THE EFFECT OF PRE-PRESS SOLVENT EXTRACTION CONDITIONS ON THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF CANOLA MEAL FOR BROILER CHICKENS AND PIGS

by

Deborah Ibukunoluwa Adewole

A thesis submitted to The Faculty of Graduate Studies of

The University of Manitoba

In partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

University of Manitoba

Winnipeg

ABSTRACT

Canola meal (**CM**) which is commonly used in poultry and swine diets as an economically viable alternative to soybean meal is mainly produced by the process called the pre-press solvent extraction. The pre-press solvent extraction does not only involve many steps, each step also involves a wide range of vital conditions including temperature, moisture and time. Variations in these processing conditions among and within processing plants may contribute to inconsistency in the chemical and nutritive compositions of the resulting meals. In addition, the desolventization/toasting step of the pre-press solvent extraction process has been implicated for reducing both the content and availability of amino acids (**AA**), especially lysine, for both broiler chickens and pigs. Canola meal end-users desire increased meal consistency for a more accurate and cost-effective feed formulation and more information on the nutritive value and heat damage of AA. The first objective of this thesis was to determine the effect of processing plant and year on the chemical composition and indicators of protein damage in CM. To achieve this, a chemical composition survey of CM from 11 processing plants that use the pre-press solvent extraction process was conducted over a 4-year period. Differences among processing plants and years were observed for protein, lysine, dietary fiber and its components, fat, and carbohydrates components. Over the 4 years, the highest variation was observed in the contents of simple sugars having a coefficient of variation (**CV**) of 41.7%), neutral detergent insoluble crude protein (**NDICP**; CV = 16.8%) and glucosinolates (CV = 35.3%), the components known to be sensitive to heat treatment. Among all AA, only lysine showed differences ($P < 0.05$) among processing plants. Lysine content averaged 18.5, 22.1, 22.9 and 20.7 g/kg in years 2011, 2012, 2013 and 2014, respectively, and was lowest in meals showing the highest NDICP and total dietary fiber values. Linear regression

equations for predicting lysine, NDICP, and total dietary fiber from neutral detergent fiber (**NDF**) or NDF and CP were developed. The second objective was to determine the effect of processing plant and pelleting on the standardized ileal digestible AA content (in broiler chickens and pigs) and AME_n in broiler chickens of CM from Canadian processing plants. To achieve this, 8 CM samples were selected and fed to the animals in semi-purified diets containing the CM samples as the only protein source. Differences in these parameters were observed among processing plants. However, the effect of pelleting may be plant-dependent. The third objective was to develop prediction equations for quick determination of indicators of CM quality including lysine, neutral detergent insoluble crude protein, dietary fiber from NDF or NDF and CP and determination of standardized ileal digestible AA content from acid detergent fiber (**ADF**) and NDF. Results showed that the NDF content of CM accurately predicted its total dietary fiber content. The standardized ileal digestible contents of some essential AA in CM can be predicted from the ADF content.

DEDICATION

To my parents and siblings who have laboured greatly to give me a sound education.

ACKNOWLEDGMENTS

I am grateful to my supervisor, Dr. Bogdan Slominski, for taking up the responsibility of supervising and mentoring me during my pursuit of a Ph.D. degree. Thanks to my Committee Members, Drs. Martin Nyachoti, Martin Scanlon, and Garry Crow for their valuable contributions to this project and my External Examiner, Dr. Doug Korver for the time and attention spent on reviewing this thesis.

My appreciation goes to Canola Council of Canada for funding this project. I am also grateful for the Cyril L. Anderson Feed-Rite Graduate Student Fellowship I received during this program.

I cannot but express my sincere gratitude to our Research Associate, Dr. Anna Rogiewicz for her guidance and support during my laboratory work and animal trials.

I appreciate the help from my lab Technician, Tom Davie, graduate Students, visiting Scientists, and staff of the Animal Science Department and T. K. Cheung Center.

Special thanks to my Darling husband, Dr. Temidayo Adewole for sacrificing his ambitions in favour of mine. His full support during this Ph.D. program is highly appreciated.

Thank you, Daniel and ‘Dara for being another source of encouragement and laughter; having to take care of you while I do a Ph.D. has earned me more virtues.

I cannot forget in a jiffy, the financial and moral supports I received from my loving siblings most especially Pastor and Dr. (Mrs.) Ige and Dr. and Mrs. Akinboro before and during this program.

I thank my Parents, Mr. I. O. Ayoade and Mrs. E. A. Ayoade for their physical, financial and spiritual investments to my future.

All Glory to God who was, is, and is to come. Maranatha!

FOREWORD

Sections of this thesis have been presented as poster and oral presentations at National and International conferences which include 13th International Rapeseed Congress, June 5 – 11, 2011, Prague, Czech Republic; 34th Western Nutrition Conference, September 24 – 26, 2013, Saskatoon, SK, Canada; Poultry Science Association (PSA) Annual Meeting, July 14 - 17, Corpus Christi, TX, USA; 14th International Rapeseed Congress, July 5 – 9, 2015, Saskatoon, SK, Canada; and 36th Western Nutrition Conference, September 29 – 30, 2015, Winnipeg, MB, Canada. This thesis was written in manuscript format and it is composed of four manuscripts, written according to the Journal of Animal Science format. All manuscripts have been or will be submitted for publications as follows:

Manuscript I. Adewole D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2016. Chemical and nutritive characteristics of canola meal from Canadian processing facilities. Anim. Feed Sci. Technol. 222:17-30 (Chapter 4)

Manuscript II. Adewole D. I., A. Rogiewicz, B. Dyck, C. M. Nyachoti, and B. A. Slominski. 2017. Standardized ileal digestible amino acid contents of canola meal from Canadian crushing plants for growing pigs. J. Anim. Sci. 95:2670-2679 (Chapter 5)

Manuscript III. Adewole D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2017. Effect of canola meal source on the standardized ileal digestible amino acids and apparent metabolizable energy contents for broiler chickens. Poult. Sci. (In press; Chapter 6).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fiber
ADM	Archer Daniels Midland
AID	Apparent ileal digestibility
AME	Apparent Metabolizable energy
AME_n	Nitrogen-corrected AME
Ala	Alanine
Arg	Arginine
Asp	Asparagine
CCC	Canola Council of Canada
CM	Canola meal
CO_2	Carbon dioxide
CP	Crude protein
Cys	Cysteine
CV	Coefficient of variation
d	day
DDGS	dried distillers' grains with solubles
DT	Desolventizer-toaster
DM	Dry matter
EL	Endogenous losses
Glu	Glutamine
Gly	Glycine
h	Hour
His	Histidine

Ile	Isoleucine
JRI	James Richardson International
L & P	Lignin and polyphenols
Leu	Leucine
Lys	Lysine
ME	Metabolizable energy
Met	Methionine
N	Nitrogen
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NIRS	Near-infrared reflectance spectroscopy
NRC	National Research Council
NSP	Non-starch polysaccharides
P	Phosphorus
Phe	Phenylalanine
Pro	Proline
R^2	Coefficient of determination
RSD	Residual standard deviation
SBC	Spent bleaching clay
SBM	Soybean meal
Ser	Serine
SID	Standardized ileal digestibility
Thr	Threonine
TMA	Trimethylamine
TME_n	Nitrogen-corrected true metabolizable energy

Tyr	Tyrosine
USDA	United States Department of Agriculture
Val	Valine
wk	week

CHAPTER 1

GENERAL INTRODUCTION

Canola is a major oilseed crop grown in the Prairie Provinces of Canada, and its coverage area is expanding. It contributes 19.3 billion dollars to the Canadian economy each year, including more than 249,000 Canadian jobs and 12.5 billion dollars in wages (LMC International, 2013). Canola crushing is an important part of the overall canola industry (Unger, 2011). Of the approximately 7 million tonnes of canola seed produced in Canada each year, about half of the seed is exported, while the other half is crushed domestically (Hickling, 2001). In Canada, extraction of oil from canola is done mainly by the pre-press solvent extraction process (Canola Council of Canada, 2015). Canola meal (**CM**), the solid component left after oil extraction, is the second most commonly used protein source for animal feed in the world after soybean meal (NSW Department of Primary Industries, 2004). The main components of CM (pre-press solvent extracted) include protein (and amino acids), carbohydrates (that is, simple sugars, sucrose, oligosaccharides, starch), dietary fiber (that is, non-starch polysaccharides, lignin with associated polyphenols, glycoproteins), fat, and ash (Khajali and Slominski, 2012).

The high protein content (35%– 45%) of the oil-free meal with a good balance of amino acids (**AA**) makes CM an attractive ingredient for feed formulations (Canola Council of Canada, 2009). The carbohydrate components of CM account for approximately one-third of the meal (Khajali and Slominski, 2012). In general, CM has a good AA profile for animal feed. Like many vegetable-based protein sources, it is limiting in lysine (Khajali and Slominski, 2012). However, it is a good source of other AA such as methionine and cysteine for pigs and poultry (Canola Council of Canada, 2009). Canola meal also contains some anti-nutritional factors which include

glucosinolates, sinapine, tannins, and phytate (Slominski et al., 2012; Adewole et al., 2016; Kasprzak et al., 2017). Although high in phytate, CM is also one of the richest sources of non-phytate (available) phosphorus (that is, 0.38% of non-phytate P vs. 0.28, 0.23, 0.09, 0.26, 0.07, and 0.13% for soybean meal, cottonseed meal, wheat, wheat bran, corn, and barley, respectively; Khajali and Slominski, 2012). Canadian solvent-extracted CM is derived from a blend of *Brassica napus* and *Brassica rapa* seeds. The majority (> 95%) of the seed produced in Canada is *Brassica napus* (Canola Council of Canada, 2015). Studies (Bell and Keith, 1991; Canola Council of Canada, 2015) have shown that there are some variabilities in the nutrient compositions of CM due to variation in environmental conditions during the growing of the canola crop or as a result of differences in cultivar and processing conditions of the seed and meal. In Canada, the extraction of oil from canola seed generally involves ten stages including cleaning, drying, conditioning, flaking, expelling, cooking, solvent extraction with hexane, desolventization/toasting, drying and then cooling and in some cases, pelleting (Newkirk et al. 2003b). Each of these steps requires wide range of temperature, moisture, and timing. For example, the desolventization and toasting stage of CM processing may last between 35 and 50 min and requires steam (15-18% moisture) and temperatures that vary from 95 to 115°C (Canola Council of Canada, 2009; Unger, 2011). Consequently, differences in processing of CM may result in variation in its nutritional composition among different processing plants because Maillard reactions may occur as a result of the combination of heat and moisture applied to the meals which contain AA and reducing sugars (Nursten, 2005; Almeida et al., 2013). Canola processing can also cause thermal degradation of 30–70% of the glucosinolates in the meal (Daun and Adolphe, 1997). Newkirk et al. (2003a) found that the desolventization and toasting process operated in Canadian oilseed processing plants reduced glucosinolates levels in CM by around 40% compared to the original

canola seed. Indeed, data from Campbell and Slominski, (1990) and Spragg and Mailler (2007) indicate that the degradation of glucosinolates during both expeller extraction and solvent extraction processing is greater than 40%, this being in the order of 50% for expeller and 80% for solvent processed meals. Excessive heating during processing can result in reduced content and digestibility of some AA, particularly lysine, by animals (Parsons et al., 1992; Anderson-Hafermann et al., 1993; Newkirk et al., 2003a; Almeida et al., 2013; Eklund et al., 2015). Examination of meal quality at various processing stages in several Canadian processing plants (Newkirk, et al., 2003b; Mosenthin et al., 2016) revealed that the desolventization and toasting process of CM significantly reduced crude protein and lysine digestibility as well as lysine content. Another factor that may contribute to the variability in the chemical and nutritive composition of CM is the practice of adding or not adding back a portion of phospholipid material and the acidulated soapstocks which are removed during oil processing (Canola Council of Canada, 2015). These additions serve to reduce the dustiness of the meal and, more importantly, increase its metabolizable energy value (Canola Council of Canada, 2009). The digestibility of AA varies between animals with the digestibility of AA in CM generally about 10% lower in poultry and pigs than soybean meal (Newkirk, 2011). Studies (Slominski, 1997; Almeida et al., 2013) have shown that excessive heat treatment reduces the digestibility of protein and AA in CM when fed to monogastric animals and that there is a significant correlation between the contents of dietary fiber components and protein (or AA) digestibility.

No study has been conducted to determine the effect of processing practices or conditions in the various crushing plants on the chemical compositions, the formation of Maillard reaction products, and standardized ileal digestible AA content in CM from Canadian processing plants. Canola meal

end-users desire increased meal consistency and more information on the nutritive value and heat damage of AA in CM (Spragg and Mailer, 2007).

It was hypothesized that there would be differences in the chemical and nutritive composition of CM from Canadian processing plants for both broiler chickens and growing pigs. Also, it would be possible to develop prediction equations for some indicators of CM quality including lysine, neutral detergent insoluble crude protein (**NDICP**), total dietary fiber and standardized ileal digestible AA content (for broiler chickens and pigs) of CM from simple chemical measures.

CHAPTER 2

LITERATURE REVIEW

2.1 RAPESEED AND CANOLA

Rapeseed belongs to one of the most widespread family of plants, the Brassicaceae (or Cruciferae) which also include cabbage, cauliflower, mustard, broccoli, and kale. Rapeseed ranks the third largest oilseed crop around the world after soybean and cottonseed (Daun, 2011). It was cultivated more than 3000 years ago in India and 2000 years ago in China and Japan. It was introduced to Canada between 1936 and early 1940's as a method of diversifying crop production, especially for the Prairie provinces (Bell, 1984). The fuel shortage caused by World War II led to the increased production of rapeseed. However, with the switch to diesel engines, and the ban of the use of rapeseed for human consumption by the United States of America in 1956, the demand for rapeseed declined (USDA, 2012). Rapeseed contains high levels of glucosinolates, which can be hydrolyzed by the enzyme myrosinase to release products with goitrogenic effects that interfere with iodine metabolism and therefore affect the functioning of the thyroid gland and consequently animal performance (Mawson et al., 1994).

In Canada, rapeseed (mainly *B. campestris*) cultivation started in 1936 during the arrival of early settlers when a Polish farmer at Shellbrook, Saskatchewan received seeds of *Brassica rapa* from a contact in Poland (Bell, 1982). These seeds became the resource material for yield and establishment trials by the Canadian Department of Agriculture. In 1942, seeds of *Brassica napus* from Argentina were introduced through the United States of America to Saskatchewan farmers who grew the crop on contract (White, 1979). For a long time, the two types of rapeseed were grown and came to be known as Polish and Argentine rape, respectively (Bell, 1982). The original interest in rapeseed production centred on its high oil content which has special properties as a

marine engine lubricant (Bell, 1982). It was later discovered that the oil contained high level of erucic acid (Bell, 1982). Following the Second World War, research reports on the use of rapeseed meal in poultry diets (Petit et al., 1944; Blakely and Anderson, 1948) indicated problems with goitrogenicity due to the presence of glucosinolates. The problem with the oil prompted rapeseed breeders to explore the possibility of reducing the erucic acid content of the oil which was about 55% (Bell, 1982). Plant breeders worked to develop rapeseed cultivars with low erucic acid content in the oil and low glucosinolate content in the meal for many years. The initial low-glucosinolates character was obtained from the Polish cultivar "Bronowski". Shortly afterwards, a "double low" cultivar of *B. campestris* was produced and by 1981, the production of high glucosinolates cultivars had nearly ceased in Canada. The first low-erucic acid rapeseed was developed in Canada by Dr. Baldour R. Stefansson of the University of Manitoba, who has been referred to as "The father of canola" because of his contribution to the development of low-erucic acid type rapeseed and Dr. Keith Downey at the Agriculture Canada Research Station in Saskatoon. In early 1960s, Dr. Stefansson surveyed over 4000 lines of rapeseed from all over the world and identified low-erucic acid lines which were then used in the breeding programs at the University of Manitoba. In 1968, the first low-erucic acid cultivars Tanka, Target and Turret were released and produced in Canada (Bell, 1984). In 1974, Dr. Stefansson released the first double zero rapeseed cultivar, Tower (Bell, 1984).

The name "Canola" was adopted in 1979 to apply in Canada to all "double low" cultivars. Canola is the registered name for rapeseed containing less than 2% of the total fatty acids in the oil as erucic acid and less than 30 μmoles of alkenyl glucosinolates per gram of oil-free dry matter of the seed. The name was used to differentiate canola from the high-glucosinolate, high-erucic acid rapeseed. In the international community, canola is also known as "double zero", "zero-zero" or

"double low" rapeseed. Before this genetic breakthrough achieved by Canadian plant breeders, rapeseed oil contained between 25 and 45% erucic acid and 100-120 μmoles of glucosinolates (Bell, 1993).

Canola is a major oilseed crop in western Canada. Its production has witnessed a steady upward movement during the past 25 years and contributes about 14% of the global vegetable oils (Gupta and Pratap 2007).

Growing conditions in the region are ideally suited to canola production and, thus, canola is commonly included in crop rotations. As it is a cool season crop (George et al., 2017), it has become one of the most valuable agricultural commodities in countries such as Canada, China, and some European countries. Canada is the leading exporter of canola seed in the world, exporting an average volume of 3.4 million tonnes annually over the period 1997-2003 (Statistics Canada, 2015).

2.2 CANOLA CROP AREAS IN CANADA

Since the Census of Agriculture began tracking the crop in 1956, the land area used to plant canola has expanded steadily, from 143,000 hectares to 9.2 million hectares in 2017 (Statistics Canada, 2017). Canola production in Canada is still overwhelmingly concentrated in the Prairies (Saskatchewan, Alberta, Manitoba and the Peace River region of British Columbia), which account for 99% of the total seeded area (Table 2.1). However, canola is also seeded in all other provinces except Newfoundland and Labrador (Statistics Canada, 2015). The growth of the canola plant is greatly influenced by weather conditions, including temperature, moisture, light, nutrition, and variety (Bita and Gerats, 2013; Zeleke et al., 2014). Research in Canada has shown that water stress and temperature are the most important environmental factors regulating growth and

development of canola in western Canada (Qaderi et al., 2012). The maturity of canola seeds varies considerably depending on location, growing season, date of seeding, and variety.

Canola production in Canada has been steadily increasing, and it is currently approximately 15 million tonnes of canola seed per year (Canola Council of Canada, 2015).

Table 2.1. Canola Crop Areas in Canada

Region	Hectares		
	2001	2006	2017
Canada	3,782,906	5,027,643	9,241,846
Newfoundland and Labrador	0	0	-
Prince Edward Island	0	64	-
Nova Scotia	0	10	-
New Brunswick	195	359	-
Quebec	3,832	6,159	15,013
Ontario	14,746	7,517	-
Manitoba	757,744	922,134	1,262,619
Saskatchewan	1,906,171	2,418,916	5,103,086
Alberta	1,076,670	1,646,468	2,804,471
British Columbia	23,548	26,018	38,445

Source: Statistics Canada, Census of Agriculture.

2.3. CANOLA CRUSHING IN CANADA

Canola oil has excellent nutritional value, due to its low saturated fatty acid content, making it the oil of choice in many applications. The seed contains approximately 42% oil and 58% meal. As a result of the high content and value of the oil, the primary focus in the past has been to maximize the value of the seed through its oil content (Newkirk, 2002).

Canola crushing is an important part of the overall canola industry in Canada (Unger, 2011). During the period from 2000 to 2010, domestic canola crushing in Canada consumed 40.5% of all Canadian canola production. Raw canola is converted to oil for use in consumer products through a process called "crushing." About fourteen canola crushing facilities currently operate in Canada, mainly in the Prairies (Figure 2.1), some new plants are under construction while others are undergoing expansion. In 2006, total canola crushing reached 3.6 million tonnes of seed, well above the average of 2.9 million tonnes in the previous five years. All of this crushing makes Canada the world's fourth-largest producer of canola/rapeseed oil, with 1.6 million tonnes of oil in 2006 (Statistics Canada, 2015).

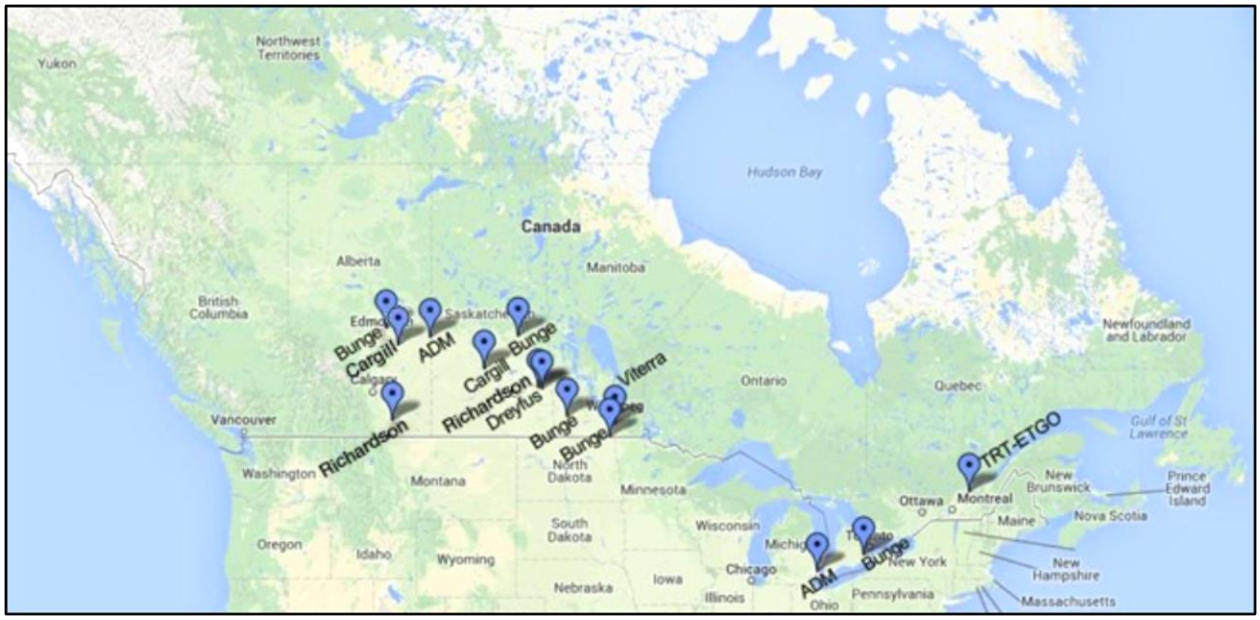

Figure 2.1. Canola Crushing Plant Locations in Canada

Source: http://www.canolacouncil.org/markets-stats/industry-overview/

2.3.1 Canola Crushing Methods

In general, three different methods for the extraction of oil are available: (1) cold pressing, (2) expeller pressing, and (3) pre-press solvent extraction.

Cold pressing or single-pass cold pressing is a method in which seeds are directed straight into the mechanical press without applying steam at a temperature range of 50 – 60℃ resulting in 50 to 70% oil extraction (Leming and Lember, 2005) leading to the production of canola cake (Kaldmae et al., 2010; Kasprzak et al., 2016). In small-scale biodiesel production facilities, canola seed is cold pressed to extract crude canola oil. Seed is subjected to mechanical pressing without preconditioning to provide external heat (Spragg and Mailer, 2007). Still, seed temperature may increase up to 65°C because of friction build-up in the press (Spragg and Mailer, 2007) or the barrel might be heated, depending on the equipment. In cold pressing, oil recovery is reduced compared with expeller pressing and solvent extraction; thus, the resulting canola cake contains more residual oil (Leming and Lember, 2005). On average (dry matter basis), cold-pressed canola cake contains 4.17 Mcal/kg of digestible energy, 2.84 Mcal/kg of NE, 0.87% of standardized ileal digestible (**SID**) lysine, 0.46% of SID methionine, and 0.79% of SID threonine for pigs (Seneviratne et al., 2011). The nutritional quality of cold-pressed canola cake varies with processing conditions, and residual glucosinolates likely do not affect nutrient digestibility (Seneviratne et al., 2011).

Expeller pressing is an old method which nowadays has been widely displaced by the use of solvent which is more efficient, especially for oilseeds with lower oil contents such as soybeans. In expeller pressing, seed is heated using steam up to 110°C before pressing and may pass through the press once or more to reach >75% oil extraction (Leming and Lember, 2005; Spragg and

Mailer, 2007) leading to the production of canola expeller meal (Toghyani et al., 2015). Expelling rapeseed/canola involves cleaning to remove debris, conditioning for about five minutes to raise the temperature to 105-108°C, flaking between rollers, cooking at approximately 130°C for 30 minutes, and then feeding into a screw-press extruder (Clandinin and Tajcnar, 1960).

The pre-press solvent extraction is a method of oil extraction by a combination of expelling and solvent extraction. It involves a two-stage oil extraction process, utilizing an initial expeller extraction operating at 100 to 120°C, resulting in the production of a seed cake with approximately 20% of oil. This then undergoes solvent oil extraction using hexane, and then a final desolventization and toasting process at temperatures of 100 to 115°C (Spragg and Mailer, 2007) leading to the production of canola meal (Eklund et al., 2015). The pre-press solvent extraction process involves the following stages – seed cleaning, moisture adjustment and storage, seed flaking, seed cooking, screw pressing, solvent extraction, desolventization and toasting, and drying, pelleting and storage (Pickard et al., 1989; Spragg and Mailer, 2007). Extraction of oil using solvents results in a meal with less than 2% residual oil, while expeller meals can contain between 8% and 15% oil due to less efficient extraction (Seneviratne et al., 2010; Spragg and Mailer, 2007). The greater residual oil content in cold-pressed and expeller-pressed canola coproducts provides more dietary energy and less AA than those in solvent-extracted canola meal (Seneviratne et al., 2010; Woyengo et al., 2010).

2.3.2 Steps Involved in Canola Crushing

The steps involved in the pre-press solvent extraction of canola are illustrated in Figure 2.2.

2.3.2.1 Seed Storage, cleaning, and moisture adjustment

Before and after arriving at the processing plants, canola seeds are subject to some factors which affect their processing and quality. Canola seeds must be matured at harvest to ensure good quality canola products. Other environmental factors that can have a significant influence on canola seed quality and the resulting oil and meal products include high chlorophyll, high moisture, and heat damage (Unger, 2011).

Canola seed is typically delivered directly from growers to the canola processing plants. Therefore, most processing plants will store seeds for 1 to 3 weeks before processing. It is important to store canola seeds at moderate temperature (usually below 18˚C in North America) to avoid insect infestations and heat damage. This is achieved by using a storage facility with moisture and temperature control (Savic et al., 2009) and by using temperature probe to monitor bulk seed temperature and sieve for insects (Burril, 2012). The use of aeration to promote uniform and cool storage conditions is an important strategy for maintaining oil and seed quality (Burril, 2012). Once the canola seeds are harvested, the moisture content is reduced for a safe storage to 5.5 – 8.5% wet basis depending on the storage temperature and the oil content (Banks 1998).

The seed delivered to the processing plant may contain foreign materials, which are removed by cleaning operations prior to processing (Canola Council of Canada, 2015). However, with the introduction of herbicide-tolerant canola varieties and hybrids, the amount of foreign material in the canola seed delivered from farms has been significantly reduced (Beckie et al., 2006).

It is very important to regulate the moisture content of the seed before any physical processing because the moisture content will affect other processes such as oil recovery, solvent recovery from the meal, oil quality, and degumming efficiency. Canola seeds are very fragile and are easily

broken into small particles when they are flaked at a low temperature (below 0°C). This impedes oil release during the pressing stage, makes solvent infiltration into the canola cell structure very difficult, and prevents the leaching of oil bodies from the canola seed particles. This results in reduced extraction efficiency and poor process economics (Unger, 2011). Therefore, the seeds are usually preheated to temperatures ranging from 30 to 90°C for 30 to 45 minutes to achieve a moisture level of 6.5 to 7.5% before flaking, especially in cold weather conditions. This also results in an improved flake formation and improved extraction efficiency (Unger, 1990).

2.3.2.2 Flaking

Flaking of canola seed is necessary to rupture the cell walls and flattening the cotyledons, thus facilitating the oil extraction process (McCurdy, 1990). The rupturing of the cell walls performs the following functions (1) allows the oil particles to move from the cellular structure to the outer surface of the flakes, where the liquid portion can be separated from the flakes, (2) allows the solvent to infiltrate into the cellular structure and leach the oil, and also allows the outward flow of the solvent from the cellular structure during the desolventization step, and (3) allows the oil particles to coalesce into larger particles for easy extraction (Unger, 1990). An optimal flake thickness ranging from 0.30 to 0.38 mm has been recommended for maximal oil yield (Canola Council of Canada, 2015; Mosenthin et al., 2016).

2.3.2.3 Cooking

Cooking of canola flakes is achieved either in a stacked cooker or a horizontal rotary conditioner. The stacked cooker has been in use for more than 50 years. It contains a series of closed cylindrical kettles stacked one on top of the other with each having a sweep type handle device for continuous mixing of the flakes while cooking (Pickard, 1993). During the past 15 years, canola processors have switched to the use of the horizontal rotary conditioner which has fewer mechanical parts and

require a lower energy input to mix the canola flakes than the stacked cooker (Unger, 2011; Mosenthin et al., 2016). Apart from equipment changes, other changes that have come to canola cooking include cooking temperature and residence time. In the 1990's, cooking of canola took 30 to 60 minutes at a temperature range of 85-90°C and at a moisture content of 6 to 10 % (Simbaya et al., 1995). However, presently, cooking of canola flakes takes 15 to 20 minutes at 80 to 105°C (Canola Council of Canada, 2015). However, these conditions still vary from one processing plant to the other. For example, an average residence time of 68 minutes was reported by Mosenthin et al. (2016).

According to Unger (2011), the cooking of canola flakes performs the following functions: (1) it allows the oil to coalesce and allows some of the protein particles to agglomerate which makes them easier to separate during the subsequent pressing operation, and (2) partially deactivates myrosinase enzyme which hydrolyses glucosinolates, in the presence of moisture to yield various sulphur-containing derivatives that could negatively impact oil colour and flavour. Myrosinase is most active at 50 to 70°C in the presence of moisture, such as in cooking operation (Youngs and Wetter, 1967).

2.3.2.4 Screw Pressing

In a canola processing plant, the screw pressing operation removes 60 to 70% of the oil from the canola flakes (Bredeson, 1983; Vadke and Sosulski, 1988; Unger, 1990). This mechanical process is achieved by the compression of the oil bearing material due to a progressive reduction in the volume enclosing this material during its forward displacement (Mosenthin et al., 2016). The pressure applied during the screw pressing operation also helps to rupture additional cellular structure that was not ruptured in the flaking process (Unger, 2011). A detailed description of the screw pressing operation has been reported by Unger (2011) and Mosenthin et al. (2016).

2.3.2.5 Solvent Extraction

The goal of canola processors is to extract oil from canola cake up to the level of 1% residual oil in the meal. To achieve this, the press cake obtained from the screw pressing operation is further extracted by a solvent extraction process. In the past, hexane had been the solvent used in the extraction of canola of the canola press cake. However, today, the solvent for canola oil extraction is rather, a mixture of 2-methioninehylpentane, 3-methioninehylpentane, hexane, and a minor amount of other solvents. This mixture is often referred to as "isohexane" which has a boiling point of 63–67°C (Unger, 2011). After leaving the solvent extractor, the resulting product, referred to as marc (solvent saturated canola cake), has less than 10% of oil (dry matter-basis) and an average temperature of 50°C (Mosenthin et al., 2016).

2.3.2.6 Desolventization and Toasting

After solvent extraction, 25 to 35% of the solvent remains in the cake. To remove this solvent, canola cake must undergo desolventization in an equipment called the desolventizer-toaster (**DT**). It is called a DT because it does not only desolventize the meal but also imparts a toasting process to deactivate the heat-labile anti-nutritional factors in the meal (Newkirk and Classen, 2002). The DT consists of an enclosed vessel with several steam-heated metal trays and applies both direct and indirect heat to the meal. The temperature in the DT is usually between 103 and 110°C, the moisture is usually 15-17% and a retention time of 30-50 minutes (Unger, 1990, 2011). With the combination of high moisture and temperature in the DT, a complete deactivation of myrosinase enzyme and a significant reduction in total glucosinolates content in the desolventized meal is achieved. This procedure is characterized by the combined application of indirect heat and direct unsaturated steam. With increasing residence time in the DT in combination with the use of unsaturated (over-heated) steam, there is a high risk of changing the physical conditions to over-

heated steam at temperatures above 100°C. This results in evaporation of water bound to marc, which, in turn, may create over-toasting conditions (Mosenthin et al., 2016). A detailed description of the DT has been reported by Mosenthin et al. (2016).

2.3.2.7 Drying and pelleting

Canola meal is usually dried at 100 to 120°C to contain only about 10% moisture. Pelleting of CM for the purpose of agglomerating smaller particles into larger ones using moisture, heat, and mechanical pressure is becoming a common practise. The advantages of pelleting include (1) pellets have a better flow property and higher bulk density than mash, thus reducing bulkiness and dustiness during transportation; (2) pelleting improves the hygienic condition of feed by reducing the activity of microorganisms e.g. *Salmonella* cross-contamination in CM; (3) pelleting can increase palatability, which enhances feed intake in animals; (4) pelleting reduces feed wastage by preventing selective feeding and sorting (Abdollahi et al., 2013); (5) the heat applied during pelleting can inactivate heat-labile anti-nutritional factors and may enhance nutrient digestibility. Pelleting involves passing mash CM from the mash bin into the feeder and conditioner. After steam injection to the CM inside the conditioner, conditioned mash flows into the pelleting chamber. Pellets are formed by passing the hot mash through a metal die followed by cooling. To optimise the conditioning process, a proper balance of heat and moisture must be obtained (moisture level of 15 – 17% and a temperature of 80 to 90°C were reported by Abdollahi et al., 2013). The heat, moisture and mechanical pressure applied during conditioning and pelleting may cause some chemical and physical alterations that may have beneficial or detrimental effects on feed components (Abdollahi et al., 2013). Pelleting may lead to the formation of Maillard reaction products which are considered unusable for the animals. The effects of pelleting on protein digestibility are inconsistent in the literature due to different processing methods and conditions

(Abdollahi et al., 2013). Huang (2015) reported that different temperatures and time of conditioning during pelleting had significant effects on nutrient profiles, rumen degradation, and intestinal digestion characteristics and neutral detergent insoluble crude protein (**NDICP**) in cattle increased with increasing pelleting temperature.

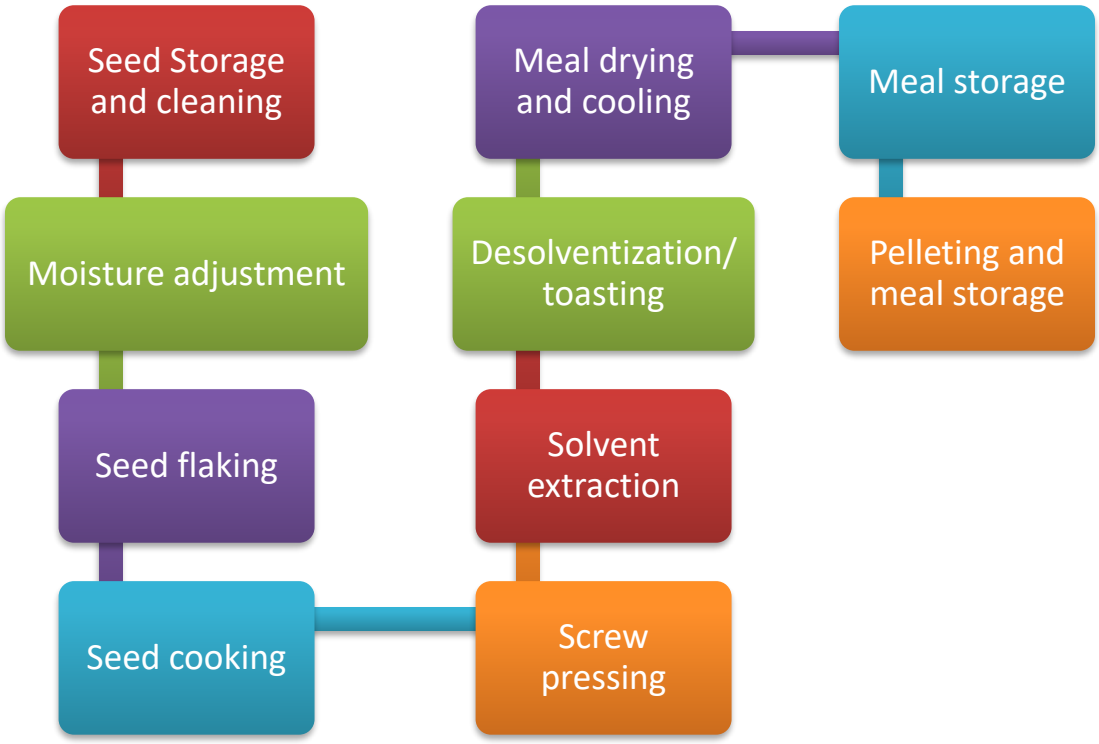

Figure 2.2. Illustration of the pre-press solvent extraction process of canola.

2.4 CANOLA MEAL: PHYSICAL AND CHEMICAL CHARACTERISTICS

Canola meal is the solid component left after oil extraction from canola seed and it is used as a protein source for animal feeding in many parts of the world. It contains high quality protein but its use in the diets of monogastric animals has been limited by the relatively high content of fiber in the meal. Canadian CM is derived from a blend of *Brassica napus* and *Brassica rapa* seeds. Canola meal is a less expensive feed ingredient than soybean meal (Gonzalez-Vega and Stein, 2012). As with any crop, the chemical composition of CM varies depending on the variety and associated method of cultivation, environmental and climatic changes during the growing season, harvest conditions (McFadden et al., 2006), and processing method including the mechanical and thermal treatments employed (Messerschmidt et al., 2014; Eklund et al., 2015).

The main components of pre-press solvent extracted CM include protein (and amino acids), carbohydrates, dietary fiber, and fat. It also contains some antinutritive factors which include glucosinolates, tannins, sinapine and phytic acid.

2.4.1 Protein and Amino Acids

Canola processing plants can have influence on the amount of protein in CM depending on the amount of gums added back to the meal. Compared to soybean meal (**SBM**), CM is lower in crude protein, however, they compare favourably with regard to AA content. In general, CM has a good AA profile for animal feed. Like many other vegetable-based protein sources, CM is limiting in lysine, however, it is a good source of other AA such as methionine and cysteine (Woyengo et al., 2010; Li et al., 2015a, b; Ivanova et al., 2016). The protein and AA compositions of CM samples used in several previous studies (year 2010 to 2015) are presented in Table 2.2. Protein content in these samples varies between 40 and 44% (dry matter basis) while lysine content varies between 2.09 and 2.87% (dry matter basis). Differences in the AA content of CM from different crushing

plants have been documented by Li et al. (2015), Adewole et al. (2016), and Wang et al. (2017). Nutritive quality of canola seed can be affected by cultivar (Grami and LaCroix, 1997), seeding date and rate (Taylor and Smith, 1992; Kirkland and Johnson, 2000), growing location (Hamama et al., 2003) and maturity at harvest (Elias and Copeland, 2001). Also, excessive heat applied during processing can lead to denaturing of proteins and possibly reduces the content and availability of AA, particularly lysine (Eklund et al., 2015; Toghyani et al., 2015). Kasprzak et al. (2017) indicated that the effect of processing conditions on the nutritive quality of CM may override that of cultivation, environment or rapeseed variety. Although heat treatment is required to facilitate oil extraction and removal of solvent from the meal, high temperature or prolonged heating has negative effects on canola protein and AA (Newkirk et al., 2003a, b). High temperature may lead to the formation of Maillard reaction products which are indicative of protein damage (Almeida et al., 2014). Protein damage results in reduced availability of AA as was demonstrated for canola (Anderson-Herfarmann et al., 1992; Almeida et al., 2014) and soybean (Parsons et al., 1991; 1992) meals.

Table 2.2. Protein and amino acid contents of canola meal, % dry matter

	Woyengo et al., 2010	Trindade Neto et al., 2012	González-Vega and Stein, 2012	Sanjayan et al., 2014	Almeida et al., 2014	Mejicanos, 2015	CCC, 2015[1]
CP	41.8	43.9	43.5	41.5	40.5	40.4	41.7
Essential Amino Acids							
Arg	2.22	2.47	2.37	2.38	2.40	2.50	2.70
His	1.11	1.21	1.13	1.08	1.10	1.29	1.38
Ile	1.54	1.69	1.64	1.37	1.50	1.33	1.42
Leu	2.61	3.02	1.67	2.94	2.90	2.66	2.22
Lys	2.18	2.69	2.87	2.26	2.09	2.21	2.42
Met	0.69	0.94	2.11	0.85	0.77	0.75	0.79
Phe	1.51	1.76	1.60	1.61	1.65	1.53	1.66
Thr	1.52	1.88	1.61	1.83	1.76	1.77	1.75
Trp	NA[2]	0.39	0.49	NA[2]	0.55	NA[2]	0.55
Val	2.03	1.96	2.17	1.82	1.98	1.82	2.02
Non-Essential Amino Acids							
Ala	1.68	1.89	1.76	1.94	1.87	1.63	1.78
Asp	2.56	3.01	2.73	3.07	2.97	2.87	2.97
Cys	0.90	1.05	0.93	0.96	0.99	0.88	0.93
Glu	6.94	7.70	6.41	7.73	6.90	7.23	7.42
Gly	1.88	2.16	2.00	2.07	2.09	2.03	2.01
Pro	2.50	2.61	2.33	2.72	2.40	2.78	2.44
Ser	1.39	1.91	1.32	1.86	1.80	1.85	1.64
Tyr	0.91	1.11	1.08	1.08	NA[2]	1.02	1.02

[1]Canola Council of Canada, 2015

[2]data not available

2.4.2 Dietary Fiber

According to the Association of Official Analytical Chemists (2001), dietary fiber is defined as the fraction of the edible part of plants, or their extracts or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine but are either completely or partially fermented in the large intestine. It is also defined as the non-digested carbohydrates and lignin that are intrinsic and intact in plants (National Academy of Science, 2002). The most widely accepted definition of dietary fiber was given by Trowell et al. (1976) as "the remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man. It is composed of cellulose, hemicellulose, oligosaccharides, pectins, gums, waxes, and lignin. Dietary fiber is one of the factors potentially limiting the use of CM in diets for poultry and swine. The NDF and total dietary fiber contents in CM are over 3 times higher than those of SBM (Adewole et al., 2016; Sotak-Peper et al., 2017; Wang et al., 2017), which is the result of a large proportion of hulls relative to seed size. The hull represents 16.8 to 21.2% of the seed mass (Carre et al., 2015; 2016), but increases to about 30% of the meal weight after solvent extraction (Mejicanos et al., 2016). The hull is the main pool for non-starch polysaccharides (**NSP**) and lignin (Slominski et al., 2012). The fiber components include lignin and associated polyphenols, NSP, cell wall protein (glycoprotein) and minerals associated with the fiber fraction (Jia et al., 2012). Non-starch polysaccharides are structural carbohydrates that are primarily located in the cell wall (Jia et al., 2012). They are composed of rhamnose, fucose, glucose, xylose, mannose, galactose, and galacturonic and glucuronic acids. Dietary fiber can also be divided into water-soluble and water-insoluble fiber components (Bach Knudsen, 2014). Soluble fiber may increase digesta viscosity and prevent contact with endogenous enzymes thereby reducing nutrient digestibility (Jha and Berrocoso, 2015). It also contributes to the reduction in the dry matter of feces and therefore, contributing to

sanitary and management problems as a result of sticky droppings from chickens. Non-soluble fiber can accelerate digesta passage rate thereby resulting in reduced time for digestion and thus reducing nutrient utilization (Khajali and Slominski, 2012).

2.4.2.1 Methodologies for Measuring Dietary Fiber

The methods for determining dietary fiber include the gravimetric, enzymatic, and chemical methods. However, at the University of Manitoba, a gravimetric-GLC method for total dietary measurement has been developed.

2.4.2.1.1 Gravimetric methods

These involve weighing of the dietary fiber after the removal of other components. They estimate total dietary fiber and water-soluble or water-insoluble fiber (Asp et al., 1983). Also, included in the gravimetric methods is a procedure where cell walls (dietary fiber) are determined as the difference between organic matter content and the sum of starch, sucrose, oligosaccharides, crude protein and crude fat contents (Aman and Hessekman, 1984). The procedures include crude fiber determination, and the detergent methods. The crude fiber method (Henneberg and Stohmann, 1859) is the oldest method of fiber determination and is still used in the proximate analysis of feeds (Bach Knudsen, 2014). The limitation of the crude fiber method is that it only measures an incomplete and variable fraction of the fibrous carbohydrate components (Hindrichsen et al., 2006). The detergent methods which were developed by Van Soest and coworkers (Van Soest, 1963, 1984; Van Soest and Wine, 1967) for the analysis of fiber-rich feedstuffs (roughages) have later also been applied to concentrated feeds (Bach Knudsen, 2014) as they provide a more satisfactory alternative to better characterize the carbohydrates in the plant cell wall (Van Soest et al., 1991. The detergent methods include the neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) determination. Neutral detergent fiber is the amount of fiber in a sample that is not soluble

in a neutral detergent solution. The major cell wall components constitute most of the NDF, including cellulose, hemicellulose, lignin, and insoluble ash (Van Soest, 1992). Acid detergent fiber is defined as the amount of fiber in a sample that is insoluble in a weak acid. It consists mainly of cellulose, lignin, and insoluble ash which are the relatively undigested components of the feed and heat-damaged protein (Moller 2009). In the NDF method, the water-soluble NSP and water-insoluble pectic substances are lost in the solution. Also, starch and protein may contaminate the NDF residue (Bach Knudsen, 1997).

2.4.2.1.2 Enzymatic-gravimetric Association of Official Analytical Chemists procedures

This was officially adopted by Association of Official Analytical Chemists (AOAC) after two international collaborative studies (Prosky et al., 1984, 1985). This method was based on earlier methods which employ various enzymes to remove starch and protein followed by isolation of the remaining residue gravimetrically with or without precipitation (Li, 1991). The enzymatic-gravimetric methods are simple, robust, with no requirement for advanced equipment and are more suited for routine analyses. However, they do not provide detailed information on fiber components (Asp, 1995; Li, 1991). There is a risk of overestimating the fiber content if other components remain in the residue (Asp, 1995).

2.4.2.1.3 Enzymatic-chemical Englyst and Uppsala procedures

These methods were originally developed for foods, but have also been used in the analysis of feedstuffs (Theander et al., 1994; 1989; Bach Knudsen, 1997). The enzymatic-chemical method was developed by Southgate (1969) and later modified by Englyst and Cummings (1988). It includes the analysis of individual soluble and insoluble NSP constituents (Bach Knudsen 2007). It is based on the initial removal of sugars and starch by specific enzymes, the precipitation and

recovery of soluble NSP by 80% ethanol, and the swelling and hydrolysis of NSP by sulphuric acid (Hindrichsen et al., 2006). Subsequently, monomeric constituents are determined by GLC or HPLC for neutral sugars, colorimetry for acidic sugars, and by gravimetry for Klason lignin (Hindrichsen et al., 2006). The Englyst methods aim at measuring only NSP as dietary fiber. This is often claimed to be an estimate of plant cell wall polysaccharides, but it should be noted that the Englyst methods are unable to differentiate between the plat cell-wall located and the added polysaccharides (Asp 1996). In the Uppsala method, a gravimetric Klason lignin determination is performed and included in the dietary fiber estimate. It is conceptually similar to the enzymatic, gravimetric methods approved by the AOAC (Asp 1996).

2.4.2.1.3 University of Manitoba method

This is a combination of gravimetric and component analysis methods which enables the recovery of NSP, lignin, cell wall protein, resistant starch, and Maillard reaction products (total dietary fiber; Slominski et al., 1994, 2006; Figure 2.3). When the NDF method is used for fiber analysis of cereal grains or protein supplements of monogastric diets, a significant underestimation of NSP and thus total dietary fiber content occurs due to the high solubility of NSP in the NDF solution and therefore losses of NSP on NDF analysis (Adewole et al., 2016). Therefore, total dietary fibre is determined by a combination of NDF and neutral detergent-soluble NSP measurements and is calculated as the sum of NDF and NDF-soluble NSP. Neutral detergent fiber-soluble NSP are calculated as total sample NSP minus NSP present in the NDF residue. Neutral detergent insoluble crude protein (**NDICP**) represents the amount of crude protein present in the NDF residue. The value for lignin with associated polyphenols is calculated by difference between the total fiber and NDICP + NSP contents. This method has been used in many studies (Slominski et al., 2012;

Sanjayan et al., 2014; Radfar et al., 2017; Adewole et al., 2016; 2017a, b). Presented in Table 2.3 are the components measured by the various procedures.

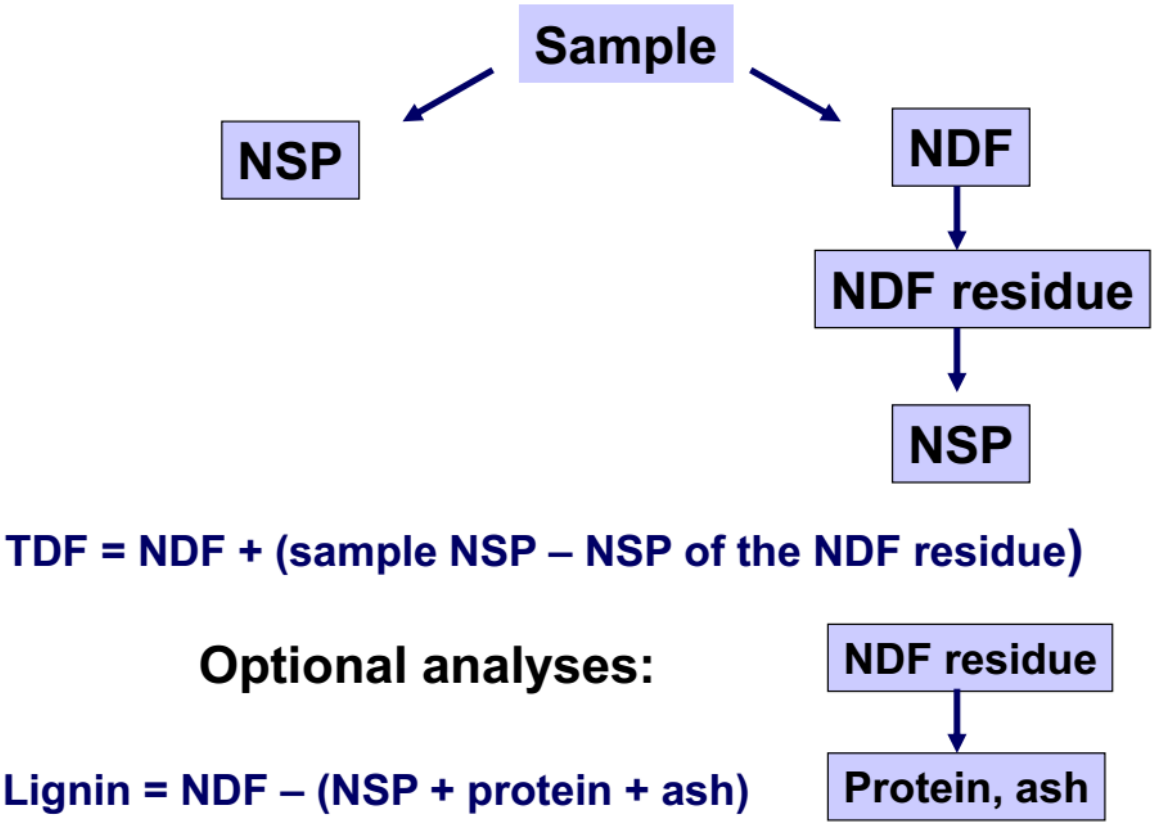

Figure 2.3. Illustration of the University of Manitoba method of dietary fiber determination. Adapted from Slominski et al., 1994, 2006. NSP = non-starch polysaccharides; NDF = neutral detergent fiber; TDF = total dietary fiber.

Table 2.3. Methods for total dietary fiber determination[1]

Component measured	AOAC gravimetric	Uppsala GLC-gravimetric	UK GLC[2]	U of M gravimetric-GLC
Non-starch polysaccharides	X	X	X	X
Lignin	X	X		X
Glycoprotein				X
Resistant starch	X		X	X
Maillard products		X		X

[1]X means component is measured by method
[2]Englyst et al. (1992; 1994)

2.4.3 Fat

Processing conditions affect the amount of oil extracted from canola seed, hence the residual oil within the finished meal. There is higher residual oil in meal resulting from expeller processing than from the solvent-extraction process. Solvent extraction provides a more efficient oil extraction process which results in more consistent finished meal oil content (Spragg and Mailer, 2007). van Barneveld (1998) and Spragg and Mailer (2007) suggested that expeller processed CM samples should be tested for crude protein and fat prior to use, while solvent extracted meals are more consistent for fat content and only protein analysis is required. However, the practice of adding back gums and soapstocks from oil refinery to finished meal in some processing plants results in an increase in oil content. This process also results in the reduction of dustiness in CM. Addition of gums and soapstocks at the rate of 0.5 to 2.0% is common in the Canadian canola industry (Hickling, 2001). In Table 2.4, the fat content of solvent-extracted CM samples from selected studies are presented. Fat content of samples in these studies varies from 1.8 to 5.5 % (dry matter basis).

Table 2.4. Fat content of solvent extracted canola meal, % dry matter

Fat content, % DM	Reference
3.1	Slominski et al., 1994
3.3-4.3	Fan et al., 1996
1.6	Mustafa et al., 2000
3.3	Thacker and Newkirk, 2005
3.9	Canola Council of Canada, 2009
5.5	Woyengo et al., 2010
3.9	Landero et al., 2011
1.8	Slominski et al., 2012
2.9	Sanjayan et al., 2014
4.1	Almeida et al., 2014
4.2	Mejicanos, 2015
4.2	Chen et al., 2015

2.4.4 Glucosinolates

Glucosinolates are a large group of sulphur-containing secondary plant metabolites, which occur in all the economically important cruciferous plants (Khajali and Slominski, 2012). Prior to the development of canola from rapeseed, the presence of glucosinolates was the major factor limiting the use of rapeseed meal in monogastric feed (Bell, 1984). The glucosinolate components of CM samples from selected studies (from 1990 to 2015) are presented in Table 2.5.

Glucosinolates in canola are composed of two main types: aliphatic and indole glucosinolates. Alipahtic glucosinolates comprise about 83% of the glucosinolates in CM (Adewole et al., 2016). They consist of gluconapin (3-Butenyl), glucobrassicanapin (4-pentenyl), progoitrin (2-hydroxy-3-butenyl), and gluconapoleiferin (2-hydroxyl-4-pentenyl). Indole glucosinolates account for the remaining 17% of glucosinolates in CM (Adewole et al., 2016). They consist of glucobrassicin (3-indolylmethyl) and 4-hydroxyglutaminecobrassicin (4-hydroxy-3-indolymethyl). Indole glucosinolates have been shown to be susceptible to thermal degradation (Campbell and Slominski, 1990). Thiocyanate ion and indoleacetonitriles have been reported as major thermal

degradation products of indole glucosinolates (Slominski and Campbell, 1987; 1989; Mosenthin et al., 2016).

It is generally believed that intact glucosinolates, per se, are non-toxic. However, they are always accompanied by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.2.1) in the seed or in the microflora of animals. In the presence of moisture and following rupture of the seed, this enzyme hydrolyses glucosinolates to yield unstable aglucones which then break down to yield a range of products, including isothiocyanates, goitrin, nitriles, and thiocyanates, that interfere with the function of thyroid gland and adversely affect growth performance in monogastric animals (Fenwick, 1982; McCurdy, 1990; Tripathi and Mishra, 2007; Khajali and Slominski, 2012). In addition to myrosinase catalyzed reaction, thermal degradation during canola seed processing and microbial fermentation in the lower gut of monogastric animals are two additional means of glucosinolate hydrolysis. High glucosinolate levels have been shown to increase mortality in poultry, as well as lower egg production and weight, and are fatal to pigs, while ruminants are comparatively more tolerant (Tripathi and Mishra, 2007). Pigs are more susceptible to the detrimental effects of glucosinolates than are poultry (Thacker and Newkirk, 2005). Newkirk and Classen (2002) fed broilers non-toasted CM containing diets which would have contained glucosinolates levels more than 3 µmol/g and observed improved growth performance compared with a toasted CM diets containing lower glucosinolate levels, whereas, pig performance either declined or did not differ from that of pigs fed toasted CM (Thacker and Newkirk, 2005). Species differences in the toxicity of glucosinolates were also reported by Bell (1993).

An average total glucosinolate content of 5.5 µmol/g, DM for Canadian CM was reported by Khajali and Slominski (2012) which was lower than most of the values presented in Table 2.5. The average value reported by Khajali and Slominski, (2012) was from more recent studies in their

laboratory. By comparison, traditional rapeseed meal contains 120-150 µmol/g of total glucosinolates (Canola Council of Canada, 2015). The low glucosinolate content of canola, compared to previous cultivars of rapeseed, constitutes the major improvement in meal quality achieved by plant breeders (Friedt and Luhs, 1999; Weber et al., 2001; Newkirk, 2011). Glucosinolates are usually measured on a molecular basis (µmol/g) rather than on a weight (mg/kg) basis. The reason for this is that glucosinolates have significantly different molecular weights, depending on the size of their aliphatic side chain (Figure 3). Since the negative effect on the animal is at the molecular level, the most accurate estimate of this effect must be gauged by expressing glucosinolate concentration on a molecular basis (Canola Council of Canada, 2015).

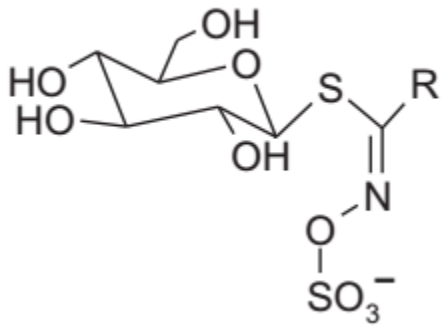

Figure 2.4. General Structure of Glucosinolates

R denotes the variable side chain from amino acids. R = 3-butenyl- (gluconapin); R = 4-pentenyl- (glucobrassicanapin); R = 2-hydrxy-3-butenyl- (progoitrin); R = 2-hydroxy-4-pentenyl- (gluconapoleiferin); R = 3-indlylmethyl- (glucobrassicin); R = 4-hydroxy-3-indolymethyl- (4-hydroxyglucobrassicin).

Table 2.5. Glucosinolates components of solvent extracted canola meal, µmol/g

Glucosinolate	1	2	3	4	5	6
Gluconapin	3.32-6.13	2.30	3.40	1.94	5.80	2.10
Glucobrassicanapin	0.45-3.04	0.50	0.67	0.34	1.50	0.30
Progoitrin	6.96-12.40	4.60	6.28	3.88	11.60	5.10
Gluconapoleiferin	0.16-1.28	0.10	0.20	0.04	0.20	0.20
Glucobrassicin	1.58-3.35	0.30	0.58	0.78	0.10	0.40
4-hydroxyglucobrassicin	0.51-1.04	3.50	4.20	1.88	7.10	1.20
Total Glucosinolates	23.8-35.1	11.4	2.56	9.72	27.1	9.2

1 = Campbell and Slominski, 1990 (Oil-free meal; 2 = Slominski et al., 1999; 3 = Newkirk et al., 2003a (as received); 4 = Newkirk and Classen, 2002 (dry matter basis); 5 = Slominski et al., 2012 (dry matter basis); 6 = Mejicanos, 2015 (as is basis).

2.4.5 Phytic Acid

Phytate or phytic acid (also known as myo-inositol-1, 2,3,4,5,6-hexakis (dihydrogen phosphate)) contributes a major proportion of the P found in plant-derived feedstuffs. Phytic acid is the primary storage form of about 80-87% of the P and probably inositol found in almost all grains or seeds. It has 12 protons and of these 6 dissociate at acidic pH, 3 at neutral pH, and the remaining 3 at basic pH, meaning that phytic acid is negatively charged at a wide range of pH conditions (figure 4; Maenz, 2001). In plant tissues phytic acid occurs as phytate (i.e., a mixed salt of cations, mainly potassium and magnesium, and to a lesser extent calcium, iron and zinc), and it is located within protein bodies (Ockenden et al., 2004; Joyce et al., 2005; Lin et al., 2005). It is well recognised that phytate reduces the availability of P and other minerals such as zinc, magnesium, and calcium as well as proteins (Cabahug et al., 1999). At neutral pH, the phosphate groups in phytic acid have either one or two negatively charged oxygen atoms, hence cations can chelate strongly between two phosphate groups or weakly with a single phosphate group (Khajali and Slominski, 2012). The ability of pigs and poultry to digest phytic acid-bound phosphorus is very low, therefore, inorganic phosphorus sources, which are expensive, are added in feeds to meet phosphorus requirements of the poultry, leading to increased cost of feeding (Selle and Ravindran, 2007, 2008). Phytic acid can reduce animal performance by reducing nutrient digestibility through binding to nutrients, digestive enzymes or both which, in turn, would result in increased endogenous losses of AA (Ravindran et al., 2000). Phytate can also change sodium partitioning and thereby may influence the capability of the gut for Na-dependent transport of nutrients including glucose and peptides (Selle et al., 2000). The phytic acid content of CM is approximately 0.64 %DM as reported by Khajali and Slominski (2012).

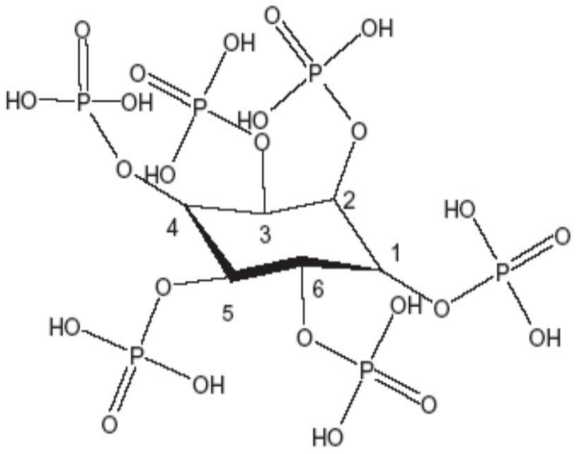

Figure 2.5. Structure of phytic acid

2.4.6 Tannins

Tannins are a family of complex phenolic polymers having molecular weights in the range of 500 to 3,000 Da (Khajali and Slominski, 2012). They are found in two groups, the hydrolysable and condensed tannins. The presence of condensed tannins in rapeseed hulls was first reported by Bate-Smith and Ribereau-Gayon (1959). Tannins are responsible for the dark colour, bitter taste, and stringency in canola products (Naczk and Shahidi, 1991). The condensed tannins are mostly found in seed coat with the brown hulls containing more than the yellow hulls (Durkee, 1971; Theander et al., 1977). The seed coat pigmentation of yellow-seeded canola is due to oxidized proanthocyanidins (condensed tannins) derived from phenylpropanoids and malonyl CoA (Akhov et al., 2009). Tannins form complexes with proteins and proteolytic enzymes in the gastrointestinal tract, thereby affecting protein digestion (Naczk and Shahidi, 1991). The removal of tannins from CM significantly increased its metabolizable energy, probably due to increased activities of endogenous enzymes. On the other hand, addition of tannic acid at 1.5 % to broiler chicken diets resulted in a severe growth depression (Leslie et al., 1976). Mansoori and Acamovic, (2007) reported that endogenous AA losses were significantly increased following 10 g per kg dietary

tannic acid addition with methionine, histidine, and lysine mostly affected and threonine, cysteine, and valine least affected. Astringency and bitter taste are additional negative effects associated with high tannin contents in CM (Simbaya, 1995). Naczk et al. (2000) reported that the total amount of tannins in rapeseed/canola hulls ranges from 1.9 to 6.2 g per 100 g of oil-free hulls. Insoluble tannins predominate in canola/rapeseed hulls and comprise of 70 to 96 % of the total tannins present. Environmental growing conditions can affect the content of tannins in canola (Naczk et al., 1998). In weaned pigs, research on the effect of tannins on growth performance has demonstrated some improvements in feed efficiency, which indicates that tannins may have beneficial and not just anti-nutritive effects (Biagi et al., 2010).

2.4.7 Sinapine

Sinapine, a choline ester of sinapic acid, accounts for approximately 1% of CM and has been implicated with the production of a "fishy" taint in brown-shelled eggs (Butler et al., 1982). It has been found that the taint is due to the presence of trimethylamine (**TMA**) in the yolk, which results from a genetic defect among laying hens of Rhode Island Red breed. This genetic imperfection obstructs the synthesis of TMA oxidase which is responsible for the conversion of TMA to the odourless N-oxide in the liver or kidney of the hens (Khajali and Slominski, 2012). Trimethylamine may be produced from either sinapine or directly from choline by the action of microorganisms in the gastrointestinal tract. The TMA oxidase deficiency is not linked to the shell color, as has sometimes been supposed, given that it was not present in a New Hampshire Red hybrid which lays brown eggs (Khajali and Slominski, 2012). Progress has been made in identifying the genetic defect that leads to the production of tainted eggs (Honkatukia et al., 2005) and the elimination of this defect through breeding could be soon achieved (Canola Council of Canada, 2009). Brand et al. (2007) reported differences in the sinapine content of different cultivars of canola, with a mean value of 9.95 mg/g and values ranging from 7.72 to 11.53 mg per

g of seed. Research in Germany is in progress to reduce the levels of sinapine in rapeseed/canola by developing low-sinapine varieties with yellow-seeded and low-fiber characteristics (Norddeutsche et al., 2010).

2.5 AMINO ACID DIGESTIBILITY IN CANOLA MEAL FOR SWINE

Knowledge on the digestibility of AA is critical for precise feed formulation and sustainable use of feed ingredients (Ravindran et al., 2017). The digestibility of most AA in CM is lower than that in SBM. Therefore, when CM replaces SBM in the diet, the overall levels of digestible AA, especially lysine and threonine, will decrease if the diet is formulated based on total AA levels (Canola Council of Canada, 2015). In some earlier feeding trials with CM and SBM in which diets were formulated to the same levels of CP, total essential AA and energy, a lower growth rate compared to SBM–fed pigs was observed (Baidoo, et al., 1987; Bell, et al., 1988; Bell, et al., 1991; McIntosh, et al., 1986). This happened because levels of digestible lysine decreased as CM inclusion level in the diets increased. Presently, swine diets are routinely formulated based on digestible as opposed to total AA. Feeding trials with CM in starter, grower and finisher pigs, in which the diets were formulated based on digestible lysine (Hickling, 1994; Hickling, 1996; King et al., 2001; Mateo et al., 1998; Mullan et al., 2000; Patience et al., 1996; Raj et al., 2000; Robertson et al., 2000; Roth-Maier, 2004; Sanjayan et al., 2014), resulted in a growth rate equivalent to what is typically found with SBM as the primary protein source, even at very high inclusion levels of CM.

Differences in AA digestibility in CM from various sources have been documented (Adewole et al., 2017). Indeed, de Lange et al. (1998) have reported that AA digestibility in CM varied among processing plants. The SID of protein and AA in CM samples from selected swine studies presented in Table 2.6. With increasing dietary inclusion of CM in swine diets, variation in

nutritive quality of CM, e.g., glucosinolates content, and protein quality is a concern (Maison and Stein, 2014; Wang et al., 2017). In the study of Wang et al. (2017) using SID AA values tabulated in NRC (2012), growth performance differed among CM sources with varying nutritional quality indicating that quality differences among CM samples affect growth performance of pigs when diets are formulated with Table values. Therefore, for accurate feed formulation, it is important to determine the AA digestibility of CM and to have a good knowledge of the variation in its nutritional value (Li et al., 2015).

Table 2.6. Standardized ileal digestibility (%) of amino acids in solvent extracted canola meal fed to growing pigs

	Woyengo et al., 2010	Trinidade Neto et al., 2012	Gonzalez-Vega and Stein, 2012	Sanjayan et al., 2014
N or CP	76.5[1]	78.0[1]	73.7[2]	79.8[2]
Essential Amino Acids				
Arg	86.2	91.0	88.0	90.3
His	78.1	87.4	79.6	87.1
Ile	78.1	82.7	76.4	79.7
Leu	79.0	82.5	78.1	80.3
Lys	66.6	81.7	67.7	78.9
Met	84.1	86.8	83.9	84.2
Phe	90.4	77.0	78.0	70.8
Thr	72.1	78.8	70.5	77.1
Trp	NR	NR	85.8	NR
Val	76.7	78.0	74.1	78.5
Non-Essential Amino Acids				
Ala	76.3	82.3	75.9	78.2
Asp	75.0	79.5	70.1	77.8
Cys	79.3	83.9	73.0	79.8
Glu	86.9	90.9	83.8	88.3
Gly	82.2	79.0	75.8	76.5
Pro	101.9	84.6	115.0	Nd
Ser	76.7	86.1	72.0	80.7
Tyr	96.3	78.0	75.7	78.7

[1]Standaridized ileal digestibility of nitrogen; [2]Standaridized ileal digestibility of crude protein; NR = not reported

2.6 AMINO ACID DIGESTIBILITY IN CANOLA MEAL FOR BROILER CHICKENS

The concept of digestible AA values is increasingly being accepted as a useful tool in poultry feed formulations (Ravindran et al., 2017). In the gastrointestinal tract of poultry, major AA absorption takes place in the small intestine, with a significant alteration of AA composition associated with microorganisms in the hindgut (Ravindran et al., 1999). Because of these factors, it is now accepted that ileal digestibility is a more reliable method for estimating AA utilization than total tract digestibility (Kong and Adeola, 2011). The apparent AA digestibility values from ileal digesta do not distinguish between AA of dietary or endogenous origins (Iyayi and Adeola, 2014). Apparent ileal AA digestibility values are not corrected for endogenous AA losses and are influenced by level of feed intake and dietary protein concentrations (Fan et al., 1994). The apparent ileal AA digestibility values of some commonly used feed ingredients for poultry have been reported (Ravindran et al., 2005). The need to correct apparent ileal digestibility values for endogenous AA losses has also been pointed out by several authors (Lemme et al., 2004; Rodehutscord et al., 2004; Adedokun et al., 2007, 2008). The standardized ileal digestible AA contents of a feed ingredient are useful for formulating balanced diets for broiler chickens because they give more accurate estimates of AA that are available in feed ingredients (Iyayi and Adeola, 2014).

Amino acids are important parts of the structure of cell walls of vegetable ingredients, where they are embedded in complex matrices with carbohydrates (Parker et al., 1999). Poultry do not express endogenous enzymes capable of digesting NSP (Bedford, 2006; Jia et al., 2012). Non-starch polysaccharides increase digesta viscosity and interfere with the contact between intestinal enzymes and dietary AA in the gastrointestinal tract, thereby decreasing their availability (Choct and Annison, 1992; Moftakharzadeh et al., 2017). The processing of CM might entrap or bind the fiber with protein, and consequently reducing its digestibility (Mateos et al., 2002; Garcia et al.,

2008). Glycoproteins, which represent the structural protein of the cell walls and Maillard reaction products, often referred to as neutral detergent insoluble nitrogen, are also poorly digested by poultry (Jia et al., 2012). The digestibility of AA in CM has been reported as lower than that of soybean meal (Adedokun et al., 2008) and this has been associated with components such as enzyme inhibitors, phenolic compounds, glucosinolates and dietary fiber (Rayner and Fox, 1976; Bell, 1993). The concentration of components in CM might differ considerably depending on the seed cultivars, growing conditions, harvesting time, seed storage conditions, seed drying temperature and further processing such as de-hulling, heat treatment, oil removal method, and pelleting (Liu et al., 2014). The variations in the nutrient contents of CM from various sources which are not only reflected in the compositions of proteins and AA, but also in the levels of NSP and other anti-nutritional factors (Choct and Annison, 1992) can cause variations in the standardized ileal digestibility of AA. Previous work has shown that the desolventization/toasting stage of pre-press solvent extraction of canola reduces the content and digestibility of AA, particularly that of lysine in poultry (Newkirk and Classen, 1999; Newkirk et al., 2000). The SID of protein and AA in CM samples from selected broiler chicken studies are presented in Table 2.7.

Table 2.7. Standardized ileal digestibility (%) of amino acids in solvent-extracted canola meal fed to broiler chickens

	Adedokun et al., 2008		Woyengo et al., 2010[3]	Kong and Adeola, 2011[4]
	NFD[1]	HDP[2]		
N or CP	NR	NR	76.2	NR
Essential Amino Acids				
Arg	85.7	86.7	79.5	80.4
His	82.5	84.5	84.0	78.6
Ile	78.0	83.5	77.9	69.2
Leu	80.2	82.0	75.6	72.0
Lys	79.2	82.0	77.3	68.0
Met	85.3	89.2	86.4	78.1
Phe	80.2	81.3	76.4	74.2
Thr	73.8	76.8	75.7	63.0
Trp	NR	90.3	NR	84.3
Val	77.5	81.2	78.9	68.7
Non-Essential Amino Acids				
Ala	80.0	82.3	77.0	NR
Asp	76.8	80.0	69.1	NR
Cys	78.0	79.3	68.1	NR
Glu	87.2	91.5	83.4	NR
Gly	78.8	80.2	79.2	NR
Pro	77.3	80.0	77.0	NR
Ser	75.8	83.3	NR	NR
Tyr	78.3	79.8	74.7	NR

[1]Nitrogen-free diet; [2]Highly digestible protein diet (contained 10% casein); [3]Substitution method and low protein casein-cornstarch based diet was fed to estimate basal endogenous AA losses; [4]Regression method; NR = not reported

2.7 PREDICTION OF STANDARDIZED ILEAL DIGESTIBLE AMINO ACID CONTENT FOR PIGS AND BROILER CHICKENS

The increasing cost of feed ingredients necessitates managing feeding as closely as possible to the requirements of animals (Bastianelli et al., 2015). Therefore, the exact knowledge of the actual nutritional value of any feed ingredient is indispensable for efficient animal production (Swiech, 2017). In vivo measurements of AA digestibility are expensive, therefore, feed ingredients can be characterized by chemical analysis of samples and by the application of prediction equations that relate the chemical composition to the nutritional properties for a category of animals (Le Goff and Noblet, 2001; Carre et al., 2014; Bastianelli et al., 2015). Swiech (2017) reported that there is a need for simple, inexpensive, rapid, and reproducible alternative methods for routine assessment of nutrient digestibility instead of direct measurements in pigs which is also applicable to broiler chickens. Ileal digestibility of protein and AA in pig feed ingredients can be estimated using alternative methods including prediction equations based on chemical composition (Swiech, 2017). Fevrier et al. (2001) compared the SID of AA and chemical composition of two oilseed meals and reported that the contents of ash, fat, N, and NDICP were useful as predictors to estimate SID of Lys, Thr, and Trp only in cotton seed meal, but not in palm kernel meals for growing pigs. Urriola et al. (2013) attempted the prediction of the concentration of standardized ileal digestible AA in distillers dried grains with solubles and reported that the use of acid detergent insoluble crude protein, KOH soluble protein, NDF, ADF, hemicellulose, CP, particle size, and colour measurements poorly predicted the concentration of standardized ileal digestible Lys, Met, Thr, and Trp for pigs. However, optical density data, along with CP concentration, accurately predicted standardized ileal digestible Lys, Thr, and Trp. Li et al. (2014) developed several equations to predict the AID and SID of N, Lys, Met, and Thr of peanut meals following a stepwise regression

procedure and reported that the SID of Lys for pigs could be predicted from the analyzed contents of NDF and Lys. Cozannet et al. (2010) reported that lysine content per unit CP could be an acceptable predictor of standardized ileal digestible Lys in wheat distillers dried grains with solubles for pigs. Kim et al. (2012) and Almeida et al. (2014) reported that the content of reactive Lys was a good predictor for the standardized ileal digestbile Lys content in distillers dried grains with solubles and CM. With the use of prediction equations, there is no need to sacrifice or to conduct ileal cannulation surgery for animals for feed evaluations (Swiech, 2017).

2.8 APPARENT METABOLIZABLE ENERGY CONTENT OF CANOLA MEAL

Canola meal is well known for having lower metabolizable energy content than that of SBM (Khajali and Slominski, 2012). Both protein supplements contain similar amounts of simple sugars (0.6%), starch (2%), and relatively high amounts of sucrose (6%; Khajali and Slominski, 2012). Soybean meal is high in oligosaccharides (5.6 vs. 2.0%), which when converted to short-chain fatty acids by microbial population of the lower gut may contribute to the overall energy content of this ingredient (Khajali and Slominski, 2012). However, CM is significantly higher in fat content, which should minimize the difference in metabolizable energy (**ME**) content between the 2 ingredients. The main reason for the lower ME of CM may be explained by the fact that the high dietary fiber content may accelerate the digesta passage rate, which in turn, may result in reduced time for digestion and thus reduced nutrient utilization (Khajali and Slominki, 2012). Fiber is poorly digested by poultry and reduces energy utilization of ingredients by accelerating the digesta passage rate, which in turn, may result in reduced time for digestion and thus reduced nutrient utilization (Khakali and Slominski, 2012). For poultry, apparent metabolizable energy (**AME**; defined as gross energy minus losses of energy in faeces, urine and gaseous products) is typically used to express the available-energy content of feed ingredients and complete diets (Sauvant et al.,

2004). The AME or true metabolizable energy (**TME**) values of ingredients are usually corrected for N retention to give nitrogen-corrected AME (**AME_n**) or TME (**TME_n**). This is done to convert all data to a basis of N equilibrium for comparative purposes (Lopez and Leeson, 2008). Retained N in the body yields energy-containing compounds with metabolites that are voided in the urine; therefore, AME values are corrected to zero N balance to adjust for the effect of differences in protein retention across birds in any assay in order to reduce the variability in estimates of AME (Leeson et al., 1977; Lopez and Leeson, 2007). When the AME value of CM was corrected for N retention, the resulting AME_n value decreased by approximately 7% (Toghyani et al., 2014), whereas that of full fat canola seed decreased by 5.7% (Toghyani et al., 2017). Nitrogen correction has also been used to reduce the variability of estimates of ME of ingredients varying in protein content (Lopez et al., 1977). In the study of Zhang and Adeola (2017), N correction resulted in a 15, 19, 10, and 4% reduction in the AME contents of CM, cottonseed meal, peanut flour meal, and bakery meal, respectively. Obviously, there was more reduction in ME of protein ingredients than that of bakery meal (energy ingredient) after N correction, because more N was retained in birds fed CM, cottonseed meal, and peanut flour meal than those fed bakery meal (Zhang and Adeola, 2017). Also, N correction allows strain comparison and accommodates any age-related effects (Lopez and Leeson, 2008). For modern broilers, the use of N correction will penalize the biological characteristics of depositing ME as protein during growth, especially with high-protein ingredients such as CM (Lopez and Leeson, 2008). In avian species, ME is corrected for N balance by subtracting 8.22 kcal of ME per g of N retention from the measured ME values (Hill and Anderson, 1958). Presented in Table 2.8 are the nitrogen corrected AME_n values of CM samples from selected studies.

Table 2.8. Nitrogen-corrected apparent metabolizable energy (AME_n) of solvent extracted canola meal for broiler chickens

AME_n of CM	Reference
1,801 kcal/kg	Woyengo et al., 2010
1,961 kcal/kg DM	Jia et al., 2012
1,875 kcal/kg	Radfar et al., 2017

2.9 MAILLARD REACTION IN CM

The Maillard reaction is the non-enzymatic browning reaction that occurs in foods, feed ingredients and even in living beings (Delgado-Andrade, 2013). It was first reported in 1912 by Louis-Camille Maillard, who described that a yellow-brown color developed after a mild heating sugars and amino acids in water. The Maillard reaction involves binding of amino groups of AA or peptides to the carbonyl group of reducing sugars such as glucose or lactose (Maillard 1912). The Maillard reaction is exceptionally widespread and occurs in foodstuffs, particularly during processing at high temperatures (roasting, baking, extruding) or during storage for prolonged periods (Nursten, 2005). It is a series of reactions that starts with the condensation of an amino group of an AA with the carbonyl group of a reducing sugar (Figure 2.5; Mauron, 1981).

2.9.1 Stages in Maillard Reaction

The stages of the Maillard reaction are illustrated in Figure 2.5 and consist of the initial, intermediate and final stages.

2.9.1.1 Initial stages

The initial stage of the Maillard reaction is characterized by the formation of glycosylamine, which is later converted to Amadori compounds in a series of reactions (Gerrard, 2002). Basically, the reaction begins with a condensation between a reducing sugar (e.g. glucose) and a compound

having a free amino group of an AA or mainly the epsilon amino group of lysine in proteins (Purlis, 2010). After dehydration of the condensation products (N-substituted glycosylamine), Schiff bases are formed which in turn may undergo successive changes (Amadori rearrangements) yielding the cyclic glycosylamine (Gerrald, 2002). Following the protonation of the ring oxygen atom, glycosylamine is converted to Amadori compounds. Each of the steps up to the formation of Amadori compounds is reversible depending on the conditions (pH, temperature, and rate of mutarotation) in which the reactions occur (Yaylayan and Huyghues-Despointes, 1994).

2.9.1.2 Intermediate Stages

The intermediate stage which is also called the Amadori rearrangement is not reversible (Nursten, 2005). It involves 3 major reactions which are: sugar dehydration, sugar fragmentation and amino acid degradation (Nurstuen, 2005).

Sugar dehydration occurs in 2 ways: under acidic conditions, there is formation of furfurals whereas under neutral or alkaline conditions and/or in the presence of amines, there is production of reductones. Reductones are products formed from sugars by the loss of only 2 molecules of water while furfurals are products formed from sugars by the loss of 3 molecules of water (Nursten, 2005). Depending on the Amadori compound, sugar fragmentation can form various end products by 2 mechanisms: retroaldolisation and oxidative fission (Nursten, 2005). Some of the products from sugar fragmentation include glycolaldehyde, acetol, ethanol, pyruvic acid, lactic acid, formic acid, and formaldehyde among others (Nursten, 2005; Almeida, 2013). Amino acid degradation (also called Strecker degradation) is a reaction in which alpha AA are oxidized to the corresponding aldehyde, giving carbon dioxide and ammonia (Nursten, 2005).

2.9.1.3 Final Stage

The final stage of Maillard reaction involves aldol condensations and an aldehyde-amine condensation reaction leading to the formation of polymeric compounds called melanoidins (Nursten, 2005) which are partially digested and absorbed in the intestines (Faist and Erbesdobler, 2001). Melanoidins are compounds generated in the late stages of the Maillard reaction from reducing sugars and proteins or AA during food processing and preservation (Wang et al., 2011) They are responsible for the formation of brown pigments in foods such as coffee, cocoa, bread, malt, and honey (Lindenmeier et al., 2002).

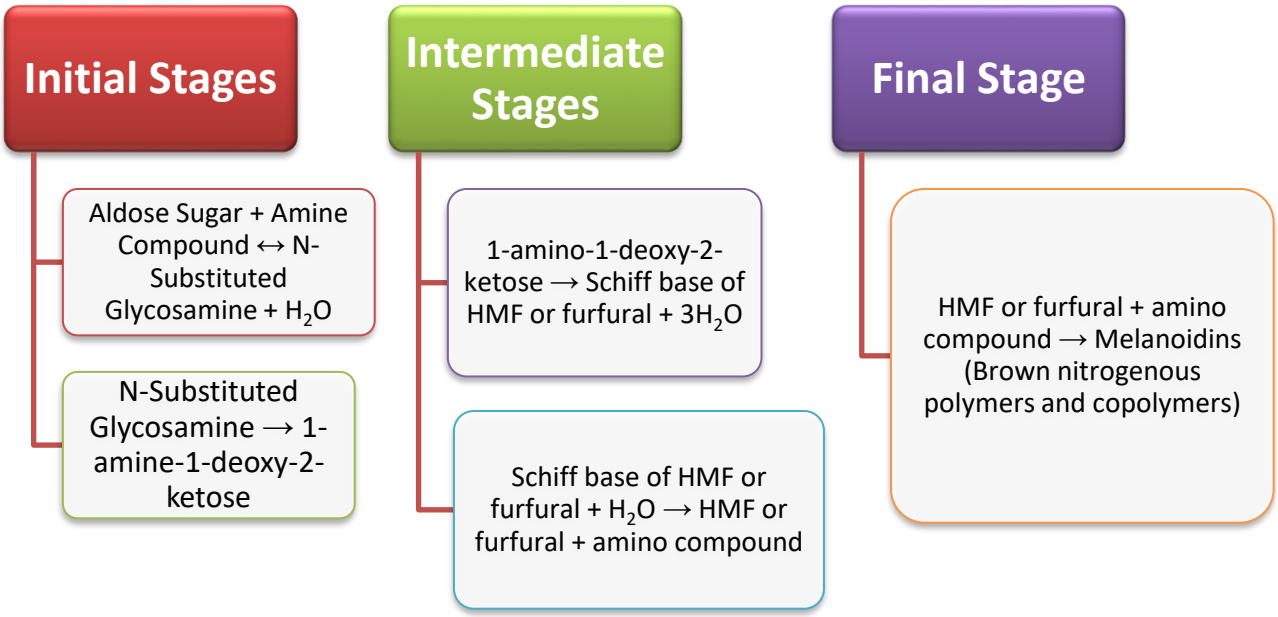

Figure 2.6. Overview of Maillard reactions, adapted from Purlis (2010)

2.9.2 Factors that may affect the kinetics of the Maillard reaction

2.9.2.1 Temperature

The Maillard reaction is a continuously ongoing reaction in that it takes place even at room temperature (Mavromichalls, 2001). These reactions happen *in vivo* at approximately 36°C (Ledi and Schleicher, 1990). At room temperature, Maillard reactions proceed very slowly. However, they increase exponentially with increase in temperature (Adrian, 1974). During the solvent extraction process, CM is heated at several stages of the process, with the desolventization/toasting utilizing the highest temperatures of 110 to 115°C (Unger, 2011). Similarly, during the production of DDGS, corn undergoes several steps under different temperatures (32-100°C) that may lead to the formation of Maillard reaction products (Pahm et al., 2008).

2.9.2.2 pH

The pH is an important factor affecting the rate of the Maillard reactions and the profile of reaction products that predominate (Newkirk, 2002). Results from many experiments have indicated that increasing pH favours the Maillard reaction (Ajandouz and Puigserver, 1999; Nie et al., 2013). The rate of reactions increases approximately linearly at pH increased from 3 to 10 (Adrian, 1974). The pH is also important in the conditions which cause sucrose to be hydrolysed into reducing sugars that react in the Maillard reactions. Sucrose is most heat labile at acidic pH so the conditions during canola processing may be well suited to sucrose hydrolysis and the subsequent Maillard reactions (Lindberg et al., 1975). The Maillard reaction itself also affects the pH. When heating glucose-Lys and glucose-Met model systems, the pH decreased as heating time increased, which was likely the result of basic amino group disappearance at the early stages of reaction (Delgado-Andrade et al., 2004).

2.9.2.3 Moisture

Water activity is an important factor for the Maillard reactions as Maillard reactions do not occur in the anhydrous state (Ames et al., 2001). Water activity is defined as the partial vapour pressure of water in a substance divided by the standard state partial vapour pressure of water. As water activity decreased, rate of Maillard reactions product formation increased (van Boekel, 2001). High levels of water inhibit the dehydration steps such as the conversion of l-amino-l-deoxy-2-ketose to furfural or reductones (Adrian, 1974). Thus, food processes that involve heating moist products at high temperatures and low humidity, such as baking, tend to promote higher levels of Maillard reactions than high humidity cooking methods such as pressure cooking (Adrian, 1974). In a mixture of casein and glucose, Maillard reactions occur optimally between 15 and 18% moisture (Lea and Hannan, 1949b). Coincidentally, the moisture content during the desolventization/toasting of CM is approximately 14 to 18%, suggesting that the processing conditions may be ideal for Maillard reactions (Newkirk, 2002).

2.9.2.4 Type of substrate

The source of the amino group for Maillard reactions is either a terminal amino group or the ε-amino group of lysine, with the reactions with lysine being most common (Adrian, 1974). Canola meal contains only tiny amounts of free monosaccharides that could be involved directly in Maillard reactions. However, it contains relatively high levels of sucrose (6.9 - 10.4%; Slominski et al., 1994). Sucrose is not a reducing sugar *per se*, but upon hydrolysis, yields a molecule of glucose and fructose which can participate in Maillard reactions. Jensen et al. (l995) showed that the sucrose content decreased with toasting, supporting the hypothesis that sucrose could indirectly be a source of carbonyl groups for the Maillard reactions during processing. Sucrose is susceptible to hydrolysis during heating especially in the presence of lysine. However, the rate of the Maillard

reactions could conceivably be limited by the rate of sucrose degradation. This has been demonstrated in experiments with albumin. After heating for one hour at 100°C at pH 5 and 14% moisture, albumin, albumin + sucrose and the albumin + glucose mixtures contained 97, 85 and 13% of the level of reactive lysine in untreated albumin, respectively (Hurrell and Carpenter, 1977).

2.9.3 Effect of Maillard Reaction on the Availability of Essential AA

Under conditions of high temperature and humidity, any compound with a free amino group is vulnerable to the Maillard reaction, and this includes free AA (Johnson *et al.*, 1977; Robbins et al., 1980), epsilon amino groups of protein-bound lysine, and free amino groups of thiamine, folacin, and gossypol (Baker, 1995). Lysine is the most susceptible AA in intact proteins because it has a free amino group at the epsilon carbon unit that is readily available to react with reducing sugars (Mavromichalls, 2001). Because destruction of lysine is often 5 to 15 times greater than that occurring with other AA (e.g., Met, Cys, Trp, Leu), changes in its bioavailability indicate both the occurrence and intensity of the Maillard reaction (Mavromichalls, 2001). Destruction of lysine is accomplished in two ways. First, lysine is bound to sugars, forming early Maillard reaction products. Although at this stage, lysine is still detected by chemical analysis, it is no longer bioavailable (Mavromichalls, 2001). Secondly, formation of late stage Maillard reaction products, such as melanoidins, reduce the amount of chemically analyzed lysine (Mavromichalls, 2001).

CHAPTER 3

HYPOTHESES AND OBJECTIVES

The studies tested the following hypotheses:

1. Canola processing plants in Canada that use the pre-press solvent extraction process produce CM that vary in chemical and nutritive composition for broiler chickens and growing pigs.
2. Pelleting of CM decreases it nutritional value for pigs and broiler chickens
3. Indicators of CM quality such as lysine, NDICP, total dietary fiber can be predicted from simple measures of NDF and CP.
4. Standardized ileal digestible AA content for broiler chickens and pigs can be predicted from simple measures of NDF and ADF.

The overall objective was to determine the effect of canola meal source (processing plant) on the chemical composition and nutritive value of CM for broiler chickens and pigs.

The specific objectives were:

1. To determine the chemical characteristics of CM from Canadian processing plants.
2. To determine the effect of CM source and pelleting on the standardized ileal digestible AA content for growing pigs.
3. To determine the effect of CM source and pelleting on the standardized ileal digestible AA and nitrogen-corrected apparent metabolizable energy contents for broiler chickens.

4. To develop prediction equations for determining indicators of CM quality which includes lysine, NDICP, total dietary fiber and standardized ileal digestible AA content-for broiler chickens and pigs.

CHAPTER 4

CHEMICAL AND NUTRITIVE CHARACTERISTICS OF CANOLA MEAL FROM CANADIAN PROCESSING PLANTS[1]

4.1 ABSTRACT

Samples of canola meal (**CM**) were collected from eleven canola processing plants in Canada over 4 successive years (2011–2014) to determine the chemical composition, indicators of protein damage and variability among processing plants over time. In each year, each processing plant provided 3 samples and all samples were subjected to a complete chemical characterization which included proximate analyses, carbohydrate, dietary fiber with its components, total phosphorus and phytate phosphorus, and amino acid (**AA**) measurements. Overall, the contents of various components of CM over the 4 years in g/kg dry matter (**DM**) were as follows: crude protein (**CP**; Nx6.25) 417; fat 35.0; sucrose 61.0; oligosaccharides 29.0; total phosphorus 11.2; phytate phosphorus 7.40; non-phytate phosphorus 3.80; neutral detergent fiber (**NDF**) 294; non-starch polysaccharides (**NSP**) 219; total dietary fiber 379; neutral detergent insoluble crude protein (**NDICP**) 54.0; lignin and polyphenols 107 and in μmol/g DM, glucosinolates 4.60. The mean AA contents were, in g/kg DM basis, Lys 21.1; Arg 22.7; His 11.7; Iso 12.5; Leu 25.9; Met 6.9; Cys 8.4; Phe 14.5; Tyr 9.60; Thr 15.5, Val 17.4, Ala 17.1, Asp 27.0, Glu 67.2, Gly 18.0, Pro 27.0, and Ser 17.8.

[1]A version of the material presented in Chapter four of this thesis has been published in Animal Feed Science and Technology. The Authors are D. I. Adewole, A. Rogiewicz, B. Dyck, and B. A. Slominski. Anim. Feed Sci. Technol. 2016. 222:17-30.

Over the 4 years, the highest variation was observed in the contents of simple sugars having a coefficient of variation (**CV**) of 41.7%, NDICP (CV = 16.8%) and glucosinolates (CV = 35.3%), the components known to be sensitive to heat treatment. Variations ($P < 0.05$) among processing plants and years were also observed for CP, ether extract, ash, total phosphorus, simple sugars, oligosaccharides, NDF, NSP, NDICP, lignin and polyphenols, total dietary fiber and glucosinolates. Among all AA, only lysine showed differences ($P < 0.05$) among processing plants. Lysine content averaged 18.5, 22.1, 22.9 and 20.7 g/kg in years 2011, 2012, 2013 and 2014, respectively, and was lowest in meals showing the highest NDICP and total dietary fiber values. Linear regression equations for predicting lysine, NDICP, and total dietary fiber from simple chemical compositions such as NDF and CP were developed. In conclusion, variations in the contents of CP, dietary fiber, NDICP, glucosinolates, and lysine among other components were observed for CM samples from processing plants in Canada. These variations were mainly associated with differences in processing conditions (particularly heat treatment) in the various processing plants. Total dietary fiber but not lysine and NDICP can be accurately predicted from NDF or NDF and CP.

***Keywords*:** canola meal; processing; dietary fiber; protein damage; glucosinolates; prediction equations

4.2 INTRODUCTION

Canola is one of the most important oilseed crops in Canada and its crushing is an important part of the overall canola industry in Canada (Unger, 2011; Canola Council of Canada, 2015). Canola meal (**CM**), which is a co-product of the canola oil extraction process accounts for approximately 60% of the whole canola seed. It is in most cases produced by pre-press solvent extraction with hexane. The pre-press solvent extraction process of canola requires a number of steps, each

involving a wide range of temperature, moisture and time. All these conditions contribute to variations in the chemical composition and nutritional value of CM. For example, in the cooker, the temperature ranges from 85 to 95°C, the moisture ranges from 4.5 to 6.0% and cooking lasts for 30 to 40 minutes. In the desolventizer/toaster (**DT**), the temperature ranges from 95 to 115°C, and the process may last for 35-50 minutes (Unger, 2011; Canola Council of Canada, 2015). Studies have shown that excessive heating during pre-press solvent extraction may result in reduced concentration of amino acids (**AA**), particularly lysine. This is because Maillard reactions may occur between AA and reducing sugars as a result of the combination of heat and moisture applied to the meal in the DT (Nursten, 2005). Earlier research from our laboratory (Slominski, 1997) showed a profound effect of moist heat treatment on CM quality. Application of temperatures higher than 110°C resulted in a significant increase in neutral detergent insoluble crude protein (**NDICP**) and dietary fiber contents and a significant decline in protein digestibility. Another study by Almeida et al. (2014) showed that autoclaving of CM at 103°C for 20, 30 and 45 minutes increased NDF and acid detergent insoluble N and reduced the standardized ileal AA digestibility when fed to growing pigs. No study has been conducted to determine the effect of processing practices or conditions in the various processing plants on the chemical compositions and formation of Maillard reaction products in CM. Canola meal end-users desire increased meal consistency and more information on the nutritive value and heat damage of AA in CM (Spragg and Mailer, 2007). Therefore, the objectives of this study were: 1) To determine the effect of processing conditions on the chemical composition and indicators of protein damage (Maillard reaction products including dietary fiber, NDICP, and lignin with associated polyphenols) in CM from Canadian processing plants, 2) To determine the variations in the chemical composition of CM produced in Canada over time, and 3) To develop prediction equations for determining the

components that can be used as indicators of heat damage including, lysine, dietary fiber, NDICP, and lignin with associated polyphenols.

4.3 MATERIALS AND METHODS

4.3.1 Canola Meal Sample Collection and Preparation

In years 2011, 2012, 2013, and 2014, CM samples were collected from 11 canola processing 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; Cargill Canola Processing, Clavet, SK. In each year, 3 samples were collected at 1-week intervals from each processing plant to minimize variability. All samples were finely ground to pass through a 1 mm sieve. It is of interest to note that the majority (> 95%) of canola seeds produced in Canada, hence used by the processing plants, belongs to the *Brassica napus* species (Canola Council of Canada, 2015). Canada grows various varieties of *Brassica napus* canola, each of which must be approved for registration and must meet or exceed checks for quality standards. This results in varying quality of seeds. These varieties are blended at the processing plant and the resulting oil and meal products must meet the criteria established by Canadian Oilseed Processors Association Trading Rules. Therefore, environmental factors can have more impact on quality than genotype. However, blending of canola seeds from different locations at the processing facilities would reduce the variability further.

4.3.2 Canola Meal Chemical Analyses

Canola meal samples were analyzed at least in duplicate for dry matter (**DM**), crude protein (**CP**), neutral detergent fiber (**NDF**), ash, fat, total and phytate phosphorus, simple sugars, oligosaccharides, sucrose, non-starch polysaccharides (**NSP**), glucosinolates, and AA.

Dry matter was determined according to AOAC (1990) method (925.09) by oven drying a 5.0 g sample at 105°C overnight. The method of Goering and Van Soest (1970) was used for the determination of NDF. Nitrogen content in CM samples and their NDF residues was determined using the combustion method (990.03; AOAC, 1990) with an N analyzer (Model CNS-2000; LECO Corp., St. Joseph, MO) and CP was calculated as N × 6.25. Ether extract in samples was determined after hexane extraction (Method 920.39; AOAC, 1990) in an Ankom extraction system (Macedon, NY, USA). The AA content was determined as described in Method 994.12 (AOAC, 1990), and as modified by Mills et al. (1989). Briefly, a 100-mg sample was digested in 4 mL of 6 M HCl in vacuo for 24 h at 110°C. The digested mixture was neutralized with 4 mL of 6.25 M NaOH and allowed to cool at room temperature. The neutralized mixture was made up to a 50-mL volume with sodium citrate buffer solution (19.6 $g \times L^{-1}$; pH 2.2) and analyzed using an AA analyzer (Sykam, Eresing, Germany). Samples for analysis of the sulphur-containing AA methionine and cysteine were subjected to performic acid oxidation before acid hydrolysis. Total phosphorus (P) was determined as described in Method 965.17 (AOAC, 2005). Phytate P was assayed using the procedure described by Haug and Lantzsch (1983). Non-phytate P was calculated by subtracting phytate P from the total P contents. Glucosinolates were analyzed using the method of Thies (1977) with some modifications (Slominski and Campbell 1987). Simple sugars (fructose and glucose), sucrose and oligosaccharides were 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 stream of air at 40°C and derivatized with a mixture of acetone: bis(trimethylsilyl) acetamide:trimethylsilyl chloride:1-methioninehylimidazole (2:1:0.1:0.05 vol/vol). The sugars in the resulting solution were determined by gas-liquid chromatography using 3% OV-7 column and Varian 430 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada).

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 composed of amylase, pullulanase 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 millilitres of water and 5 mL of myo-inositol (internal standard) solution were then added and the mixture was boiled for 2 h. One millilitre 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-methioninehylimidazole. Component neutral sugars were separated using SP-2340 column and Varian CP-3380 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada).

It is well known that the detergent methods, including NDF, were originally developed by Van Soest' group for forage analysis (Van Soest, 1963, 1984; Van Soest and Wine, 1967). When the same NDF method is used for fiber analysis of cereal grains or protein supplements of monogastric diets, a significant underestimation of NSP and thus total dietary fiber content occurs due to the high solubility of NSP in the NDF solution and therefore losses of NSP on NDF analysis

(Slominski et al., 1994). Therefore, total dietary fibre 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.

4.3.3 Statistical Analysis

All data were subjected to analysis of variance using the GLM procedure of SAS. The effects of processing plant and year were incorporated into the statistical procedure using this model:

$$Y_{ij} = \mu + P_i + Qj + E_{ij}$$

Where Y_{ij} = chemical composition of CM from the ith processing plant in the jth year

μ = population mean

P_i =effect of processing plant (fixed effect) i= 1-11

Q_j = effect of year (fixed effect) j=1-4

E_{ij} = error deviation in the ith processing plant within the jth year.

Means were compared using the Tukey's studentized range test when there was a significant difference at $P < 0.05$ between means. Coefficient of variation (**CV**) was determined using descriptive statistics of the GLM procedure of SAS. Scatter plots of Excel software were used to determine the correlations between total dietary fiber and NDICP, dietary fiber and lysine, NDF and lysine, lysine and NDICP, glucosinolates and Lys, and NDF and total dietary fiber. The REG procedure of SAS was used to develop prediction equations for Lys, NDICP, and dietary fiber

(dependent variables). The independent variables used in the regression equations were NDF (single linear regression) and NDF + CP (multiple regression). Statistical significance was considered at $P < 0.05$.

4.4 RESULTS

4.4.1 Effect of Processing Plant and Year on the Chemical and Nutritive Composition of CM

As presented in Table 4.01, there was variation ($P < 0.05$) among processing plants in the contents of CP (402 – 429 g/kg DM), ether extracts (26.0 – 43.0 g/kg DM), ash (71.0 – 79.0 g/kg DM), and total P (10.6 – 11.6 g/kg DM). However, there were no processing plant differences in phytate and non-phytate P contents. Canola meal from Plants 1, 4 and 10 had similar and highest CP contents while those from Plants 7 and 11 were significantly lower ($P < 0.05$) in CP than Plants 1, 4 and 10. The overall mean CP for CM from all processing plants over the 4-year period was 417 g/kg DM. Plant 2 had the highest ether extract content of 43.0 g/kg DM while Plant 8 had the lowest ether extract content of 26.0 g/kg DM. Ether extract contents in CM from Plants 8 and 10 were significantly lower than those from other Plants. The overall mean ether extract content was 35.0 g/kg DM. Canola meal from Plant 6 had the highest ash content of 79.0 g/kg DM while that from Plant 8 had the lowest content of 71.0 g/kg DM. Apart from CM from Plants 6 and 8, other CM samples had similar ash content. The overall mean ash was 75.0 g/kg DM. There were differences ($P < 0.05$) in the total P content among processing plants. The overall mean total P content was 11.2 g/kg DM. Non-phytate P contents were similar among processing plants despite differences in total P content.

Year 2013 had the highest CP content and significantly higher than other years (Table 4.02). Ether extract in 2014 was 28.0 g/kg DM and significantly lower than in other years. Ash

content was higher in 2011 than in other years. Total P was significantly higher ($P < 0.05$) in year 2013 than in other years.

Table 4.01. Effect of processing plant on crude protein (CP), ether extract (EE), ash, total phosphorus (P), phytate P, and non-phytate P (NPP) contents of canola meal (g/kg, dry matter basis)[1]

Processing Plant	CP	EE	Ash	Total P	Phytate P	NPP
1	425[a]	39.7[ab]	77.3[ab]	11.3[abc]	7.5	3.8
2	410[ab]	43.4[a]	78.0[ab]	10.6[c]	7.0	3.6
3	413[ab]	36.3[b]	76.6[abc]	11.0[abc]	7.4	3.6
4	429[a]	32.1[ab]	74.1[ab]	11.6[ab]	7.7	3.9
5	420[ab]	37.9[ab]	77.0[ab]	11.3[abc]	7.4	3.9
6	421[ab]	35.5[ab]	78.9[a]	11.0[abc]	7.5	3.5
7	403[b]	30.3[ab]	73.7[ab]	11.4[ab]	7.6	3.8
8	417[ab]	26.2[b]	70.9[b]	10.8[dbc]	7.4	3.4
9	416[ab]	38.6[ab]	74.2[ab]	11.1[abc]	7.2	3.9
10	426[a]	26.6[b]	73.0[ab]	11.6[a]	7.7	3.9
11	402[b]	38.9[ab]	72.5[ab]	11.3[abc]	7.4	3.9
Mean	417	35.1	75.1	11.2	7.4	3.8
SEM[2]	4.00	3.20	1.60	0.20	0.15	0.10
CV[3]	1.93	18.1	4.17	2.77	4.36	6.43

[a-d]Means followed by different letters within columns are significantly different ($P<0.05$).
[1]Number of observations contributing to each mean = 4.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.02. Effect of year on crude protein (CP), ether extract (EE), ash, total phosphorus (P), phytate P, and non-phytate P (NPP) contents of canola meal (g/kg, dry matter basis)[1]

Year	CP	EE	Ash	Total P	Phytate P	NPP
2011	406[c]	37.2[a]	80.2[a]	10.1[c]	6.9[b]	3.2[b]
2012	417[b]	39.7[a]	71.8[b]	11.2[b]	6.8[b]	4.4[a]
2013	437[a]	35.8[a]	73.6[b]	12.2[a]	8.0[a]	4.2[a]
2014	406[c]	27.6[b]	74.8[b]	11.2[b]	7.9[a]	3.3[b]
Mean	417	35.1	75.1	11.2	7.4	3.8
SEM[2]	2.40	1.90	0.90	0.09	0.09	0.09
CV[3]	1.93	18.1	4.17	2.77	4.36	6.43

[a-c]Means followed by different letters within columns are significantly different ($P<0.05$).
[1]Number of observations contributing to each mean =11.
[2]Standard error of the mean.
[3]Coefficient of variation.

As illustrated in Table 4.03, there were differences ($P < 0.05$) among processing plants in the contents of simple sugars (1.7 – 5.6 g/kg DM), and oligosaccharides (25.6 – 32.3 g/kg DM). Plant 5 had the highest content of simple sugars which was significantly higher than those of samples from plants 3, 7, 8, 9 and 10. Canola meal from plant 10 had the lowest simple sugars content. There was no difference in sucrose content of CM among processing plants. Higher contents of oligosaccharides were recorded for CM from plants 10 and 11 than that from plant 4. Canola meal samples from other plants had similar oligosaccharides content. The mean simple sugars, sucrose and oligosaccharides contents were 2.9, 61.0 and 29.1 g/kg DM, respectively.

Samples from year 2012 had higher simple sugars content than those from other years (Table 4.04). Samples from year 2014 had higher sucrose content than those from 2011 and 2013 (Table 4.04).

Table 4.03. Effect of processing plant on the carbohydrate contents of canola meal (g/kg, dry matter basis)[1]

Processing Plants	Simple sugars[2]	Sucrose	Oligosaccharides[3]
1	3.3[ab]	60.2	29.7[ab]
2	3.2[ab]	60.5	28.4[ab]
3	2.1[b]	58.5	28.3[ab]
4	3.8[ab]	57.4	25.6[b]
5	5.6[a]	58.9	27.8[ab]
6	2.9[ab]	59.2	29.1[ab]
7	1.8[b]	63.3	29.6[ab]
8	2.5[b]	62.9	29.4[ab]
9	2.3[b]	59.4	29.3[ab]
10	1.7[b]	64.3	31.2[a]
11	2.8[ab]	61.8	32.3[a]
Mean	2.9	60.6	29.1
SEM[4]	0.61	1.40	1.13
CV[5]	41.7	4.74	7.54

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

Table 4.04. Effect of year on the carbohydrate contents of canola meal (g/kg, dry matter basis)[1]

Year	Simple sugars[2]	Sucrose	Oligosaccharides[3]
2011	2.0[b]	56.5[c]	24.0[c]
2012	4.5[a]	60.0[b]	30.5[ab]
2013	2.6[b]	56.0[c]	29.8[b]
2014	2.6[b]	69.8[a]	32.3[a]
Mean	2.9	60.6	29.1
SEM[4]	0.37	0.90	0.68
CV[5]	41.7	4.74	7.54

[a-c]Means followed by different letters within columns are significantly different ($P<0.05$).
[1]Number of observations contributing to each mean = 11.
[2]Includes fructose and glucose.
[3]Includes raffinose and stachyose.
[4]Standard error of the mean.
[5]Coefficient of variation.

As documented in Table 4.05, there were differences ($P < 0.05$) among processing plants in the contents of NDF (269-339 g/kg DM), NDICP (41.0-75.0 g/kg DM), lignin and polyphenol (97-121 g/kg DM), and total fiber (347-424 g/kg DM). There was no difference in NSP content (207- 228 g/kg DM) of CM among processing plants and the mean NSP content was 219 g/kg DM. Meals from Plants 1 and 10 had the lowest NDF content which were not significantly different from those from other Plants except for Plants 2 and 9 which had the highest NDF contents. The mean NDF content was 294 g/kg DM. Neutral detergent insoluble crude protein contents were different among processing Plants. Canola meal from plants 1, 3, 4, 10 and 11 were significantly lower in NDICP content than those from Plants 2 and 9 but were not different from Plants 5, 6, 7 and 8. The lowest NDICP value of 41.0 g/kg DM was recorded for CM from Plant 10 while the highest value of 75.0 g/kg DM was recorded for CM from Plant 9. Highest lignin and polyphenol content (121 g/kg DM) was recorded for CM from Plant 9 while the lowest (97.0 g/kg DM) was recorded for CM from Plant 10. Canola meal from Plants 1 and 10 had significantly lower lignin and polyphenol contents than that from Plant 9. Total dietary fiber content was highest in CM from Plant 9 (424 g/kg DM) and lowest in CM from Plant 1 (347 g/kg DM). Other Plants had similar total fiber contents. The mean NDICP, lignin and polyphenols and total fiber contents were 54.0, 107, and 379 g/kg DM, respectively.

As illustrated in Table 4.06, 2013 samples had lower NDF content (274 g/kg DM) than those from other years. Samples from 2014 had higher (233 g/kg DM) NSP content than those from other years. Lignin and polyphenols contents in 2011 and 2014 samples were higher than those from 2012 and 2013. Samples from year 2014 had higher ($P < 0.05$) total dietary fiber content than preceding years.

Table 4.05. Effect of processing plant on neutral detergent fiber (NDF), non-starch polysaccharides (NSP), neutral detergent insoluble crude protein (NDICP), lignin and polyphenols (L & P) and total fiber contents of canola meal (g/kg, dry matter basis)[1]

Processing Plant	NDF	Dietary fiber fraction			
		NSP[2]	NDICP	L & P	Total fiber
1	269[c]	207	43.8[c]	98[b]	347[c]
2	316[ab]	217	73.0[ab]	117[ab]	402[ab]
3	280[bc]	212	44.4[c]	109[ab]	363[bc]
4	285[bc]	213	48.8[c]	107[ab]	369[bc]
5	286[bc]	213	55.9[abc]	103[ab]	371[bc]
6	286[bc]	219	54.1[abc]	103[ab]	375[bc]
7	298[bc]	224	51.0[bc]	106[ab]	380[abc]
8	298[bc]	226	54.2[abc]	108[ab]	386[abc]
9	339[a]	225	75.0[a]	121[a]	424[a]
10	276[c]	228	40.9[c]	97[b]	366[bc]
11	302[bc]	224	50.0[c]	113[ab]	388[abc]
Mean	294	219	53.7	107	379
SEM[3]	7.30	4.60	4.50	4.20	9.20
CV[4]	4.95	4.21	16.8	7.84	4.85

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

Table 4.06. Effect of year on neutral detergent fiber (NDF), non-starch polysaccharides (NSP), neutral detergent insoluble crude protein (NDICP), lignin and polyphenols (L & P) and total dietary fiber contents of canola meal (g/kg, dry matter basis)[1]

Year	NDF	Dietary fiber fraction			
		NSP[2]	NDICP	L & P	Total fiber
2011	293[a]	213[b]	44.8[b]	116[a]	374[b]
2012	300[a]	220[b]	60.1[a]	96[b]	376[b]
2013	274[b]	209[b]	52.7[ab]	93[b]	355[b]
2014	309[a]	233[a]	54.7[a]	124[a]	411[a]
Mean	294	219	53.7	107	379
SEM[3]	4.40	2.80	2.70	2.50	5.60
CV[4]	4.95	4.21	16.8	7.84	4.85

[a-b]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 11.
[2]Includes arabinose, xylose, mannose, galactose, glucose and uronic acids.
[3]Standard error of the mean.
[4]Coefficient of variation.

4.4.2 Effect of Processing Plant and Year on AA Content of CM

Except for lysine, there were no differences in AA contents among processing plants (Table 4.07). Plant 1 had the highest lysine content and significantly higher lysine content than Plants 2, 4 and 9. Other Plants had similar lysine contents. The mean lysine content was 21.1 g/kg DM.

There were differences ($P < 0.05$) among years for all AA (Table 4.08). Generally, AA contents were significantly lower in samples from 2011 than those from other years.

4.4.3 Effect of Processing Plant and Year on Glucosinolates Content of CM

As presented in Table 4.09, there were differences ($P < 0.05$) in the contents of 3-butenyl, 2-hydroxy-3-butenyl, total aliphatics, 4-hydroxy-3-indolylmethioninehyl, total indoles and total glucosinolates among processing plants. The content of aliphatic glucosinolates ranged from 1.59 µmol/g DM (Plant 9) to 7.14 µmol/g DM (Plant 1), while the content of indoles ranged from 0.26 µmol/g DM (Plant 9) to 2.48 µmol/g DM (Plant 1). Consequently, total glucosinolates ranged from 1.90 µmol/g DM (Plant 9) to 9.70 µmol/g DM (Plant 1). The total glucosinolates contents of CM from Plants 1 and 3 were significantly higher than those of plants 4, 5, and 9. The overall mean total glucosinolates content was 4.6 µmol/g DM.

There were variations among years ($P < 0.05$) in the contents of 3-butenyl, 3-indlolylmethioninehyl, 4-hydroxyl-3-indolylmethioninehyl and total indoles. However, there were no differences among years in the total aliphatics and total glucosinolates contents (Table 4.10).

Table 4.07. Effect of processing plant on amino acid contents of canola meal (g/kg, dry matter basis)[1]

Processing Plant	Arg	His	Ile	Leu	Lys	Met	Cys	Phe	Thr	Tyr	Val	Ala	Asp	Glu	Gly	Pro	Ser
1	23.9	12.1	12.9	26.7	22.9[a]	7.0	8.6	15.0	16.0	10.0	17.9	17.6	28.2	69.7	18.8	27.9	18.3
2	22.6	11.8	12.5	26.2	20.3[b]	6.8	8.2	14.7	15.4	9.70	17.5	17.3	27.2	67.3	18.0	27.3	17.8
3	23.2	11.7	12.5	25.7	21.5[ab]	7.2	8.7	14.5	15.6	9.70	17.4	17.1	27.6	67.0	18.3	26.8	18.0
4	22.1	11.3	12.1	25.3	20.7[b]	6.4	8.1	14.1	14.9	9.10	16.9	16.6	26.4	65.2	17.3	26.5	17.3
5	23.5	12.3	12.9	27.0	21.5[ab]	7.0	8.5	15.1	16.2	10.0	18.0	17.7	26.6	70.7	19.0	28.2	18.6
6	22.9	11.6	12.6	26.0	20.8[ab]	7.0	8.6	14.7	15.2	9.50	17.8	17.3	27.1	67.7	17.9	26.9	17.6
7	22.5	11.4	12.4	25.6	21.0[ab]	7.1	8.5	14.4	15.4	9.50	17.4	16.8	26.8	65.5	17.9	26.1	17.6
8	22.1	11.3	12.0	25.0	20.7[ab]	7.2	8.8	14.0	15.3	9.30	16.7	16.6	26.4	64.8	17.5	26.4	17.4
9	22.4	11.8	12.8	26.1	20.0[b]	6.7	8.2	14.5	15.5	9.60	17.6	17.4	27.2	67.7	18.0	27.3	17.9
10	22.1	11.5	11.9	25.4	21.1[ab]	6.5	8.1	14.1	15.1	9.20	16.8	17.0	26.7	65.9	17.6	26.7	17.4
11	22.9	11.8	12.5	26.2	21.6[ab]	6.6	7.4	14.6	15.7	9.60	17.3	17.3	27.3	67.7	18.3	27.0	18.0
Mean	22.7	11.7	12.5	25.9	21.1	6.9	8.4	14.5	15.5	9.60	17.4	17.1	27.0	67.2	18.0	27.0	17.8
SEM[2]	0.54	0.23	0.33	0.61	0.48	0.35	0.32	0.35	0.36	0.22	0.44	0.39	0.87	1.58	0.43	0.63	0.43
CV[3]	4.75	3.98	5.24	4.68	4.60	10.20	7.71	4.75	4.69	4.65	5.07	4.57	6.40	4.70	4.76	4.65	4.78

[a-b]Means followed by different letters within columns are significantly different ($P<0.05$).
[1]Number of observations contributing to each mean = 4.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.08. Effect of year on amino acid contents of canola meal (g/kg, dry matter basis)[1]

Year	Arg	His	Ile	Leu	Lys	Met	Cys	Phe	Thr	Tyr	Val	Ala	Asp	Glu	Gly	Pro	Ser
2011	18.1^{c}	8.90^{c}	11.8^{b}	23.7^{b}	18.5^{c}	5.60^{b}	7.80^{b}	12.9^{c}	14.3^{c}	8.60^{c}	14.7^{c}	14.7^{c}	24.3^{b}	57.1^{c}	18.0^{b}	22.4^{d}	15.1^{d}
2012	24.7^{a}	13.2^{a}	13.6^{a}	27.4^{a}	22.1^{a}	7.20^{a}	8.60^{a}	15.4^{a}	18.2^{a}	10.5^{a}	18.8^{a}	16.8^{b}	29.4^{a}	72.5^{a}	20.6^{a}	27.3^{b}	19.0^{b}
2013	25.4^{a}	13.0^{a}	11.9^{b}	28.4^{a}	22.9^{a}	7.30^{a}	8.80^{a}	16.0^{a}	13.0^{d}	9.60^{b}	19.1^{a}	22.3^{a}	28.7^{a}	74.7^{a}	15.2^{c}	33.6^{a}	20.3^{a}
2014	22.7^{b}	11.7^{b}	12.4^{b}	24.2^{b}	20.7^{b}	7.30^{a}	8.40^{ab}	13.9^{b}	16.5^{b}	9.60^{b}	17.0^{b}	14.7^{c}	25.8^{b}	64.5^{b}	18.5^{b}	24.7^{c}	16.9^{c}
Mean	22.7	11.7	12.5	25.9	21.1	6.90	8.40	14.5	15.5	9.60	17.4	17.1	27.0	67.2	18.0	27.0	17.8
SEM[2]	0.33	0.14	0.20	0.37	0.29	0.21	0.20	0.21	0.22	0.13	0.27	0.24	0.52	0.95	0.26	0.38	0.26
CV[3]	4.753	3.983	5.243	4.679	4.598	10.191	7.709	4.746	4.691	4.649	5.073	4.567	6.397	4.697	4.759	4.645	4.779

[a-d]Means followed by different letters within columns are significantly different (P<0.05).
[1]Number of observations contributing to each mean = 11.
[2]Standard error of the mean.
[3]Coefficient of variation.

Table 4.09. Effect of processing plant on glucosinolate contents of canola meal (µmol/g, dry matter basis)[1]

Processing Plant	Aliphatic glucosinolates					Indole glucosinolates			Total glucosinolates
	3-Butenyl	4-pentenyl	2-hydroxy-3-butenyl	2-hydroxy-4-pentenyl	Total aliphatics	3-indolylmethioninehyl	4-hydroxy-3-indolylmethioninehyl	Total indoles	
1	2.13^{a}	0.22	4.59^{a}	0.21	7.14^{a}	0.23	1.78^{a}	2.48^{a}	9.70^{a}
2	0.69^{bcd}	0.02	1.68^{bc}	0.08	2.49^{bcd}	0.09	0.31^{bc}	0.51^{bcd}	3.00^{bcd}
3	1.52^{abc}	0.17	3.68^{ab}	0.17	5.54^{ab}	0.18	0.80^{abc}	1.21^{b}	6.70^{ab}
4	0.47^{d}	0.05	1.33^{c}	0.11	1.96^{cd}	0.13	0.32^{bc}	0.33^{cd}	2.30^{cd}
5	0.59^{cd}	0.02	1.27^{c}	0.09	1.96^{cd}	0.09	0.15^{c}	0.20^{d}	2.30^{cd}
6	0.74^{bcd}	0.09	2.00^{bc}	0.09	2.92^{bcd}	0.13	0.32^{bc}	0.38^{bcd}	3.30^{bcd}
7	1.35^{abcd}	0.15	3.47^{ab}	0.10	5.06^{abc}	0.18	0.82^{abc}	1.02^{bcd}	6.20^{abc}
8	1.01^{bcd}	0.14	2.95^{abc}	0.10	4.19^{abcd}	0.15	0.67^{abc}	0.94^{bcd}	5.20^{bcd}
9	0.41^{d}	0.05	1.05^{c}	0.09	1.59^{d}	0.08	0.18^{c}	0.26^{d}	1.90^{d}
10	1.64^{ab}	0.68	3.55^{ab}	0.61	6.48^{a}	0.63	1.42^{ab}	1.12^{bc}	6.30^{abc}
11	0.94^{bcd}	0.08	2.64^{abc}	0.09	3.75^{abcd}	0.17	0.43^{bc}	0.56^{bcd}	4.30^{bcd}
Mean	1.04	0.15	2.56	0.16	1.99	0.19	0.65	0.82	4.60
SEM[2]	0.202	0.139	0.418	0.15	0.705	0.141	0.242	0.170	0.820
CV[3]	38.8	182.3	32.6	192.4	36.0	152.0	74.1	41.7	35.3

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

Table 4.10. Effect of year on glucosinolate contents of canola meal (µmol/g, dry matter basis)[1]

Year	Aliphatic glucosinolates					Indole glucosinolates			Total glucosinolates
	3-butenyl	4-pentenyl	2-hydroxy-3-butenyl	2-hydroxy-4-pentenyl	Total aliphatics	3-indolylmethioninehyl	4-hydroxy-3-indolylmethioninehyl	Total indoles	
2011	0.88[b]	0.08	2.11	0.31	3.39	0.18[ab]	0.78[ab]	0.88[ab]	4.30
2012	1.41[a]	0.10	3.03	0.07	4.61	0.12[ab]	0.56[ab]	0.61[b]	5.20
2013	1.08[ab]	0.29	2.89	0.19	4.46	0.40[a]	1.02[a]	1.01[a]	5.10
2014	0.79[b]	0.08	2.22	0.05	3.20	0.05[b]	0.25[b]	0.78[ab]	4.00
Mean	1.04	0.15	2.56	0.16	1.99	0.19	0.65	0.82	4.60
SEM[2]	0.122	0.084	0.252	0.091	0.425	0.085	0.146	0.103	0.490
CV[3]	38.8	182.3	32.6	192.4	36.0	152.0	74.1	41.7	35.3

[a-b]Means followed by different letters within columns are significantly different ($P<0.05$).
[1]Number of observations contributing to each mean =11.
[2]Standard error of the mean.
[3]Coefficient of variation.

4.4.4 Some Notable Correlations in the Chemical Compositions of CM

It is worthy of note that CM from Plant 9 had the highest contents of NDICP (75.0 g/kg DM), lignin with polyphenols (121 g/kg DM), NDF (339 g/kg DM), and total dietary fiber (424 g/kg DM; Table 5). However, it had the lowest content of glucosinolates (1.9 µmol/g DM) and lysine (20.0 g/kg DM; Tables 4.01 and 4.07). Canola meal from Plant 1 had the lowest content of total dietary fiber (347 g/kg DM) and the highest content of glucosinolates (9.7 µmol/g DM; Tables 4.01 and 4.05; Fig. 4.1).

Canola meal samples from plant 10 had the highest CP content (426 g/kg DM), highest total phosphorus content (11.6 g/kg DM), highest sucrose content (64.0 g/kg DM), highest NSP content (228 g/kg DM), lowest NDICP content (41.0 g/kg DM, and lowest lignin with polyphenols content (97.0 g/kg DM).

There were positive correlations between dietary fiber and NDICP contents (R^2 = 0.79; Figure 4.2), between glucosinolates and lysine (R^2 = 0.56; Figure 4.3) and between NDF and total dietary fiber (R^2 = 0.97; Figure 4.4). Also, there were negative correlations between total dietary fiber and lysine (R^2 = 0.64; Figure 4.5), lysine and NDICP (R^2 = 0.48; Figure 4.6), and lysine and NDF (R^2 = 0.53; Figure 7). Linear regression equations for predicting lysine, NDICP, and total dietary fiber from simple chemical compositions such as NDF and CP, were developed and are presented in Table 4.11. The R^2 values ranged from 0.16 to 0.98 and all P values were less than 0.03. Example calculations for lysine, NDICP, and total dietary fiber contents of CM obtained from 11 processing plants in year 2014 were presented in Table 4.12 using the developed prediction equations.

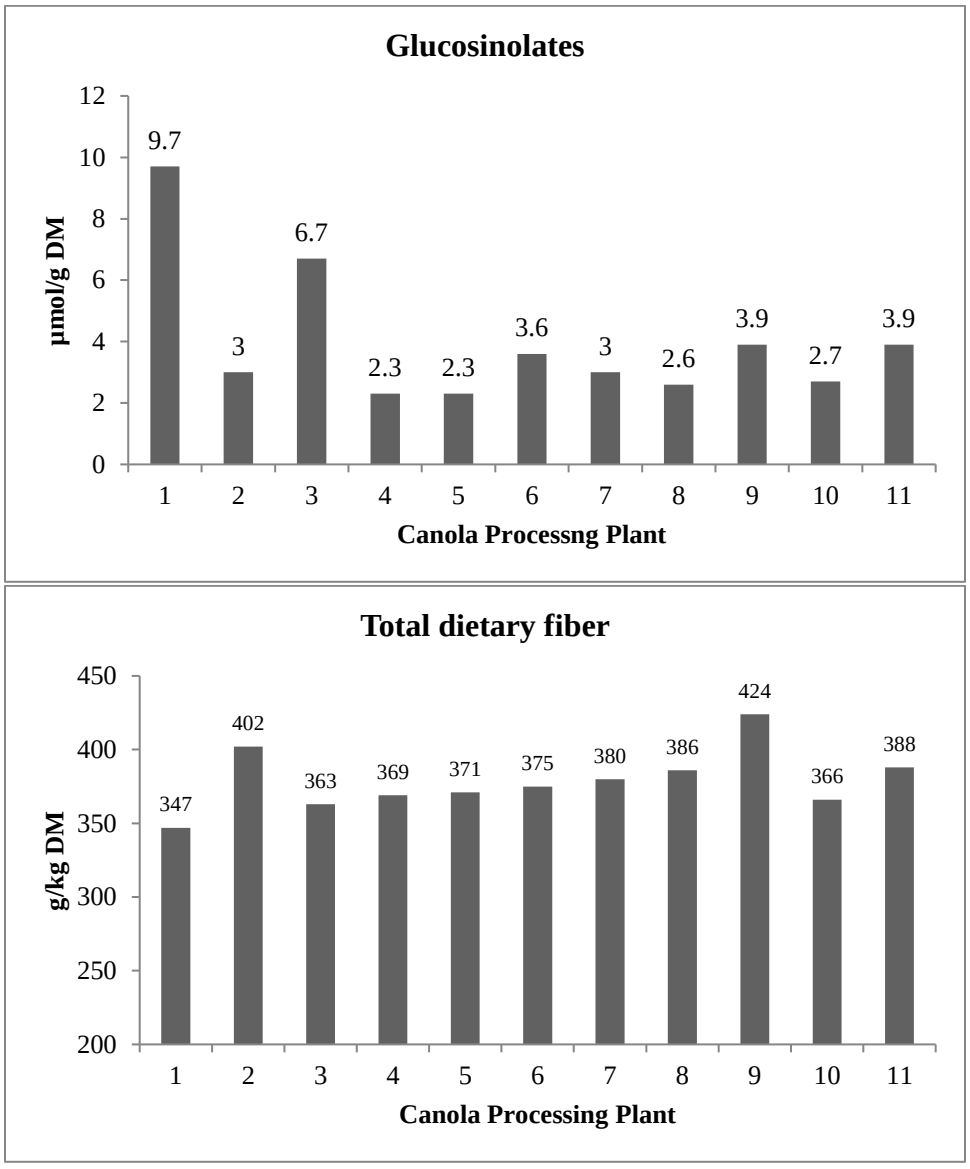

Figure 4.01. Relationship between glucosinolates and total dietary fiber contents in canola meal from Canadian processing plants

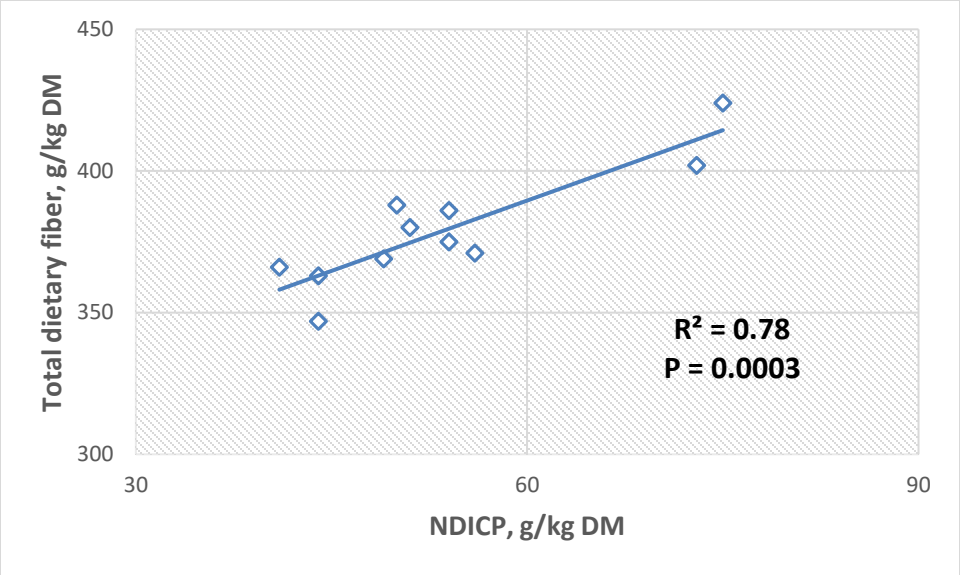

Figure 4.02. Relationship between total dietary fiber and neutral detergent insoluble crude protein (NDICP) contents in canola meal from Canadian processing plants

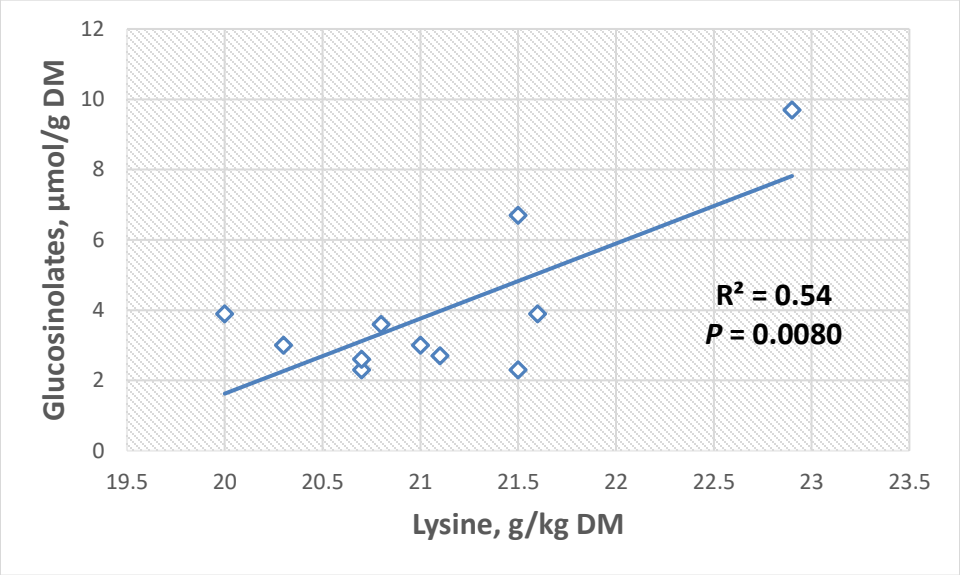

Figure 4.03. Relationship between total glucosinolates and lysine contents in canola meal from Canadian processing plants

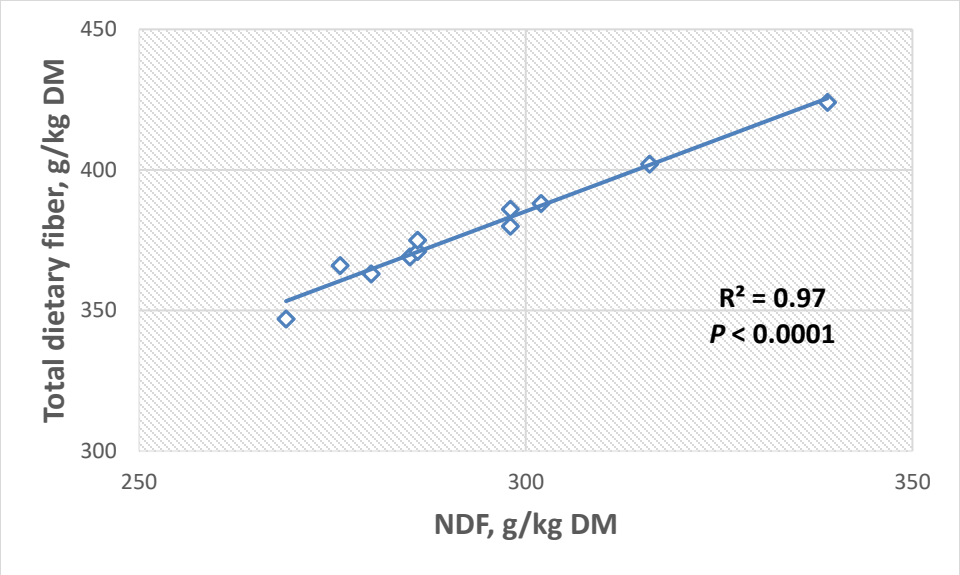

Figure 4.04. Relationship between total dietary fiber and NDF contents in canola meal from Canadian processing plants

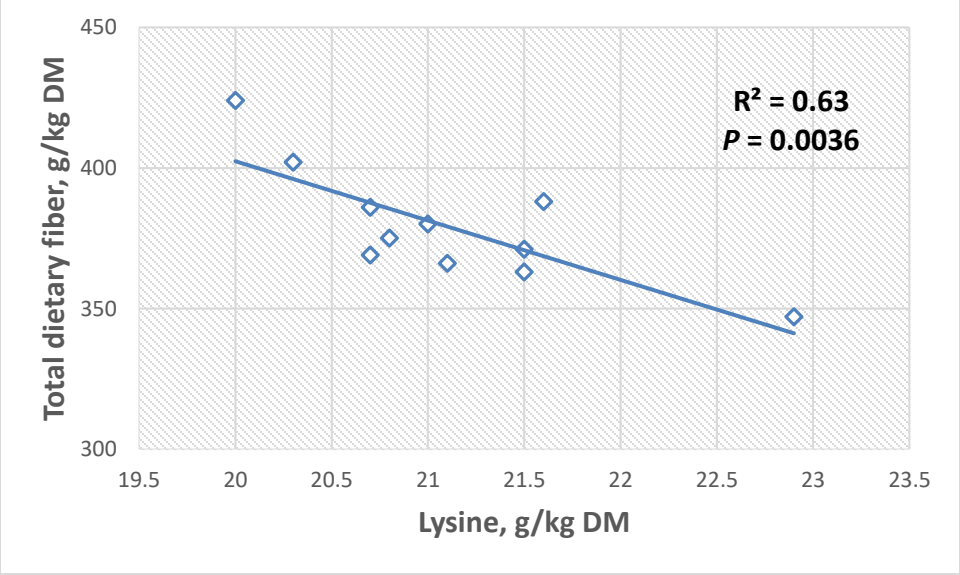

Figure 4.05. Relationship between total dietary fiber and lysine contents in canola meal from Canadian processing plants

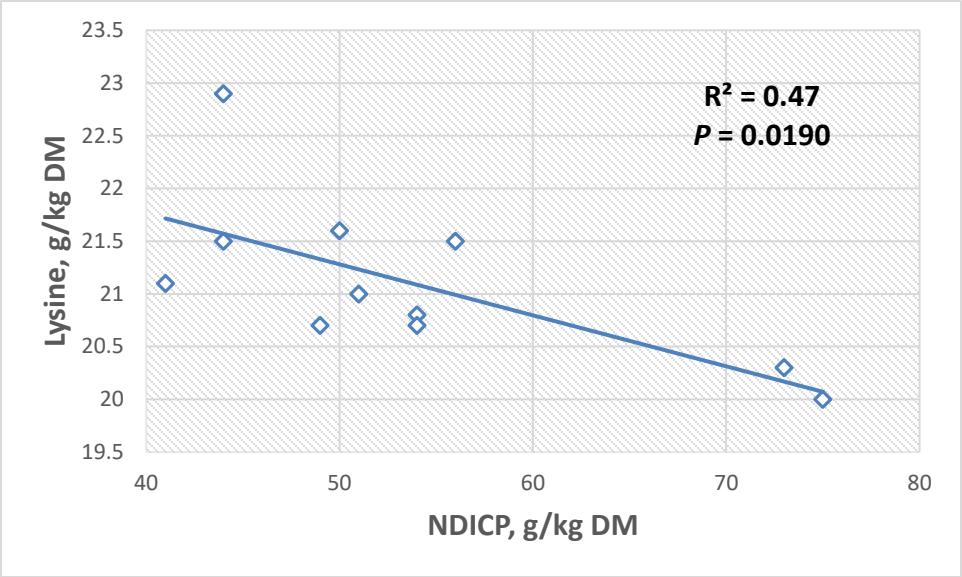

Figure 4.06. Relationship between lysine and neutral detergent insoluble crude protein (NDICP) contents in canola meal from Canadian processing plants

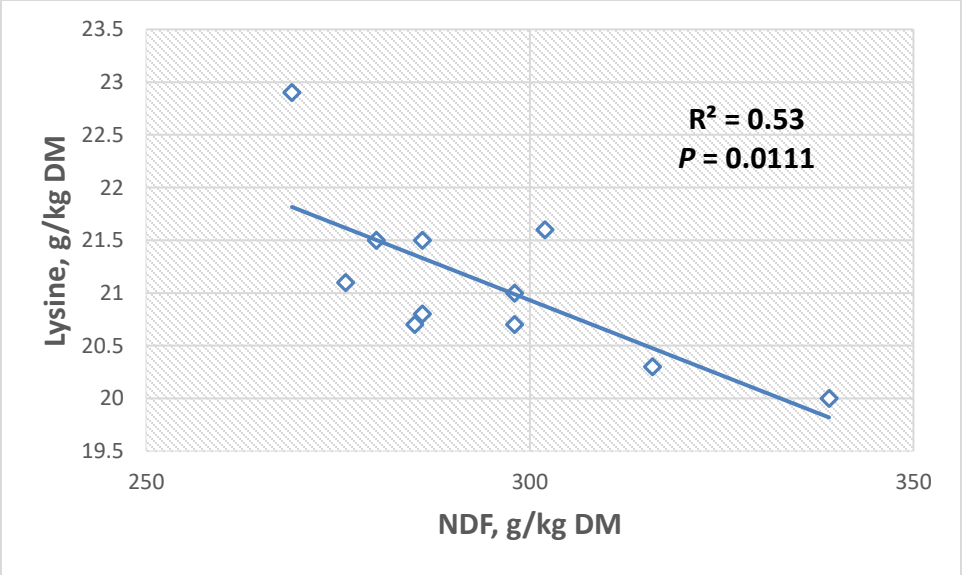

Figure 4.07. Relationship between lysine and NDF contents in canola meal from Canadian processing plants

Table 4.11. Prediction of lysine, neutral detergent insoluble crude protein (NDICP), and total dietary fiber contents of canola meal (g/kg dry matter basis)[1]

#	Equation	RSD[2]	R^2	*P*-value
1	Lys = 29.49 - 0.029NDF	0.56	0.53	0.0100
2	Lys = 41.49 - 0.034 NDF – 0.025CP	0.52	0.59	0.0270
3	NDICP = -93.06 + 0.499NDF	5.18	0.81	0.0002
4	NDICP = -213.95 + 0.552NDF + 0.252CP	4.74	0.84	0.0007
5	Total dietary fiber = 76.18 + 1.030NDF	3.52	0.97	<0.0001
6	Total dietary fiber = 39.74 + 1.046NDF + 0.076CP	3.46	0.98	<0.0001

[1] n=11 i.e., 11 observations were used in the regression analysis. Each observation represents the mean value of the samples obtained from each plant over the 4-year period.

[2]Residual standard deviation

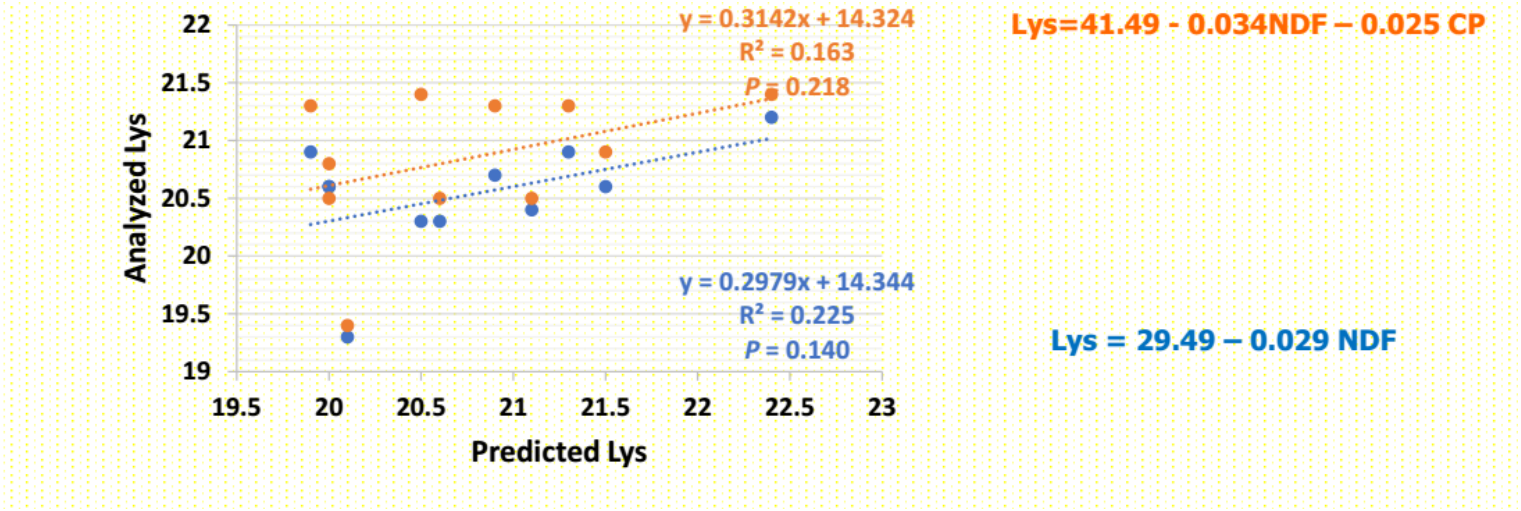

Figure 4.08. Relationship between analyzed and predicted lysine contents of canola meal. The chemical composition of CM from 11 processing plants in year 2013 were used as validation dataset.

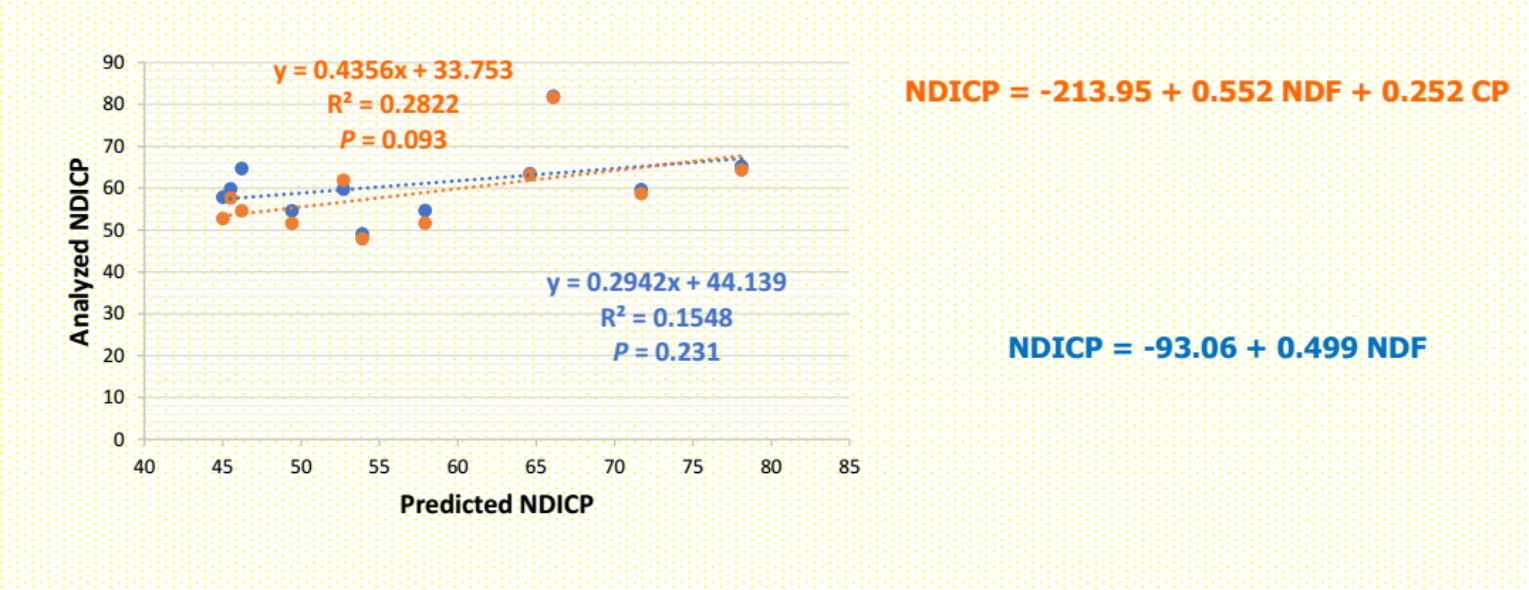

Figure 4.09. Relationship between analyzed and predicted neutral detergent insoluble crude protein (NDICP) contents of canola meal. The chemical composition of CM from 11 processing plants in year 2013 were used as validation dataset.

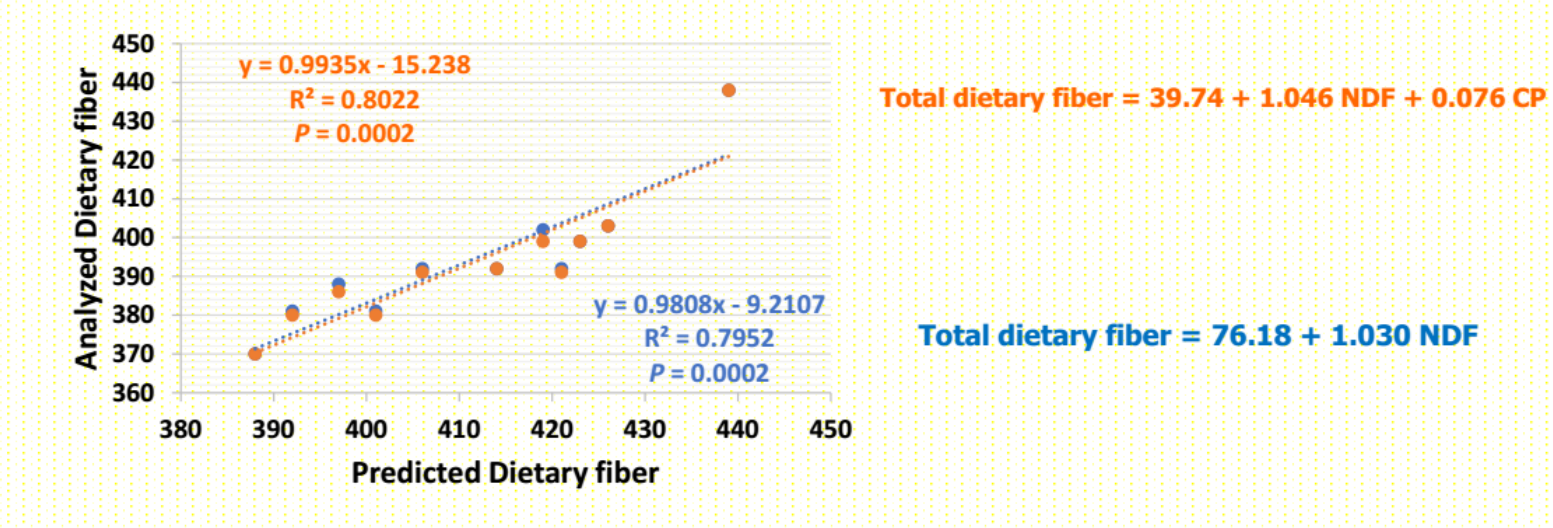

Figure 4.10. Relationship between analyzed and predicted neutral detergent insoluble crude protein (NDICP) contents of canola meal. The chemical composition of CM from 11 processing plants in year 2013 were used as validation dataset.

4.5.5 Equations for Predicting lysine, Neutral Detergent Insoluble Crude Protein (NDICP), and Total Dietary Fiber Contents of CM

As illustrated in Table 4.11, all the equations had significant *P*-values. As presented in Figure 4.08 to 4.10, regression equations comparing analyzed and predicted lysine and NDICP were not significant and had very low coefficient of determination ($\mathbf{R^2}$). Predicted values of lysine and NDICP did not compare favourably with analyzed values as observed from the non-significant *P*-values (0.09 to 0.44) and very low R^2 (0.07 to 0.28). However, predicted total dietary using equations 5 and 6 compared favourably with analyzed values ($P < 0.001$; $R^2 = 0.80$). The chemical composition of CM samples obtained in year 2013 were used as validation dataset.

4.5 DISCUSSION

The pre-press solvent extraction of CM consists of 10 basic stages which are: 1) Cleaning and drying of the seed to approximately 6% moisture, 2) Preconditioning (heating) 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 reduce the oil content to approximately 15 to 20%, 6) Solvent extraction with hexane to remove additional oil, 7) Desolventization in the DT to remove hexane from the oil-extracted meal, 8) Cooling, 9) Drying with forced air (some plants add back gums and screenings at this point), and 10) Grinding and in some cases, pelleting (Unger, 2011; Canola Council of Canada, 2015). Each plant in Canada uses similar equipment. However, there are differences in how the equipment is being operated to extract oil. Processing conditions of CM may not be consistent among processors and this may cause some variability in the nutritive composition (Spragg and Mailer, 2007). Some

of these variations may result from heat damage. Heat and pressure applied through canola processing is recognised to have detrimental effects upon meal quality with excessive heat damaging protein and reducing its availability for animals (Newkirk, 2002; Khajali and Slominski, 2012). Maillard reactions may occur as a result of the combination of heat and moisture applied at some stages of CM processing. Canola meal contains both AA and reducing sugars, the components required for the Maillard reactions to occur. Under conditions of elevated temperature and moisture, any compound with a free amino group is vulnerable to the the Maillard reactions, and this includes free AA, epsilon amino groups of protein-bound lysine, and free amino groups of thiamine, folacin, and gossypol (Macromichalls, 2001). Lysine is the most susceptible AA in intact proteins because it has a free amino group at the epsilon carbon which is readily available to react with reducing sugars (Macromichalls, 2001).

The CP content ranged from 404 to 429 g/kg DM with an overall average of 420 g/kg DM which is similar to that of 418.5 g/kg DM reported by Bell and Keith (1991), 405.6 g/kg DM reported by Khajali and Slominski (2012), and that of 411.0 g/kg DM listed by NRC (2012).

4.5.1 Effect of Processing Plant and Year on the Chemical and Nutritive Composition of CM

Variations in the CP content of the CM in this study may be mainly due to variations in the CP content of the canola seed feedstock as a result of changes in the growing and harvesting conditions (Bell and Keith, 1991). Protein content in itself is not a good indicator of CM quality but protein digestibility is a more important parameter. However, variation in the protein content is regarded as a limiting factor in the value of CM (Spragg and Mailer, 2008).

Variations among processing plants and years in the content of ether extract could be a consequence of different processing practices among the processing plants in terms of adding back any by-products of seed cleaning (i.e., screenings and other dockage) and oil-refining (i.e., gums,

soapstocks, phospholipids, etc.) into the meal to increase the energy content of the meal and to reduce dustiness (Canola Council of Canada, 2015). Without adding the various by-products, the fat content of the meal is approximately 10 – 20 g/kg (Matthaus, 2012). Based on the results of the present study and the oil content ranging from 26 to 43 g/kg DM, it would appear evident that most of the processing plants, if not all of them would add the by-products of oil-refining to the meal.

The variation in the NDF, lignin and dietary fiber contents could be a consequence of the Maillard reactions. Excessive heating may lead to the formation of Maillard reaction products which are indicative of protein damage and would lead to the increase in the analyzed NDF, ADF, and lignin contents and reduced protein digestibility (Slominski, 1997; Almeida et al., 2014). The analyzed concentration of lignin may increase because some melanoidins, which are polymers originating from the Maillard reaction, may be analyzed as lignin (Miao et al., 1994). Consequently, heat treatment of feed ingredients is expected to increase the analyzed values of NDF and total dietary fiber (Almeida et al., 2014). Mustafa et al. (2000) reported that CM obtained after the stages of desolventization/toasting and drying processes had similar NDF content, which was higher than that of the solvent-extracted meal prior to desolventization/toasting. This indicates that the variation in the NDF content among processing plants is probably due to differences in heat treatment applied during the processing of the meal. McKinnon et al. (1995) also found that moderate heat input increased the NDF content of CM with little or no impact on acid detergent fiber and acid detergent lignin contents.

There were differences in the contents of simple sugars (having the highest CV of 41.7%) and oligosaccharides among processing plants and among years. However, sucrose content did not differ among processing plants. There is lack of information on the regional and seasonal effects

on the carbohydrate contents of canola seeds or meal. It is well known that the Maillard reaction involves the binding of amino group of AA to the carbonyl group of reducing sugars such as glucose and fructose (Maillard, 1912). Although, the deterioration of protein quality as a result of AA losses during Maillard reactions is of more concern to nutritionists and producers, sugar losses are greater and more intense than AA losses (Adrian 1974).

Differences in the content of total P among processing plants and year can be explained by differences in growing conditions of the canola seed feedstock used in the various processing plants. However, the phytate and non-phytate P contents were similar between processing plants.

4.5.2 Effect of Processing Plant and Year on the AA content of CM

Amino acid content was generally lower in samples from year 2011 than those from other years. This could be attributed to the lower CP content observed in CM from year 2011 than those from other years which could be as a result of variations in the growing and soil conditions among the years. There were variations in lysine content among processing plants, whereas other AA were similar among processing plants. Lysine is the primary AA involved in the early stage of Maillard reactions due to the presence of the highly reactive ε-amino group (Mauron, 1981). Most of the AA are susceptible to advanced Maillard reactions such as Strecker degradation which leads to the formation of dark brown pigments (Mauron, 1981). The sparge steam in the desolventizer/toaster introduces both heat and moisture and therefore has the potential to promote the Maillard reaction (Newkirk et al., 2003b). In this context, maximal losses of lysine, in the presence of reducing sugars occur between 15 and 18 % moisture contents (Mauron, 1981) which coincided with the level of moisture in the meal exiting the DT in the study of Newkirk et al. (2003b). Newkirk et al. (2003a) also confirmed that the final stages of meal processing, and in particular,

desolventization/toasting reduce meal AA content. The lysine content during desolventization/toasting of sunflower seed has also been shown to be reduced indicating that the effect is not confined to canola processing (San Juan and Villamide, 2001). As lysine is the AA most vulnerable to heat damage, its availability should be a sensitive indicator of the effects of heat treatment.

4.5.3 Effect of Processing Plant and Year on the Glucosinolates Content of CM

There were variations ($P < 0.05$) among processing plants in the contents of both aliphatic and indole glucosinolates as a result of differences ($P < 0.05$) in some individual components (i.e., 3-butenyl, 2-hydroxy-3-butenyl and 4-hydroxy-3-indolylmethioninehyl). The total glucosinolate content ranged from 2.0 to 10.1 µmol/g DM which is much lower than 30 µmol/g aliphatic glucosinolates in air-dried oil-free meal as the maximum level for canola definition. Total glucosinolates had a high coefficient of variation (**CV**) of 35.3 %. The coefficient of variation is a measure of spread that describes the amount of variability relative to the mean. The highest glucosinolates content was found in CM from Plant 1 which happened to have the lowest dietary fiber content, while the lowest glucosinolates content was found in CM from Plant 9 which happened to have the highest dietary fiber content (Figure 4.01). This suggests that the increase in fiber content might have been caused by CM overheating, which also reduced the glucosinolates content to 2.0 µmol/g DM. This supports the earlier reports that application of high temperature would destroy the glucosinolates in CM (Campbell and Slominski, 1990). Glucosinolates are a group of structurally related sulphur-containing substances which yield a range of aglucone and sulphur-containing compounds upon hydrolysis. In the presence of moisture and following rupture of the cells, the glucosinolates are hydrolysed by myrosinase to yield a range of products including isothiocyanates, goitrin, and nitriles (Hanschen and Schreiner, 2017). In addition to myrosinase

catalysed reaction, thermal degradation during CM processing is another means of glucosinolates decomposition (Campbell and Slominski, 1990). As reported by Slominski and Campbell (1987), indole glucosinolates which represent a significant portion of the total glucosinolates content of CM have been shown to be more susceptible to thermal degradation than the aliphatic ones. This could be the reason why, in the current study, all components of indole glucosinolates were different among years whereas only one component of aliphatics (3-butenyl) was different among years. Major decomposition of indole glucosinolates would occur in the DT with little or no effect during meal drying (Campbell and Slominski, 1990). The average value for total glucosinolates observed in the current study was 4.9 µmol/g DM which is similar to that of 4.95 µmol/g DM reported by Gorski et al. (2017) but lower than that of 7.2 µmol/g DM reported by Newkirk et al. (2003a). The level of glucosinolates in CM produced in Canada has continued to decrease due to selection pressure by canola breeders. The low glucosinolates content of canola, compared to previous cultivars of rapeseed, constitutes the major improvement in CM quality achieved by plant breeders.

4.5.4 Some Notable Correlations in the Chemical Compositions of CM

Lysine, NDICP (also known as glycoprotein), glucosinolates, and dietary fiber components have been used as indicators of protein damage in canola and other oilseed meals (Campbell and Slominski, 1990; Newkirk, 2002; Newkirk et al., 2003a; b; Almeida, 2013). Therefore, they were also used as indicators of protein damage in the current study. The positive correlation between the total dietary fiber and NDICP (also referred to as glycoprotein; Figure 4.2) contents shows that the NDICP is contributing to the increase in total dietary fiber and it is an indication that the total dietary fiber increased as a result of protein damage caused by meal overheating. The NDICP is a measure of CM protein and AA digestibility. Newkirk et al. (2000) found that neutral detergent

insoluble nitrogen (expressed as a percentage of total protein) values below 10 % are indicative of CM with greater than 85% lysine availability. Glucosinolates are heat-sensitive, therefore, the correlations between the glucosinolates and lysine contents observed in the current study (Figure 4.3) further suggested that CM from some processing plants was over-heated which led to the losses of lysine. The positive correlation between NDF and total dietary fiber (Figure 4.4) indicates that NDF can be used to predict the total dietary fiber content in CM. The negative relationships between dietary fiber and lysine, lysine and NDICP, and NDF and lysine (Figures 4.5, 4.6, and 4.7) also suggest that a second-stage of Maillard reaction which leads to reduction in lysine concentrations may be occurring in the CM from some of the processing plants.

4.5.5 Equations for Predicting lysine, Neutral Detergent Insoluble Crude Protein (NDICP), and Total Dietary Fiber Contents of CM

All the equations were significant ($P < 0.05$). The R^2 obtained for equations 1 (0.53), and 2 (0.59) were relatively low and indicate poorer fit than those of other equations. This suggests that 53 or 59%, respectively, of the observed variable variations were explained by the equations. Traditionally, the R^2 value has been used as the primary measure of model performance in the literature (Meloche et al., 2014; Rochell et al., 2011; Anderson et al., 2012; Kerr et al., 2013). For equations 1 and 2, the residual standard deviation (**RSD**) values, which are measures of the accuracy of the dependent variable being measured, were below 10% of the expected mean values. This implies that more than 90% of the expected mean values fall within the equation. Validation of equations 1 and 2 using a section of the dataset (Year 2013 samples) in the current study showed R^2 values of 0.225 and 0.163, respectively (Figure 4.08). This shows that though the equations may not give accurate predictions of the Lys content of CM, they will be useful in ranking CM quality. Equations 3 and 4 seem to provide information about NDICP due to the observed relatively

high R^2 ranging from 0.81 to 0.84 and low RSD values of 4.74 to 5.18 (which were less than 10% of the expected mean values). However, they may not accurately predict NDICP because the predicted and analyzed values did not compare favourably as observed from the validation *P*-values (0.09 to 0.23) and R^2 (0.15 to 0.28) values (Figure 4.09). Equations 5 and 6 provide accurate prediction for the total dietary fiber content of CM as observed from the high R^2 (0.97 and 0.98, respectively) and significant *p*-values. Validation of equations 5 and 6 showed high R^2 values of 0.795 and 0.802, respectively (Figure 4.10) showing that the predicted dietary fiber values compare favourably with analyzed values. The inclusion of CP slightly improved the accuracy of all the prediction equations increasing the R^2 value from 0.53 to 0.59 (Equations 1 and 2), 0.81 to 0.84 (Equations 3 and 4) and 0.97 to 0.98 (Equations 5 and 6). This agrees with the report of Li et al. (2014) that the inclusion of CP contents significantly improved the accuracy of the prediction equations for digestible energy content of peanut meal by increasing R^2 value from 0.67 to 0.91 and 0.92. These equations will enable CM users to simply predict total dietary fiber from NDF or NDF and CP contents without the need for any further analysis. Total dietary fiber analysis is a complex analysis which involves a number of other assays (including NSP analysis).

4.6 CONCLUSIONS

There were variations among processing plants and years in the contents of CP, NDF, ether extract, total phosphorus, simple sugars, oligosaccharides, total dietary fiber, glucosinolates, NDICP, lignin with polyphenols and lysine of CM produced by the pre-press solvent extraction process. It is possible that the high total dietary fiber with the corresponding high NDICP and low glucosinolates with the corresponding low lysine observed in the CM from some processing plants were caused by meal overheating and the consequent formation of Maillard reaction products. The

yearly variations in the contents of CP could be due to differences in growing and soil conditions during canola growing seasons in addition to the variations in processing conditions. Total dietary fiber but not lysine and NDICP can be accurately predicted from NDF or NDF and CP.

CHAPTER 5

STANDARDIZED ILEAL DIGESTIBLE AMINO ACIDS CONTENTS OF CANOLA MEAL FROM CANADIAN CRUSHING PLANTS FOR GROWING PIGS[2]

5.1 ABSTRACT

Studies have shown that there are variations in the chemical composition of canola meal (**CM**) produced in Canada, some of which may result from differences in processing conditions among the canola seed crushing plants. Therefore, it is necessary to determine the variability in the nutritive value of CM as its end-users desire increased meal consistency and more information on any potential variability in amino acid (**AA**) digestibility. Thus, 18 ileal cannulated barrows (average initial BW = 23.3 kg) were used to determine the apparent (**AID**), standardized ileal digestibility (**SID**) of N and AA and standardized ileal digestible N and AA contents in CM from 6 processing plants in Canada. Two processing plants provided CM in both mash and pellet forms while the other four provided it in mash form only, giving a total of 8 CM samples. The barrows were fed 9 corn starch-based diets. The first 8 diets were formulated to contain the CM samples as the only source of AA. The ninth diet was a casein-corn starch diet to determine ileal endogenous AA losses. All diets contained 0.3 % titanium dioxide as an indigestible marker. Pigs were housed individually in pens and were fed the 9 diets in a completely randomized design for 3 periods to give 6 observations per diet. Each experimental period lasted for 7 d consisting 5 d adaptation and

[2]A version of the material presented in Chapter five of this thesis has been published in Journal of Animal Science. The Authors are D. I. Adewole, A. Rogiewicz, B. Dyck, C. M. Nyachoti, and B. A. Slominski. J. Anim. Sci. 2017 95:2670-2679.

2 d ileal digesta collection at 2 h intervals from 8:00 to 20:00 h on d 6 and 7. There was an effect ($P < 0.05$) of CM source on (i) the AID of Met, Cys, and Val; (ii) the SID of Met and Cys; and (iii) the standardized ileal digestible content of all AA except His. The SID values for Arg, Lys, Met and Thr averaged 87.2, 78.2, 84.5 and 74.1%, respectively. The standardized ileal digestible contents of Arg, Lys, Met and Thr averaged 2.10, 1.71, 0.50 and 1.01%, respectively. Pelleting reduced ($P < 0.05$) the standardized ileal digestible content of all AA. There was an interactive effect of CM source and pelleting on the standardized ileal digestible contents of N, Arg, Leu, Ala, and Glu. In conclusion, there were variations in the standardized ileal digestible AA contents of CM from different sources in Canada but canola meal source and pelleting had minimal effect on the AID and SID values of CM. Pelleting may reduce the standardized ileal digestible content of AA in CM.

Keywords: amino acid, canola meal source, digestibility, pelleting, pig

5.2 INTRODUCTION

Solvent-extracted canola meal (**CM**), which is widely used as an AA source in swine diets, is the main co-product of the canola seed crushing industry. Bell and Keith (1991) reported differences in AA contents of CM produced in Western Canada, which resulted from differences in processing or environmental conditions. In a recent survey of CM from 11 processing plants by Adewole et al. (2016), differences in lysine, glucosinolates, neutral detergent insoluble crude protein (**NDICP**), and total dietary fiber were also observed. Moreover, significant correlations were observed between lysine and some other heat-sensitive components of CM including glucosinolates, NDICP, and total dietary fiber. This suggests that the late stage Maillard reaction loss of lysine may be occurring in CM from some crushing plants. During the first stages of the Maillard reaction, AA are still detectable by chemical analysis, but are no longer bioavailable

(Almeida, 2013). Destruction of lysine is often 5 to 15 times as much as that occurring with other AA (Mavromichalis, 2001). Therefore, a decrease in lysine content and availability indicate both the occurrence and intensity of the Maillard reaction (Adrian, 1966). It has been demonstrated that thermal over-processing of high-protein materials reduces lysine availability (Mavromichalis, 2001). Variability in the standardized ileal digestible AA contents of CM produced in Canada has not been determined for growing pigs. Pelleting CM reduces bulkiness, dustiness, and wastage during transportation. The heat applied during pelleting of CM in the crushing plants may further decrease AA digestibility. Therefore, the objectives of this study were to determine the effects of CM source and pelleting on the standardized ileal digestible AA contents of CM produced in Canada for growing pigs and to propose prediction equations for determining standardized ileal digestible AA contents of CM from simple measures of NDF and ADF.

5.3 MATERIALS AND METHODS

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

5.3.1 Canola Meal, Experimental Diets and Animals

Canola meal samples were collected from 6 canola crushing plants in Canada. The plants were chosen based on the differences in chemical and nutritive composition of CM produced in those processing plants over two consecutive years (Adewole et al., 2016). Two crushing plants provided CM samples in both mash and pellet forms whereas the others provided samples in mash form only, giving a total of 8 CM samples (Tables 5.1 and 5.2). The CM samples are named CM1, CM2, CM3, CM4, CM5 mash, CM5 pellets, CM6 mash and CM6 pellet. The 2 pelleted CM samples were ground in a hammer mill through a 2-mm screen before diet preparation. The experimental

diets included 8 cornstarch-based diets in which the CM samples were the only source of AA and a casein-cornstarch diet to determine ileal endogenous AA losses (Table 5.3). All diets contained 0.3% titanium dioxide as an indigestible marker and were fed in mash forms.

It is of interest to note that the majority (>95%) of canola seed produced and processed in Canada belongs to the *Brassica napus* species (Canola Council of Canada, 2015). Canada grows various varieties of *Brassica napus* canola, each of which must meet or exceed checks for quality standards and be approved for registration. These varieties are all blended prior to processing at the crushing plants. The blending of canola seeds from different locations at crushing plants would minimize variability and the resulting oil and meal products must meet criteria established by the Canadian Oilseed Processors Association.

Eighteen Genesus (Yorkshire-Landrace female x Duroc male) barrows were obtained from the University of Manitoba Glenlea Swine Research Unit at an average initial BW of 15.5 ± 0.80 (mean ± SD) kg and fitted with a simple T-cannula at the distal ileum as described by Nyachoti et al. (2002). Pigs were housed individually in pens (1.47 x 1.14 m) with smooth sides and plastic-covered, expanded metal sheet flooring in a temperature controlled room (22 to 24°C) and were allowed a recovery period of 10 d during which they were fed a commercial grower diet before the commencement of the experiment. At the commencement of the experiment, pigs had an average BW of 23.3 ± 1.3 (mean ± SD) kg.

5.3.2 Experimental Design and Procedure

The experiment was designed and conducted as an incomplete block design with 3 periods. Pigs were weighed at the beginning of each period and were fed at 2.6 times maintenance energy requirement based on their BW at the beginning of each experimental period. Two equal meals were offered at 0800 and 1600 h as dry mash. Each experimental period lasted 7 d each, in which

pigs were allowed to acclimatize to their respective diets for 5 d. On d 6 and 7, ileal digesta were collected continuously for 12 h (0800 to 2000 h) into plastic bags as described by Nyachoti et al. (2002). Collection bags contained 10 mL of 10% (vol/vol) formic acid to minimize bacterial activities. Every 1 to 2 h, or whenever the bags were three-quarters full, digesta were removed and stored in a freezer at –20°C until further analyses.

5.3.3 Sample Preparation and Chemical Analyses

Digesta samples were thawed and pooled within each pig and period, homogenized in a heavy-duty blender (Waring Commercial, Torrington, CT), and a 200-g sample of digesta was lyophilized for later analyses. Canola meal samples, test diets, and digesta samples were finely ground and were analyzed for AA and DM. Diets and digesta samples were analyzed for titanium after the samples were ashed at 600°C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian, Palo Alto, CA) according to the method of AOAC (2005, Method 985.01). Canola meal samples were analyzed for CP, NDF, ADF, ash, fat, total and phytate P, simple sugars, oligosaccharides, sucrose, non-starch polysaccharides (**NSP**), and glucosinolates.

Dry matter was determined according to AOAC (1990, Method 925.09) by oven drying a 5-g sample at 105°C overnight. The method of Goering and Van Soest (1970) was used for the determination of NDF and ADF. Nitrogen content in CM, NDF residues, diets and digesta was determined using the combustion method (990.03; AOAC, 1990) with an N analyzer (Model CNS-2000; LECO Corp., St. Joseph, MO) and CP was calculated as N × 6.25. Ether extract in samples was determined after hexane extraction (Method 920.39; AOAC, 1990) in an Ankom extraction system (Macedon, NY, USA).

The AA content was determined as described in Method 994.12 (AOAC, 1990), and as modified by Mills et al. (1989). Briefly, a 100-mg sample was digested in 4 mL of 6 M HCL in vacuo for 24 h at 110°C. The digested mixture was neutralized with 4 mL of 6.25 M NaOH and allowed to cool at room temperature. The neutralized mixture was made up to a 50-mL volume with sodium citrate buffer solution (19.6 $g \times L^{-1}$; pH 2.2) and analyzed using an AA analyzer (Sykam, Eresing, Germany). Samples for analysis of S-containing AA (Met and Cys) were subjected to performic acid oxidation before acid hydrolysis. Tryptophan was not determined.

Total P was determined as described in method 965.17 (AOAC, 2005). Phytate P was assayed using the procedure described by Haug and Lantzsch (1983). Non-phytate P was calculated by subtracting phytate P from total P. Glucosinolates were analyzed using the method of Thies (1977) with some modifications (Slominski and Campbell, 1987). Simple sugars (fructose and glucose), sucrose and oligosaccharides were determined by gas-liquid chromatography according to the procedure described by Slominski et al. (2004). Briefly, 200 mg of CM sample was extracted with 5 mL of 80% ethanol and 1 mL of myo-inositol and was 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 v/v). The sugars in the resulting solution were determined by gas-liquid chromatography using 3% OV-7 column and Varian 430 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada).

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 dimethylsulfoxide and incubated overnight at 45°C with a solution of starch-

degrading enzymes composed of amylase, pullulanase 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 millilitres of water and 5 mL of myo-inositol (internal standard) solution were then added and the mixture was boiled for 2 h. One millilitre 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 fibre 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 represented the amount of CP present in the NDF residue. The value for lignin with associated polyphenols was calculated by difference between the total fiber and NDICP + NSP contents.

5.3.4 Calculations and statistical analysis

The AID of nutrients was calculated using the indicator method as described by Nyachoti et al. (1997). Standardized ileal digestibility (**SID**) of N and AA were calculated using this equation: SID, % = [AID + (EL/N_{Diet})] × 100, where SID represents the SID (%) of N or AA; EL is the basal endogenous loss of N or AA obtained from feeding a low-protein (5%), casein-based diet to growing pigs (mg/kg DMI); and N_{Diet} is the dietary content of N or AA (mg/kg DM).

Endogenous losses (**EL**) of CP and AA were calculated from the casein-based diet using the following equation: EL, mg/kg DMI = ($Ti_{diet}/Ti_{digesta}$) × $N_{digesta}$, where EL represents basal

endogenous losses of N or AA; Ti_{diet} represents the dietary titanium content; and $Ti_{digesta}$ represents the titanium content in digesta.

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) by the following mixed-effects model:

$Y_{ij} = \mu + D_i + P_j + E_{ij}$,

where Y_{ij} = AID, SID or standardized ileal digestible content of CM of the ith diet within the jth period; μ = overall mean; D_i = effect of diet (fixed) i =1 - 8; P_j = effect of period (random) j=1- 3; E_{ij} = error of the ith diet within the jth period. Means were compared using the Tukey's studentized range test when there was a significant difference at $P < 0.05$ between means. Pig was the experimental unit. Contrast statements were used to determine the difference in AID, SID and standardized ileal digestible content of AA between mash and pelleted CM samples. The contrast results show that pelleting reduced AID, SID, or standardized ileal digestible AA contents of CM in one of the CM samples but had no effect on the other. Therefore, the 4 dietary treatments that contained either CM5 or CM6 mash or pellets were further compared as a 2 x 2 factorial arrangement to investigate the interactive effects of CM and pelleting. The REG procedure of SAS was used to develop prediction equations for determining standardized ileal digestible AA contents from the NDF and ADF components.

5.4 RESULTS

The analyzed chemical compositions of the CM samples are presented in Tables 5.1 and 5.2. There were variations in the contents (%, DM) of fat (1.6-4.9), NDF (24.9-32.4), ADF (16.9-20.1), total dietary fiber (32.5-39.3), lysine (1.66-2.45), methionine (0.46-0.72), threonine (0.99-1.72) and other AA. Pelleted CM had lower AA concentrations compared with the mash forms even though the CP contents were similar.

The ingredient and calculated and analyzed compositions of experimental diets are presented in Table 5.3. The NDF content of diets ranged from 10.8 to 14.0 % DM and the lysine content ranged from 0.93 to 1.10% DM.

As presented in Table 5.4, there were differences ($P < 0.05$) among CM samples in the AID values for Met, Cys, and Val. Pelleting reduced ($P < 0.05$) the AID of His, Ile, Leu, Phe, Thr, Val, Asp, Ser, and Tyr. There was no interaction between CM source and pelleting on the AID value of any AA.

Table 5.1. Chemical composition of canola meal (CM) used in the study (%, DM basis) [1]

Components	CM1	CM2	CM3	CM4	CM5		CM6	
	mash	mash	mash	mash	mash	pellets	mash	pellets
Crude protein	39.8	40.5	39.6	39.2	43.6	40.9	39.2	41.4
Fat	3.9	4.9	2.9	1.6	2.5	3.4	3.4	3.6
Carbohydrates								
Sucrose	6.2	6.3	6.7	6.6	7.0	6.9	6.4	6.3
Oligosaccharides[2]	3.3	2.6	3.0	3.0	2.8	2.8	2.6	2.7
Simple Sugars[3]	0.1	0.5	0.5	0.3	0.4	0.3	0.4	0.3
Dietary fiber fractions								
Acid detergent fiber (ADF)	19.3	18.6	16.9	18.8	17.1	18.8	19.4	20.1
Neutral detergent fiber (NDF)	25.7	32.4	31.5	30.6	24.9	26.7	27.8	28.8
Total dietary fiber	33.8	38.8	37.5	39.3	32.5	34.5	36.1	34.7
Glycoproteins (NDICP)[4]	2.5	6.8	6.3	4.5	3.8	3.2	3.6	3.4
Non-starch polysaccharides	22.5	19.5	19.2	22.4	20.3	19.9	21.1	19.0
Lignin and polyphenols	8.8	12.5	12.0	12.4	8.4	11.4	11.4	12.3
Phosphorus (P)	1.26	1.11	1.16	1.10	1.28	1.20	1.21	1.23
Phytate P	0.86	0.68	0.73	0.64	0.81	0.78	0.78	0.78
Non-phytate P	0.41	0.44	0.42	0.35	0.46	0.43	0.43	0.38
Glucosinolates, µmol/g DM[5]	10.1	3.0	2.0	2.4	4.5	4.4	3.2	5.9

[1]CM1to CM6 are canola meal samples from crushing plants 1 to 6; Samples were analyzed at least in duplicates.
[2]Includes raffinose and stachyose.
[3]Includes fructose A, glucose A + fructose B and glucose B.
[4]Neutral detergent insoluble crude protein.
[5]Includes 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, 2-hydroxy-4-Pentenyl, 2-hydroxyl-4-pentenyl, 3-indolymethyl and 4-hydroxy-3-indolylmethyl.

Table 5.2. Amino acid composition of canola meal (CM) used in the study (%, DM basis)[1]

Item	CM1	CM2	CM3	CM4	CM5		CM6	
	mash	mash	mash	Mash	Mash	pellets	mash	pellets
Indispensable AA								
Arg	2.55	2.48	2.66	2.55	2.79	2.56	2.26	1.84
His	1.17	1.27	1.37	1.28	1.36	1.30	1.19	0.96
Ile	1.28	1.30	1.27	1.30	1.35	1.20	1.02	0.83
Leu	2.59	2.83	2.96	2.90	3.09	2.86	2.51	1.97
Lys	2.24	2.21	2.30	2.25	2.45	2.30	2.01	1.66
Met	0.72	0.56	0.64	0.55	0.64	0.61	0.56	0.46
Cys	0.90	0.73	0.70	0.71	0.65	0.52	0.88	0.68
Phe	1.50	1.60	1.66	1.62	1.74	1.61	1.38	1.09
Thr	1.72	1.37	1.41	1.35	1.35	1.36	1.23	0.99
Val	1.72	2.02	1.98	2.05	2.14	1.93	1.62	1.34
Dispensable AA								
Ala	1.63	2.11	2.30	2.25	2.41	2.28	1.98	1.52
Asp	2.84	2.86	3.03	2.89	3.09	2.96	2.58	2.02
Glu	9.61	7.17	7.70	7.32	7.62	7.27	6.56	5.19
Gly	1.93	1.61	1.65	1.56	1.59	1.54	1.42	1.15
Pro	2.65	3.11	3.30	3.27	3.62	3.39	2.94	2.22
Ser	1.81	1.98	2.13	2.02	2.14	2.18	1.85	1.46
Tyr	1.03	0.98	1.02	0.99	1.06	0.97	0.84	0.68

[1]CM1to CM6 are canola meal samples from processing plants 1 to 6; Samples were analyzed at least in duplicates.

Table 5.3. Ingredient, and calculated, and analyzed composition of experimental diets containing different samples of canola meal (CM)

Ingredient	Diets[1]								
	CM1	CM2	CM3	CM4	CM5		CM6		Casein
	mash	mash	mash	mash	Mash	pellets	Mash	pellets	
Ingredient composition, %									
Canola meal	50.0	47.0	46.0	45.0	42.0	43.0	47.0	47.0	-
Canola oil	-	-	-	-	-	-	-	-	2.0
Corn starch	47.0	50.0	51.0	52.0	55.0	54.0	50.0	50.0	69.0
Sucrose	-	-	-	-	-	-	-	-	20.0
Casein	-	-	-	-	-	-	-	-	5.0
Calcium carbonate	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	1.0
Monocalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.2
Vitamin/Mineral premix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Iodized salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Titanium dioxide	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Calculated composition									
CP, %	18.5	18.2	18.1	18.1	18.3	18.1	18.2	18.1	4.4
ME, kcal/kg	3,195	3,236	3,250	3,263	3,303	3,290	3,236	3,236	3,837
Ca, %	0.66	0.63	0.62	0.61	0.60	0.60	0.63	0.63	0.62
Available P, %	0.41	0.40	0.39	0.36	0.39	0.38	0.40	0.38	-
D Lys, %	0.77	0.72	0.71	0.69	0.65	0.66	0.72	0.72	0.35
D Met, %	0.56	0.53	0.52	0.50	0.47	0.48	0.53	0.53	0.13
Analysed composition, % DM									
CP	20.3	19.1	18.0	17.8	17.1	17.4	18.4	18.7	4.0
NDF	12.5	14.0	12.4	13.2	10.8	10.8	13.1	12.1	-
Ash	5.5	5.0	5.2	4.7	4.7	5.0	5.0	4.8	-
Lys	0.95	0.93	1.00	1.00	1.10	0.91	1.10	0.99	0.33
Met	0.38	0.34	0.36	0.28	0.34	0.33	0.27	0.26	0.09

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; All diets were fed in mash forms

[2]Supplied per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 825, IU; vitamin E, 40 IU; vitamin K, 4 mg; thiamine, 1 mg; riboflavin, 5 mg; niacin, 35 mg; pantothenic acid, 15; vitamin B_{12}, 25 g; biotin 200 g; folic acid, 2 mg; Cu, 15 mg; iodine, 0.21 mg; Fe, 100 mg; Mn, 20 mg; Se, 0.15 mg; and Zn, 100 mg.

Table 5.4. Effect of canola meal source and pelleting on the apparent ileal N and AA digestibility of canola from different sources for growing pigs[1]

Item	CM1 mash	CM2 mash	CM3 mash	CM4 mash	CM5 mash	CM6 mash	SEM[2]	CM5 pellets	CM6 pellets	P-value[3]	
										pelleting	CM * Pelleting
N	67.2	59.8	63.3	62.4	63.3	64.8	1.85	59.0	66.4	0.543	0.107
Indispensable AA											
Arg	81.2	78.6	83.5	82.3	81.4	83.6	1.27	79.2	81.4	0.115	0.728
His	45.7	38.1	48.2	46.8	50.2	51.0	4.11	36.8	47.8	0.048	0.134
Ile	72.1	69.3	76.8	78.0	76.7	76.6	1.86	72.6	70.2	0.009	0.530
Leu	74.6	73.3	77.9	78.4	77.2	78.2	1.57	72.7	74.6	0.023	0.723
Lys	72.7	67.3	71.5	73.4	73.0	73.4	1.92	67.0	70.9	0.058	0.330
\Met	85.9[a]	80.2[ab]	84.1[a]	77.2[b]	81.7[ab]	78.1[b]	1.50	77.1	77.0	0.125	0.317
Cys	76.6[a]	53.2[c]	66.1[ab]	61.2[bc]	62.9[bc]	57.8[bc]	3.56	54.1	64.9	0.266	0.614
Phe	76.4	76.2	79.3	78.6	78.9	79.2	1.56	73.5	75.4	0.006	0.619
Thr	64.5	63.2	67.6	66.8	69.4	68.7	2.29	60.4	66.2	0.012	0.131
Val	69.7[ab]	67.6[b]	73.8[ab]	74.7[a]	74.2[ab]	73.7[ab]	1.95	69.8	68.1	0.014	0.750
Dispensable AA											
Ala	72.7	68.8	74.1	74.5	73.9	74.4	1.81	67.7	72.2	0.060	0.219
Asp	67.4	60.9	67.1	67.7	67.6	68.1	2.16	59.6	65.6	0.045	0.142
Glu	81.9	79.1	83.2	83.4	82.5	83.9	1.19	78.9	82.6	0.097	0.217
Gly	66.9	58.4	66.9	66.7	64.2	68.2	2.91	57.0	64.2	0.114	0.233
Pro	63.6	57.0	63.7	60.1	55.2	62.9	6.03	52.9	63.7	0.495	0.713
Ser	67.2	64.2	69.8	68.5	69.3	69.9	2.19	60.4	67.9	0.028	0.072
Tyr	72.7	69.2	74.5	73.8	73.7	73.6	1.61	66.2	71.3	0.023	0.144

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; Number of observations contributing to each mean = 5.
[2]Standard error of the mean for CM1 to 5 mash.
[3]p-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.
[a-c]Means followed by different letters within rows are significantly different ($P<0.05$); Include only mash CM samples.

The ileal endogenous N and AA losses determined using low-protein diet are presented in Table 5.5. The means had standard deviations ranging from 0.016 (Tyr) to 0.229 (Pro).

Table 5.5. Ileal endogenous N and AA losses in growing pigs fed a casein-cornstarch diet

Item	Ileal endogenous losses (g/kg DM)	
	Mean[1]	SD[2]
N	2.15	0.107
Indispensable AA		
Arg	0.64	0.032
His	1.17	0.057
Ile	0.36	0.017
Leu	0.67	0.034
Lys	0.70	0.038
Met	0.14	0.006
Cys	0.22	0.008
Phe	0.36	0.019
Thr	0.63	0.032
Val	0.52	0.027
Dispensable AA		
Ala	0.58	0.030
Asp	1.20	0.057
Glu	1.60	0.079
Gly	1.24	0.080
Pro	2.89	0.229
Ser	0.81	0.039
Tyr	0.31	0.016

[1]n = 6.
[2]Standard deviation

Table 5.6 shows the SID values of N and AA in CM fed to growing pigs. There were differences ($P < 0.05$) among CM sources in the SID values (%) of only Met and Cys. Among mash samples, CM1 had the highest SID value for N, Met, and Cys while CM2 had the lowest value for N and Cys. Canola meal 4 had the lowest SID value for Met. Pelleting reduced ($P < 0.05$) the SID of Ile, Phe, Thr, and Val. There was no interaction between CM source and pelleting on the SID of N or any AA.

Standardized ileal digestible contents of N and AA are presented in Table 5.7. There was an effect of CM source ($P < 0.05$) on the standardized ileal digestible content of N and all AA except His. Among mash samples, CM1 had the highest standardized ileal digestible contents of N, Cys, Thr, and Gly while CM6 had the lowest standardized ileal digestible contents of Arg, Ile, Lys, Phe, Val, Asp, Glu, and Tyr. Pelleting reduced ($P < 0.05$) the standardized ileal digestible content of all AA but not of N. There were interactive effects of CM source and pelleting in the standardized ileal digestible contents of N, Arg, Leu, Ala, and Glu.

Table 5.6. Effect of canola meal source and pelleting on the standardized ileal N and AA digestibility of canola meal from different sources for growing pigs[1]

Item	CM1 mash	CM2 mash	CM3 mash	CM4 mash	CM5 mash	CM6 mash	SEM[2]	CM5 pellets	CM6 pellets	P-value[3]	
										Pelleting	CM * Pelleting
N	76.2	68.6	72.1	71.2	72.7	73.3	1.85	68.7	74.9	0.600	0.124
Indispensable AA											
Arg	87.6	84.7	89.0	88.2	86.8	88.9	1.27	85.4	87.3	0.296	0.792
His	68.0	58.7	67.8	67.3	70.3	69.9	4.11	59.3	69.5	0.170	0.124
Ile	78.6	76.1	82.5	83.9	82.3	82.1	1.86	79.0	77.1	0.027	0.646
Leu	80.6	79.2	83.2	83.3	82.7	83.3	1.57	79.1	80.5	0.065	0.751
Lys	80.0	74.8	78.4	79.4	79.6	79.9	1.92	74.6	78.6	0.132	0.378
Met	89.5[a]	84.2[abc]	87.9[ab]	82.0[c]	85.8[abc]	83.1[bc]	1.50	81.2	82.2	0.142	0.311
Cys	81.9[a]	59.2[bc]	71.6[ab]	68.1[bc]	69.1[ab]	65.2[bc]	3.56	60.2	72.5	0.273	0.577
Phe	82.0	80.8	84.4	84.8	83.8	84.1	1.56	79.4	80.8	0.020	0.677
Thr	73.8	70.8	76.0	76.3	76.8	76.4	2.29	69.0	74.0	0.034	0.167
Val	76.5	73.5	79.8	81.9	80.0	79.5	1.95	76.3	75.0	0.043	0.847
Dispensable AA											
Ala	80.8	76.6	81.3	82.0	81.0	81.5	1.81	76.2	79.9	0.155	0.285
Asp	77.3	70.4	76.0	76.9	76.1	76.8	2.16	69.8	74.9	0.121	0.198
Glu	87.1	84.1	87.9	88.3	87.3	88.5	1.19	84.5	87.6	0.214	0.295
Gly	82.0	73.1	80.6	80.8	77.8	81.5	2.91	72.7	78.6	0.298	0.298
Pro	87.9	79.9	85.6	83.0	76.9	83.6	6.03	78.5	85.9	0.820	0.871
Ser	75.5	73.8	78.2	78.4	78.5	78.9	2.19	71.2	77.3	0.084	0.117
Tyr	80.2	76.9	81.3	80.9	80.6	80.4	1.61	74.2	78.6	0.058	0.197

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; Number of observations contributing to each mean = 5.
[2]Standard error of the mean for CM1 to 5 mash.
[3]p-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.
[a-c]Means followed by different letters within rows are significantly different (P<0.05); Include only mash CM samples.

Table 5.7. Effect of canola meal source and pelleting on the standardized ileal digestible N and AA contents (%) of canola meal from different sources for growing pigs[1]

Item	CM1 mash	CM2 mash	CM3 mash	CM4 mash	CM5 mash	CM6 mash	SEM[2]	CM5 pellets	CM6 pellets	P-value[3]	
										pelleting	CM * Pelleting
N	4.84^{a}	4.49^{c}	4.78^{ab}	4.49^{c}	5.06^{a}	4.53^{b}	0.120	4.62	4.62	0.192	0.033
Indispensable AA											
Arg	2.23^{b}	2.10^{bc}	2.37^{ab}	2.25^{b}	2.42^{a}	2.00^{c}	0.032	2.19	1.60	<0.001	0.026
His	0.80	0.75	0.92	0.86	0.96	0.83	0.052	0.77	0.66	0.001	0.712
Ile	1.01^{b}	0.99^{bc}	1.04^{ab}	1.09^{a}	1.11^{a}	0.84^{c}	0.025	0.95	0.64	<0.001	0.389
Leu	2.09^{c}	2.25^{b}	2.46^{a}	2.44^{ab}	2.55^{a}	2.09^{c}	0.045	2.26	1.58	<0.001	0.023
Lys	1.79^{ab}	1.65^{bc}	1.80^{ab}	1.81^{ab}	1.95^{a}	1.61^{c}	0.044	1.72	1.29	<0.001	0.371
Met	0.64^{a}	0.44^{cd}	0.55^{b}	0.47^{c}	0.55^{b}	0.48^{c}	0.009	0.55	0.37	<0.001	0.092
Cys	0.73^{a}	0.43^{d}	0.50^{c}	0.47^{cd}	0.45^{cd}	0.59^{b}	0.027	0.32	0.50	<0.001	0.608
Phe	1.23^{bc}	1.29^{b}	1.39^{ab}	1.37^{ab}	1.45^{a}	1.16^{c}	0.025	1.27	0.89	<0.001	0.052
Thr	1.27^{a}	0.97^{c}	1.07^{b}	1.03^{bc}	1.04^{bc}	0.94^{c}	0.034	0.94	0.73	<0.001	0.073
Val	1.32^{bc}	1.49^{b}	1.58^{ab}	1.68^{a}	1.71^{a}	1.29^{c}	0.039	1.47	1.01	<0.001	0.583
Dispensable AA											
Ala	1.32^{c}	1.62^{b}	1.86^{ab}	1.84^{ab}	1.95^{a}	1.61^{b}	0.039	1.74	1.20	<0.001	0.047
Asp	2.19^{abc}	2.01^{c}	2.30^{a}	2.22^{ab}	2.35^{a}	1.98^{cd}	0.064	2.07	1.51	<0.001	0.215
Glu	6.02^{c}	6.04^{c}	6.76^{a}	6.46^{ab}	6.66^{a}	5.81^{cd}	0.086	6.15	4.53	<0.001	0.0004
Gly	1.66^{a}	1.11^{c}	1.32^{b}	1.22^{bc}	1.28^{b}	1.15^{bc}	0.048	1.14	0.93	<0.001	0.340
Pro	2.35^{b}	2.46^{ab}	2.82^{a}	2.70^{a}	2.80^{a}	2.45^{ab}	0.192	2.69	1.92	0.025	0.352
Ser	1.40^{bc}	1.46^{b}	1.67^{a}	1.58^{ab}	1.71^{a}	1.46^{ab}	0.044	1.49	1.12	<0.001	0.339
Tyr	0.83^{a}	0.75^{b}	0.83^{a}	0.80^{ab}	0.86^{a}	0.68^{c}	0.019	0.72	0.53	<0.001	0.888

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; Number of observations contributing to each mean = 5.
[2]Standard error of the mean for CM1 to 5 mash.
[3]p-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.
[a-c]Means followed by different letters within rows are significantly different (P<0.05); Include only mash CM samples.

Linear regression equations for predicting standardized ileal digestible Arg, Lys, His, Met, Thr, Ile, Leu, Phe, Val, Asp, Glu, Ser, and Tyr contents from a simple measure of ADF were presented in Table 5.8. Regression equations predicting standardized ileal digestible AA contents from ADF were significant ($P < 0.05$; except for Met and Thr) and had R^2 values ranging from 0.37 to 0.72. However, regression equations predicting standardized ileal digestible AA contents from NDF were not significant ($P = 0.374$ to 0.995) and had very low R^2 values (0.00 to 0.13; data not shown).

Table 5.8. Prediction of standardized ileal digestible amino acid contents of canola meal in growing pigs from a simple measure of ADF (n=8)[1]

#	Equation	R^2	P-value	RSD^2
1	SID ARG = 5.707 - 0.192 ADF	0.68	0.011	0.156
2	SID LYS = 4.181 - 0.133 ADF	0.57	0.031	0.139
3	SID HIS = 2.183 - 0.073 ADF	0.78	0.004	0.047
4	SID MET = 1.154 - 0.035 ADF	0.23	0.244	0.076
5	SID THR = 1.951 - 0.051 ADF	0.15	0.345	0.147
6	SID ILE = 2.865 - 0.102 ADF	0.55	0.035	0.111
7	SID LEU = 6.585 - 0.235 ADF	0.73	0.007	0.172
8	SID PHE = 3.792 - 0.136 ADF	0.73	0.007	0.099
9	SID VAL = 5.315 - 0.197 ADF	0.68	0.012	0.162
10	SID ASP = 5.526 - 0.185 ADF	0.63	0.018	0.169
11	SID GLU = 15.743 - 0.521 ADF	0.71	0.008	0.395
12	SID SER = 4.164 - 0.144 ADF	0.80	0.003	0.086
13	SID TYR = 2.051 - 0.069 ADF	0.58	0.029	0.072

[1]SID = standardized ileal digestible; ADF = acid detergent fiber.
[2]Residual standard deviation

5.5. DISCUSSION

The pigs remained healthy and consumed their meal allowances throughout the experiment. The results of this research indicate that there were variations in the chemical composition and standardized ileal digestible AA contents of CM from different processing plants. However, variations in the AID and SID values were minimal (only observed for the sulfur AA). The SID of Met ranged from 82.0 to 89.5%, while that of Cys ranged from 59.2 to 81.9% for mash CM samples This shows the impact of the variability in the total AA content of CM on the standardized ileal digestible AA contents. The differences in chemical composition of CM could either be as a result of differences in crushing conditions (particularly for the components that are sensitive to heat treatment e.g. glucosinolates, lysine, NDICP, and dietary fiber fractions) or variations in the cultivar composition of the canola seeds (Kasprzak et al., 2016). Environmental factors including soil conditions, fertilizer application, and weather conditions could be responsible for differences in CP and fat content of canola seeds as dryer temperature increases the CP content and cooler temperature increases the oil content (Fayyaz-ul-Hassan etal., 2005).

The commercial CM samples were obtained from processing plants in various locations in Canada but the growing locations of the canola seeds that were used by the crushing plants were unknown. The cultivars also could not be identified as canola seeds are usually blended prior to crushing. It is possible that some crushing plants sourced canola seeds from a different region. The blending of CM by the crushing plants is expected to eliminate cultivar differences. However, because CM has gone through heat treatment, there is the possibility that the AA content and digestibility may be reduced through Maillard reactions (Cromwell et al., 2003). It is well known that overheating of CM during crushing can lead to losses in the content and digestibility of AA (Mosenthin et al., 2016). Research has shown that SID of lysine in CM decreased during solvent

extraction processing (Almeida et al., 2014). This suggests a potential negative effect of the desolventization-toasting process (Khajali and Slominski, 2012). Similarly, Newkirk et al. (2003) found that AID coefficient of lysine was significantly reduced (from 0.87 to 0.79) in desolventized and toasted CM. Lysine damage due to heat treatment in the desolventizer/toaster was well supported in our previous study (Adewole et al., 2016) by a positive relationship between lysine and heat sensitive glucosinolate contents as well as a negative relationship between lysine and NDF and total dietary fiber contents.

The general steps for solvent extraction of oil from seeds include 1) seed flaking and cooking at 80 to 105°C for 15 to 20 min to rupture the seed coat and cells, resulting in increased oil availability; 2) pressing of the flaked and cooked seeds to remove some oil (this process results in an increase in meal temperature to 100 to 120°C); 3) solvent extraction of the pressed seeds to remove additional oil; 4) desolventization and toasting of the extracted meal at 95 -115°C for 30 min to remove the solvent; and 5) meal drying (Newkirk, 2009). The major product of the canola crushing industry is the oil, therefore the processing (i.e. desolventization and drying) of the CM by-product may not be as carefully controlled as that of the oil refinery.

The differences in AID values of Met, Cys, and Val in the current study are consistent with the results of Fan et al (1996) who reported variations ($P < 0.05$) in the AID (%) of Arg (79.4 to 84.4), Ile (66.2 to 74.5), Leu (69.8 to 77.1), Met (77.3 to 82.4), Cys (67.7 to 75.6), Val (65.3 to 72.3) and Glu (78.3 to 83.0) in CM produced in Western Canada. The reason for the differences in the AID values among CM samples has been attributed to differences in tannin content, NDF content, fiber sources and hulls content in different cultivars of CM (Fan et al., 1996). Tannins may bind to protein (AA) forming complexes resistant to proteolytic enzymes, or bind directly to enzyme proteins (Eggum and Christensen, 1975). The CM samples used in the current study varied

widely in their contents (%, DM) of NDF (24.9-32.4), NSP (19.0-22.5) and total dietary fiber (32.5-39.3). The differences in those components among CM samples could have been responsible for the observed differences in AID values. However, the differences in AID may also be related to heat treatment (Khajali and Slominski, 2012).

The values for the ileal endogenous AA losses (g/kg DMI) of Arg (0.64), Cys (0.22) and Thr (0.61) observed in this study are similar to those (0.62, 0.29 and 0.63 g/kg DMI, respectively) reported by Opapeju et al. (2006). Kiarie and Nyachoti, (2007) reported 0.34 and 0.12 g/kg DMI as ileal endogenous losses of Ile and Met. These are consistent with the values 0.36 and 0.14 g/kg DMI observed in the current study. Similarly, Woyengo et al. (2010) reported 0.66 and 0.36 g/kg DMI for Leu and Phe which is consistent with the values 0.67 and 0.36 g/kg DMI observed in this study. The ileal endogenous losses value (g/kg DMI) of Lys (0.70) observed in this study is consistent with that (0.63) reported by Yang et al. (2010). Of all analyzed AA, Pro made the largest contribution (2.89 g/kg DMI) to ileal endogenous AA losses and Met made the lowest contribution (0.14 g/kg DMI). The current data are in agreement with previous studies in which Pro constituted the largest proportion of ileal endogenous AA losses in swine (Opapeju et al., 2006; Woyengo et al., 2010).

Other than the SID of Met and Cys, there were no differences among CM samples in the SID of AA. The reason for the differences among processing plants in the SID values of the sulfur AA (Met and Cys) but not for other AA could be because CM is a rich source of the sulfur AA and are therefore more available for the Maillard reaction. The average SID values (87, 78, 85 and 72 %) observed in this study are similar to that (86, 79, 84 and 72 %) reported by Woyengo et al (2010) and that (85, 74, 85, and 70 %) reported by (NRC) 2012 for Arg, Lys, Met and Thr, respectively. On average, the standardized ileal digestible AA content (%) observed in this study

for indispensable AA were 2.1 for Arg, 0.83 for His, 0.97 for Ile, 2.24 for Leu, 1.7 for Lys, 0.5 for Met,0.49 for Cys, 1.27 for Phe, 1.01 for Thr and 1.46 for Val. In addition to processing conditions, differences in standardized ileal digestible AA contents in the current study may result from a large number of factors such as variety of canola, fertilizer application, and environmental conditions which alter the total and relative amounts of major seed proteins (albumins and globulins), resulting in differences in AA composition and digestibility (Sauer and Ozimek, 1986).

Pelleting reduced the standardized ileal digestible content of all AA and there were CM source and pelleting interactive effects on the standardized ileal digestible N and AA contents. The heat, moisture, timing, and mechanical pressure applied during conditioning and pelleting may cause some chemical and physical alterations that may have beneficial or detrimental effects on feed components (Abdollahi et al., 2013). Depending on heat treatment intensity associated with pelleting, significant improvements in nutrient digestibility have been reported for feed ingredients (Wondra et al., 1995; Gall et al., 2008). Moderate pelleting temperatures over a very short time are expected to reduce diet particle size and disrupt cell wall structures thus improving nutrient digestibility. It is possible that the heat treatment intensity applied during the pelleting of the CM samples used in the current study differed from one source to the other. This explains the reason for the CM source-pelleting interaction observed for the standardized ileal digestible AA contents of CM in the current study.

The regression equations indicate that ADF (but not NDF; data not shown) could be a more suitable predictor of standardized ileal digestible AA contents in CM because the equations involving ADF had higher R^2 values and significant p-values than those involving NDF values except for the equations predicting standardized ileal digestible Met and Thr which had low R^2 values of 0.23 and 0.15, respectively. This is probably because advanced glycation products of the

late stage Maillard reaction are concentrated in the ADF fraction (Cozannet et al., 2010; Pelletier et al., 2010). These products have higher influence on AA digestibility than products from early stages of Maillard reaction (Rooijen et al., 2014), indicating that AA involved in early stages of Maillard reaction may still be utilized. This supports the report of Broesder et al. (2013) that heat damage of feed ingredients is associated with an increase in ADF value which results in a decrease in N digestibility in cattle and sheep. Moreover, the concentration of ADF was increased in dark-coloured distillers dried grains with solubles, which suggested a greater degree of heat damage in such ingredients (Cromwell et al., 1993). In addition, Almeida (2013) observed a negative correlation between the concentration of SID AA and ADF content of CM in growing pigs and concluded that SID AA may be best predicted by equations that include the concentration of acid detergent insoluble N in the model. Having confirmed the variations in the standardized ileal digestible AA contents among CM sources, the prediction equations provide a means of quick determination of standardized ileal digestible AA for a more accurate, cost-effective, and environmental friendly swine feed formulation. The low R^2 values observed for the equations predicting standardized ileal digestible Met and Thr may imply that Met and Thr could have a different behaviour from other AA with regard to the fiber content. Similarly, low R^2 values have been reported when predicting standardized ileal digestible Met from chemical composition of cottonseed meal (Fevrier et al., 2001). Equations predicting standardized ileal digestible Arg, Lys, His, Ile, Phe, Val, Asp, Glu, Ser, and Tyr from ADF content had R^2 ranging from 0.57 to 0.80 showing that 57 to 80% of the observed variable variations were explained by the model. For equations predicting standardized ileal digestible Arg, Lys, His, Leu, and Phe, the RSD values were less than 10% of the expected mean values, implying that more than 90% of the expected mean values fall within the equations. The RSD which is also known as the root mean square error

provides an indication of the error term associated with the model. Using prediction equation reflects more accurate nutrient content prior to diet formulation allowing for more efficient diet formulation. For example, the standardized ileal digestible content of Arg and Lys for CM in the current study vary from 1.60 to 2.42 and 1.29 to 1.95%, respectively. Whereas, NRC (2012) reports average values of 1.93 and 1.53 as standardized ileal digestible Arg and Lys contents, respectively. The standardized ileal digestible Arg values obtained in the current study ranged from 82 to 125% of the tabulated NRC (2012) value while that of Lys ranged from 84 to 128% of the tabulated NRC (2012). It is therefore obvious that the use of values tabulated in NRC (2012) will over- or under-estimate the standardized ileal digestible AA contents in CM. In the study of Wang et al. (2017) using standardized ileal digestible AA contents tabulated in NRC (2012), growth performance differed among CM sources with varying nutritional quality indicating that quality differences among CM samples affect growth performance of pigs when diets are formulated with table values. Therefore, the prediction equations developed in the current study will be more accurate compared to the use of tabulated standardized ileal digestible AA values for feed formulation.

In conclusion, the standardized ileal digestible AA contents of CM vary widely with source. Pelleting reduced the standardized ileal digestible AA content of CM but its effect may be plant-dependent. Standardized ileal digestible Arg, Lys, His, Ile, Phe, Val, Asp, Glu, Ser, and Tyr contents in CM for growing pigs can be predicted from a simple measure of ADF. However, these prediction equations need to be validated using an external data set.

CHAPTER 6

EFFECTS OF CANOLA MEAL SOURCE ON THE STANDARDIZED ILEAL DIGESTIBLE AMINO ACIDS AND APPARENT METABOLIZABLE ENERGY CONTENTS FOR BROILER CHICKENS[3]

6.1 ABSTRACT

Two studies were conducted to determine the effect of canola meal (**CM**) source on the standardized ileal digestible amino acids (**AA**) and nitrogen-corrected apparent metabolizable energy (**AME_n**) contents in CM from 6 processing plants in Canada. Two processing plants provided CM in both mash and pellet forms while the others provided CM in mash form only, giving a total of 8 CM samples. In the standardized ileal digestibility (**SID**) assay, 280 birds were housed 7 per cage and fed a commercial starter diet from 1 to 14 d of age followed by the test diets from 15 to 21 d. Diets were formulated to contain CM as the only source of protein. Chromic oxide (0.3 %) was included in all diets as an indigestible marker. Diets were randomly assigned to 5 cages of 7 birds. On d 21, birds were euthanized by CO_2 asphyxiation and digesta samples were collected from the terminal ileum for SID of AA. In the AME_n assay, 330 birds were housed 6 per cage and fed a commercial starter diet from 1 to 14 d of age followed by the experimental diets from d 15 to 19. The 8 experimental diets were formulated by mixing 30% of CM with 70% of a corn-soybean meal-based diet. There were differences ($P < 0.05$) among mash CM samples in the standardized ileal digestible AA and AME_n contents in CM. Standardized ileal digestible contents

[3]A version of the material presented in Chapter six of this thesis has been published in Poultry Science. The authors are D. I. Adewole, A. Rogiewicz, B. Dyck, and B. A. Slominski. 2017 (In Press).

of Arg, Lys, Met, and Thr averaged 2.17, 1.74, 0.53, and 1.03%, respectively. There were pelleting and CM-pelleting interactive effects on the standardized ileal digestible AA and AME_n contents in CM. There were differences (P<0.05) among mash CM samples in the AME_n contents with values ranging from 1,691 to 2,041 kcal/kg DM. In conclusion, there were variations in the standardized ileal digestible AA and AME_n contents of CM. Pelleting reduced the standardized ileal digestible content of AA but its effect may be source-dependent. Regression equations for predicting standardized ileal digestible AA contents from a simple measure of ADF were developed.

Key words: canola meal, amino acid, digestibility, AME_n, broiler chickens

6.2 INTRODUCTION

Canola meal (**CM**), a by-product of the canola seed-crushing industry to extract oil is widely used as a source of amino acids (**AA**) in poultry diets. The most commonly used method to obtain canola oil is pre-press solvent extraction in which CM is subjected to higher temperatures (up to 160°C) compared to the expeller-extracted CM which is subjected to moderate temperatures of 95 to 115°C during the oil extraction process (Canola Council of Canada, 2009). Higher processing temperature, which causes overheating of oilseed meals has been reported to reduce the availability of lysine and partial destruction of cystine and perhaps other AA through Maillard reactions (Hurrel, 1990; Parsons et al., 1992). We have recently evaluated the chemical composition and nutritive value of solvent-extracted CM for pigs and observed variability in the heat-sensitive chemical constituents (lysine, glucosinolates, neutral detergent insoluble crude protein (**NDICP**), lignin, and total dietary fiber) and standardized ileal digestible AA contents (Adewole et al., 2016; 2017a). However, there is limited information about the variability in the

standardized ileal digestibility (**SID**) of AA and standardized ileal digestible AA contents of CM for broiler chickens. The digestive tract in pigs differs from that in poultry, as there was lack of a strong relationship between protein digestibility of feedstuffs in pigs and poultry (Roosenstein et al., 2000), therefore, the process of protein digestion in CM is expected to be different in pigs and broiler chickens. Opapeju et al. (2006) reported that true ileal AA digestibilities of different batches of dry extruded-expelled soybean meal were different in poultry and pigs. In a study by Palacios et al. (2004), pigs and chicks utilized nutrients in soybean meal differently with chicks having greater relative growth rate than pigs. Similarly, Green and Kiener (1989) reported greater true digestibility of AA for soybean meal in cecetomized roosters compared with pigs, although in the same study, true AA digestibilities in meat and rapeseed meals were similar in both species. It is possible that different feed ingredients induce endogenous nutrient losses in pigs and poultry differently.

The dietary fiber components of CM have been shown to be inversely related its energy digestibility in broiler chickens (Khajali and Slominski, 2012). Toghyani et al. (2014) reported differences in the AME_n contents of expeller-extracted CM of varying chemical compositions and subjected to different processing conditions for broiler chickens. To the best of our knowledge, the possible variations in AME_n content in solvent-extracted CM from different sources for broiler chickens have not been investigated.

Pelleting of CM to reduce bulkiness, dustiness and wastage during transportation and feeding is becoming a common practice (Huang, 2015). The heat applied during pelleting of CM may reduce energy and nutrient digestibility. Therefore, the objectives of this study were to determine whether the variations in the standardized ileal digestible AA with CM sources and pelleting obtained in the pig study would also apply to broiler chickens and to propose prediction

equations for standardized ileal digestible AA contents of CM from simple measures of neutral detergent (**NDF**) and acid detergent fiber (**ADF**).

.

6.3 MATERIALS AND METHODS

6.3.1 Canola Meal Samples

The CM samples used in this study were the same as those used in Chapter 5 of this thesis. The analyzed chemical compositions of the samples were presented in Tables 5.1 and 5.2 (Chapter 5).

6.3.2 Standardized ileal AA digestibility Assay

The experimental diets included 8 CM, sucrose-based diets in which the CM samples were the only sources of AA (Table 6.1). All diets were formulated based on the analyzed chemical composition (Tables 5.1 and 5.2) and contained 0.3% chromic oxide as an indigestible marker. Two hundred and eighty 1-d-old male broiler chicks were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and were housed in electrically heated Alternative Design Super Brooders (Alternative Design Manufacturing & Supply, Inc., Siloam Springs, AR) under a controlled environment. Room temperature was maintained at 32, 28, and 24°C during wk 1, 2, and 3, respectively. From d 1 to 14, birds were fed a chick starter diet that met or exceeded NRC (1994) recommendations for broiler chicks in mash form. On d 14, birds were fasted for 3 h, weighed, and distributed at 7 birds/cage based on BW. In a completely randomized design, five replicate cages were assigned to each of the 8 test diets which were fed on d 15 to 21. Birds had *ad libitum* access to feed and water throughout the study period. On d 21, birds were euthanized by CO_2 asphyxiation and the digesta from the terminal ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileocecal junction) were collected by gently squeezing the contents of

the ileum into sample bags. Digesta from birds within a cage were pooled into 1 bag and frozen immediately after collection and subsequently freeze-dried. The dried ileal digesta were stored in airtight bags at -4°C until needed for chemical analysis.

6.3.3 Apparent Metabolizable Energy (AME_n) Assay

The diets included a complete corn-soybean meal-based basal diet formulated to meet NRC (1994) nutrient requirements and 8 additional diets that contained 30% of the same CM samples used in the digestibility study and 70% of the basal diet (Table 6.2). All diets contained 0.3% chromic oxide as an indigestible marker. Three hundred and fifteen 1-d-old male Ross-308 broiler chickens were obtained from a local hatchery (Carlton Hatchery, Grunthal Manitoba, Canada). Birds were individually weighed upon arrival and were then divided into 63 groups of 5 birds balanced for BW. Each group was housed in a cage in an electrically heated Alternative Design Super Brooders (Alternative Design Manufacturing & Supply, Inc., Siloam Springs, AR) under a controlled environment. The brooder and room temperature were set at 32 and 29°C, respectively, during the first week. Room temperature was maintained at 28 and 24°C during week 2 and 3, respectively. From 1 to 14 d of age, birds were fed a chick starter diet that met or exceeded NRC (1994) recommendations for broiler chicks in mash form. On d 14, the 9 experimental diets were randomly assigned to the 63 groups (7 groups per diet) from d 14 to 19 of age. Fresh water and feed were available to all chicks for *ad libitum* intake throughout the experimental period. On d 19, excreta samples were obtained from each cage and were frozen and freeze-dried for determination of AME_n content. The experimental protocol was reviewed and approved by the Animal Care Protocol Management and Review Committee of the University of Manitoba, and birds were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

6.3.4 Chemical Analysis

Canola meal samples, test diets, and digesta samples were finely ground and were all analysed for AA. Canola meal samples were analyzed for CP, neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), ash, fat, total and phytate phosphorus, simple sugars, oligosaccharides, sucrose, non-starch polysaccharides (**NSP**), and glucosinolates. Canola meal and diet samples were analyzed for DM. Diets (from digestibility and AME_n studies), digesta and excreta samples were analysed for chromium after the samples were ashed at 600°C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian, Palo Alto, CA) according to the method of AOAC (2005, method 985.01). Diets (from the AME_n study) and excreta samples were analyzed for gross energy using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL) and N.

The contents of DM, NDF, ADF, ether extract, AA, total and phytate P, glucosinolates, simple sugars, sucrose, oligosaccharides, NSP, and total dietary fiber were determined as previously described by Adewole et al. (2016; 2017a).

6.3.5 Standardized Ileal AA Digestibility and AME_n Calculations

The apparent ileal digestibility (**AID**) coefficient of AA was calculated using the indigestible marker using the formula by Moughan et al. (1992):

$AID = [(AA/Cr)_d – (AA/Cr)_{id}/(AA/Cr)_d] \times 100,$

Where $(AA/Cr)_d$ = ratio of AA to chromium (Cr) content in diet and $(AA/Cr)_{id}$ = ratio of AA to Cr content in ileal digesta.

Basal endogenous loss estimates determined previously in our laboratory with N-free diet were used for the correction of AID coefficients. The SID coefficients were calculated as follows:

SID, % = AID + [(basal IAA_{end}/AA_{diet}) x 100], Where IAA_{end} = basal ileal endogenous amino acid loss and AA_{diet} = amino acids in diet. The endogenous ileal AA losses (g/kg of DM intake) used to calculate the SID values are presented in Table 6.5.

Standardized ileal digestible AA contents were calculated by multiplying the standardized ileal digestibility coefficients with the AA values.

Nitrogen retention and AME_n values of test ingredients were calculated as described by Leeson and Summers (2001).

6.3.6 Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) by the following model:

$Y_{ij} = \mu + D_{ij} + E_{ij}$,

where Y_{ij} = AID, SID, standardized ileal digestible AA content or AME_n of mash CM of the jth cage in the ith diet; μ = overall mean; D_i = effect of diet i =1 - 8; E_{ij} = error deviation of the jth cage in the ith diet. Means were compared using the Tukey's studentized range test when there was a significant difference at $P < 0.05$ between means. Cage was the experimental unit. Contrast statements were used to determine the difference in AID, SID, standardized ileal digestible AA content or AME_n between mash and pelleted CM samples. The contrast results show that pelleting reduced the AID, SID, and standardized ileal digestible AA contents in one of the CM samples and increased them in the other sample. Therefore, the 4 dietary treatments that contained either CM5 or CM6 mash or pellets were further compared as a 2 x 2 factorial arrangement to investigate the interactive effects of CM source and pelleting. The REG procedure of SAS was used to develop prediction equations for determining standardized ileal digestible AA contents from chemical compositions. Statistical significance was considered at $P \leq 0.05$.

6.4 RESULTS

The analyzed chemical and AA compositions of CM samples are presented in Tables 5.1 and 5.2 (Chapter 5). The CM samples used in this study vary in chemical compositions with CM5 mash having the highest CP, non-phytate P, Lys, and Arg contents of 43.6, 0.46, 2.45, and 2.79%, DM, respectively. Canola meal 2 mash had the highest NDF (32.4%, DM) and fat (4.9%, DM) contents. Pelleted CM samples had lower AA contents compared with their mash forms except for Ser and Thr in CM5. Also, CM6 (both mash and pellets) had extremely lower AA contents than other CM samples. The fat (1.6-4.9%, DM) and NDF (24.9-32.4 %, DM) contents of the CM samples were highly variable.

Table 6.1. Ingredient and analyzed compositions (%) of experimental diets used in the standardized ilea amino acid digestibility assay[1]

Ingredient	Diets							
	CM1	CM2	CM3	CM4	CM5		CM6	
	mash	mash	mash	Mash	mash	pellets	mash	pellets
Canola meal	59.6	56.9	55.9	54.86	50.6	52.4	56.9	57.1
Canola oil	6.4	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Sucrose	30.05	33.10	34.04	34.97	39.24	37.47	33.09	32.81
Calcium carbonate	0.90	0.98	0.96	0.87	1.06	0.98	0.96	0.89
Dicalcium phosphate	1.25	1.22	1.30	1.50	1.30	1.35	1.25	1.40
Mineral premix[2]	0.50	0.50	0.50	0.50	0.5	0.50	0.50	0.50
Vitamin premix[3]	1.00	1.00	1.0	1.00	1.0	1.00	1.00	1.00
Chromium oxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100	100
Analysed composition[4]								
Methionine	0.35	0.33	0.30	0.33	0.31	0.32	0.31	0.37
Cysteine	0.50	0.47	0.48	0.48	0.43	0.44	0.50	0.48
Lysine	1.09	1.01	1.03	1.01	1.01	1.01	1.01	1.14
Arginine	1.17	1.07	1.17	1.09	1.07	1.11	1.23	1.13
Threonine	0.79	0.73	0.75	0.70	0.69	0.70	0.76	0.80

[1]Diets were formulated based on analyzed chemical composition of ingredients.

[2]Provided the following per kilogram of diet: Mn, 70 mg; Cu, 10 mg; Fe, 80 mg; Zn, 80 mg; Se, 0.3 mg; I, 0.5 mg; Na, 1.7 g.

[3] provided the following per kilogram of diet: vitamin A, 8,255 IU; vitamin D_3, 3,000 IU; vitamin E, 30.0 IU; vitamin B_{12}, 0.013 mg; vitamin K, 2.0 mg; niacin, 24.5 mg; choline, 1,081 mg; folic acid, 4.0 mg; biotin, 0.25 mg; riboflavin, 6.0 mg.

[4]Dry matter basis.

Table 6.2. Ingredient, and calculated and analyzed compositions of the basal diet used in the AME_n assay

Component	Basal Diet
Ingredient composition, %	
Corn	35.1
Wheat	28.0
Soybean meal	24.0
Fish meal	6.98
Canola oil	2.80
Calcium carbonate	1.10
Monocalcium phosphate	0.50
DL-Methionine	0.12
Mineral premix[1]	0.50
Vitamin premix[2]	1.00
Calculated composition	
Crude protein, % DM	22.3
AME_n, Kcal/kg DM	3,102
Calcium, % DM	1.03
Available P, % DM	0.50
Met + Cys, % DM	0.86
Lys, % DM	1.56
Thr, % DM	1.00
Analysed composition	
Crude protein, %	23.7
AME_n, Kcal/kg DM	3,052

[1]Provided the following per kilogram of diet: Mn, 70 mg; Cu, 10 mg; Fe, 80 mg; Zn, 80 mg; Se, 0.3 mg; I, 0.5 mg; Na, 1.7 g

[2] provided the following per kilogram of diet: vitamin A, 8,255 IU; vitamin D_3, 3,000 IU; vitamin E, 30.0 IU; vitamin B_{12}, 0.013 mg; vitamin K, 2.0 mg; niacin, 24.5 mg; choline, 1,081 mg; folic acid, 4.0 mg; biotin, 0.25 mg; riboflavin, 6.0 mg

6.4.1 Apparent and Standardized Ileal Digestibility of CM

Table 6.3 shows the AID of AA. There were differences ($P < 0.05$) among mash CM samples in the AID of all AA except arginine, phenylalanine, threonine, valine, cysteine, and tyrosine. Among mash CM samples, the AID values of lysine ranged from 77.4% (CM6 mash) to 81.1 % (CM5 mash). Differences in AID values among mash CM samples ranged from 2.6 (phenylalanine) to 9.5 (histidine) percentage units for indispensable AA and 3.1 (glutamic acid) to 6.2 (aspartic acid and serine) percentage units for dispensable AA. Among mash CM samples, CM5 mash had the highest ($P < 0.05$) AID values for all AA except threonine and glycine. There was no effect of pelleting except for isoleucine and valine. However, there was CM-pelleting interactive effect ($P < 0.05$) on the AID of arginine, isoleucine, leucine, phenylalanine, valine, and tyrosine.

Table 6.4 shows the SID of AA in CM fed to broiler chickens. Differences ($P < 0.05$) existed among mash CM samples for the SID of all AA except isoleucine, phenylalanine, threonine, valine, and tyrosine. Among mash CM samples, CM5 mash had the highest ($P < 0.05$) SID values for all AA except isoleucine, phenylalanine, and tyrosine. Differences in SID values among mash CM samples ranged from 2.9 (arginine) to 9.5 (histidine) percentage units for indispensable AA and 3.2 (glutamic acid) to 8.7 (tyrosine) percentage units for dispensable AA. Similar to the AID results, CM5 pellets had the highest SID values for all AA. However, CM6 pellets had the lowest SID values for most AA. There was no pelleting effect on SID values except for isoleucine and valine. However, there was plant-pelleting interaction for the SID of arginine, isoleucine, valine, and tyrosine.

As presented in Table 6.5, there were differences ($P < 0.05$) among CM samples in the standardized ileal digestible contents of all AA. Canola meal 5 mash had the highest standardized ileal digestible content of all AA except methionine, cysteine, threonine, and glycine in which CM1mash had the highest values. Canola meal 6 pellets had the lowest ($P < 0.05$) standardized ileal digestible content of all AA except cysteine in which CM5 pellets had the lowest ($P < 0.05$). There was CM-pelleting interaction for the SID contents of all AA except cysteine.

Table 6.3. Effect of canola meal source and pelleting on the apparent ileal amino acid digestibility for broiler chickens fed canola meal from different sources[1]

Component	CM1 mash	CM2 mash	CM3 mash	CM4 mash	CM5 mash	CM6 mash	SEM[2]	CM5 Pellets	CM6 pellets	P-values[3]	
										Pelleting	CM * pelleting
Indispensable AA											
Arg	87.7	86.5	85.6	86.1	88.4	86.3	0.58	89.5	84.6	0.512	0.009
His	59.6^{ab}	52.2^{b}	57.5^{ab}	54.1^{ab}	60.9^{a}	51.4^{b}	1.72	63.1	54.1	0.472	0.994
Ile	77.0	79.4	81.9	77.8	80.3	79.7	0.86	81.7	73.3	0.013	<0.001
Leu	82.3^{ab}	82.3^{ab}	81.9^{ab}	81.8^{ab}	84.7^{a}	81.7^{ab}	0.70	85.5	79.8	0.099	0.012
Lys	79.6^{ab}	78.1^{ab}	79.2^{ab}	77.7^{b}	81.1^{a}	77.4^{b}	0.90	82.1	76.0	0.356	0.072
Met	90.5^{ab}	88.4^{abc}	85.4^{c}	88.0^{abc}	91.4^{a}	86.9^{bc}	0.75	91.3	88.7	0.156	0.249
Phe	83.2	83.5	84.6	82.9	85.4	82.8	0.69	86.8	80.4	0.365	0.011
Thr	71.8	70.6	74.8	68.7	72.8	68.8	1.09	74.7	66.8	0.607	0.058
Val	74.4	76.7	76.9	75.8	78.2	77.0	1.03	79.6	70.3	0.017	<0.001
Dispensable AA											
Ala	81.4^{ab}	79.5^{b}	82.1^{ab}	81.2^{ab}	84.4^{a}	79.5^{b}	0.75	84.6	78.9	0.105	0.264
Asp	76.6^{a}	71.8^{b}	74.7^{ab}	72.8^{ab}	77.1^{a}	70.9^{b}	1.01	78.0	71.1	0.279	0.467
Cys	73.3	69.3	71.4	68.6	72.0	68.3	1.35	76.3	70.5	0.012	0.163
Glu	87.6^{ab}	86.4^{abc}	86.8^{abc}	85.8^{bc}	88.9^{a}	85.8^{bc}	0.60	89.2	86.0	0.262	0.586
Gly	78.6^{ab}	76.1^{abc}	80.1^{a}	74.2^{bc}	79.2^{ab}	74.8^{abc}	1.00	80.0	74.1	0.492	0.258
Pro	74.9^{ab}	73.4^{ab}	72.2^{b}	73.3^{ab}	77.3^{a}	72.4^{b}	1.02	78.0	73.3	0.269	0.769
Ser	74.4^{ab}	71.7^{bc}	73.4^{ab}	72.5b^{abc}	76.5^{a}	70.3^{c}	1.02	77.6	70.4	0.260	0.398
Tyr	77.6	77.2	81.6	75.7	79.0	77.0	0.92	80.7	73.3	0.223	0.003

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; Number of observations contributing to each mean = 5.
[2]Standard error of the mean for CM1 to 5 mash.
[3]p-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.
[a-c]Means followed by different letters within rows are significantly different (P<0.05); Statistical analysis include only mash CM samples.

Table 6.4. Effect of canola meal source and pelleting on the standardized ileal amino acid digestibility for broiler chickens fed canola meal from different sources[1]

Component	EL, g/kg DMI[2]	CM1 mash	CM2 mash	CM3 mash	CM4 mash	CM5 mash	CM6 mash	SEM[3]	CM5 pellets	CM6 pellets	P-values[4]	
											Pelleting	CM * Pelleting
Indispensable AA												
Arg	0.145	89.0^{ab}	87.8^{ab}	86.9^{b}	87.4^{ab}	89.8^{a}	87.5^{ab}	0.57	90.8	85.8	0.554	0.025
His	0.083	60.9^{ab}	53.6^{b}	60.0^{ab}	55.4^{ab}	62.3^{a}	52.8^{b}	1.63	64.4	55.3	0.254	0.929
Ile	0.117	79.1	81.4	84.8	80.0	82.4	81.4	0.78	83.9	75.2	0.021	<0.001
Leu	0.207	83.9^{ab}	84.0^{ab}	84.1^{a}	83.5^{ab}	86.4^{a}	83.1^{ab}	0.64	87.2	81.3	0.416	0.077
Lys	0.095	80.5^{ab}	79.0^{ab}	80.9^{ab}	78.6^{ab}	82.0^{a}	78.2^{ab}	0.81	83.0	76.8	0.846	0.178
Met	0.046	91.8^{ab}	87.8^{abc}	87.0^{c}	89.4^{abc}	92.8^{a}	88.4^{bc}	0.76	92.7	90.0	0.269	0.199
Phe	0.120	84.8	83.5	86.8	82.9	85.4	82.8	0.71	86.8	80.^{c}	0.575	0.037
Thr	0.345	76.2	75.4	74.8	73.6	77.8	73.4	1.02	79.7	71.1	0.884	0.108
Val	0.177	76.6	78.8	79.7	77.9	80.4	78.8	0.96	81.8	72.3	0.023	0.001
Dispensable AA												
Ala	0.169	83.4^{ab}	81.8^{bc}	84.4^{b}	83.0^{bc}	86.3^{a}	81.2^{bc}	0.68	86.5	80.6	0.836	0.607
Asp	0.314	78.8^{a}	74.1^{b}	77.8^{a}	75.1^{ab}	79.4^{a}	73.0^{b}	0.96	80.3	73.2	0.621	0.739
Cys	0.141	76.4^{a}	69.3^{b}	75.3^{a}	68.6^{b}	72.0^{ab}	68.3^{b}	1.32	76.3	70.5	0.058	0.501
Glu	0.395	88.7^{ab}	87.6^{abc}	88.2^{ab}	86.9^{bc}	90.1^{a}	86.9^{bc}	0.51	90.3	87.1	0.692	0.925
Gly	0.203	80.8^{ab}	78.5^{abc}	82.9^{a}	76.7^{bc}	81.7^{a}	77.1^{bc}	0.91	82.5	76.3	0.968	0.432
Pro	0.267	79.5^{ab}	75.1^{ab}	74.0^{b}	74.9^{ab}	79.1^{a}	74.0^{b}	0.97	79.7	74.8	0.495	0.932
Ser	0.315	77.7^{ab}	75.0^{bc}	77.0^{ab}	75.7^{abc}	79.9^{a}	73.4^{bc}	0.93	80.9	73.4	0.627	0.652
Tyr	0.559	96.8	89.7	93.2	88.6	92.1	88.1	1.08	94.1	84.8	0.493	0.044

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; number of observations contributing to each mean =5.

[2] EL = Ileal endogenous amino acid losses values from a previous experiment; DMI = dry matter intake.

[3]Standard error of the mean for CM1 to 5 mash.

[4]P-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.

[a-c]Means followed by different letters within rows are significantly different (P<0.05); includes only mash CM samples.

Table 6.5. Effect of canola meal source and pelleting on the standardized ileal digestible amino acids contents for broiler chickens fed canola meal from different sources[1]

Component	CM1 mash	CM2 mash	CM3 mash	CM4 mash	CM5 mash	CM6 mash	SEM[2]	CM5 Pellets	CM6 pellets	P-values[3]	
										pelleting	CM * pelleting
Indispensable AA											
Arg	2.27^{bc}	2.18^{c}	2.31^{b}	2.22^{bc}	2.50^{a}	1.97^{d}	0.01	2.32	1.58	<0.001	<0.001
His	0.71^{bcd}	0.68^{cd}	0.82^{a}	0.71^{bcd}	0.85^{a}	0.63^{d}	0.01	0.84	0.53	<0.001	<0.001
Ile	1.01^{b}	1.05^{b}	1.07^{ab}	1.04^{b}	1.11^{a}	0.83^{c}	0.01	1.01	0.62	<0.001	0.003
Leu	2.17^{cd}	2.38^{bc}	2.49^{ab}	2.43^{bc}	2.67^{a}	2.09^{c}	0.01	2.49	1.60	<0.001	<0.001
Lys	1.81^{b}	1.74^{c}	1.86^{b}	1.77^{bc}	2.01^{a}	1.57^{d}	0.01	1.91	1.27	<0.001	<0.001
Met	0.66^{a}	0.50^{d}	0.55^{c}	0.49^{d}	0.59^{b}	0.49^{d}	0.00	0.57	0.41	<0.001	<0.001
Phe	1.27^{bc}	1.34^{b}	1.45^{a}	1.34^{b}	1.48^{a}	1.15^{c}	0.00	1.39	0.88	<0.001	<0.001
Thr	1.31^{a}	1.03^{b}	1.14^{ab}	1.00^{b}	1.05^{b}	0.90^{c}	0.01	1.08	0.70	<0.001	<0.001
Val	1.32^{c}	1.59^{b}	1.58^{b}	1.60^{b}	1.72^{a}	1.28^{d}	0.01	1.58	0.97	<0.001	0.018
Dispensable AA											
Ala	1.36^{e}	1.72^{c}	1.94^{ab}	1.86^{b}	2.08^{a}	1.97^{d}	0.01	1.98	1.22	<0.001	<0.001
Asp	2.24^{ab}	2.12^{b}	2.35^{a}	2.17^{b}	2.45^{a}	0.63^{d}	0.02	2.38	1.48	<0.001	<0.001
Cys	0.69^{a}	0.50^{cd}	0.53^{bc}	0.49^{cd}	0.47^{d}	0.83^{c}	0.00	0.40	0.48	<0.001	0.076
Glu	6.13^{cd}	6.28^{c}	6.79^{ab}	6.36^{c}	6.86^{a}	2.09^{c}	0.01	6.50	4.52	<0.001	<0.001
Gly	1.586^{a}	1.26^{b}	1.37^{b}	1.19^{c}	1.30^{b}	1.57^{d}	0.00	1.27	0.88	<0.001	<0.001
Pro	2.03^{cd}	2.34^{b}	2.45^{ab}	2.45^{ab}	2.86^{a}	0.49^{d}	0.01	2.71	1.66	<0.001	<0.001
Ser	1.41^{b}	1.48^{b}	1.65^{ab}	1.53^{b}	1.74^{a}	1.15^{c}	0.00	1.68	1.07	<0.001	0.001
Tyr	0.92^{b}	0.88^{bc}	0.89^{b}	0.87^{bc}	0.98^{ab}	0.90^{c}	0.00	0.91	0.58	<0.001	0.001

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; number of observations contributing to each mean = 5.
[2]Standard error of the mean for CM1 to 5 mash.
[3]p-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.
[a-d]Means followed by different letters within rows are significantly different (P<0.05); include only mash CM samples.

Linear regression equations for predicting standardized ileal digestible arginine, lysine, histidine, methionine, threonine, isoleucine, leucine, phenylalanine, valine, asparagine, glutamine, and serine from a simple measure of ADF are presented in Table 6.8. Regression equations predicting standardized ileal digestible AA contents from NDF were not significant ($P > 0.05$) and had very low R^2 values (0.00 - 0.29; data not shown).

Table 6.6. Prediction of standardized ileal digestible amino acid contents of canola meal for broiler chickens from a simple measure of acid detergent fiber (n=8)[1]

#	Equation	R^2	P-value	RSD^2
1	SID ARG = 5.807 - 0.195 ADF	0.60	0.025	0.192
2	SID HIS = 2.133 - 0.075 ADF	0.53	0.042	0.085
3	SID LEU = 6.672 - 0.234 ADF	0.62	0.021	0.222
4	SID ILE = 2.917- 0.104 ADF	0.45	0.070	0.138
5	SID LYS = 4.592 - 0.153 ADF	0.53	0.039	0.171
6	SID PHE = 3.954 - 0.143ADF	0.66	0.014	0.123
7	SID MET = 1.078 - 0.029 ADF	0.19	0.277	0.072
8	SID THR = 2.253 - 0.065 ADF	0.14	0.370	0.198
9	SID GLU = 16.185 - 0.536 ADF	0.60	0.023	0.521
10	SID ASP = 6.092 - 0.212 ADF	0.52	0.044	0.245
11	SID SER = 4.424 - 0.157 ADF	0.67	0.014	0.134

[1]SID = standardized ileal digestible; ADF = acid detergent fiber.
[2]Residual standard deviation.

6.4.2 AME_n Content of CM

As presented in Table 6.7, there were differences (P = 0.001) among CM samples in the AME_n values. Among mash CM samples the AME_n contents (kcal/kg DM) ranged from 1,691 (CM3 mash) to 2,041 (CM1 mash). There was no effect (P = 0.477) of pelleting on the AME_n content. However, there was CM-pelleting interaction (P = 0.005) on the AME_n content as evident by the fact that pelleting had more profound effect on CM6 (1,590 versus 1,871 kcal/kg) than CM5 (1,812 versus 1,871 kcal/kg).

Table 6.7. Effect of canola meal source and pelleting on nitrogen corrected apparent metabolizable energy (AME_n) contents for broiler chickens fed canola meal from different sources[1]

Canola meal	AME_n, kcal/kg DM
CM1 mash	2,041[a]
CM2 mash	1,888[ab]
CM3 mash	1,691[b]
CM4 mash	1,727[b]
CM5 mash	1,694[b]
CM6 mash	1,871[ab]
SEM[2]	60.88
CM5 pellets	1,812
CM6 pellets	1,590
Pelleting[3]	0.477
CM * Pelleting[3]	0.005

[1]CM1 to CM6 are diets containing canola meal samples 1 to 6; number of observations contributing to each mean = 7.
[2]Standard error of the mean for CM1 to 5 mash.
[3]p-values from a 2x2 factorial analysis of CM5 and CM6 mash versus pellets.
[a-b]Means followed by different letters within a column are significantly different ($P<0.05$); include only mash CM samples.

6.5 DISCUSSION

The results of this study indicate that there is a high degree of variability in the AID, SID, standardized ileal digestible AA and AME_n contents among sources of CM available to the feed industry, especially for broilers. The range of CP content (39.2-43.6 %, DM) of the CM samples used in the current study was close to the average of 41.1 %, DM reported by NRC (2012) for solvent extracted CM. The variations in chemical composition (particularly the components that are sensitive to heat treatment e.g. glucosinolates, lysine, NDICP, and dietary fiber fractions) among the CM samples and the extremely lower AA content in CM6 (both mash and pellets) could be as a result of differences in canola seed crushing conditions especially, the desolventization/toasting process (Newkirk et al., 2003 a,b). It has been demonstrated that applying high temperatures may be beneficial because it would destroy the glucosinolates and improve the CM flavor and palatability (Jenson et al., 1995). However, overheating of CM during processing could lead to Maillard reactions which result in the formation of carbohydrate-protein complexes, which are neutral detergent insoluble (Van Soest, 1994). It has also been reported that overheating of soybean and other protein ingredients reduces their nutritional value due to Maillard reaction and partial destruction of cystine and certain other AA (Evans and Butts, 1949; Carpenter, 1960; Hulshof et al., 2016). The possibility of lysine damage due to heat treatment was well supported in our previous study (Adewole et al., 2016) by a positive relationship between lysine and heat sensitive glucosinolate contents, as well as a negative relationship between lysine and NDF and total dietary fiber contents. Therefore, the high variations in the NDF (24.9 - 34.6%) and NDICP (2.51 - 7.26%) contents of the CM samples used in the current study suggest the presence of Maillard reaction products. Classen et al. (2004) also reported increased NDICP in CM after desolventization/toasting.

The variations among CM samples in the AID and SID of most AA are expected because of the variations in dietary fiber fractions (including NDICP) among the CM samples. Among the indispensable AA, only the AID of arginine, histidine, leucine, lysine, and methionine varied among CM sources. This supports the report of Cromwell et al. (1993) that lysine, arginine, and cystine were more sensitive to overheating than were other AA and that lysine and the sulfur AA concentrations were highly predictive of chick growth performance. Newkirk et al. (2003a, b) reported greater AID of AA for non-toasted than toasted CM fed to broiler chickens and also attributed it to Maillard reactions. Classen et al. (2004) also reported that Maillard reactions occurred in CM during the desolventization and toasting process when the meal temperature and moisture content were at least 105°C and 10%, respectively. The moisture content in CM before desolventization/toasting step is approximately 7% (Newkirk et al., 2003a). However, during desolventization/toasting, the solvent is removed from the meal by infusing hot steam into the desolventizer/toaster which increases meal moisture content to as high as 15-18% (Spragg and Mailer, 2007).

The SID of AA in CM for broiler chickens has been reported in few studies (Adedokun et al., 2008; Woyengo et al., 2010). The SID values obtained in the current study was slightly higher than those reported by Woyengo et al. (2010) but similar to those reported by Adedokun et al. (2008) for solvent extracted CM. This could be because Woyengo et al. (2010) used the substitution method for the determination of AID and SID whereas the direct method was employed in the current study and in the study of Adedokun et al. (2008). The direct procedure is normally considered when the test feedstuff can be formulated as the sole source of AA in the diet (Adeola, 2001) and should be used with feedstuffs that have a relatively high protein content (at least 18%, as-is basis) such as CM (Gabert et al., 2001). This procedure is relatively easy and

simple, only one diet is needed and the determined diet digestibility equals that of the test ingredient (Zhang and Adeola, 2017). However, in the case of cereal grains which have relatively low protein content, the test ingredients can only be formulated to replace a portion of the basal diet to provide the AA, the difference method, including substitution can be applied to get equally robust values (Gabert et al., 2001; Zhang and Adeola, 2017). Woyengo et al. (2010) reported lower AID and SID of AA in solvent extracted CM than in expeller extracted meal and attributed the reduction to the desolventization and toasting of the meal during solvent extraction, leading to Maillard reactions. In the current study, the differences in the standardized ileal digestible AA contents among CM samples reflect differences in total content of AA and differences in standardized ileal digestibility of AA.

The regression equations indicate that ADF (but not NDF; data not shown) could be a suitable predictor of standardized ileal digestible AA contents in CM because the equations involving ADF had higher R^2 values and significant p-values than those involving NDF values. This supports the report of Cromwell et al. (1993) that among other chemical constituents (fat, dietary fiber, ADF, NDF, and ash) of dried distillers' grains with solubles, only ADF seemed to be related to nutritional value. The relationship of increased ADF concentration of feed ingredient with reduced nutritional value and growth performance in broiler chickens is plausible, because ADF is associated with the more indigestible components of feed (Goering and Van Soest, 1970). This also supports the report of Adewole et al. (2017a) that significant relationships were obtained between the ADF content of CM and the standardized ilea digestible AA contents. Equations predicting standardized ileal digestible arginine, lysine, histidine, phenylalanine, asparagine, glutamine, and serine from ADF content had R^2 ranging from 0.52 to 0.67 showing that 52 to 67% of the of the observed variable variations were explained by the model. Also, equations predicting

standardized ileal digestible arginine, lysine, leucine, and phenylalanine had RSD values that were less than 10% of the expected mean values which implies that more 90% of the expected mean values fall within the equation.

The variation in the AME_n content among CM samples observed in the current study may be due to variations in chemical compositions which include fat (2.9 - 4.9% DM), sucrose (6.2 – 7.0% DM), NDF (24.9 – 34.6% DM) and ADF (17.4 – 21.0% DM). The fiber content in CM has been shown to be inversely related to energy digestibility of the meal (Downey and Bell, 1990). High fiber content in CM sample could accelerate digesta transit time thereby reducing the contact between nutrients and endogenous enzymes or limited the capacity of the gut to use complex carbohydrates, which could translate to higher excreta output (Toghyani et al., 2014) and thereby reducing nutrient digestibility. Adeola and Ileleji (2009) obtained 589 kcal/kg higher AME in corn distillers grains with solubles, which had 50 and 45% lower contents of NDF and ADF, respectively, than corn distillers' grains without solubles. Overheating of CM during processing causes the formation of Maillard reaction products, thus adding to the lignin content, which could be manifested in the ADF and NDF fractions (Molero-Vilchez and Wedzicha, 1997). Among the CM samples used in the current study, CM1 mash had the lowest NDF (25.7%, DM) and glycoprotein (also known as neutral detergent insoluble CP; 2.5%, DM) contents. Interestingly, CM1 mash also had the highest AME_n content (2,041 kcal/kg). This is consistent with the report of Toghyani et al. (2014) that NDF had the strongest correlation with AME_n. It is generally accepted that fiber and fat contents may have the greatest impact on AME_n of CM (Toghyani et al., 2014). The fiber and fat contents were not significantly correlated with AME_n in the current study, probably because of small number of samples and because the fat contents were rather consistent among the CM samples. The mean AME_n value of 1,789 kcal/kg (DM basis) obtained

in the current study is comparable to that of 1,801 kcal/kg (DM basis) obtained by Woyengo et al. (2010) for solvent extracted CM.

In conclusion, there were differences in the AID and standardized ileal digestible AA contents for broiler chickens among CM from different sources. Pelleting may reduce the standardized ileal digestible AA content of CM but its effect may be source-dependent. Nutritionists should therefore be cautious of the source of data for standardized ileal digestible AA and AME_n contents when formulating diets to reduce feed costs, N excretion and enhance performance. Standardized ileal digestible lysine, leucine, phenylalanine, and arginine of CM for broiler chickens can be predicted from a simple measure of ADF. Acid detergent fiber is a simple, fast, and inexpensive chemical analysis that can be easily obtained by nutritionists and animal producers. However, validation of the equations using independent samples is necessary to gain more confidence in using them.

CHAPTER 7

GENERAL DISCUSSION

There are about 14 major commercial canola crushing plants in Canada. In this thesis, samples were collected through Canola Council of Canada from 11 crushing plants. It was a blind study as the specific crushing plants from which samples originated were not known to the investigators.

All the crushing plants involved in this study use the pre-press solvent extraction process which is currently the most effective method of extracting oil from canola seeds (Spragg and Mailer, 2007; Seneviratne et al., 2010). The steps of pre-press solvent extraction process of canola seed have been detailed by Unger (1990). Briefly, canola seed is preconditioned prior to processing by heating at 30 to 40°C for 30 to 45 min to prevent shattering and to improve oil extraction. Canola seed is then flaked to break the hulls and rupture the oil cells. Following flaking, the seed is cooked (75 to 85°C for 20 to 40 min) by passing through a series of steam heated cooking units. Screw pressing of the cooked flakes reduces the oil content by 60 to 70% and produces press-cake. The remaining oil in the press-cake is then removed by solvent extraction. The solvent is then removed from the solvent-extracted meal in the DT by heating the meal to 103 to 107°C for 30 to 40 min. Pelleting of CM is becoming a common practise for the purposes of reducing the bulkiness and dustiness during transportation and reducing *Salmonella* cross-contamination by means of heat treatment. Some pellet mills use 70 – 75˚C while some use 80˚C or higher.

Heating in the cooker and the DT is likely to reduce protein quality of the different intermediate canola products at various stages of processing and eventually the quality of the final meal. Processing conditions applied in canola seed processing have been identified as reducing

CM protein quality (Mosenthin et al., 2016). Desolventization is the process of removing hexane used to extract the oil in the DT. It is called a DT because it does not only desolventizes the meal but also imparts a toasting process to reduce the level of heat-labile anti-nutritional factors in the meal (Newkirk and Classen, 2002). High processing temperature has been reported to reduce the nutritive value of feedstuffs (Almeida et al., 2014). The major issues of relevance for the oilseed processing industry are the effects of temperature applied via steam and pressure within both the expeller and desolventization operations (Toghyani et al., 2014; Barekatain et al., 2017; Kasprzak et al., 2017; Toghyani et al., 2017).

In the current thesis, the actual conditions of processing (including pelleting) were not obtained from the processing plants because such information was limited due to commercial confidentiality. The lack of information regarding processing conditions from the various processing plants is a major limitation of this study. However, based on the available data in Canada, as well as information from other countries, there is a clear link between processing conditions and meal quality. It is recognised that processing plants are operated with different conditions applied in terms of seed conditioning, expeller temperatures, solvent extraction and desolventization and toasting. Conditions used in the different processing plants could be different due to the type and brand of equipment used, the age of equipment (new versus old plants), and the preferences of the plant engineers in facilitating the most cost-effective crushing operation. To date, there has not been a readily available method of assessing the impact of on-site changes to processing conditions upon CM quality. In Canada, where most plants use the solvent extraction process, industry results have identified that the major heat damage is occurring within the DT (Classen et al., 2004). Research has suggested that further work should be done to assess the nutritive values of CM to better guide its use in formulating effective animal diets.

We hypothesised that there would be differences among processing plants and years in the chemical compositions of CM produced by the pre-press solvent extraction method as well as differences in nutritive compositions for broiler chickens and growing pigs. Variability in chemical composition of CM was previously assessed by Bell and Keith (1991) but there has not been a comparable survey since then. The survey of Bell and Keith (1991) was based on limited numbers of samples, limited number of analyses, and did not provide any year-to-year variation in commercial CM. Improved methodology for the determination of CP, total dietary fiber and its components, AA, and glucosinolates are all suggestive of a need for periodic reassessment of the chemical composition of CM. In the fourth chapter of this thesis, it was revealed that there were differences in the chemical compositions (crude protein, fat, total P, NDF, total dietary fiber, NDICP, glucosinolates, simple sugars, oligosaccharides, and AA) of CM among processing plants in Canada using the same extraction method and among the years considered. Significant correlations were also observed between lysine and other heat-sensitive components which include glucosinolates, NDF, NDICP, and total dietary fiber. It is important to note that after the first 2 years (2011 and 2012) of the chemical composition survey which was reported in Chapter 4 of this thesis, recommendations regarding processing conditions were given through Canola Council of Canada to Plants 2 and 9 which produced relatively poor quality CM based on the chemical composition (mainly dietary fiber components and lysine) results from 2011 and 2012. It was interesting to see that during the following year (2013), there was a drastic reduction in the contents of dietary fiber and its components in those plants (see Appendix IV to VI). The improvement in the contents of dietary fiber components in the CM samples from those 2 plants following recommendations could be because of lower temperature used in the DT.

Accurate knowledge of the digestible AA contents would be very useful for a more accurate and cost-effective diet formulation and in the ranking of feedstuffs (Sauer and Ozimek, 1986). Chapters 5 and 6 of this thesis revealed that there were differences among processing plants in the nutritional value of CM for pigs and broiler chickens, respectively. These chapters also demonstrated that pelleting may reduce the nutritional value of CM for both broiler chickens and growing pigs. The effect of pelleting may be plant-dependent, showing that pelleting *per se* may not be the issue but the conditions under which the pelleting is done. Although, standardized ileal digestibility of AA in CM have been previously determined in pigs and broiler chickens (as discussed in the literature review), the current study was the first that determined the effect of CM source on the standardized ileal digestible AA contents in pigs or broiler chickens. Stein et al. (2014) and Sotak-Peper et al. (2017) have reported the effect of distilers' dried grains with solubles and soybean meal sources, respectively, on their standardized ileal AA digestibility values.

Two species of non-ruminant animals (pigs and broiler chickens) were used in this research. The two species showed differences in digestive physiology (Crevieu-Gabriel et al., 1999). Body temperature is slightly higher in chickens, about 40°C as opposed to 37°C in pigs. The pH values along the digestive tract are not the same (Braude et al., 1976) and depending on the pH, proteins have various structures which can lead to various susceptibilities to hydrolysis. The transit time to the end of the small intestine is shorter in chickens, about 4 h for soluble compounds and 6 h for insoluble compounds (Sklan et al., 1975), than in pigs which have a transit time of about 5 h for soluble compounds and 14 h, 30 min for insoluble compounds (Clemens et al., 1975). Reports from previous studies (Green and Kiener 1989; Palacios et al., 2004; Opapeju et al., 2006) have suggested that the possibility of using poultry as a model for pigs is dependent on the type of feed ingredient, as different feed ingredients induce endogenous nutrient losses in

pigs and poultry differently. However, the close similarities in the standardized ileal digestible AA contents between broiler chickens and pigs observed in Chapters 5 and 6 of this thesis is worthy of note. It suggests that broiler chickens could be a useful model for growing pigs when determining digestible AA contents for CM. Amino acid digestibility measurements in pigs are more expensive and logistically more challenging than those in broilers. The measurement of ileal AA digestibility in pigs requires the use of cannulated pigs, which is time-consuming and may cause discomfort in the animals (Roosenstein et al., 2000). Chickens are less expensive, can be used expeditiously and are subjected to little discomfort (Roosenstein et al., 2000). For similar reasons, Zijlstra et al. (2011) have attempted to predict the digestible energy content of barley for pigs from the its AME_n values from broiler chickens. Presented in Figures 7.1 to 7.3 are the relationships between standardized ileal digestible lysine, methionine, and arginine contents of CM in broiler chickens and growing pigs (data were obtained from Chapters 5 and 6 of the current thesis).

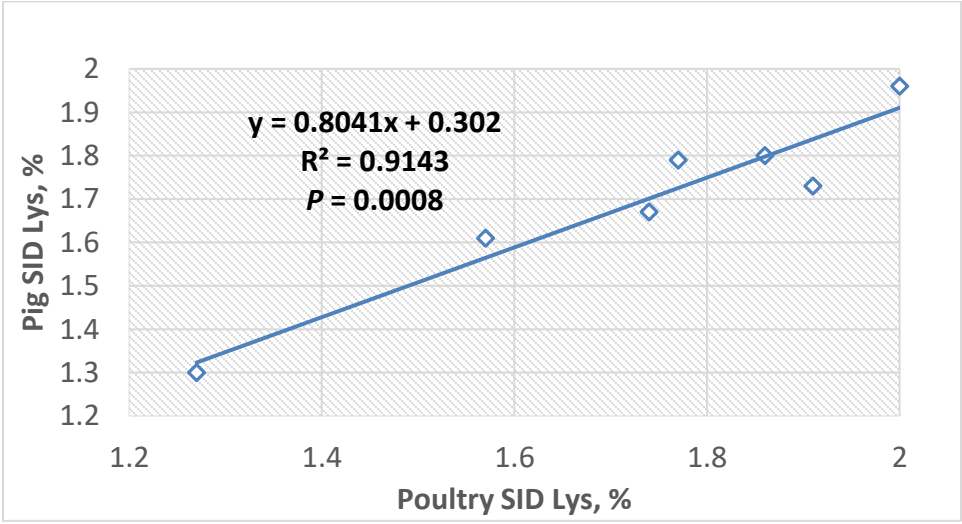

Figure 7.1 Relationship between standardized ileal digestible lysine content for broiler chickens and pigs (Source: Chapters 5 and 6 of the current thesis).

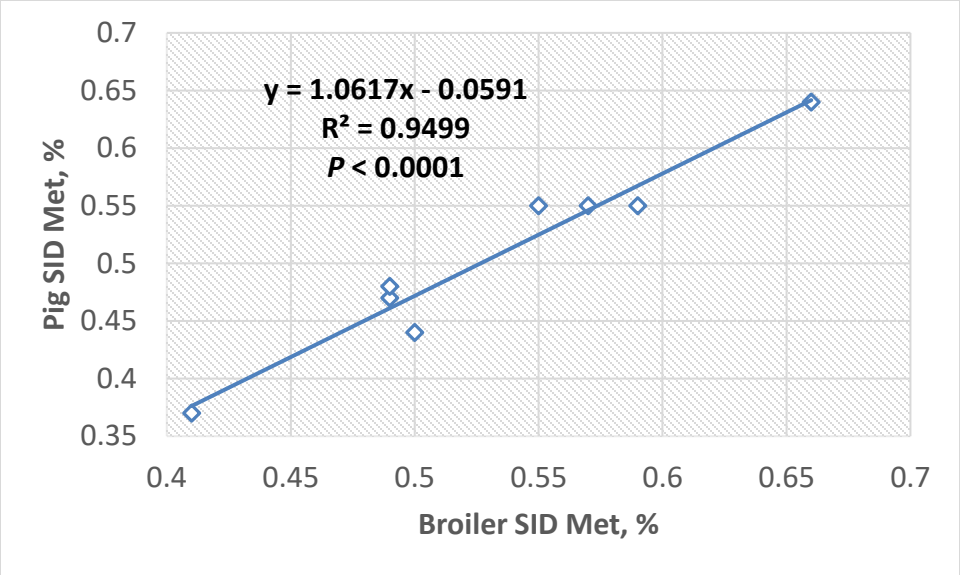

Figure 7.2 Relationship between standardized ileal digestible methionine content for broiler chickens and pigs (Source: Chapters 5 and 6 of the current thesis).

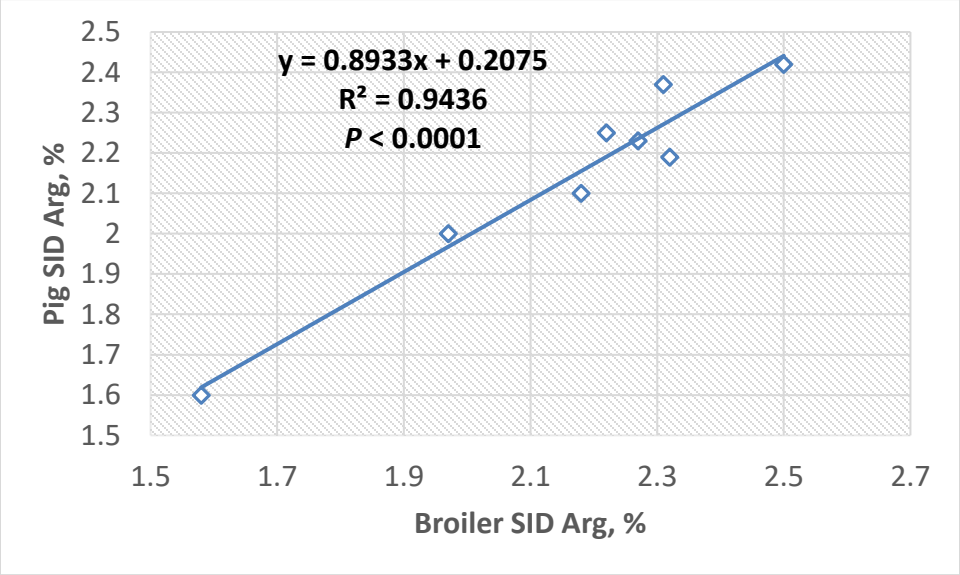

Figure 7.3 Relationship between standardized ileal digestible arginine content for broiler chickens and pigs (Source: Chapters 5 and 6 of the current thesis).

Previous studies (Newkirk and Classen, 1999, Newkirk et al., 2003a, Newkirk et al., 2003b) have shown that the desolventization/toasting stage of pre-press solvent extraction of canola reduces the content and digestibility of AA. Mustafa et al. (2000) also reported that the DT increased the NDF content and reduced CP solubility and in vitro crude protein degradability of CM and this suggested that the increase in NDF content is mainly due to the increase in the level of NDICP following heat treatment in the DT. Results from this thesis did not only corroborate those previous findings but also gave further insight as to the occurrence of Maillard reaction in the DT that causes the reduction in the content and digestibility of AA and a corresponding increase in the contents of dietary fiber and its components. The correlations among the heat-sensitive components observed in this thesis suggested that Maillard reaction may be occurring during processing and this may be responsible for most differences in the chemical composition of CM among processing plants and even among years.

Even though the pre-press solvent extraction process is currently the most effective method of extracting oil from canola seeds, the loss in value of the meal during processing would indicate that the process should be modified in some plants to prevent destruction of AA through Maillard reaction (Newkirk et al., 2003b). Clearly, there is a prospect of adjusting processing conditions to produce a more consistent high quality CM. This would improve protein quality and at the same time, reduce negative impacts of the anti-nutritional components (Spragg and Mailer, 2007). Toasting *per se* truly reduces the glucosinolates content of the meal but in light of the low glucosinolates levels in current varieties of canola, it may not be necessary. Reducing glucosinolates in CM by toasting can potentially be more disadvantageous than beneficial to the nutritional value of the meal (Newkirk et al., 2003a). The sparge steam in the DT introduces both heat and moisture and therefore has the potential to promote Maillard reactions. Optimal

conditions for the Maillard reactions have been defined as being between 15 and 18% moisture in combination with elevated temperatures (Mauron, 1981). Desolventization without sparge steam resulted in a light-coloured meal and appeared to reduce, if not eliminate toasting (Newkirk and Classen, 2002). It also resulted in high body weight of broiler chickens which suggested that there may be no anti-nutritional factors in CM that need to be reduced by the toasting process for broiler chickens (Newkirk and Classen, 2002). However, there was poorer performance when pigs (especially during the growing period) were fed non-toasted CM compared to toasted CM and this would relate to the higher glucosinolates content of the non-toasted meal (Thacker and Newkirk, 2004). It would appear that pigs are more susceptible to the harmful effects of glucosinolates that poultry. Therefore, for pigs, it may be more advisable to reduce the maximum inclusion level of CM in the diets rather than reducing the content and availability of AA of the meal by overheating (Newkirk et al., 2003a). The standardization of the desolventization step among processing plants may be beneficial to feed manufacturers and the livestock industry, as it may create a product that is less variable in AA composition and digestibility (Almeida et al., 2014).

Another factor responsible for differences between the chemical compositions of CM among processing plants may be the addition of screenings or gums to the meal in the DT. The screenings obtained from cleaning the seed prior to processing are often added back to the meal in the DT as a means of disposal (Newkirk et al., 2003a). The composition of these screenings could affect the fat and protein contents of the meal.

Differences in environmental conditions among regions within Canada and among years causing differences in the chemical compositions of canola seeds may also be responsible for differences in the chemical composition of CM.

In Chapter 6 of this thesis, it was established that there were differences among processing plants in the AME_n content of CM. This difference may be related to the contents of fat, carbohydrates or fiber. However, it was observed that those parameters were poorly correlated with AME_n content of CM. It is expected that the fat content should have the greatest effect on AME_n content. The goal of all canola processors is to extract oil from canola seeds to as low as 1% residual oil in the meal. However, the addition of gums and soapstocks from oil refinery to CM increases the fat content of the meal and this may cause variations in the fat and hence, the AME_n content of CM. The gums and soapstocks are a complex fraction containing various components, with soapstocks accounting for 5 – 10% of the crude oil mass with a high concentration of free fatty acids and the rest is made up of triglycerides, phospholipids, and minor amounts of other oil-derived compounds. The entire fraction commonly known as "gums and soapstocks" also include spent bleaching clay (SBC), a bentonite product from canola oil refining (Blair et al., 1986), being added back to CM. In this context, there has recently been some deliberations as to the quality and potential effects of SBC in animal nutrition (Blair et al., 1986; Keith and Bell, 1986). Additional analyses (such as acid hydrolysed ether extract) may have to be conducted in order to determine any potential contribution of gums and soapstocks to the energy content of CM.

A dilemma facing commercial nutritionists is the inability to rapidly and easily detect batches of ingredients that reduced AA digestibility due to overheating (Parsons 1996). Therefore, having established the fact that there were differences among processing plants and years in the chemical and nutritive compositions of CM for both broiler chickens and pigs in the current study, we explored a method for quick determination of chemical and nutritive value of the meal which is the development of prediction equations. We developed prediction equations for determining

lysine, total dietary fiber and NDICP from a simple measure of NDF or a combination of NDF and CP. For lysine, the equations had low R^2 and predicted values did not compare favourably with analyzed value. The equations seem to provide information about NDICP due to the observed relatively high R^2 ranging from 0.81 to 0.84 and low RSD values of 4.74 to 5.18. However, they may not accurately predict NDICP because the predicted and analyzed values did not compare favourably as observed from the validation *p*-values (0.09 to 0.23) and R^2 (0.15 to 0.28) values. However, the equations provide accurate prediction for the total dietary fiber content of CM as observed from the high R^2 (0.97 and 0.98, respectively) and significant *p*-values in the validation analysis. These equations will enable CM users to simply predict total dietary fiber from NDF or NDF and CP contents without the need for any further analysis. Total dietary fiber analysis is a complex analysis which involves a number of other assays, including NSP analysis which takes approximately 3 days to complete (Slominski et al., 2006).

In addition, equations for predicting standardized ileal digestible AA contents for pigs and broiler chickens from a simple measure of ADF were developed. Increasing the number of observations in the study and using CM samples that vary more widely in ADF composition might increase the accuracy of the equations. Including other parameters like CP and AA compositions may also improve the accuracy of the equations. In the current study only the ADF content was used as the predictor because it was thought that the choice of a prediction equation to estimate standardized ileal digestible AA should be dependent on the simplicity and the cost of the analytical procedures involved. Acid detergent fiber is a simple, fast, and inexpensive chemical analysis that can be easily obtained by nutritionists and animal producers. A successful prediction model should contain as few variables as possible to allow for practical application (Meloche et al., 2014). When developing models intended to predict nutrient digestibility on the basis of

chemical composition, it is advantageous to select the least expensive, most accurate, and least time-consuming chemical components (Meloche et al., 2014). Analysis for proximate composition are assumed to be relatively simple and are widely utilized in the poultry industry to assess nutrient variability among ingredient sources (Meloche et al., 2014).

Another method for rapid determination of standardized ileal digestible AA content which was not explored in the current study is the Near-Infrared Reflectance Spectroscopy (**NIRS**) with calibration equations predicting nutritional value from feed spectrum within a specific family of ingredients. The NIRS is a promising technology which is based on the absorption of infrared light by the chemical bonds of organic molecules. The infrared colour of samples provides information about its composition. The mechanism and underlying principles of NIRS have been described previously by Kempen and Jackson (1996). The NIRS method has been used to predict essential AA contents of various protein ingredients (Fontaine et al., 2001), feed digestibility from pig feaces (Bastianelli et al., 2014), and digestible energy content of barley for pigs (Zijlstra et al., 2011). Information regarding the use of NIRS in poultry feed is limited.

In conclusion, this thesis further established the fact that there are differences in the chemical and nutritive values for broiler chickens and pigs of CM from processing plants in Canada. Covering a wide range of chemical analyses including proximate analysis, dietary fiber and its components, carbohydrates and its components and AA compositions and presenting significant correlations between the heat-sensitive chemical components of CM, it surpasses previous reports in providing insight into the occurrence of Maillard reaction during the pre-press solvent extraction process. Also, it demonstrated that pelleting *per se* may not affect the nutritional value of CM for either broiler chickens or growing pigs but the conditions under which the pelleting is done would be of importance. Finally, this thesis developed prediction equations for

quick determination of total dietary fiber contents of CM from a simple measure of NDF or NDF and CP, and standardized ileal digestible AA content for broiler chickens and pigs from a simple measure of ADF. These equations will serve as useful tools in feed formulation and in ranking of CM quality.

CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTION

CONCLUSIONS

1. There were differences in the contents of crude protein, lysine, fat, total phosphorus, NDF, NSP, total dietary fiber, lignin and polyphenols, neutral detergent insoluble crude protein, simple sugars and oligosaccharides, and glucosinolates of CM among processing plants and among years.
2. Lysine was the only AA that differs among processing plants.
3. There were significant correlations among the heat-sensitive components of CM (total dietary fiber, NDF, NDICP, glucosinolates, and lysine).
4. The greatest variations in chemical composition were observed in simple sugars, NDICP, and glucosinolates, the components that are sensitive to heat treatment.
5. There was an effect of CM source on the standardized ileal digestible AA contents of CM among processing plants in both broiler chickens and growing pigs and on the AME_n content for broiler chickens.
6. Pelleting may reduce the standardized ileal digestible AA contents of CM for broiler chickens and growing pigs. It may also reduce its AME_n content for broiler chickens.
7. The interactive effect of CM source and pelleting on the standardized ileal digestible AA contents of CM in broiler chickens and growing pigs and also on the AME_n contents in broiler chickens shows that the effect of pelleting on the nutritive of CM may be plant dependent.

8. From simple measure of NDF or NDF and CP total dietary fiber but not lysine and NDICP content of CM can be accurately predicted.
9. Neutral detergent fiber content of CM do not provide accurate prediction of it standardized ileal digestible AA content for either broiler chickens or growing pigs.
10. Prediction equations for determining standardized ileal digestible AA contents in pigs and broiler chickens may not be useful for accurate feed formulation for ranking of CM quality
11. Broiler chickens may be a useful model for determining standardized ileal digestible AA contents of CM in pigs.

FUTURE DIRECTIONS

1. The use of enzyme and AA supplementation to mitigate low AA availability for broiler chickens and pigs in some CM samples that might be heat-damaged.
2. The effect of CM source on energy digestibility in growing pigs. We desired to conduct an energy digestibility experiment in pigs as it was done for poultry in the current study. However, there was not enough CM feedstuff from the various processing plants.
3. The use of near NIRS technology to predict the standardized ileal digestible AA in CM for broiler chickens and pigs as a measure of quality for animal feeding. Development of NIRS calibrations for CM application by industry will allow for rapid assessment of meal quality. The wide array of data on the chemical composition of CM available from the current thesis makes it easy to conduct an NIRS study.

CHAPTER 9

LITERATURE CITED

Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Anim. Feed Sci. Technol. 179:1-23.

Adedokun, S. A., M. S. Lilburn, C. M. Parsons, O. Adeola, T. J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86: 2590-2597.

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535–2548.

Adeola O. Digestion and balance techniques in pigs. In: Lewis AJ, Southern LL, editors. Swine Nutrition. CRC Press; Washington, DC: 2001. pp. 903–916.

Adeola, O., and K. E. Ileleji. 2009. Comparison of two types in the determination of metabolizable energy content of corn distillers dried grains with solubles for boiler chickens by the regression method. Poult. Sci. 88:579-585.

Adewole, D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2016. Chemical and nutritive characteristics of canola meal from Canadian processing facilities. Anim. Feed Sci. Technol. 222:17-30.

Adewole, D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2017a. Standardized ileal digestible amino acid contents of canola meal from Canadian crushing plants for growing pigs. J. Anim. Sci. 95:2670-2679.

Adewole, D. I., A. Rogiewicz, B. Dyck, and B. A. Slominski. 2017b. Effects of canola meal source on the standardized ileal digestible amino acids and apparent metabolizable energy contents for broiler chickens. Poult. Sci. (In press).

Adrian, 1. 1974. Nutritional and physiological consequences of the Maillard reaction. World Rev. Nutr. Diet. 19:71-122.

Adrian, J., R. Frangne, L. Petit, B. Godon, and J. Barbier. 1966. Nutritional repercussion of the soluble products formed during Maillard reaction. Ann. Nutr. Aliment. 20:257-277.

Ajandouz, E. H. and A. Puigserver. 1999. Nonenzymatic browning reaction of essential amino acids: Effect of pH on caramelization and Maillard reaction kinetics. J. Agric. Food Chem. 47:1786-1793.

Almeida, F. N. 2013. Effects of the Maillard reactions on chemical composition and amino acid digestibility of feed ingredients and on pig growth performance. PhD Dissertation, Univesity of Illinois at Urbana-Champaign, Urbana, Illinois.

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

Aman, P. and K. Hesselman. 1984. Analysis of starch and other main constituents of cereal grains. Swedish J. Agric. Sci. Res. 14:135-139.

Ames, J. M, R. C. E. Guy, and G. J. Kipping. 2001. Effect of pH, Temperature, and Moisture on the Formation of Volatile Compounds in Glycine/Glucose Model Systems. J. Agric. Food Chem. 49:4315–4323.

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

Anderson, P. V., B. J. Kerr, T. E. Weber, C. J. Ziemer, and G. C. Shurson. 2012. Determination and prediction of digestible and metabolizable energy from chemical analysis of corn coproducts fed to finishing pigs. J. Anim. Sci. 90:1242–1254.

Asp, N. G., C. G. Johanson, H. Hallmer, and M. Siljestrom. 1983. Rapid enzymatic assay of soluble and insoluble dietary fiber. J. Agric. Food Chem. 31:476-482.

Asp, N. G. 1996. Classification and methodology of food carbohydrates as related to nutritional effects. Am. J. Clin. Nutr. 61 (Suppl.) 93OS-7S.

Asp, N. G. 1996. Dietary carbohydrates: Classification by chemistry an physiology. Food Chem. 57:9-14.

Assadi, E., H. Janmohammadi, A. Taghizadeh, and S. Alijani. 2011. Nutrient composition of different varieties of full-fat canola seed and nitrogen-corrected true metabolizable energy of full-fat canola seed with or without enzyme addition and thermal processing. J. Appl. Poult. Res. 20:95–101.

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.

Bach Knudsen, K. E. 1997. Carbohydrates and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319-338.

Bach Knudsen, K. E. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93:2380-2393.

Baidoo, S.K., F.X. Aherne, B.N. Mitaru and R. Blair. 1987. Canola meal as a protein supplement for growing-finishing pigs. Anim. Feed Sci. Technol. 18:37–44.

Banks, H. J. 1998. Effect of storage conditions on quality change in canola, stored Grain Research Laboratory, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601

Baker, D. H. 1995. 'Vitamin bioavailability'. In: Baker, D. H. and Lewis A. J., Bioavailability of nutrients for animals. San Diego CA, Academic Press, 399-431.

Bastianelli, D., L. Bonnal, Y. Jaguelin-Peyraud, and J. Noblet. 2015. Predicting feed digestibility from NIRS analysis of pig faeces. Animal 9:781-786.

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

Beckie, H. J., K. N. Harker, L. M. Hall, S. I. Warwick, A. Legere, P. H. Sikkema, G. W. Clayton, A. G. Thomas, J. Y. Leeson, G. Seguin-Swartz, and M. -J. Simard. 2006. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86:1243-1264.

Bedford, M. R. 2006. Effect of non-starch polysaccharides on avian gastrointestinal function

G.C. Perry (Ed.), Avian gut function in health and disease, CAB International, Wallingford, UK. pp. 159-170.

Bell J. M., M. O. Keith, and D. S. Hutcheson. 1991. Nutritional evaluation of very low glucosinolate canola meal. Can. J. Anim. Sci. 71:497–506.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73:689–697.

Bell, J. M. and M. O. Keith. 1991. A survey of variation in the chemical composition commercial canola meal produced in Western Canadian crushing plants. Can. J. Anim. Sci.71:469-480.

Bell, J. M. 1982. From rapeseed to canola: a brief history of research for superior meal and edible oil. Poult. Sci. 61: 613-622.

Bell, J. M. 1983. Factors affecting the nutritional value of canola meal – A review. Can. J. Anim. Sci. 73:679-697.

Bell, J. M., l984. Nutrients and toxicants in rapeseed meal: a review. J. Anim. Sci. 58:996-1010.

Bell, J. M., M. O. Keith and C. S. Darroch. 1988. Lysine supplementation of grower and finisher pig diets based on high-protein barley, wheat and soybean meal or canola meal, with observations on thyroid and zinc status. Can. J. Anim. Sci. 68:931–940.

Benhke, K. C. 1996. Feed manufacturing technology: current issues and challenges. Anim. Feed Sci. Technol. 62:49-57

Biagi G, I. Cipollini, B. R. Paulicks, F. X. Roth. 2010. Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. Arch. Anim. Nutr. 64:121–35.

Blakely, R. M. and R. W. Anderson. 1948. Studies with rapeseed oilcake meal I. The effects of various levels of rapessed oilcake meal intake diet on the weight of the thyroid glands of turkey poults. J. Sci. Agric. 28:393-397.

Brand T. S., N. Smith, and L. C. Hoffman. 2007. Anti-nutritional factors in canola produced in Brassica juncea yellow. J. Anim. Sci. 92:3895–3905.

Braude, R., R. J. Fulford, and A. G. Low. 1976. Studies on digestion and absorption in the intestine of growing pigs. Measurements of the flow of digesta and pH. Br. J. Nutr. 36:497-510.

Bredeson, D. K. 1983. Mechanical oil extraction. J. Am. Oil Chem. Soc. 60:211-213.

Broesder, J. T., S. A. Gunter, L. J. Krysl, and M. B. Judkins. 1992. Digestion of ruminal masticate dried by threonine different methods. Anim. Feed Sci. Technol. 37:323-331.

Burill, P. 2012. Better canola. Canola technology updates for growers and advisors. Safe storage, Vol. 2.

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

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40:660-666.

Campbell, L. D., and B. A. Slominski. 1990. Extent of thermal decomposition of indole glucosinolates during the processing of canola seed. J. Am. Oil Chem. Soc. 67:73–75.

Canadian Council on Animal Care. 1993. Guide to the care and use of Experimental Animals. 2nd ed. Vol. 1. Can. Counc. Anim. Care, Ottawa, Ontario, Canada.

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

Canola Council of Canada. 2009. Canola meal Feed Industry Guide, 4th ed. Publication of Canola Council of Canada, Winnipeg, MB, Canada.

Canola Council of Canada. 2011. What is Canola. http://www.canolacouncil.org/oil-andmeal/what-is-canola/#OfficialDefinition. Accessed on September 15.2015.

Canola Encyclopedia. 2014. Canola Council of Canada. http://www.canolacouncil.org/canola-encyclopedia/crop-development/growth-stages/ Accessed on April 6.2017.

Carre, B. M. Lessire, and H. Juin. 2014. Prediction of the net energy value of broiler diets. Animal 8:1395-1401.

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

Carré, P, M. Citeau, G. Robin, and M. Estorges. 2016. Hull content and chemical composition of whole seeds, hulls and germs in cultivars of rapeseed (*Brassica napus*). OCL 23: A302.

Carre, B. and J. M. Brillouet. 1986. Yield and composition of cell wall residues isolated from various feedstuffs used for non-ruminant farm animals. J. Sci. Food Agric. 37:341-351.

Carpenter, K. J. 1960. The estimation of the available lysine in animal-protein foods. Biochem. J. 77:604.

Chen, X., C. Parr, P. Utterback, and C. M. Parsons. 2015. Nutritional evaluation of canola meals produced from new varieties of canola seeds for poultry. Poult. Sci. 94:984-991.

Choct, M. and G. Annison. 1990. Anti-nutritive activity of wheat pentosans in broiler diets. Br. Poult. Sci. 30:81- 82.

Clandinin, D. R., and E. W. Tajcnar 1960. Effects of variations in cooking and conditioning temperatures used during expeller processing of rapeseed on the fat and lysine content of rapeseed oil meals. Poult. Sci. 39:291-293.

Clandinin, D. R., A. R. Robbiee, J. S. Slinger, and J. M. Bell. 1981. Canola meal for livestock and poultry. In: Canola meal for livestock and poultry. Clandinin, D. R. (editor). Canola Council of Canada. Publ. No. 59. Winnipeg, Manitoba.

Clandinin, D. R., A. R. Robbiee, J. M. Bell, and J. S. Slinger. 1986. Composition of canola meal. In: Canola meal for livestock and poultry. Clandinin, D. R. (editor). Canola Council of Canada. Publ. No. 59 (revised edition). Winnipeg, Manitoba.

Classen, H. L., Newkirk, R. K., and D. D. Maenz. 2004. Effects of conventional and novel processing on the feed value of canola meal for poultry. Proc. Aust. Poult. Sci. Sym. 16, 1-8.

Clemens, E. T. and C. E. Stevens. 1975. Sites of organic acid production and pattern of digesta movements in the gastrointestinal tract of swine. J. Nutr. 105:759-768.

Cozannet, P., Y. Primot, C. Cady, J. P. Metayer, P. Callu, M. Lessire, F. Skiba, and J. Noblet. 2010. Ileal digestibility of amino acids in wheat distillers dried grains with solubles for pigs. Anim. Feed Sci. Technol. 158: 177-186.

Crevieu-Gabriel, I., J. Gomez, J. Caffin, and B. Carre. 1999. Comparison of pig and chicken pepsins for protein hydrolysis. Repr. Nutr. Dev. 39:443-454.

Cromwell, G.L., Herkelman, K.L., Stahly, T.S.1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. J. Anim. Sci. 71:679–686.

Daun, J. K. 2011. Origin, distribution, and production. In: Canola chemistry, production, processing, and utilization. Daun, J. K., Michael Eskin, E. N., and Hickling D. AOAC Press, Urbana, IL 61802.

Daun, J. K. and D. Adolphenylalanine. 1997. A revision to the canola definition. GCIRC Bulletin. July 1997. Pages 134–141.

de Lange, C. F. M., V. M. Gabert, D. J. Gillis, F. Patience. 1998. Digestible energy contents and apparent ileal amino acid digestibilities in regular or partial mechanically dehulled canola meal samples fed to growing pigs. Can. J. Anim. Sci., 78: 641-648.

Delgado-Andrade, C. 2013. Maillard reaction products: some considerations on their health effects. Clinical Chemistry and Laboratory Medicine, 52(1), pp. 53-60. Retrieved 20 Apr. 2016, from doi:10.1515/cclm-2012-0823.

Delgado-Andrade, C., I. Seiquer, R. Nieto, and M. P. Navarro. 2004. Effects of heated glucose-Lys and glucose-methionine model-systems on mineral solubility. Food Chem. 87:329-337.

DeSmet B. 2010. Rapeseed Plant Part 1: Preparation; Paul Ellis of DeSmet Ballestra (Rosedowns).

Downey, R. K. and J. M. Bell. 1990. New developments in canola research. Pages 37-46 In: Canola and Rapeseed. Production, Chemistry, Nutrition and processing Technology. F. Shahidi, ed. Van Nostrand Reinhold, New York, NY.

Kerr, B. J., W. A. Dozier III, and G. C. Shurson. 2013. Effects of reduced-oil corn distillers dried grains with solubles composition on digestible and metabolizable energy value and prediction in growing pigs. J. Anim. Sci. 91:3231–3243.

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

Eggum, B. O. and K. D. Christenson. 1975. Influence of tannin on protein utilization of feedstuffs with special reference to barley. Breeding for seed improvement using nuclear techniques. International Atomic Energy Agency, Vienna 135-143.

Eklund, M., N. Sauer, F. Schone, U. Messerschmidt, P. Rosenfelder, J. K. Htoo, and R. Mosenthin. 2015. Effect of processing of rapeseed under defined conditions in a pilot plant on chemical composition and standardized ileal amino acid digestibility in rapeseed meal for pigs. J. Anim. Sci. 93:2813-2815.

Elias, S. G. and L. O. Copeland. 2001. Physiological and harvest maturity of canola in relation to seed quality. Agron. J. 93:1054-1058

Englyst, H. N. and J. H. Cummings. 1988. Improved method for the determination of dietary fiber as non-starch polysaccharides in plant foods. J. Assoc. Off. Anal. Chem. 71:808-814.

Englyst, H. N., and J. H. Cummings. 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.

Englyst, H. N., and J. H. Cummings. 1988. Improved method for the determination of dietary fiber as non-starch polysaccharides in plant foods. J. Assoc. Off. Anal. Chem. 71:808-814.

Englyst, H. N., M. E. Quiqley, G. J. Hudson, and J. H. Cummings. 1992. Determination of dietary fibre as non-starch polysaccharides by gas-liquid chromatography. Analyst 117:1707-1714.

Englyst, H. N., M. E. Quiqley, and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119:1497-1509.

Evans, R. J., and H. A. Butts. 1949. Inactivation of amino acids by autoclaving. Science 109:569.

Fairfield, D., Thomas, H., Garrison, R., Bliss, J., Behnke, K., 2005. Pelleting. In: Schofield, E.K. (Ed.), Feed Manufacturing Technology V. American Feed Industry Association, Arlington, pp. 142–167.

Faist, V., and H. F. Erbersdobler. 2001. Metabolic transit and in vivo effects of melanoidins and precursor compounds deriving from the Maillard reaction. Ann. Nutr. Metab. 45:1-12.

Falk, D. 1985. Pelleting cost centre. In: Mcellhiney, R.R. (Ed.), Feed Manufacturing Technology III. American Feed Manufacturers Association, Arlington, pp.167–190.

Fan, M.Z., W.C. Sauer, R.T. Hardin, K.A. Lien.1994. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. J. Anim. Sci. 72: 2851-2859.

Fan, M. Z., W. C. Sauer, and V. M. Gabert. 1996. Variability of apparent ileal amino acid digestibility in canola meal for growing-finishing pigs. Can. J. Anim. Sci. 76:563-569.

Fayyaz-ul-Hassan, H. Ali, M. A. Cheema, and A. Manaf. 2005. Effects of environmental variation on oil content and fatty acid composition of canola cultivars. J. res. Sci. 16:65-72.

Fenwick, G. R. 1982. The assessment of a new protein source-Rapeseed. Proc. Nutr. Soc. 41:277-288.

Fevrier, C., Y. Lechevestrier, Y. Lebreton, and Y. Jaguelin,-Peyraud. 2001. Prediction of the standardized ileal true digestibility of amino acids from the chemical composition of oilseed meals in the growing pig. Anim. Feed Sci. Technol. 90:103-115.

Fontaine, J., J. Horr, and B. Schirmer. 2001. Near-Infrared reflectance spectroscopy enables the fast and accurate prediction of the essential amino acid contents in soy, rapeseed meal, sunflower meal peas, fishmeal, meat meal products, and poultry meal. J. Agric. Chem. 49:57-66.

Friedt W. and W. Lühs. 1999. Breeding of rapeseed (*Brassica napus*) for modified seed quality: synergy of conventional and modern approaches. In: Wratten N, Salisbury BA (eds) Proceedings of the 10th International Rapeseed Congress, 26–29 Sept, GCICRC, Canberra, Australia, p 76.

Garcıa, M., R. Lazaro, M. A. Latorre, M. I. Gracia, and G. G. Mateos. 2008. Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. Poult. Sci. 87:940–948.

George, N.A., J. Hollingsworth, W.-R. Yang, and S.R. Kaffka. 2017. Canola and camelina as new crop options for cool-season production in California. Crop Sci. 57:1–20.

Gerrard, J. A. 2002. New aspects of an ageing chemistry – recent developments concerning the Maillard reaction. Aust. J. Chem. 55:299-310.

Goering, H. K., and P. J. Van Soest. 1970. Forage fibre analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379, ARS-USDA, Washington, DC.

Goering, H. K., and P. J. Van Soest. 1970. Forage fibre analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379, ARS-USDA, Washington, DC.

González-Vega, J. C., and H. H. Stein. 2012. Amino acid digestibility in canola-, cottonseed- and sunflower-products fed to finishing pigs. J. Anim. Sci. 90:4391-4400.

Gorski, M. C. Foran, P. Utterback, and C. M. Parsons. 2017. Nutritional evaluation of conventional and increased-protein, reduced-fiber cnola meal fed to broiler chickens. Poult. Sci. 96:2159-2167.

Grami, B. and L. J. LaCroix. 1977. Cultivar variation in total nitrogen uptake in rape. Can. J. Plant Sci. 57:619-624.

Green, S. and T. Kiener. 1989. Digestibilities of nitrogen losses amino acids in soya-bean, sunflower, meat and rapeseed meals measured with pigs and poultry. Anim. Prod. 48:157-179.

Gupta S. K. and A. Pratap. 2007. History, origin and evolution. In: Gupta SK (ed) Advances in botanical Research-rapeseed breeding, vol 45. Academic, Amsterdam.

Hanschen, F. S. and M. Schreiner. 2017. Isothiocyanates, nitriles, and epithionitriles from glucosinolates are affected by genotype and developmental stage in *Brassica oleracea* varieties. Front. Plt. Sci. Vol. 8. doi.org/10.3389/fpls.2017.01095.

Hamama A. A., H. L. Bhardwaj, and D. E. Starner. 2003. Genotype and growing location effects on phytosterols in canola oil. J. Am. Oil Chem. Soc. 80:1121–1126

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

Henneberg, W. and F. Stohmann. 1859. Uber das Erhaltungsfutter volljahrigen Rindviehs. J. Landwirtsch 3:485-551.

Hickling, D. 1994. Canola meal hog-feeding trials in Western Canada. Canola Council of Canada. Winnipeg, MB, Canada.

Hickling, D. 1996. Canola meal hog-feeding trials in Mexico. Canola Council of Canada. Winnipeg, MB, Canada.

Hickling, D., 2001. Cano1a Meal Feed Industry Guide. Canola Council of Canada, Winnipeg, Manitoba, Canada. http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx. Accessed on September 18.2015.

Hill, F. W. and D. L. Anderson. 1958. Comparison of metabolizable energy and productive determinations with growing chicks. J. Nutr. 64:587-603

Hindrichsen, I K, M. Kreuzer, J. Madsen, K. E. Bach Knudsen. 2006. Fiber and lignin analysis in concentrate, forage, and feces: Detergent versus enzymatic-chemical method. J. Dairy Sci. 81:2168-2176.

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

Hurrell, R. F. 1984. Reactions of food proteins during processing and staorage and their nutritional consequences. In Developments in Food Proteins, Vol. 3, pp 213 -244 (B. J. F. Hudson, editor). London: Applied Science Publishers.

Hurrell, R. F., and K. J. Carpenter, 1977. Mechanisms of heat damage in proteins. 8. The role of sucrose in the susceptibility of protein in foods to heat damage. Br. 1. Nutr.38:285-297.

Hurrel, R. F. 1990. Influence of the Maillard reaction on the nutritional value of foods. Pages 245-258 In: The Maillard reation in food processing, Human Nutrition and Physiology Birkhatiser Verlag, Basel, Switzeland.

Huang, X. 2015. Improvements in nutritive value of canola meal with pelleting. M.Sc. dissertation, University of Saskatchewan, Saskatoon, Canada.

Hulshof, T. G., P. Bikker, A. F. B. van der Poel, and W. H. Hendriks. 2016. Assessment of protein quality of soybean meal and 00-rapeseed meal toasted in the presence of lignosulfonate by amino acid digestibility in growing pigs and Maillard reaction products. J. Anim. Sci. 94:1020-1030.

Ivanova, P, V. Chalova, G., Uzunova, L. Koleva, and I. Manolov 2016. Biochemical characterization of industrially produced rapeseed meal as a protein source in food industry Agric. Agric. Sci. Proc.10: 55-62.

Iyayi, E. A. and O. Adeola. 2014. Standardized ileal amino acids digestibility of feedstuffs in broiler chickens. Eur. Poult. Sci. 78.

Jensen, S. K., Y. G. Liu, and B. O. Eggun. 1995. The effect of heat treatment on glucosinolates and nutritional value of rapeseed meal in rats. Anim. Feed Sci. Technol. 53: 17-28.

Jha, R. and J. D. Berrocoso. 2015. Review: Dietary fiber utilization and its effects on physiological functions and gut health of swine. Animal 9:1441-1452.

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

Johnson, G. H., D. H. Baker, and E. G. Perkins. 1977. Nutritional implications of the Maillard reaction: the availability of fructose-phenylalanine to the chick. J. Nutr. 107:1659-1664.

Jongbloed, A. W., Z. Mroz, and P. A. Kemme. 1992. The effect of supplementary Aspergillus niger phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and phytic acid in different sections of the alimentary tract. J. Anim. Sci. 70:1159-1168.

Joyce, C., A. Deneau, K. Peterson, I. Ockenden, V. Raboy, and J. N. A. Lott. 2005. The concentrations and distributions of phytic acid phosphorus and other mineral nutrients in wild-type and low phytic acid Js-12-LPA wheat (Triticum aestivum) grain parts. Can. J. Bot. 83:1599-1607.

Kaldmae, H., R. Leming, M. Kass, M., A. Lember, S. Tolp, and O. Kart. 2010. Chemical composition and nutritional value of heat-treated and cold-pressed rapeseed cake. Vet. Med. Zoot. 49:55-60.

Kasprzak, M. M., J. G. M. Houdijk, S. Kightley, O. A. Olukosi, G. A. White, P. Carre, and J. Wiseman. 2016. Effects of rapessed variety and oil extraction method on the content and ileal digestibility of crude protein and amino acids in rapeseed cake and softly processed rapeseed meal fed to broiler chickens. Anim. Feed Sci. Technol. 213:90-98.

Kasprzak, M. M., J. G. M. Houdijk, O. A. Olukosi, H. Appleyard, G. A. White, S. P. J. Kightley, P. Carre, and J. Wiseman. 2017. The influence of oil extraction process of different

rapeseed varieties on the ileal digestibility of crude protein and amino acids in broiler chickens. Anim. Feed Sci. Technol. 227:68-74.

Khajali, F. and B. A. Slominski. 2012. Factors affecting the nutritive value of canola meal for poultry. Poult. Sci. 91:2564-2575.

Kiarie, E., and C. M. Nyachoti. 2007. Ileal digestibility of amino acids in co-extruded peas and full fat canola for growing pigs. Anim. Feed Sci. Technol. 139:40–51.

Kim, B. G., D. Y. Kill, Y. Zhang, and H. H. Stein. 2012. Concentrations of analyzed or reactive lysine, but not crude protein, may predict the concentration of digestible lysine in distillers dried grains with solubles (DDGS) fed to pigs. J. Anim. Sci. 90:3796-3808.

Kirkland, K. J. and Johnson, E. N. 2000**.** Alternative seeding dates (fall and April) affect *Brassica napus* canola yield and quality. Can. J. Plant Sci. 80: 713–719.

King, R.H., P.E. Eason, D.K. Kerton and F.R. Dunshea. 2001. Evaluation of solvent-extracted canola meal for growing pigs and lactating sows. Aust. J. Agric. Res. 52:1033–1041.

Kong, C. and O. Adeola. 2011. Protein utilization and amino acid digestibility of canola meal in response to phytase in broiler chickens. Poult. Sci. 90:1508-1515.

Landero, J. L., E. Beltranena, M. Cervantes, A. Morales, and R. T. Zijlstra. 2011. The effect of feeding solvent-extracted canola meal on growth performance and diet nutrient digestibility in weaned pigs. Anim. Feed Sci. Technol. 170:136–140.

Lea, C. H., and R. S. Hannan, 1949. The effect of activity of water, of pH and of temperature on the primary reaction between casein and glutaminecose. Biochem. Biophys. Acta. 3:313-325.

Leeson, S., K. N. Boorman, D. Lewis, and D. H. Shrimpton. 1977. Metabolizable energy studies with turkeys: Nitrogen correction factor in metabolizable energy determinations. Br. Poult. Sci. 18:373–379.

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

Ledl, F., and E. Schleicher. 1990. New aspects of the Maillard reaction in foods and in the human body. Angewandte Chem. Int. Ed. Engl. 29:565-594.

Le Goff G. and J. Noblet. 2001. Comparative digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci. 79:2418-2427.

Leming, R., and A. Lember. 2005. Chemical composition of expeller-extracted and cold-pressed canola meal. Agraarteadus 16:103–109.

Lemme, A., V. Ravindran, and W. L. Bryden. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World Poult. Sci. J. 60: 423-437.

Leslie, A. J., J. D. Summers, R. Grandhi, and S. Leeson. 1976. Arginine-lysine relationship in rapeseed meal. Poult. Sci. 55:631–637.

Li, B. W. 1991. Dietary fiber methodologies: Status and controversial issues. Agris Records FAO

Li, P. L., F. L. Wang, F. Wu, J. R. Wang, L. Liu, and C. H. Lai. 2015a. Chemical composition, energy and amino acid digestibility in double-low rapeseed meal fed to growing pigs. J. Anim. Sci. Biotechnol. 6:1-10.

Li, P. L., F. Wu, Y. F. Chen, J. R. Wang, P. P. Guo, Z. C. Li, L. Liu, and C. H. Lai. 2015b. Determination of the energy content and amino acid digestibility of double-low rapeseed cakes fed to growing pigs. Anim. Feed Sci. Technol. 210:243-253.

Lin, L., I. Ockenden, and J. N. A. Lott. 2005. The concentrations and distributions of phytic acid phosphorus and other mineral nutrients in wild-type and low phytic acid 1-1 (lpa1-1) corn (Zea mays L.) grain parts. Can. J. Bot. 83:131-141.

Lindberg, B., I. Lonngren, and S. Svensson, 1975. Specific degredation of polysaccharides. Adv. Carbohyd. Chern. Bioch. 31: 185-201.

Lindenmeier, M., V. Faist, and T. Hofmann. 2002. Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. J. Agric. Food Chem. 50, 6997-7006.

LMC International. 2013. The economic impact of canola on the Canadian economy. Report for canola Council of Canada, Winnipeg, Canada.

Lomer, M. C., R. P. Thompson, J. Commisso, C. L. Keen, and J. J. Powell. 2000. Determination of titanium dioxide in foods using inductively coupled plasma emission spectrometry. Analyst (Lond.) 125:2339–2343.

Lopez, G. and S. Leeson. 2007. Relevance of nitrogen correction for assessment of metabolizable energy with broilers to forty-nine days of age. Poult. Sci. 86:1696–1704.

Lopez, G. and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Maison, T. and H. H. Stein. 2014. Digestiblity by growing pigs of amino acids in canola meal from North America and 00-rapeseed expellers from Europe. J. Anim. Sci. 92:3502-3514.

Maenz, D. D. 2001. Enzymatic and other characteristics of phytases as they relate to their use in animal feeds. Page 61- 84 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G.G. Partridge, ed. CABI Publishing, UK.

Maillard, L. C. 1912. Action des acides amines sur les sucres; formation des melanoidines par voie methodique. Academic Science Comptes Rendus, 154, 66–68.

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

Mateo, J.P., O.B.N. Malingan and D. Hickling. 1998. Canola meal (Brassica napus) and feed peas for growing-finishing pigs: An on-farm feeding trial. Philippine J. Vet. Anim. Sci. 24:27–35.

Mateos, G. G., R. P. Lazaro, and M. I. Gracia. 2002. The feasibility of using nutritional modifications to replace drugs in poultry feeds. J. Appl. Poult. Res. 11:437–452.

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, K. Heaney, Z. Zduncyk, and H. Kozlowska. 1994. Rapeseed meal glucosinolates and their antinutritional effects. Part 3: Animal growth and performance. Mol. Nutr. Food Res J. 38:167–77.

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

Mcfadden, A., R. J. Mailer, and P. A. Thacker. 2006. Australian Canola, Vol 12. Australian Oilseeds Federation.

McIntosh, M.K., S.K. Baidoo, F.X. Aherne and J.P. Bowland. 1986. Canola meal as a protein supplement for 6- to 20-kilogram pigs. Can. J. Anim. Sci. 66:1051–1056.

McKinnon, J. J., J. A. Olubobokum, A. F. Mustafa, R. D. H. Cohen, and D. A. Christensen. 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.

Mejicanos, G. 2015. Tail-end dehulling of canola meal: Chemical composition and nutritive value of dehulled meal for broiler chickens and weaned pigs. M.Sc. Thesis, Department of Animal Science, University of Manitoba,Winnipeg, Manitoba, Canada.

Mejicanos, G., N. Sanjayan, I. H. Kim and C. M. Nyachoti. 2016. Recent advances in canola meal utilization in swine nutrition. J. Anim. Sci. Technol. 58:7.

Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W. A. Dozier. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. Poult. Sci. 93:1428-1439.

Mendoza, O. 2014. Development and validation of prediction equations for the metabolizable energy content of distillers dried grains with solubles from different sources for pigs. Ph.D. Dissertation, University of Illinois at Urbana-Champaign.

Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilisation and broiler chicken performance. Poult. Sci. 84:37–47.

Meng, X., B. A. Slominski, L. D. Campbell, W. Guenter and O. Jones. 2006. The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part 1: Canola seed. Poult. Sci. 85:1025–1030.

Messerschmidt, U. M. Eklund, N. Sauer, V. T. S. Rist, P. Rosefelder, H. K. Spindler, J. K. Htoo, F. Schone, and R. Mosenthin. 2014. Chemical composition and standardized ileal amino acid digestibility in rapeseed meals sourced from German oil mills for growing pigs. Anim. Feed Sci. Technol. 187:68-76.

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

Mills, P. A., R. G. Rotter, and R. R. Marquardt. 1989. Modification of glucosamine method for the quantification of fungal contamination. Can. J. Anim. Sci. 56:1105–1107.

Moftakharzadeh, S. A., M. Hossein, and S. Mahmud. 2017. Effect of using the Matrix Values for NSP-degrading enzymes on performance, water intake, litter moisture and jejunal digesta viscosity of broilers fed barley-based diet. Acta Sci. Anim. Sci. 39: 65-72.

Molero-Vilchez, M. D. and B. L. Wedzicha. 1997. A new approach to study the significance of amadori compounds in the Maillard reaction. Food Chem. 58:249-254.

Moller, J. 2009. Gravimetric determination of acid detergent fiber and lignin in feed: Interlaboratory study. J. AOAC Int. 92:74-90.

Mosenthin, R. U. Messerschmidt, N. Sauer, P. Carre, A. Quinsac, and F. Schone. 2016. Effect of the desolventizing/toasting process on chemical composition and protein quality of rapeseed meal. J. Anim. Sci. Biotechnol. 7:36-48.

Mosenthin, R., W. C. Sauer, R. Blank, J. Huisman, and M. Z. Fan. 2000. The concept of digestible amino acids in diet formulation for pigs. Livest. Prod. Sci. 64:265-280.

Moughan, P. J, G. Schuttert, and M. Leenaars. 1992. Endogenous amino acid flow in the stomach and small intestine of young growing rat. J. Sci. Food Agric. 60:437-442.

Mroz, Z., A. W. Jongbloed, and P. A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. J. Anim. Sci. 72:126-132.

Mullan, B. P., J. R. Pluske, J. Allen and D.J. Harris. 2000. Evaluation of Western Australian canola meal for growing pigs. Aust. J. Agric. Res. 51:547–553.

Mustafa, A. F., D. A. Christensen, J. J. McKinnon, and R. Newkirk. 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.

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

Naczk, M. R. and F. Shahidi. 1991. Critical evaluation of quantification methods of rapeseed tannins. Proc. 8th international Rapeseed Congress. D. I. Mcgregor (editor). Saskatoon, Saskachewan, Canada. pp 1385-1390.

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

Newkirk, R. W. 2002. The effects of processing on the nutritional value of canola meal for broiler chickens. Ph.D. dissertation, University of Saskatchewan, Saskatoon, Canada.

Newkirk, R. W. 2009. Canola Meal Feed Industry Guide. 4th ed. Publication of Canola Council of Canada, Winnipeg, Manitoba, Canada.

Newkirk, R. W. and H. L. Classen. 1999. The effects of standard oilseed extraction and processing on the nutritional value of canola meal for broiler chickens. Poult. Sci. 78(Suppl. 1):39. (Abstr.)

Newkirk, R. W., and H. L. Classen. 2002. The effects of toasting canola meal on body weight, feed conversion efficiency, and mortality in broiler chickens. Poult. Sci. 81:815–825.

Newkirk, R. W., H. L. Classen, and M. J. Edney. 2003a. 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., H. L. Classen, T. A. Scott, and M. J. Edney. 2003b. The availability and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. 83:131-139.

Newkirk, R. W., H. L. Classen, T. A. Scott, M. J. Edney, D. R. Declercq, and A. J. Siemens. 2000. Prediction of lysine availability in canola meal. XXI World's Poultry Congress. Abstracts and Proceedings. Montreal Canada.

Newkirk, R. 2011. Meal nutrient composition. In: Canola: chemistry, production, processing, and utilization. J. K. Daun, N. A. Michael Eskin, and D. Hickling (editors). AOAC Press, Urbana IL. 61802.

Nie, S., J. Huang, J. Hu, Y. Zhang, S. W. C. Li, M. Marcone, and M. Xie. 2013. Effect of pH, temperature, and heating time on the formation of furan in sugar–glycine model systems. Food Sci. Hum. Wel. 2: 87-92.

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

National Research Council. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

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

NSW Department of Primary Industries. 2014. Variability of quality traits in canola seed, oil and meal-a review. Jamie Ayton/Horticulture, Wagga Wagga Agricultural Institute. Available at www.dpi.nsw.gov.au

Nursten, H. 2005. The Maillard Reaction. Chemistry, Biochemistry, and Implications. Royal Society of Chemistry, Cambridge, UK.

Nyachoti, C. M., C. F. M. de Lange, and H. Schulze. 1997. Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. J. Anim. Sci. 75:3206–3213.

Nyachoti, C. M., E. M. McNeilage-Van de Wiele, C. F. M. de Lange, and V. M. Gabert. 2002. Evaluation of the homoarginine technique for measuring true ileal amino acid digestibilities in pigs fed a barley-canola meal-based diet. J. Anim. Sci. 80:440–448.

Ockenden, I., J. A. Dorsch, M. M. Reid, L. Lin, L. K. Grant, V. Raboy, and J. N. A. Lott. 2004. Characterization of the storage of phosphorus, inositol phosphate 123 and cations in grain tissues of four barley (Hordeum vulgare L.) low phytic acid genotypes. Plant Sci. 167:1131-1142.

Opapeju, F. O., A. Golian, C. M. Nyachoti and L. D. Campbell. 2006. Amino acid digestibility in dry extruded-expelled soybean meal fed to pigs and poultry. J. Anim. Sci. 84:1130-1137.

Pahm, A. A., C. Pedersen, and H. H. Stein. 2008. Application of the reactive Lys procedure to estimate Lys digestibility in distillers dried grains with solubles fed to growing pigs. J. Agric. Food Chem. 56:9441-9446.

Palacios, M. F., R. A. Easter, K. T. Soltwedel, C. M. Parsons, M. W. Douglas, T. Hymowitz, and J. E. Pettigrew. 2004. Effects of soybean variety and processing on growth performance of young chicks and pigs. J. Anim. Sci. 82:1108-1114.

Parker M. L., A. Grant, N. M. Rigby, P. S. Belton, and J. N. R. Taylor. 1999. Effects of popping on the endosperm cell walls of sorghum and maize. J. Cereal Sci. 30:209–216.

Parsons, C. M., K. Hashimoto, K. J. Wedekind, and D. H. Baker. 1991. Soybean protein solubility in potassium hydroxide: An in vitro test of an in vivo protein quality. J. Anim. Sci. 69:2918–2924.

Parsons, C. M., K. Hashimoto, K. J. Wedekind,Y. Han, and D. H. Baker. 1992, Effect of overprocessing on availability of amino acids and energy in Soybean meal. Poult. sci. 71: 133-140.

Parsons, C. M. Digestible amino acids for poultry and swine. Anim. Feed Sci. Technol. 59: 147-153.

Pastuszewsk, B., G. Jablecki, L. Buraczewska, P. Dakowski, M. Taciak, R. Matyjek, and A. Ochtabinska. 2003. The protein value of differently processed rapeseed solvent meal and cake assessed by in vitro methods and in tests with rats. Anim. Feed Sci. Technol. 106:175-188.

Patience, J. F., D. Gillis and C. F. M. de Lange. 1996. Dehulled canola meal for growing-finishing pigs. Monograph No. 96-02. Prairie Swine Centre. Saskatoon, Canada.

Petit, J. A., S. J. Slinger, E. V. Evans, and F. N. Marcellus. 1944. The utilization of sunflower seed oilmeal, wheat distllers's dried grains and rapeseed oilmeal in poultry rations. J. Sci. Agric. 24:201-213.

Pickard, M. D. 1993. Processing of canola seed for quality meal. In: Canola meal: feed industry guide. Hickling, D. (edit). Canola Council of Canada. Winnipeg, Manitoba.

Pickard, M. D., C. G. Youngs, L. R. Wetter, and G. S. Boulter. 1989. Processing of canola seed for quality meal. Pages 3-4 in: Canola Meal for Livestock and Poultry. D. R. Clandinin, ed.Canola Council of Canada, Winnipeg, MB, Canada.

Proski, L., N. -G. Asp, I. Furda, J. W. DeVries, T. F. Schweizer, and B. F. Harland. 1984. Determination of total dietary fiber in foods, food products, and total diets: Interlaboratory study. J. Assoc. Off. Anal. Chem. 67:1044-1052.

Proski, L., N. -G. Asp, I. Furda, J. W. DeVries, T. F. Schweizer, and B. F. Harland. 1985. Determination of total dietary fiber in foods and food products: Collaborative study. J. Assoc. Off. Anal. Chem. 68:677-679.

Purlis, E. 2010. Browning development in bakery products - A review. J. Food Eng. 99:239–249.

Radfar, M., A. Rogiewicz, and B. A. Slominski. 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.

Raj, S., H. Fandrejewski, D. Weremko, G. Skiba, L. Buraczewska, T. Zebrowska and I. K. Han. 2000. Growth performance, body composition, and protein and energy utilisation of pigs fed ad libitum diets formulated according to digestible amino acid content. Asian-Aus. J. Anim. Sci. 13:817–823.

Ravindran, V., S. Cabahug, G. Ravindran and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci.78:699–706.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on nutrient digestibility and retention. B. Poult. Sci. 41:193-200.

Ravindran, V., L. I. Hew, G. Ravindran, W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. Anim. Sci. 81: 85-97.

Ravindran, V., O. Adeola, M. Rodehutscord, H. Kluth, J. D. van der Klis, E. van Eerden, and A. Helmbrecht. 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – Results of collaborative studies and assay recommendations. Anim. Feed Sci. Techno. 225:62-72.

Rayner, C. J. and M. Fox. 1976. Amino acid digestibility studies of autoclaved rapeseed meals using an in vitro enzymatic procedure. J. Sci. Food Agric. 27:643-648.

Robbins, K. R., D. H. Baker, and J. W. Finley. 1980. Studies on the utilization of lysinoalanine and lanthionine. J. Nutr. 110:907-915.

Robertson, W. M., M. E. R. Dugan, S. J. Landry, K. Erin, G. Clayton and S. Jaikaran. 2000. Evaluation of live performance, carcass composition and meat quality of market hogs fed diets with various combinations of peas, canola meal and soybean meal with wheat or corn as the cereal base. Lacombe Research Station. Agriculture and Agri-Food Canada.

Rochell, S. J., B. J. Kerr, and W. A. Dozier. 2011. Energy determination of corn co-products fed to broiler chicks from 15 to 24 days of age, and use of composition analysis to predict nitrogen-corrected apparent metabolizable energy. Poult. Sci. 90:1999–2007.

Rodehutscord, M., M. Kapocius, R. Timmler, A. Dieckmann. 2004. Linear regression approach to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45: 85-92.

Roosenstein, A. M., J. W. Cone, and A. C. Beynen. 2000. An attempt to predict protein digestibility of feedstuffs in pigs by using the values for poultry. Neth. J. Agri. Sci. 48:341-347.

Roth-Maier, D. A., B. M. Böhmer and F. X. Roth. 2004. Effects of feeding canola meal and sweet lupin (L. uteus, L. angustifolius) in amino acid balanced diets on growth performance and carcass characteristics of growing-finishing pigs. Anim. Res. 53:21–34.

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

Sanjayan, N., J. M. Heo and C. M. Nyachoti. 2014. Nutrient digestibility and growth performance of pigs fed diets with different levels of canola meal from Brassica napus black and and *Brassica juncea* yellow. J. Anim. Sci. 92:3895–3905.

Sauer, W. C. and L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical applications. A review. Livest. Prod. Sci. 15:367-388.

Sauvant, D., J. M. Perez, and G. Tran. 2004. "Tables of composition and nutritional value of feed materials," Second/Ed. Wageningen Academic Publishers, Netherlands.

Savic, T. B., T. Kricka, N. Voca, V. Jurisic, and A. Matin. 2009. Effect of storage temperature on rapeseed quality. Agric. Conspec. Sci. 74:143-147.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilization. Nutr. Res. Rev. 13: 255-278.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. A review. Anim. Feed Sci. Technol. 35:1-41.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99-122.

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

Seneviratne, R. W., M. G. Young, E. Beltranena, L. A. Goonewardene, R. W. Newkirk, and R. T. Zijlstra. 2010. The nutritional value of expeller-pressed canola meal for grower-finisher pigs. J. Anim. Sci. 88:2073–2083.

Simbaya, J. 1995. Potential for improved canola meal utilization by monogastric animals. Ph.D. dissertation, University of Manitoba, Winnipeg, Manitoba, Canada.;

Simbaya, J., B. a Slominski, G. Rakow, L. D. Campbell, R. K. Downey, and J. M. Bell. 1995. Quality Characteristics of Yellow-Seeded Brassica Seed Meals: Protein , Carbohydrates, and Dietary Fiber Components. J. Agric. Food Chem. 43:2062–2066.

Sklan, D., D. Dubrov, U. Eisner, and S. Hurwitz. 1975. ^{51}Cr-EDTA, ^{91}Y and ^{141}Ce as nonabsorbed reference substances in the gastrointestinal tract of the chicken. J. Nutr. 105:1549-1552.

Skoch, E.R., K. C. Behnke, C. W. Deyoe, and S. F. Binder. 1981. The effect of steam-conditioning rate on the pelleting process. Anim. Feed Sci. Technol. 6, 83–90.

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

Slominski B. A. and L. D. Campbell. 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. 2015. Canola science cluster research report. Canola Council of Canada.

Slominski, B. A. 1997. Developments in the breeding of low fiber rapeseed/canola. J. Anim. Feed Sci. 6:303-317.

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., D. Boros, L. D. Campbell, W. Guenter, and O. Jones. 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., J. Simbaya, L. D. Campbell, G. Rakow, and W. Guenter. 1999. Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. Anim. Feed Sci. Technol. 78:249-262.

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

Slominski, B. A., Meng, X., Campbell, L. D., Guenter, W., Jones, O., 2006. The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part II: Flaxseed. Poult. Sci. 85:1031-1037.

Slominski, B.A. and L. D. Campbell. 1989. Indoleacatonitriles- thermal degradation products of indoleglucosinolates in commercial rapeseed (brassica napus) meal. J. Sci. Food Agric. 47: 75-84.

Slomiski B. A., W. Jia, A. Rogiewicz, C. M. Nyachoti, and D. Hickling. 2012. Low-Fiber Canola. Part 1. Chemical and Nutritive Composition of the meal. J. Agric. Food Chem. 60:12225-12230.

Smallman, C. 1996. Maximising conditioning potential. Feed Milling Int. 190:15–16.

Snee, R. D. 1977. Validation of regression models: methods and examples. Technometrics. 19:415–428.

Sotak-Peper, K. M., J. C. Gonzalez-Vega, and H. H. Stein. 2017. Amino acid digestibility in soybean meal sourced different regions of the United States and fed to pigs. J. Anim. Sci. 95:771-778.

Southgate, D. A. T. 1969. Determination of carbohydrates in foods. II. Unavailable carbohydrates. J. Sci. Fd. Agric. 20:331-335.

Spragg, J. and R. Mailer. 2007. Canola Meal Value Chain Quality Improvement. A Final Report Prepared for AOF and Pork CRC. JCS Solutions Pty Ltd., BerwickVic., AU.

Spragg, J. and R. Mailer. 2008. Canola Meal Value Chain Quality Improvement. A Final Report Prepared for AOF and Pork CRC. JCS Solutions Pty Ltd., BerwickVic., AU.

Statistics Canada. 2015. Canola: a Canadian success story. Edited by Luc Casséus. http://www.statcan.gc.ca/pub/96-325-x/2007000/article/10778-eng.htm, Accessed Dec., 2015.

Stefansson B. R, F. W. Hougen, and R. K. Downey. 1961. Note on the isolation of rape plants with seed oil free from erucic acid. Can. J. Plant Sci. 41:218–9.

Stein, H. H., M. L. Gibson, C. Pedersen, and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grains with solubles fed to growing pigs. J. Anim. Sci. 84:853-860.

Swiech, E. 2017. Alternative prediction methods of protein and energy evaluation of pig feeds. J. Anim. Sci. Biotecnol. 8:39

Thacker, P. A. and R. W. Newkirk. 2005. Performance of growing-finishing pigs fed barley-based diets containing toasted or non-toasted canola meal. Can. J. Anim. Sci. 85:53–59.

Taylor, A. J. and C. J. Smith. 1992. Effect of sowing date and seeding rate on yield and yield components of irrigated canola (Brassica napus L.) grown on a red-brown earth in south-eastern Australia. Just. J. Agric. Res. 43:1629-1641.

Theander, O. and P. Aman. 1974. Carbohydrates in rapeseed and turnip rapeseed meals. Proceedings to the 4th International Rapeseed congress. Giessen, Germany. pp 429438.

Theander, O. and P. Aman. 1979. Studies on dietary fiber. 1. Analysis and chemical characterization of water-soluble and water-insoluble dietary fibers. Swedish J. Agric. Res. 9:97-106.

Theander, O., P. G. Aman, F. Miksche, and S. Yasunda. 1977. Carbohydrates, polyphenols and lignin in seed hulls of different colours from turnip rapeseed. J. Agric. Food Chem. 25:270-273.

Theander, O., P. Åman, E. Westerlund, and H. Graham. 1989. Plant cell walls and monogastric diets. Anim. Feed Sci. Technol. 23:205-225.

Theander. O., P. Aman, E. Westerlund, and H. Graham. 1994. Enzymatic/chemical analysis of dietary fiber. J. AOAC Int. 77:703-709.

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

Toghyani, M., N. Rodgers, M. R. Barekatain, P. A. Iji, and R. A. Swick. 2014. Apparent metabolizable energy value of expeller-extracted canola meal subjected to different processing conditions for growing broiler chickens. Poult. Sci. 93:2227-2236.

Toghyani, M., N. Rodgers, P. A. Iji, and R. A. Swick. 2015. Standardized ileal amino acid digestibility of expeller-extracted canola meal subjected to different processing conditions for starter and grower broiler chickens**.** Poult. Sci. 94:992-1002.

Toghyani, M, R. A. Swick, and R. Barekatain. 2017. Effect of seed source and pelleting temperature during steam pelleting on apparent metabolizable energy value of full-fat canola seed for broiler chickens. Poult. Sci. 96:1325-1333.

Trindade Neto, M. A., F. O. Opepaju, B. A. Slominski and C. M. Nyachoti. 2012. Ileal amino acid digestibility in canola meal from yellow- and black-seeded Brassica napus and Brassica juncea fed to growing pigs. J. Anim. Sci. 90:3477–3484.

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

Trowell, H., D. A. T. Southgate, T. M. S. Wolever, A. R. Leeds, M. A. Gassull, and D. J. A. Jenkins. 1976. Dietary fibre redefined. Lancet 1:967.

Tuohy, K. M., D. J. S. Hinton, S. J. Davies, M. J. C. Crabbe, G. R. Gibson, and J. M. Ames. 2006. Metabolism of Maillard reaction products by the human gut microbiota – implications for health. Mol. Nutr. Food Res. 50:847-857.

Unger, E. H. 2011. Processing. In: Daum, J.K., Eskin, N.A.M., Hickling, D.(Eds.), Canola: Chemistry, Production, Processing, and Utilization. AOCS Press, Urbana, IL, USA, pp. 163–188.

Unger, H. E. 1990. Commercial processing of canola and rapeseed: crushing and oil extraction. In Shahidi, F. (Ed.), Canola and Rapeseed Production, Chemistry, Nutrition, and Processing Technology. Van Nostrand Reinhold, New York, pp. 235-360.

Urriola, P. E., L. J. Johnson, H. H. Stein, and G. C. Shurson. 2013. Prediction of the concentration of standardized ileal digestible amino acids in distillers dried grains with solubles. J. Anim. Sci. 91:4389-4396.

USDA. Economic Research Serinevice. Soybeans and oil crops, canola. 2012.

Vadke VS, Sosulski FW. 1988. Mechanics of oil expression from canola. J Am Oil Chem Soc. 65:1169–1176.

Valle-Riestra, J., and R. H. Barnes. 1970. Digestion of heat-damaged egg albumen by the rat. J. Nutr. 100:873-882.

van Barneveld, R.J. 1998. Influence of oil extraction method on the nutritional value of canola meal for growing pigs. DAS 38/1188 Final Report PRDC and AOF.

Van Boekel, M. A. J. S. 2001. Kinetic aspects of the Maillard reaction: A critical review. Nah. Food 45:150-159.

Van Kempen, T. and D. Jackson. 1996. NIRS may provide rapid evaluation of amino acids. Feedstuffs. 68:12-15.

Van Kempen, T. and P. H. Simmins. 1997. Near-Infrared Reflectance Spectroscopy in precision feed formulation. J. Appl. Poult. Res. 6:471-477.

Van Rooijen, C., G. Bosch, P. A. Wierenga, W. H., Hendriks, and A. F. B. van der Poel. 2014. The effect of steam pelleting of a dry dog food on the Maillard reaction. Anim. Feed. Sci. Technol. 198:238-247.

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination fiber and lignin. J. AOAC 46:829-835.

Van Soest, P. J. and R. H. Wine. 1967. Use of detergents in the analysis fibrous feeds. IV. Determination of plant cell-wall constituents. J. AOAC 50:50-55.

Van Soest, P. J. 1984. Some physical characteristics of dietary fibers and their influence on the microbial ecology of the human colon. Proc. Nutr. Soc. 43:25-33.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74:3583-3597.

Van Soest, P. J. 1992. "Nutritional ecology of the ruminant," Second/Ed. Cornell University Press, United States of America.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, p. 194.

Wang, H.-Y., H. Qian, and W.-R. Yao. 2011. Melanoidins produced by the Maillard reaction: Structure and biological activity. Food Chem. 128:573–584.

Wang, L. F., E. Beltranena, and R. T. Zijlstra. 2017. Diet nutrient digestibility and growth performance of weaned pigs fed *Brassica napus* canola meal varying in nutritive quality. Anim. Feed Sci. Technol. 223:90-98.

Weber S., M. K. Zarhloul, and W. Friedt. 2001. Modification of oilseed quality by genetic transformation. In: Esser K, Kadereit JW, Lüttge U, Runge M (eds) Progress in botany, vol. 62. Springer, Berlin Heidelberg New York, pp 140–174.

White, J.W. 1979. Production of rapeseed in Canada. In: The story of rapeseed in the Western Cape areas of South Africa. South Afr. J. Anim. Sci. 37:45–50.

Woyengo, T. A., E. Kiarie and C. M. Nyachoti. 2010. Energy and amino acid utilization in expeller-extracted canola meal fed growing pigs. J. Anim. Sci. 88:1433-1441.

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2010. Metabolizable and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poult. Sci. 89:1182-1189.

Yang, Y., E. Kiarie, B. A. Slominski, A. Brûlé-Babel, and C. M. Nyachoti. 2010. Amino acid and fiber digestibility, intestinal bacterial profile, and enzyme activity in growing pigs fed dried distillers grains with solubles-based diets. J. Anim. Sci. 88:3304–3312.

Yaylayan, V. A., and A. Huyghues-Despointes. 1994. Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. Crit. Rev. Food Sci. Nutr.34:321-369.

Youngs, C.G. and L.R. Wetter. 1967. Micro determination of the major individual isothiocyanates and oxazolidinethiones in rapeseed; J. Am. Oil Chem. Soc. 44:551–555.

Zhang, F. and O. Adeola. 2017. Energy values of canola meal, cottonseed meal, bakery meal, and peanut flour meal for broiler chickens determined using the regression method. Poult. Sci. 96:397-404.

Zijlstra, R. T., M. L. Swift, L. F. Wang, T. A. Scott, and M. U. Edney. 2011. Near-Infrared reflectance spectroscopy accurately predicts the digestible energy content of barley for pigs. Can. J. Anim. Sci. 91:301-304.

APPENDICES

Appendix I. Chemical composition of expeller-extracted canola meal (CM) from a Canadian processing plant over a 4-year period (%, DM basis) [1]

Components	2011	2012	2013	2014	Average
Crude protein	36.9	38.4	40.6	35.6	37.9
Fat	13.0	11.9	12.1	11.2	12.0
Ash	7.8	7.6	7.5	7.0	7.5
Carbohydrates					
Sucrose	4.7	6.1	5.3	6.0	5.3
Oligosaccharides[2]	1.5	3.0	3.0	2.4	2.5
Dietary fiber fractions					
Neutral detergent fiber (NDF)	32.0	26.9	30.9	36.3	31.5
Total dietary fiber	40.1	38.2	37.7	43.9	40.0
Non-starch polysaccharides	19.7	21.3	18.0	19.7	19.7
Glycoproteins (NDICP)[3]	5.7	5.4	6.6	6.9	6.2
Lignin and polyphenols	14.7	11.5	13.1	16.7	14.0
Phosphorus (P)	1.00	1.03	0.96	1.01	1.00
Phytate P	0.62	0.57	0.73	0.70	0.66
Non-phytate P	0.38	0.46	0.23	0.31	0.34
Glucosinolates, μmol/g DM[4]	11.1	11.6	12.3	7.4	10.6

[1] Values are means of three canola meal samples.
[2]Includes raffinose and stachyose.
[3]Neutral detergent insoluble crude protein.
[4]Includes 3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, 2-hydroxy-4-Pentenyl, 2-hydroxyl-4-pentenyl, 3-indolymethyl and 4-hydroxy-3-indolylmethyl.

Appendix II. Amino acid composition of expeller-extracted canola meal (CM) from a Canadian processing plant over a 4-year period (%, DM basis) [1]

Item	2011	2012	2013	2014	Average
Indispensable AA					
Arg	1.79	2.36	2.50	2.04	2.17
His	0.85	1.23	1.26	1.04	1.10
Ile	1.18	1.37	1.23	1.12	1.23
Leu	2.32	2.59	2.82	2.16	2.47
Lys	1.56	1.95	2.06	1.65	1.80
Met	0.41	0.71	0.65	0.70	0.62
Cys	0.66	0.84	0.75	0.81	0.76
Phe	1.26	1.46	1.54	1.23	1.37
Thr	1.38	1.69	1.25	1.45	1.44
Val	1.46	1.87	1.90	1.54	1.69
Dispensable AA					
Ala	1.42	1.58	2.26	1.32	1.64
Asp	2.35	2.67	2.76	2.32	2.52
Glu	5.45	6.82	7.42	5.72	6.35
Gly	1.74	1.93	1.39	1.65	1.68
Pro	2.11	2.52	3.45	2.20	2.57
Ser	1.44	1.71	1.98	1.47	1.65
Tyr	0.83	0.97	0.90	0.83	0.88

[1] Values are means of three canola meal samples.

Appendix III. True metabolizable energy (TME_n) contents of solvent-extracted canola meal derived from three Canadian processing plants[1,2]

Plant	TME_n, kcal/kg DM
CM1	2,267
CM2	2,201
CM3	2,297

[1]CM1 to CM3 are canola meal samples from plants 1 to 3.

[2]Nitrogen-corrected true metabolizable energy contents of canola meals were determined at the University of Illinois using the assay procedure described by Parsons (1985). Briefly, 30 g of each canola meal samples (B. napus black, and B. juncea yellow) were precision-fed to 2 groups of 5 individually caged cecectomized Single Comb White Leghorn roosters after 24 h of feed withdrawal. Excreta were then collected during the next 48 h. The excreta samples were frozen, freeze-dried, weighed to determine total output and ground to pass through 1-mm sieve. Feed and excreta were analyzed for nitrogen (990.03, AOAC) and for gross energy using an adiabatic bomb calorimeter. TME_n was calculated as described by Parsons et al. (1992). Endogenous corrections for energy were made using roosters fed 50 mL of a 500 g/kg glucose solution (25 g of dry glucose) for 48 h to determine the endogenous excretion of energy and nitrogen.

Appendix IV. Chemical composition of canola meals from different crushing plants (% DM; Mean ± SD)

Sample	Crude Protein	Ether Extract	Glucosinolates	Ash	Total P	Non-phytate P
Plant 1						
2011	40.8 ±0.54	4.0 ±0.19	8.3 ±0.38	8.1 ±0.03	1.00 ±0.03	0.37 ±0.01
2012	42.0 ±0.61	4.5 ±0.20	9.8 ±1.42	7.7 ±0.12	1.17±0.03	0.54 ±0.03
2013	45.4 ±0.37	3.9 ±0.23	12.0 ±1.00	7.1 ±1.47	1.10 ±0.05	0.31 ±0.09
2014	41.5 ±0.43	3.5 ±0.15	8.5 ±0.26	8.0 ±0.17	1.12 ±0.17	0.41 ±0.04
Mean	**42.4**	**4.0**	**9.7**	**7.7**	**1.10**	**0.41**
Plant 2						
2011	39.3 ±1.94	4.8 ±0.23	2.0 ±0.38	8.5 ±0.40	0.93 ±0.04	0.34 ±0.05
2012	41.0 ±0.28	4.6 ±0.17	3.6 ±1.46	7.3 ±0.30	1.04 ±0.03	0.48 ±0.03
2013	42.9 ±0.59	4.6 ±0.69	3.2 ±0.12	7.9 ±0.09	1.04 ±0.03	0.31 ±0.07
2014	40.9 ±0.59	3.5 ±0.58	3.2 ±0.42	7.5 ±0.16	1.11±0.02	0.40 ±0.05
Mean	**41.0**	**4.4**	**3.0**	**7.8**	**1.03**	**0.38**
Plant 3						
2011	39.4 ±0.28	3.6 ±0.28	4.5 ±0.84	8.3 ±0.41	0.95 ±0.04	0.32 ±0.03
2012	42.2 ±0.63	4.1 ±1.01	7.7 ±1.07	7.1 ±0.66	1.12 ±0.02	0.53 ±0.02
2013	43.2 ±0.48	3.8 ±0.09	7.5 ±0.86	7.8 ±0.13	1.10 ±0.04	0.23 ±0.03
2014	40.6 ±0.57	3.1 ±0.39	7.0 ±1.54	7.5 ±0.19	1.10 ±0.01	0.42 ±0.04
Mean	**41.3**	**3.6**	**6.7**	**7.7**	**1.07**	**0.37**
Plant 4						
2011	42.1 ±0.37	3.7 ±0.24	2.7 ±0.22	7.9 ±0.09	1.04 ±0.03	0.41 ±0.05
2012	42.5 ±0.23	3.9 ±0.28	1.8 ±0.55	7.0 ±0.05	1.12 ±0.14	0.50 ±0.18
2013	44.7 ±1.57	3.3 ±0.41	1.8 ±0.16	7.4 ±0.06	1.17 ±0.04	0.29 ±0.05
2014	41.0 ±0.08	2.0 ±0.49	2.8 ±0.75	7.1 ±0.06	1.15 ±0.03	0.42 ±0.05
Mean	**42.9**	**3.2**	**2.3**	**7.4**	**1.12**	**0.41**
Plant 5						
2011	40.8 ±0.64	4.1 ±1.51	3.2 ±1.16	7.6 ±0.13	1.00 ±0.01	0.39 ±0.06
2012	41.3 ±0.33	4.9 ±0.18	3.0 ±0.76	7.0 ±0.60	1.13 ±0.02	0.51 ±0.01
2013	44.6 ±0.23	3.5 ±0.26	2.1 ±0.30	8.0 ±0.73	1.09 ±0.03	0.23 ±0.14
2014	41.1 ±0.24	2.7 ±0.08	0.8 ±0.10	8.2 ±0.61	1.17 ±0.03	0.45 ±0.01
Mean	**41.9**	**3.8**	**2.3**	**7.7**	**1.10**	**0.41**
Plant 6						
2011	41.3 ±0.13	3.8 ±0.61	1.6 ±0.21	8.9 ±0.27	1.03 ±0.05	0.39 ±0.03
2012	42.2 ±0.48	3.6 ±0.28	3.5 ±0.69	7.4 ±0.06	1.11 ±0.01	0.48 ±0.06
2013	44.4 ±0.29	3.8 ±0.95	4.9 ±0.31	7.6 ±0.33	1.07 ±0.02	0.28 ±0.03
2014	40.6 ±0.65	3.0 ±0.46	3.2 ±0.82	7.8 ±0.34	1.10 ±0.05	0.36±0.11
Mean	**42.1**	**3.6**	**3.3**	**7.9**	**1.08**	**0.37**
Plant 7						
2011	38.7 ±0.12	3.9 ±0.07	6.6 ±0.78	7.7 ±0.13	1.04 ±0.01	0.41 ±0.04
2012	40.3 ±0.60	4.2 ±0.55	9.3 ±2.38	7.0 ±0.22	1.19 ±0.03	0.53 ±0.06
2013	42.5 ±0.52	3.4 ±0.16	3.1 ±0.22	7.4 ±0.70	1.12 ±0.02	0.28 ±0.14
2014	39.6 ±0.51	0.6 ±0.08	5.8 ±1.41	7.4±0.06	1.10 ±0.01	0.38 ±0.02
Mean	**40.3**	**3.0**	**6.2**	**7.4**	**1.11**	**0.40**
Plant 8						
2011	41.0 ±0.46	1.5 ±0.32	2.6 ±0.79	8.0 ±0.24	1.00 ±0.04	0.33 ±0.02
2012	42.6 ±0.53	2.7 ±1.22	5.2 ±0.75	6.8 ±0.12	1.12 ±0.02	0.52 ±0.03
2013	42.0 ±0.62	3.9 ±0.70	8.2 ±0.12	6.9 ±0.31	1.04 ±0.02	0.20 ±0.07
2014	41.2 ±0.27	2.4 ±0.11	4.6 ±1.15	6.7 ±0.29	1.07 ±0.01	0.32 ±0.02
Mean	**41.7**	**2.6**	**5.2**	**7.1**	**1.06**	**0.35**
Plant 9						

2011	40.8 ±3.08	4.7 ±0.66	0.9 ±0.11	7.9 ±0.51	1.01 ±0.03	0.39 ±0.05
2012	41.0 ±0.91	4.0 ±0.79	2.0 ±1.34	7.4 ±0.08	1.10 ±0.02	0.48 ±0.03
2013	44.2 ±0.55	3.0 ±0.30	3.1 ±0.58	7.0 ±0.13	1.11 ±0.11	0.39 ±0.17
2014	40.5 ±0.10	3.8 ±0.46	1.6 ±0.39	7.4 ±0.27	1.13 ±0.01	0.44 ±0.03
Mean	**42.0**	**3.9**	**2.0**	**7.4**	**1.07**	**0.42**
Plant 10						
2011	42.4 ±0.22	3.1 ±0.36	7.4 ±4.26	7.8 ±0.21	1.08 ±0.02	0.46 ±0.04
2012	43.3 ±0.09	2.7 ±0.11	6.2 ±0.28	7.3 ±0.52	1.16 ±0.01	0.50 ±0.03
2013	44.0 ±0.70	2.8 ±0.39	5.2 ±1.14	6.8 ±0.83	1.16 ±0.06	0.26 ±0.02
2014	40.6 ±0.85	2.3 ±0.35	3.6 ±1.15	7.4 ±0.28	1.15 ±0.01	0.42 ±0.04
Mean	**42.6**	**2.7**	**6.3**	**7.3**	**1.13**	**0.41**
Plant 11						
2011	40.1 ±0.69	4.0 ±0.33	4.6 ±1.31	7.8 ±0.16	1.04 ±0.03	0.39 ±0.04
2012	40.6 ±0.33	4.4 ±0.09	5.4 ±2.12	7.1 ±0.09	1.11 ±0.07	0.50 ±0.06
2013	43.0 ±0.69	3.4 ±0.33	4.4 ±1.46	7.1 ±0.16	1.10 ±0.05	0.24 ±0.06
2014	37.3 ±0.54	3.8 ±0.16	2.9 ±0.89	7.1 ±0.32	1.12 ±0.03	0.39 ±0.04
Mean	**40.2**	**3.9**	**4.3**	**7.3**	**1.09**	**0.38**

Appendix V. Carbohydrates and fiber compositions of canola meal from different crushing plants (% DM; Mean ±SD)

Sample	Simple sugars	Sucrose	Oligo-saccharides	NDF	Dietary Fiber Components			
					NSP	Glyco-protein	Lignin	Total
Plant 1								
2011	0.2 ±0.15	5.5 ±0.27	1.8 ±0.32	27.2 ±0.92	19.5 ±0.73	3.0 ±0.66	11.6 ±0.30	34.1 ±0.84
2012	0.8 ±0.45	6.0 ±0.44	3.2 ±0.29	28.4 ±1.52	21.1 ±0.68	5.8 ±1.33	8.7 ±2.59	35.7 ±2.06
2013	0.1 ±0.01	5.6 ±0.23	2.9 ±0.11	23.3 ±0.55	19.1 ±1.73	3.4 ±0.18	7.7 ±0.42	30.1 ±1.91
2014	0.2±0.01	7.0±0.21	3.2±0.05	29.5±1.01	22.9±0.24	5.4±1.00	11.1±1.28	39.3±0.80
Mean	**0.3**	**6.0**	**2.8**	**27.1**	**20.7**	**4.4**	**9.8**	**34.8**
Plant 2								
2011	0.2 ±0.02	5.2 ±0.08	1.5 ±0.09	31.9 ±0.90	22.3 ±0.31	6.3 ±0.33	12.4 ±1.07	41.0 ±1.54
2012	0.4 ±0.02	6.7 ±0.49	3.3 ±0.24	31.8 ±2.19	21.2 ±0.63	7.1 ±1.24	11.3 ±0.86	39.6 ±1.51
2013	0.3 ±0.09	5.2 ±0.18	2.7 ±0.08	30.9 ±0.82	19.6 ±0.60	7.9 ±0.40	10.2 ±1.17	37.7 ±1.14
2014	0.4±0.20	7.6±0.97	3.4±0.44	34.7±1.40	23.7±1.03	7.8±0.52	12.8±1.50	44.3±1.81
Mean	**0.3**	**6.1**	**2.7**	**32.3**	**21.7**	**7.3**	**11.7**	**40.7**
Plant 3								
2011	0.2 ±0.03	5.3 ±1.25	1.7 ±0.28	27.6 ±1.34	21.6 ±0.93	3.1 ±0.55	11.3 ±0.29	35.9 ±1.49
2012	0.5 ±0.15	6.2 ±0.01	2.9 ±0.07	28.2 ±2.81	21.7 ±1.36	4.8 ±1.87	9.0 ±1.89	35.6 ±2.30
2013	0.1 ±0.02	5.3 ±0.26	2.9 ±0.35	26.7 ±0.95	19.4 ±0.67	4.0 ±0.28	10.3 ±0.26	33.7 ±0.46
2014	0.2±0.01	7.0±0.20	3.3±0.37	30.9±2.49	22.1±0.37	5.8±1.09	13.0±0.80	40.9±1.57
Mean	**0.2**	**5.9**	**2.7**	**28.4**	**21.2**	**4.4**	**10.9**	**36.5**
Plant 4								
2011	0.2 ±0.04	5.4 ±0.80	1.3 ±0.24	26.4 ±0.74	21.0 ±0.19	3.5 ±0.23	10.5 ±0.37	35.0 ±0.79
2012	0.9 ±0.30	6.4 ±1.17	2.9 ±0.34	28.7 ±1.91	21.0 ±0.98	5.2 ±0.28	9.6 ±2.83	35.7 ±1.69
2013	0.4 ±0.03	5.6 ±0.23	2.8 ±0.10	27.9 ±1.81	20.6 ±1.38	5.5 ±0.65	9.3 ±0.98	35.5 ±2.16
2014	0.3±0.12	6.3±0.22	2.9±0.35	29.1±1.46	21.8±0.58	5.1±0.90	12.8±0.97	39.7±1.92
Mean	**0.4**	**5.7**	**2.3**	**28.3**	**21.3**	**4.9**	**10.7**	**36.8**
Plant 5								
2011	0.4 ±0.08	5.8 ±0.26	1.6 ±0.20	30.0 ±2.58	21.8 ±0.26	4.9 ±1.61	11.4 ±0.26	38.1 ±2.14
2012	0.8 ±0.52	6.0 ±0.36	2.9 ±0.18	28.2 ±1.22	21.1 ±0.77	5.1 ±0.43	9.4 ±1.10	35.6 ±1.48
2013	0.4 ±0.00	5.5 ±0.13	3.0 ±0.10	25.5 ±1.44	20.7 ±0.24	5.1 ±0.45	8.1 ±0.41	34.0 ±0.76
2014	0.7±0.11	6.8±0.24	2.9±0.09	31.4±0.9	21.7±0.76	7.2±1.09	12.2±0.42	41.1±0.57
Mean	**0.6**	**5.9**	**2.6**	**28.8**	**21.3**	**5.6**	**10.3**	**37.2**
Plant 6								
2011	0.3 ±0.10	5.5 ±0.49	1.7 ±0.43	29.7 ±0.87	21.9 ±0.51	5.2 ±0.67	11.8 ±0.13	38.8 ±0.22
2012	0.6 ±0.67	6.5 ±0.49	2.9 ±0.12	29.5 ±1.96	22.0 ±0.58	6.1 ±1.12	8.7 ±1.88	37.3 ±1.71
2013	0.2 ±0.07	5.7 ±0.14	3.1 ±0.13	25.5 ±0.48	21.6 ±0.61	5.4 ±0.06	7.9 ±0.07	34.8 ±0.58
2014	0.1±0.00	6.9±0.25	3.2±0.01	31.0±0.66	22.3±0.94	4.9±0.90	12.3±0.49	40.1±1.40
Mean	**0.3**	**5.9**	**2.7**	**28.9**	**21.9**	**5.4**	**10.3**	**37.8**
Plant 7								
2011	0.1 ±0.01	6.1 ±0.13	1.7 ±0.18	28.7 ±1.74	20.3 ±0.87	3.8 ±0.53	10.8 ±0.76	34.9 ±0.95
2012	0.4 ±0.34	7.0 ±0.21	3.3 ±0.31	30.1 ±3.18	22.7 ±1.07	5.6 ±1.94	9.1 ±1.82	37.4 ±3.03
2013	0.2 ±0.02	5.6 ±0.11	3.0 ±0.12	30.2 ±2.16	23.4 ±0.67	6.5 ±1.23	10.1 ±1.20	40.1 ±1.20
2014	0.1±0.01	7.0±0.2	3.1±0.19	30.8±0.42	23.0±0.61	4.5±0.48	12.4±0.51	40.0±0.58
Mean	**0.2**	**6.3**	**2.8**	**29.9**	**22.4**	**5.1**	**10.6**	**38.1**
Plant 8								
2011	0.2 ±0.04	6.3 ±0.32	1.6 ±0.15	31.3 ±2.66	21.2 ±2.20	5.2 ±1.20	12.2 ±1.00	38.6 ±3.60
2012	0.6 ±0.18	6.3 ±0.05	3.1 ±0.03	29.4 ±2.74	23.0 ±1.09	5.4 ±1.36	8.8 ±0.83	37.2 ±2.10
2013	0.2 ±0.02	5.7 ±0.25	3.1 ±0.10	27.9 ±1.48	21.9 ±1.57	4.6 ±0.55	9.8 ±0.82	36.3 ±0.88
2014	0.2±0.01	7.1±0.35	3.2±0.32	32.6±1.00	24.2±0.49	6.5±0.94	12.3±0.43	43.0±0.88
Mean	**0.3**	**6.3**	**2.8**	**30.3**	**22.6**	**5.4**	**10.8**	**38.8**
Plant 9								

2011	0.2 ±0.10	5.2 ±0.41	0.7 ±0.69	35.6 ±2.51	22.0 ±0.41	7.9 ±0.79	14.1 ±0.48	44.0 ±1.63
2012	0.4 ±0.19	6.6 ±0.40	3.3 ±0.11	36.5 ±3.84	23.3 ±0.80	9.6 ±1.81	12.3 ±1.79	45.2 ±2.50
2013	0.3 ±0.02	5.7 ±0.11	2.9 ±0.21	28.3 ±0.52	20.4 ±1.20	5.9 ±0.53	10.1 ±0.41	36.4 ±1.26
2014	0.2±0.12	7.1±0.32	3.4±0.27	33.0±1.21	24.2±0.16	6.6±1.06	12.0±0.84	42.8±1.77
Mean	**0.2**	**5.9**	**2.5**	**33.4**	**22.5**	**7.5**	**12.1**	**41.9**
Plant 10								
2011	0.1 ±0.02	6.3 ±0.44	1.8 ±0.14	25.7 ±0.65	22.1 ±1.70	2.7 ±0.55	10.1 ±0.23	35.0 ±1.74
2012	0.3 ±0.21	7.2 ±0.26	3.4 ±0.06	28.0 ±1.57	21.7 ±1.69	5.0 ±0.90	7.8 ±1.48	34.5 ±2.39
2013	0.2 ±0.07	6.1 ±0.32	3.1 ±0.35	26.5 ±0.13	21.9 ±1.00	4.4 ±0.26	8.8 ±0.94	35.1 ±0.27
2014	0.2±0.04	7.0±0.52	3.5±0.28	30.7±1.51	25.4±0.52	4.6±0.45	12.2±1.43	42.1±2.16
Mean	**0.2**	**6.4**	**2.9**	**27.6**	**22.8**	**4.1**	**9.7**	**36.6**
Plant 11								
2011	0.1 ±0.01	5.7 ±0.17	1.5 ±0.14	29.0 ±1.58	21.0 ±0.36	4.0 ±1.22	11.7 ±0.22	36.7 ±1.03
2012	0.4 ±0.04	6.5 ±0.08	3.2 ±0.15	31.5 ±1.35	22.6 ±2.01	6.3 ±1.67	10.9 ±0.68	39.8 ±2.05
2013	0.3 ±0.03	5.6 ±0.08	3.0 ±0.11	28.7 ±2.01	21.6 ±0.85	5.1 ±0.82	10.0 ±0.95	36.7 ±2.02
2014	0.4±0.13	7.2±0.11	3.4±0.08	31.6±0.61	24.5±1.84	4.6±0.36	12.7±0.16	41.9±1.59
Mean	**0.3**	**6.2**	**3.0**	**30.2**	**22.4**	**5.0**	**11.3**	**38.8**

Appendix VI. Amino acid content of canola meals from different crushing plants (% DM)

Sample	Lys	Arg	His	Ile	Leu	Met	Cys	Phe	Tyr	Thr	Val
Plant 1											
2011	2.03±0.17	1.86±0.19	0.92±0.09	1.22±0.19	2.43±0.28	0.63±0.05	0.82±0.01	1.32±0.15	0.91±0.08	1.47±0.12	1.52±0.21
2012	2.31±0.12	2.52±0.14	1.32±0.02	1.41±0.16	2.77±0.13	0.71±0.09	0.85±0.04	1.57±0.07	1.07±0.04	1.83±0.07	1.93±0.14
2013	2.58±0.03	2.78±0.01	1.37±0.04	1.29±0.06	2.97±0.09	0.74±0.10	0.90±0.11	1.69±0.03	1.01±0.01	1.41±0.05	2.03±0.10
2014	2.24±0.01	2.38±0.01	1.22±0.01	1.23±0.00	2.49±0.02	0.74±0.03	0.86±0.02	1.43±0.01	0.99±0.02	1.70±0.01	1.69±0.00
Mean	**2.29**	**2.39**	**1.21**	**1.29**	**2.67**	**0.70**	**0.86**	**1.50**	**0.99**	**1.60**	**1.79**
Plant 2											
2011	1.70±0.18	1.78±0.14	0.88±0.07	1.18±0.05	2.38±0.21	0.46±0.02	0.70±0.01	1.28±0.10	0.86±0.07	1.42±0.13	1.45±0.07
2012	2.13±0.05	2.38±0.03	1.31±0.03	1.32±0.03	2.68±0.06	0.68±0.12	0.79±0.10	1.51±0.02	1.03±0.02	1.78±0.03	1.83±0.04
2013	2.25±0.05	2.56±0.07	1.31±0.04	1.24±0.06	2.92±0.06	0.83±0.01	0.94±0.03	1.63±0.02	0.97±0.02	1.29±0.03	2.01±0.07
2014	2.06±0.07	2.32±0.05	1.22±0.02	1.27±0.03	2.51±0.04	0.76±0.01	0.86±0.01	1.44±0.01	1.01±0.00	1.69±0.05	1.74±0.04
Mean	**2.03**	**2.26**	**1.18**	**1.25**	**2.62**	**0.68**	**0.82**	**1.47**	**0.99**	**1.54**	**1.75**
Plant 3											
2011	1.87±0.20	1.83±0.21	0.88±0.07	1.20±0.14	2.38±0.23	0.61±0.05	0.79±0.09	1.29±0.11	0.86±0.09	1.45±0.13	1.48±0.16
2012	2.29±0.07	2.56±0.06	1.33±0.02	1.38±0.11	2.78±0.10	0.74±0.02	0.88±0.02	1.56±0.08	1.06±0.04	1.82±0.04	1.88±0.14
2013	2.33±0.06	2.54±0.07	1.30±0.03	1.14±0.06	2.76±0.13	0.79±0.05	0.96±0.04	1.54±0.07	0.97±0.02	1.33±0.04	1.82±0.08
2014	2.16±0.10	2.39±0.10	1.18±0.02	1.29±0.10	2.44±0.07	0.74±0.03	0.86±0.04	1.45±0.10	1.01±0.08	1.68±0.05	1.79±0.13
Mean	**2.16**	**2.33**	**1.17**	**1.25**	**2.59**	**0.72**	**0.87**	**1.46**	**0.98**	**1.53**	**1.74**
Plant 4											
2011	1.77±0.02	1.71±0.08	0.85±0.02	1.12±0.07	2.27±0.09	0.47±0.02	0.77±0.02	1.22±0.02	0.80±0.04	1.36±0.03	1.39±0.10
2012	2.23±0.05	2.51±0.05	1.32±0.01	1.40±0.02	2.81±0.02	0.68±0.07	0.83±0.06	1.58±0.03	1.06±0.03	1.85±0.01	1.93±0.02
2013	2.07±0.26	2.38±0.28	1.21±0.15	1.08±0.12	2.65±0.33	0.80±0.07	0.94±0.06	1.48±0.17	0.85±0.08	1.18±0.15	1.76±0.19
2014	2.00±0.10	2.25±0.11	1.13±0.05	1.23±0.04	2.37±0.13	0.60±0.09	0.69±0.11	1.35±0.07	0.94±0.05	1.59±0.07	1.68±0.09
Mean	**2.02**	**2.21**	**1.13**	**1.21**	**2.53**	**0.64**	**0.81**	**1.41**	**0.91**	**1.49**	**1.69**
Plant 5											
2011	2.08±0.11	2.11±0.04	1.02±0.03	1.35±0.03	2.71±0.01	0.50±0.09	0.75±0.04	1.48±0.01	0.99±0.01	1.63±0.03	1.70±0.04
2012	2.12±0.08	2.39±0.06	1.31±0.01	1.29±0.10	2.67±0.09	0.77±0.02	0.87±0.00	1.49±0.07	1.02±0.03	1.79±0.04	1.78±0.13
2013	2.40±0.04	2.66±0.01	1.38±0.01	1.24±0.09	2.93±0.11	0.79±0.03	0.94±0.03	1.65±0.05	1.01±0.04	1.40±0.05	1.98±0.11
2014	1.99±0.02	2.25±0.06	1.20±0.02	1.27±0.02	2.50±0.01	0.74±0.02	0.84±0.02	1.42±0.01	0.97±0.01	1.68±0.01	1.75±0.02
Mean	**2.15**	**2.35**	**1.23**	**1.29**	**2.70**	**0.70**	**0.85**	**1.51**	**1.00**	**1.62**	**1.80**
Plant 6											
2011	1.87±0.13	1.84±0.12	0.91±0.08	1.15±0.03	2.37±0.17	0.60±0.07	0.78±0.07	1.31±0.09	0.87±0.08	1.45±0.14	1.46±0.04
2012	2.22±0.08	2.53±0.04	1.34±0.02	1.45±0.10	2.82±0.06	0.74±0.08	0.89±0.03	1.60±0.06	1.07±0.03	1.83±0.05	2.02±0.04
2013	2.36±0.08	2.65±0.09	1.34±0.07	1.25±0.04	2.98±0.09	0.77±0.03	0.95±0.04	1.68±0.06	0.99±0.04	1.29±0.06	2.02±0.04
2014	1.88±0.26	2.12±0.30	1.07±0.15	1.19±0.16	2.24±0.32	0.69±0.03	0.81±0.04	1.28±0.18	0.88±0.12	1.49±0.22	1.62±0.21
Mean	**2.08**	**2.29**	**1.16**	**1.26**	**2.60**	**0.70**	**0.86**	**1.47**	**0.95**	**1.51**	**1.78**
Plant 7											

2011	1.81±0.04	1.69±0.02	0.83±0.01	1.11±0.02	2.20±0.00	0.61±0.01	0.79±0.04	1.21±0.01	0.81±0.01	1.36±0.03	1.41±0.03
2012	2.22±0.07	2.43±0.06	1.29±0.01	1.35±0.11	2.70±0.09	0.73±0.02	0.85±0.02	1.53±0.05	1.05±0.04	1.80±0.03	1.87±0.14
2013	2.28±0.04	2.56±0.04	1.30±0.01	1.25±0.05	2.88±0.04	0.76±0.02	0.90±0.01	1.63±0.02	0.99±0.05	1.35±0.04	1.97±0.05
2014	2.09±0.04	2.32±0.03	1.16±0.01	1.24±0.03	2.44±0.04	0.74±0.01	0.86±0.01	1.38±0.03	0.94±0.02	1.66±0.02	1.69±0.04
Mean	**2.10**	**2.25**	**1.14**	**1.23**	**2.56**	**0.71**	**0.85**	**1.44**	**0.95**	**1.54**	**1.74**
Plant 8											
2011	1.83±0.09	1.77±0.03	0.88±0.02	1.15±0.08	2.34±0.05	0.66±0.11	0.85±0.09	1.27±0.02	0.85±0.02	1.42±0.05	1.44±0.11
2012	2.28±0.04	2.52±0.07	1.34±0.01	1.39±0.10	2.79±0.08	0.73±0.03	0.86±0.06	1.57±0.05	1.07±0.02	1.86±0.02	1.91±0.13
2013	2.05±0.22	2.21±0.24	1.16±0.10	1.00±0.17	2.45±0.30	0.73±0.01	0.91±0.03	1.40±0.20	0.86±0.11	1.11±0.13	1.64±0.23
2014	2.11±0.09	2.30±0.09	1.16±0.08	1.24±0.02	2.42±0.11	0.77±0.00	0.89±0.02	1.38±0.06	0.95±0.03	1.73±0.20	1.70±0.03
Mean	**2.07**	**2.20**	**1.14**	**1.20**	**2.50**	**0.72**	**0.88**	**1.40**	**0.93**	**1.53**	**1.67**
Plant 9											
2011	1.67±0.08	1.75±0.08	0.87±0.04	1.24±0.13	2.38±0.16	0.46±0.02	0.72±0.01	1.26±0.07	0.85±0.04	1.40±0.08	1.50±0.14
2012	2.05±0.11	2.36±0.03	1.31±0.03	1.36±0.03	2.66±0.03	0.72±0.04	0.85±0.04	1.50±0.01	1.01±0.01	1.78±0.02	1.88±0.04
2013	2.27±0.15	2.57±0.17	1.34±0.06	1.27±0.06	2.93±0.03	0.73±0.06	0.87±0.05	1.66±0.02	1.00±0.01	1.34±0.05	1.99±0.02
2014	2.01±0.05	2.26±0.02	1.19±0.00	1.25±0.10	2.47±0.10	0.77±0.03	0.85±0.04	1.38±0.02	0.96±0.01	1.70±0.06	1.68±0.08
Mean	**2.00**	**2.24**	**1.18**	**1.28**	**2.61**	**0.67**	**0.82**	**1.45**	**0.96**	**1.55**	**1.76**
Plant 10											
2011	1.74±0.18	1.64±0.23	0.80±0.11	1.07±0.16	2.15±0.32	0.55±0.12	0.81±0.04	1.15±0.16	0.76±0.11	1.28±0.18	1.34±0.21
2012	2.27±0.10	2.44±0.13	1.34±0.02	1.28±0.15	2.71±0.13	0.79±0.01	0.90±0.01	1.51±0.10	1.04±0.05	1.83±0.07	1.77±0.20
2013	2.26±0.11	2.44±0.13	1.28±0.03	1.15±0.04	2.82±0.07	0.53±0.04	0.68±0.03	1.56±0.03	0.93±0.03	1.25±0.02	1.90±0.05
2014	2.15±0.05	2.29±0.08	1.19±0.02	1.26±0.07	2.47±0.08	0.74±0.03	0.85±0.02	1.42±0.05	0.96±0.01	1.68±0.02	1.72±0.09
Mean	**2.11**	**2.21**	**1.16**	**1.19**	**2.54**	**0.65**	**0.81**	**1.41**	**0.92**	**1.51**	**1.68**
Plant 11											
2011	2.00±0.16	1.91±0.12	0.94±0.07	1.19±0.10	2.47±0.13	0.63±0.07	0.78±0.03	1.35±0.09	0.90±0.05	1.51±0.09	1.48±0.10
2012	2.21±0.04	2.47±0.02	1.29±0.02	1.38±0.03	2.71±0.02	0.68±0.05	0.84±0.04	1.52±0.03	1.04±0.01	1.80±0.02	1.89±0.02
2013	2.37±0.04	2.62±0.07	1.34±0.02	1.24±0.08	2.98±0.14	0.58±0.06	0.67±0.09	1.63±0.06	0.99±0.04	1.35±0.06	1.93±0.10
2014	2.05±0.06	2.17±0.06	1.12±0.02	1.18±0.09	2.34±0.11	0.73±0.03	0.83±0.05	1.34±0.06	0.91±0.02	1.60±0.05	1.62±0.10
Mean	**2.16**	**2.29**	**1.17**	**1.25**	**2.62**	**0.66**	**0.78**	**1.46**	**0.96**	**1.57**	**1.73**