
Methionine + cysteine	0.88	0.82	0.74
Lysine	1.21	1.03	1.06
Threonine	0.97	0.71	0.99

[1]Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride).

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg pantothenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg endox, 11 mg virginamycin, 99 mg monensin sodium.

TABLE 4.4. Composition and calculated analysis of experimental diets used in the growth performance study

Item	Starter phase (1-10 d of age)				Grower phase (11-24 d of age)				Finisher phase (25-35 d of age)			
	Control	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow	Control	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow	Control	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow
Ingredient (%)												
Wheat	30.0	24.0	25.0	27.0	30.1	25.0	25.8	26.0	34.0	15.0	22.3	23.1
Corn	35.9	35.0	36.0	34.0	38.6	37.0	38.0	38.0	37.6	48.9	44.1	43.5
Soybean meal	21.5	11.7	10.0	10.0	19.2	9.7	7.5	8.0	18.0	10.0	7.2	7.5
Fish meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	2.0	2.0	2.0	2.0
B. napus, black	-	15.0	-	-	-	15.0	-	-	-	15.0	-	-
B. napus, yellow	-	-	15.0	-	-	-	15.0	-	-	-	15.0	-
B. juncea, yellow	-	-	-	15.0	-	-	-	15.0	-	-	-	15.0
Canola oil	3.0	4.9	4.8	4.4	3.2	5.1	4.9	4.4	4.1	5.1	5.2	4.9
Cacium carbonate	1.36	1.21	1.29	1.23	1.07	0.95	1.01	0.94	1.22	1.10	1.16	1.07
Dicalcium phosphate	1.10	1.00	0.95	0.93	0.90	0.78	0.75	0.72	1.14	1.05	1.0	1.01
DL-Methionine	0.08	0.07	0.08	0.08	0.04	0.02	0.04	0.03	0.05	0.02	0.05	0.04
L-Lysine	0.39	0.44	0.51	0.50	0.24	0.29	0.36	0.35	0.27	0.27	0.36	0.35
Threonine	0.18	0.18	0.23	0.17	0.11	0.10	0.16	0.09	0.10	0.08	0.15	0.08
Mineral premix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis (% unless specified)												
ME (kcal/kg)	3003	3001	3005	3004	3052	3046	3047	3047	3099	3087	3084	3094
Crude protein	22.1	22.0	22.0	22.0	21.1	21.1	21.0	21.1	19.0	19.0	18.9	19.0
Calcium	1.05	1.05	1.05	1.05	0.90	0.90	0.90	0.90	0.85	0.85	0.85	0.85
Non-phytate P	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42
Methionine	0.47	0.47	0.47	0.47	0.42	0.42	0.42	0.42	0.38	0.38	0.38	0.38
Methionine + cysteine	0.76	0.79	0.79	0.77	0.71	0.73	0.73	0.71	0.66	0.66	0.68	0.65
Lysine	1.27	1.27	1.27	1.27	1.10	1.10	1.10	1.10	0.97	0.97	0.97	0.97
Threonine	0.83	0.83	0.83	0.83	0.73	0.73	0.73	0.73	0.65	0.65	0.65	0.65
Glucosinolates (µmol/g)	0.00	1.07	1.97	1.68	0.00	1.07	1.97	1.68	0.00	1.07	1.97	1.68

[1]Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride).

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg panthotenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mgaaaaa thiamine, 125 mg endox, 11 mg virginamycin, 99 mg monensin sodium

4.3.7 Animal Care

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care with the animal protocols approved by the Animal Care and Use Committee of the University of Manitoba. For the TME_n study, birds housing, handling and euthanasia were approved by the University of Illinois Animal Care Committee.

4.3.8 Statistical Analysis

The data were tested by the GLM procedure of the SAS program. Means were separated by Tukey's honestly significant difference. All statements of significance are based on $P \leq 0.05$. Results were presented as means and standard errors of the means.

4.4 RESULTS AND DISCUSSION

4.4.1 Chemical Composition of Canola Meals

Table 4.5 shows the chemical composition of canola meals. Crude protein content of *B. juncea* was higher than those of yellow and black *B. napus* (47.2 versus 43.4 and 41.1 % DM). As documented earlier, crude protein content of conventional CM varies from 38.0 to 43.5 % (dry matter basis) and in this context the conventional CM used in the current study was within that range (Bell and Keith, 1991; Simbaya et al., 1995; Slominski et al., 1999; Kong and Adeola, 2011; Woyengo et al., 2011; Slominski et al., 2012; Zhou et al., 2013). However, protein content in *B. juncea* meal was higher than that in studies by Slominski et al. (1999) and Zhou *et al*. (2013) but similar to that reported by

Slominski et al. (2012). Yellow *B. napus*, on the other hand, had the lowest protein content in comparison with the same meal type analyzed earlier (Simbaya et al., 1995; Slominski et al., 1999), although in the more recent study (Slominski et al., 2012) yellow *B. napus* contained 49.8 % DM, which demonstrates the inferior quality of the meal used in the current study. Such variation could be the result of changes in soil and environmental conditions.

Ether extract was higher in black *B. napus* in comparison with yellow *B. napus* and *B. juncea* (5.1 vs 3.5 and 4.0 % DM). Ether extract variation is the reflection of the oil refining by-products, including gums and soap stocks being added back to the meal during processing (Bell, 1984; CCC, 2015). On average, either extract was close to the NRC value of 3.8% (as-fed basis).

The sucrose content was higher in yellow *B. napus* in comparison with *B. juncea* and black *B. napus* (10.1 vs 8.0 and 6.6% DM). This is in agreement with the earlier studies (Slominski et al., 1994; Simbaya et al., 1995; Slominski et al., 2012) showing higher sucrose content of meals derived from yellow-seeded *B. napus* canola. Sucrose is highly digestible and this would have a positive impact on the available energy values of the meal (Simbaya et al., 1995). In addition, yellow *B. napus* had lower oligosaccharide content in comparison with the two other meals.

In agreement with the earlier studies on yellow canola germplasm characterization, ADF, NDF, and TDF contents were lower in meals from yellow-seeded canola in comparison with their black-seeded counterpart (Slominski et al., 1994; Simbaya et al., 1995; Jiang et al., 1999; Slominski et al., 2012; Zhou et al., 2013). Total dietary fiber content of both yellow *B. napus* and *B. juncea* meals was lower than that of

black *B. napus* (29.8 and 28.9 vs. 35.0% DM), primarily due to their lower lignin and polyphenol contents (3.1 and 5.3 vs. 10.1% DM). In this context, selection for yellow seed coat color, a visual marker of lower polyphenol and/or proanthocyanidins content represents a major agronomic trait for *Brassica* crop improvement as it is linked to increased seed oil, protein and sucrose contents at the expense of fiber components (Stringam et al., 1974; Theander et al., 1977; Simbaya et al., 1995; Newkirk et al., 1997; Slominski et al., 1999).

Yellow *B. napus*, however, had the highest content of NSP and glycoprotein associated with the fiber fraction. Earlier studies from this laboratory showed yellow *B. napus* to have lower TDF in comparison with *B. juncea* mostly due to the lower lignin and polyphenols, and glycoprotein contents (Slominski *et al*., 1994; Simbaya *et al*., 1995; Slominski *et al*., 2012). In the current study, however, the content of glycoproteins in yellow *B. napus* was slightly higher than that of *B. juncea* meal, although the level of lignin was in fact much higher in *B. juncea*. Such discrepancy could be due to the seed size of the material used for processing and thus different proportions of embryo and hull fractions contributing to the total fiber content. In this context, it has been demonstrated that *B. juncea* seed could be of small size with the hull fraction contributing more lignin to the total fiber content of the meal (Slominski et al., 2012).

In the current study, CM from yellow-seeded *B. napus* had higher phytate P and the lowest non-phytate P contents in comparison with its black-seeded counterpart and *B. juncea* meals (Table 4.5). In addition, both yellow *B. napus* and *B. juncea* meals contained more glucosinolate than their conventional black-seeded counterpart (Table 4.6). The glucosinolate content is in agreement with previous studies with the aliphatic 3-

butenyl glucosinolate (gluconapin) predominating in of *B. juncea* meal and 2-hydroxy-3-butenyl (progoitrin) and hydroxyglucobrassicin in *B. napus* species (Slominski et al., 1999; Slominski et al., 2012; Thacker and Widyaratne, 2012; Zhou et al., 2013).

It is generally believed that any potential negative effect of glucosinolates would be directly related to their break down products, including goitrin and isothiocyanates. It is therefore of importance that the myrosinase enzyme is effectively inactivated by heat-treatment in the crushing operation of canola seed. This was the case in the current study with no myrosinase activity detected in all CM samples evaluated (data not shown).

The results of amino acid analysis are shown in Table 4.7. Although somewhat lower in lysine, *B. juncea* contained the highest contents of arginine, methionine, and threonine due to the highest CP content among the meals evaluated. When amino acids were expressed in g/16 g N, yellow-seeded *B. napus* showed the highest value for lysine and cystine while black-seeded *B. napus* had the highest value for methionine.

Overall, among the three types of meal evaluated, yellow-seeded *B. juncea* appeared to have superior quality characteristics with intermediate quality characteristic observed for yellow *B. napus.* In comparison with the conventional *B. napus* canola, yellow *B. napus* and *B. juncea* contained more crude protein, more sucrose and less total dietary fiber. Lower fiber content was reflected in a lower content of lignin with associated polyphenols. The glucosinolates content of all canola meals was low although the meals from the new types of yellow-seeded *B. napus* and *B. juncea* contained more glucosinolates than their conventional black-seeded counterpart.

TABLE 4.5. Chemical composition of meals derived from black- or yellow-seeded *B. napus* canola and canola quality *B. juncea* (% DM basis)[1]

Component	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow
Crude protein	41.1	43.4	47.2
Ether extract	5.1	3.5	4.0
Carbohydrates			
Simple sugars[2]	0.31	0.22	0.28
Sucrose	6.6	10.1	8.0
Oligosaccharides[3]	3.1	2.8	3.1
Starch	0.4	0.5	0.8
Fiber fractions			
Acid detergent fiber (ADF)	20.1	9.3	9.9
Neutral detergent fiber (NDF)	25.2	19.0	18.5
Total Dietary Fiber (TDF)	35.0	29.8	28.9
Non starch polysaccharides	21.8	22.8	20.4
Arabinose	4.7	5.6	4.8
Xylose	1.9	2.2	1.8
Mannose	0.4	0.5	0.3
Galactose	1.6	1.9	1.5
Glucose	6.7	6.5	6.2
Uronic acid	6.2	6.1	5.7
Lignin and polyphenols	10.1	3.1	5.3
Glycoprotein (NDICP)[4]	3.2	3.9	3.2
Ash	8.5	7.3	8.0
Total phosphorus (P)	1.17	1.23	1.22
Phytate P	0.63	0.95	0.64
Non-phytate P	0.54	0.28	0.58
Glucosinolates (µmol/g)	7.9	14.6	12.5

[1]Samples were analyzed in duplicates.
[2]Includes glucose and fructose.
[3]Includes raffinose and stachyose.
[4]Neutral detergent insoluble crude protein.

TABLE 4.6. Glucosinolate content of meals derived from black- or yellow-seeded *B. napus* canola and canola quality *B. juncea* (µmol/g DM basis)

Component	*B. napus*, black	*B. napus*, yellow	*B .juncea*, yellow
Sinigrin (2-propenyl)	0.0	0.0	0.2
Gluconapin (3-butenyl)	1.6	4.9	9.8
Glucobrassicanapin (4-penetyl)	0.2	1.0	0.5
Progoitrin (2-hydroxy-3-butenyl)	3.8	4.3	0.5
Glucobrassicin (3-indolylmethyl)	0.4	0.7	0.0
Hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl)	1.7	3.7	0.6
Total	7.9	14.6	12.5

[1] Samples were analysed in duplicates.

TABLE 4.7. Amino acid content of meals derived from black- or yellow-seeded *B. napus* canola and canola-quality *B. juncea* (DM basis)[1]

Amino acid	% DM			g/16 g N		
	B. napus, black	*B. napus*, yellow	*B. juncea*, yellow	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow
Alanine	1.66	1.75	1.98	4.03	4.03	4.19
Arginine	2.50	2.72	3.32	6.09	6.26	7.04
Aspartic acid	2.95	3.03	3.83	7.17	6.97	8.12
Cystine	0.89	0.98	0.82	2.16	2.26	1.73
Glutamic acid	7.09	7.63	8.25	17.25[a]	17.57	17.47
Glycine	2.06	2.12	2.46	5.01	4.89	5.22
Histidine	1.25	1.27	1.43	3.04	2.94	3.02
Isoleucine	1.34	1.45	1.67	3.26	3.34	3.53
Leucine	2.71	2.89	3.35	6.60	6.65	7.10
Lysine	2.28	2.54	2.30	5.55	5.85	4.87
Methionine	0.80	0.71	0.83	1.94	1.64	1.75
Phenylalanine	1.55	1.67	1.85	3.76	3.86	3.92
Proline	2.76	3.02	2.87	6.72	6.96	6.08
Serine	1.90	1.98	2.18	4.63	4.56	4.62
Threonine	1.82	1.94	2.11	4.42	4.47	4.48
Tyrosine	1.04	1.12	1.26	2.53	2.59	2.68
Valine	1.81	1.92	2.16	4.40	4.44	4.57

[1]Samples were analyzed in duplicates.

4.4.2 Apparent (AME_n) and True (TME_n) Metabolizable Energy Contents

As shown in Table 4.8, CM effect on AME_n was significant as determined with broiler chickens (14 - 19 d of age). *B. juncea* meal increased AMEn values ($P<0.05$) to 2178 kcal/kg DM. Similarily, enzyme (multi-carbohydrase) supplementation significantly increased AME_n values from 1953 to 2082 kcal/kg DM. When the effect of meal and enzyme interaction was considered a trend ($P=0.09$) was observed and the AME_n values for yellow *B. napus* and *B. juncea* increased by 14.2 and 8.2 %, respectively, following enzyme addition.

Canola meal had a significant effect on TME_n, and as shown in Table 4.8, the increased value for *B. juncea* meal in comparison with *B. napus* black was observed. Enzyme supplementation showed a trend ($P=0.08$) in improving the TME_n values by 3.2%.

Irrespective of canola species or enzyme supplementation, and in agreement with the current study, the AME_n values from earlier studies have been reported to be between 2000 to 2050 kcal/kg (NRC, 1994; Newkirk et al., 1997; Mandal et al., 2005).

It is well known that metabolizable energy is related to the total dietary fiber (Newkirk et al., 1997; Jia et al., 2012), residual oil (Toghyani et al., 2014), digestible carbohydrates such as sucrose (Slominski *et al*., 1999), and glucosinolates contents of CM (Mandal et al., 2005; Khajali and Slominski, 2012). In the earlier study from this laboratory, yellow *B. napus* with the lowest dietary fiber and highest sucrose and protein contents showed the highest AME_n value (Jia *et al*., 2012). Similarly, Newkirk et al. (1997) reported a negative relationship between dietary fiber and AME_n content of *B. juncea*.

TABLE 4.8: Effect of canola type and enzyme supplementation on apparent (AME_n)[1] and true (TME_n)[2] metabolizable energy content (kcal/kg DM)

Effect		AME_n (kcal/kg DM)	TME_n (kcal/kg DM)
Meal			
	B. napus, black	1876[b]	2435[b]
	B. napus, yellow	1998[b]	-
	B. juncea, yellow	2178[a]	2622[a]
	SEM	48.7	31.0
Enzyme[3]			
	-	1953[b]	2489
	+	2082[a]	2569
	SEM	40.3	37.0
Meal × Enzyme			
	B. napus, black	1902[b]	2368[b]
	B. napus, black + enzyme	1851[b]	2502[ab]
	B. napus, yellow	1865[b]	-
	B. napus, yellow + enzyme	2131[ab]	-
	B. juncea, yellow	2092[ab]	2609[a]
	B. juncea, yellow + enzyme	2264[a]	2635[a]
	SEM	71.9	52.4
Effects and their significance			
Meal		0.001	0.003
Enzyme		0.03	0.08
Meal × Enzyme		0.09	0.28

[1]Each diet was fed to 6 replicate cages of 6 birds each.
[2]Each diet was fed to 5 individually caged cecectomized roosters.
[3]Enzyme supplement provided 1,700 U of cellulase, 1,100 U of pectinase, 1,200 U of xylanase, 360 U of glucanase, 240 U of mannanase, 30 U of galactanase, 1,500 U of amylase, 120 U of protease per kg of diet.
[ab]Means within columns with no common letters differ significantly ($P<0.05$)

Earlier research from this laboratory showed a similar effect of enzyme supplementation on *B. juncea* meal (Jia et al., 2012). Some other studies showed a negligible effect of enzyme addition on the AME_n content of rapeseed meal (Zobac et al., 1998; Mandal et al., 2005). According to Kocher et al. (2000), enzyme supplementation could even have a negative effect on the AME_n contents, which signifies the importance of using specific enzyme to target NSP structures in CM. Although the enzyme used in this study was

specifically selected to target NSP of CM, its effect seem to be different in canola species due to potentially different NSP structure in such meals.

4.4.3 Standardized Ileal Amino Acid Digestibility of Canola Meals

Standardized ileal amino acid digestibility of yellow *B. napus*, *B. juncea*, and the conventional black-seeded *B. napus* canola as determined with broiler chickens (21 d of age) were, on average, 82.5, 83.2, and 81.8%, respectively. As illustrated in Table 4.9, similar SID values for all amino acids were observed for the three meals. These values are close to the values determined earlier for the conventional CM (Adedokun et al., 2007; Woyengo et al., 2010; Kong and Adeola, 2011; Kim et al., 2012; Kong and Adeola, 2013). In terms of SID amino acid contents, however, distinct differences were observed among the meals with *B. juncea* meal containing the highest SID contents with the exception of lysine, cystine and proline being the highest (P<0.05) in yellow *B. napus*.

Earlier studies demonstrated that the SID values are better estimates of amino acid digestibility and could be additively used to formulate poultry diets to minimize the cost of feeding and nitrogen excretion (Ravindran et al., 1999; Lemme et al., 2004; Ravindran and Hendriks, 2004; Stein et al., 2005; Garcia et al., 2007).

TABLE 4.9. Standardized ileal digestibility (SID) of amino acids and SID amino acid contents of meals derived from black- or yellow-seeded *B. napus* canola and canola-quality *B. juncea* for broiler chickens (%)[1]

Amino acid	SID (% DM)			SID content (% DM)		
	B. napus, black	*B. napus*, yellow	*B. juncea*, yellow	*B. napus*, black	*B. napus*, yellow	*B. juncea*, yellow
Alanine	83.3	85.0	84.8	1.38^{c}	1.49^{b}	1.68^{a}
Arginine	88.8	89.2	90.1	2.22^{c}	2.43^{b}	2.99^{a}
Aspartic acid	78.7	78.5	82.8	2.32^{b}	2.38^{b}	3.17^{a}
Cystine	76.1	77.2	74.4	0.68^{b}	0.76^{a}	0.61^{c}
Glutamic acid	88.6	88.7	89.0	6.28^{b}	6.76^{ab}	7.34^{a}
Glycine	80.8	80.3	81.3	1.66^{b}	1.71^{b}	2.00^{a}
Histidine	61.7	61.0	64.1	0.77^{b}	0.78^{ab}	0.91^{a}
Isoleucine	78.8	83.0	80.2	1.05^{b}	1.20^{ab}	1.34^{a}
Leucine	83.8	85.5	84.8	2.27^{c}	2.47^{b}	2.84^{a}
Lysine	80.3	81.1	79.2	1.83^{b}	2.06^{a}	1.82^{b}
Methionine	91.8	90.5	91.2	0.73^{b}	0.64^{c}	0.75^{a}
Phenylalanine	84.7	86.4	86.3	1.31^{c}	1.45^{b}	1.60^{a}
Proline	76.6	77.2	78.4	2.12^{c}	2.33^{a}	2.25^{b}
Serine	77.9	77.5	79.9	1.48^{b}	1.53^{b}	1.74^{a}
Threonine	76.2	76.2	77.6	1.39^{c}	1.48^{b}	1.64^{a}
Tyrosine	89.6	89.7	92.4	0.93^{c}	1.01^{b}	1.17^{a}
Valine	76.4	80.3	77.6	1.38^{b}	1.55^{ab}	1.67^{a}

[1]Samples were analyzed in duplicates.

[a,b] Means within columns with no common letters differ significantly (P<0.05).

4.4.4 Growth Performance Study with Broiler Chickens

Growth performance of chickens fed the Control and 15% canola meal diets is shown in Table 4.10. As the diets were formulated based on determined digestible amino acids and available energy contents, feed intake and FCR were not significantly different among treatments in the starter phase (1-10 d of age). However, BWG decreased significantly (P<0.05) for the diet containing yellow *B. napus*. In the grower phase (11-24 d of age), BWG averaged 992, 1037, 985, and 1036 for the Control, black and yellow *B. napus*, and *B. juncea* meals, respectively, with the conventional black *B. napus* and *B. juncea* meals being significantly higher (P<0.05) than those of Control and yellow *B. napus*. As well, yellow *B. napus* had significantly higher FCR than other treatments in the grower phase. In the finisher phase (25-35 d of age), chicks fed diets containing yellow *B. napus* had significantly lower (P<0.05) feed intake compared to the Control, and lower BWG compared to the Control and *B. juncea* diets, which resulted in the highest FCR for this meal. Overall, only chicks fed diets containing yellow *B. napus* meal showed some reduction in growth performance which could be attributed to different chemical composition and levels of anti-nutritional factors such as NSP, phytic acid, and glucosinolates in this meal. In fact, the meal from yellow-seeded *B. napus* had the highest content of NSP, glucosinolates, and phytate (Table 4.5) which, either individually or additively, could have negatively influenced the growth performance.

It is well known that water-soluble NSP increase digesta viscosity and, therefore, interfere with energy and other nutrients' utilization (Graham and Aman, 1991; Chibowska et al., 2000; Kocher et al., 2000; Meng et al., 2005; Thacker and Petri, 2011;

Gopinger et al., 2014). Although the water-soluble NSP were not determined in the current study, earlier research from this laboratory demonstrated that yellow *B. napus* meal contained 2.8% of soluble NSP which was significantly higher than the values of 1.8 and 2.2% determined for the conventional black-seeded *B. napus* and *B. juncea* canola, respectively (Jia et al., 2012).

Glucosinolates are always accompanied by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) in the seed and in the presence of moisture and following rupture of the seed, are hydrolyzed to a range of products including isothiocyanates, goitrin, nitriles, and thiocyanates which interfere with the function of thyroid gland and along with the pungency of isothiocyanates and bitterness of goitrin adversely affect growth performance (Fenwick, 1982; McCurdy, 1990; Tripathi and Mishra, 2007; Mailer et al., 2008). Although the myrosinase enzyme is effectively inactivated by heat-treatment applied in the crushing operation of canola seed, some thermal decomposition of glucosinolates and the formation of similar breakdown products may occur (Campbell and Slominski, 1990). In broiler chickens, the growth depression would be minimal at the glucosinolate level of 4 µmol/g diet. However, when the levels of glucosinolates increased to 6-10 µmol/g some reduction in growth has been observed with the level of glucosinolates above 10 µmol/g resulting in a severe growth depression (Mawson et al., 1994). It has been indicated that the glucosinolate level should be less than 2.5 µmol/g diet in young poultry with less tolerance to this compounds (Mushtaq *et al*., 2007). Considering a conservative 2.5 µmoles per gram of diet as the maximum level of glucosinolate inclusion, it would appear that yellow *B. napus* canola diets used in the

TABLE 4.10. Effect of canola type on growth performance of broiler chickens (1-35 d of age)[1]

Item	Feed intake (g/bird)				Body weight gain (g/bird)				Feed conversion ratio (g feed/g gain)			
	1-10 d	11-24 d	25-35 d	1-35 d	1-10 d	11-24 d	25-35 d	1-35 d	1-10 d	11-24 d	25-35 d	1-35 d
Control	281	1431^{a}	1879^{a}	3562^{a}	211^{a}	992^{b}	1126^{a}	2323^{a}	1.33	1.44^{a}	1.67^{c}	1.53^{ab}
B. napus, black	275	1398^{ab}	1804^{ab}	3467^{ab}	206^{a}	1037^{a}	1052^{bc}	2297^{a}	1.34	1.35^{c}	1.71^{ab}	1.51^{b}
B. napus, yellow	262	1371^{b}	1780^{b}	3378^{b}	191^{b}	985^{b}	1016^{c}	2187^{b}	1.37	1.39^{b}	1.75^{a}	1.54^{a}
B. juncea, yellow	269	1408^{ab}	1839^{ab}	3482^{ab}	203^{a}	1036^{a}	1084^{ab}	2314^{a}	1.32	1.36^{bc}	1.70^{bc}	1.50^{b}
SEM	5.3	11.5	19.5	31.8	2.3	9.0	10.8	17.6	0.03	0.01	0.01	0.01

[1]Each diet was fed to seven replicate pens of 50 birds.

[a,b]Means within columns with no common letters differ significantly (P<0.05).

current study were still below this level (i.e., 2.2 µmol/g diet) and should have a minimal negative effect on growth performance. For some reason, yellow-seeded *B. napus* canola used in the current study was much higher in phytic acid content than its black-seeded counterpart and *B. juncea* meal. It is well known that phytate chelates cations such as calcium, iron, zinc and copper as well as nitrogen and amino acids (Cowieson et al., 2003; Mushtaq et al., 2007). Phytate also inhibits digestive enzymes such as pepsin and trypsin especially in the post-hatched chicks with less developed digestive tract which could limit their growth (Pallauf and Rimbach, 1997; SariCiqek and Serdar, 2006).

4.5 Conclusions

It would appear evident that breeding for low-fiber canola would result in quantitative changes as evidenced by increased oil, protein, and sucrose contents and decreased fiber content in the seed. Among the fiber components, lignin and polyphenols associated with the hull fraction of the seed would have a minimal or no antinutritive effect on growth performance of broiler chickens.

Canola meal could be used effectively in broiler chicken rations at 15% in all 3 phases of growth when diets are formulated based on determined nutrient availability data. However, the contents of anti-nutritional factors such as dietary fiber, glucosinolates, or phytate would have to be considered when formulating diets for broiler chickens.

4.6 ACKNOWLEDGEMENTS

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5. MANUSCRIPT III

Degradation of non-starch polysaccharides of canola meal by exogenous enzymes and its effect on growth performance of broiler chickens

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5.1 ABSTRACT: To target the complex structure of non-starch polysaccharides of CM, four different *in vitro* enzyme incubation studies were conducted. In the *in vitro* Experiments 1 and 2, samples of *B. napus* and *B. juncea* canola were incubated for 16 hours with 0.5% of carbohydrase preparations CX A (cellulase and xylanase A), CX B (cellulase and xylanase B), P A (pectinase A); P B (pectinase B), M (mannanase), XG (xylanase and glucanase); GP (galactanase and pectinase) individually and in combination. Enzyme preparation GP demonstrated a significant (P<0.05) NSP degradation of up to 40% that was further increased using a combination of GP and CX B. The next two *in vitro* experiments were carried out to investigate the effect of enzyme concentration and incubation time on NSP degradation. Enzyme combination GP and CX B with the highest activity towards NSP depolymerization was chosen to be used in the broiler chicken experiments. In the first *in vivo* experiment, broiler chickens were fed 30% of *B. napus* and *B. juncea* meals without and with enzyme supplementation from 4 to 19 d of age. A significantly lower BWG and FI and higher intestinal viscosity (P<0.05) were observed in birds fed the enzyme-supplemented *B. juncea* diet. However, no significant difference in FCR was observed. The higher digesta viscosity leading to the lower BWG in birds fed *B. juncea* meal and enzyme could be explained by the production of water-soluble NSP, resulting from their incomplete degradation under the conditions of the gastrointestinal tract. In the second *in vivo* experiment, growth performance, dietary AME_n and NSP digestibility in broiler chickens fed diets containing 15% of CM without and with enzyme supplementation were determined in the starter (0-10 d) and grower (11-24 d) phases of the experiment. No significant differences in BWG,

FI, FCR and dietary AME_n were observed. Digestibility of NSP was significantly higher in birds fed *B. napus* canola and enzyme (18.3 vs 6.7 %) in comparison with the no-enzyme treatment, although it was not translated into any visible improvement in growth performance.

Keywords: Canola meal, NSP degradation, enzyme supplementation, broiler chicken

5.2 INTRODUCTION

High protein content (approximately 40%), a well-balanced amino acid profile and their availability makes canola meal (CM) one of the most valuable protein sources used in poultry diets (Bell, 1993; Newkirk, 2009). However, the presence of anti-nutritional factors including non-starch polysaccharides (NSP) limits its usage in monogastric diets (Slominski and Campbell, 1990; Bell, 1993, Bedford and Morgan, 1996; Kocher et al., 2000). Unlike starch, NSP are not digestible by endogenous enzymes of poultry and may negatively affect nutrient utilization and animal performance (Bedford and Morgan, 1996; Pustjens et al., 2012).

Anti-nutritional effects of NSP are due to several factors, including water-holding capacity which increases gut viscosity and acts as a physical barrier between digestive enzymes and nutritients, and may increase passage rate of digesta and thus reduce nutrient availability and absorption by entrocytes (Antoniou *et al*., 1981; White *et al*., 1981; Annison, 1991; Jensen et al., 1995; Choct, 2002). A negative relationship between fiber content and energy digestibility has also been observed (Downey and Bell, 1990; Ahmad et al., 2007; Mushtaq et al., 2007).

Non-starch polysaccharide constituent sugars of CM account for 18% of the meal

with the high concentration of arabinose, uronic acids, and glucose followed by galactose, xylose and mannose (Slominski and Campbell, 1990; Bell, 1993). In addition to cellulose, the major non-cellulosic polysaccharides of CM are pectic polysaccharides, including rhamnogalacturonan, homogalacturonan, and xylogalactouronan with arabinose, galactose and xylose residues side chains. Other polysaccharides such as arabinan, arabinogalactans, galactans, galactomannans and mannans are also present (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Bacic et al., 1988; Slominski and Campbell, 1990; Daveby and Aman, 1993; Meng and Slominski, 2005; Putsjens et al., 2013).

Various enzymes have been used to improve the nutritive value of feedstuffs for poultry. However, their mode of action is still not fully understood (Bedford and Schulze, 1998; Meng et al., 2005, Slominski, 2011). By solubilizing NSP, enzymes eliminate the encapsulating effect of cell walls and may hydrolyze carbohydrate-protein complexes which would lead to improvements in energy and amino acid utilization (Bedford and Classen, 1992; Meng et al, 2005). Enzymatic hydrolysis of NSP may also produce oligosaccharides and low-molecular weight polysaccharides with prebiotic properties which modify gut microflora and intestinal metabolic and morphological responses, and thus may facilitate gut development and health (Choct et al., 1999; Bedford, 2000; Engberg et al., 2004; Gao et al., 2007; Kiarie et al., 2007; Jia et al., 2009).

Targeting NSP of CM with enzymes would appear to be more challenging than that of cereal grains due to the complex nature of such polymers. Therefore, a diversified combination of enzymes to target the various NSP structures of CM is needed (Slominski, 2011). In earlier research from this laboratory, a significant depolymerization

of CM NSP was achieved *in vitro* when combinations of carbohydrate enzymes were used in concert (Meng et al., 2005). In some studies, the use of enzyme cocktails in poultry diets failed to improve growth performance when high dietary levels of CM were used, although the NSP digestibility was improved (Slominski and Campbell, 1990; Simbaya et al., 1996; Kocher et al., 2000, 2001; Mushtaq et al., 2007). Only a few studies on the effect of enzymes on NSP depolymerization and the effects of enzyme cocktails on growth performance of poultry are published (Meng et al., 2005; Liu et al., 2013; Smeets et al., 2014). Therefore, the objective of this study was to determine the composition of NSP in two types of CM and to evaluate the optimal level and combination of several carbohydrase enzymes for their ability to depolymerize the cell wall structures of CM in vitro. The effect of a selected multi-carbohydrase supplement on growth performance, NSP digestibility and dietary AME_n contents of CM fed to broiler chickens was also investigated.

5.3 MATERIALS AND METHODS

5.3.1 In vitro Enzyme Evaluation

A series of *in vitro* incubation studies was carried out to degrade the NSP structures of *B. juncea* and the conventional *B. napus* canola. In Experiment 1, various enzyme preparations, including CX A (cellulase, xylanase A), CX B (cellulase, xylanase B), PA (pectinase A), M (mannanase), XG (xylanase, glucanase), GP (galactanase, pectinase) were used individually and in combination with *B. juncea* meal serving as a substrate. In Experiment 2, the experimental preparation GP was evaluated alone and in combination with CX B, P A, and P B using both the conventional and *B. juncea* CM as substrates. In both experiments enzyme concentration was 0.5% and the incubation time

of 16 hours was used. In the third *in vitro* experiment, a GP enzyme with the highest affinity towards CM NSP was used for incubation with *B juncea* and *B napus* meals for 5 and 16 hours. To explore the possibility of further NSP depolymerization, combinations of GP with CX B and PA at different concentrations were also used in Experiment 4. To mimic the digesta transit time, the meals were incubated for 5 hours.

The *in vitro* enzyme evaluation method used in all four experiments was based on the procedure described by Meng et al. (2005). Briefly, each test enzyme solution was mixed with 0.1g of CM in 0.1 M sodium acetate buffer (pH 5.2; 8 ml final volume). The mixture was then incubated for 16 h at 40°C in the incubator-shaker at the speed of 210 RPM. Ethanol was then added to 80% of its final concentration, the contents were mixed and left for 1 h at room temperature, and then centrifuged at 2200 rpm for 15 minutes. The supernatant that contained enzyme hydrolysis products was discarded and the residue was subjected to NSP analysis as described by Englyst and Cummings (1984) with modifications (Slominski and Campbell, 1990). Component neutral sugars were separated using an SP-2340 column and Varian CP-3380 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada). Uronic acids were determined using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK). The reduced recovery of component sugars in the enzyme treated samples in comparison to that of the control treatment was an indicator of the degree of NSP degradation.

Enzyme preparations were provided by Canadian Bio-Systems Inc., Calgary, Alberta, Canada.

5.3.2 Broiler Chicken Growth Performance Experiment 5

A 2 × 2 factorial arrangement of treatments was used to evaluate the effect of canola meals and enzyme supplementation on growth performance of broiler chickens in a short-term trial (4-19 d of age). Based on the results of *in vitro* studies, a combination of GP and CX B carbohydrases was used at a dietary level of 0.02%.

One-day-old male Ross-308 broiler chickens were obtained from a local commercial hatchery. The birds were held in electrically heated battery brooders (16 Cage Super Brooder, Alternative Design Mfg., Siloam Springs, AR) for a 4-day pre-experimental period and were fed commercial chick starter crumbles (21% protein). On day 5, birds were fasted for 4 h, and then randomly distributed among 9 replicates (pens) of 5 birds each. Experimental diets contained 30% of CM and were formulated to contain 2950 kcal/kg ME and 22% CP (Table 5.1). Birds had free access to water and feed and were provided with continuous light. Body weight (BW) and feed intake (FI) were recorded at the end of the experiment on day 19. Feed conversion ratio (FCR) values were calculated. On day 19, 5 birds were randomly selected from each treatment and euthanized by cervical dislocation. Fresh digesta (1.5 g) from the jejunum was collected and centrifuged at 9961×g for 10 minutes and viscosity of the supernatant was determined at 40ºC using Brookfield digital viscometer (DV-II+LV model; Brookfield Engineering Laboratories Inc., Stoughton, MA, USA).

5.3.3 Broiler Chicken Growth Performance Experiment 6

A 2 × 2 factorial arrangement of treatments was used to evaluate the effect of CM and enzyme supplementation on growth performance, dietary AME_n content and NSP digestibility in broiler chickens fed diets in the starter (0-10 d) and grower (11-24 d) phases of the experiment. Based on the results of in vitro studies, a combination of GP

and CX B carbohydrases was used at the dietary level of 0.02%.

TABLE 5.1. Composition of experimental diets used in Experiment 5.

Item	*B. napus* meal	*B. juncea* meal
Ingredient (%)		
Corn	46.3	48.0
Soybean meal	14.6	13.1
B. napus meal	30.0	-
B. juncea meal	-	30.0
Canola oil	5.0	4.7
Cacium carbonate	1.2	1.2
Dicalcium phosphate	1.15	1.15
DL- Methionine	0.05	0.04
Mineral premix[1]	0.5	0.5
Vitamin premix[2]	1.0	1.0
Titanium oxide	0.3	0.3
Total	100	100
Calculated composition (% unless specified)		
Metabolizable energy (kcal/kg)	2,954	2,952
Crude protein	22.1	22.1
Calcium	1.02	1.02
Non-phytate P	0.41	0.40
Methionine	0.51	0.51
Methionine + cysteine	0.97	0.97
Lysine	1.17	1.16
Threonine	0.89	0.89
Analyzed composition (%)		
Crude Protein	20.8	20.8

[1]Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride)

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg panthotenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg endox, 11 mg virginamycine, 99 mg monensin sodium

One-day-old male Ross 308 were obtained from a commercial hatchery and randomly assigned to 7 replicates (pens) of 5 birds each per treatment. Wheat/corn/SBM/CM diets were formulated based on Ross 308 breeder recommendation

and digestible amino acids but with the reduced AME_n (-150 kcal/kg), and crude protein and amino acids (-5%) contents. The diets supplied 2850 kcal/kg ME and 20.9% CP in the starter and 2900 kcal/kg ME and 20.0% CP in the grower phase of the experiment. Each diet contained 3 g/kg of chromium oxide (Cr_2O_3) as an internal marker (Table 5.2). Birds had free access to water and feed and were provided with continuous light. Body weight and FI were recorded at the end of each phase and FCR was calculated. At the end of the experiment, excreta samples were collected from each pen, freeze dried, ground to pass through 1mm sieve and pooled to provide 4 replicates per treatment. Duplicate samples of diet and excreta were analyzed for chromium using the procedure described by Williams et al. (1963), nitrogen using a combustion method of AOAC (968.06), and a nitrogen analyzer model TruSpecN (Leco Corp., St. Joseph, MI, USA), and gross energy (Parr 6300 Calorimeter, Parr Instrument Co., Moline, IL, USA). Non-starch polysaccharides were determined as described before. Dietary AME_n and nitrogen retention were calculated as described by Leeson and Summers (2001). Apparent total tract digestibility of NSP was calculated using the following equation (Meng et al., 2005):

NSP digestibility (%) = $\{1-[(Cr_2O_{3\%\ diet}/Cr_2O_{3\%\ excreta}) \times (NSP_{\%excreta}/NSP_{\%diet})]\} \times 100$

5.3.4 Animal Care

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care with the animal protocols approved by the Animal Care and Use Committee of the University of Manitoba.

TABLE 5.2. Ingredients and nutrient composition of experimental diets used in Experiment 6.

Item	Starter phase (1-10 d of age)		Grower phase (11-24 d of age)	
	B. napus	*B. juncea*	*B. napus*	*B. juncea*
Ingredient (%)				
Wheat	34.0	35.0	32.0	33.0
Corn	27.0	28.0	31.1	33.0
Soybean meal	14.5	13.0	13.0	11.0
B. napus meal	15.0	-	15.0	-
B. juncea meal	-	15.0	-	15.0
Canola oil	3.5	3.0	4.0	3.0
Cacium carbonate	1.55	1.55	1.40	1.40
Dicalcium phosphate	1.36	1.33	1.15	1.10
DL- Methionine	0.10	0.11	0.05	0.06
L-Lysine	0.52	0.58	0.35	0.42
Threonine	0.19	0.18	0.11	0.11
Mineral premix[1]	0.5	0.5	0.5	0.5
Vitamin premix[2]	1.0	1.0	1.0	1.0
Chromium oxide	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0
Calculated nutrient composition (% unless specified)				
Metabolizable energy (kcal/kg)	2843	2855	2908	2897
Crude protein	20.9	20.9	20.0	19.9
Calcium	1.00	1.00	0.90	0.90
Non-phytate P	0.45	0.45	0.40	0.40
Methionine	0.45	0.45	0.40	0.40
Methionine + cysteine	0.77	0.75	0.71	0.69
Lysine	1.21	1.21	1.04	1.04
Threonine	0.79	0.79	0.69	0.69
Analysed composition (% unless specified)				
Crude protein	21.0	21.1	19.4	19.7
Glucosinolates (μmol/g)	0.96	1.0	0.78	0.90

[1]Provided per kg of diet: 70 mg Mn (as manganese oxide), 80 mg Zn (as zinc oxide), 80 mg Fe (as ferrous sulphate), 10 mg Cu (as copper sulphate), 0.3 mg Se (as sodium selenite), 0.5 mg Iodine (as calcium iodate), 337 g Na (as sodium chloride)

[2]Provided per kilogram of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 11 mg panthotenic acid, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin, 4 mg pyridoxine, 4 mg thiamine, 125 mg endox, 11 mg virginamycine, 99 mg monensin sodium

5.3.5 Statistical Analyses

The data were tested by the GLM procedure of SAS. Means were separated by Tukey's honestly significant difference. All statements of significance are based on $P \leq 0.05$. Results were presented as means and standard error of the means.

5.4 RESULTS

5.4.1 In vitro Enzyme Evaluation

The result of different carbohydrase evaluation using *B. juncea* meal as a substrate are shown in Table 5.3. Total NSP content of CM averaged 171 g/kg with arabinose, uronic acid and glucose component sugars predominating followed by galactose, xylose and small amount of mannose. When compared to the control treatment, incubation with the enzyme preparation GP reduced ($P<0.05$) the recovery of component sugars by 40%. Other carbohydrases, including CX A, CX B, PA, M and XG did not have any significant effect on the component sugar recovery. As well, no further reduction in NSP degradation for GP in combination with other enzymes was observed. For xylose, a combination of GP + CX B showed the lowest recovery among the enzymes evaluated which was lower ($P<0.05$) than that of GP alone. Similarly, uronic acid recovery was reduced significantly using GP + CX B and GP + CX A compared to the control.

In Experiment 2 (Table 5.4), GP as well as CX B and pectinases P A and P B were further evaluated using both *B. juncea* and *B. napus* meals. Total NSP recovery was reduced ($P<0.05$) when GP was used in both meals. Interestingly, a combination of GP

TABLE 5.3. Screening of different carbohydrase enzymes for their activity towards canola meal non-starch polysaccharides (NSP) degradation (g/kg) (Experiment 1).

Enzyme	NSP component sugar (g/kg)						Total
	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	
None	56.4^{a}	17.0^{a}	3.3^{a}	17.4^{a}	34.8^{a}	42.6^{ab}	171.4^{a}
Cellulase/xylanase A (CX A)	51.6^{ab}	15.3^{ab}	2.9^{abc}	16.4^{a}	26.0^{a}	42.6^{ab}	154.7^{a}
Cellulase/xylanase B (CX B)	41.9^{b}	14.0^{abcd}	2.9^{abc}	16.1^{a}	20.4^{a}	45.0^{a}	140.3^{ab}
Pectinase A (P A)	46.0^{ab}	15.0^{abc}	2.8^{abc}	15.0^{a}	22.5^{a}	42.0^{abc}	143.3^{ab}
Mannanase (M)	48.3^{ab}	14.4^{abcd}	3.2^{a}	15.7^{a}	27.8^{a}	43.3^{ab}	152.8^{a}
Xylanase/glucanase (XG)	43.9^{b}	14.6^{abcd}	2.4^{c}	15.0^{a}	19.9^{a}	44.9^{a}	140.7^{ab}
Galactanase/pectinase (GP)	22.7^{c}	12.1^{cdef}	2.9^{abc}	10.1^{b}	19.1^{a}	36.0^{bcd}	102.8^{c}
GP + CX A	22.7^{c}	10.7^{efg}	2.7^{abc}	9.9^{b}	18.0^{a}	33.2^{cd}	97.2^{c}
GP + CX B	17.9^{c}	8.3^{g}	2.5^{bc}	8.5^{b}	18.2^{a}	32.0^{d}	87.4^{c}
GP + P A	24.5^{c}	12.7^{bcde}	3.2^{a}	10.9^{b}	23.8^{a}	37.5^{abcd}	112.5^{bc}
GP + M	21.6^{c}	10.7^{efg}	3.1^{ab}	9.8^{b}	18.0^{a}	38.0^{abcd}	101.3^{c}
GP + XG	23.7^{c}	11.6^{def}	2.9^{abc}	10.7^{b}	18.0^{a}	35.8^{bcd}	102.7^{c}
GP + CX A + P A	22.3^{c}	10.0^{efg}	2.7^{abc}	9.9^{b}	16.3^{a}	35.6^{bcd}	96.7^{c}
GP + CX B + P A	19.7^{c}	9.5^{fg}	2.7^{abc}	9.2^{b}	20.5^{a}	36.0^{bcd}	97.7^{c}
GP + CX A + P A + P B	21.5^{c}	9.7^{efg}	3.0^{abc}	9.7^{b}	17.0^{a}	38.1^{abcd}	99.1^{c}
SEM	2.00	0.55	0.13	0.44	3.60	1.56	5.71

^{a,b}Means within columns with no common letters differ significantly (P<0.05).

TABLE 5.4. Degradation of non-starch polysaccharides (NSP) following incubation of *B. napus* and *B. juncea* canola meal with different carbohydrase enzymes (g/kg) (Experiment 2).

Canola meal	Enzyme	NSP component sugar (g/kg)						Total
		Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	
B. napus[1]	None	39.6^{a}	16.3^{a}	3.7^{a}	14.3^{a}	62.0^{a}	48.7^{abc}	184.6^{a}
	Galactanase/pectinase (GP)	21.4^{c}	10.7^{c}	3.8^{a}	9.7^{b}	53.9^{ab}	43.4^{bcd}	142.9^{b}
	Cellulase/xylanase B (CX B)	34.3^{b}	13.7^{b}	3.7^{a}	13.8^{a}	60.1^{a}	52.7^{a}	178.3^{a}
	Pectinase A (P A)	38.6^{a}	15.9^{a}	3.6^{ab}	13.5^{a}	62.9^{a}	49.8^{ab}	184.3^{a}
	Pectinase B (P B)	36.1^{ab}	15.5^{a}	3.6^{ab}	13.3^{a}	63.5^{a}	41.0^{d}	173.0^{a}
	GP + CX B	17.4^{d}	7.2^{d}	3.2^{c}	8.4^{bc}	35.9^{c}	40.6^{d}	112.7^{c}
	GP + P A	22.0^{c}	10.6^{c}	3.6^{ab}	9.8^{b}	43.6^{bc}	42.1^{cd}	131.7^{b}
	GP + P B	22.1^{c}	10.5^{c}	3.9^{a}	9.9^{b}	51.9^{ab}	40.0^{d}	138.3^{b}
	GP + CX B + P A	17.0^{d}	7.2^{d}	3.1^{c}	8.2^{bc}	36.4^{c}	42.2^{cd}	114.1^{c}
	GP + C + P B	15.2^{d}	6.0^{d}	3.3^{bc}	7.5^{c}	34.5^{c}	40.1^{d}	106.6^{c}
	SEM	0.65	0.33	0.07	0.31	2.13	1.35	2.28
B. juncea	None	37.2^{a}	15.6^{a}	2.8^{ab}	12.5^{a}	74.3^{a}	41.0^{ab}	183.3^{a}
	Galactanase/pectinase (GP)	20.8^{c}	10.5^{b}	3.0^{a}	9.0^{b}	52.7^{b}	34.3^{bcd}	130.2^{b}
	Cellulase/xylanase B (CX B)	32.3^{b}	14.4^{a}	2.8^{ab}	12.3^{a}	76.7^{a}	40.1^{abc}	178.6^{a}
	Pectinase A (P A)	36.1^{a}	15.7^{a}	2.8^{ab}	12.1^{a}	73.6^{a}	41.4^{a}	181.7^{a}
	Pectinase B (P B)	34.1^{ab}	14.3^{a}	2.7^{ab}	11.8^{a}	71.2^{a}	42.9^{a}	176.8^{a}
	GP + CX B	20.3^{c}	11.2^{b}	2.9^{a}	8.8^{bc}	52.9^{b}	33.0^{d}	129.1^{b}
	GP + P A	20.1^{c}	10.3^{bc}	2.9^{a}	8.7^{bc}	52.4^{b}	37.1^{abcd}	131.3^{b}
	GP + P B	20.6^{c}	10.8^{b}	3.0^{a}	8.9^{b}	53.5^{b}	32.3^{d}	129.0^{b}
	GP + CX B + P A	16.2^{d}	7.8^{d}	2.4^{b}	7.4^{d}	37.1^{c}	33.4^{cd}	104.2^{c}
	GP + C + P B	16.3^{d}	8.3^{cd}	2.7^{ab}	7.7^{cd}	38.9^{c}	36.3^{abcd}	110.1^{c}
	SEM	0.56	0.36	0.08	0.21	1.27	1.24	2.59

[1] Statistical analysis was tested within each canola type.

[a,b]Means within columns with no common letters differ significantly (P<0.05).

+ CX B and GP + CX B + P A and P B further reduced total NSP recovery in *B. napus* canola in comparison with GP used alone. The same effect was observed using GP with P A and P B in *B. juncea* meal. The recovery of individual component sugars arabinose, xylose and glucose followed the same trend as total sugars for both *B. napus* and *B. juncea* meals.

In the next two *in vitro* experiments, different concentrations of enzymes GP in combination with CX B and P A were used following 5 hours incubation to mimic the digesta transit time in birds' gastrointestinal tract. Therefore, both canola meals were incubated for 5 and 16 hours using two concentrations of GP enzyme: 0.5 and 0.05% (Table 5.5). In *B. napus* CM, total NSP recovery was reduced (P<0.05) by 45.2, 24.5, 25.8 and 11.2% when 0.5% and 16h, 0.05% and 16h, 0.5% and 5h and 0.05% enzyme and 5 hours incubation time were used, respectively. In this type of canola even reducing the concentration to 0.05% and incubation time to 5 h still showed a significant effect on NSP depolymerization. In *B. juncea* CM, contrary to *B. napus* meal, the use of lower enzyme concentration and shorter incubation time did not show any significant NSP degradation. In Experiment 4, various concentrations of GP in combination with CX B and P A were used in 5 h incubation time. Similarly to experiment 3, only enzyme concentrations higher than 0.5% showed a significant NSP depolymerization when compared with the control and there was no effect when the enzyme concentrations were lower than 0.5% (Table 5.6).

5.4.2 Broiler Chicken Growth Performance Experiment 5

Growth performance of broiler chickens fed diets containing 30% of CM without and with enzyme supplementation is presented in Table 5.7. Both canola types and

TABLE 5.5. Degradation of non-starch polysaccharides (NSP) following incubation of canola meal with different concentrations of GP (galactanase/pectinase) carbohydrase for 16 or 5 hours (g/kg) (Experiment 3).

Canola meal	Enzyme concentration (%)	Time of incubation	NSP component sugar (g/kg)						
			Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	Total
Control	-	-	37.9^{a}	16.2^{a}	3.7^{a}	13.6^{a}	54.4^{a}	59.0^{ab}	184.7^{a}
B. napus[1]	0.5	16	17.9^{d}	9.4^{c}	2.9^{b}	9.5^{d}	12.4^{b}	49.4^{b}	101.3^{d}
	0.05	16	32.1^{b}	13.9^{b}	3.0^{b}	12.3^{b}	21.8^{b}	56.5^{ab}	139.5^{c}
	0.5	5	26.6^{c}	13.2^{b}	3.6^{a}	11.3^{c}	26.5^{b}	56.1^{ab}	137.1^{c}
	0.05	5	36.3^{a}	15.8^{a}	3.5^{a}	13.0^{ab}	34.2^{ab}	60.8^{a}	163.5^{b}
SEM			0.67	0.33	0.05	0.14	4.66	1.91	3.44
Control	-	-	51.0^{a}	15.8^{a}	2.5^{a}	16.2^{a}	19.1^{a}	63.5^{a}	168.2^{a}
B. juncea	0.5	16	24.0^{d}	7.9^{d}	2.5^{a}	11.0^{c}	9.1^{c}	55.1^{a}	109.7^{d}
	0.05	16	44.1^{b}	13.9^{b}	2.4^{a}	14.9^{ab}	13.4^{b}	61.4^{a}	150.1^{bc}
	0.5	5	36.3^{c}	12.2^{c}	2.7^{a}	13.3^{b}	12.2^{b}	58.7^{a}	135.4^{c}
	0.05	5	50.8^{a}	15.2^{a}	2.5^{a}	16.2^{a}	14.7^{b}	66.0^{a}	165.4^{ab}
SEM			0.60	0.22	0.06	0.31	0.54	1.95	2.86

[1] Statistical analysis was tested within each canola type.

[a,b]Means within columns with no common letters differ significantly (P<0.05).

TABLE 5.6. Degradation of non-starch polysaccharides (NSP) following incubation of *B. juncea* meal with different enzyme concentrations for 5 hours (g/kg) (Experiment 4).

Enzyme	Enzyme concentration (%)	NSP component sugar (g/kg)						
		Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	Total
None (Control)	-	35.9[a]	14.4[a]	2.5[c]	11.8[a]	72.6[a]	44.0[abc]	181.1[a]
Galactanase/pectinase (GP) + Cellulase/xylanase B (CX B)	1.000	20.8[e]	9.6[e]	3.1[a]	8.4[f]	49.1[b]	36.8[d]	127.7[e]
	0.500	25.2[d]	11.7[d]	2.8[bc]	9.4[e]	56.1[ab]	42.3[c]	147.4[cde]
	0.250	29.8[c]	12.3[bcd]	2.7[bc]	10.4[cd]	62.8[ab]	42.9[bc]	160.9[abc]
	0.125	33.5[ab]	13.5[abc]	2.8[bc]	11.3[ab]	68.8[a]	46.7[a]	176.5[a]
	0.050	34.5[a]	13.7[ab]	2.6[c]	11.5[a]	70.0[a]	44.7[abc]	176.9[a]
Galactanase/pectinase (GP) + Cellulase/xylanase B (CX B) + Pectinase A (P A)	1.000	20.9[e]	9.4[e]	3.2[a]	8.4[f]	49.4[b]	38.0[d]	129.1[de]
	0.500	25.2[d]	11.4[d]	3.0[ab]	9.7[de]	57.7[ab]	43.0[abc]	149.8[bcd]
	0.250	29.4[c]	12.1[cd]	2.7[bc]	10.3[cd]	62.2[ab]	46.3[ab]	162.9[abc]
	0.125	31.3[bc]	13.3[abc]	2.6[c]	10.7[bc]	65.7[ab]	43.6[abc]	167.0[abc]
	0.050	34.3[ab]	13.8[ab]	2.5[c]	11.5[a]	61.6[ab]	46.2[ab]	169.8[ab]
SEM		0.57	0.26	0.05	0.13	2.97	0.68	3.84

[a,b]Means within columns with no common letters differ significantly ($P<0.05$).

enzyme addition had significant effect on BWG and a trend (P = 0.07) was observed for the interaction. Diets containing *B. juncea* meal reduced BWG (P<0.05) in comparison to those fed *B. napus*. When supplemented with enzyme, further reduction in BWG of birds fed *B. juncea* meal was observed (P<0.05). Feed intake was affected by the type of CM and was reduced (P<0.05) in birds fed *B. juncea* meal in comparison to *B. napus*. Enzyme supplementation didn't have a significant effect on FI for both meals. However, birds fed *B. juncea* meal showed the lowest (P<0.05) FI when the diet was supplemented with enzyme. Feed conversion ratio was not different among treatments. Similarly to BWG, intestinal digesta viscosity was affected by canola type and enzyme supplementation with a trend (P<0.1) being observed for the meal × enzyme interaction. Highest digesta viscosity was demonstrated in the birds fed *B. juncea* meal supplemented with enzyme, which was higher (P<0.05) than that of birds fed *B. napus* diets, regardless of enzyme supplementation.

5.4.3 Broiler Chicken Growth Performance Experiment 6

Growth performance of broiler chickens fed diets containing 15% CM and the effect of enzyme supplementation is presented in Table 5.8. Neither canola type and enzyme supplementation, nor their interaction showed any significant effect on BWG, FI, FCR and dietary AME_n values in the starter and grower phases of the experiment.

Both enzyme and meal × enzyme interaction showed a trend (P=0.05) in improving total tract NSP digestibility values. Total digestibility value was improved (P<0.05) in birds fed *B. napus* meal and enzyme in comparison with the same meal with no enzyme added (18.3 vs 6.7%) but no differences were observed in birds fed *B. juncea* meal without and with enzyme supplementation. In terms of component sugars, arabinose,

TABLE 5.7. Effect of canola type and enzyme supplementation on growth performance of broiler chickens fed diets contanining 30% canola meal from 5-19 d of age (Experiment 5).[1]

Effect		Feed intake (g/bird/14d)	Body weight gain (g/bird/14d)	Feed conversion ratio (g feed/g gain)	Viscosity (mpa.s)
Meal					
	B. napus	728.2[a]	511.8[a]	1.42	1.46[b]
	B. juncea	674.3[b]	466.2[b]	1.45	1.76[a]
	SEM	7.92	3.71	0.02	0.06
Enzyme[2]					
	-	710.3	496.9[a]	1.43	1.48[b]
	+	692.3	481.0[b]	1.44	1.74[a]
	SEM	7.92	3.71	0.02	0.06
Meal × Enzyme					
	B. napus	732.0[a]	514.9[a]	1.42	1.41[b]
	B. napus + enzyme	724.4[ab]	508.6[a]	1.43	1.51[b]
	B. juncea	688.5[bc]	478.9[b]	1.44	1.55[b]
	B. juncea +enzyme	660.1[c]	453.4[c]	1.46	1.97[a]
	SEM	11.19	5.25	0.02	0.09
Effects and their significance					
Meal		<0.000	<0.000	0.343	0.002
Enzyme		0.118	0.005	0.600	0.006
Meal × Enzyme		0.354	0.078	0.713	0.077

[1]Each diet was fed to nine replicate cages of 5 birds each.

[2]Galactanase/pectinase (GP) + cellulase/xylanase B(CX B) carbohydrase was used at the dietary level of 0.02%.

[a,b]Means within columns with no common letters differ significantly (P<0.05).

TABLE 5.8. Effect of canola type and enzyme supplementation on growth performance and dietary apparent metabolizable energy (AME_n) content of broiler chickens fed diets contanining 15% canola meal from 1-24 d of age[1] (Experiment 6).

Effect	Feed intake (g/bird)			Body weight gain (g/bird)			Feed conversion ratio (g feed/g gain)			Dietary AME_n[3]
	1-10 d	11-24 d	1-24 d	1-10 d	11-24 d	1-24 d	1-10 d	11-24 d	1-24 d	(kcal/kg DM)
Meal										
B. napus	269	1173	1442	205	808	1013	1.32	1.45	1.43	3051
B. juncea	261	1199	1459	208	819	1026	1.26	1.47	1.42	3047
SEM	4.27	17.29	19.84	5.26	14.19	18.11	0.02	0.01	0.01	11.33
Enzyme[2]										
-	265	1206	1472	208	826	1034	1.28	1.46	1.42	3053
+	264	1165	1429	205	800	1005	1.30	1.46	1.43	3045
SEM	4.27	17.29	19.84	5.26	14.19	18.11	0.02	0.01	0.01	11.33
Meal × Enzyme										
B. napus	271	1206	1477	208	820	1029	1.30	1.47	1.44	3031
B. napus + enzyme	267	1139	1406	202	795	997	1.33	1.43	1.41	3071
B. juncea	260	1207	1467	208	832	1039	1.26	1.45	1.41	3076
B. juncea +enzyme	261	1190	1452	208	805	1013	1.26	1.48	1.44	3019
SEM	6.04	24.46	28.06	7.43	20.07	25.61	0.03	0.01	0.01	
Effects and their significance										
Meal	0.18	0.30	0.54	0.71	0.60	0.60	0.06	0.28	0.96	0.97
Enzyme	0.87	0.10	0.14	0.67	0.21	0.27	0.62	0.80	0.96	0.78
Meal × Enzyme	0.66	0.31	0.33	0.66	0.97	0.92	0.73	0.02	0.09	0.01

[1]Each diet was fed to 7 replicate pens of 5 birds each.

[2]Galactanase/pectinase (GP) + Cellulase/xylanase B (CX B) carbohydrase was used at the dietary level of 0.02%.

[3]Means of 4 pooled excreta samples of 5 birds each.

TABLE 5.9. Effect of canola type and enzyme supplementation on total tract non-starch polysaccharides (NSP) digestibility of broiler chickens fed 15% canola meal from1-24 d of age[1] (Experiment 6)

Effect	NSP component sugars digestibility (%)[2]						
	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	Total
Meal							
B. napus	10.5	10.1	36.1	3.4	17.2	10.3	12.5
B. juncea	7.7	9.2	34.0	1.9	18.5	11.4	12.1
SEM	2.36	2.16	1.97	2.54	1.63	2.11	1.85
Enzyme							
-	4.2^{b}	6.9	31.9^{b}	-2.3^{b}	16.8	8.3	9.5
+	14.0^{a}	12.3	38.1^{a}	7.6^{a}	18.9	13.5	15.0
SEM	2.55	2.33	2.13	2.74	1.75	2.28	1.99
Meal × Enzyme							
B. napus	1.9^{b}	3.7	30.2^{b}	-4.5^{b}	13.1	6.4	6.7^{b}
B. napus + enzyme	19.1^{a}	16.4	41.9^{a}	11.35^{a}	21.2	14.2	18.3^{a}
B. juncea	6.5^{ab}	10.1	33.7^{ab}	-0.03^{ab}	20.5	10.1	12.4^{ab}
B. juncea +enzyme	9.0^{ab}	8.3	34.3^{ab}	3.75^{ab}	16.5	12.7	11.8^{ab}
SEM	3.34	3.05	2.79	3.59	2.30	2.98	2.61
Effects and their significance							
Meal	0.47	0.75	0.50	0.74	0.66	0.68	0.87
Enzyme	0.01	0.09	0.04	0.02	0.31	0.11	0.06
Meal × Enzyme	0.06	0.04	0.08	0.13	0.03	0.42	0.05

[1]Each diet was fed to 7 replicate pens of 5 birds each.

[2]Means of 4 pooled excreta samples of 5 birds each.

[a,b]Means within columns with no common letters differ significantly (P<0.05).

mannose and galactose digestibilities showed significant enzyme effect and the meal × enzyme interaction showed similar trend as that of total NSP. No differences in xylose, glucose and uronic acid digestibilities were observed among the treatments (P>0.05).

5.5 DISCUSSION

Total NSP content of *B. napus* and *B. juncea* CM in all four *in vitro* experiments were in agreement with the values determined earlier (Meng et al., 2005, Meng and Slominski, 2005). It is generally believed that the relative ratio of different component sugars indicates the predominant polysaccharide present, which, in turn, could be used to select the most effective enzyme combinations (Aulrich and Flachowsky, 1998; Gao et al., 2011; Liu et al., 2013). In agreement with earlier studies (Huisman et al., 1998; Slominski and Campbell, 1990; Meng et al., 2005), the component sugar profile demonstrated high uronic acids, arabinose, xylose and galactose contents, which are characteristic of pectic polysaccharides. Other polysaccharides include cellulose, arabinans, arabinogalactans, galactans, glucoxylan and glucoarabinoxylans (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Daveby and Aman, 1993; Bach Knudsen, 1997). In agreement with Meng et al., (2005), small amounts of mannose indicate the presence of β-mannans and/or galactomannans as minor components of CM cell wall structure.

Enzymes degrade CM cell wall structure to simple sugars, oligosaccharides and low molecular weight polysaccharides, all contributing to reduced recovery of NSP in the *in vitro* studies (Slominski et al., 1993; Meng et al., 2005). The complex heterogeneous structure of CM NSP makes it difficult to determine the mode of action of various enzymes. However, the GP (galactanase/pectinase) enzyme showed the highest affinity

towards the cell wall structure. This enzyme seems to depolymerize the galactan and arabinogalactan side chains rather than the main rhamnogalacturonan backbone of pectic polysaccharides. This is illustrated by the reduced recovery of arabinose and galactose with no significant effect of this enzyme towards uronic acids. In experiment 1, CX B (cellulase/xylanase B) combined with GP enzyme reduced recovery of xylose and uronic acids further. Similar effects were observed when GP and CX B were used in combination with two pectinases P A and P B when reduced recovery of arabinose, xylose, galactose, glucose, and uronic acids was observed. This combination seemed to contain multiple activities towards main and side chains of pectic polysaccharides as well as other polysaccharides. In this context, multiple carbohydrases used in combination were effective in NSP degradation of various polysaccharides of CM, peas and soybean meal (Meng et al., 2005).

The current study clearly demonstrated that enzyme concentrations as low as 0.05% would decrease the effect of enzyme addition. The 0.02% enzyme concentration that was used in the *in vivo* experiments was below that level, however, considering the industry standards and enzyme usage of maximum 0.01%, enzyme inclusion rate of 0.05% would not be feasible.

As demonstrated earlier, to maximize the extent of NSP hydrolysis, relatively high concentrations of enzymes are needed to access the complex, and for the most part water-insoluble NSP structures (Castanon et al., 1997; Meng et al., 2005, Meng et al., 2006). According to Castanon et al. (1997), high levels of enzymes were needed to hydrolyze both water-soluble and water-insoluble NSP in rye, while increasing the enzyme level in barley didn't show the same effect. When in the current study the enzyme

incubation time was reduced to 5 h to simulate the digesta transit time, the degree of NSP hydrolysis decreased, which would be due to the relatively slow process of water-insoluble NSP depolymerization.

According to Castanon et al. (1997), enzyme supplementation may solubilize water-insoluble NSP, which would be further hydrolyzed to low-molecular weight carbohydrates. When enzymes are used at lower levels the water-soluble NSP would increase without further hydrolysis, accumulate in the hindgut (Bedford et al., 1991), and as a consequence could increase digesta viscosity (Pettersson and Aman, 1989). This could explain the increased digesta viscosity when high level of *B. juncea* meal and enzyme were used in the current study. Increased digesta viscosity could reduce substrate diffusion, effective digestive enzyme-substrate interaction, and consequently nutrient availability (Ikegami et al., 1990; Choct and Annison, 1992) leading to reduced BWG in birds fed the *B. juncea* diet supplemented with enzyme. It is also possible, however, that the less than optimal performance of birds fed *B. juncea* meal could be explained by high aliphatic glucosinolate content of this meal in comparison with *B. napus* canola (Slominski et al., 1999; Jia et al., 2012). This effect could be more pronounced in younger birds (Ahmad et al., 2007) which was the case in the current study. This could also explain lack of effect of *B. juncea* meal on broiler performance when lower concentration of this meal was used in the growth performance study (Table 5.8).

The current study demonstrated a significant NSP depolymerization *in vitro* and improved NSP digestibility *in vivo* in birds fed *B. napus* CM, however such effects did not translate into any visible effects on growth performance when 15% CM was used. This might be due to limited contribution of volatile fatty acids, the byproduct of NSP

fermentation in the lower gut, to available energy content of CM (Moran et al., 1982; Meng and Slominski, 2005). When high concentration of CM was used, the enzyme used in this study seemed to depolymerized CM NSP to a limited extent with a potential production of water-soluble NSP, which consequently could increase gut viscosity and affect BWG, especially in birds fed *B. juncea* meal.

5.6 CONCLUSIONS

A combination of GP + CX B enzymes showed the highest affinity towards CM NSP among the enzymes evaluated *in vitro*. Broiler chickens fed diets containing 30% of *B. juncea* meal supplemented with the same enzyme combination demonstrated higher digesta viscosity resulting in impaired BWG. This could be due to insufficient level of enzyme and/or minimal enzyme efficacy under the conditions of the gastrointestinal tract (i.e., pH). Enzyme supplementation in birds fed 15% of *B. napus* meal increased NSP digestibility significantly but did not affect the growth performance and dietary AME_n values, most likely due to limited contribution of NSP hydrolysis products to the available energy content of CM.

5.7 ACKNOWLEDGEMENTS

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6. GENERAL DISCUSSION

Chemical characterization of meals derived from yellow-seeded *B. juncea* and the conventional black-seeded *B. napus* canola was conducted and is presented in Manuscript 1. There was no difference in crude protein, ether extract, simple sugars and oligosaccharide contents between the meals. *B. juncea* meal had the higher sucrose and lower fiber content in comparison to its black-seeded counterpart, which is in agreement with the data presented in Manuscript 2 and the earlier reports, demonstrating a positive correlation between the percentage of yellow seeds in the sample and the concentration of sucrose and fiber in yellow-seeded canola (Slominski *et al*., 1994; 1999; 2012). In the second set of samples used in the study (Manuscript 2), crude protein of *B. juncea* was higher than those of yellow and black *B. napus* (47.2 vs. 43.4 and 41.1 % DM). Higher protein content of *B. juncea* meal and the lower protein content of *B. napus* yellow in this study in comparison with those reported earlier (Simbaya et al., 1995; Slominski et al. 1999; Zhou et al., 2013) could be the result of soil and environmental changes. Ether extract values reported in both Manuscripts 1 and 2 were close to the NRC value of 3.8% (as-fed basis) with the variation being the reflection of the oil refining by-products, including gums and soapstocks, added back to the meal during processing (Bell, 1984; CCC, 2015).

Total dietary fiber contents of *B. juncea* reported in Manuscript 1 and of both yellow *B. napus* and *B. juncea* meals reported in Manuscript 2 were lower than that of black *B. napus*, primarily due to their lower lignin and polyphenol contents with polyphenols (i.e., proanthocyanidins) rather than lignin contributing to this fraction of fiber. In this context, selection for yellow seed coat color, a visual marker of lower

polyphenol and/or hull proanthocyanidins content represents a major agronomic trait for *Brassica* crop improvement as it is linked to increased seed oil, protein and sucrose contents at the expense of fiber components (Simbaya et al., 1995; Newkirk et al., 1997; Slominski et al., 1999; 2012). The level of lignin was in fact much higher in *B. juncea* which could be due to the *B. juncea* small seed size with the hull fraction contributing more lignin to the total fiber content of the meal (Slominski et al., 2012). Lower ADF and NDF values of yellow-seeded canola reported in Manuscripts 1 and 2 are reflection of the thinner seed coat in this species (Stringam et al., 1974; Theander et al., 1977; Slominski and Campbell, 1990; Slominski *et al*., 1994; Slominski et al., 2012; Zhou et al., 2013). Meals derived from yellow-seeded *B. juncea* and *B. napus* canola had a higher glucosinolate content than *B. napus* black, although the values were still within the definition of CM. Different glucosinolate contents in meals from various crushing plants across Canada has been observed, indicating the effect of processing on glucosinolate content (Adewole *et al*., 2016). In agreement with previous studies, different glucosinolate profile of *B. juncea* was reported in both Manuscripts 1 and 2 with the aliphatic 3-butenyl glucosinolate (gluconapin) predominating (Slominski *et al*., 1999; Slominski *et al*., 2012; Thacker and Widyaratne, 2012; Zhou *et al*., 2013). The implication of such difference on the nutritive value of the meal is not clear. It is generally believed that any potential negative effect of glucosinolates would be directly related to their break down products, including isothiocyanates and goitrin.

Contrary to earlier studies, *B. juncea* showed similar or higher AA contents, including lysine, than the conventional meal (Slominski *et al*., 1999; 2012), which is a desirable characteristic of this meal. When amino acids were expressed in g/16 g N

(Manuscript 2), yellow-seeded *B. napus* showed the highest values for lysine and cystine while black-seeded *B. napus* had the highest value for methionine.

Overall, among the three types of meal evaluated, yellow-seeded *B. juncea* appeared to have superior quality characteristics with intermediate quality characteristic observed for yellow-seeded *B. napus.*

Total dietary fiber (Newkirk et al., 1997; Jia et al., 2012), ether extract (Toghyani et al., 2014), digestible carbohydrates such as sucrose (Slominski *et al*., 1999), and glucosinolates (Mandal et al., 2005; Khajali and Slominski, 2013) are some of the factors that are responsible for the difference in AME_n values of CM. However, a short experimental period of test material consumption in the AME_n assay, could minimize the negative effect of CM glucosinolates. As demonstrated in both Manuscripts 1 and 2, the AME_n values are in agreement with the earlier studies (NRC, 1994; Newkirk et al., 1997; Mandal et al., 2005). Similarly to an earlier study (Jia *et al*., 2012), enzyme addition increased energy values of *B. juncea* meal by 12.6 and 8.2%, respectively, in studies reported in Manuscript 1 and 2. However, other studies showed a negligible (Zobac et al., 1998; Mandal et al., 2005) or negative (Kocher et al., 2000) effect of enzyme addition on the AME_n content of rapeseed meal, which signifies the importance of using specific enzyme to target NSP structures of CM. Higher TME_n values for the yellow-seeded canola were demonstrated which could be justified by the difference in carbohydrate and oil contents of the meal (Manuscripts 1 and 2).

Total standardized ileal amino acid digestibility for the three canola meals were between 81.8 to 83.2% and are close to the values determined earlier for the conventional

CM (Adedokun et al., 2007; Woyengo et al., 2010; Kong and Adeola, 2011; Kim et al., 2012; Kong and Adeola, 2013).

When diets containing 30% of CM were fed to broiler chickens, birds fed a *B. juncea* diet had significantly lower feed intake and body weight gain than those fed the conventional meal, although no difference in FCR was observed. The gut viscosity values were low with no significant difference between the treatments and, thus, would be considered to have a minimal effect on growth performance. The negative effect of *B. juncea* meal on growth performance could be due to the high aliphatic glucosinolate content of *B. juncea* meal (Slominski *et al*, 1999; Jia *et al*., 2012). Glucosinolates may reduce bird appetite and voluntary feed intake thus adversely affect feed intake and growth performance of the chicken (Mushtaq *et al*., 2007; Tripathi and Mishra, 2007; Min *et al*., 2011). The adverse affects of glucosinolates on liver function and metabolic activities increase the expenditure of energy and other nutrients in visceral organs for the maintenance at the expense of growth and consequently would decrease BWG of broiler chickens (Mailer *et al*., 2008; Woyengo *et al*., 2011). As birds used in the current study were younger than 18 days old, the negative effect of glucosinolates, especially the aliphatic ones of *B. juncea* meal, could have been more pronounced.

When diets containing 15% CM were fed to chickens (Manuscript 1), *B. juncea* canola gave the lowest BWG, which as mentioned earlier could be attributed to the higher aliphatic glucosinolate content of *B. juncea* meal (Slominski *et al*., 1999; Jia *et al*., 2012). In the study reported in Manuscript 2, only chicks fed diets containing yellow *B. napus* meal showed some reduction in growth performance. Different chemical composition and several anti-nutritional factors such as higher fiber, glucosinolates, and

phytate content in this meal could be responsible for the lower BWG. It is worth to mention that the glucosinolate level of *B. napus* yellow was still below a conservative level of 2.5 µmoles per gram of the broiler chicken diet (Mushtaq *et al*., 2007) and should have a minimal negative effect on growth performance. In agreement with the earlier studies from this laboratory (Jia et al., 2012), greater response of *B. juncea* meal to the multicarbohydase supplementation was demonstrated.

Total NSP content and component sugar profile of *B. napus* and *B. juncea* meals observed in the current study (Manuscript 3), were in agreement with some earlier reports (Huisman et al., 1998; Slominski and Campbell, 1990; Meng et al., 2005), demonstrating high uronic acids, arabiose, xylose and galactose contents characteristic of pectic polysaccharides. Other polysaccharides included cellulose, arabinans, arabinogalactans, galactans, glucoxylan, glucoarabinoxylans and β-mannans (or galactomannans) as minor components of CM cell wall structure (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Daveby and Aman, 1993; Bach Knudsen, 1997; Meng et al., 2005). A new galactanase/pectinase enzyme (GP) showed the highest affinity towards the cell wall structure, depolymerizing the galactan and arabinogalactan side chains rather than the rhamnogalacturonan backbone of pectic polysaccharides, which is illustrated by the reduced recovery of arabinose and galactose with no significant effect of this enzyme towards uronic acids. When combined with another enzyme containing cellulase and xylanase activities (CX B), the enzyme seemed to contain multiple activities towards main and side chains of pectic polysaccharides as well as other polysaccharides. It is well known that relatively high concentrations of enzymes are needed to maximize the extent of NSP hydrolysis and to access the water-insoluble NSP of CM (Castanon et al., 1997;

Meng et al., 2005, Meng et al., 2006). Therefore, when the enzyme incubation time was reduced to 5 h, the degree of NSP hydrolysis decreased due to a relatively slow process of water-insoluble NSP depolymerization.

When broiler chickens were fed diets containing 30% of CM without and with enzyme supplementation, *B. juncea* meal reduced BWG significantly in comparison to *B. napus*. When supplemented with enzyme, further reduction in BWG of birds fed *B. juncea* meal was observed. Similarly, highest digesta viscosity was demonstrated in birds fed *B. juncea* meal supplemented with enzyme, which was significantly higher than that of birds fed *B. napus* diets, regardless of enzyme supplementation. Increased digesta viscosity could reduce nutrient availability (Ikegami et al., 1990; Choct and Annison, 1992) leading to reduced BWG in birds fed the *B. juncea* diet supplemented with enzyme. This could be due to the increase in the levels of water-soluble NSP and their accumulation in the hindgut (Bedford et al., 1991) in situations when lower levels of enzymes are used.

The minimal determined effect of *B. juncea* meal on broiler performance when lower concentration (15%) of this meal was used could be explained by the lower concentration of dietary glucosinolates (Slominski et al., 1999; Jia et al., 2012). Total tract NSP digestibility was significantly improved in birds fed 15% *B. napus* meal and enzyme in comparison with the same meal with no enzyme added (18.3 vs 6.7%) (Manuscript 3). However, such effects did not translate into the visible effect in growth performance. This might be due to the limited contribution of volatile fatty acids, the byproduct of NSP fermentation in the lower gut, to available energy content of CM (Moran et al., 1982; Meng and Slominski, 2005). Similarly, previous research has shown

the positive effect of multicarbohyrase enzyme in depolymerizing CM cell wall polysaccharides *in vitro* (Slominski and Campbell, 1990; Simbaya *et al*., 1996; Meng *et al*., 2005) with less promising results *in vivo* due to the short transit time of digesta and less favorable conditions for the enzymes in the gastrointestinal tract (Shires *et al*., 1987; Simbaya *et al*., 1996).

7. CONCLUSIONS AND FUTURE RESEARCH

Conclusions

1. It would appear that breeding for low-fiber canola would result in quantitative changes as evidenced by increased oil, protein, and sucrose contents and decreased fiber content in the seed.
2. Higher glucosinolate content and their different profile of *B. juncea* meal could cause a significant reduction in BWG of young broilers.
3. Canola meal could be used effectively in broiler chicken rations at 15% in all 3 phases of growth when diets are formulated based on determined nutrient availability data.
4. Multicarbohydrase enzyme addition significantly increased the metabolizable energy content and body weight gain of birds fed *B. juncea* meal.
5. A combination of galactanase/pectinase and cellulase/xylanase enzymes showed the highest acctivity towards NSP depolymerization among the enzymes evaluated *in vitro*.
6. Broiler chickens fed diets containing 30% of *B. juncea* meal and supplemented with the same enzyme combination demonstrated higher digesta viscosity resulting in impaired BWG. This could be due to the low enzyme efficacy due to less than optimum conditions in the gastrointestinal tract, leading to the incomplete conversion of NSP to their low-molecular weight analogs.
7. Enzyme supplementation in birds fed 15% of *B. napus* meal increased NSP digestibility significantly but did not affect the growth performance and dietary

AME_n values, most likely due to limited contribution of NSP hydrolysis products to the available energy content of CM.

Future research

1. Current study shows that chemical characterization of meals derived from the canola seeds crushed in 2 consecutive years 2010 and 2011 was different due to changes in soil, environmental and processing conditions. This suggests the importance of characterizing canola meals produced under different environmental and processing conditions in the future studies.
2. Current study suggest that anti-nutritional factors of CM, particularly GLS, DF and phytate, could be responsible for lower BWG of birds. Evaluation and consideration of such factors when higher quantities of CM are fed to poultry is recommended.
3. Through the years of breeding, GLS content of canola meals has been reduced to quantities <20μmole/g. However, this research shows that these values may still be detrimental to birds. Future canola breeding programs focusing on reducing GLS levels even further would be beneficial. Special attention to the GLS profile is suggested.
4. When enzyme addition is considered, using specific enzyme cocktail to target the complex NSP structures of CM is suggested.
5. A new galactanase/pectinase enzyme cocktail, depolymerizing the galactan and arabinogalactan side chains of canola cell walls was identified in this study. Higher concentrations of this enzyme blend should be evaluated in the future

studies so as to maximize the extent of NSP hydrolysis and to facilitate the prebiotic effect of NSP hydrolysis products in poultry.

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