High inclusion levels of canola meal in broiler chicken nutrition

A Thesis

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ABSTRACT

Canola meal (**CM**) is a less expensive protein ingredient than soybean meal (**SBM**) for livestock feed formulation. Canola processing has a major impact on the nutritional quality of the meal, limitations in the dietary inclusions are associated with reduced meal quality because of overheating during processing. Crude protein (**CP**) and amino acid (**AA**) digestibility are affected by differences in processing methods and conditions causing a variation in the chemical and nutritive value of the meal. As a result, protein quality of CM can be verified using standardized ileal digestibility (**SID**) of AA in young broiler chickens to ascertain values that are reliable for efficient feed formulation when considering high inclusion levels of CM in poultry diet. Two separate experiments were conducted to measure SID of AA of CM sourced from 14 crushing plants across Canada, as well as to determine the effect of varying inclusion levels of CM on growth performance and nutrient digestibility of young broiler chickens. There were significant variations in SID of Lys (86.3 vs. 74.2%), Trp (94.5 vs. 76.7%) and Thr (87.3 vs.71.6%) of CM for broiler chickens. Whereas, Met (91.8 vs. 87.4%) and Cys (81.8 vs. 73.7%) were not significantly affected but Arg, which is known as a conditionally essential AA, varied significantly (92.0 vs. 83.8%). Lysine (1.97 vs. 1.53%), Thr (1.64 vs. 1.24%) and Met (0.68 vs. 0.46%) as well as other AA varied significantly in their standardized ileal digestible contents, while a regression analysis showed a significant relationship among AA suspected to be heat sensitive. In the growth performance experiment, overall result revealed that neutral detergent fiber (**NDF**) content of diet differed substantially with increased levels of CM but bird performance was not significantly affected. Energy utilization varied with age of birds while NDF digestibility was not affected by incremental levels of CM inclusion.

DEDICATION

This thesis is dedicated to God, my parent, siblings, friends and colleagues who nurtured me to be what I am today.

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FOREWORD

Some part of this thesis has been presented as poster presentation at the Animal Nutrition Conference of Canada in Edmonton Alberta, May 2-3, 2018. This thesis was written in manuscript format and it is made up of two manuscripts. All manuscripts will be submitted for publication.

Manuscript 1. S.A. Ariyibi., A. Rogiewicz., B.A. Slominski (2018). Standardized ileal amino acid digestibility of canola meal in broiler chickens in relation to its source. (Chapter 3)

Manuscript 2. S.A. Ariyibi., A. Rogiewicz., B.A. Slominski (2018). High inclusion levels of canola meal in broiler chicken nutrition. (Chapter 4)

LIST OF ABBREVIATIONS

AA	Amino acid
AAFC	Agriculture and Agri-Food Canada
AB	Alberta
ADF	Acid detergent fiber
AMD	Archer Daniels Midland
AIAAD	Apparent ileal amino acid digestibility
AID	Apparent ileal digestibility
AME	Apparent Metabolizable energy
AME_n	Nitrogen-corrected AME
ANOVA	Analysis of variance
Ala	Alanine
AOAC	Association of Official Analytical Chemists
AR	Arizona
Arg	Arginine
Asp	Aspartic acid
BWG	Body weight gain
℃	Celsius degrees
CA	California

CCAC	Canadian Council on Animal Care
CF	Crude fiber
CM	Canola meal
CO_2	Carbon dioxide
CP	Crude protein
Cr_2O_3	Chromium (III) oxide
Cys	Cystine
DM	Dry matter
EECM	Expeller-extracted CM
FCR	Feed conversion ratio
FI	Feed intake
G	Gram
GE	Gross energy
GLM	Generalized linear model
Glu	Glutamine
Gly	Glycine
HCL	Hydrochloric acid
His	Histidine
IEL	Ileal endogenous losses

IL	Illinois
Ile	Isoleucine
Kcal	Kilocalorie
Kg	Kilogram
Leu	Leucine
Lys	Lysine
MB	Manitoba
ME	Metabolizable energy
Met	Methionine
MI	Michigan
MT	Metric ton
NaOH	Sodium hydroxide
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NSP	Non-starch polysaccharides
NRC	National Research Council
NSW	New South Wales
ON	Ontario
Phe	Phenylalanine

Pro	Proline
QC	Québec
R^2	Coefficient of determination
SAS	Statistical Analysis System
SBM	Soybean meal
SECM	Solvent-extracted CM
Ser	Serine
SIAAD	Standardized ileal amino acid digestibility
SID	Standardized ileal digestibility
SK	Saskatchewan
TDF	Total dietary fiber
Thr	Threonine
TME_n	Nitrogen-corrected true ME
Trp	Tryptophan
Tyr	Tyrosine
USA	United States of America
Val	Valine

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CHAPTER 1

GENERAL INTRODUCTION

Agriculture and Agri-Food Canada has predicted that the rise in the consumption of poultry products is expected to continue over the next ten years (AAFC, 2016). It has led to an increased demand for conventional feedstuff, making them more expensive, thereby prompting a search for alternative feed ingredients. Soybean meal (SBM) remains the major protein source for poultry and pigs. This is due to its balanced amino acid (AA) profile which meets nutritional requirements of livestock (Stein et al., 2007). The cost incurred by farmers while using SBM in diet formulation is substantial and has made many livestock producers (poultry and swine) to search for alternative, cost effective protein sources.

Canola meal (CM) is an alternative protein source that is available and more affordable than SBM when used in formulation of poultry and swine diets. It is a co-product of the canola crushing industry and is considered as a cost-effective substitute for SBM in broiler diets. Its crude protein (CP) content is between 40-43% DM while apparent metabolizable energy (AME) value for broiler chicken averaged 1875 kcal/kg DM (Adewole et al., 2016; Radfar et al., 2017). Canola meal has lower AA digestibility and higher fiber content when compared to SBM. However, CM has a well-balanced AA profile for poultry, which makes it to favorably complement SBM in diet formulation. Canola meal has higher methionine and cysteine contents but lower arginine and lysine contents when compared to SBM (Khajali and Slominski, 2012). The presence of antinutritional factors such as glucosinolates, condensed tannins, sinapine, phytic acid have limited the use of CM in monogastric feed formulation. Variability exists in the nutrient composition of CM due to differences in environmental and harvesting conditions, but mainly due to processing of the seed and meal (NSW Department of Primary Industries, 2014). Over the years improved

genetics have helped to discover superior variety of seed, this in addition to favorable processing techniques, have led to the production of meal that contains low levels of anti-nutritional components (Bell, 1982). In Canada, canola seed is traditionally processed using pre-press solvent extraction method to separate the oil from the meal. In 2015, Canada was the third producer of CM in the world (4,570 MT) followed by China (11,379 MT) and European Union (13,714 MT) (Index mundi, 2016). There are 14 canola crushing plants in Canada with the capacity to crush about 10 million tonnes of canola seed and the production of 3 million tonnes of canola oil and 4 million tonnes of CM annually (Canola Council of Canada, 2017). A smaller sector of the Canadian crushing industry still utilizes expeller extraction methods where oil from seed is physically extracted after heat pre-conditioning. These smaller oil seed processing facilities are located where canola is not commonly grown and so limited in availability. Seeds are processed mainly for bio-diesel thus, production is not capital intensive in comparison with solvent extraction. The nutritional profile of expelled-extracted CM (EECM) are not very different form solvent-extracted CM (SECM) except that it contains more fat and has higher energy value (Spragg and Mailer, 2007). Although SECM is commonly used in poultry diets to economically substitute for SBM, it may not fully replace SBM due to lower energy content, lower nutrient utilization, presence of anti-nutritional factors and Maillard reaction products associated with processing. Various approaches, including breeding of yellow-seeded canola, dehulling and enzyme supplementation to enhance nutrient utilization by monogastric animals have been employed to improve the overall nutritive value of CM (Khajali and Slominski, 2012).

A study by Adewole et al. (2016) has revealed variations in the nutritional quality of CM sourced from crushing plants across Canada. The impact of differences in the processing conditions between crushing plants on nutrient digestibility and growth performance of broiler

chickens fed CM remains an interesting area of research. Determination of standardized ileal digestibility (SID) of AA of CM obtained from all crushing plants across Canada would provide nutritionist and industry with valuable information on the nutritive values of CM. The nutritive value of CM can be affected by application of heat and moisture beyond the optimum temperature and time during processing, which results in Maillard reaction. This, in turn reduces the concentration and digestibility of AA, particularly lysine, which is susceptible to heat damage.

In Western Canada, CM has been commonly used as a source of protein for poultry. Dietary inclusion levels of CM, however, have been historically limited to 5-10%, mainly due to concerns related to high fiber content and presence of glucosinolates. The lower cost per gram of key available AA and phosphorus coupled with meal availability and various approaches to improve the overall nutritive value of CM have lead nutritionists to re-consider the inclusion levels of CM in broiler chicken nutrition. The key to feeding high inclusion levels of CM in poultry is to balance the diets on an available AA and energy contents. Standardized ileal digestible (SID) AA values are used in diet formulation because they are additive, and their estimate closely meets the nutritional requirements of birds (Lemme et al., 2004). When broiler chicken diets are formulated based on SID AA values, CM can be included at much higher levels without negatively affecting performance (Newkirk and Classen, 2002; Ramesh et al., 2006). The objective of this thesis was to measure ileal digestible AA content of CM samples sourced from 14 crushing plants across Canada, as well as to determine the effect of varying inclusion levels of CM on growth performance and nutrient digestibility of young broiler chickens.

The hypothesis for this study are stated below;

- Canola meal samples obtained from different crushing plants in Canada will have different SID AA values.
- High inclusion levels of CM in broiler chicken diets formulated based on SID AA and AME_n content will not affect growth performance.

CHAPTER 2

LITERATURE REVIEW

2.1 The origin and use of canola seed

Canola is an improved variety of rapeseed which was developed using traditional plant breeding techniques to have low erucic acid content (<2%) in oil and low level of glucosinolates (<30µmol/g) in the oil free portion. The new varieties (low glucosinlate and erucic acid content) were developed in Canada hence, the name "canola" coined from the root word Canada and "ola" meaning "oil low acid", which differentiate it from the high glucosinolate, high erucic acid rapeseed (Bell, 1982). The major focus of canola producers is to grow the crop for oil production which makes it the leading oil seed crop in Canada with the production of 15 million tonnes per annum and contributing about 22% to the worlds production record (Iji et al., 2017). However, the goal is to increase the production to 26 million tonnes per year in response to rising world demand for oil seeds (Canola Council of Canada 2015).

Canola meal is a by-product obtained after oil extraction from canola seed. During processing in the crushing plants, oil is extracted and used for human consumption while the seed solids are processed into co-product that is an excellent feed ingredient for livestock. Canola meal contains high protein and a well-balanced AA profile for poultry and swine (Canola Council of Canada 2015; Wickramasuriya et al., 2015). This makes it a commonly used protein supplement in animal diet, and its availability and relatively low cost makes it an economically viable alternative replacement for SBM in poultry and swine diets. However, the inclusion rate of CM in diets has been limited due to higher fiber, lesser protein and energy (Kocher et al., 2000) as compared to SBM. There is an established relationship that exist between fiber, energy and protein content of CM, which to a large extent affects CP and energy digestibility of non-ruminants.

2.2 Chemical composition and nutritive value of canola meal

2.2.1 Chemical composition

Canola meal is a good source of nutrients for poultry and swine. Three common varieties of canola *Brassica napus*, *Brassica rapa* and *Brassica juncea* are processed in Canadian plants, although *Brassica napus* has been a prevalent species produced and crushed in Canada. The main components of CM include protein and AA, carbohydrates (simple sugars, sucrose, oligosaccharides and starch), fiber (non-starch polysaccharide, lignin, polyphenols and glycoproteins) and fat (phospholipids, glycolipids, triglycerides and free fatty acids) (Khajali and Slominski 2012). It is also a good source of minerals (calcium, iron, manganese, and selenium) and vitamin B compared with SBM or other feed ingredients (Newkirk, 2009). It has sufficiently high amount of total phosphorus (1.12%) in form of phytate (0.74%) and non-phytate (0.38%) phosphorus (Adewole et al., 2016). Phytate (a complex of inositol and phosphorus) makes minerals unavailable and is considered an anti-nutritional factor due to its impact on bioavailability of zinc, calcium and iron. The readily digestible form of phosphorus (non-phytate phosphorus) is more available in CM compared to SBM, making it a good supplement or alternative for SBM. Canola meal, in addition to vitamin B6, is higher in choline, biotin, folic acid, niacin, riboflavin, thiamine than SBM (Canola Council of Canada 2015). Glucosinolates are the major anti-nutritional factors present in CM. They are not toxic *per se* but when they become hydrolyzed by enzyme myrosinase (present in plant tissue), they are broken down into toxic metabolites (Bell 1993). They can also be converted into toxic substance by excessive heating during oil extraction process and the action of microbial myrosinase in the gastro-intestinal tract. A survey carried out to determine the chemical composition of CM obtained from 11 crushing plants across Canada, revealed the average glucosinolates level to be 4.6µmol/g DM (Adewole et al., 2016). Other anti-nutritive

components present in CM, include sinapine (1.1% DM) most abundant phenolic compound in canola responsible for off flavor and tannins (complex polyphenols that impair enzymatic digestion). There are variations in the nutritional composition of CM because of differences in cultivar, processing of seed and meal as well as environmental conditions during growing and harvesting of the crop (Liu et al., 2014). Oil and fat content of the CM varies with the type of processing methods, where the amount of heat applied during processing contributes to the degradation of heat sensitive nutrients, especially some AA.

2.2.2 Energy

Relatively low metabolizable energy is the major limiting factor in the nutritive value of CM. This is often associated with high fiber content as fiber dilutes the energy (Zhang et al., 2013). Differences in canola cultivars, age of the birds being fed (energy utilization differs with ages of birds) and seed processing methods influences the caloric value of CM. The energy content of CM is directly related to residual oil content of the meal, thus expeller-extracted CM is higher in ME because it often contains more oil compared to pre-press solvent extracted CM. A study to investigate the effect of processing conditions and chemical composition on apparent metabolizable energy AME_n value of expeller-extracted CM for broilers by Toghyani et al. (2014) reported that AME_n value varied remarkably among expeller-extracted CM samples and that chemical constituents, particularly fiber component, may influence the AME_n values. The overall nutritive value has been improved through plant breeding aimed at reduction of fiber and glucosinolates levels. Jia et al. (2012) reported that yellow-seeded *B. napus* CM that contained increased protein and reduced fiber contents in comparison to the conventional *B. napus* black and yielded higher AME_n and amino acid digestibility in broiler chickens. Some by-products of oil

refining (gums and soap stocks) are added back to the meal during the processing of canola seed which increases the energy content of the meal (Canola Council of Canada, 2015).

2.2.3 Protein and amino acids

Canola meal ranks second, after SBM, as the most commonly used protein source for animal feed in the world. Its high protein content of about 41.5% of oil free meal coupled with a well-balanced amino acid profile makes it a commonly considered ingredient in poultry feed formulation (Radfar et al., 2017). In comparison with SBM, CM has more sulphur containing AA (methionine and cysteine) but less arginine and lysine. Both can complement each other when used to formulate poultry diet (Khajali and Slominski, 2012). Basically, the growing conditions determine the protein content of CM, but protein quality of CM is closely linked to the processing methods and conditions.

Canola seed is processed using mechanical and/or thermal means as they are exposed to several steps leading to separation of oil from seed. It has been demonstrated that CP and AA availability in the meal are affected by differences in processing methods and conditions such that excessive heat treatment leads to a reduction of the nutritive value of the meal. The severity of protein damage is in stages and noticeable with the increased formation of glycoproteins resulting from Maillard reaction. This unavailable form of protein is measured as neutral detergent insoluble CP (NDICP) and is responsible for a reduction in total protein digestibility. The amount and duration of heat applied during processing can lead to destruction of heat sensitive AA. Some AA, especially lysine, can be turned to biologically unavailable derivatives (un-reactive lysine) during heat processing (Kim et al., 2012). The reduction in digestibility of AA is influenced by overheating during the desolventizing and toasting stages of CM processing. Newkirk et al. (2003) revealed that CM still maintains its quality until it reaches the desolventizing/toasting phase

where temperature of up to 107°C can causes some protein damage. Adewole et al. (2016) revealed negative relationships between dietary fiber and lysine, and between lysine and NDICP, suggesting that CM from some Canadian processing facilities could be undergoing an intermediate stage of Maillard reaction which is indicative of reduced lysine concentration. Traditional toasting causes meal to become darker in color, and it concerns feed manufactures who prefer a lighter colored ingredient for feed production (Canola Council of Canada 2015). Special attention to the cooking step and heat supply while crushing canola seed using solvent extraction process can increase AA content and nutritive value of the meal.

2.2.4 Dietary fiber

Canola seeds are smaller and contain more oil than soybean, thus the resulting meal contains relatively larger proportion of dietary fiber. Based on the research by Adewole et al., (2016), average Canadian CM contains, on DM basis, 29.4% of neutral detergent fiber (NDF) and 37.9% of total dietary fiber (TDF). Neutral detergent fiber and TDF content of CM are three times higher than that of SBM (Adewole et al., 2016; Wang et al., 2017).

Canola fiber remains a major reason for holding back the increase of utilization of CM in diet for broiler chickens. This is because fiber is associated with the risk of negative impact on availability and digestibility of nutrients, especially AME_n, AA and minerals. Generally, dietary fiber has a negative impact on feed intake and nutrient digestibility (Mateous et al., 2012), with the soluble fiber portion being particularly obstructive on nutrient breakdown and digestibility due to their viscous properties limiting endogenous enzymatic activities (Jha and Berrocoso, 2015). Canola fiber, however is associated with the hull fraction, thus less water-soluble and biologically active (Slominski, 2015).

Different approaches have been used to reduce fiber content and to improve the overall nutritive value of CM. These include optimizing processing conditions during extraction, breeding for thinner seed coat, dehulling and use of exogenous enzymes to optimize nutrient utilization by monogastric animals (Khajali and Slominski, 2012).

Processing conditions and temperature used during oil extraction might affect the quality of CM. In addition to the fiber components present in the seed, the TDF content in the meal can be affected in the pre-press solvent oil extraction process. The overheating of the meal in the desolventizer-toaster might increase the dietary fiber content due to protein damage and formation of advanced Maillard reaction products, proteins-like components which resemble lignin and are neutral detergent insoluble (Bell, 1993). In the study by Adewole et al. (2017) the high variation in the NDF and NDICP contents of CM from different processing plants across Canada was observed. The high content of the TDF fractions, including NDICP, in different CM samples indicates the presence of Maillard reaction products and negatively affects the SID of all AA. The amount of fiber derived from AA damage is an indicator of the quality of CM.

Kong and Adeola (2016) compared nutrient content and digestibility of expelled-extracted CM with solvent-extracted CM using broiler chickens. They demonstrated that the NDF value of the latter meal was higher than that of the former one because of greater fat and lower NDF contents in the expelled meal. However, digestible energy content of a diet decreased linearly and was closely related to higher levels of fiber in both CM types compared to a typical corn-SBM diet. This would suggest that a relationship exist between fiber and energy content of CM. In addition, the condition of processing could have an impact on energy metabolism and nutrient utilization.

In the study by Gorski et al. (2017), the chemical composition of new reduced-fiber, increased-protein CM revealed its superior quality over the conventional CM. However, when included in the diets, the difference in broiler chicken growth performance was not observed. Similarly, the meals of newly developed yellow-seeded *B. napus* canola and canola quality *B. juncea* mustard are lower in dietary fiber contents than conventional CM. However, when used in the diets formulated based on digestible AA and available energy contents, no differences in growth performance parameters in broiler chickens and turkeys were observed when compared to those fed SBM diets (Rogiewicz et al. 2016).

Dehulling is another process to reduce fiber fraction that dilutes available nutrients. Hansen et al. (2017) revealed that fractionation of rapeseed meal by balling and sieving classification reduced fiber and increased CP contents resulting in a higher digestibility of CP and AA. A study conducted by Mejicanos et al. (2017) using sieving technology revealed that in comparison with the parent meal, dehulled fractions of CM contained less dietary fiber and more protein. However, when fed to young broilers and weaned pigs, no difference in growth performance was observed. It could be concluded that canola fiber simply dilutes nutrients and have a minimal effect on nutrient utilization.

Total dietary fiber of CM is composed mainly of non-starch polysaccharides (NSP). The CM quality could be improved by using the cell-wall degrading exogenous enzymes which have a potential to depolymerize NSP, thus contribute to release of additional energy (Meng and Slominski, 2005).

2.3 Canola seed processing

2.3.1 Pre-press solvent extraction

Canola seed is commercially processed using the pre-press solvent extraction method of separating oil from seeds. Processing conditions have a major impact on the nutritional quality of the meal. It is therefore, important to understand the strength and weakness of this feed ingredient to maximize its use in feed formulation. There are different steps involved in the separation and extraction of oil from the seed. The following explains the processes involved in the pre-press solvent extraction of oil (Figure 2.1).

Seed cleaning, preconditioning and flaking: The extraction process begins with seed cleaning, followed by pre-conditioning the seed at about 75-78℃ for 30 minutes after which flaking of the seed is achieved by passing through a roller mill. This is done to breakdown seed cell walls and ensure the appropriate flake size (0.30-0.38mm) for easy oil extraction.

Seed Cooking: The flakes are rapidly heated to and sustained between 80-90℃ so that myrosinase enzyme present in seed can be inactivated. This would prevent production of undesirable metabolites that affect the quality of oil and meal. The cooking lasts for about 15-20 minutes.

Pressing: The cooked canola seed flakes go through the expeller where the oil is mechanically removed by screw pressing. This is done to remove around 50-60% of the seed oil content while avoiding excess pressure and temperature to maximize the output of the expellers. This process produces a press-cake that is ready for extraction with hexane.

Solvent Extraction: This is done to remove the remaining oil (18-20%) from the pressed cake. It is the efficient secondary extraction which uses hexane, a solvent specifically refined for vegetable

oil industry. This procedure last for about 90 minutes at a temperature of between 50-60℃ until a residual oil content of less than 1% is obtained.

Desolventizing and Toasting: These involves the removal of solvent by injecting live steam through the meal (toasting). The meal is heated for about 45 minutes to 95-115℃ and moisture increases to 12-18%. Air is then blown through it to cool and dry the meal to about 10-12% moisture content. Finally, the meal is ground using hammer mill to uniform texture and is either pelleted or stored in mash form. Thermal processing of CM can destroy as much as 30-70% of glucosinolates, but care should be taken not to overheat the meal as this can affect meal quality and nutrient digestibility.

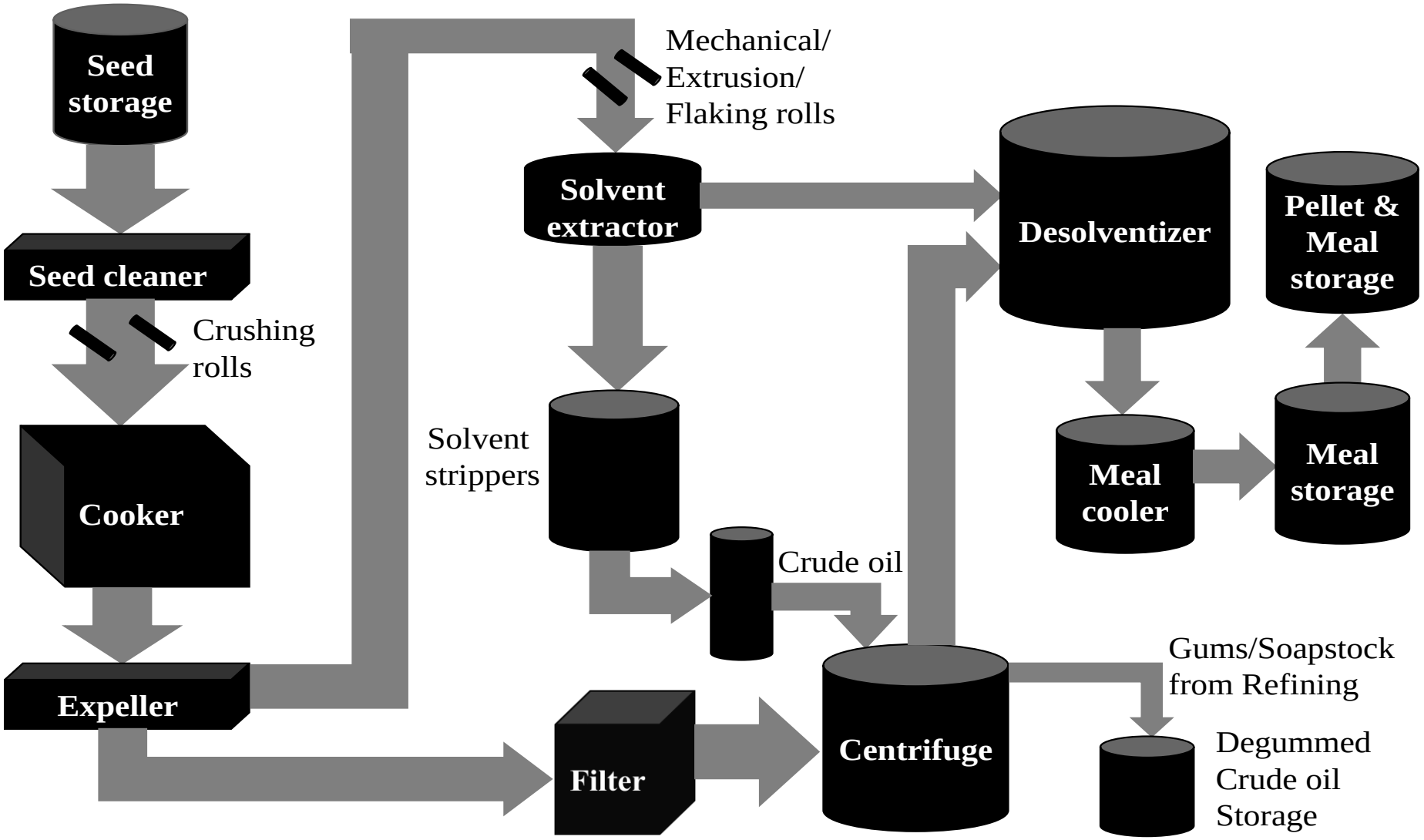


Figure 2. 1. Flow diagram of Canola oil extraction and meal processing (adapted from Canola Council of Canada, 2015)

2.3.2 Expeller pressing

Extraction of oil can also be achieved through "expeller/double" pressing. Some processing facilities in Canada use this method. This processing method gained more interest lately, particularly in the smaller scale operations. Canola flakes are expelled twice after heat pre-conditioning. This process does not involve the solvent extraction, desolventization/toasting, and drying and cooling stages. Expeller-extracted CM usually contains around 12% more oil compared to the solvent-extracted meal. However, temperatures used for expeller processing can vary between processing plants. Temperatures between 95-130℃, as observed by Mailer (2004), lead to differences in the nutritional content and protein and AA digestibilities. The resulting meal with higher oil content (8-15%) contains higher metabolizable energy as compared to the traditional pre-press solvent extraction process (Kong and Adeola, 2016).

2.3.3 Cold pressing

The cold press oil extraction process does not require heat pre-conditioning. In this process, mechanical separation is achieved by slowly pushing the cake through a screw barrel to limit mechanically generated heat to not more than 60℃. The oil content of cold pressed CM usually ranges between 12-20% of fat. Feed additives such as fat stabilizer and/or anti-oxidants help to maintain the quality of fat in cake or meal overtime.

2.4 Moist heat treatment of oilseed crops

Heat treatment offers many benefits for the further utilization of oilseed crops. It is one of the methods used for protein denaturation with the intent of increasing AA availability, digestion and absorption. Moist heat treatment facilitates the separation of oil from seed during the oil extraction and production of the meal suitable for livestock nutrition (Chemat, 2017). It helps to inactivate anti-nutritional and organic components of meal/oil in the process of oil extraction.

Bioavailability of protein in feed ingredients and AA profile are strong quality determinants for protein supply and utilization in monogastric animal nutrition (Conde-Aguilera et al., 2014). At the cooking stage of canola processing, heating between 80-90°C helps to deactivate myrosinase enzyme which can convert glucosinolates into toxic metabolites thereby reducing CM quality (Canola Council of Canada, 2015). Due to moist heat treatment, there is a decline in the glucosinolates content of CM. Newkirk and Classen, (2002) reported that glucosinolates content of solvent extracted co-products is lower compared to expeller or cold pressed co-products due to removal of some of these toxic compounds by steaming before desolventization.

Overheating remains the noticeable challenge as a large variation exists in the nutritional quality of products (meals) subjected to heat treatment. These variations are linked to differences in the processing conditions which are known to negatively affect digestibility of protein and nutritional quality. The application of heat and moisture during the desolventization/toasting of CM beyond the optimum temperature and time results in Maillard reaction. This reduces the concentration and digestibility of AA, particularly lysine, which is susceptible to heat damage (Mosenthin et al., 2016). Maillard products are formed through a reaction between free amino group of some AA and reducing sugars thereby binding them up and preventing liberation during digestion. Oftentimes and in relation to heat treatment, lysine is considered the most reactive AA. However, some other key AA such as threonine (THR), arginine (ARG), tryptophan (TRP) and glutamine (GLU), belonging to the class of α-AA, could be affected by heat treatment and their side chains enables them to take part in this reaction.

Chemical changes due to Maillard reactions do not completely explain the reduction in AA digestibility (Salazar-Villanea et al., 2016) as changes in the structure of protein (secondary and tertiary) also affects digestion processes. Physical changes seem to occur before chemical changes

during toasting and a simultaneous occurrence have a confounding influence on protein digestibility (Gerrard et al., 2012). Protein aggregation involves inter and intra molecular hydrogen bonding of amide groups (lysine, arginine and histidine), evident with the appearance of beta-sheets which increases with prolonged toasting time. Protein precipitation and/or gelation leads to loss of protein functional properties with a reduction in water holding capacity. Protein degradation from excessive heat results in destruction of AA, hydrolysis of peptide bonds and other reactions that decrease digestibility.

Other thermally induced reactions (depending on type and structure) occur due to modification of side chains, denaturation and/or re-folding of proteins alongside formation of new bonds and interactions. Cross-links of peptide chains (iso-peptides) are formed as temperature increases (Gerrard, 2002). They are known to limit the activities of protein enzyme digestion and absorption of another AA, the best example being lysinoalanine. The interconversions that occour between AA from one form to another is called racemization. Processes with heat treatment favour the forward reaction (L-amino acid to D-amino acid) as higher temperature increases racemization (Varga-Visi et al., 2009). Consequently, AA become optically inactive and their nutritional value is reduced due to decreased digestion by protease. Disulfide bond formation and hydrogen sulfide liberation involves lateral association of protein molecules that occurs through a chain reaction of sulfhydryl group with disulfide groups. Sulfhydryl groups of proteins are lost due to oxidation of cystic acid and/or splitting of hydrogen sulfide (Tang et al., 2009). Majority of the hydrogen sulphide comes from sulfhydryl groups and this could be more evident with sulphur containing AA as their losses increase with increasing temperatures.

2.5 Amino acid digestibility and bioavailability of canola meal for poultry

Amino acids are organic substances having both amino and acid groups. Majority of AA, except glycine, have an asymmetric carbon and are optically active. They have different biochemical properties and functions due to variation in their side chains. There are more than 300 naturally existing AA, but only 20 are responsible for protein synthesis.

Amino acid digestibility involves enzymatic breakdown and microbial fermentation of protein in feed ingredients ingested and absorbed from the gastro-intestinal tract. Availability of AA is defined as those amino and acid groups in a form suitable for digestion absorption and utilization (Rutherfurd and Moughan, 2012). The knowledge of AA availability in feed stuffs, is important in the determination of dietary protein quality. A higher confidence level is reached using these values as poultry diets are more efficiently formulated combined with dietary accommodation of elevated inclusion levels of unconventional feed ingredients and better reliability in the prediction of growth performance (Bryden and Li, 2010).

Poultry feed formulations have shifted focus from true AA digestibility, that uses adult roosters for *in vivo* digestibility determination, to SID of AA, the method that uses growing broiler chickens (Garcia et al., 2007; Bandegan et al., 2010). This is because SID of AA allows birds to exhibit their natural feeding behavior and better describes the additivity of feed ingredients used in diet formulations. Also, the over-estimation or under-estimation that occurs due to endogenous AA secretions, influence of microbial fermentation in the hind-gut and AA of urinary source are either corrected or eliminated. Standardized ileal digestibility assay is becoming the most recognized, reliable and consistent *in vivo* digestibility studies in poultry nutrition (Ullah et al., 2016).

The nutritive value of processed feed ingredients is influenced by AA content and digestibility. As demonstrated by Adewole et al. (2016 and 2017), CM obtained from different processing plants across Canada differ in CP and AA contents. These variations may largely be influenced by the processing conditions. When evaluating alternative co-products such as CM, digestible content of essential AA and not total AA content is useful in the determination of protein quality. Woyengo et al. (2010) concluded that the expeller-extracted CM had greater digestible AA and AME_n contents compared with pre-pressed solvent extracted CM when fed to broiler chickens. As earlier explained, solvent-extracted CM is subjected to higher temperature during desolventizing/toasting whereas, expeller-extracted CM do not undergo this stage of processing. Double pressing minimizes the detrimental effect of processing temperature on CM nutritional quality because the meal is not heated for desolventizing (Canola Council of Canada, 2015). This further explains the impact of processing methods on nutrient content and digestibility of meal in broiler chickens. Also, some processing plants subject CM to other heat-induced processes such as pelleting which could potentially affect nutrient content and their availability (Liu et al., 2014). A study conducted by Kasprzak et al. (2016) revealed that CP and AA content of rapeseed was consequentially affected by variety and processing methods used. They further observed that a mild processing condition relating to heat treatment during hexane extraction yielded a co-product with greater AA and CP digestibility compared to the cold-press as apparent ileal digestible (AID) and SID AA differed significantly between both processing methods.

2.6 High inclusion levels of canola meal in poultry diets

Solvent-extracted CM is recognized as a suitable alternative protein source for poultry. This is because CM is readily available in Canada and reasonably low in price compared to SBM such that the cost of poultry feed formulation could be reduced substantially when SBM is replaced with increased levels of CM. However, in practice dietary inclusion is still below 10%, although several studies have been conducted and concluded that it is safe to increase CM inclusion levels in broiler chicken diets. Limitations in the inclusion levels have been associated with issues relating to lower AME_n and AA digestibility, higher glucosinolates and fiber contents compared with SBM (Kocher et al., 2000; Meng and Slominski, 2005). Other anti-nutritional components of CM, tannins and sinapine negatively affect the nutritive value due to their binding with proteins and impairing enzymatic activities in the gut so that there is a reduction in the availability of nutrients (Khajali and Slominski, 2012).

Some studies have evaluated different inclusion levels of CM in broiler chicken diets but results have not been consistent regarding optimal inclusion levels while not compromising broiler chicken performance. Mushtaq et al. (2007) tested 20 and 30% inclusion of CM in starter (1-21 days) and finisher (21-42 days) diets of broiler chickens and observed a significant reduction in body weight gain (BWG) with 30% CM during the starter phase. The CM and consequently the experimental diet was high in glucosinolates, this may be responsible for reduced performance as younger birds have lower tolerance to higher level of glucosinolates. It might not be an issue anymore since the quality of CM has been improved substantially due to extensive plant breeding towards lowering glucosinolates level. Canola meal from Canadian processing facilities have very low levels of glucosinonates (4.6μmol/g) (Adewole et al., 2016). Researchers have tested CM at the levels from 10-20% in broiler starter and grower diet without a decline in performance given

that diets are formulated based on digestible AA content (Canola Council of Canada, 2015). Thus, considering the results from previous experiments with a well formulated diet (based on available and digestible content), CM inclusion could be raised up to 30%. Waldroup et al. (2011) stated that performance was not affected with a 25% CM inclusion in starter, grower, finisher diet of broiler chickens. Another study revealed that feed intake (FI) of broilers was not affected by CM inclusion levels but BWG and feed conversion ratio (FCR) were negatively affected by 20% CM inclusion (Payvastagan et al., 2012). The same author concluded that FCR increased by addition of 20% CM in a broiler starter diet (1 to 21 days), but there was no effect of varying inclusion levels of CM on BWG and FCR during the finisher phase (21 to 42 days) and for the entire trial (Payvastagan et al., 2013). Furthermore, there was an increase in BWG of broilers (7-14 days) that were fed diet containing 16% CM while FI of chickens (14-21 days) increased with a 23% inclusion level (Gopinger at al., 2014). A diet containing 15% CM did not adversely affect growth performance of broiler chickens while a significant increase in FI was recorded when 20% of CM was included in the diet (Rabie et al., 2015). Two recent studies showed that feeding graded levels (0, 10, 20, 30%) of CM did not affect FI of birds but there was a linear decrease in BWG and FCR (Aljuobori et al., 2016; Payvastagan et al., 2017). Aljuobori et al. (2016) used extruded CM for their study, thus the meal contained more oil, less crude fiber (CF) and higher glucosinolates content. Clearly, the poor performance (BWG and FCR) could be caused by high glucosinolates content. Additionally, extrusion treatment of CM did not lead to an improvement in performance of broiler chickens (de Vries et al., 2014). In the second study by Payvastagan et al. (2017), locally processed expeller-extracted CM with 23.5μmol/g glucosinolates and 12.9% CF content was used. Thus, the diet with 30% of CM contained around 7μmol/g glucosinolates and 5.5% CF. The excess of CF and high dietary levels of glucosinolates explain the negative effect on BWG and FCR. A

level of glucosinolates above 8μmol/g of diet may result in severe growth depression. However, the effect on growth performance would be mild at levels of 4μmol/g while some decline in growth could be observed at levels between 6-10μmol/g. A level above 10μmol/g of diet can cause a serious retardation in growth of broiler chickens (Tripathi and Mishra, 2007).

It appears that several factors are responsible for inconsistencies in the results obtained for performance parameter (FI, BWG and FCR) of broilers fed high inclusion levels of CM. The most prominent issue relates to the experimental design as graded inclusion levels of CM are not taken into consideration for the different stages of broiler chicken development. Oftentimes the same inclusion levels are applied in test diets irrespective of growth phase whereas dietary fiber tolerance will vary with birds of different age. Young broiler chickens are sensitive to high amount of fiber because they require a minimum amount in the diet for the digestive organs to function properly. They react differently to fiber inclusions depending on their age, type and amount present in the diet. Moderate levels enhance growth performance while high amounts of fiber may result in reduced BWG of broiler chickens (Walugembe et al., 2014).

Also, the type of CM used could have an impact on the performance of broiler chickens. Depending on the methods of processing (solvent, expeller, extruded or cold pressed), CM could vary in the glucosinolates contents as the dietary provision for monogastic animals should be maintained at a safe level of 2-2.5μmol/g feed (Woyengo et al., 2017). A restricted amount of less than 4μmol/g have been recommended for broiler chickens, which represents the level under which the adverse effect on growth performance will not occur (Khajali and Slominski, 2012). The adverse effect of high glucosinolates levels are often linked with bitter taste, this could be the case in pig nutrition because birds have poor receptors for bitter taste (Go, 2006). High glucosinolates level could affect thyroid functions, however, Woyengo et al. (2011) demonstrated that increased

dietary level of expeller-extracted CM did not negatively affect thyroid function and serum biochemical parameters in broiler chickens.

It is important to balance the diet based on SID AA and available energy content which is the key to incorporate high inclusion levels of CM in broiler chicken diets. Huang et al. (2006) reported the average apparent ileal digestibility of 15 AA to be 0.90 and 0.87 for layers and broilers, respectively. This difference is substantial; thus, digestible AA instead of total AA content of feed ingredients should be considered when formulating diets for broiler chickens. The effect of high dietary levels of CM on growth performance of poultry were evaluated by Rogiewicz et al. (2015 and 2016). They demonstrated that a diet containing 15% CM for broiler chickens (1-35 days) was not different from the control SBM diet for BWG and FCR. A diet with 20% CM inclusion for turkeys (1-56 days) resulted in similar BWG and FCR compared with the control SBM diet. There were no significant differences in hen-day production, egg quality parameters, FI and efficiency when up to 20% CM was included in the laying hen diet for 24 weeks. These cross-species study imply that CM with low glucosinolate content can be safely used to formulate poultry diets at the levels between 15-20% without affecting growth and animal health.

Based on the literature review, it could be concluded that when diets are precisely formulated (i.e. on the basis of apparent metabolizable energy and standardized ileal digestible amino acids), the high inclusion levels of canola meal in poultry diets are feasible without compromising growth performance. This conclusion should become a general recommendation for poultry nutritionists. Future research could focus on the optimizing high inclusion level of CM for poultry in different stage of development, and on the potentially beneficial impact of high level of dietary fiber derived from canola meal on the gut health of broiler chicken.

CHAPTER 3

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY OF CANOLA MEAL IN BROILER CHICKENS IN RELATION TO ITS SOURCE

3.1 Abstract

Excessive heat treatment during the desolventizing/toasting stage of pre-press solvent extraction of canola seeds might reduce digestibility of amino acids (AA) and contribute to variation in the nutritive value of canola meal (CM). Canola meal could be used at high inclusion levels for broilers if diets are formulated based on available energy and AA contents. The experiment was carried out to determine the standardized ileal digestibility (SID) of AA for CM sourced from 13 plants across Canada using prepress solvent extraction and 1 plant using expeller pressing. In this study, one-day-old broiler chickens were fed a pre-experimental starter diet from 1 to 14 d of age followed by the test diets from 15 to 21d. Fourteen test diets were formulated to contain 22% of CP with CM as a sole source of AA. One group received a casein-corn starch diet for the determination of endogenous AA losses. All diets contained Cr_2O_3 (0.3%) as an indigestible marker. On d 21, birds were euthanized and the ileal digesta samples were collected, freeze-dried and analyzed for AA and chromium contents. The ANOVA testing using the MIXED procedure of SAS revealed significant variations ($P < 0.05$) in SID of Lys (86.3 vs. 74.2%), Trp (94.5 vs. 76.7%) and Thr (87.3 vs.71.6%) of CM for broiler chickens. Whereas, Met (91.8 vs. 87.4%) and Cys (81.8 vs. 73.7%) were not significantly ($P > 0.05$) affected but Arg, which is known as a conditionally essential AA, varied significantly (92.0 vs. 83.8%). Lysine (1.97 vs. 1.53%), Thr (1.64 vs. 1.24%), Trp (0.40 vs. 0.33%) and Met (0.68 vs. 0.46%) as well as other AA varied significantly ($P < 0.05$) in their standardized ileal digestible contents. Regression analysis showed

a significant relationship ($P < 0.05$) among those AA suspected to be affected by heat treatment. Variations in SID of AA of CM could result from differences in processing conditions.

3.2 Introduction

Pre-pressed solvent extracted canola meal (CM) is a co-product of the Canadian oil crushing industry. It is suitable for use in the formulation of poultry diet because of a well-balanced amino acid (AA) profile. Although limited by energy and some anti-nutritive factors such as glucosinolates and fiber, CM is still recognized as the most valuable alternative that favorably complements soybean meal (SBM), known to contain less methionine and cysteine (Khajali and Slominski, 2012).

Canola meal is commercially produced using the pre-press solvent extraction process. A smaller sector of the Canadian processing industry utilizes expeller extraction method to separate oil from the seeds. The pre-press solvent extraction process involves moist heat treatment which may impact the nutritive value of the meal. Protein degradation following excessive heat treatment may lead to the reduction in the content of available AA.

The knowledge of AA availability in feed-stuffs is an important consideration in the determination of dietary protein quality. Poultry feed formulations have shifted focus from true AA digestibility to standardized ileal digestibility (SID) of AA as there were clear differences between both AA digestibility assays (Garcia et al., 2007; Bandegan et al., 2010). Standardized ileal AA digestibility determination have become a widely acceptable method of choice in the poultry and swine nutrition. The AA digestibility of CM can be affected by different processing conditions during the pre-press solvent extraction process. Adewole et al. (2016 and 2017) revealed significant variations in the nutritional quality of CM sourced from some Canadian processing facilities and examined the effect of CM source on SID AA and AME_n contents for

broiler chickens. However, the impact of crushing plant variation on AA availability and digestibility for broiler chickens remains an important area for research. Therefore, the objective of this study was to determine the standardized ileal AA digestibility of CM sourced from all 14 canola processing facilities in Canada and to understand the relationships that exist between heat sensitive AA, including lysine, threonine, arginine, tryptophan and glutamine.

3.3 Materials and Methods

3.3.1 Canola meal samples

Canola meal samples were sourced from 14 canola crushing plants in Canada which include: 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; and Lloydminster, SK; Louis Dreyfus company Yorkton, SK; Richardson Oilseed Ltd Yorkton, SK and Lethbridge, AB; Cargill Canola Processing, Clavet, SK; Camrose AB Viterra Becancour, QC. One expeller-extracted CM sample was collected from Viterra Canola Processing, St. Agathe, MB.

It is of interest to note 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, all which must be approved for registration and must meet or exceed checks for quality standards. This results in differences in the chemical composition of CM. However, all varieties of canola are blended during crushing and the resulting oil and meal products must meet certain criteria for Canadian Oilseed Processors Association Trading Rules. The blending of canola seeds from different locations would minimize the variability.

3.3.2 Diets

A three-week study was conducted to determine the SID AA of 13 solvent-extracted CM (SECM) and 1 expeller-extracted CM. From day 1 to 16 chicks were fed a commercial broiler starter diet in mash form that met or exceeded NRC (1994) recommendation for broiler chickens (Table 3.1). Experimental CM-corn starch-based diets with CM samples serving as the only source of protein and AA were fed to broilers from day 16 to 21. Composition of experimental diets are presented in Table 3.2. A highly digestible protein casein (Sigma Aldrich)-corn starch based diet was used for the determination of endogenous AA losses (Table 3.3). Test diets were formulated to reach 22% CP, 2700-2800 Kcal/kg ME, 1% Ca, 0.45% available P and contained 0.30% chromic oxide as an indigestible marker.

3.3.3 Animal care and housing

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009) with the animal protocols approved by the Animal Care Use Committee of the University of Manitoba. A total of 361 one-day-old male Ross 308 broiler chickens were obtained from a local hatchery (Carlton Hatchery, Grunthal MB, Canada) and were housed in electrically heated Alternative Design Super Brooders (Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR) under a controlled environment. Room temperature was maintained at 32, 28, and 24°C for week 1, 2, and 3, respectively. On day 16, birds were fasted for 3 hours, weighed and were randomly assigned into experimental treatments, with 5 birds/cage and 5 cages per treatment. Experimental diets were fed for 5 days ensuring birds had unrestricted access to feed, light and water throughout the trial.

Table 3. 1 Ingredients and calculated composition of starter diet used in the pre-experimental period (%).

Ingredient	Diet
Corn	49.60
Soybean meal	35.00
Canola meal	5.00
Vegetable oil	5.47
Calcium carbonate	1.73
Monocalcium carbonate	1.45
L-Lysine	0.07
DL-Methionine	0.16
Threonine	0.02
Mineral premix[1]	0.50
Vitamin premix[2]	1.00
Total	100
Calculated composition	
Crude protein	22.00
ME, Kcal/kg DM	3104
Calcium	1.06
Non-phytate phosphorus	0.45
Lysine	1.16
Methionine	0.45

[1] Provided per kg of diet: 70 mg Mn, 80 mg Zn, 80 mg Fe, 10 mg Cu, 0.3 mg Se, 0.5 mg I, 337 g Na.
[2] Provided per kg of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin.

Table 3. 2 Ingredients and analyzed compositions (%) of experimental diets used in the standardized ileal amino acid digestibility study.

Ingredient	Canola meal from different crushing plants (CM 1-14)[1]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14[2]
Canola meal	59.50	60.10	60.10	55.50	59.35	60.00	60.00	58.90	58.00	54.25	59.00	59.53	58.00	62.90
Cornstarch	15.60	15.35	15.36	17.61	15.71	15.35	15.35	15.92	16.35	18.14	15.82	15.55	16.32	13.94
Sucrose	15.70	15.35	15.36	17.62	15.71	15.35	15.35	15.83	16.35	18.13	15.82	15.55	16.33	13.94
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Calcium carbonate	1.25	1.20	1.24	1.29	1.21	1.15	1.15	1.15	1.22	1.18	1.14	1.08	1.15	1.14
Mono calcium phosphate	1.15	1.20	1.14	1.18	1.22	1.35	1.35	1.40	1.28	1.50	1.42	1.49	1.40	1.28
Mineral Premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.30	0.30	0.30	0.30	0.30	0.30	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	100	100	100	100	100	100
Calculated composition														
Crude protein	22.00	22.00	22.00	22.02	22.02	22.03	22.03	22.02	22.04	22.00	22.02	22.00	22.01	22.00
ME, Kcal/kg DM	2777	2767	2768	2842	2779	2765	2765	2782	2798	2854	2780	2770	2797	2720
Calcium	1.06	1.05	1.06	1.05	1.05	1.06	1.06	1.06	1.06	1.06	1.06	1.05	1.05	1.06
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed composition														
Methionine	0.39	0.40	0.45	0.41	0.43	0.43	0.41	0.40	0.44	0.46	0.38	0.45	0.46	0.42
Cysteine	0.45	0.47	0.50	0.46	0.48	0.50	0.47	0.46	0.50	0.53	0.44	0.51	0.51	0.49
Lysine	1.23	1.20	1.32	1.06	1.15	1.26	1.30	1.35	1.19	1.24	1.21	1.16	1.15	1.02
Arginine	1.19	1.22	1.30	1.11	1.11	1.25	1.28	1.33	1.22	1.27	1.20	1.18	1.20	1.17
Threonine	0.92	0.98	1.01	0.95	0.89	0.99	0.97	1.04	0.96	0.97	0.84	0.89	0.97	0.88
Tryptophan	0.23	0.24	0.26	0.26	0.29	0.28	0.22	0.23	0.27	0.27	0.27	0.30	0.30	0.28

[1] Prepress solvent-extracted canola meal from plant 1 to 13 [2] Expeller-extracted canola meal [3] Provided per kg of diet: 70 mg Mn, 80 mg Zn, 80 mg Fe, 10 mg Cu, 0.3 mg Se, 0.5 mg I, 337 g Na. [4] Provided per kg of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin.

Table 3. 3 Composition of highly digestible protein (HDP) diet used in the study (%).

Ingredients	HDP Diet
Sucrose	43.10
Corn-starch	33.10
Casein[1]	10.00
Cellulose	6.00
Vegetable oil	2.00
Calcium carbonate	1.85
Monocalcium phosphate	2.15
Mineral premix[2]	0.50
Vitamin premix[3]	1.00
Cr_2O_3	0.30
Total	100
Calculated composition	
Crude protein	8.87
ME, Kcal/kg DM	3401
Calcium	1.13
Non-phytate phosphorus	0.49

[1] Casein from bovine milk, Sigma Aldrich.
[2]Provided per kg of diet: 70 mg Mn, 80 mg Zn, 80 mg Fe, 10 mg Cu, 0.3 mg Se, 0.5 mg I, 337 g Na.
[3] Provided per kg of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin.

3.3.4 Sample collection and chemical analyses

On day 21, birds were euthanized by CO_2 asphyxiation and the digesta were collected from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileocecal junction) by carefully squeezing the content into sample bags. Digesta of birds among cages within the same treatment were pooled, frozen immediately and freeze-dried. The dried ileal digesta were stored in airtight plastic bags at room temperature until they were used for chemical analysis.

Canola meal samples, test diets and digesta samples were finely ground using IKA-Analytical mill (IKA A11 Basic, USA) and were analyzed for CP, AA and DM content. Diets and digesta samples were analyzed for chromium after the samples were ashed at 600℃ for 12 hours in a muffle furnace, using coupled plasma mass spectrometry (ICP-AES; Vista, Varian, Palo Alto, CA) according to the method of AOAC (2005; method 985.01). Dry matter was determined according to AOAC 2005 method 930.15 by oven drying a 5.0g sample at 105℃ overnight. Nitrogen content of samples were determined by combustion method (990.03; AOAC, 1990) using LECO N analyzer (model TruSpec N Leco Corp., St. Joseph, MI, USA) and CP was calculated as N x 6.25. Samples for AA analysis were prepared according to the AOAC (2005) official procedures (994.12), alternative 3 and 1 (sulfur AA). Briefly, a 100-mg sample was digested in 4 mL of 6 M HCl *in vacuo* for 24 hours at 110℃. 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 × L^{-1}; pH 2.2) and then analyzed using an AA analyzer (S4300, Sykam GmbH, Eresing, Germany). Samples for analysis of S-containing AA (Met and Cys) were subjected to performic acid oxidation before hydrolysis. Samples for tryptophan analysis were hydrolyzed with 25% NaoH at 120℃ for 20 hours (Hugli and Moore, 1972) and were analyzed using Sykam AA analyzer.

3.3.5 Calculation and Statistical analysis

Standardized ileal digestible AA was calculated using the formulas below;

- Ileal Endogenous Losses (IEL)

  $$IEL\ (\%) = [AA_{digesta} \times (Cr_2O_{3\ diet}/Cr_2O_{3\ digesta})]$$

- Apparent ileal amino acid digestibility

  $$AIAAD\ (\%) = [1 - (Cr_2O_{3\ \%\ diet}/Cr_2O_{3\ \%\ digesta}) \times (AA_{digesta}/AA_{diet})] \times 100$$

- Standardized ileal amino acid digestibility (SIAAD)

  $$SIAAD\ (\%) = AIAAD\ (\%) + [(IEL) \div (AA_{diet})] \times 100$$

The standardized digestibility values were multiplied by the respective AA contents to calculate the quantity of standardized digestible AA present.

Data were analyzed using the Mixed procedure of SAS, version 9.2 (SAS Institute Inc., Cary, NC). Means were separated using Turkey's studentized range test at a significant level of $P \leq 0.05$. A regression analysis was carried out to compare SID of select AA suspected to be heat sensitive.

3.4 Results and Discussion

The analyzed AA contents of CM samples sourced from all processing facilities across Canada are presented in Table 3.4. There were noticeable variations in AA content as well as the sum (indispensable and dispensable AA) of CM among processing facilities. Canola meal 10 had the highest content for all indispensable and dispensable AA except Ile, Trp, Cys, Pro and Tyr, CM6 and CM8 had the lowest content for majority of the AA except CM2, CM9, CM12, CM13 which were low in Met, Val, Trp, Asp and Tyr, respectively. The AA content of expeller-extracted CM was different when compared to those of solvent-extracted CM.

Table 3. 4 Analyzed amino acid content of canola meals (CM) used in digestibility study (% DM).

	Canola meal from different crushing plants (CM 1-14)[1]													
Amino acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14[2]
Indispensable AA														
Arginine	2.27	2.22	2.23	2.33	2.16	2.28	2.33	2.13	2.19	2.52	2.40	2.31	2.28	2.11
Histidine	1.21	1.23	1.17	1.23	1.18	1.17	1.18	1.10	1.29	1.32	1.23	1.21	1.28	1.19
Isoleucine	1.35	1.45	1.36	1.43	1.30	1.27	1.35	1.37	1.28	1.45	1.40	1.41	1.53	1.29
Leucine	2.62	2.69	2.63	2.76	2.58	2.49	2.65	2.71	2.63	2.94	2.77	2.72	2.81	2.54
Lysine	2.12	2.09	2.14	2.08	2.02	2.01	2.15	2.19	2.05	2.28	2.20	2.03	2.07	1.65
Methionine	0.70	0.51	0.64	0.56	0.57	0.62	0.59	0.55	0.66	0.75	0.64	0.67	0.66	0.60
Phenylalanine	1.44	1.53	1.48	1.54	1.47	1.43	1.50	1.50	1.50	1.62	1.53	1.50	1.60	1.45
Threonine	1.71	1.75	1.69	1.75	1.68	1.62	1.66	1.64	1.75	1.87	1.79	1.72	1.78	1.64
Valine	1.77	1.86	1.76	1.86	1.70	1.74	1.79	1.69	1.69	1.88	1.79	1.84	1.84	1.67
Tryptophan	0.41	0.42	0.44	0.43	0.39	0.39	0.43	0.43	0.44	0.46	0.46	0.38	0.42	0.42
Dispensable AA														
Alanine	1.69	1.78	1.71	1.76	1.71	1.59	1.72	1.58	1.75	1.90	1.81	1.77	1.81	1.70
Aspartic acid	2.73	2.90	2.69	2.88	2.73	2.86	2.74	2.92	2.86	3.08	2.94	2.87	2.97	2.66
Cystine	0.77	0.88	0.66	0.69	0.69	0.66	0.72	0.59	0.71	0.82	0.71	0.74	0.73	0.67
Glutamic acid	6.82	6.72	6.70	7.20	6.80	6.62	6.84	6.56	6.94	7.79	7.19	6.81	7.26	6.50
Glycine	1.94	1.95	1.90	2.00	1.93	1.84	1.97	1.87	1.95	2.13	2.02	1.94	2.02	1.87
Proline	2.30	2.41	2.28	2.39	2.26	2.52	2.35	3.43	2.32	2.60	2.38	2.39	2.47	2.26
Serine	1.76	1.85	1.74	1.82	1.76	1.71	1.69	1.67	1.85	2.00	1.88	1.81	1.91	1.72
Tyrosine	0.96	1.02	0.94	1.01	0.99	1.08	1.03	1.02	0.99	1.06	0.99	1.05	1.08	0.99
Total content	34.6	35.3	34.2	35.7	33.9	33.9	34.7	35.0	34.9	38.5	36.1	35.2	36.5	32.9

[1] Prepress solvent-extracted canola meal from plants 1 to 13

[2] Expeller-extracted CM

Table 3. 5 Effect of canola meal (CM) source on the standardized ileal amino acid (AA) digestibility for broiler chickens fed pre-press solvent extracted CM (%).

	Indispensable AA									
CM	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine	Valine	Tryptophan
1	87.18^{bcde}	81.04^{bcd}	85.93^{bcde}	84.36^{bcde}	81.51^{abc}	90.65	84.39^{bc}	77.25^{bcd}	81.72^{bcdef}	86.41^{bc}
2	85.67^{cde}	72.82^{d}	83.54^{cde}	82.78^{cde}	77.01^{cde}	88.73	83.07^{bc}	74.20^{bcd}	79.73^{bcdef}	84.95^{bc}
3	87.59^{bcd}	80.25^{bcd}	86.46^{bcde}	83.96^{bcde}	81.35^{abc}	87.36	83.52^{bc}	77.48^{bcd}	82.25^{bcde}	84.94^{bc}
4	86.21^{bcde}	82.18^{abcd}	81.34^{e}	82.54^{bcde}	76.55^{cde}	90.58	82.51^{bc}	77.25^{bcd}	77.35^{def}	85.53^{bc}
5	83.78^{e}	77.33^{bcd}	82.77^{de}	81.32^{e}	75.98^{de}	88.13	80.97^{c}	76.04^{bcd}	78.66^{cdef}	83.51^{cd}
6	88.27^{bcd}	84.12^{abc}	85.61^{bcde}	84.78^{bcde}	80.29^{bcd}	90.07	85.10^{bc}	78.74^{bcd}	82.11^{bcde}	84.71^{bc}
7	89.53^{ab}	82.69^{bc}	88.09^{abc}	86.15^{abcd}	83.85^{ab}	91.82	86.42^{ab}	79.82^{abcd}	84.10^{abc}	86.47^{bc}
8	89.05^{abc}	85.08^{ab}	87.39^{abcd}	86.35^{abc}	82.98^{ab}	90.58	86.37^{ab}	80.29^{abc}	83.29^{bcd}	86.82^{bc}
9	88.95^{abc}	85.12^{ab}	88.95^{ab}	87.26^{ab}	81.83^{abc}	90.27	86.37^{ab}	81.65^{ab}	85.13^{ab}	89.19^{ab}
10	91.95^{a}	92.48^{a}	91.68^{a}	90.13^{a}	86.34^{a}	90.46	90.26^{a}	87.26^{a}	89.42^{a}	94.49^{a}
11	85.97^{cde}	80.33^{bcd}	82.31^{de}	81.28^{e}	77.76^{cde}	88.09	81.77^{bc}	71.63^{d}	76.03^{f}	76.69^{e}
12	85.46^{de}	77.30^{bcd}	82.26^{e}	81.21^{e}	75.61^{de}	88.92	81.96^{c}	71.95^{d}	77.43^{def}	78.95^{de}
13	84.08^{e}	74.67^{cd}	82.30^{de}	81.95^{de}	74.23^{e}	87.38	82.11^{bc}	73.35^{cd}	78.39^{cdef}	82.20^{cd}
Mean[1]	87.21	81.19	85.28	84.16	79.64	89.46	84.22	77.45	81.20	84.99
SEM[2]	0.885	1.989	1.249	1.061	1.173	1.099	1.054	1.866	1.350	0.885

[1] Mean of amino acid digestibility values across 13 prepress solvent-extracted canola meals

[2] Standard error of the mean; Number of observations contributing to each mean = 5

[a-f] Means within a column with no common letters differ significantly ($P < 0.05$).

Table 3. 6 Effect of canola meal (CM) source on the standardized ileal amino acid digestibility for broiler chickens fed pre-press solvent-extracted CM (%).

	Dispensable AA							
CM	Alanine	Aspartic acid	Cystine	Glutamic acid	Glycine	Proline	Serine	Tyrosine
1	84.63^{bcd}	78.11^{bcd}	79.10	91.24^{bcd}	78.20^{bcde}	76.51^{abc}	85.14^{bcde}	80.27^{bcd}
2	82.68^{cde}	73.66^{bcde}	77.96	89.92^{bcd}	75.35^{bcde}	72.82^{cd}	81.38^{bcde}	78.75^{bcd}
3	84.09^{bcd}	77.82^{bcde}	77.21	91.63^{bcd}	78.20^{bcde}	78.54^{abc}	84.34^{bcde}	79.24^{bcd}
4	83.28^{bcde}	76.96^{bcde}	81.79	91.28^{bcd}	77.09^{bcde}	79.57^{abc}	85.49^{bcde}	77.09^{bcd}
5	82.59^{cde}	74.59^{bcde}	73.68	89.77^{bcd}	74.35^{cde}	69.59^{d}	81.98^{bcde}	75.71^{cd}
6	85.05^{bcd}	78.27^{bcd}	79.97	92.00^{bc}	78.56^{bcd}	78.86^{abc}	85.71^{bcde}	81.27^{abc}
7	86.09^{bc}	79.56^{abc}	79.98	93.02^{ab}	79.87^{bc}	80.98^{a}	86.68^{abcd}	82.92^{ab}
8	86.79^{ab}	80.35^{ab}	78.28	93.10^{ab}	81.23^{ab}	80.99^{a}	87.21^{abc}	81.85^{ab}
9	88.30^{ab}	80.92^{ab}	76.81	93.38^{ab}	80.34^{abc}	79.15^{ab}	88.75^{ab}	82.18^{ab}
10	92.30^{a}	86.99^{a}	79.99	95.88^{a}	86.20^{a}	80.92^{a}	93.86^{a}	86.35^{a}
11	78.18^{e}	71.60^{cde}	75.86	88.58^{cd}	72.84^{de}	75.40^{abcd}	79.59^{de}	75.29^{d}
12	80.23^{de}	69.75^{e}	75.89	88.16^{d}	72.52^{e}	74.38^{bcd}	79.21^{e}	77.79^{bcd}
13	81.60^{cde}	70.27^{de}	74.10	88.19^{d}	73.12^{de}	73.74^{bcd}	80.78^{cde}	76.10^{cd}
Mean[1]	84.29	76.83	77.74	91.24	77.53	77.03	84.62	79.60
SEM[2]	1.248	1.853	1.874	0.870	1.338	1.399	1.717	1.458

[1] Mean of amino acid digestibility values across 13 prepress solvent-extracted canola meals

[2] Standard error of the mean; Number of observations contributing to each mean = 5

[a-e] Means within a column with no common letters differ significantly ($P < 0.05$).

The observed variation in the analyzed CM samples are consistent with those reported by Adewole et al. (2016). These variations may largely be due to processing conditions employed by canola processors. Depending on the processing technique employed (prepress solvent extraction, double or cold pressing), canola undergoes different oil removing stages of cooking, pressing, solvent extraction and desolventizing/toasting. Subjecting canola seed/cake to these physical treatments of pressing and heating, coupled with increased residence time during toasting, have a major impact on the nutritional quality of the meal (Spragg and Mailer, 2007). Usually, CM is produced in Canada using prepress solvent extraction process to ensure that majority of the oil is removed. This process includes, but is not limited to, pre-conditioning, cooking, pressing, solvent extraction and desolventizing/toasting, all of which involve heat treatment. However, the damaging effect of heat is most significant during the desolventizing/toasting stage where hexane from de-oiled meal is removed at high temperature for a longer time frame (Gonzalez-Vega et al., 2011). The amount and duration of heat applied during processing can lead to destruction of heat sensitive AA. Some AA, especially lysine, can be turned to biologically unavailable derivatives (un-reactive lysine) during heat processing. Table 3.5 and 3.6 show the SID of AA for CM fed to broiler chickens. There were significant differences ($P < 0.05$) among CM samples for SID of all AA except Met and Cys. CM10 had the highest digestibility value for all AA ($P < 0.05$), except Met and Cys of which CM4 and CM7 had the highest digestibility, ($P > 0.05$) respectively. Canola meal 11 and 12 appeared to have the lowest digestible values ($P < 0.05$) for Thr, Val, Trp, Ala, Tyr and Leu, Asp, Glu, Gly, Ser respectively. Four AA: Arg, Phe, Cys and Pro were least digestible in CM5. Canola meal 13 had the lowest SID ($P < 0.05$) for Lys while CM2, CM3 and CM4 had the lowest SID for His, Met and Ile, respectively. As presented in Tables 3.7 and 3.8, there were differences ($P < 0.05$) among CM samples in the SID content of all AA. Canola meal 10 had the

highest SID content ($P < 0.05$) for all AA except Cys and Trp. Canola meal 5 had significantly lower SID Arg, Ile, Leu, Lys, Phe, Val and Pro content while CM12 was low in SID Thr, Trp, Asp, Glu, Gly and Ser content ($P < 0.05$). Canola meal 2 had the lowest content for SID of His and Met, CM6, CM8 and CM11 had the lowest values ($P < 0.05$) for SID Ala, Cys and Tyr, respectively. The total digestible AA content was highest in CM10 but lowest in CM5 (34.6 and 27.3 %DM), respectively.

The variations observed in the digestibility values and SID content of AA for broiler chickens agree with those previously observed by Adewole et al. (2017). The variation in processing conditions across Canadian processing facilities might have been responsible for the differences observed in the current study and hence inconsistent digestibility values for canola co-products. Crude protein and AA digestibility of CM could be affected by the variation in the length of time spent in the desolventizer/toaster (Kasprzak et al., 2016). Eklund et al. (2015) reported a linear decrease in digestible lysine content of CM for pigs from 1.95 to 1.72% DM when increasing interval of residence times alongside direct and indirect heat application. The severity of protein damage due to excessive heat is in stages and noticeable with the increased formation of glycoproteins resulting from Maillard reaction (Purlis, 2010). This non-enzymatic irreversible reaction involves the bonding of reducing sugar with free and/or epsilon amino groups under the influence of heat and moisture that may occur at some stages of CM processing (Jaeger et al., 2010; Mavromichalis, 2001). Desolventizing/toasting of canola is usually achieved at temperatures between 95-115℃ with moisture content of about 12-18% (Daun et al., 2011). However, temperature and moisture above certain optimum needed for hexane removal (i.e., 103-105℃) may be capable of inducing Maillard reaction between sugars and protein so that the content of AA is reduced in the meal.

It is well known that reduction in digestibility of AA is, in part, influenced by overheating during the desolventization/toasting stages of CM processing. Newkirk et al. (2003) revealed that CM still maintain its quality until it reaches the desolventizing/toasting phase where temperature of up to 107℃ can cause the protein damage. Kasprzak et al. (2017) examined the influence of two oil extraction processes on CP and AA digestibility of two double-low variety of rapeseed for broiler chickens. The process that involved hexane extraction and desolventization lead to a substantial variation in chemical composition of the meal. Irrespective of variety, there was a reduced standardized and apparent ileal digestibility value of AA in the meal, adding that a reduction in heating intensity (soft processing) could be a way to improve digestibility and nutritional value of meal. An earlier report by Eklund et al. (2015) stated that reducing the residence time in the desolventizer/toaster produced CM with greater SID Lys for pigs.

Table 3. 7 Effect of canola meal (CM) source on the standardized ileal digestible amino acid content for broiler chickens fed pre-press solvent extracted meal (% DM).

	Indispensable AA									
CM	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine	Valine	Tryptophan
1	1.98^{cd}	0.98^{c}	1.16^{cd}	2.21^{cde}	1.73^{bc}	0.64^{b}	1.22^{fg}	1.32^{bc}	1.45^{bc}	0.36^{cde}
2	1.90^{ef}	0.89^{c}	1.22^{bc}	2.22^{cd}	1.61^{de}	0.46^{g}	1.27^{bcdef}	1.29^{bc}	1.49^{b}	0.37^{bcde}
3	1.96^{cdef}	0.94^{c}	1.18^{cd}	2.21^{cde}	1.74^{bc}	0.56^{de}	1.24^{defg}	1.31^{bc}	1.45^{bc}	0.40^{a}
4	2.00^{bc}	1.01^{bc}	1.16^{cde}	2.28^{bc}	1.59^{de}	0.51^{f}	1.28^{bcdef}	1.35^{bc}	1.44^{bcd}	0.38^{abc}
5	1.81^{g}	0.91^{c}	1.08^{f}	2.10^{e}	1.53^{e}	0.50^{f}	1.19^{g}	1.28^{c}	1.34^{d}	0.34^{ef}
6	2.01^{bc}	0.98^{bc}	1.09^{ef}	2.11^{de}	1.61^{de}	0.56^{de}	1.22^{fg}	1.28^{c}	1.43^{bcd}	0.36^{de}
7	2.09^{b}	0.97^{c}	1.19^{bcd}	2.29^{bc}	1.80^{b}	0.54^{e}	1.30^{bcd}	1.32^{bc}	1.51^{b}	0.38^{abcd}
8	1.90^{f}	0.94^{c}	1.20^{bcd}	2.34^{b}	1.81^{b}	0.50^{f}	1.30^{bc}	1.32^{bc}	1.41^{bcd}	0.39^{abc}
9	1.95^{cdef}	1.10^{b}	1.14^{def}	2.29^{bc}	1.68^{cd}	0.59^{c}	1.30^{bcde}	1.43^{b}	1.44^{bcd}	0.39^{abcd}
10	2.32^{a}	1.22^{a}	1.33^{a}	2.65^{a}	1.97^{a}	0.68^{a}	1.47^{a}	1.64^{a}	1.68^{a}	0.40^{ab}
11	2.07^{b}	0.99^{bc}	1.15^{cdef}	2.26^{bc}	1.71^{bcd}	0.57^{cde}	1.25^{cdefg}	1.29^{c}	1.36^{cd}	0.39^{abc}
12	1.98^{cde}	0.94^{c}	1.16^{cd}	2.20^{cde}	1.54^{e}	0.59^{c}	1.23^{efg}	1.24^{c}	1.42^{bcd}	0.33^{f}
13	1.91^{def}	0.96^{c}	1.25^{b}	2.30^{bc}	1.54^{e}	0.58^{cd}	1.32^{b}	1.30^{bc}	1.45^{bc}	0.37^{bcde}
Mean[1]	1.99	0.99	1.18	2.27	1.68	0.56	1.28	1.34	1.45	0.37
SEM[2]	0.016	0.024	0.015	0.024	0.022	0.006	0.013	0.029	0.021	0.006

[1] Mean of amino acid digestibility values across 13 prepress solvent-extracted canola meals

[2] Standard error of the mean; Number of observations contributing to each mean = 5

[a-g] Means within a column with no common letters differ significantly ($P < 0.05$).

Table 3. 8 Effect of canola meal (CM) source on the standardized ileal digestible amino acid content for broiler chickens (% DM).

	Dispensable AA								
CM	Alanine	Aspartic acid	Cystine	Glutamic acid	Glycine	Proline	Serine	Tyrosine	Total AA Content[1]
1	1.43^{cde}	2.13^{bcde}	0.61^{bc}	6.23^{cdef}	1.52^{bcd}	1.76^{cd}	1.50^{c}	0.77^{ef}	29.0
2	1.48^{bc}	2.14^{bcde}	0.69^{a}	6.04^{f}	1.47^{bcd}	1.75^{d}	1.50^{c}	0.80^{cdef}	28.6
3	1.44^{cde}	2.10^{cde}	0.51^{ef}	6.14^{def}	1.48^{bcd}	1.79^{cd}	1.47^{c}	0.75^{f}	28.6
4	1.47^{bcd}	2.22^{bcde}	0.56^{cde}	6.57^{b}	1.54^{bc}	1.90^{bcd}	1.56^{bc}	0.78^{def}	29.6
5	1.42^{cde}	2.03^{de}	0.51^{ef}	6.10^{ef}	1.43^{cd}	1.57^{e}	1.44^{c}	0.75^{f}	27.3
6	1.35^{e}	2.24^{bcd}	0.53^{de}	6.09^{f}	1.45^{cd}	1.99^{ab}	1.47^{c}	0.88^{ab}	28.6
7	1.48^{bc}	2.18^{bcde}	0.57^{cd}	6.36^{bcde}	1.57^{b}	1.90^{bc}	1.47^{c}	0.86^{bc}	29.8
8	1.37^{de}	2.35^{b}	0.46^{f}	6.11^{ef}	1.51^{bcd}	1.87^{bcd}	1.46^{c}	0.84^{bcd}	29.1
9	1.55^{b}	2.32^{bc}	0.55^{de}	6.48^{bc}	1.57^{b}	1.84^{cd}	1.64^{b}	0.81^{cde}	30.0
10	1.76^{a}	2.68^{a}	0.65^{ab}	7.47^{a}	1.84^{a}	2.11^{a}	1.88^{a}	0.92^{a}	34.6
11	1.42^{cde}	2.10^{cde}	0.53^{de}	6.37^{bcd}	1.48^{bcd}	1.80^{cd}	1.50^{c}	0.74^{f}	28.9
12	1.42^{cde}	2.00^{e}	0.56^{cde}	6.00^{f}	1.41^{d}	1.77^{cd}	1.43^{c}	0.82^{cde}	28.0
13	1.47^{bc}	2.09^{cde}	0.54^{de}	6.40^{bc}	1.48^{bcd}	1.83^{cd}	1.54^{bc}	0.82^{bcde}	29.1
Mean[2]	1.47	2.20	0.56	6.34	1.52	1.84	1.53	0.81	29.3
SEM[3]	0.019	0.049	0.012	0.053	0.024	0.029	0.027	0.012	-

[1] Total digestible content for indispensable and dispensable amino acids

[2] Mean of amino acid digestibility values across 13 prepress solvent-extracted canola meals

[3] Standard error of the mean; Number of observations contributing to each mean = 5

[a-f] Means within a column with no common letters differ significantly ($P < 0.05$).

Table 3. 9 Standardized ileal digestibility values and digestible amino acid content of expeller-extracted CM for broiler chickens

Amino acids	SID AA (%)	SID AA Content (% DM)
Indispensable AA		
Arginine	86.67	1.83
Histidine	77.17	0.92
Isoleucine	83.35	1.08
Leucine	83.06	2.11
Lysine	74.93	1.23
Methionine	90.61	0.54
Phenylalanine	84.67	1.23
Threonine	74.42	1.22
Valine	79.13	1.32
Tryptophan	78.66	0.38
Dispensable AA		
Alanine	81.70	1.39
Aspartic acid	74.22	1.97
Cystine	80.85	0.54
Glutamic acid	88.76	5.77
Glycine	75.67	1.42
Proline	73.87	1.67
Serine	82.63	1.42
Tyrosine	77.56	0.77
Total digestible content	-	26.8

The SID values and digestible AA content of expeller-extracted CM fed to broiler chickens are presented in Table 3.9. The SIAAD and AA content of CM 14 (expeller-extracted CM) was excluded from the statistical analyses, only one sample was collected and evaluated from one crushing plant. Thus, the comparison to the CM 1-13 only demonstrates numerical differences. In general, the SID values of AA were not very different when compared with each of the solvent-extracted meals. Methionine had the highest while Pro had the lowest digestibility value. A comparison of SID content of AA obtained from expeller-extracted CM with each of those from 13 solvent-extracted CM revealed a slight difference in digestibility, however, there were some similarities with CM5 and CM12 for majority of the AA. The total digestible AA content of expeller-extracted CM is somewhat close to the one obtained from CM5.

Expeller-extracted CM often contains higher oil content, variation in cooking conditions with the intent of extracting more oil often results in variability of available AA (Mailer, 2004). Meal temperature during expeller extraction of canola could go up to 160°C, heat generated during the expelling process may be a contributing factor to this high temperature. However, heating is for a short period of time, but protein quality could be compromised if cooling is not achieved immediately after oil extraction (Canola Council of Canada, 2015). Toghyani et al. (2015) in their study with broiler starter and grower concluded that processing conditions during oil extraction could have a significant impact on CP and AA digestibility of expeller-extracted CM due to changes in the chemical composition and its effect on digestibility of some AA. Apart from increase in energy, the protein quality is affected because of nutrient dilution from higher oil content. This nutrient encapsulating effect of oil coupled with excess temperature during processing (cooking and pressing) might be an explanation for slight differences observed in the available and digestible content of AA from expeller-extracted CM when compared to those of

solvent-extracted CM. Interestingly, the strong similarities observed for digestible AA contents among those ones not of expeller-extracted meals suggests that an expelled meal could be affected the same way as potentially overheated solvent-extracted meal, because CM5 and CM12 were on the low end with respect to digestible AA content.

The relationships between SID of AA is presented in Table 3.10. A relationship exists among AA ($R^2 > 0.5$) except for Met and Cys, which were poorly correlated with other AA ($R^2 < 0.48$), but correlated with each other ($R^2 = 0.59$). Lysine, the first limiting and most heat sensitive AA as well as Arg, a conditionally essential AA, were strongly linked with every other (R^2 from 0.56 to 0.91 and 0.57 to 0.91), respectively. Threonine being the third limiting and more heat sensitive after Lys, was strongly related with other AA (R^2 from 0.76 to 0.98) with exception of Pro ($R^2 = 0.49$). Oftentimes and in relation to heat treatment, Lys is considered the most reactive AA. However, some other key AA such as Thr, Arg, Trp and Glu, belonging to the class of alpha and epsilon AA, could be affected by heat treatment and their side chain enables them to take part in side chain reactions. It was interesting to discover the linear relationships that exists among all AA except Met and Cys. This non-relationship of Met and Cys with other AA could be a further validation of the lack of difference observed in AA digestibility values of CM fed to broiler chickens. The strong relationship between Lys and Arg as well as some other AA may be a strong indication that not only Lys is affected by heat treatment. Mosenthin et al. (2016) reported lower content of Lys, reactive Lys and their corresponding ratios with CP for rapeseed meal exposed to wet toasting conditions with increasing residence time in the desolventizer/toaster. This reduction was associated with a reduced digestibility of CP and other AA, specifically *in-vivo* digestion of Lys, Cys and Arg. The graphs showing the relationships among AA suspected to be more sensitive to heat are presented in Figures 3.01 to 3.10. The relationships between Thr and Glu, Lys and Arg,

Thr and Trp, Arg and Glu are the strongest while those of Trp and Arg as well as Lys and Trp appears to be the weakest.

Table 3. 10 Relationships between standardized ileal digestibility of amino acid (R-squared values)

Amino acid	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Trp	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
Arg	-	0.791	0.846	0.897	0.910	0.461	0.924	0.769	0.803	0.576	0.733	0.814	0.413	0.860	0.871	0.750	0.796	0.900
His	0.791	-	0.618	0.725	0.693	0.392	0.722	0.764	0.592	0.484	0.642	0.779	0.317	0.767	0.766	0.637	0.800	0.624
Ile	0.846	0.618	-	0.931	0.895	0.282	0.895	0.808	0.965	0.683	0.834	0.804	0.144	0.826	0.844	0.456	0.775	0.891
Leu	0.897	0.725	0.931	-	0.846	0.403	0.976	0.908	0.947	0.797	0.926	0.884	0.271	0.915	0.940	0.576	0.910	0.929
Lys	0.912	0.693	0.895	0.846	-	0.404	0.841	0.739	0.819	0.564	0.703	0.828	0.304	0.837	0.850	0.609	0.741	0.837
Met	0.461	0.392	0.282	0.403	0.404	-	0.437	0.384	0.285	0.325	0.371	0.425	0.594	0.464	0.426	0.477	0.439	0.479
Phe	0.924	0.722	0.895	0.976	0.841	0.437	-	0.848	0.908	0.725	0.866	0.825	0.310	0.865	0.907	0.591	0.857	0.955
Thr	0.769	0.764	0.808	0.908	0.735	0.384	0.848	-	0.878	0.889	0.966	0.959	0.288	0.959	0.954	0.494	0.981	0.804
Val	0.803	0.592	0.965	0.947	0.819	0.285	0.908	0.878	-	0.808	0.924	0.837	0.173	0.858	0.885	0.420	0.836	0.915
Trp	0.574	0.484	0.683	0.797	0.560	0.325	0.725	0.891	0.808	-	0.939	0.842	0.281	0.832	0.850	0.327	0.880	0.714
Ala	0.733	0.642	0.834	0.926	0.703	0.371	0.866	0.966	0.924	0.939	-	0.903	0.250	0.915	0.930	0.436	0.942	0.851
Asp	0.814	0.779	0.804	0.884	0.828	0.425	0.825	0.959	0.837	0.842	0.903	-	0.379	0.984	0.972	0.551	0.962	0.791
Cys	0.413	0.317	0.144	0.271	0.304	0.594	0.310	0.288	0.173	0.281	0.250	0.379	-	0.400	0.387	0.568	0.370	0.341
Glu	0.860	0.767	0.826	0.915	0.833	0.464	0.865	0.961	0.858	0.831	0.915	0.983	0.400	-	0.978	0.614	0.960	0.841
Gly	0.871	0.766	0.844	0.940	0.850	0.426	0.907	0.954	0.885	0.850	0.930	0.972	0.387	0.978	-	0.609	0.960	0.870
Pro	0.750	0.637	0.456	0.576	0.609	0.477	0.591	0.494	0.420	0.327	0.436	0.551	0.568	0.609	0.614	-	0.574	0.553
Ser	0.796	0.800	0.775	0.910	0.741	0.439	0.857	0.981	0.836	0.880	0.942	0.962	0.370	0.960	0.960	0.574	-	0.797
Tyr	0.900	0.624	0.891	0.929	0.837	0.479	0.955	0.804	0.915	0.714	0.851	0.791	0.341	0.841	0.870	0.553	0.797	-

Figure 3. 1. Graph showing correlation between digestible Threonine and Glutamine

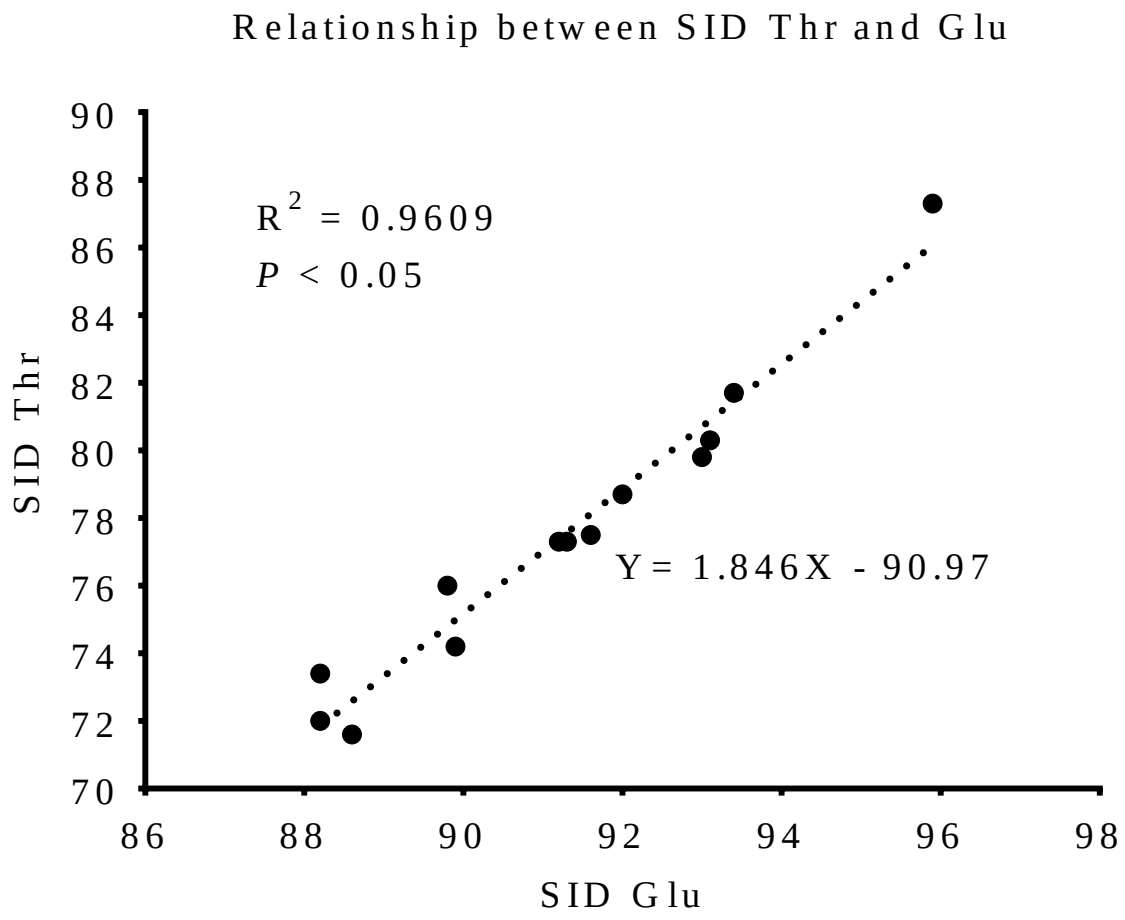


Figure 3. 2. Graph showing correlation between digestible Lysine and Arginine

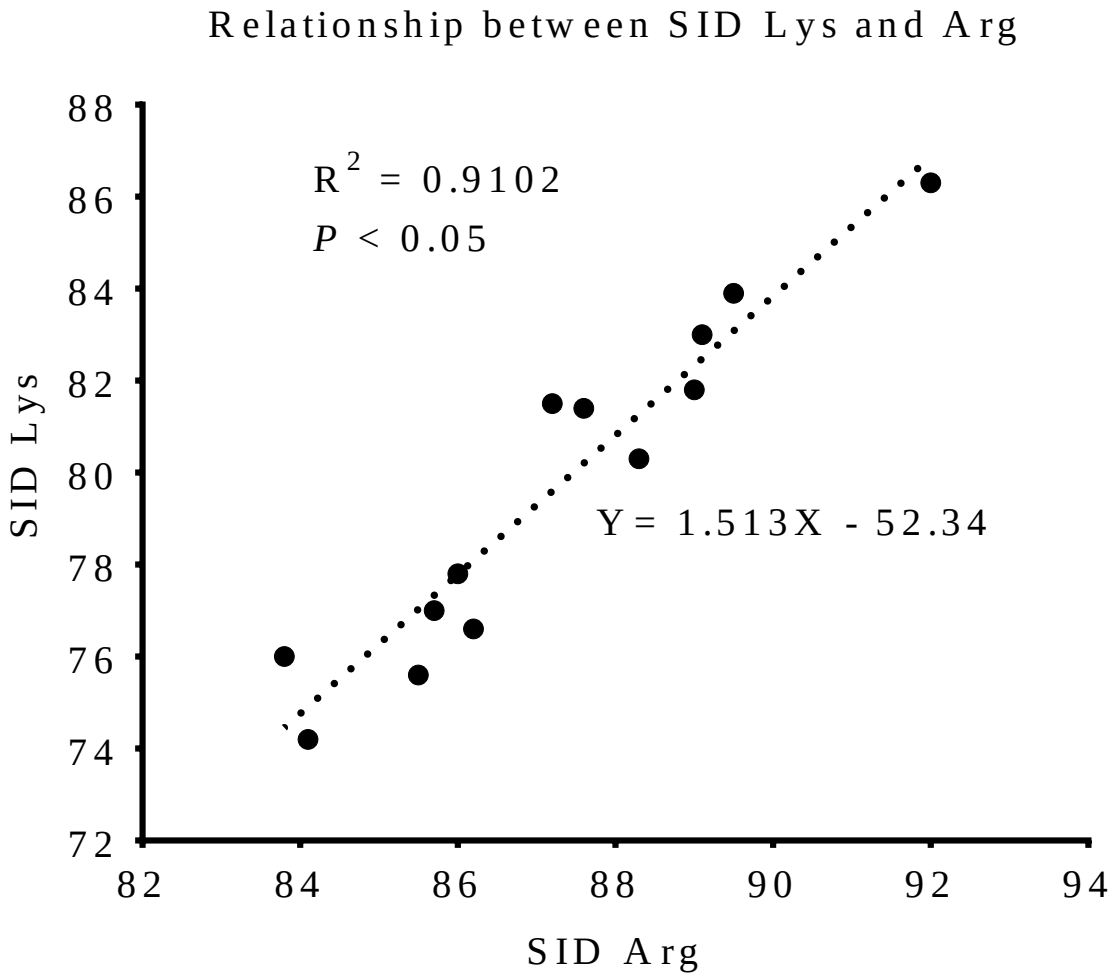


Figure 3. 3. Graph showing correlation between digestible Threonine and Tryptophan

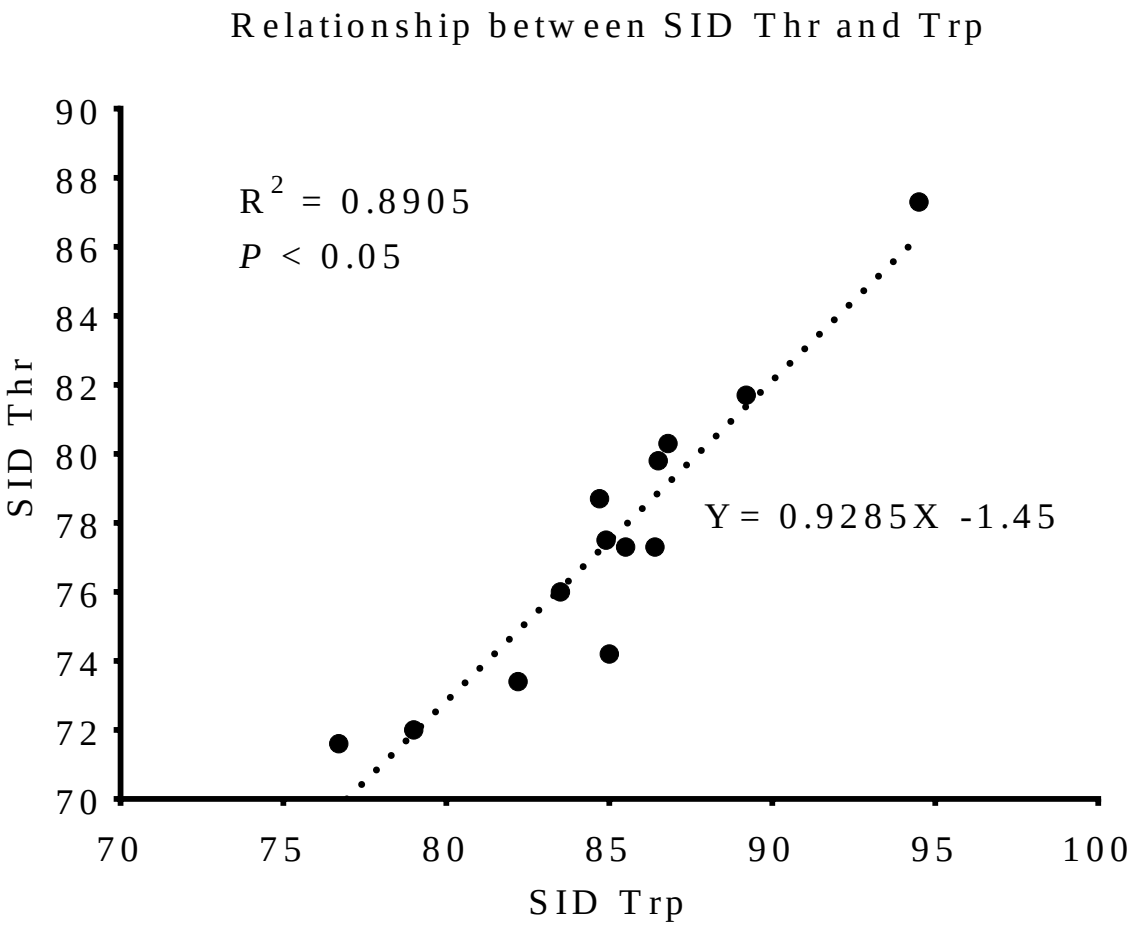


Figure 3. 4. Graph showing correlation between digestible Arginine and Glutamine

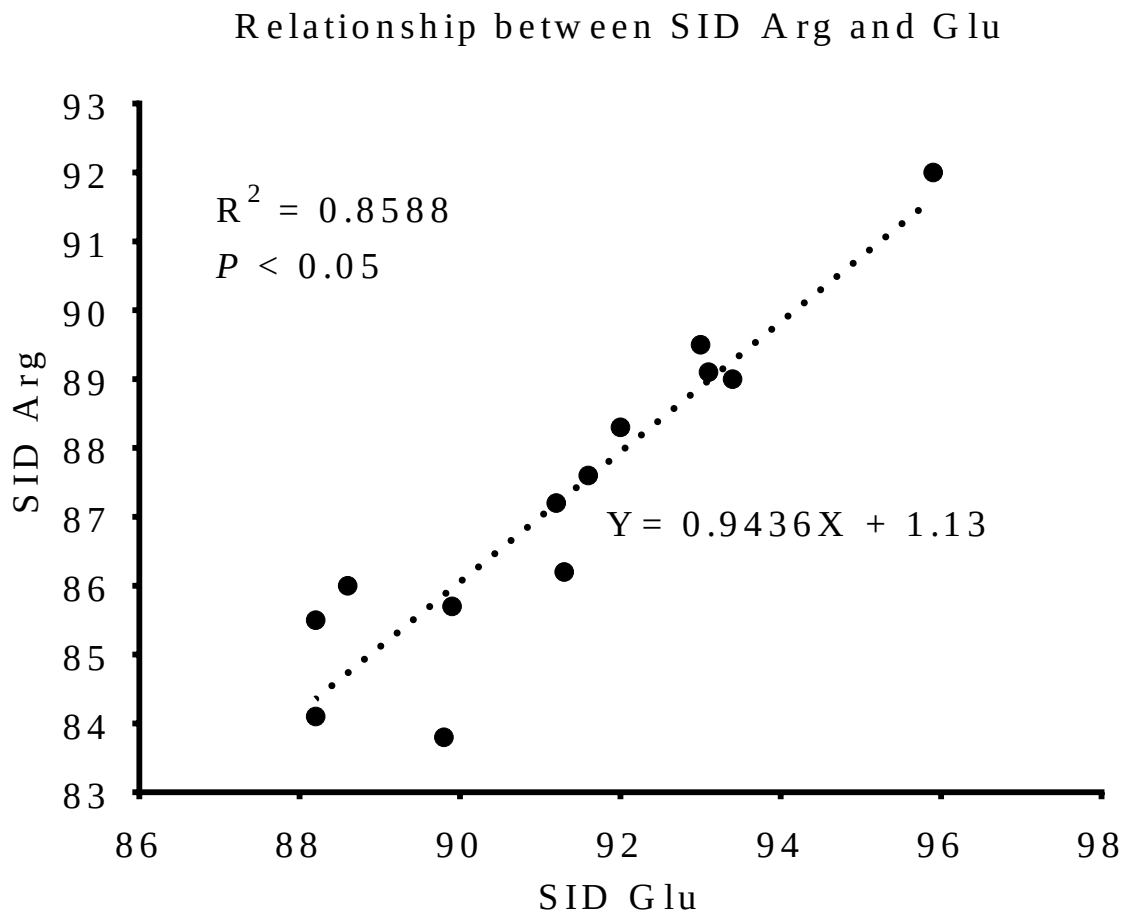


Figure 3. 5. Graph showing correlation between digestible Lysine and Glutamine

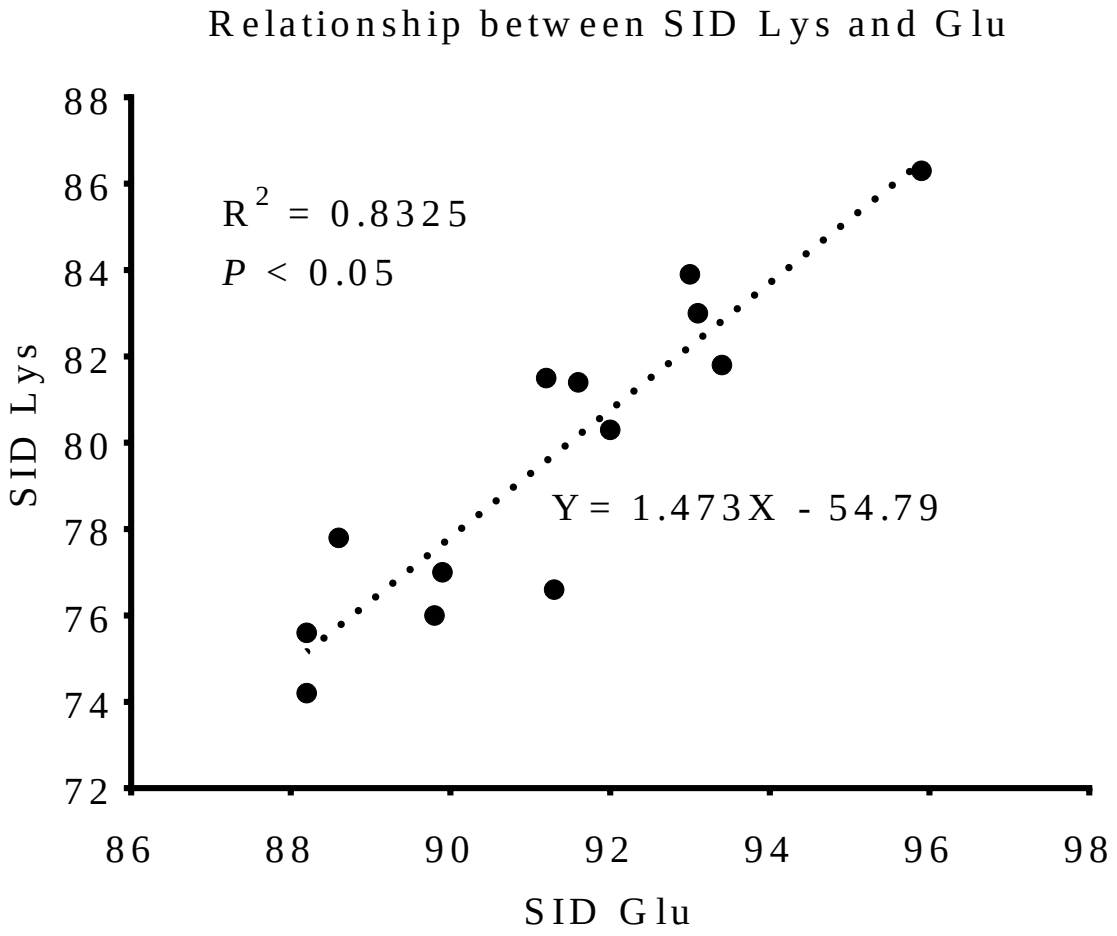


Figure 3. 6. Graph showing correlation between digestible Tryptophan and Glutamine

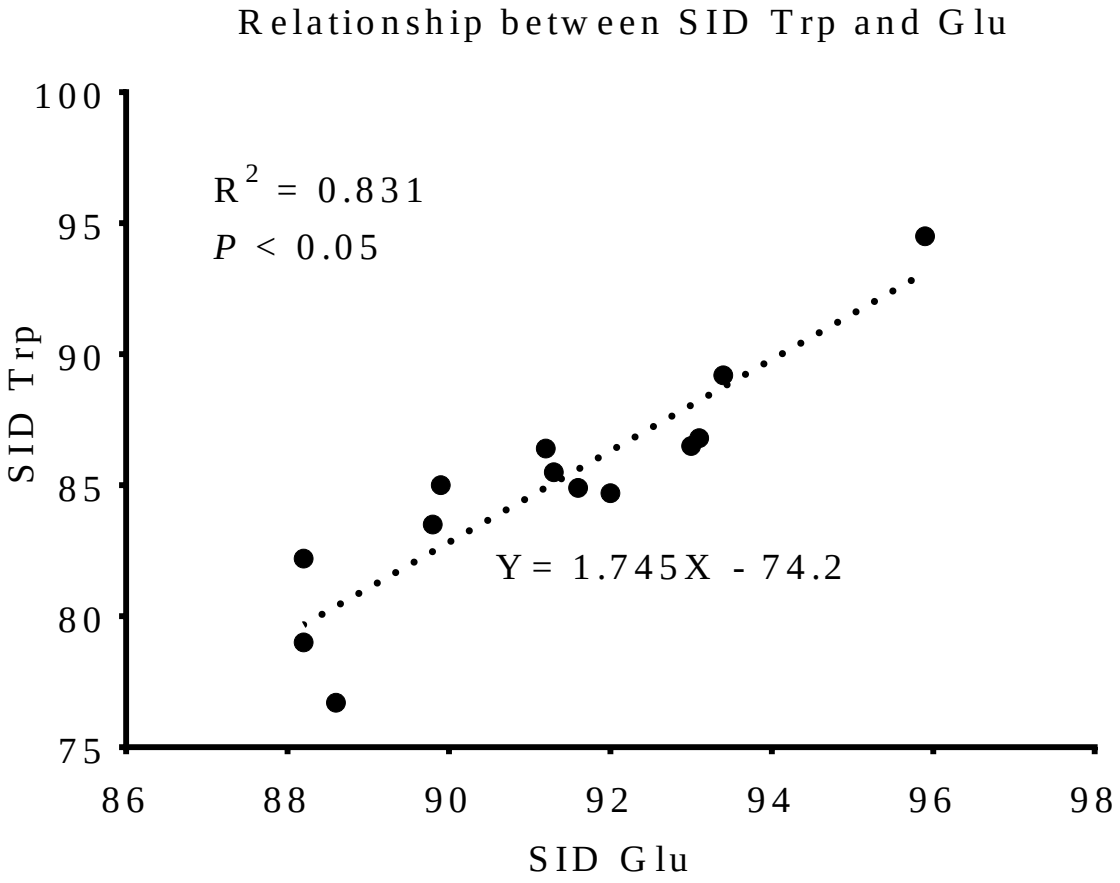


Figure 3. 7. Graph showing correlation between digestible Threonine and Arginine

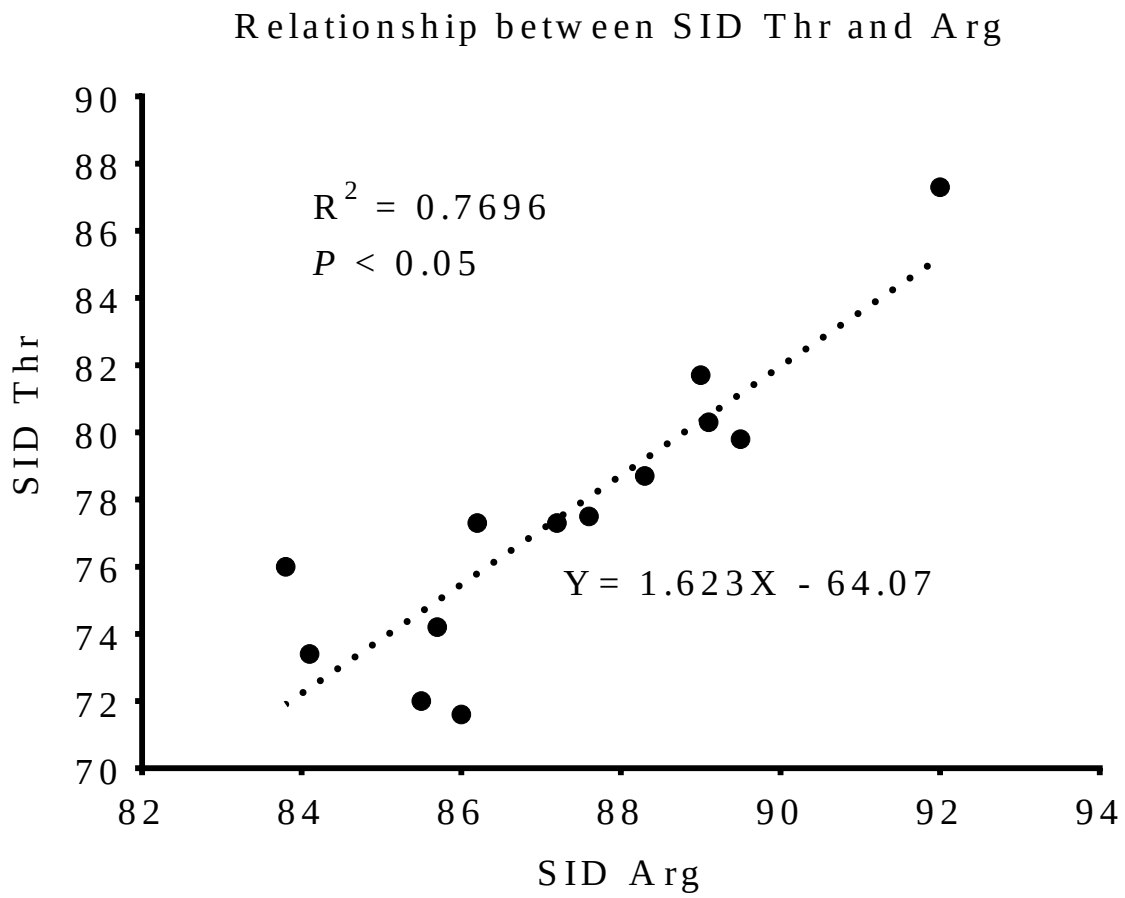


Figure 3. 8. Graph showing correlation between digestible Lysine and Threonine

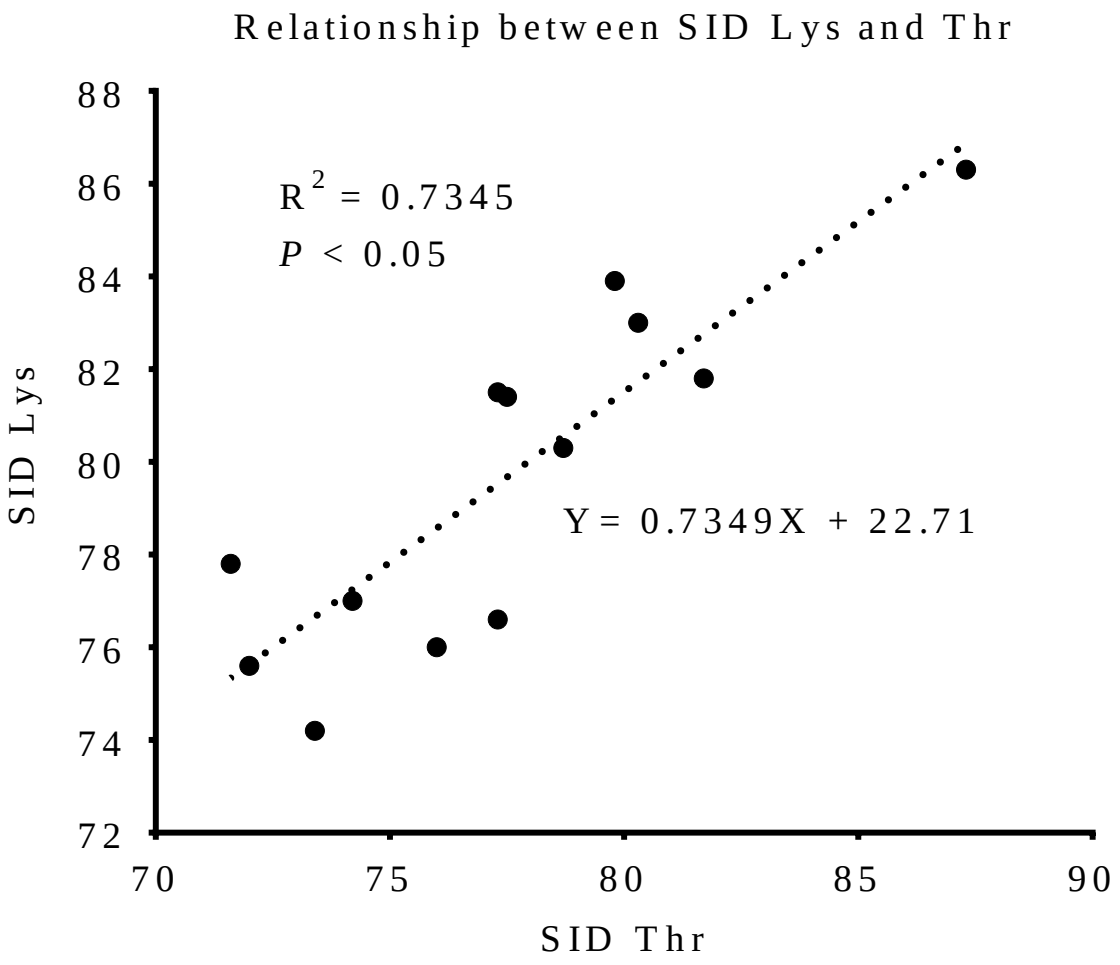


Figure 3. 9. Graph showing correlation between digestible Tryptophan and Arginine

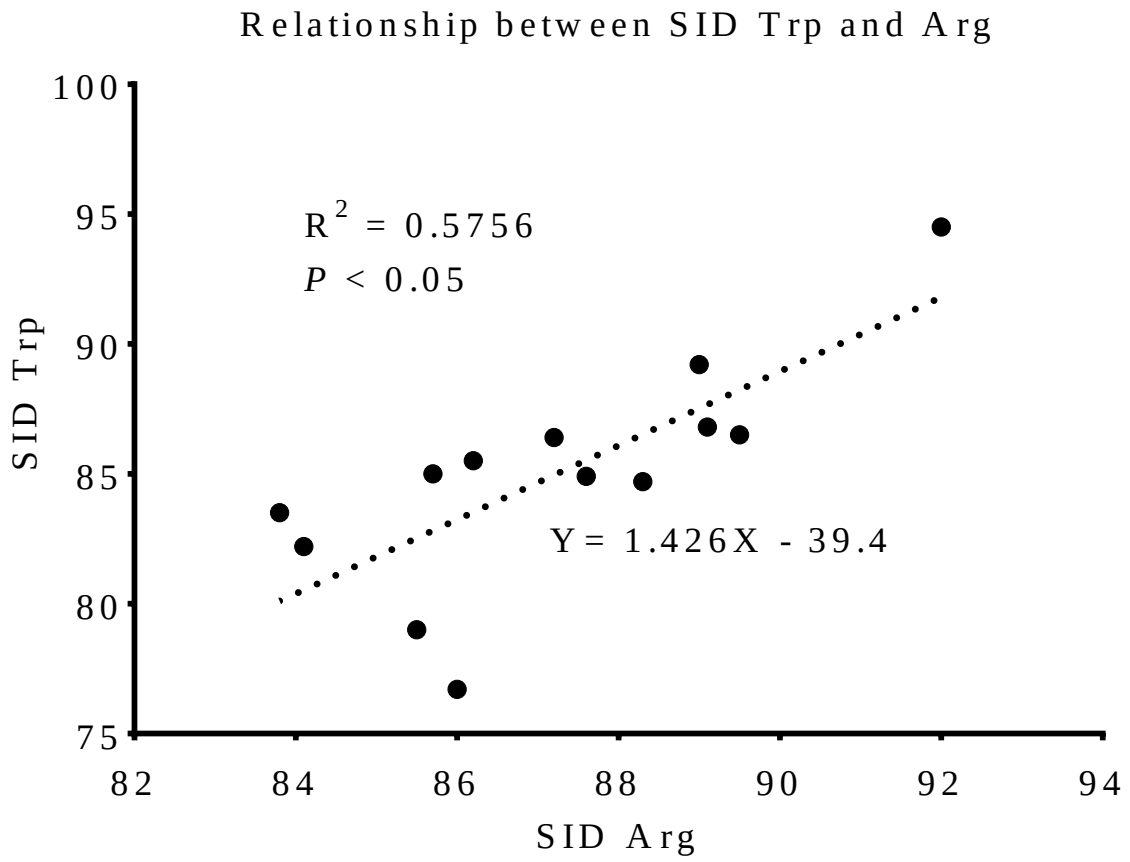
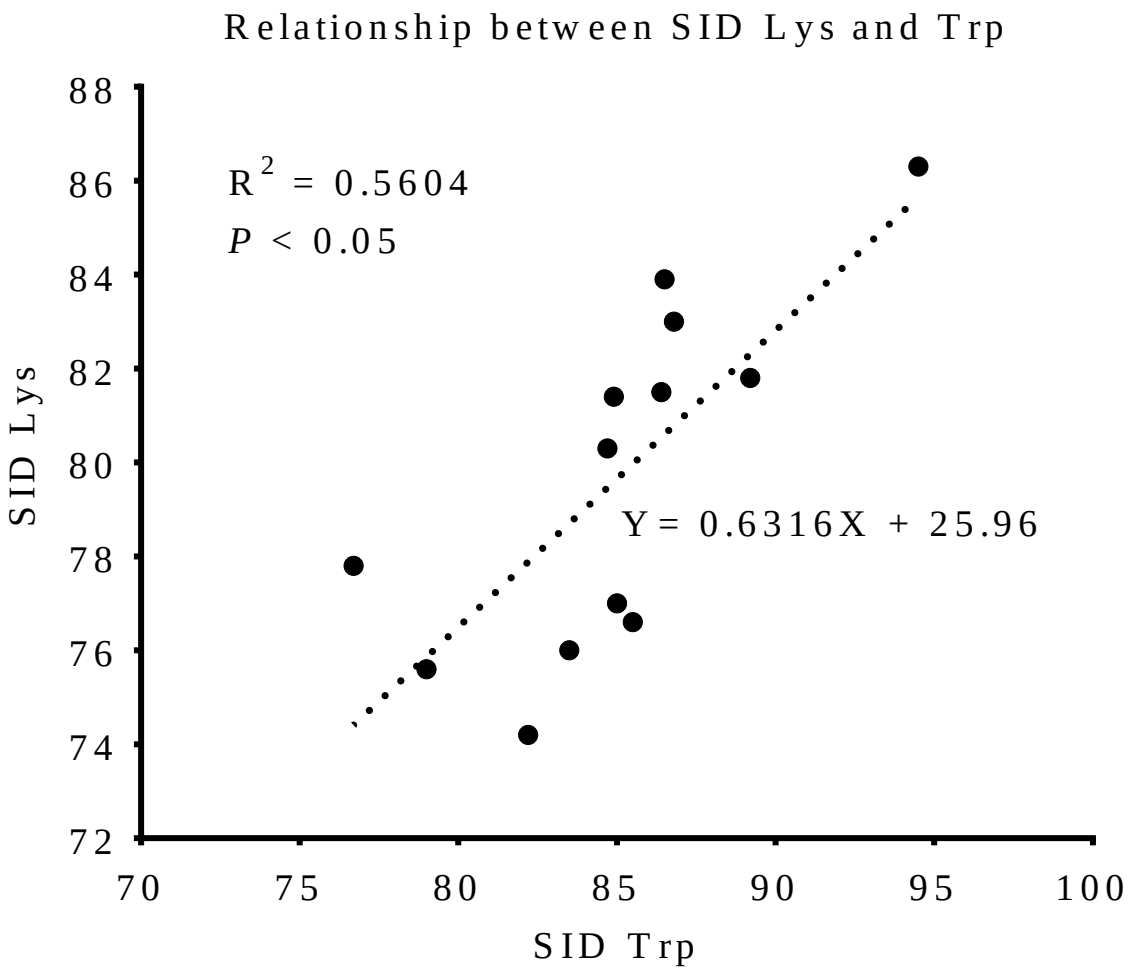


Figure 3. 10. Graph showing correlation between digestible Lysine and Tryptophan



Chemical changes due to Maillard reaction do not completely explain the reduction in AA digestibility (Salazar-Villanea et al., 2016) as changes in the structure of protein (secondary and tertiary) also affect digestion processes. Physical changes seem to occur before chemical changes during toasting and a simultaneous occurrence have a confounding influence on protein digestibility (Gerrard et al., 2012). These physical changes of protein in the form of aggregation (inter and intra molecular hydrogen bonding), precipitation and degradation (hydrolysis of peptide bonds) resulting from excessive heat treatment could be responsible for destruction and reduced digestibility of AA. Salazar-Villanea et al. (2016) reported a linear decrease in the AA content of Ala, Asp, Glu, Gly, Lys and Arg with increasing toasting time of rapeseed meal. A two-step enzymatic digestibility method used for *in-vitro* digestibility study also revealed a linear decrease in the rate of protein hydrolysis with increasing toasting time. They also demonstrated a linear relationship between toasting time and secondary protein structure, as well as a significant correlation between this protein structure and Lys content. Racemization is the interconversions that occur between one form of AA to another, higher temperature increases the forward reaction of L-AA to D-AA such that AA becomes optically inactive and nutritional value reduced due to decreased protease digestion (Varga-Visi et al., 2009). This might be the reasons for the strong association among AA in the current study.

3.5 Conclusions

It is evident from the current study that SID of AA and standardized ileal digestible contents of AA varies among CM sourced from different processing facilities across Canada, variations may largely be due to differences in temperature applied during desolventizing/toasting of meal. There was a strong relationship between SID of AA, this could be an indication that other AA apart from Lys are susceptible to damage through heat treatment. Apart from Maillard

formation of secondary protein structures, other heat induced chemical reactions could be responsible for differences in AA digestibility.

CHAPTER 4

HIGH INCLUSION LEVELS OF CANOLA MEAL IN BROILER CHICKEN NUTRITION

4.1 Abstract

Solvent-extracted canola meal (CM) is commonly used source of protein for poultry. Dietary inclusion level of CM, however, has been historically limited to 5-10% due to concerns related to high fiber content and the presence of glucosinolates. A four-week feeding trial was conducted to determine the effect of varying inclusion levels of CM on the growth performance of broiler chickens. The experiment comprised of four growth phases of six treatment groups: pre-starter (0, 3, 6, 9, 12, 15 % of CM), starter (0, 4, 8, 12, 14, 18 % of CM), grower 1 (0, 5, 10, 15, 20, 25 % of CM) and grower 2 (0, 6, 12, 18, 24, 30 % of CM), each lasting for one week. Diets were balanced for SID AA contents by replacing soybean meal (SBM) in a corn-SBM basal diet with graded levels of CM. One-day-old broiler chickens (housed 5 birds/cage) were allotted into the six treatment groups with ten replicates per treatment. Body weight gain (BWG) and feed intake (FI) of birds were monitored weekly and feed conversion ratio (FCR) was calculated. Completely randomized design using the GLM procedure of SAS was used. Overall, the result showed that although neutral detergent fiber (NDF) content of diets differed substantially with increased levels of CM, bird performance was not significantly ($P > 0.05$) affected by CM inclusion levels. Irrespective of the phase and CM inclusion levels, FI, BWG, and FCR averaged 2,019 g/bird/28 d, 1,387 g/bird/28 d, and 1.46 g/feed/g gain and were similar to 1,921 g/bird/28 d, 1,325 g/bird/28 d, and 1.45 g feed/g gain for the control treatment, respectively. Determined apparent metabolizable energy (AME_n) values of diets containing CM differed with age of birds. Incremental levels of CM inclusion didn't have a significant ($P > 0.05$) effect on NDF digestibility.

It could be concluded that CM can effectively replace SBM when used up to 30% in broiler chicken diets assuming the diets are formulated on a digestible AA basis. Canola fiber has minimal effect on nutrient utilization as seen from the excellent performance of broiler chickens fed diets of different fiber content.

4.2 Introduction

Solvent-extracted canola meal (CM) is commonly used to substitute for soybean meal (SBM) in poultry diet. This could be considered as an economically viable alternative but may not fully replace SBM in ration formulation due to lower energy, protein and higher fiber contents (Kocher et al., 2000). The relatively low metabolizable energy (ME) is usually related to higher fiber content as this dilutes available energy (Zhang et al., 2013). Canola fiber may also influence the digestibility and availability of other nutrients such as amino acid (AA) and minerals.

Breeding of new varieties, de-hulling, or enzyme supplementation have been used to improve the overall nutritive value of CM (Khajali and Slominski, 2012). Furthermore, balancing diets on digestible AA and available energy content is the key to feeding high inclusion levels of CM in broiler chicken diets. When such diets are formulated based on digestible AA contents, inclusion levels could be higher without negatively affecting performance (Newkirk and Classen, 2002; Ramesh et al., 2006). Also, the glucosinolates content of CM obtained from Canadian processing facilities is low, suggesting that a reduced performance may not be due to high glucosinolates content.

Several studies have examined the effect of graded levels of CM on growth performance of broiler chicken (Naseem et al., 2006; Waldroup et al., 2011; Woyengo at al., 2011; Gopinger et al., 2014 and Ahmed et al., 2015). However, result have not been consistent regarding optimal CM inclusion as one or more performance parameter are compromised due to high inclusion levels. It

appears that the different phases of broiler chicken growth are not considered when raising the inclusion levels of CM in broiler chicken diet. Dietary fiber allowance of broiler chickens could be of importance when the same inclusion levels are applied in diets for chickens of different ages. It has been found that high amounts of fiber in poultry diets may result in reduced performance in broiler chickens (Walugembe et al., 2014). As such, there is a scarcity of information on the graded levels of CM with respect to differences in age of broiler chickens. Therefore, the aim of this study was to determine the effect of graded levels of CM on growth performance and energy and fiber digestibility of young broiler chickens at different stages of growth.

4.3 Materials and Methods

4.3.1 Experimental diets

Conventional CM was obtained from Bunge canola processing plant, Altona, MB, Canada. A four-week feeding trial was conducted to determine the varying inclusion levels of CM on the growth performance and nutrient digestibility of broiler chickens. Twenty-four test diets were balanced for SID AA and available energy content by replacing SBM with graded levels of CM. The experimental diets were fed from one day of age. All diets, including the control corn/SBM/wheat diet, were formulated to meet the nutritional requirements for different phases of broiler chicken growth using the breeder recommendation. Each diet contained 0.30% chromic oxide as an internal marker. The experiment had four phases namely: pre-starter, starter, grower 1 and grower 2, each lasting for one week (Tables 4.1 and 4.2).

Table 4. 1 Ingredients and analyzed composition (%) of diets used in the pre-starter and starter phases of experiment.

Ingredient	Pre-starter (0-7 days)						Starter (8-14 days)					
	1	2	3	4	5	6	1	2	3	4	5	6
Canola meal	0.00	3.00	6.00	9.00	12.00	15.00	0.00	4.00	8.00	12.00	14.00	18.00
Corn	44.15	43.96	43.40	43.00	42.25	41.31	48.00	47.72	47.00	46.55	46.50	46.00
Soybean meal	35.10	32.92	31.00	28.63	26.50	24.50	33.00	29.72	26.84	23.85	22.55	19.55
Wheat	12.00	11.00	10.00	9.50	9.00	8.50	10.00	9.00	8.00	7.00	6.00	5.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.20	2.30	2.30	2.40	2.40
Vegetable oil	1.81	2.21	2.75	3.05	3.48	3.97	2.25	2.68	3.26	3.73	4.07	4.62
Calcium carbonate	1.44	1.42	1.40	1.37	1.35	1.32	1.51	1.48	1.43	1.41	1.38	1.35
Mono calcium phosphate	1.32	1.31	1.29	1.27	1.25	1.24	1.20	1.16	1.14	1.11	1.08	1.05
L-Lysine	0.17	0.17	0.17	0.19	0.19	0.19	0.14	0.15	0.16	0.18	0.17	0.18
DL-Methionine	0.17	0.17	0.16	0.16	0.16	0.15	0.07	0.07	0.06	0.06	0.05	0.05
Threonine	0.04	0.04	0.03	0.03	0.02	0.02	0.03	0.02	0.01	0.01	0.00	0.00
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.30	0.30	0.30	0.30	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	100	100	100	100
Calculated composition												
Crude protein	23.0	23.0	23.0	23.0	23.0	23.0	22.0	22.0	22.0	22.0	22.0	22.0
ME, kcal/kg DM	2950	2950	2950	2950	2950	2950	3000	3000	3000	3000	3000	3000
Calcium	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Available phosphorus	0.48	0.48	0.48	0.48	0.48	0.48	0.45	0.45	0.45	0.45	0.45	0.45
Lysine	1.40	1.40	1.40	1.40	1.40	1.40	1.32	1.32	1.32	1.32	1.32	1.32
Methionine	0.59	0.59	0.59	0.59	0.59	0.59	0.48	0.48	0.48	0.48	0.48	0.48
Threonine	0.91	0.91	0.91	0.91	0.91	0.91	0.86	0.86	0.86	0.86	0.86	0.86
Arginine	1.45	1.45	1.45	1.45	1.45	1.45	1.34	1.34	1.34	1.34	1.34	1.34
Analyzed composition												
Dry matter	91.5	91.9	91.8	92.0	92.1	92.3	91.6	91.7	91.9	91.7	92.3	92.1
Crude protein	23.9	23.8	23.4	23.0	23.0	23.5	22.8	23.1	22.4	21.5	22.3	22.2
Neutral detergent fiber	8.5	8.7	8.8	8.7	10.3	10.3	7.9	8.7	9.2	10.0	10.9	11.4

[1] Provided per kg of diet: 70 mg Mn, 80 mg Zn, 80 mg Fe, 10 mg Cu, 0.3 mg Se, 0.5 mg I, 337 g Na. [2] Provided per kg of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin.

Table 4. 2 Ingredients and analyzed composition (%) of diets used in grower 1 and grower 2 phases of experiment.

Ingredient	Grower 1 (15-21 days)						Grower 2 (22-28 days)					
	1	2	3	4	5	6	1	2	3	4	5	6
Canola meal	0.00	5.00	10.00	15.00	20.00	25.00	0.00	6.00	12.00	18.00	24.00	30.00
Corn	53.00	52.32	51.00	50.20	49.50	48.80	59.20	57.66	56.10	54.65	53.00	52.00
Soybean meal	28.53	24.45	21.00	17.12	13.00	9.10	26.00	21.30	16.52	12.10	8.00	3.20
Wheat	8.00	7.00	6.00	5.00	4.00	3.00	4.00	3.50	3.00	2.00	1.00	0.00
Fish meal	3.50	3.80	3.90	4.00	4.30	4.50	4.00	4.10	4.30	4.40	4.40	4.50
Vegetable oil	2.84	3.40	4.16	4.83	5.46	5.96	3.30	3.77	4.53	5.38	6.25	7.01
Calcium carbonate	1.22	1.16	1.12	1.08	1.04	0.98	1.16	1.10	1.04	1.00	0.94	0.89
Mono calcium phosphate	1.00	0.95	0.90	0.85	0.77	0.74	0.80	0.76	0.68	0.63	0.58	0.54
L-Lysine	0.08	0.09	0.09	0.10	0.12	0.12	0.00	0.01	0.03	0.04	0.04	0.06
DL-Methionine	0.03	0.03	0.03	0.02	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Threonine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.30	0.30	0.30	0.30	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	100	100	100	100
Calculated composition												
Crude protein	21.0	21.0	21.0	21.0	21.0	21.0	20.0	20.0	20.0	20.0	20.0	20.0
ME, kcal/kg DM	3100	3100	3100	3100	3100	3100	3150	3150	3150	3150	3150	3150
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.85	0.85	0.85	0.85	0.85	0.85
Available phosphorus	0.44	0.44	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40	0.40	0.40
Lysine	1.21	1.21	1.21	1.21	1.21	1.21	1.09	1.09	1.09	1.09	1.09	1.09
Methionine	0.45	0.45	0.45	0.45	0.45	0.45	0.41	0.41	0.41	0.41	0.41	0.41
Threonine	0.80	0.80	0.80	0.80	0.80	0.80	0.73	0.73	0.73	0.73	0.73	0.73
Arginine	1.23	1.23	1.23	1.23	1.23	1.23	1.13	1.13	1.13	1.13	1.13	1.13
Analyzed composition												
Dry matter	92.4	92.2	92.5	92.4	92.6	92.5	91.6	92.1	92.2	91.7	92.0	92.5
Crude protein	21.3	21.3	21.0	21.0	21.0	21.1	20.6	20.5	20.7	20.4	20.7	20.2
Neutral detergent fiber	8.2	9.4	10.1	11.4	13.8	14.2	8.5	9.8	10.9	12.5	13.0	13.9

[1] Provided per kg of diet: 70 mg Mn, 80 mg Zn, 80 mg Fe, 10 mg Cu, 0.3 mg Se, 0.5 mg I, 337 g Na. [2] Provided per kg of diet: 8250 IU vitamin A, 3000 IU vitamin D3, 30 IU vitamin E, 0.13 mg vitamin B12, 2 mg vitamin K3, 6 mg riboflavin, 40.3 mg niacin, 1301 mg choline, 4 mg folic acid, 0.25 mg biotin.

4.3.2 Animal care and housing

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009) with the animal protocols approved by the Animal Care Use Committee of the University of Manitoba. A total of 300 one-day-old Ross 308 broiler chickens were obtained from a local hatchery (Carlton Hatchery, Grunthal MB, Canada). All birds were randomly assigned to 10 cages of 5 birds each per treatment. Birds were housed in electrically heated Alternative Design Super Brooders (Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR) under a controlled environment with unrestricted access to feed and water. Room temperature was maintained at 32, 28, 24 and 20°C for week 1, 2, 3 and 4 respectively. Feed intake and BWG of birds were recorded weekly with cage being the experimental unit. Feed conversion ratio was calculated. Experimental diets were changed weekly. Growth performance parameters for the entire trial from 1 to 28 days of age were also calculated.

4.3.3 Sample collection and chemical analyses

Excreta samples from each cage were collected at the end of starter and grower 2 phases over a 3-hour period; immediately frozen at -20°C and freeze-dried. Samples from the same cage were pooled to yield 5 replicates per treatment and were finely ground to determine AME_n of diet and fiber digestibilities. Duplicate samples of diets and excreta were analyzed for chromium and nitrogen contents. Nitrogen content was determined by combustion method (990.03; AOAC, 2005) using LECO N analyzer (model TruSpec N Leco Corp., St. Joseph, MI, USA). Chromium content were analyzed after samples were ashed at 600°C for 12 hours in a muffle furnace, using coupled plasma mass spectrometry (ICP-AES; Vista, Varian, Palo Alto, CA, USA) according to the method of AOAC (2005, method 985.01). Gross energy (GE) of diet and excreta samples were estimated using an adiabatic bomb calorimeter (model 6400; Parr Instruments Co., Moline, IL, USA), which

had been calibrated using benzoic acid as a standard. Nitrogen retention and AME_n values of diets were calculated as described by Leeson and Summers (2001). The method of Goering and Van Soest (1970) was used to determine NDF content of samples.

4.3.4 Calculations and Statistical analysis

Apparent metabolizable energy AME_n of diet was calculated using the formulas below:

- AME_n of diet (kcal/kg) =

$$GE_{\text{kcal/kg diet}} - [GE_{\text{kcal/kg excreta}} \times (Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{excreta}})]$$

$$- 8.22 \times \{N_{\%\ \text{diet}} - [N_{\%\ \text{excreta}} \times (Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{excreta}})]\}$$

- NDF digestibility (%) =

$$[1 - (Cr_2O_{3\ \%\ \text{diet}}/Cr_2O_{3\ \%\ \text{excreta}}) \times (NDF_{\text{excreta}}/NDF_{\text{diet}})] \times 100$$

Data were analyzed using the Mixed procedure of SAS, version 9.2 (SAS Institute Inc., Cary, NC). Means were separated using Turkey's studentized range test at a significant level of $P \leq 0.05$.

4.4 Results and Discussion

The effects of incremental levels of CM on growth performance of broiler chickens fed pre-starter (0-7 days) and starter (8-14 days) diets are shown in Tables 4.3 and 4.4. There were no significant differences ($P > 0.05$) between treatments for FI, BWG and FCR. Although NDF content of diets differed substantially with increasing levels of CM (Tables 4.1 and 4.2), all diets containing CM had similar effect as corn-SBM diet on growth performance of broiler chickens in pre-starter (0-7 days) and starter (8-14 days) phases of experiment.

Table 4. 3 Effect of incremental levels of CM on growth performance of broiler chicken in the pre-starter phase of experiment *(0-7 days).*

CM inclusion level (%)	Feed intake (g/bird/week)	Body weight gain (g/bird/week)	Feed conversion ratio (g feed/g gain)
0	132.5	105.9	1.26
3	132.3	106.3	1.25
6	134.0	113.6	1.18
9	136.2	113.9	1.20
12	137.6	117.7	1.17
15	139.2	120.4	1.16
SEM[1]	2.14	3.46	0.025
P-value[2]	0.141	0.022	0.024

[1] Standard error of the mean; Number of observations contributing to each mean = 9

[2] Overall *P*-value of incremental levels

Table 4. 4 Effect of incremental levels of CM on growth performance of broiler chicken in the starter phase of the experiment *(8-14 days).*

CM inclusion level (%)	Feed intake (g/bird/week)	Body weight gain (g/bird/week)	Feed conversion ratio (g feed/g gain)
0	408.5	316.7	1.29
4	418.2	320.7	1.31
8	427.2	342.2	1.25
12	436.6	334.4	1.31
14	430.9	331.1	1.30
18	437.9	341.3	1.28
SEM[1]	7.62	7.39	0.019
P-value[2]	0.067	0.091	0.261

[1] Standard error of the mean; Number of observations contributing to each mean = 9

[2] Overall *P*-value of incremental levels

The overall analysis of variance indicated statistical significance for BWG and FCR with no separation of means by Turkey's studentized range test, notwithstanding, there was a trend towards increase in BWG (P = 0.022) with increasing levels of CM inclusion, and that corresponds to a better FCR (P = 0.024) of broiler chickens fed pre-starter diets (0-7 days). The effect of incremental levels of CM on growth performance of broiler chickens fed grower 1 (15-21 days) and grower 2 (22-28 days) diets are shown in Tables 4.5 and 4.6. There were no differences (P > 0.05) in FI of broiler chickens fed grower 1 and grower 2 diets except for 10% of CM in grower 1 and 12% CM in grower 2 diets. Feed intake was significantly higher (P < 0.05) than the control corn-SBM diet (596.0 vs 543.0 g/bird/week and 894.1 vs 837.1 g/bird/week) although this significance was not indicated by the overall analysis of variance (P = 0.08) for birds that were fed grower 2 diets (22-28 days). This could be due to variation within replicates. In comparison with the corn-SBM diet, there were no differences (P > 0.05) in BWG and FCR of broiler chickens except for 25% CM inclusion in grower 1 diet which differed significantly for FCR (P < 0.05). Nevertheless, there were no mean separations for BWG of broiler chickens fed grower 2 diets (22-28 days). The effect of incremental levels of CM on growth performance of broiler chickens for the entire trial (1-28 days) is shown in Table 4.7. There were no differences between treatment means (P > 0.05) for FI, BWG and FCR when compared with the control corn-SBM diet, whereas, a 6-12% CM inclusion was significantly higher (P < 0.05) in FI (2051 vs 1921 g/bird/week) and BWG (1431 vs 1325 g/bird/week) than the control but not different from the other treatments, as reflected by an appreciable FCR. The treatment effects indicated by the overall analysis of variance for BWG and FCR of pre-starter diet and BWG of grower 2 diets likely relate to contrast(s) other than pairwise comparisons. The overall analysis of variance didn't show a statistical significance but Turkey's

studentized range test separated the means for grower 2 FI. This may be because the mean separation tool is highly conservative while the overall analysis of variance is liberal.

Table 4. 5 Effect of incremental levels of CM on growth performance of broiler chicken in the grower 1 phase of experiment *(15-21 days).*

CM inclusion level (%)	Feed intake (g/bird/week)	Body weight gain (g/bird/week)	Feed conversion ratio (g feed/g gain)
0	543.0[b]	374.1	1.46[b]
5	567.7[ab]	387.6	1.46[ab]
10	596.0[a]	411.9	1.45[b]
15	575.1[ab]	393.4	1.46[ab]
20	571.4[ab]	389.0	1.47[ab]
25	587.4[ab]	381.7	1.54[a]
SEM[1]	11.36	9.42	0.019
P-value[2]	0.037	0.121	0.019

[1] Standard error of the mean; Number of observations contributing to each mean = 9

[2] Overall *P*-value of incremental levels; [a,b] Means within a column with no common letters differ significantly (P < 0.05).

Table 4. 6 Effect of incremental levels of CM on growth performance of broiler chicken in the grower 2 phase of experiment *(22-28 days).*

CM inclusion level (%)	Feed intake (g/bird/week)	Body weight gain (g/bird/week)	Feed conversion ratio (g feed/g gain)
0	837.1[b]	528.1	1.59[ab]
6	867.5[ab]	555.7	1.56[b]
12	894.1[a]	563.3	1.59[ab]
18	874.7[ab]	556.5	1.58[b]
24	865.9[ab]	533.2	1.63[ab]
30	866.2[ab]	520.4	1.67[a]
SEM[1]	12.65	11.38	0.019
P-value[2]	0.080	0.047	0.003

[1] Standard error of the mean; Number of observations contributing to each mean = 9

[2] Overall *P*-value of incremental levels; [a,b] Means within a column with no common letters differ significantly (P < 0.05).

Similarly to the current study, Perez-Maldonado et al. (2001), formulated broiler starter and finisher diets based on digestible AA using 10, 20, 30, 40% CM dietary inclusions from four different sources. Aside from the source that was high in total condensed tannins, other CM sources with their corresponding graded levels lead to a significant improvement in FCR. The results from the current study aligns with a study by Newkirk and Classen (2002) who reported that a substitution of SBM with 60% of toasted and untoasted CM up to 20% of diet resulted in BWG of broiler chickens not different from the control. This implies that CM may be added in diets of broiler chickens above 5-10% inclusion without compromising performance. Canola meal that was used to formulate diets with similar energy and crude protein content for broiler chicken at 20% inclusion resulted in performance parameters that were significantly superior than the diets containing 0, 5, 10 and 15% of CM (Baloch et al., 2003). Also, Naseem et al. (2006) demonstrated that CM can be incorporated into broiler chicken diet at 25% inclusion without compromising performance. In contrast to the present study, Mushtaq et al. (2007) tested 20 and 30% inclusion of CM in the starter (1-21 days) and finisher (21-42 days) diets for broiler chickens formulated based on the digestible AA content, and observed a significant reduction in BWG with 30% CM during the starter phase. The CM and consequently the experimental diets were high in glucosinolates, which possibly was responsible for reduced performance, as younger birds have lower tolerance to higher levels of glucosinolates. However, results of our study are well supported by Waldroup et al. (2011) who concluded that there was no significant negative effect of CM inclusions as high as 25% on growth performance of broiler starter and grower when compared to the control corn-SBM diet. A fairly recent study by Gopinger et al. (2014) showed that CM inclusions up to 16.4% and 22.9% (that corresponds to the period when our starter and grower 1 diets were fed) resulted in a statistically significant increase in daily BWG and FI, respectively,

but the response to other performance parameters did not differ considerably. The same study reported a significant improvement in FCR and increased daily BWG for diets up to 20% and 30% CM inclusion during a growing period similar to the time we fed our grower 2 diet. We did notice some trend towards improvement of broiler chicken performance within each phase around the highlighted inclusion rates that were also not different from the control treatment. Nevertheless, contrary to earlier studies and our results, An et al. (2016) stated that there was a linear decrease in daily BWG and FI during the starter phase of broilers fed increasing levels of CM in their diet. The growth performance in the grower phase was not affected by the high inclusion of CM. A 15% inclusion affected daily BWG but not final BWG, which is contrary to the current study as CM was fed up to 15% and 18% in the pre-starter and starter phase, respectively, with no negative effect on performance parameters. Lack of consistency from the above results might be due to diets formulations based on the nutritive values of CM determined *in vivo* using adult roosters (i.e., TME_n) as opposed to those using young broiler chickens (AME_n). In another study Gorski et al. (2017), FI and BWG were reduced significantly with dietary CM inclusions at 20, 30 and 40% during the starter phase. This observation is not in line with our current results for the starter phase where FI and BWG of birds from treatments with CM inclusions above 10% were higher, but not significantly different from the control treatment. In the grower phase of the study by Gorski et al. (2017), there were no significant differences in growth performance among dietary treatments (0, 10, 20 and 30% of CM). However, in the current study, FI and FCR in the 10% (grower 1) and 12% (grower 2) CM diets were significantly higher than the control but not different from the other treatments. The results of the second experiment by Gorski et al. (2017) agreed with ours where they reported no effect on performance at 8% inclusion during the starter phase as well as when graded levels of 8, 16 and 24% during the grower phase were used.

Table 4. 7 Effect of incremental levels of CM on growth performance of broiler chicken for the entire experiment *(1-28 days)*

CM inclusion level (%)	Feed intake (g/bird/week)	Body weight gain (g/bird/week)	Feed conversion ratio (g feed/g gain)
0	1921[b]	1325[b]	1.45[ab]
3-6	1986[ab]	1370[ab]	1.45[ab]
6-12	2051[a]	1431[a]	1.43[b]
9-18	2023[ab]	1398[ab]	1.45[b]
12-24	2006[ab]	1371[ab]	1.46[ab]
15-30	2031[ab]	1364[ab]	1.49[a]
Average[1]	2019	1387	1.46
SEM[2]	26.2	22.0	0.009
P-value[3]	0.017	0.036	0.003

[1] Average of 6 treatment means [2] Standard error of the mean; Number of observations contributing to each mean = 9

[3] Overall *P*-value of incremental levels; [a,b] Means within a column with no common letters differ significantly (P < 0.05).

Table 4. 8 Effect of incremental levels of CM on energy value and neutral detergent fiber (NDF) digestibility in the starter phase of the experiment *(8-14 days).*

CM inclusion level (%)	AME_n (Kcal/kg DM)	NDF (% DM)
0	3325	10.74
4	3271	9.60
8	3261	12.35
12	3210	8.90
14	3260	9.90
18	3265	6.22
SEM[1]	20.7	2.584
P-value[2]	0.221	0.682

[1] Standard error of the mean; Number of observations contributing to each mean = 5

[2] Overall *P*-value of incremental levels

Table 4. 9 Effect of incremental levels of CM on energy value of diet and neutral detergent fiber (NDF) digestibility in the grower 2 phase of the experiment *(22-28 days).*

CM inclusion level (%)	AME_n (Kcal/kg DM)	NDF (% DM)
0	3614^{ab}	21.20^{ab}
6	3548^{bc}	24.30^{ab}
12	3490^{c}	13.62^{b}
18	3654^{a}	27.74^{a}
24	3625^{a}	25.12^{ab}
30	3595^{ab}	25.75^{ab}
SEM[1]	16.6	2.846
P-value[2]	0.001	0.025

[1] Standard error of the mean; Number of observations contributing to each mean = 5

[2] Overall *P*-value of incremental levels; [a-c] Means within a column with no common letters differ significantly ($P < 0.05$).

Table 4. 10 Determined AME_n values of CM diets fed to chickens of different age (kcal/kg DM)

CM inclusion level (%)	Starter diet (8-14 days)		CM inclusion level (%)	Grower 2 diet (22-28 days)	
	Calculated AME_n	Determined AME_n		Calculated AME_n	Determined AME_n
0	3000	3325	0	3150	3614
3	3000	3271	6	3150	3548
6	3000	3261	12	3150	3491
9	3000	3210	18	3150	3654
12	3000	3260	24	3150	3626
15	3000	3265	30	3150	3595
Mean	3000	3265	Mean	3150	3588

The effect of incremental levels of CM on energy value of diet and NDF digestibility in broiler chickens fed starter and grower 2 diets are shown in Tables 4.8 and 4.9. There were no differences between treatment means ($P > 0.05$) for AME_n values and NDF digestibility of broiler chickens fed starter diets (8-14 days). There were also no differences between treatment means ($P > 0.05$) for dietary AME_n values and NDF digestibility of broiler chickens fed grower 2 diet (22-28 days) when compared to the control corn-SBM diet. However, a 12% CM inclusion had a significantly different and lowest AME_n value ($P < 0.05$) compared with the control (3614 vs 3491 kcal/kg DM) as well as the lowest NDF digestibility. The reason for this is not clear as levels higher than 12% were not significantly different from the control. It may be due to an error in the sample collection rather than an effect of increasing CM inclusion. In agreement with our findings, Toghyani et al. (2016) revealed that the crude fiber (CF), NDF and acid detergent fiber (ADF) contents of CM supplemented diets were higher than the control SBM diet but dietary treatments did not have any significant impact on energy utilization of broiler chickens examined at 24 and 35 days of age. In the study by Zhang and Adeola (2016), CM supplemented at 12.5 and 25% inclusions in broiler chicken diets (21-28 days) did not have any effect on growth performance but energy digestibility was reduced. Several studies have always attributed a depression in growth performance of broilers fed diets containing CM to high fiber content and reduced energy utilization, because of the viscous property of dietary fiber thereby limiting nutrient digestibility. This may not necessarily be applicable to the properties of CM fiber, which is less water-soluble. As results from the current study revealed, there were no significant negative effects of increasing dietary CM inclusion on energy and fiber digestibility of broiler starters specifically. Mejicanos et al. (2017) revealed that diets containing dehulled CM with less fiber and more protein when fed to broiler chickens and pigs had no significant impact on growth performance. Also, Gorski et al.

(2017) reported that a test CM had reduced fiber content, increased CP, AA and metabolizable energy contents than the conventional CM. This enabled additional replacement of SBM with the test CM in diets of broiler chickens while still maintaining optimal growth performance.

The calculated and determined AME_n values for starter and grower 2 diets with respect to CM inclusion levels (Table 4.10) show that determined AME_n value of diets containing CM differ with the age of broiler chickens indicating that the age of bird influenced the energy digestibility. This could be supported by Batal and Parsons (2002) who reported that AME_n and AA digestibility of corn-SBM increase with age of broiler chickens between 0-14 days. Although our data is representative of the starter and grower phase, this gives an insight into the differences we recorded. Jimenez-Moreno et al. (2015) stated that the response of broiler chickens to different dietary fiber sources with respect to growth performance differs with composition of basal diet, age, genetics and health. The nutritive value of feed components used for the diet formulation should be taken from those determined *in vivo* using the birds in comparable age or stage of development.

Increased dietary levels of CM as reflected by an excellent broiler chicken performance from our study was possible through a careful feed formulation that considered different stages of broiler chicken development. The reference diet and those containing CM were formulated using the available energy and digestible AA contents determined *in vivo* using broiler chickens as opposed to those obtained with adult roosters. The understanding that CM inclusions could influence dietary fiber content of diet and that birds of different ages will respond to it differently was also was useful in the study. Also, putting into perspective the end users of our research, we made a friendly consultation with commercial nutrition experts who shared their ideas on the industrial standards regarding nutrient requirements of broiler chickens of different age. Results

from the current study suggest it is safe to go limits above the current practice without compromising broiler chicken performance.

4.5 Conclusions

There were no negative effects of increased dietary CM inclusion on growth performance of broiler chickens of different ages. Regardless of the phase and CM inclusion levels, the average of six treatment means for FI, BWG and FCR were similar to the control treatment at 28 days of age. Although NDF content of diet differed substantially, AME_n of diet and NDF digestibility were not significantly affected by graded levels of CM inclusion. Canola meal may effectively replace SBM up to 30% in broiler chicken diets, provided diets are formulated on available energy and digestible AA contents.

GENERAL CONCLUSION

In relation to different CM sources, the current study demonstrated significant variations in the standardized ileal digestible AA content for broiler chickens. The nutritive value of expeller-extracted CM could be similar to those from potentially overheated solvent-extracted meals if processing conditions are not well managed. The relationships that exist between SID of AA including heat sensitive AA, such as threonine, arginine, glutamine and tryptophan, may be an indication that other AA apart from lysine undergo protein degradation.

Graded levels of CM substantially influenced NDF content of diet for broiler chickens. However, this did not significantly impact chicken performance at different and/or entire stages of growth. The benefit of formulating diet on available energy and digestible AA content can be observed as the current study demonstrated no negative effects of increasing dietary CM inclusion on energy and fiber digestibility of broiler chickens. It was evident that energy digestibility is influenced by age of bird.

The results from the current study provided useful information regarding AA, AME_n of diet and NDF digestibilities of CM, which are major factors of consideration in livestock feed formulation. Considering graded levels of CM in the performance trial, dietary inclusions with respect to different stages of growth of broiler chickens resulted in optimum performance. This implies that farmers could take advantage of this economically viable alternative feed ingredient, by increasing inclusions to a safe limit for effective production.

Future research could be directed towards better understanding of other reactions apart from Maillard, that could potentially affect AA bioavailability and digestibility resulting from moist heat treatment of oil seed crops.

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