Chemical Composition and Available Energy Contents of Canola Meal from Canadian Crushing Plants

by

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ABSTRACT

Samples of canola meal (CM) were collected from thirteen canola crushing plants in Canada. On average, the contents of various components in % dry mater (DM) were as follows: crude protein (CP) 40.3; ether extract (EE) 2.9; acid hydrolyzed EE (AEE) 6.0; sucrose 5.92; oligosaccharides 3.22; total phosphorus (P) 1.13; non-phytate P 0.33; neutral detergent fiber 30.7; total dietary fiber 40.0; neutral detergent insoluble CP 5.0; lignin and polyphenols 11.7; lysine 2.19; arginine 2.28; methionine 0.74; cystine 0.92 and threonine 1.57 and in μmol/g DM, glucosinolates 2.75. The apparent metabolizable energy content of CM averaged 1,789 kcal/kg. Digestibilities of EE and AEE averaged 81 and 53%, respectively. In summary, variations in the chemical composition of CM would indicate different processing practices between crushing plants. Low digestibility of AEE would indicate that soapstocks and gums poorly digested and contribute less energy to the AME_n content of CM than EE.

Keywords: Canola meal, Chemical composition, AME_n, Fat digestibility

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LIST of ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
AEE	Acid hydrolyzed ether extract
AID	Apparent ileal digestibility
AME	Apparent metabolaizable energy
AME_n	Apparent metabolizable energy, nitrogen corrected
Ala	Alanine
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Arg	Arginine
Asp	Aspartate
ATTD	Apparent total tract digestibility
Ca	Calcium
CM	Canola meal
CP	Crude protein
Cr	Chromium
CV	Coefficient of variation
Cys	Cysteine
DEB	Dietary electrolyte balance
DM	Dry matter
DT	Desolventizer-toster
GE	Gross energy
Glu	Glutamate
GLS	Glucosinolates
Gly	Glycine
His	Histidine
Ile	Isoleucine
Lys	Lysine
Leu	Leucine
ME	Metabolizable energy
Met	Methionine
NDICP	Neutral detergent insoluble crude protein
NDF	Neutral detergent fiber
NE	Net energy
NRC	National Research Council
P	Phosphorus
Phe	Phenylalanine
R^2	Coefficient of determination
SAS	Statistical Analysis System
SBM	Soybean meal
SBC	Spent bleaching clay
SEM	Standard error of mean
Ser	Serine
Trp	Tryptophan

Tyr	Tyrosine
Val	Valine

CHAPTER 1: INTRODUCTION

Canola is a major oil-seed crop in Western Canada and was developed by Canadian plant breeders in 1970s to produce lines with low levels of erucic acid in the oil and low levels of glucosinolates in the non-oil part of the seed (Thomas, 2005; Newkirk, 2009). Canola is the second in the world production in terms of oilseed crop with 3 million tons of canola oil each year and 4 million tons of canola meal (CM) produced in Canada (Canola Council of Canada, 2015). In Europe, rapeseed varieties with low levels of erucic acid (<2%) and glucosinolates (<30 μmol/g) are called "double-zero" or "double-low" rapeseed while in North America and Australia such varieties are called canola (Shahidi, 1990; Spragg and Mailer, 2007; Newkirk, 2009). New varieties of canola are continuously being developed to improve yield, disease and insect resistance, and oil and meal quality (Thomas, 2005). For CM, efforts have been directed towards increasing the concentration of CP, AA, vitamins, and minerals, and reducing the concentration of fiber and glucosinolates. At the same time, the digestibility of AA, energy, and carbohydrates is being improved (Newkirk, 2009). Consequently, the meal is becoming widely accepted as a source of protein for animal feeds (Anon, 1992).

Canola could be fed in poultry diets as full fat seeds (Meng *et al*, 2006; Talebali and Farzinpour, 2005), meals (Mushtaq *et al*, 2007) and oils (Thacker and Campbell, 1994). Canola seed, meal and crude oil are considered a good source of energy for poultry (Canola Council of Canada, 2015). The oil extraction methods including expelling and pre-press solvent extraction influence the nutritional value of CM. Pre-press solvent extracted CM is one of the most commonly used protein sources in animal nutrition (Canola Council of Canada, 2009). In addition, co-products from oil refining such as gums and soapstocks may be added back to the meal to reduce the dustiness and increase the energy density of CM. These additions may increase the total oil

content in CM by 1 to 2% (Spragg and Mailer, 2007, Newkirk, 2009). Soapstocks are formed by refining crude or degummed vegetable oils with alkali to produce sodium soaps which are separated from the oil by centrifugation. Usually, soapstocks will account for 5 to 10% of the crude oil mass, although higher values can be reached when the crude oil has a high concentration of free fatty acids (Dowd, 1996).

In this study, it was hypothesized that the nutritive value of CM can be diminished by the processing conditions used in the canola processing plants. In addition, gums and soapstocks from oil refining can increase the apparent metabolizable energy (AME_n) values of CM but would also contribute to the variability in the nutritive value of CM.

The goal of this study was to evaluate the chemical composition and nutritive value of pre-press solvent extracted meals of black-seeded *B. napus* canola from different crushing plants across Canada. Following chemical characterization, samples of CM of different nutritive profile were evaluated further to determine apparent metabolizable energy (AME_n) content and fat, and total phosphorus digestibilities by broiler chickens. In addition, the contribution of gums and soapstocks to the AME_n content of CM for poultry was determined.

CHAPTER 2: LITERATURE REVIEW

2.1. Composition of Canola Meal

2.1.1 Energy

Canola meal is known to contain a lower metabolizable energy (ME) content relative to soybean meal (SBM) despite having a higher amount of oil (Slominski and Khalaji, 2012; Thacker, 1990). The AME_n value of CM for poultry has been reported to be 2000 kcal/kg, and lower than 2440 kcal/kg reported for soybean meal (NRC, 1994). Slominski et al. (1999) suggested the TME_n (kcal/kg DM) in *Brassica napus and Brassica juncea* (yellow-seeded canola) as 2320 and 2152 kcal/kg, respectively and the TME_n (kcal/kg DM) for brown-seeded CM to be 2192 kcal/kg for broilers. There are differences in chemical composition of CM and SBM making it difficult to identify the components responsible for this difference in energy availability. Dietary fiber can explain the lower ME of CM compared to SBM. In CM, high dietary fiber content may accelerate the digesta passage rate which may result in lower digestion and nutrient utilization (Newkirk, 2009; Rogiewicz et al., 2012). The energy content of CM may vary as a result of the oil extraction method. For example, expelled CM contains residual oil at the average level of 9.7 % whereas the pre-press solvent extracted meal contain 3.2 % (NRC, 2012). In addition, gums and soapstocks which are added back to into the meal (around 1.5-2%) may contain approximately 50 % of canola oil and such oil is expected to increase the ME values of the CM (Bell, 1999). Reducing the fiber content of canola by breeding yellow-seeded lines, dehulling the seed prior to oil extraction and use of dietary enzyme are among ways to improve AMEn of CM (Khajali and Slominski 2012).

2.1.2 Protein Content

The concentrations of crude protein (CP) and amino acids (AA) in canola and rapeseed meals are influenced by environmental factors, growing conditions, variety, and amount of residual oil

and carbohydrates in the meal may influence the CP and AA contents (Bell and Keith, 1991; Bell, 1993; Spragg and Mailer, 2007; Newkirk, 2009). The varieties of canola seeds that contain a greater concentration of CP and AA may consequently result in more CP and AA in the final meals (Bell, 1993; Slominski et al., 2012). Gums, phospholipids and waxes removed from the oil during processing are added back to the CM in desolventization step and may also affect the protein content of CM (Summers and Leeson, 1977, Canola Council of Canada, 2015). Additionally, the screenings produced when canola seed is cleaned at the processing plant are similarly added back to the meal (Hickling, 2001).

2.1.3 Amino Acid Composition

Canola meal has been used extensively as a suitable alternative source of protein, with a good balance of amino acids, in livestock diets (Canola Council of Canada, 2015). When compared with SBM (Table 2.1), CM protein has relatively high concentration of Met, Cys and Thr, but less Lys and Trp (Newkirk, 2009; Khajali and Slominski, 2012). In addition, CM is known for its lower and less consistent amino acid digestibility compared to the SBM.

Table 2.1 Amino acid composition of soybean and canola meal (% of CP)[1]

Indispensable AA	Soybean meal (dehulled)	Canola meal
Arg	7.23	6.08
His	2.68	2.85
Ile	4.48	3.79
Leu	7.58	6.53
Lys	6.20	5.52
Met	1.38	1.89
Cys	1.47	2.29
Met + Cys	2.85	4.19
Phe	5.03	3.95
Tyr	3.33	2.83
Phe + Tyr	8.36	6.77
Thr	3.90	4.13
Trp	1.38	1.15
Val	4.67	4.75

[1] NRC, 2012.

2.1.4 Carbohydrates

Carbohydrates in Canola could be categorized into soluble sugars, insoluble carbohydrates, and fiber (Barthet and Daun, 2011). The concentration of soluble carbohydrates in mature seeds is approximately 10% of the oil-free weight, with sucrose ranging from 3.9 to 9.8%, stachyose from 0.8 to 1.6%, raffinose from 0.3 to 2.6%, fructose from 0.1 to 0.5%, and glucose from 0.1 to 0.4% (Barthet and Daun, 2011). Hemicellulose concentration was suggested to be around 3%, and cellulose and starch from 4 to 5%, and 1%, respectively (Salunkhe et al., 1992). The concentrations of crude fiber and neutral detergent fiber (NDF) in CM range from 10 to 12%, and 22 to 30%, respectively. These concentrations range from 7 to 12% and 24 to 28% for crude fiber and NDF in canola expelled meal (Sauvant et al., 2004; Spragg and Mailer, 2007).

2.1.5 Fiber Fractions and their Content

Fiber content of CM is three times higher than that of SBM (Bell, 1993), which results in its lower metabolizable energy (Newkirk et al., 1997). This high concentration of fiber in CM is the result of a large proportion of hull in the seed. Canola seed is smaller (~ 2 mm diameter) than soybean seed (~ 5 mm dimeter) which result in a higher specific surface area and high proportion of hull. The hull signifies 16.8 to 21.2 % of the canola seed mass (Carre et al., 2015), but after oil extraction, it increases to up to about 30 % of the meal weight.

Dietary fibers are grouped into soluble and insoluble fibers depending on their solubility in water. In CM, water-insoluble fiber accounts for 15.0-28.6% and water-soluble fiber for 2.0-12.6% (Jensen et al., 1995b). Fiber principally includes NSP, lignin with associated polyphenols, glycoproteins and minerals bound to fiber components (Simbaya, 1996).

Canola contains a higher proportion of oil in the seed (~42%) compared to soybean (~18%), which following its removal concentrates the hull and other fibre components in the meal. The hulls comprise between 19.6 and 30.4% of the meal after oil extraction (Jensen et al., 1995).

2.1.6 Dietary Electrolyte Balance (DEB)

To attain maximum broiler performance and livability, optimal dietary electrolyte balance is important to poultry (Saedi and Khajali, 2010; Khajali and Saedi, 2011). Canola meal is a good source of most of the minerals (Bell, 1993). Canola meal is richer in Ca, P, S, Mg, Mn and Se compared to SBM, although K and Cu contents are lower. Even though the availability of most of the minerals is low in CM due to the presence of phytic acid and high fibre content, high amounts of available Ca, Mg and P are present in CM (Table 4) (Bell, 1993; Khajali and Slominski, 2012).

High levels of sulfur in CM are well known to cause leg abnormality in broiler chickens as sulfur interferes with calcium absorption (Summers et al., 1992; Summers, 1995). In addition, approximately 75% of the total sulphur in SBM exists in organic form (that is, sulfur amino acids), whereas for CM it is around 60%. Based on Summers et al. (1990), sulfur from inorganic sources (i.e., sulfuric acid) would be more toxic than that from an organic source such as cysteine. The selection pressure by plant breeders to further reduce the glucosinolate content of Canola would also result in the reduced sulfur content of the meal, which would ultimately improve the DEB (Khalaji and Slominski, 2002). Less potassium in CM compared to SBM (11.4 vs. 19.6 g/ kg) may result in a lower dietary electrolyte balance (DEB) or dietary cation-anion difference (DCAD) in CM. Feed intake in broilers is positively correlated with DEB and consequently commonly observed decrease in feed intake when including CM in broiler feeds could be related to the cation and anion levels in the diet suggesting that higher levels of dietary cations will correct the problem (Newkirk, 2009; Khajali et al., 2011).

As for vitamins, CM is a considered as a rich source of biotin, niacin, choline, thiamin, Vitamin B6 and niacin than SBM (Khalaji et al., 2011).

2.1.7 Phosphorus Content, Digestibility and Relative Bioavailability in Canola Meal for Broilers

Phosphorus (P) is considered as the second most ample mineral in the body and is extremely important for bone mineralization, phospholipid support of membranes, and energy storage as ATP (Lisegang et al., 2002; Viveros et al., 2002). Swine NRC (2012) reported 1.08% (as-is basis) of total P content for conventional black-seeded solvent extracted CM whereas the Poultry NRC (1994) reported 1.17% (as-is basis) of P content. Slominski et al. (2012) reported 1.30% (% DM) of phosphorus content in conventional CM and 1.24% (% DM) for high protein yellow-seeded

Canola. The total P and non-phytate P levels reported by Poultry NRC (1994) for CM are 1.17 and 0.30% (as-is basis), respectively suggesting that P digestibility in poultry is 25.6% (as-is basis). Enami (2011) estimated P bioavailability to be approximately 30 to 50 % of the total P of CM due to the high amounts of phytate-bound P in comparison to the total P content.

Table 2.2 Electrolyte and vitamin composition of canola meal compared to soybean meal (% DM)[1]

Component	Canola meal	Soybean meal
Minerals, %		
Calcium	0.7	0.3
Phosphorus	1.2	0.7
Magnesium	0.6	0.3
Sodium	0.08	0.01
Potassium	1.29	2.0
Sulphur	0.72	0.48
Vitamins, mg/kg		
Biotin	1.0	0.3
Folic acid	2.3	1.3
Niacin	169.5	2.9
Pantothenic acid	9.5	16.0
Riboflavin	3.7	29
Thiamine	5.2	4.5

[1] Bell, 1993; Khajali and Slominski, 2012.

2.2 Anti-nutritional Factors

2.2.1 Glucosinolates

Glucosinolate is a term used for a wide variety of secondary sulphur-containing plant metabolites which are mainly found in the order *Brassicales*. Plants of the family *Brassicaceae* including the genus *Brassica* are placed in this order (rapeseed, mustard, and cabbage) (Chen, 2001; Khalaji and Slominski, 2012).

More than 120 different glucosinolates have been identified up to this date (Chen and Andreasson, 2001) based on different side-chain structure. There are two types of glucosinolates in CM: dominant aliphatic glucosinolates and indole (indolyl) glucosinolates (Slominski and Campbell, 1987). Canola glucosinolates include 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 latter two being the predominant indole glucosinolates in canola seeds (Bell, 1984; Slominski and Campbell, 1987; Shahidi and Gabon, 2007). It is generally believed that intact glucosinolates do not cause any toxic effects to animals, but, the break down products of glucosinolates either by enzyme myrosinase or by non-enzymatic factors can cause harmful effects to animals. In the seed, glucosinolates are present in vacuoles and are separated from myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), which is located in the myrosin cells. In the presence of moisture and following the seed rupture, glucosinolates come into contact with myrosinase, which leads to their hydrolysis to unstable aglucens (Bones et al., 1991). Consequently, glucans break down to yield isothiocyanates, goitrin, nitriles, and thiocyanates. In addition, non-enzymatic factors including heat, low pH, anatomical and physiological structure of the gastrointestinal tract, digesta transit time and microbial activity cause break down of GLS with the production of harmful break-down products (Bell, 1993).

2.2.2 Effect of Glucosinolates in Poultry

Toxic effects of glucosinolates relate to the content, extent of hydrolysis and composition of the hydrolysis products. It has been suggested that feeding of high-glucosinolate rapeseed meal to poultry results in reduced plasma thyroid hormone concentration, enlarged thyroids (goitre; Elwinger, 1986), enlarged livers (Campbell and Smith, 1979; Vermorel et al., 1986), hemorrhagic liver syndrome (Campbell and Slominski, 1991), and reduced growth rate (Campbell and Smith, 1979).

Hydrolysis products of glucosinolates have a bitter taste with anti-nutritional and goitrogenic effects negatively affecting thyroid hormone synthesis, appetite, feed intake and

growth performance (Slominski and Rakowska, 1985; McCurdy, 1990; Seskeviciene et al., 2004; Tripathi and Mishra, 2007; Woyengo et al., 2011). Low thyroid activity causes reduced metabolic rate, increased fat deposition and consequently results in growth depression in poultry (Thomas et al., 1983; Pearson et al., 1983; Whittow 2000). Glucosinolates derivatives (higher than 30 μmole/g) can lead to hypothyroidism, which may reduce thyroid hormones and change the ratio between triiodothyronine (T3) and thyroxin (T4) in the blood (Adibmoradi and Pedram 2007). Fenwick and Curtis (1980), suggested that feed intake problems related to glucosinolates are more severe in laying hens and turkey than in broilers. Opposite results with negative effect of glucosinolates on feed intake and body weight gain in broiler chickens were documented in several studies (Leeson et al, 1987; Marangos et al, 1974). Woyengo et al. (2011) observed the increase in the liver size and hepatic metabolic activity in birds fed expeller-extracted CM with potentially active myrosinase. This further changed nutrient metabolism in the liver and consequently reduced feed intake. Increases in hepatic enzyme activity resulting from liver hypertrophy were demonstrated in several studies (Slominski and Campbell, 1991; Newkirk and Classen, 2002). Studies by Ibrahim and Hill (1980) and Martland et al. (1984) showed that laying hens fed high levels of glucosinolates showed high mortality due to hemorrhagic liver syndrome and liver hypertrophy resulting in reduced egg production. These adverse effects limit the inclusion rate of CM in laying hen diets to 10% (Mawson et al., 1994).

Detoxification of glucosinolates by different methods was showed to be effective in reducing their toxic effects. Chemical treatment (Das and Singhal, 2001), soaking (Tyagi et al, 1997), water extraction, heat treatment (Jensen et al, 1995), toasting and pelleting are some of the methods of detoxifying glucosinolates. The effectiveness of detoxification of glucosinolates using these methods varies. Wet heating/pressure cooking and dry heating are two methods of heat

treatment of glucosinolates, in which pressure cooking is more effective than dry heating (Jensen et al, 1995; Subuh et al, 1995). Heating rapeseed meal at of 100°C for 30 min was found to reduce total glucosinolates by 50μmol/g and maintained the protein quality (Jensen et al, 1995).

In the past three decades, breeding programs have reduced the glucosinolates content of CM to less than 30 μmol/g of aliphatic glucosinolates in the oil-free meal (Canola Council of Canada, 2015). In addition, glucosinolates in CM are reduced further during pre-press solvent extraction process due to heat treatment and myrosinase inactivation (Canola Council of Canada, 2015). Newkirk and Classen (2002) showed that solvent-extracted CM has less bitter taste in comparison with its expeller-pressed counterpart, suggesting the effect of desolventization on the loss of glucosinolates hydrolysis products. Later studies confirmed these results demonstrating higher glucosinolates content in expeller-pressed meal which lacks the desolventization-toasting step in meal preparation (Glencross et al., 2004; Spragg and Mailer, 2007).

2.2.3 Sinapine

Sinapine, the choline ester of sinapic acid (Butler et al., 1982), is considered the most abundant phenolic ester in rapeseed. Canola meal contains approximately 1% of sinapine (Canola Council of Canada, 2015). Sinapine has been associated with the production of a "fishy" taint in brown-shelled eggs. Sinapine is a bitter phenolic compound and therefore it would contribute to the unpleasant and bitter taste of different products (Naczk et al., 1998). Progress has been made in identifying the genetic defect that leads to the production of tainted eggs (Honkatukia et al., 2005) In addition; research in Germany is underway aiming to reduce the levels of sinapine in rapeseed/canola by developing low-sinapine varieties with yellow seeded and low-fiber characteristics (Norddeutsche Pflanzenzucht and Lembke, 2010).

2.2.4 Phytates

Phytic acid [myo-inositol (1,2,3,4,5,6-hexakis dihydrogen phosphate)] is the major storage form of P in grains and oil seeds. It is considered as an anti-nutritional factor even though its role in animal nutrition is not completely understood (Khalaji and Slominski, 2012). Phytic acid has a strong capacity to form insoluble complexes with proteins and several minerals such as Ca, Fe, Zn, Mn, Mg to make them biologically unavailable for animals (Cabahug et al., 1999). The phytic acid content of CM is around 2.5% on dry matter basis (Canola Council of Canada, 2009) and higher than that of SBM (~1.5%, De Boland et al., 1975). Summers et al. (1983) reported the proportion of phytate P in the total P content of Brassica meals from 36 to over 70%.

Poultry lack the ability to hydrolyse the majority of phytate in spite of having endogenous intestinal phytase (Maenz and Classen, 1998). As a result, the bound P is poorly digested (Nahashon et al., 1994). In addition, some of the protein bound to phytate is poorly digested too, for which diminishes the availability of some amino acids. As reviewed by Woyengo and Nyachoti (2013), phytic acid can affect animal performance by reducing nutrient digestibility through binding to nutrients, the digestive enzymes or both which, as a result, would increase endogenous losses of amino acids. Adhikari et al. (2013) conducted a study with CM from *B. napus* black and *B. juncea* Canola and reported true total tract digestibility of phosphorus values of 33.3 and 32.0 %, respectively. Standardized total tract digestibility values were reported to be 31.0 and 28.3 %. It has been suggested that phytate also reduces the metabolizable energy content of CM, but the reason for the effect is unknown (Newkirk and Classen, 2001).

2.2.5 Tannins

Tannins which are complex polyphenolic compounds with the molecular weights of 500 to 3,000 Da are also present in CM. They are mostly found in the hulls with dark-colored hulls containing more tannins than yellow hulls. Two types of tannins exist: hydrolysable and condensed fractions (Yapar and Clandinin, 1972). Bate-Smith and Ribereau-Gayon (1959) were first to report condensed tannins in rapeseed hulls which was verified by Durkee (1971), who identified cyanidin, pelargonidin, and *n*-butyl derivative of cyaniding in the hydrolysis products of rapeseed hulls. Khajali and Slominski (2012) reported that in addition to giving the CM a dark color, tannins may bind with protein and proteolytic enzymes in gastrointestinal tract and reduce protein digestibility. Mansoori and Acamovic (2007) also suggested that water-soluble tannins could be responsible for poor growth performance of broiler chickens in their study. However, 70 to 96% of the total tannins in canola are water-insoluble which are located in the hulls therefore could have minimal effect on the nutritive value of CM (Khajali and Slominski, 2012).

2.3 Canola Seed Processing

The chemical composition and nutritive value of CM depends mainly on the processing conditions during the canola oil extraction process. The processing methods used to extract the oil from canola seeds consists of seed cleaning, flaking, cooking, hexane extraction, and processing of oil (Unger, 2011).

In general, there are two types of oil extraction methods: processing with or without solvent extraction (Adams et al., 2006). Oil may be expelled from the seeds by cold-pressing or double pressing methods if solvent extraction is not used (Spragg and Mailer, 2007, Newkirk,

2009). Canola meal composition as affected by different extraction procedures is shown in Table 2.3.

Table 2.3 Composition of full fat canola seeds, expeller and pre-press solvent extracted canola meals[1]

Component	Full fat seeds[2]	Pre-press solvent extracted meal[3]	Expeller meal[4]
Moisture (%)	6.2	12.0	5.0
Crude protein (%)	18.4	36.7	34.8
Oil (%)	40.5	3.3	9.5
Linoleic acid (%)	8.3	0.7	1.9
Ash (%)	3.8	6.7	6.2
Crude fiber (%)	8.9	11.2	11.8
Neutral detergent fiber (%)	17.9	25.4	
Acid detergent fiber (%)	12.7	16.2	
Sinapine (%)		1.0	
Phytic acid (%)		2.3	
Glucosinolates (μmoles/g)		4.2	

[1]Canola Council of Canada (2015).
[2] Feedipedia, 2015.
[3] Slominski, 2015; Broderick, 2015.
[4] Slominski, 2015; Broderick, 2015.

2.3.1 Solvent Extraction Process

In Canada, canola seed is traditionally processed using pre-press solvent extraction and includes expelling prior to solvent extraction. Pre-press solvent extraction process (Figure 2.1) of Canola involves various stages including: 1) Cleaning (according to grading standards based on maximum moisture content, seed damage and chlorophyll level), and drying of the seed to 6-7% moisture, 2) Preconditioning (heating), 3) Flaking, 4) Cooking, 5) Expelling, 6) Solvent extraction, 7) Desolventization in the desolventizer-toaster (DT), 8) Cooling, 9) Drying, and 10) Grinding, and in some cases pelleting (Unger, 2011; Canola Council of Canada, 2015). In the pre-press

solvent extraction process, a portion of oil is extracted by using screw presses and then the remaining oil is removed by solvent extraction. Canola seeds are preheated at 35ºC in order to prevent shattering while flaking (Unger, 1990). Flaking ruptures, the seed cell walls. In the next step, flakes are cooked-conditioned in order to thermally rupture oil cells resulting in reduced oil viscosity and enhanced coalescing of oil droplets, increased diffusion rate of prepared oil cake and denatured hydrolytic enzymes (myrosinase). The seed expelling step which removes up to 60-70% of oil is followed by solvent extraction with hexane. Desolventization-toasting removes the hexane by heating the meal up to 100-110˚C. Toasting the meal leads to the decrease in glucosinolate content. After desolventization step, the CM contains 1% of residual oil and 15 to 18% of moisture. (Canola Council of Canada, 2015).

Soapstocks and gums are the portion of phospholipid materials in crude canola oil and are removed during processing. In Canada, these by-products are added back into the meal during desolventization-toasting at the level of 1-2%. This decreases the dustiness of the meal, increases the phospholipid content and the metabolizable energy content of the meal (Canola Council of Canada, 2015).

2.3.2 Effect of Processing on Quality of Canola Meal

The processing conditions which is used to extract canola oil influences the quality of the CM. Newkirk (2009) considered 3 processing key factors to affect quality of CM which included temperature, moisture, and additives (gums and soapstocks). While the temperature (90°C) during processing can be helpful in deactivating myrosinase enzyme in the seed to avoid break down of glucosinolates to toxic aglucones, there are some adverse effects of temperature during processing as well. Amino acids, especially lysine, threonine and methionine are susceptible to damage in the presence of heat (Hurrell, 1984). Excess heat results in decrease in amino acid digestibility in

broiler chickens (Anderson-Haferman et al, 1993; Zhang and Parsons, 1994). A decrease in protein quality and digestibility is due to the Maillard reaction involving amino acids, especially lysine (Newkirk and Classen 1999). Schumacher DT is the most popular design in North America. The solvent extracted meal entering the DT is yellow in color, but after exit, CM is brown in color, which is indicative of Maillard reaction. The Maillard reaction is a chemical reaction between amino acids and reducing sugars and CM contains both the required components for this reaction to occur. Any compound with a free amino group, including free amino acids, epsilon amino groups of protein-bound lysine, and free amino groups of thiamine, folacin, and gossypol is exposed to the Maillard reaction under conditions of elevated temperature and moisture (Mavromichalls, 2001). Therefore, the nutritive value of CM, can be affected by processing conditions (Adewole, et al., 2015). Extreme heating during pre-press solvent extraction may reduce digestibility of AA, especially lysine. Adewole et al. (2015) also suggested that dietary fiber and low glucosinolate content observed in some canola processing plants could have been caused due to CM overheating. In addition, oil refining by products such as gums and soap stocks which are added back to the meal may increase the oil content of CM by 1 to 2% (Spragg and Mailer, 2007, Newkirk, 2009; Barthet and Daun, 2011).

2.4 Soapstocks and Gums

Edible oils are produced primarily from oilseeds. Free fatty acids (FFA), mono-, di-, and triacylglycerides (MAGs, DAGs, and TAGs), phosphatides, pigments, sterols, tocopherols, glycerol, hydrocarbons, vitamins, protein fragments, trace amounts of metals and glycolipids are the main component of crude oils extracted from oilseeds (Cheryan, 1998). The initial step in producing edible oils is the separation of the oil from other solid components of the seeds which

are achieved by pressing followed by solvent extraction (Younget al., 1994). In the next step, this crude oil is refined to remove free fatty acids and other non-TAG components which mainly contribute to undesirable flavor, odor and appearance. Soapstocks, deodorizer distillates and acidic water are by-products produced from crude oil refining processes.

2.4.1 Characteristics of Soapstocks

Soapstocks are formed by refining crude oils with alkali to produce sodium soaps, which are later separated from the oil by centrifugation. Usually, soapstocks will account for 5 to 10% of the crude oil mass, although higher values can be reached when the crude oil has a high concentration of free fatty acids (Dowd, 1996). Depending on the refining method and the equipment used, soapstocks consist of 70-95% water and 5-30% of fatty material with 60-70% of the fatty material being in the form of sodium soaps of fatty acids and the rest in the form of triglycerides, phospholipids, and negligible amounts of other oil-derived compounds (Mag et al., 1983).

Soapstocks are being used in animal feed as an additive, especially in pig and poultry diets. Because of the high concentration of fatty acids, soapstocks can be used in a similar way to fat in animal diets. In addition, it can be used as a good nutrient source for microorganisms, feedstock for chemical reactions in fertilizer production, and as fatty acid source for biodiesel and animal feedstock.

When soapstocks are added to the meal, they increase the energy density of the diet, reduce dust, improve the palatability, help heat stress conditions, and improve pelleting of feeds by reducing particle separation. However, it has been suggested to add soapstocks in small amounts (approximately 3.5%) as long chain fatty acids are difficult to digest by animals (Johnson & McClure, 1973).

The percentage of polyunsaturated fatty acids contained in a soapstocks mixture is one of the parameters that has to be taken into consideration as the percentage of polyunsaturated fatty acids in the diet can affect a broilers taste. It has been documented by Pardio et al. (2005) that the addition of up to 1% of polyunsaturated fatty acids from soybean soapstocks to broilers' diet from one to seven weeks of age increased their weight by approximately 1.7 g. Soybean oil soapstocks are considered to be a proper alternative for soybean or corn oil in soybean meal-sorghum animal diets (Bartov et al., 1974) and does not affect the composition of hen egg yolk (Pardio et al., 2005). However, Bruce et al. (2006) observed decreased amino acid digestibility and consequent growth performance problem in pigs when added soybean soapstocks to diets.

2.5 Spent Bleaching Clay (SBC)

Vegetable oils for human consumption are also subjected to a bleaching process during refining to remove non-glyceride components and impurities including carotenoids, chlorophylls, trace metals, free fatty acids and hydro-peroxides to ensure that the oil meets the necessary requirements for colours. In addition, bleaching improves product shelf-life which is attained by mixing the oil with bleaching earth, typically bentonite clay, under a vacuum for 15– 30 min (Bergaya et al., 2006; Zschau, 2001). In the next step, the oil/bleaching earth mix is filtered to separate the treated oil from the waste clay. Bleaching clay may also be referred to as "bleaching earth", "Fullers earth" or "Friedland clay", and usually contains 20–40% (w/w) of residual vegetable oil and pigments, oxidation products, free fatty acids (FFA), phosphatides and trace metals (Pollard et al., 1991; Pollard et al., 1993). Spent bleaching clay (SBC) is intended for use in all livestock species as a source of fats and oils to support the nutritional requirements of the animal. This fat additive was shown to contribute 3230 kcal/kg to poultry metabolic energy, 2698

kcal/kg to swine net energy and 2.1 Mcal/kg to dairy net energy of lactation when SBC was included in canola and soybean meal at a maximum level of 1.0%. The current threshold for SBC added to vegetable protein meals in the USA is 0.2% and 1.0% in the European Union. No threshold currently exists for meal sold in Canada (Canola Council of Canada, 2015) but the Canadian Oilseed Processors Association (COPA) has been working closely with the Canadian Food Inspection Agency (CFIA) to establish a 1.0% inclusion by weight (basis of oil-free clay) threshold for spent bleaching clay (SBC) to be added to CM at a 0.2% inclusion rate (Canola Council of Canada, 2016).

2.6 Methods of Fat Analysis

The procedures used for fat analysis include ether extraction and acid-ether extraction. Extraction in ether (or hexane) is the conventional method of fat analysis but complications with low oil recovery from fat-filled milk powders and newly introduced calcium (Ca) salts of fatty acids in the United Kingdom led to regulatory changes in oil extraction procedures from many compound feeds and feeds of animal origin (Sanderson, 1986). These changes included boiling the sample in 3*N* HCl, followed by washing with water prior to the ether extraction step. This adjustment resulted in consistently higher oil values than ether extraction alone. The acidified ether extraction (modified Werner-Schmidt method) adapted from AOAC 954.02 (Horwitz, 2000) is indicated as the recommended procedure for fat analysis of Ca soap feed supplements (Palmquist and Jenkins, 2003). In a study by Sukhija and Palmquist (1988), extraction with petroleum ether/10% glacial acetic acid increased the weight of oil recovered from alfalfa hay, corn silage, and low- and high fat concentrate mixes. Also, fatty acid analysis of the acid extracts resulted in

higher fat content than without acid treatment, and the proportion of fatty acids in the acidified ether extract was shown to be higher for all samples except corn silage.

In the solvent extraction method, some of the Ca soaps and the phospholipids may not be extracted, whereas the acid hydrolysis method is believed to result in a higher fat extraction. Solvent extraction of fat without acid hydrolysis will, therefore, result in the lower values. Kil et al. (2010) found lower fat digestibility values in their samples using acid hydrolysis method comparing to studies in which samples were analyzed by ether extraction without acid treatment. It has been suggested that the underestimation of the concentration of fat in the ileal and fecal samples by the ether extraction procedure is greater than for the diet samples as the concentration of Ca soaps is likely greater in ileal and fecal samples than in diets and feed ingredients, which may result in the higher calculated digestibility values (Kil et al., 2010).

2.7 Conclusions

Feed represents 60-70% of the costs of poultry production and, therefore, it is important to use the lower cost feed ingredients. The term Canola has been recognised as a name for "double zero" cultivars of rapeseed. Canola is considered as the most important oilseed grown in Western Canada with approximately 18 million tons produced annually and represents one of the main success stories of Canadian agricultural research with the reduction of erucic acid to less than 2% of oil and aliphatic glucosinolates to less than 30 µmol/g of meal.

Knowledge of the characteristics of CM is critical for diet formulation. Canola meal is an excellent source of amino acids and its protein is known for its low antigenicity. However, lower metabolizable energy, high levels of fiber, phytate and other anti-nutritional factors restrict its use to less than full replacement of SBM in poultry diet. Despite containing approximately 45% of

protein, CM is low and variable in amino acid digestibility, especially lysine (NRC, 1994) which led to studies on the effect of processing on the nutritional value of CM for non-ruminants.

Canola meal quality may be affected by the method and condition used for oil extraction. Low yield of oil from seed expelling made pre-press solvent extraction process the most common method of oil extraction as it results in higher yields of oil and meal quality. However, pre-press solvent extraction process may decrease protein and amino acids digestibilities, especially lysine, due to meal desolventization/toasting. Maillard reaction which is promoted by the elevated temperature and moisture in DT leads to the formation of aldose products of AA which are not efficiently utilized by animals.

Gums and soapstocks are added back to the meal in the pre-press solvent extraction process. This addition reduces the dustiness of the meal as well as increases the total oil content of CM by 1 to 2%.

CHAPTER 3: MATERIALS AND METHODS

3.1 Sample Collection and Preparation

Canola meal samples were collected from 13 canola crushing plants in Canada which included: Bunge Oilseed Processing at Altona, MB; Harrowby, MB; Nipawin, SK; Fort Saskatchewan, SK and Hamilton, ON; Archer Daniels Midland (ADM) Agri-Industries Ltd. at Windsor, ON; Lloydminster, SK and Yorkton, SK; James Richardson International *(*JRI) Canola Oil Processing Plant at Yorkton, SK and Lethbridge, AB; Cargil Canola Processing, Clavet, SK; Camrose, AB. Each crushing plant provided three samples which were collected within 1 week intervals in 2015. There is no other information about sampling procedures as this study was carried out as a blind survey.

3.2 Chemical Analyses of Canola Meal

Canola meal samples were analyzed in duplicate for DM (AOAC (1990) method 925.09), ash (Method 942.05; AOAC Int., 2007), gross energy (GE) by bomb calorimetry (Model 6300, Parr Instruments, Moline, IL), ether extract (EE) (Method 920.39; AOAC, 1990), acid-hydrolyzed ether extract (AEE), which was determined by acid hydrolysis using 3*N* HCl (Sanderson, 1986) followed by fat extraction with ether (hexane) (Method 920.39; AOAC, 1990) using an Ankom extraction system (Macedon, NY, USA). The samples were also analyzed for CP by combustion (method 990.03; AOAC, 1990) with an N analyzer (Model CNS-2000; LECO Corp., St. Joseph, MO), amino acids (AA) (994.12) using an amino acid analyzer (S4300, Sykam GmbH, Eresing, Germany), crude fiber (CF) (Method 978.10; AOAC Int., 2007), NDF (Goering and Van Soest (1970), total phosphorus (P) as described in Method 965.17 (AOAC, 2005), and phytate (Haug and Lantzsch (1983). Carbohydrate profile (glucose, fructose, sucrose, raffinose, and stachyose)

was determined by gas−liquid chromatography according to the procedure described by Slominski et al. (2004). Briefly, 0.2 g of sample was extracted with 5 ml of 80% ethanol and 1 ml of myo-inositol for 3h and then centrifuged. The supernatant was dried under a stream of air at 40°C and derivatized with a mixture of acetone:bis (trimethylsilyl) acetamide:trimethylsilyl chloride:1-methylimidazole (2:1:0.1:0.05 vol/vol). The sugars were determined by gas-liquid chromatography using 3% OV-7 column and Varian 430 gas chromatograph (Agilent Technologies, Mississauga, ON, Canada). Glucosinolates were determined using the method of Thies (1977) with some modifications (Slominski and Campbell 1987). Briefly, 100 mg of canola samples was weighed into 15 mL centrifuge tubes, and after adding two milliliters of methanol, 1.0 mL of benzyl glucosinolate (internal standards, 0.5 mM), and 0.1 mL of lead-barium acetate were extracted for 3 h at room temperature, and then centrifuged. Two milliliters of supernatant were transferred to a DEAE-Sephadex column, washed with 1 mL of 67% methanol, water, and pyridine acetate, and then were incubated at room temperature overnight after adding purified sulfatase solution. Desulfated glucosinolates were then eluted with 2 mL of 60% methanol, evaporated under air and the dry residue was trimethylsilylated for 30 min at room temperature. The trimethylsilyl derivatives of desulfoglucosinolates were then separated by gas-liquid chromatography using a glass column packed with 2% OV-7 (Slominski and Campbell, 1987). Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with modifications (Slominski et al., 2006). In brief, a 100-mg sample was treated with dimethylsulphoxide and incubated overnight at 45°C with a solution of starch-degrading enzymes amylase, pullulonase and amyloglucosidase (Sigma, St. Louis, MO). Ethanol was then added, the mixture left for 1 h, centrifuged and the supernatant discarded. The dry residue was

dissolved in 1 mL of 12M H_2SO_4 and incubated for 1 h at 35°C. Six mL of water and 5 mL of myo-inositol (internal standard) solution were then added and the mixture was boiled for 2 h. One mL of the hydrolysate was then taken and neutralized with 12 M ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetic anhydride in the presence of 1-methylimidazole. Component neutral sugars were separated using SP-2340 column and Varian CP-3380 gas chromatograph (Agilent Technologies, Mississauga, ON, Canada).

Due to the high solubility of NSP in the NDF solution and therefore losses of NSP on NDF analysis, total dietary fiber was determined by a combination of NDF and neutral detergent-soluble NSP measurements and was calculated as the sum of NDF and NDF-soluble NSP (Slominski et al., 1994, 2006). Neutral detergent fiber-soluble NSP were calculated as total sample NSP minus NSP present in the NDF residue. Neutral detergent insoluble crude protein (NDICP) represented the amount of crude protein present in the NDF residue. The value for lignin with associated polyphenols was calculated by difference between the total fiber and NDICP + NSP contents.

3.3 Apparent Metabolizable Energy (AME_n) Assay with Broiler Chickens

Broiler chickens were assigned at random to each of forteen tretments treatments to evaluate the effect of canola meals on metabolizable energy (AME_n) content. A basal diet composed of practical feed ingredients was used and was formulated to provide 3104 kcal/kg ME and 22.1 % protein (Table 3.1). Thirteen experimental diets composed of 70% of basal diet and 30% of test ingredient were used in this experiment. 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. Each treatment had 6 replicate pens of 5 birds per pen. Birds were

provided with continuous light, had free access to water, and were fed corn/soybean meal basal diet formulated to provide 3104 kcal/kg ME and 22.1% protein (Table 3.1). 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 the 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 ICP and AOAC 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). Animal procedures in the current study 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.

3.4 Ether Extract (EE) Digestibility, Acid-hydrolyzed Ether Extract (AEE) Digestibility and Phosphorus (P) Digestibility in Broiler Chickens

Duplicate samples of diets and excreta obtained from broiler chicken assay were analyzed for EE (Method 920.39; AOAC, 1990) and AEE (Sanderson, 1986) in an Ankom extraction system (Macedon, NY, USA). Total phosphorus (P) was analyzed as described in Method 965.17 (AOAC, 2005) for all diet and excreta samples in order to measure the P digestibility.

Table 3.1 Composition and calculated analysis of a basal diet used in the apparent metabolizable energy (AME_n) assay

Item	%
Ingredient, %	
Corn	57.47
Soybean meal	30.60
Fish meal	5.00
Vegetable oil	2.85
Calcium carbonate	1.52
Monocalcium phosphate	0.87
DL-Methionine	0.10
L-Lysine	0.05
Threonine	0.04
Mineral premix[1]	0.5
Vitamin premix[2]	1.0
Total	100.0
Calculated analysis (% unless specified)	
Metabolizable energy (kcal/kg)	3104
Crude protein	22.1
Calcium	1.05
Non-phytate P	0.45
Methionine	0.53
Methionine + cysteine	0.58
Lysine	1.28
Threonine	0.88

[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.

3.5 Calculation of Apparent Metabolizable Energy (AMEn)

ME of basal diet = $GE_{Diet} - (GE_{Excreta}/ g_{Diet} + N$ correction)

ME of test diet= $GE_{Diet} - (GE_{Excreta}/ g_{Diet} + N$ correction)

where,

ME (kcal/kg) = Metabolizable energy content of the diet;

GE diet and GE excreta (kcal/kg) = Gross energy of the diet and excreta, respectively

N correction= $N_{retained}$ x 8.22

N retained (g/kg) is the N retained by the broilers per kilogram of diet consumed

The retained nitrogen was calculated as:

$N_{Retained} = N_{Diet} - (N_{Excreta} \times Cr_{Diet}/Cr_{Excreta})$

where,

N_{Diet} and $N_{Excreta}$ (%) = N contents of the diet and excreta, respectively

$CrO2_{Diet}$ and $CrO2_{Excreta}$ (mg/g) = Chromium oxide concentration of the diet and excreta, respectively

The AME_n of the test ingredient was calculated as follows:

ME of the basal diet – [(ME of the basal diet - ME of the test diet)/0.3)]

3.6 Calculation of Fat Digestibility

Fat content of the basal diet – [(Fat content of the basal diet – Fat content of the test diet)/0.3)]

3.7 Calculation of Phosphorus (P) Digestibility

P content of the basal diet – [(P content of the basal diet – P content of the test diet)/0.3)]

3.8 Statistical Analysis

All data were analyzed by ANOVA using the General Linear Models (GLM) procedure (SAS Institute, 1989). Means were separated by Tukey's Honestly Significant Difference. Differences were considered significant when $P<0.05$. Means, standard deviations (SD), coefficients of variation (CV) between treatments, and linear regressions equations for some chemical compositions were calculated using the standard methods.

CHAPTER 4: RESULTS

4.1 Effect of Crushing Plant on the Chemical and Nutritive Composition of Canola Meal

As presented in Table 4.1, there were variations ($P < 0.05$) between crushing plants in the contents of CP (Nx6.25) (from 38.9. to 43.1% DM), EE (from 2.44 to 3.77% DM), AEE (from 3.53 to 5.77 % DM), ash (from 7.57 to 8.57% DM), total P (from1.05 to 1.20% DM), phytate P (from 0.73 to 0.89% DM) and NPP (from 0.25 to 0.40% DM). Canola meal from plant 10 had the highest CP contents with the meals from plants 1, 2, 3, 5, 7 and 12 being significantly lower ($P < 0.05$). The overall mean CP content of CM from all crushing plants was 40.3% DM.

The mean ether extract content was 2.91% DM. Plant 2 had the highest EE content of 3.77% DM which was not significantly different from samples from other plants except for the meal from plant 6 which had the lowest EE content of 2.44% DM. Meals from plant 12 had the highest AEE content of 5.77 % DM while those from plant 5 had the lowest AEE content of 3.53% DM. The different contents of EE and AEE in CM are shown in Figure 4.1.

Canola meal from plant 6 had the highest ash content of 8.57% DM while those from plants 10 and 4 were significantly lower. Meals from plant 10 had the lowest content of 7.57% DM. The mean ash content was 8.08% DM.

There were differences ($P < 0.05$) in the total P, phytate P and non-phytate P contents of CM between crushing plants. Canola meal from plants 4 and 1 had the highest contents of total P which was significantly higher than those from plants 10 and 12 (1.05 % DM). Meal from plant 4 had the highest P content of 1.20% DM among crushing plants. The overall mean total P content was 1.13 % DM. The phytate P contents of meals from plants 2 and 3 were significantly lower than that from plant 11 which had the highest phytate P content of 0.89% DM. Plant 3 had the lowest phytate P content of 0.73% DM. Canola meals from plant 4 and 12 contained the highest

(0.40% DM) and lowest (0.25% DM) contents of NPP, respectively. The overall mean phytate P and NPP contents were 0.80 and 0.33% DM respectively.

As documented in Table 4.2, there were differences ($P < 0.05$) between crushing plants in the contents of simple sugars (from 0.23 to 0.63% DM), sucrose (from 5.63 to 6.21% DM) and starch (from 1.17 to 1.85% DM). Meals from plant 13 had the highest (0.63% DM) content of simple sugars whereas those from plant 9 had the lowest content of 0.23 % DM. Higher contents of sucrose were recorded for CM from plants 1 and 9 than that from plant 11. No difference in oligosaccharides content of CM was observed between crushing plants in this study. Samples from plants 10 and 13 were significantly lower in starch content than that from plant 6 which had the highest (1.85% DM) starch content. The mean simple sugars, sucrose, oligosaccharides and starch contents of CM were 0.39, 5.92 and 3.22 and 1.50% DM, respectively.

As shown in Table 4.3, there were significant differences ($P < 0.05$) between crushing plants in the contents of NDF (from 28.3 to 34.0% DM), NDICP (from 3.41 to 7.45% DM), lignin and polyphenols (from10.2 to 13.9% DM), and total dietary fiber (from 37.4 to 43.3% DM). Meals from plant 1 and 6 were significantly lower in NDF content than that from plant 10 which had the highest content of NDF between crushing plants. The mean NDF content was 30.7% DM. There was no significant difference between crushing plants in the content of NSP, which averaged 23.3% DM (from 22.4 to 24.2% DM). Canola meal from plant 2 contained the highest NDICP (7.45% DM), which was significantly higher than those from plants 1, 3, 5, 8 and 10. Lowest lignin and polyphenol content (10.2% DM) was recorded for CM from plant 7 which was not significantly different from other plants except for those samples from plants 12 and 3. The highest (13.9% DM) lignin and polyphenol contents was observed for CM from plant 12. Total dietary fiber content was highest in CM from plant 12 (43.3% DM) which was significantly higher than

those from plants 1, 4 and 8. The lowest total dietary fiber content in CM was observed for plant 1. The mean NDICP, lignin and polyphenols and total fiber contents were 4.98, 11.7, and 40.0% DM, respectively.

4.2 Effect of Crushing Plant on Amino Acids Content of Canola Meal

The contents of amino acids of 13 different crushing plants are shown in Table 4.4. There were plant effects on asparagine, glutamine, proline, alanine, cysteine, tyrosine, phenylalanine and lysine contents in this study. Plants 1 and 2 had the lowest asparagine contents which were not significantly different from other plants except for plant 10 which had the highest asparagine content between crushing plants. Canola meal from plant 2 had the lowest glutamine content between crushing plants which was significantly lower than those from plants 4 and 10. The highest proline content was observed for meal from plant 10, which was significantly higher than those from plants 5 and 6. Plants 8 had the highest alanine content which was significantly higher than those from plants 1 and 2. Meals from plants 2 and 5 had the lowest cysteine content between crushing plants which was not significantly different from other plants except for plant 10. The lowest amount for tyrosine was recorded for plant 2 which was significantly lower than that from plant 6. Meals from plants 4 and 10 were significantly higher in phenylalanine content than that from plant 2 but not for samples from other plants. Sample from plant 8 had the highest lysine content which was significantly higher than those from plants 2 and 13. Canola meal from plant 2 contained the lowest amount (2.03% DM) of lysine among the crushing plants with the mean lysine content of 2.19% DM. The mean asparagine, glutamine, proline, alanine, cysteine, tyrosine, phenylalanine contents were 2.83, 6.71, 2.78, 1.93, 0.92, 1.01 and 1.58% DM, respectively. There

was no plant effect on amino acids threonine, serine, valine, methionine, ileucine, leucine, histidine and NH_3 between crushing plants.

4.3 Effect of Crushing Plant on Glucosinolates Content of Canola Meal

As shown in Table 4.5, there was significant plant effect ($P < 0.05$) on glucosinolate content of CM from 13 crushing plants. Canola meal from plant 3 contained the highest amount (5.70 µmol/g DM) of glucosinolate while samples from plant 13 was found to contain the lowest glocosinolates content. The mean glucosinolate content was 2.75 µmol/g DM.

4.4 Apparent Metabolizable Energy (AME_n) Content

There was significant plant effect ($P < 0.05$) on AME_n content of CM in this study but the Tukey's test was not able to detect the differences between means (Table 4.6). Plant 12 had the highest content of AME_n between crushing plants. On average, CM showed an AME_n value of 1,789 kcal/kg which ranged from 1,618 kcal/kg (plant 9) to 1,995 kcal/kg (plant 12).

4.5 Canola Meal Ether Extract (EE) and Acid-hydrolyzed Ether Extract (AEE) Digestibilities in Broiler Chickens

The results of the apparent digestibility of EE fat, AEE fat, gums and soapstocks are shown in Table 4.7. There were significant differences ($P < 0.05$) between crushing plants in EE digestibility and digestible EE content in this study. Meal from plant 10 had the lowest EE digestibility. Canola meal from plant 1 had the highest (90.9%) EE digestibility between crushing plants. The mean EE digestibility was 81.1%. Regarding digestible EE content, CM from plant 2

had the highest (2.81%) digestible EE content between crushing plants, which was significantly higher than other plants except for those samples from plants 1,12 and 13. Meals from plant 10 had the lowest digestible EE content among crushing plants with the mean digestible EE content of 2.1%. There was significant ($P < 0.05$) plant effect on AEE digestibility and digestible AEE content of CM. Plant 11 had the lowest digestible AEE content and was significantly lower than those from plants 9 and 12. Canola meal from plant 12 contained the highest (4.2%) digestible AEE content. There were significant differences ($P < 0.05$) between crushing plants in the content of digestible gums and soapstocks. Plant 9 produced meals with the highest digestible gums and soapstocks content.

4.6 Canola Meal Phosphorus Digestibility in Broiler Chickens

There were significant differences ($P < 0.05$) between crushing plants in P digestibility, amount of P retained by the body, and digestible P content (Table 4.8). Canola meal from plant 3 had the lowest P digestibility, amount of retained P, and digestible P content, which was significantly lower than those from plants 6, 10, and 11. The highest P digestibility, and digestible P content were observed for meals from plants 10, and 6 and 10, respectively. The mean amount of retained P, CM P digestibility and digestible P content were 0.38, 25.7 and 0.26 %, respectively.

4.7 Some Notable Correlations Between Chemical Components of Canola Meal

There were positive relationships ($P < 0.05$) between NDF and total dietary fiber contents ($R^2 = 0.85$; Figure 4.2) and between dietary fiber and NDICP contents ($R^2 = 0.66$; Figure 4.3). Also, there was a negative correlation ($P < 0.05$) between lysine and NDICP contents ($R^2 = 0.45$; Figure 4.4). No significant relationship was observed between total dietary fiber and lysine ($R^2 = 0.28$; Figure 4.5) and lysine and NDF ($R^2 = 0.05$; Figure 4.6). There was a positive relationship ($P < 0.05$) between AME_n and EE contents of CM from different crushing plants ($R^2 = 0.48$; Figure 4.7). No significant relationship was observed between AME_n and AEE ($R^2 = 0.24$; Figure 4.8), AME_n and carbohydrate ($R^2 = 0.0042$; Figure 4.9) and AME_n and CP contents ($R^2 = 0.15$; Figure 4.10).

Table 4.1 Effect of canola meal source on crude protein (CP), ether extract (EE), ash, total phosphorous (P), phytate P, and non-phytate P (NPP) contents (% DM)[1].

Crushing Plant	CP	EE	Ash	Total P	Phytate P	NPP
1	39.6^{c}	3.28^{ab}	8.39^{ab}	1.17^{a}	0.79^{ab}	0.38^{ab}
2	38.9^{c}	3.77^{a}	8.16^{ab}	1.12^{abcd}	0.77^{b}	0.35^{abcd}
3	39.6^{c}	2.63^{ab}	8.10^{ab}	1.12^{abcd}	0.73^{b}	0.39^{ab}
4	42.5^{ab}	2.98^{ab}	7.86^{b}	1.20^{a}	0.80^{ab}	0.40^{a}
5	39.5^{c}	2.62^{ab}	8.17^{ab}	1.15^{abc}	0.79^{ab}	0.36^{abc}
6	39.7^{bc}	2.44^{b}	8.57^{a}	1.12^{abcd}	0.81^{ab}	0.31^{abcd}
7	39.5^{c}	2.98^{ab}	8.10^{ab}	1.10^{bcd}	0.79^{ab}	0.31^{abcd}
8	40.4^{abc}	3.03^{ab}	8.07^{ab}	1.08^{cd}	0.79^{ab}	0.29^{bcd}
9	40.7^{abc}	2.75^{ab}	8.16^{ab}	1.15^{abc}	0.80^{ab}	0.35^{abcd}
10	43.1^{a}	2.47^{ab}	7.75^{b}	1.05^{d}	0.78^{ab}	0.27^{cd}
11	40.4^{abc}	2.45^{ab}	7.97^{ab}	1.17^{ab}	0.89^{a}	0.28^{cd}
12	39.5^{c}	3.34^{ab}	7.86^{ab}	1.05^{d}	0.80^{ab}	0.25^{d}
13	40.9^{abc}	3.08^{ab}	7.94^{ab}	1.14^{abc}	0.84^{ab}	0.30^{abcd}
Mean	40.34	2.91	8.08	1.13	0.80	0.33
SEM[2]	0.56	0.24	0.12	0.016	0.02	0.18
CV[3]	3.10	13.8	2.75	4.05	4.60	14.66
P-value	0.0003	0.01	0.006	0.0001	0.02	0.0001

[a-d]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 3.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.2 Effect of canola meal source on carbohydrate content (% DM)[1].

Crushing Plant	Simple sugars[2]	Sucrose	Oligosaccharides[3]	Starch
1	0.31^{def}	6.21^{a}	3.04	1.24^{ab}
2	0.41^{cd}	5.83^{ab}	3.06	1.54^{ab}
3	0.25^{ef}	6.00^{ab}	3.44	1.68^{ab}
4	0.43^{bcd}	6.12^{ab}	3.01	1.53^{ab}
5	0.49^{abc}	5.87^{ab}	3.21	1.64^{ab}
6	0.41^{bcd}	6.17^{ab}	3.20	1.85^{a}
7	0.35^{def}	6.02^{ab}	2.93	1.72^{ab}
8	0.32^{def}	5.70^{ab}	3.43	1.39^{ab}
9	0.23^{f}	6.21^{a}	2.95	1.52^{ab}
10	0.37^{cde}	5.70^{ab}	3.32	1.19^{b}
11	0.37^{cde}	5.63^{b}	3.48	1.50^{ab}
12	0.55^{ab}	5.78^{ab}	3.38	1.53^{ab}
13	0.63^{a}	5.65^{ab}	3.37	1.17^{b}
Mean	0.39	5.92	3.22	1.50
SEM[4]	0.02	0.10	0.03	0.12
CV[5]	38.5	3.70	6.19	13.7
P-value	0.0001	0.001	0.12	0.01

[a-f]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean =3.
[2]Includes fructose and glucose.
[3]Includes raffinose and stachyose.
[4]Standard error of the mean.
[5]Coefficient of variation.

Table 4.3 Effect of canola meal source on neutral detergent fiber (NDF), non-starch polysaccharides (NSP), neutral detergent insoluble crude protein (NDICP), lignin and polyphenols (L&P) and total fiber contents (% DM)[1].

Crushing Plant	NDF	Dietary fiber fraction			
		NSP[2]	NDICP	L & P	Total fiber
1	28.3^{c}	22.5	3.80^{b}	11.1^{bc}	37.3^{c}
2	33.3^{ab}	22.9	7.45^{a}	12.6^{ab}	42.9^{ab}
3	30.9^{abc}	23.2	4.05^{b}	11.9^{abc}	39.1^{abc}
4	30.2^{abc}	22.4	5.14^{ab}	11.0^{bc}	38.5^{bc}
5	30.2^{abc}	23.1	4.68^{b}	11.8^{abc}	39.5^{abc}
6	28.9^{c}	23.1	5.25^{ab}	11.0^{bc}	39.4^{abc}
7	30.2^{abc}	24.2	5.11^{ab}	10.2^{c}	39.5^{abc}
8	29.8^{bc}	23.3	3.41^{b}	11.4^{bc}	38.1^{c}
9	33.0^{ab}	24.0	6.01^{ab}	12.5^{abc}	42.6^{ab}
10	30.3^{abc}	23.1	4.62^{b}	11.4^{bc}	39.1^{abc}
11	30.3^{abc}	23.3	4.18^{b}	12.4^{abc}	39.8^{abc}
12	34.0^{a}	23.9	5.50^{ab}	13.9^{a}	43.3^{a}
13	30.2^{abc}	23.6	5.53^{ab}	11.6^{abc}	40.7^{abc}
Mean	30.7	23.3	5.0	11.7	40.0
SEM[3]	0.74	0.39	0.50	0.45	0.86
CV[4]	5.48	2.39	21.3	7.92	4.65
P-value	0.0002	0.07	0.0009	0.001	0.0005

[a-c]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 3.
[2]Includes arabinose, xylose, mannose, galactose, glucose and uronic acids.
[3]Standard error of the mean.
[4]Coefficient of variation.

Table 4.4 Effect of canola meal source on amino acid content (% DM)[1].

Crushing Plant	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG
1	2.63^{b}	1.47	1.78	6.37^{ab}	2.76^{ab}	1.64	1.82^{bc}	0.88^{ab}	1.77	0.73	1.33	2.67	0.98^{ab}	1.53^{ab}	1.24	2.18^{ab}	2.20
2	2.63^{b}	1.47	1.78	6.15^{b}	2.80^{ab}	1.59	1.76^{c}	0.83^{b}	1.72	0.70	1.25	2.56	0.95^{b}	1.47^{b}	1.22	2.03^{b}	2.14
3	2.85^{ab}	1.58	1.92	6.59^{ab}	2.90^{ab}	1.69	1.89^{abc}	0.92^{ab}	1.81	0.77	1.30	2.72	1.04^{ab}	1.62^{ab}	1.25	2.32^{ab}	2.34
4	2.99^{ab}	1.62	1.97	7.11^{a}	2.91^{ab}	1.79	2.03^{ab}	0.97^{ab}	1.91	0.77	1.43	2.87	1.02^{ab}	1.65^{a}	1.29	2.29^{ab}	2.39
5	2.71^{ab}	1.55	1.86	6.55^{ab}	2.52^{b}	1.69	1.89^{abc}	0.86^{b}	1.79	0.73	1.31	2.69	1.03^{ab}	1.58^{ab}	1.22	2.11^{ab}	2.14
6	2.78^{ab}	1.53	1.86	6.57^{ab}	2.53^{b}	1.64	1.88^{abc}	0.89^{ab}	1.78	0.73	1.30	2.68	1.09^{a}	1.55^{ab}	1.21	2.08^{ab}	2.23
7	2.94^{ab}	1.62	1.97	6.78^{ab}	2.90^{ab}	1.74	1.96^{abc}	0.91^{ab}	1.83	0.69	1.31	2.77	0.97^{ab}	1.55^{ab}	1.27	2.25^{ab}	2.33
8	2.96^{ab}	1.63	1.95	6.91^{ab}	2.83^{ab}	1.77	2.08^{a}	0.94^{ab}	1.93	0.72	1.41	2.81	1.03^{ab}	1.63^{ab}	1.27	2.33^{a}	2.36
9	2.83^{ab}	1.60	1.96	6.86^{ab}	2.94^{ab}	1.73	2.02^{ab}	0.87^{ab}	1.87	0.68	1.38	2.75	0.98^{ab}	1.61^{ab}	1.24	2.19^{ab}	2.27
10	3.05^{a}	1.62	1.99	7.20^{a}	2.99^{a}	1.77	2.03^{ab}	1.01^{a}	1.91	0.79	1.40	2.86	1.02^{ab}	1.66^{a}	1.29	2.30^{ab}	2.37
11	2.85^{ab}	1.58	1.94	6.84^{ab}	2.68^{ab}	1.74	1.89^{abc}	0.95^{ab}	1.80	0.76	1.34	2.74	0.98^{ab}	1.54^{ab}	1.25	2.25^{ab}	2.37
13	2.83^{ab}	1.58	1.91	6.63^{ab}	2.71^{ab}	1.70	1.94^{abc}	0.94^{ab}	1.82	0.74	1.34	2.76	1.01^{ab}	1.61^{ab}	1.26	2.15^{ab}	2.22
14	2.79^{ab}	1.56	1.91	6.70^{ab}	2.71^{ab}	1.71	1.92^{abc}	0.96^{ab}	1.76	0.75	1.27	2.72	1.00^{ab}	1.58^{ab}	1.30	2.04^{b}	2.22
Mean	2.83	1.57	1.91	6.71	2.78	1.71	1.93	0.92	1.82	0.74	1.34	2.74	1.01	1.58	1.25	2.19	2.28
SEM[3]	0.07	0.04	0.05	0.18	0.08	0.04	0.04	0.02	0.04	0.03	0.04	0.06	0.02	0.03	0.03	0.05	0.29
CV[4]	4.55	3.33	3.66	4.28	5.37	3.33	4.73	5.57	3.49	4.50	4.11	2.97	3.65	3.45	2.23	4.69	3.90
P-value	0.01	0.01	0.09	0.02	0.004	0.1	0.004	0.007	0.1	0.5	0.1	0.1	0.07	0.01	0.7	0.03	0.3

[a-c]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 3.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.5 Glucosinolate contents of canola meals from different crushing plants (µmol/g DM)[1].

Plant	Gluconapin	Glucobrassica-napin	Progoitrin	Glucobrassicin	OH-Glucobrassicin	Total
1	0.58	0.08	1.89	0.19	0.47	3.18[d]
2	0.42	0.00	1.15	0.18	0.57	2.33[ef]
3	1.06	0.09	3.46	0.22	0.77	5.70[a]
4	0.40	0.00	0.94	0.21	0.44	1.99[fg]
5	0.27	0.03	0.85	0.17	0.35	1.66[g]
6	0.43	0.07	1.35	0.18	0.49	2.52[e]
7	0.92	0.12	2.70	0.18	0.60	4.52[b]
8	0.74	0.14	2.03	0.16	0.82	3.89[c]
9	0.52	0.08	1.41	0.16	0.36	2.54[e]
10	0.52	0.11	1.31	0.10	0.62	2.67[e]
11	0.71	0.16	1.81	0.18	0.64	3.49[d]
12	0.12	0.00	0.40	0.12	0.52	1.14[h]
13	0.00	0.00	0.09	0.00	0.08	0.17[i]
Mean	-	-	-	-	-	2.75
SEM[2]	-	-	-	-	-	1.39
CV[3]	-	-	-	-	-	50.5
P-value	-	-	-	-	-	0.0001

[a-i]Means followed by different letters within a column are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 3.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.6 Effect of canola meal source on apparent metabolizable energy (AME_n) content (Kcal/kg, as-is basis)[1].

Crushing Plant	AME_n
1	1967
2	1944
3	1730
4	1664
5	1805
6	1761
7	1659
8	1793
9	1618
10	1732
11	1643
12	1995
13	1940
Mean	1789
SEM[2]	80.1
CV[3]	7.43
P-value	0.006

[1]Number of observations contributing to each mean = 3.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.7 Effect of canola meal source on ether extract (EE) and acid-hydrolyzed ether extract (AEE) digestibilities in broiler chickens (%, as-is basis)[1].

Crushing Plant	EE digestibility	Digestible EE content	AEE digestibility	Digestible AEE content	Digestible soapstocks and gums content
1	90.8[a]	2.73[ab]	64.2	3.28[ab]	0.56[ab]
2	82.7[ab]	2.88[a]	55.1	4.09[ab]	1.27 [ab]
3	87.9[a]	2.10[cde]	32.3	2.05[ab]	0.0 [b]
4	80.3[ab]	2.18[bcd]	41.2	2.88[ab]	0.7 [ab]
5	81.3[ab]	1.94[cde]	50.8	3.0[ab]	1.06 [ab]
6	80.9[ab]	1.80[de]	50.1	2.76[ab]	1.06 [ab]
7	72.3[ab]	1.98[cde]	63.4	3.84[ab]	1.87 [ab]
8	73.0[ab]	2.04[cde]	41.3	3.0[ab]	0.96 [ab]
9	82.3[ab]	2.07[cde]	72.8	4.45[a]	2.38[a]
10	65.3[b]	1.49[e]	64.9	3.64 [ab]	2.15 [ab]
11	80.9[ab]	1.80[ed]	46.2	1.67[b]	0.0[b]
12	89.7[a]	2.75[ab]	63.2	4.18[a]	1.43 [ab]
13	87.1[a]	2.47[abc]	43.7	2.43[ab]	0.0[b]
Mean	81.1	2.20	53.0	3.17	1.03
SEM	4.43	0.40	8.46	0.81	0.76
CV	8.70	18.5	21.7	25.6	73.6
P-value	0.005	0.0001	0.04	0.005	0.007

[a-e]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 3.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.8 Effect of canola meal source on total phosphorus (P) digestibility in broiler chickens (%, as is basis)[1].

Crushing Plant	P retained	P digestibility, %	Digestible P content
1	0.37^{ab}	22.3^{ab}	0.24^{ab}
2	0.35^{bc}	18.6^{ab}	0.19^{ab}
3	0.30^{c}	7.4^{b}	0.07^{b}
4	0.36^{abc}	15.6^{ab}	0.17^{ab}
5	0.37^{ab}	24.4^{ab}	0.25^{ab}
6	0.39^{ab}	35.2^{a}	0.36^{a}
7	0.39^{ab}	30.0^{ab}	0.30^{ab}
8	0.37^{ab}	28.9^{ab}	0.29^{ab}
9	0.38^{ab}	24.1^{ab}	0.26^{ab}
10	0.40^{ab}	36.8^{a}	0.36^{a}
11	0.40^{ab}	31.4^{a}	0.34^{a}
12	0.39^{ab}	29.7^{ab}	0.29^{ab}
13	0.41^{a}	29.3^{ab}	0.31^{ab}
Mean	0.38	25.7	0.26
SEM	0.01	7.45	0.05
CV	7.58	31.9	31.3
P-value	0.0001	0.003	0.01

[a-c]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 3.
[2]Standard error of the mean.
[3]Coefficient of variation.

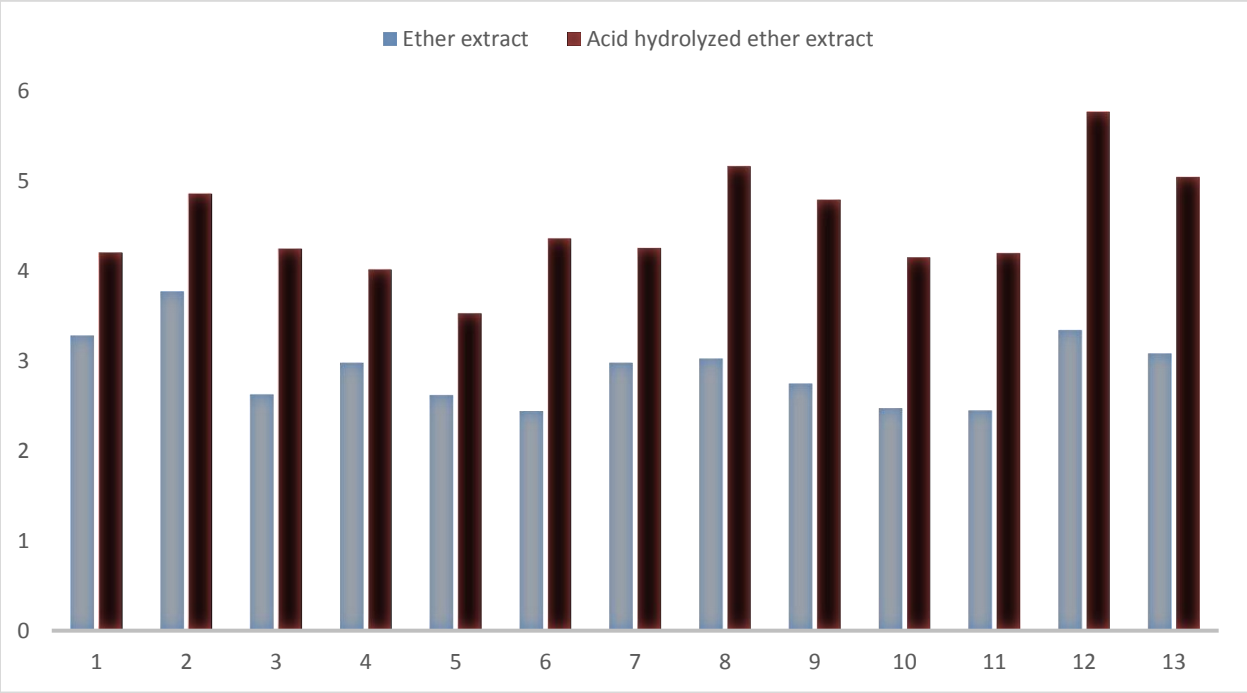

Figure 4.1 Ether extract and acid-hydrolyzed ether extract contents of canola meal from 13 crushing plants (% DM).

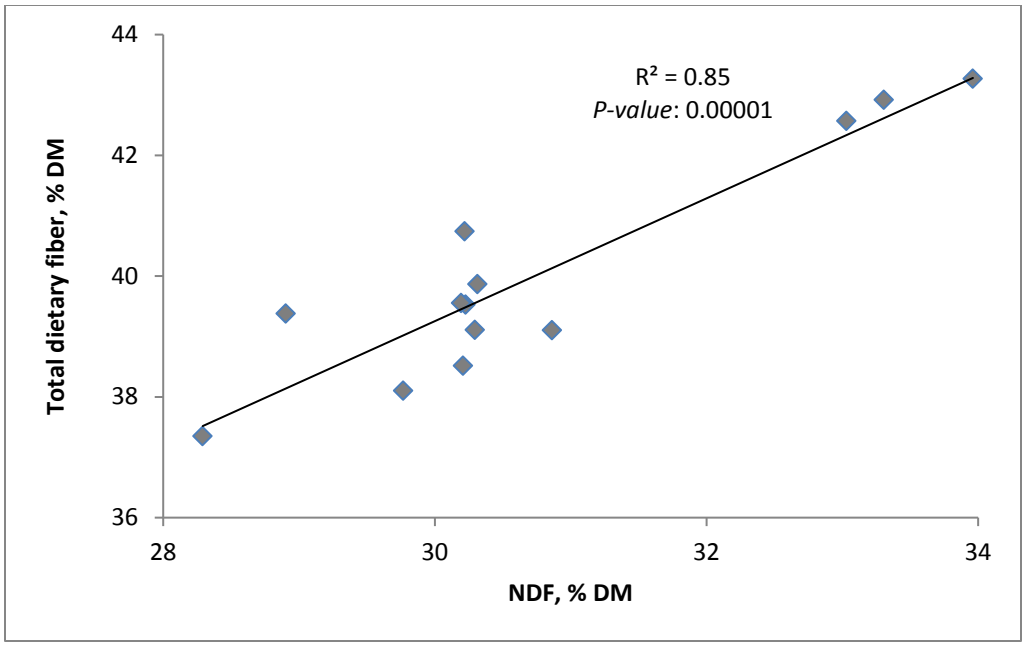

Figure 4.2 Relationship between total dietary fiber and neutral detergent fiber (NDF) contents of canola meal. The number of observations contributing to each point on the graph is 3.

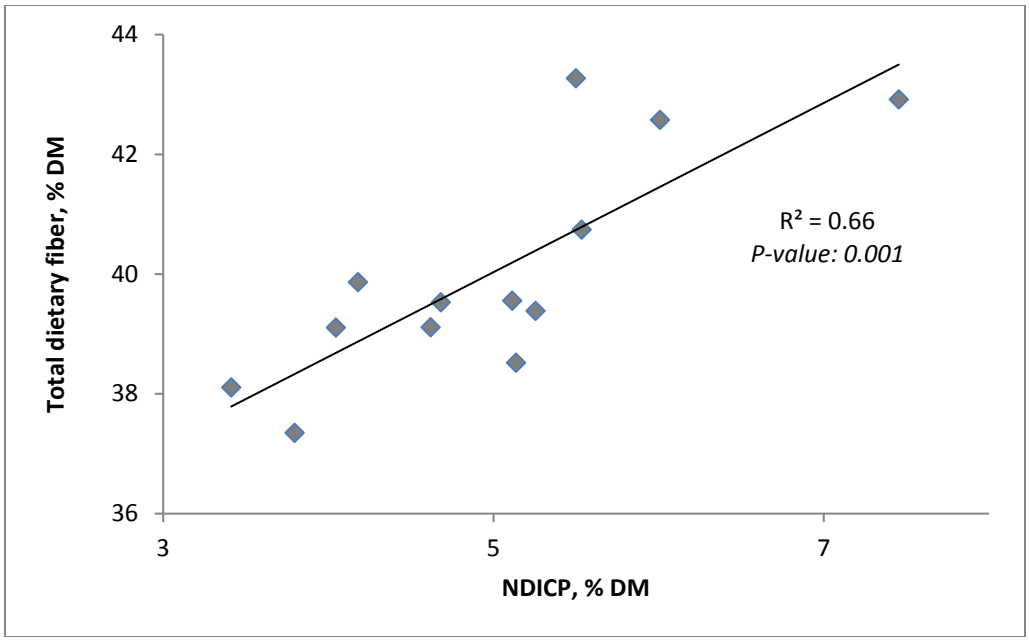

Figure 4.3 Relationship between total dietary fiber and neutral detergent insoluble crude protein (NDICP) contents of canola meal from 13 crushing plants. The number of observations contributing to each point on the graph is 3.

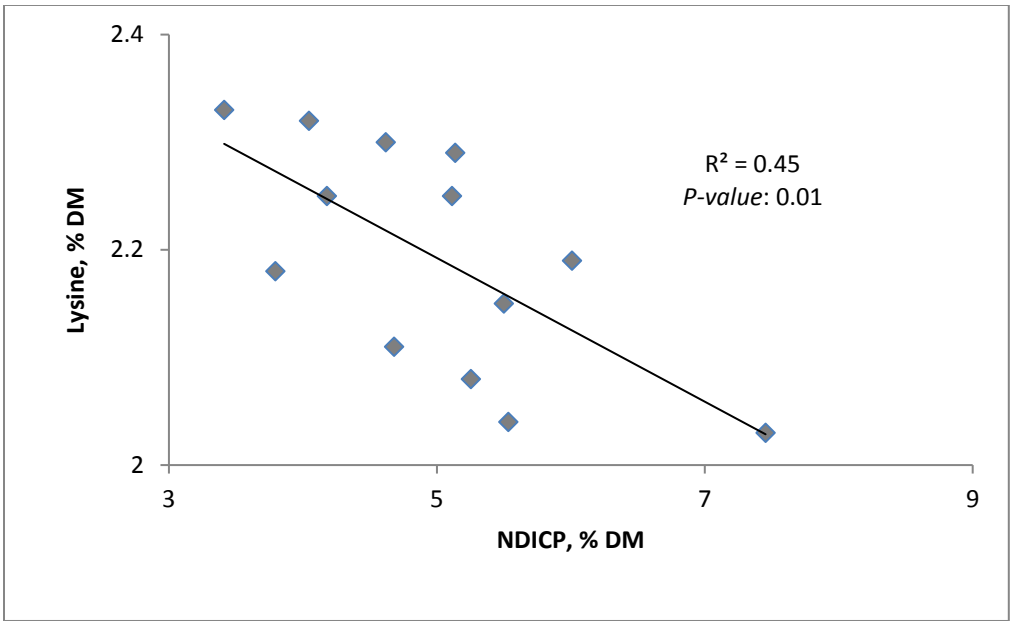

Figure 4.4 Relationship between lysine and neutral detergent insoluble crude protein (NDICP) contents of canola meal. The number of observations contributing to each point on the graph is 3.

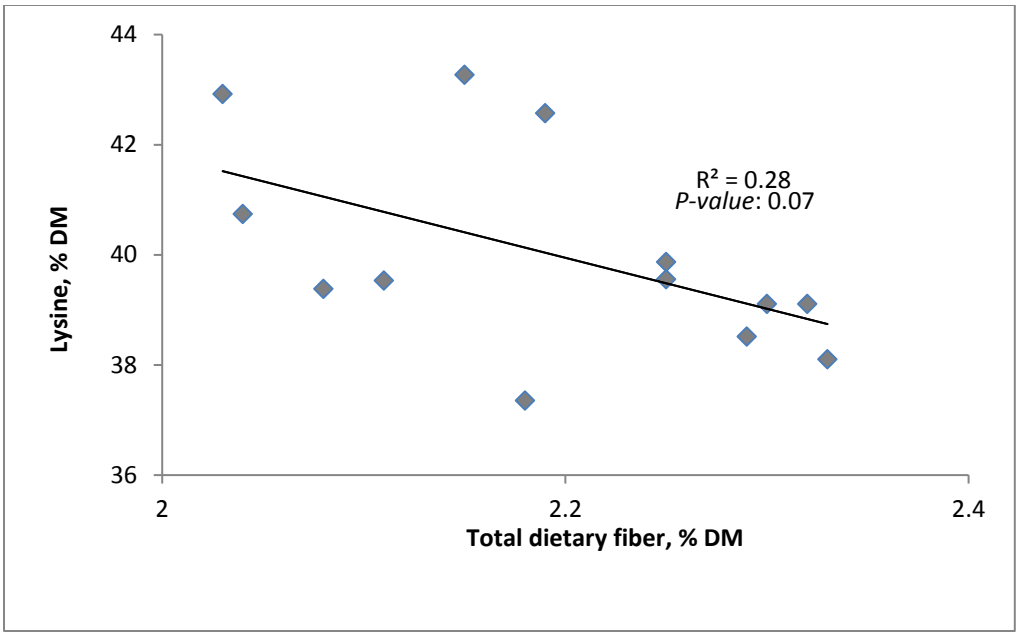

Figure 4.5 Relationship between total dietary fiber and lysine contents of canola meal. The number of observations contributing to each point on the graph is 3.

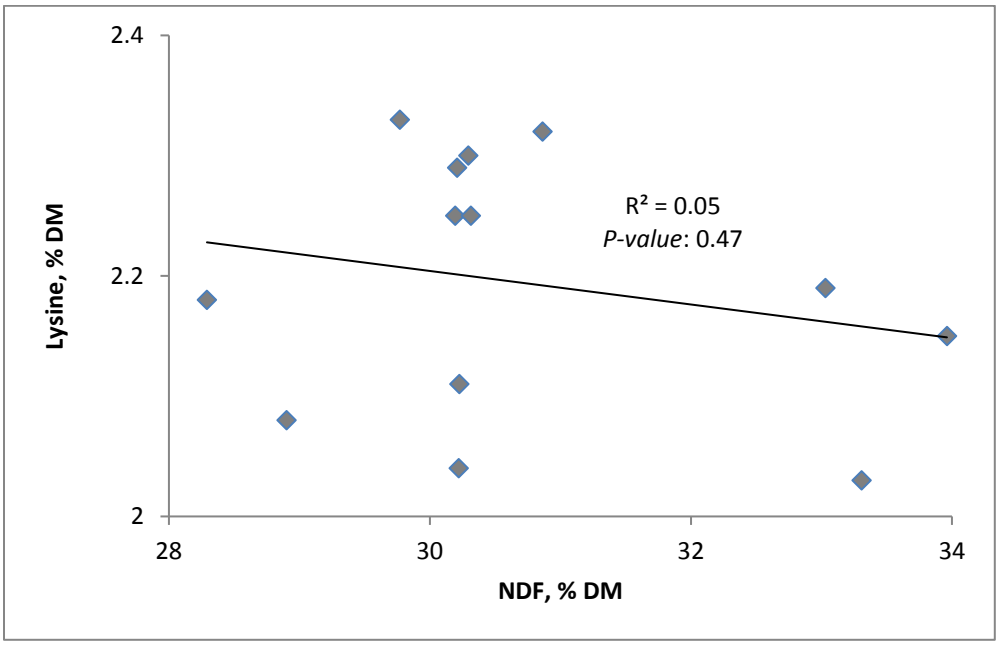

Figure 4.6 Relationship between lysine and neutral detergent finer (NDF) contents of canola meal. The number of observations contributing to each point on the graph is 3.

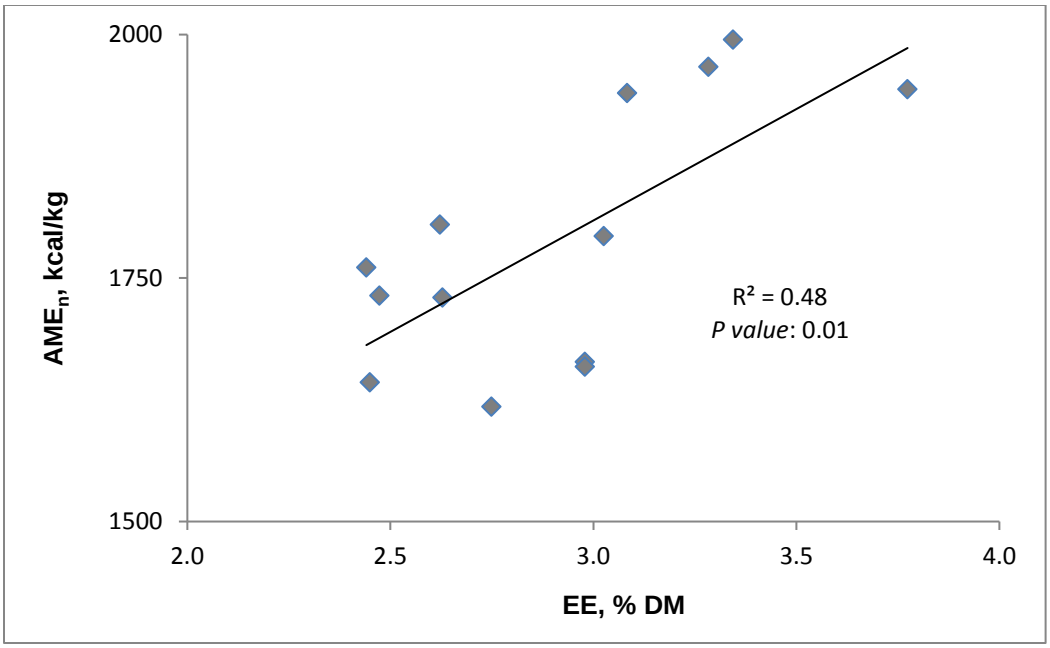

Figure 4.7 Relationship between apparent metabolizable energy (AME_n) and ether extract (EE) contents of canola meal. The number of observations contributing to each point on the graph is 3.

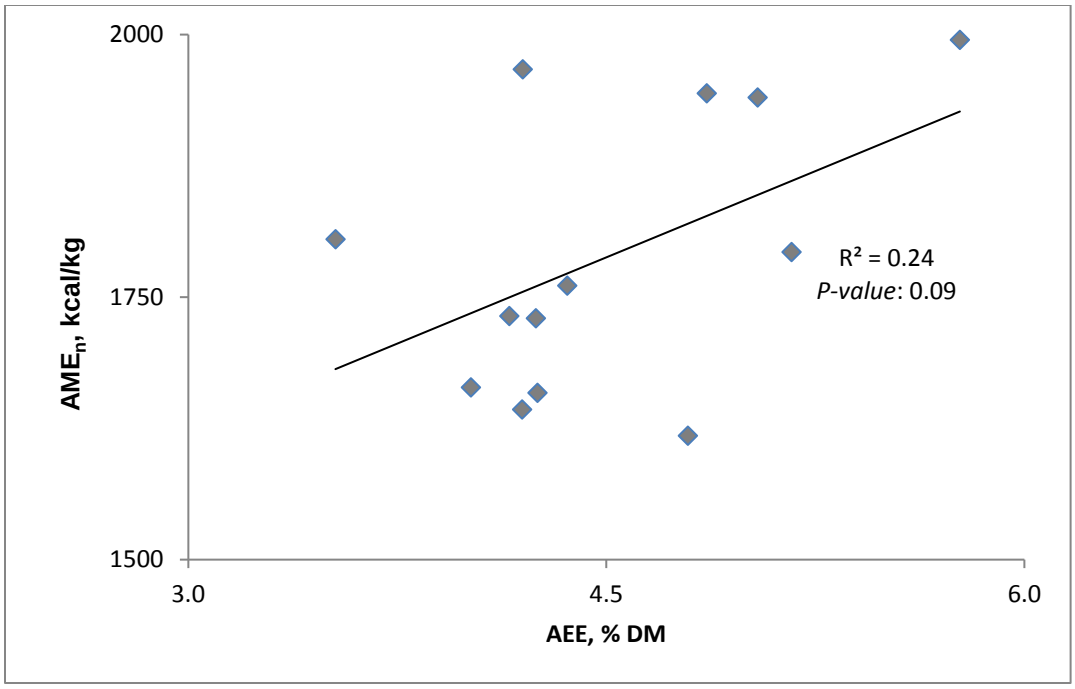

Figure 4.8 Relationship between apparent metabolizable energy (AME_n) and acid-hydrolyzed ether extract (AEE) contents of canola meal. The number of observations contributing each point on the graph is 3.

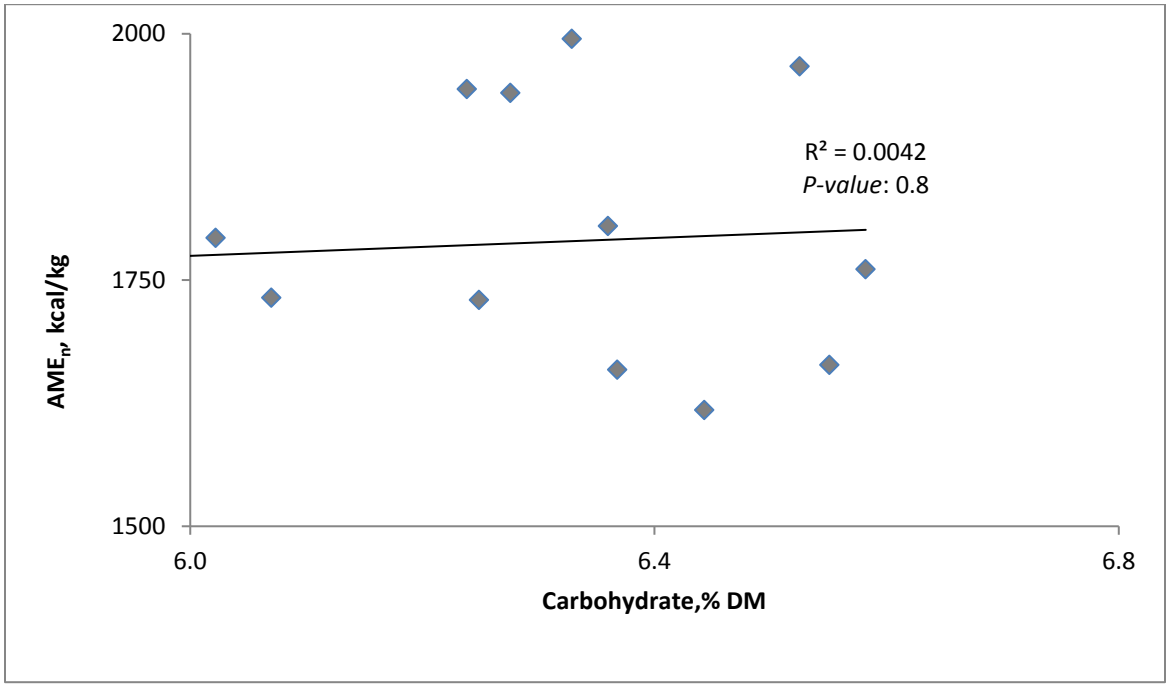

Figure 4.9 Relationship between apparent metabolizable energy (AME_n) and carbohydrate contents of canola meal. The number of observations contributing each point on the graph is 3.

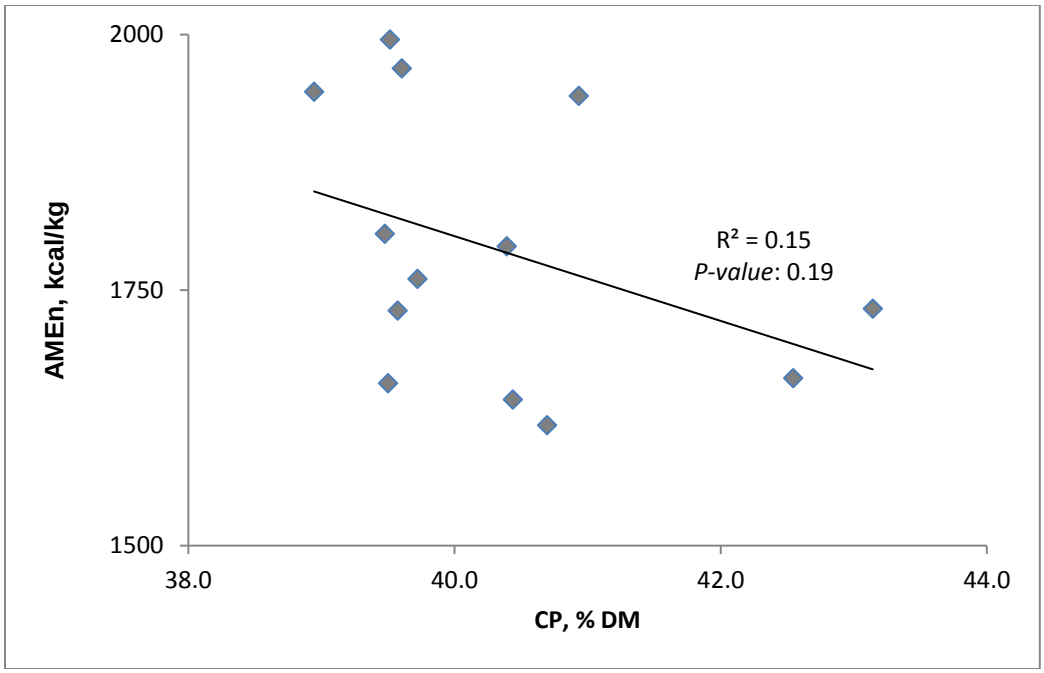

Figure 4.10 Relationship between apparent metabolizable energy (AME_n) and crude protein (CP) contents of canola meal. The number of observations contributing to each point on the graph is 3.

CHAPTER 5: DISCUSSION

Canola, the low glucosinolate, low erucic acid form of rapeseed *(Brassica napus)* is an important oilseed crop in Western Canada and the meal has an excellent balance of amino acids, low levels of glucosinolates, and is used in animal production as a source of protein. There are several methods for processing canola and it has been suggested that processing may affect the quality of protein in CM. Amino acids, in particular lysine and methionine are susceptible to damage during meal processing (Hurrell, 1984). Pre-press solvent extraction of Canola is the traditional method of processing and consists of 10 basic stages including: 1) Cleaning and drying of the seed to approximately 6% moisture, 2) Preconditioning (heating) in order to prevent shattering of the seeds during flaking, 3) Flaking by passing through a roller mill, 4) Cooking to deactivate myrosinase enzyme and to reduce oil viscosity and thereby coalesce the oil, 5) Expelling to decrease the oil content to approximately 15 – 20%, 6) Solvent extraction with hexane to remove additional oil, 7) Desolventization in the DT which removes hexane from the oil-extracted meal, 8) Cooling, 9) Drying with forced air (some plants add back gums and screenings at this step), and 10) Grinding and pelleting in some cases (Unger, 2011; Canola Council of Canada, 2015). Processing conditions of CM may not be consistent among processors which might cause some variability in the nutritive composition (Spragg and Mailer, 2007). Moreover, during pre-press solvent extraction, the meal is heated at different stages which may affect its quality. Canola meal contains both amino acids and reducing sugars, the components required for Maillard reaction to occur. Therefore, Maillard reactions may occur in the presence of heat and moisture applied at some stages of canola seed processing. Lysine, in principle, is the most susceptible amino acid because it has a free amino group at the epsilon carbon which is readily available to react with reducing sugars (Macromichalls, 2001).

5.1 Effect of Crushing Plant on the Chemical and Nutritive Composition of Canola Meal

The chemical composition and nutritive value of CM varies depending on variations in concentrations of nutrients in the seeds as well as differences in oil extraction procedures (Bell and Keith, 1990; Bell, 1993; Barthet and Duan, 2011, Newkirk, 2011). In the current study, the average concentrations of DM and ash in CM were in agreement with the values reported by Rostagno et al. (2011) and NRC (2012). The CP content ranged from 38.9 to 43.1 % DM with an overall average of 40.3 % DM which is similar to that of 41.8 % DM reported by Bell and Keith (1991), 40.6 % DM reported by Khajali and Slominski (2012), 41.1 % DM listed by NRC (2012) and 42.0 reported by Adewole et al. (2016).

Variations observed in the CP content of CM in this study (Table 4.1) may be mainly due to variations in the CP content of the canola seed feedstock which may be a consequence of differences in the growing conditions (Bell and Keith, 1991; Adewole et al., 2016). The average concentration of EE in canola meal in this study was in agreement with the study by Radfar et al. (2017) but slightly lower than the value of 3.8 % (as fed basis) or 3.5 % DM reported earlier by NRC (1994) and Adewole et al. (2016), respectively. However, EE concentration was higher than the value of 1.8 % DM reported earlier by Slominski et al. (2012) indicating that no oil refining by-products were added back to the meals. Likewise, differences in the content of total phosphorus, phytate phosphorus and non-phytate phosphorus between crushing plants can be explained by differences in growing conditions of the canola seed feedstock used in the various crushing plants across Canada (Adewole et al., 2016).

In Canada, by-products of seed cleaning (i.e., screenings and other dockage) and oil-refining (i.e., gums, soapstocks, phospholipids, etc.) are added back into the meal to increase the energy content of the meal and to reduce dustiness (Canola Council of Canada, 2015) which may

explain the variations observed between crushing plants in the content of ether extract in current study (Table 4.1). It has been suggested that the fat content of the meal is approximately 1-2% without adding the various by-products (Matthaus, 2012). On average, fat content of CM was 2.91 % in the present study documenting that most of the crushing plants, if not all of them would add the by-products of oil-refining to the meal.

There was no difference in oligosaccharide content between the conventional CM (Table 4.2) which is in agreement with the earlier reports from this laboratory (Slominski et al., 1994, 1999, 2012; Radfar et al., 2017).

Excessive heating during canola processing may lead to the formation of Maillard reaction products which are indicative of protein damage and would lead to the increase in the NDF, ADF, and lignin contents and reduced protein digestibility (Slominski, 1997; Almeida et al., 2014). Moreover, glycoproteins represent the structural protein of the cell walls and potentially the Maillard reaction products formed during the desolventization and toasting resulting in the neutral detergent insoluble protein formation (Van Soest, 1994). Consequently, high glycoprotein content is indicative of Maillard reaction with the Maillard reaction products contributing to the high dietary fibre content known to be poorly digested by poultry (Jia et al., 2012). Therefore, the variation in the NDF, lignin and total dietary fiber contents in the present study could be a consequence of Maillard reaction during processing. Melanoidins, which are polymers originating from Maillard reaction, may be analyzed and identified as lignin and may result in increased analyzed concentration of lignin (Miao et al., 1994). Accordingly, heat treatment of feed ingredients is expected to increase the analyzed values of NDF and total dietary fiber (Almeida et al., 2014). Several studies showed that heat treatment applied during the desolventization/toasting and drying steps of meal processing increases the NDF content of the meal. In a study by Mustafa

et al. (2000), CM obtained after desolventization/toasting and drying stages had higher NDF content than that of the solvent-extracted meal prior to desolventization/toasting. Therefore, it can be concluded that the variation in the NDF content between crushing plants is mainly due to differences in heat treatment applied during the desolventization/toasting and drying of the meal. McKinnon et al. (1995) also suggested the increased NDF content of CM as a consequence of heat. However, they reported little or no impact of moderated heat on acid detergent fiber and acid detergent lignin contents of CM.

5.2 Effect of Crushing Plant on the Amino Acids Content of Canola Meal

There were statistically significant differences in the content of asparagine, glutamine, proline, alanine, cysteine, tyrosine, phenylalanine and lysine between different crushing plants across Canada. However, there was no variation between crushing plants in the content of other amino acids. Most of the amino acids are susceptible to advanced Maillard reactions including Strecker degradation which leads to the formation of dark brown pigments (Mauron, 1981). However, the presence of the highly reactive ε-amino group suggests lysine as the primary amino acid involved in the early stages of Maillard reactions. (Mauron, 1981). Therefore, its availability could be a sensitive indicator of the effects of heat treatment. Newkirk et al. (2003b) reported that prior to desolventization/toasting, processing had no effect on amino acid content. However, desolventization-toasting changed the colour of the yellow meal to a brownish colour indicating that Maillard browning reactions may have occurred (Hurrell, 1984). At the same time, the lysine content of the meal was reduced suggesting that the color change was due to the formation of brown pigments during Maillard reactions. It has been suggested that Maillard reaction is likely promoted by the elevated temperature and moisture conditions during desolventization-toasting

(Mauron, 1981). The meal is heated indirectly on heated trays and directly with steam (sparge steam) during desolventization-toasting. The spurge steam has the potential to promote Maillard reactions as it introduces both heat and moisture. In another study by San Juan and Villamide (2001), the lysine content of sunflower meal has also been shown to be reduced during desolventization/toasting which indicate that the effect is not limited to canola processing.

5.3 Effect of Crushing Plant on the Glucosinolates Content of Canola Meal

There were significant differences ($P < 0.05$; Table 4.5) in the contents of total glucosinolates between different crushing plants. Total glucosinolates ranged from 0.17 µmol/g DM (Plant 13) to 5.70 µmol/g DM (Plant 3) which are well within the acceptable limit (< 30 µmol/g of air-dried, oil-free meal) for CM. The overall mean total glucosinolates content was 2.75 µmol/g DM. The results are in agreement with findings of Adewole et al. (2016) who observed different glucosinolate contents in CM from different crushing plants across Canada indicating the effect of processing on their content.

Glucosinolates which are a group of structurally related sulphur-containing compounds are hydrolyzed by myrosinase enzyme in the presence of moisture and yield a range of products including isothiocyanates, goitrin, and nitriles (Bell, 1984). Additionally, thermal degradation during CM processing is suggested as another method of glucosinolate decomposition (Campbell and Slominski, 1990). Slominski and Campbell (1987) documented that indole glucosinolates which represent a substantial portion of the total glucosinolates content of CM have been shown to be more susceptible to thermal degradation than the aliphatic ones with the main decomposition of indole glucosinolates occurring in the desolventizer-toaster with little or no effect during meal drying .The conditions involved in the various stages of the crushing process, including time,

temperature and moisture, are adequate to effect thermal decomposition of glucosinolates and affect the nutritive value of the meal produced (Campbell and Slominski, 1990). The average value for total glucosinolates observed in the current study was 2.75 µmol/g DM which is lower than those of 4.9 and 7.2 µmol/g DM reported by Adewole et al. (2016) and Newkirk et al. (2003a), respectively.

5.4 Apparent Metabolisable Energy (AME_n) Contents

Apparent metabolisable energy values in the current study are in agreement with the mean value of 1874 Kcal/kg reported in earlier study (Radfar et al., 2017). Ether extract, sucrose, fibre and glucosinolate contents are considered to be some of the factors contributing in the difference in AME_n values of CM (Mandal et al., 2005; Jia et al., 2012). However, contrary to earlier studies (Hijikuro and Takemasa, 1985; Lessire et al., 1986) the glucosinolate content of CM does not seem to have any negative effect on AME_n in this study. In a study by Classen et al. (1991), 16% improvement in AME_n of CM with very low level of glucosinolate was found compared to the commercial CM fed to broilers. However, Bell et al. (1991) suggested that generally the effect of fiber on AME_n is more pronounced. Toghyani et al. (2014) reported that depending on processing conditions and chemical composition of expeller extracted CM fed to broilers, ileal digestible energy, apparent ME (AME), and AME_n values vary considerably between the samples. We concluded that differences (Table 4.6) between crushing plants in the content of AME_n in this study could result from different processing practices.

5.5 Ether Extract (EE) and Acid-hydrolyzed Ether Extract (AEE) Digestibilities of Canola Meal in Broiler Chickens

The procedure used to analyze for fat may influence the calculated values of fat digestibility (Kil et al., 2010.) Therefore, the digestibility values for AEE observed in the current study were different from those of crude fat determined without acid hydrolysis (EE).

In the ether extraction method, some of the Ca soaps and the phospholipids may not be solubilized, whereas the acid hydrolysis procedure is believed to result in a more complete fat extraction. Solvent extraction of fat without acid hydrolysis will therefore result in the lower fat values. Apparent EE digestibility of CM in this study is 81.1% which was greater than that of 53% for AEE digestibility. This is in agreement with Kil et al. (2010) who found lower digestibility values using acid hydrolysis method comparing to studies in which samples were analyzed without using acid hydrolysis. They concluded that the concentration of Ca soaps is likely greater in ileal and fecal samples than in diets and feed ingredients, including CM. The underestimation of the concentration of fat in the ileal and fecal samples may result in greater calculated fat digestibility values. Jorgensen et al. (2000) and Seneviratne et al. (2011) found greater than 90% ileal digestibility when using canola oil as a supplementary fat source in their study. Various factors have been suggested to impact fat digestibility and its contribution to AME_n.

Standardized fat digestibility data have documented that the AME or TME of lipids may not be explained in some cases by digestibility values as some of the energy attributed to the lipid source derives from the basal diet (Lessire et al., 1986) which occurs in part due to increased digestion and absorption of the basal diet in the presence of supplemental lipid. The level of interactions between basal diet and supplemental lipid sources differ due to the basal diet type. It has been shown that corn-based basal diet resulted in higher TME values for tallow than several

wheat-based basal diets (Sibbald and Kramer, 1978). In addition, lipid content and profile of the basal diet can affect this interaction. Protein level of the diet has been suggested to influence fat digestibility. In one study, chicks fed soy oil, tallow, and 1:1 mixture of the two were assayed for AME in diets that contained either 24 or 34% protein. The results showed that higher protein containing diet yielded higher AME values than the lower protein basal diet, and this effect was more pronounced for tallow (Sibbald et al., 1961). Dietary NDF may decrease apparent digestibility of fat (Bach Knudsen and Hansen, 1991) and may have a negative influence on the utilization of nutrients in broilers (Pettersson and Aman 1989; Choct and Annison 1990; Choct and Annison 1992). In the current study, however, we found no correlation between fat digestibility and NDF.

Mineral level of the diet also may affect lipid utilization, likely through the formation of insoluble soaps (Edwards et al., 1960). Rising et al. (1990) showed that the addition of 3.8% calcium in the form of ground limestone to wheat diets containing 3, 6, and 9% of animal fat resulted in a 14% decrease in apparent lipid digestibility.

In Canada, soapstocks and gums are increasingly being used as an animal feed additive, mainly in pig and poultry diets. Adding gums, which mostly consist of phospholipids and soapstocks, which are oil-rich components, increases the energy content of the meal and reduces dustiness. In addition, it can improve the palatability, help heat stress conditions, and improve pelleting of feed products by reducing feed particle separation (NRC, 2015). Pardio et al. (2001) have studied the effect of different soapstocks (corn, sunflower, canola, and soybean) on production performance and broiler skin pigmentation and concluded that addition of soybean soapstocks improved live body weight gain of birds and produced the highest percentage of skin pigmentation. However, Pardio et al. (2005) found no significant effect on egg quality parameters

when soybean soapstocks were added to the layer diets. In another study by Bruce et al. (2006), the addition of soybean soapstocks to pigs and roasters' diets decreased the amino acid digestibility and growth performance. In the current study, AEE digestibility was lower than that of EE (Table 4.7) which would indicate that soapstocks and gums are poorly digested and contribute less energy to the AME_n content of CM than EE.

5.6 Canola Meal Phosphorus Digestibility in Broiler Chickens

Comprehensive knowledge regarding the availability of P from feed raw materials is required to optimize the supply of dietary phosphorus (P) to animals. There is extensive confusion regarding the current terminology used to describe available P (available P, nonphytate P, retainable P) in feed ingredients. Various proposed definitions of available P and different approaches to determine P availability have made it difficult to compare published data and to compile comprehensive feeding tables for use by the feed industry. This complicates issues related to feeding costs and sustainable handling of the limited global phosphate reserves. According to Rodehutscord (2009), measurement of digestible P may be the preferable method to assess P availability for poultry. Similarly, WPSA (2013) suggested using digestible P for assessing dietary P levels and provided a protocol for estimating digestible P in feedstuffs.

Few studies to date have provided detailed information on apparent P digestibility in double zero rapeseed meal and true P digestibility (TPD) of CM for pigs (Akinmusire and Adeola, 2009; Rodehutscord et al., 1997). Unfortunately, there is lack of such information for poultry. In the current study, the diet was formulated to contain 1.0 and 0.45% of Ca and non-phytate P respectively in growing chicks according to recommendations of NRC (1994). However, the concentration of dietary P used to determine the digestibility of P in feed ingredients was not

important as apparent P digestibility was not affected by the inclusion level of P as documented by Stein et al. (2008). In current study, the retained dietary P concentrations (0.38%) (Table 4.8) were slightly lower than the calculated value of 0.45%; Table 3.1. It is important to mention that phosphorus output in the excreta is the sum of undigested dietary and endogenous P, P used by the hindgut microflora, and P excreted via urine. In the present study, apparent digestible P content of CM and retainable P content in the body were different than the nonphytate P contents in the meal. Mutucumarana et al. (2014) documented that the P evaluation system based on nonphytate P is not reflective of P availability in both corn and CM. The values or true digestible P and retainable P contents were considerably higher than the non-phytate P contents in both corn and CM, signifying that a portion of phytate-bound P is being used by broiler chickens. Apparent P digestibility of CM (Table 4.8) were significantly different between crushing plants which might be due to different P content of CM reflecting different growing conditions. Retainable P contents in this study was 0.38 % which was in agreement with 0.39% of Waldroup et al. (2000). Therefore, it can be suggested that P requirement for broilers should be lower than the generally accepted value of 0.45%.

5.7 Some Notable Correlations in the Chemical Compositions of Canola Meal

The strong positive correlation between NDF and total dietary fiber (Figure 4.2) suggests that NDF can contribute to the prediction of the total dietary fiber content in CM. As shown in Figure 4.3, there was a positive relationship between the total dietary fiber and NDICP (also referred to as glycoprotein) contents indicating that NDICP is contributing to the increase in total dietary fiber suggesting that the total dietary fiber content increased as a consequence of protein damage caused by CM overheating. Earlier study by Adewole et al. (2016) demonstrated the same positive correlation between the total dietary fiber and NDICP. In addition, Newkirk et al. (2000)

documented that neutral detergent insoluble nitrogen content (expressed as a percentage of total protein) below 10% is indicative of CM with greater than 85% lysine availability. The negative relationship between lysine and NDICP (Figures 4.4) also suggests that a second-stage of Maillard reaction which leads to a reduction in lysine concentration may be occurring in the CM from some of the crushing plants.

The correlation between EE and AME_n contents observed in the current study (Figure 4.7) further confirmed that fat content of CM has the greatest effect on AME_n content of CM among the contributing factors studied. Carbohydrates were poorly correlated with AME_n (Figure 4.9), probably because they are not affected by processing conditions and their content is rather constant among crushing plants in the current study.

5.8 Conclusions

There were statistically significant variations between crushing plants in the contents of CP, NDF, ether extract, total phosphorus, phytate phosphorus, non-phytate phosphorus, simple sugars, sucrose, starch, total dietary fiber, glucosinolates, NDICP, lignin and polyphenols, phenylalanine and lysine of CM produced in different regions of Canada. It was concluded that the high total dietary fiber with the corresponding high NDICP observed in CM from some crushing plants may be a consequence of meal overheating and the consequent formation of Maillard reaction products. Ether extract was found to have significant effect on AME_n content of CM compared to the other contributing factors studied. In addition, soapstocks and gums were found to be poorly digested and contribute less energy to the AME_n content of CM than EE.

CHAPTER 6: REFERENCES

Adams, J., Cassarino, C., Lindstrom, J., Eslin, L., Lux, S. M., Holcomb, H., 2006. Canola oil fuel cell demonstration: volumeIII-technical, commercialization, and application issues associated with harvested biomass. Available at: ttp://dodfuelcell.cecer.army.mil/library_items/ CanolaVol_III.pdf Accessed Feb. 7, 2011.

Adewole, D.I., Rogiewicz, A., Dyck, B., Nyachoti, C.M, Slominski B.A., Effect of processing conditions and nutritive value of canola meal for broiler chickens and pigs. Saskatoon, SK: Proc. Intl. Rapeseed Congr; 2015. July 2015.

Adhikari, P.A., Heo, J.M., Nyachoti, C.M., 2015. True and standardized total tract phosphorus digestibility in canola meals from Brassica napus black and Brassica juncea yellow fed to growing pigs. J Anim Sci. 93, 209–16.

Adibmoradi, M., and Pedram, G., 2007. Effects of levels of canola meal on morphology of thyroid gland in broiler chicks. World's Poult. Sci. Assoc. 16th Europeans symposium on Poultry Nutrition. 29-31.

Akinmusire, A. S., and O. Adeola. 2009. True digestibility of phosphorus in canola and soybean meals for growing pigs: Influence of microbial phytase. J. Anim. Sci. 87, 977–983.

Almeida, F.N., Htoo, J.K., Thomson, J., Stein, H.H., 2014. Effects of heat treatment on the pigs. apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Anim. Feed Sci. and Technol. 187, 44–52.

Anderson-Hafermann, C., Zhang, Y., Parsons, C. M., 1993. Effects of processing on the nutritional quality of canola meal. Poult. Sci. 72, 326-333.

Anon, 1992. From Rapeseed to Canola, The Billion Dollar Success Story. National Research Council Publications, Ottawa.

Association of Official Analytical Chemists, 1990. Official Methods of Analysis. 15th ed. Assoc. off. Anal. Chem., Washington, DC.

Association of Official Analytical Chemists, 2005. Official Methods of Analysis of AOAC International, 18th ed.; AOAC International: Gaithersburg, MD.

Barthet, V. J., Daun, J. K., 2011. Seed morphology, composition, and quality. Pages 119-162 in Canola: Chemistry, Production, Processing, and Utilization. J. K. Daun, N. A. M. Eskin, and D. Hickling, eds. AOCS Press, Urbana, IL.

Bate-Smith, E.C., Ribereau-Gayon, P., 1959. Leucoanthocyanidins in seeds. Qual. Plant. Mater. Veg. 5, 189–198.

Bartov, I., Lipstein, B., Bornstein, S.,1974. Differential effects of dietary acidulated soybean oil soapstock, cottonseed oil soapstock and tallow on broiler carcass fat characteristics. Poult. Sci. 53, 115–124.

Bell, M., Keith, M. O. 1991. A survey of variation in the chemical composition of commercial canola meal

Bell, J.M., 1993. Factors affecting the nutritional value of canola meal: a review. Can J. Anim. Sci. 73, 689–97.

Bergaya, F., Theng, B.K.G., Lagaly, G., 2006. Handbook of Clay Science. Elsevier Ltd.

Bones, A., Thangstad, O.P., Haugen, O.A., Espevik, T., 1991. Fate of myrosin cells: characterization of monoclonal antibodies against myrosinase. J Exp Bot, 42, 1541–1549.

Bredeson, D. K., 1983. Mechanical oil extraction. JAOCS 60:211-213.

Broderick, G.A., 2015. Canola science cluster research report. Canola Council of Canada.

Bruce, K. J., Karr-Lilienthal, L. K., Zinn, K. E., Pope, L. L., Mahan, D. C., Fastinger, N. D., et al. (2006). Evaluation of the inclusion of soybean oil and soybean processing by-products to soybean meal on nutrient composition and digestibility in swine and poultry. J. Anim Sci. 84, 1403–1414.

Butler, E.J, Pearson, A.W, Fenwick, G.R., 1982. Problems which limit the use ofrapeseed meal as a protein source in poultry diets. J. Sci. Food Agric. 33, 866–75.

Campbell, L. D., Slominski, B.A., 1991. Nutritive quality of low-glucosinolatecanola meal for laying hens. In: Proc. 8th Int. Rapeseed Congress. 442-447. Saskatoon, SK.

Campbell, L.D., Smith, T. K., 1979. Responses of growing chickens to high dietary contents of rapeseed meal. Br. Poult. Sci. 20:231-237.

Canola Council of Canada, 2015. Canola meal feed industry guide, 5th Edition. Publication of Canola Council of Canada, Winnipeg, MB, Canada.

Carre, P., Citeau, M., Quinsac, A., 2015. Composition of kernel and hull fractions obtained from rapeseed dehulling. Saskatoon, SK: Proc. Intl. Rapeseed Congr; July 2015.

Chen, S., Andreasson, E., 2001. Update on glucosinolate metabolism and transport. Plant Physiol Biochem.39, 743–58.

Cheryan, M. 1998. Ultrafiltration and microfiltration handbook (2nd ed.). Lancaster, PA: Technomic Pub. Co., p. 527.

Das, M.M., Singhal, K.K., 2001. Effect of feeding chemically treated mustard cake on growth, thyroid and liver function and carcass characteristics in kids. Small. Rumin. Res. 56, 31– 38.

De Boland, A.R., Gan1er, G.B. O'Dell, B.L., 1975. Identification and properties of phytate in cereal grains and oilseed products. 1. Agric. Food Chern. 23, 1186-1189.

Dowd, M.K., 1996. Compositional characterization of cottonseed soapstocks. Journal of the American Oil Chemists' Society. 73, 1287–1295.

Durkee, A.B., 1971. The nature of tannins in rapeseed (*Brassica campestris*). Phytochemistry 10, 1583–1585.

Enami HR. 2011. A Review of using canola/rapeseed meal in aquaculture feeding. J Fish Aqua Sci. 6, 22–36.

Englyst, H. N., Cummings, J. H., 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as aditol acetates. Analyst 109, 937-942.

Feedipedia. 2015. Canola science cluster research report. Canola Council of Canada.

Fenwick, G. R., and R. F. Curtis. 1980. Rapeseed meal and its use in poultry diets. A review. Anim. Feed Sci. Technol. 5:255-298.

Fenwick, G.R., Griffiths, N.M., Heaney, R.K., 1982. Bitterness in Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*): The role of glucosinolates and their breakdown products. J. Sci. Food Agric. 34, 73-80.

Glencross, B., Hawkins, W., Curnow, J., 2004. Nutritional assessment of Australian canola meal. Part I. Evaluation of canola oil extracted methods and meal processing conditions on the digestible value of canola meal fed to the red seabream (*Pagrus auratus*, Paulin). Aquacult. Res. 35, 15–24.

Haug, W., Lantzsch, H.J., 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34, 1423–1426.

Hickling, D., 2001. Cano1a Meal Feed Industry Guide. Canola Council of Canada, Winnipeg.

Hijikuro, S., Takemasa, M., 1985. Metabolizable energy values of rapeseed meal produced in Japan and Canada. Jap. Poult. Sci. 22, 33–37.

Honkatukia, M., Reese, K., Presinger, R., Tuiskula-Haavisto, M., Weigend, S., Rito, J., Maki-Tanila, A., Vikki, J., 2005. Fishy taint in chicken eggs is associated with a substitution within a conserved motif of the *FMO3* gene. Genomics 86:225–232.

Horwtitz, W., 2000. Official Methods of Analysis. 4.5.01. Crude fat for ether extract in animal feed. 41.1.33. Polymers and oxidation products of heated vegetable oils. 17th ed. Assoc. Office. Anal. Chem., Washington, DC.

Hurrell, R.F., 1984. Reactions of food proteins during processing and storage and their nutritional consequences. Dev. Food Prot. 3, 213-244.

Ibrahim, I.K., Hill, R., 1980. The effects of rapeseed meals from brassica napus varieties and the variety tower on the production and health of laying fowl. Br. Poult. Sci. 21, 42-430.

Jensen, S.K., Liu, Y.G., Eggum, B.O., 1995. The influence of feed size and hull content on the composition and digestibility of rapeseed in rats. Anim. Feed. Sci. Technol. 54, 9-19.

Jensen, S. K., Liu, Yong-Gang, Eggum, B.O., 1994. The effect of heat treatment on glucosinolates and nutritional value of rapeseed meal in rats. Anim. Feed Sci. Technol. 53, 17-28.

Jia, W., Mikulski, D., Rogiewicz, A., Zdunc´ızyk, Z., Jankowski, J., Slominski, B.A. 2012. Low-fiber canola. Part 2. Nutritive value of the meal. J. Agric. Food Chem. 60, 12231–12237.

Johnson, R.R., McClure, K.E., 1973. High fat rations for ruminants. Effects of fat added to corn plant material prior to ensiling on digestibility and voluntary intake of silage. J. Anim. Sci. 36, 397–406.

Jørgensen, H., Fernández, J. A., 2000. Chemical composition and energy value of different fat source for growing pigs. Acta Agric. Scand. A Anim. Sci. 50,129–136.

Khajali, F., Saedi, M., 2011. The effect of low chloride and high bicarbonate diets on growth, blood parameters, and pulmonary hypertensive response in broiler chickens reared at high altitude. Arch. Geflugelkd. 75, 235–238.

Khajali, F., Slominski, B.A., 2012. Factors that affect the nutritive value of canola. Pout. Sci. 91, 2564–75.

Kil, D. Y., Sauber, T. E., Jones, D. B. Stein, H.H. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fi ber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88, 2959–2967.

Leeson, S., Atteh, J.O., Summers, J.D., 1987. The replacement value of canola meal for soybean meal in poultry diets. Can. J.Anim. Sci. 67, 151–158.

Leeson, S., Summers, J.D., 2001. Scott's Nutrition of the Chicken, 4th rev. ed. University Books, Canada.

Lessire, M., Baudet, J.J., Larbier, M., 1986. Nutritional value of high- or low-glucosinolate rapeseed meals, produced from whole or dehulled seeds. In: Proc. 7th. Euro. Poul. Conf., Paris, pp. 254–257.

Liesegang, A., Urspring, R., Gasser, J., Sassi, M.L., Risteli, J., Riond, J. L., Wanner. M., 2002. Influence of dietary phosphorus deficiency with or without addition of fumaric acid to a diet in pigs on bone parameters. J. Anim. Physiol. A. Nutr. 86:1-16.

Maenz, D.D., Classen, H.L., 1998. Phytase activity in the small intestinal brush border membrane of the chicken. Poult. Sci. 77, 557-563.

Mag, T.K., Green, D.H., Kwong, A.T, 1983. Continuous acidulation of soapstock and recovery of acid oil. Journal of the American Oil Chemists' Society. 60, 1008–1011.

Mandal, A.B., Elangovan, A.V., Tyagi, P.K., Tyagi, P.K., Johri, A.K., Kaur, S., 2005. Effect of enzyme supplementation on the metabolisable energy content of solvent-extracted rapeseed and sunflower seed meals for chicken, guinea fowl and quail. Brit. Poult. Sci. 46, 75–79.

Mansoori, B., Acamovic, T., 2007. The effect of tannic acid on the excretion of endogenous methionine, histidine and lysine with broilers. Anim. Feed Sci. Technol. 134, 198–210.

Marangos, A.G., Hill, R., Laws, B.M., Muschamp, D. 1974. The influence of three rapeseed meals and a mustard meal on egg and broiler production. Brit. Poult. Sci. 15, 404–415.

Martland, M.F., Butler, E.J. Fenwick, G.R., 1984. Rapeseed induced liver haemorrhage, reticulolysis and biochemical changes in laying hens: the effects of feeding high and low glucosinolate meals. Res. Vet. Sci. 36, 298–309.

Matthaus, B., 2012. Oil Technology. In: Gupta, S. K. Technological Innovations in major oil crops Volume 2. Springer, New York Dordrecht Heideiberg London.

Mauron, J., 1981. The Maillard reaction in food: a critical review from the nutritional standpoint. Progr. Food Nutr. Sci. 45, 5-35.

Mavromichalis, I., 2001. The Maillard reaction in feed manufacturing. Feed Technol. 5, 13–14.

Mawson, R., Heaney, R. K. Zdunczyk, Z., Kozlowska, H., 1994. Rapeseed meal-glucosinolates and their antinutritional effects. Part 5. Anim. Reprod. 38, 588–598.

Meng, Slominski, B.A., Campbell, L.D., Guenter, W., Jones, O. 2006. The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part I: Canola Seed. Poult. Sci. 85, 1025-1030.

McCurdy, S.M., 1990. Effects of processing on the functional properties of canola/rapeseed protein. J. Am. Oil Chem. Soc. 67, 281–284.

McKinnon, J.J., Olubobokum, J.A., Mustafa, A.F., Cohen, R.D.H., Christensen, D.A., 1995. Influence of dry heat treatment of canola meal on site and extent of nutrient disappearance in ruminants. Anim. Feed Sci. Technol. 56, 243–252.

Miao, Y., Amari, M., Yoshizaki, S., 1994. Mechanism of spontaneous heating of hay part 2 chemical changes in spontaneous heated hay. Trans. Am. Soc. Agric. Eng. 37, 1567–1570.

Mushtaq, T., Sarwar, M., Ahmad, G., Mirza, M.A, Nawaz, H.M., Mushtaq, H., Noreen, U., 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broilers. Poult. Sci. 86, 2144 -2151.

Mustafa, A.F., Christensen, D.A., McKinnon, J.J., Newkirk, R.W., 2000. Effects of stage of processing of canola seed on chemical composition and in vitro protein degradability of canola meal and intermediate products. Can. J. Anim. Sci. 80, 211-214.

Mutucumarana, R.K., Ravindran, V., Ravindran, G. Cowieson, A.J., 2014Measurement of true ileal digestibility and total tract retention of phosphorus in corn and canola meal for broiler chickens. Poult. Sci. 93, 412–419.

Naczk, M., Amarowicz, A., Sullivan, A., Shahidi, F.,1998. Current research developments on polyphenolics of rapeseed/canola: a review. Food Chem. 62, 489–502.

Naczk, M., Amarowicz, R., Pink, D., Shahidi, F., 2000. Insoluble condensed tannins of canola/rapeseed. J. Agric. Food Chem. 48, 1758–1762.

Nahashon, S.N., Nakaue, H. S. Mirosh, L.W., 1994. Production variables and nutrient retention in Single Comb White Leghorn laying pullets fed diets supplemented with direct-fed microbials. Poult. Sci. 73, 1699-1711.

Newkirk, R.W., Classen, H.L., 2001. The non-mineral nutritional impact of phytate in canola meal fed to broiler chicks. Anim. Feed Sci. Techno. 91, 115-128.

Newkirk, R.W., Classen, H.L., Edney, M.J. 2003. Effects of prepress-solvent extraction on the nutritional value of canola meal for broiler chickens. Anim.Feed Sci. Technol. 104, 111-119.

Newkirk, R.W., Classen, H.L., Tyler, R.T., 1997. Nutritional evaluation of low glucosinolate mustard meals (*Brassica juncea*) in broiler diets. Poult. Sci. 76, 1272-1277.

Newkirk, R., 2009. Canola meal. Feed Industries Guide, 4th edition. Canadian International Grains Institute, Winnipeg, Manitoba.

Newkirk, R., 2011. Meal nutrient composition. Pages 229-244 in Canola: Chemistry, Production, Processing, and Utilization. J. K. Daun, N. A. M. Eskin, D. Hickling, eds. AOCS Press, Urbana, IL.

Norddeutsche Pflanzenzucht H.G., Lembke, K.G., Hohenlieth. GABIKanada (CGAT): YelLowSin – Functional genomic approaches for the development of yellow seeded, low-sinapine oilseed rape (Canola; Brassica napus) Teilprojekt F. 2010. http://edok01.tib.uni hannover.de/edoks/e01fb10/635444097l.pdf Accessed on September 22.2015.

NRC. 1994. Nutrient Requirements of Poultry, 9th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient requirements of swine. 11th ed. National Academy Press, Washington DC.

Statistics Canada, 2001. Cereals and oilseeds review. Agriculture Division, Statistics Canada, Ottawa.

Palmquist, D.L., Jenkisen, T.C., 1980. Fat in lactation ration. Review. J. Dairy Sci. 63, 1-14.

Pardio, V. T., Landin, L. A., Waliszewski, K. N., Badillo, C., Perez-Gil, F., 2001. The effect of acidified soapstocks on feed conversion and broiler skin pigmentation. Poultry Science, 80, 1236–1239.

Pardio, V.T., Landin, L.A., Waliszewski, K.N., Perez-Gil, F., Diaz, L., Hernandez, B., 2005. The effect of soybean soapstock on the quality parameters and fatty acid composition of the hen egg yolk. Poultry Science, 84, 148–157.

Pearson, A.W., Greenwood, N.M., Butler, E.J. and Fenwick, G.R. 1983. Biochemical changes in layer and broiler chickens when fed on a high-glucosinolate rapeseed meal. Br. Poult. Sci. 24, 417–427.

Radfar, M., Rogiewicz, A., Slominski, B.A., 2017. Chemical composition and nutritive value of canola-quality Brassica juncea meal for poultry and the effect of enzyme Supplementation. Anim.Feed Sci. Technol. 225, 97–108.

Rodehutscord, M., 2009. Approaches and challenges for evaluating phosphorus sources for poultry. Pages 2–6 in Proc. 17th Eur. Sym. Poult. Nutr. WPSA UK branch, Edinburgh, UK.

Rogiewicz, A., Nurnberg, L., Slominski, B.A., 2012. The effect of prepress-solvent extraction on the chemical and nutritive composition of Canola Meal. Brazil: Proc. 24th W. Poult. Cong. Salvador.

Sanderson, P., 1986. Anew method of analysis of feeding stuffs for the determination of crude oils and fats. Pages 77-80 in Recent Advances in Animal Nutrition- 1986. W. Haresign and D.J.A. Cole, ed. Butterworths, London.

Saedi, M., Khajali, F., 2010. Blood gas values and pulmonary hypertension as affected by dietary sodium source in broiler chickens reared at cool temperature in a high-altitude area. Acta Vet. Hung. 58, 379–388.

San Juan, L.D., Villamide, M.J., 2001. Nutritional evaluation of sunflower products for poultry as affected by the oil extraction process. Poult. Sci. 80, 431-437.

Shahidi, F., 1990. Rapeseed and canola: Global production and distribution. Pages 3-13 in Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing technology. F. Shahidi, ed.Van Nostrand Reinhold, New York, NY.

Salunkhe, D.K., Chavan, J.K., Adsule, R.N., Kadam, S.S., 1992. Rapeseeds: Page 59-96. In World oilseeds: chemistry, technology, and utilization. Van Nostrand Reinhold, New York, NY.

Sauvant, D., Perez, J.M., Tran, G., 2004. Tables of composition and nutritional value of feed materials. 2nd ed. Wageningen Academic Publishers, Amstelveen, Netherlands.

Seneviratne, R. W., Beltranena, E., Newkirk, R.W., Goonewardene, L. A., Zijlstra, R. T.., 2011. Processing conditions affect nutrient digestibility of cold-pressed canola cake for grower pigs. J.Anim. Sci. 89, 2452–2461.

Seskeviciene, J., Jeroch, H., Westermeir, C., 2004. Effects of high amounts of rapeseed cake and peas in diet for broiler chickens. Bodenkultur. 54, 181–186.

Simbaya J., 1996. Potential for irnproved utilkation of canola meal by monogastric animais. Ph.D. Thesis, University of Manitoba, Winnipeg, Canada.

Slominski, B.A., Rakowska, M., 1985. Influence of 1-cyano-2-hydroxy- 3-butene and intact glucosinolates on the nutritive value of rapeseed meal. Hod. Rosl. Aklim. Nasien. 29, 17–25.

Slominski, B.A., Campbell, L.D., 1987. Gas chromatographic determination of indole glucosinolates- A re-examination. J. Sci. Food Agric. 40, 131–143.

Slominski, B.A., Campbell, L.D., 1990. Nonstarch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J Sci Food Agric. 53,175–84.

Slominski, B.A., Campbell, L.D., Guenter, W., 1994. Carbohydrates and dietary fiber components of yellow- and brown-seeded canola. J. Agric. Food Chem.42, 704–707.

Slominski, B.A., Malgorzata, C., Guenter, W. and Campbell, L.D. 1999a. Nutritive value of enzyme treated canola meal. Proceedings of 10th International rapeseed congress.

Slominski, B. A., Boros, D., Campbell, L. D., Guenter, W., Jones, O., 2004. Wheat by-products in poultry nutrition. Part 1. Chemical and nutritive composition of wheat screenings, bakery by-products and wheat mill run. Can. J. Anim. Sci. 84, 421-428.

Slominski, B.A., 2015. Canola science cluster research report. Canola Council of Canada.

Spragg, J. C., Mailer, R.J., 2007. Canola meal value chain quality improvement: A final report prepared for AOF and CRC. Project code: 1B-103-0506. Available at: http://www.porkcrc.com.au/Final_Report_1B-103.pdf Accessed Jan. 9, 2011.

Stein, H.H., Kadzere, C.T., Kim, S.W. Miller, P.S., 2008. Influence of dietary phosphorus concentration on the digestibility of phosphorus in monocalcium phosphate by growing pigs. J.Anim. Sci. 86, 1861–1867.

Subuh, A.M.H., Rowan, T.G. Lawrence, T.L.J., 1995. Toxic moieties in ruminal and duodenal digesta and in milk, and hepatic integrity in cattle given diets based on rapeseed meals of different glucosinolate content either untreated or treated with heat or formaldehyde. Anim. Feed Sci. Technol. 52, 51–61.

Sukhija, P.S., Palmquist, D.L., 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36, 1202-1206.

Summers, J. D., Spratt, D., Bedford, and M., 1990. Factors influencing the response to calcium supplementation of canola meal. Poult. Sci. 69, 615–622.

Summers, J.D., Bedford, M., Spratt, D. 1992. Sulphur and calcium supplementation of soybean and canola meal diets. Can. J. Anim. Sci. 72, 127-133.

Summers, J. D. 1995. Canola meal and acid-base balance. Anim. Feed Sci. Technol. 53, 109–115.

Summers, J.D., Leeson, S., 1977. Performance and carcass grading characteristics of broiler chickens fed rapeseed gums. Can. J. Anim. Sci. 57, 485-488.

Talebali, H. Farzinpour, A., 2005. Effect of different levels of full fat canola seeds as a replacement for soybean meal on the performance of broiler chickens. Int. J. Poult. Sci. 3, 982-985.

Thacker, P. A., 1990. Canola meal. In: Thacker, P. A.; Kirkwood, R. N.(Eds) Nontraditional feed sources for use in swine production. Butterworth Publishers, Stoneham, Massachusetts, pp. 69–7.

Thacker, P.A., Campbell, G.L., Xu, Y. 1994. Composition and nutritive value of acidulated fatty acids, degummed canola oils and tallow as energy sources for starting broiler chicks. Anim.Feed Sci. Technol. 46, 251-260.

Thies, W., 1977. Analysis of glucosinolates in seeds of rapeseed (Brassica napus): concentration of glucosinolates by ion exchange. Z. Pflanzenzuchtg. 79, 331-335.

Thomas, V.M., Katz, R.J., Auld, D.A., Petersen, C.F., Sauter, E.A. Steel, E.E. 1983. Nutritional value of expeller rape and safflower oil seeds meals for poultry. Poult. Sci. 62, 882–886.

Thomas, P. 2005. Review of University of Alberta Canola Breeding Program. Available at: http://www.acidf.cafilesfocuscanola.pdf Accessed Jan. 5, 2011.

Tripathi, M. K., Mishra, A. S., 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132, 1–27.

Tyagi, A.K., 2002. Influence of water soaking of mustard cake on glucosinolate hydrolysis. Anim. Feed Sci.Technol. 99, 215–219.

Unger, E. H. 1990. Commercial processing of canola and rapeseed: Crushing and oil extraction. In: F. Shahidi (Ed.) Canola and rapeseed production, chemistry, nutrition and processing technology. pp. 235-249. Van Nostrand Reinhold, New York.

Unger, E. H., 2011. Processing. Pages 163-188 in Canola: Chemistry, Production, Processing, and Utilization. J. K. Daun, N. A. M. Eskin, D. Hickling, eds. AOCS Press, Urbana, IL.

Vermorel, M., Heaney, R. K., Fenwick, G.R., 1986. Nutritive value of rapeseed meal: Effects of individual glucosinolates. 1. Sci. Food Agric. 37, 1197-1202.

Viveros, A., A. Brenes, I. Arija, and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enxyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci. 81:1172-1183.

Whittow, G.C., 2000. Sturkie's avian physiology San Diego: Academic Press. 303-304 pp.

Woyengo, T.A., Kiarie, E., Nyachoti, C.M., Energy and amino acid utilization in expeller extracted canola meal fed to growing pigs. J Anim Sci. 2010, 88:1433–41.

Woyengo, R.A. Kiarie, E., Nyachoti, C.M., 2011.Growth performance, organ weights and blood parameters of broilers fed containing expellere-extracted canola meal. Poult. Sci. 90, 2520-2527.

Woyengo, T.A., Nyachoti, C.M., 2013. Review: anti-nutritional effects of phytic acid in diets for pigs and poultry – current knowledge and directions for future research. Can J Anim Sci. 93, 9–21.

WPSA (World's Poultry Science Association). 2013. Determination of phosphorus availability in poultry. World's Poult, Sci. J. 69, 687-698.

Young, F. V. K., Biernoth, C. P. E., Krog, N., Davidson, N. G. J., & Gunstone, F. D. (1994). Processing of fats and oils, p. 58.

Yapar, Z., Clandinin, D. R., 1972. Effect of tannins in rapeseed meal on its nutritional value for chicks. Poult. Sci. 51, 222–228.

Zhang, Y. E., Parsons, C.M., 1994. Effects of over processing on the nutritional quality of sunflower meal. Poult. Sci. 73, 436-442.

Zschau, W., 2001. Bleaching of edible fats and oils. Eur. J. Lipid Sci. Technol. 103, 505–551.