DETERMINATION OF METABOLISABLE ENERGY AND DIGESTIBLE AMINO ACIDS OF CANOLA MEALS AND CANOLA SEED FOR BROILERS

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ABSTRACT

Nutrient composition, apparent metabolisable energy (AME) and ileal amino acid (AA) digestibility of two canola meals (CM1 and CM2) and one canola seed (CS) sample were evaluated using laboratory analyses and animal studies. The AME assay was conducted with broilers using the classical total excreta collection between day 18 and 25 post-hatch. A maize-soybean meal basal diet was formulated and a test diet, containing CM or CS, was developed by replacing (w/w) 30% of the basal diet with CM or CS. The AME of CM and CS was calculated based on the difference between the AME values of basal and test diets. Ileal protein and amino acid (AA) digestibility of CM and CS were determined using direct method. In this method, the assay diets were formulated with the CM or CS serving as the sole source of AA. All diets contained titanium dioxide as an indigestible marker. The ratios between the titanium and AA in the diet and digesta were used to calculate the digestibility. The crude protein of CM1, CM2 and CS were determined to be 411, 393 and 235 g/kg (as received basis), respectively. Compared to both the CMs, CS had the highest neutral detergent fibre (NDF) and lowest total dietary fibre (TDF) value. Calcium was the major mineral in CS (17.7 g/kg), while major mineral in both the CM samples was potassium (13.6 and 13 g/kg, respectively). The overall concentration was low in CS compared to both the CM. Differences ($P < 0.05$) were observed in the AME and apparent metabolisable energy corrected for nitrogen (AMEn) value of CM and CS. The AMEn content of CM1, CM2 and CS were 7.22 and 6.78 and 10.29 MJ/kg DM, respectively. A tendency ($P = 0.052$) was observed for effect of dietary treatment on standardised ileal digestibility coefficient (SIDC) of protein and AA. The CS had highest ($P < 0.05$) digestibility compared to both CM samples, and no differences ($P > 0.05$) were observed between the two CM samples. The standardised ileal digestible protein content was highest ($P < 0.05$) in CM1 (293

g/kg), followed by CM2 (279 g/kg) and CS (176 g/kg). The digestible AA content, in general, followed the similar trend as digestible protein. In conclusion, the present study showed that the nutrient composition, AMEn, and standardised ileal digestibility of protein and AA vary between CM and CS samples. CM and CS are attractive feed ingredients for poultry and with careful considerations CS and CM can be used as a partial replacement for SBM in poultry diets. CS has high AMEn content, therefore, can be used as a potential energy source, while CM has high digestible AA content, therefore, can be added as a protein source in poultry diets.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fibre
AID	Apparent ileal digestibility
AIDC	Apparent ileal digestibility coefficient
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
BW	Body weight
Ca	Calcium
CAID	Coefficient of apparent ileal digestibility
CF	Crude fibre
CM	Canola meal
CME	Expeller-extracted canola meal
CMS	Solvent-extracted canola meal
CP	Crude protein
CS	Canola seed
D	Day
DM	Dry matter
FCR	Feed conversion ratio
FFCS	Full-fat canola seed
g	Gram
GE	Gross energy
GLS	Glucosinolates
h	Hours
HEAR	High erucic acid rapeseed
K	Potassium
kg	Kilogram
KOH	Potassium hydroxide
LEAR	Low erucic acid rapeseed
LSD	Least significant difference
ME	Metabolisable energy
min	Minutes

MJ	Mega joule
mm	Millimetre
N	Nitrogen
NDF	Neutral detergent fibre
NDIN	Neutral detergent insoluble nitrogen
NSP	Non-starch polysaccharides
P	Phosphorous
RSM	Rapeseed meal
SBM	Soybean meal
SID	Standardised ileal digestibility
SIDC	Standardised ileal digestibility coefficient
TDF	Total dietary fibre
Ti	Titanium
TiO_2	Titanium oxide
TMA	Trimethylamine

CHAPTER 1

INTRODUCTION

The demand for feed and raw material has increased significantly over the decades and will continue to increase in the future due to the increase in demand for meat products, especially poultry meat. Ever-increasing cost of conventional ingredients has motivated poultry nutritionist to explore the use of locally available feed ingredients such as canola meal (CM)/canola seed (CS) instead of soybean meal (SBM), which is conventional protein source in poultry feed with excellent amino acid (AA) profile. CM/CS is one of the alternative feed ingredients that remain under-utilised in poultry feed due to numerous problems in practical implementation. Presence of anti-nutritional factors such as glucosinolates, erucic acid, phytate and sinapine make it less preferred as a poultry feed ingredient (Bell, 1984). However, the development of low glucosinolates and low erucic acid cultivars of canola has resulted in increased usage of CM in poultry diets in recent years (Canola Council of Canada, 2015).

Concentration of nutrients (energy and protein) and the digestibility of AAs in CM/CS are important factors that can affect the inclusion of these ingredients in broiler diets. The concentration of nutrients in CM is largely affected by its fibre content, which is higher than that of SBM. Seed genotype, growing conditions and harvesting time can affect seed composition and, therefore, affect meal quality (Khajali and Slominski, 2012). Similarly, oil removal from canola and processing of the meal include numerous steps that could affect meal quality. Nutritive value of CS can be calculated from nutritive value in CM and oil by assuming that approximately 57% of the seed is meal and 43% is oil. However, the estimation of energy value of CS cannot be calculated based on this assumption as it is not processed in the same manner as of CM (Newkirk, 2009). Crushing

of whole CS reduces its particle size and thereby increase its energy digestibility (Newkirk, 2009). Regrettably, growing conditions and processing effects on CM quality have received little attention in broiler nutrition and only limited studies have been conducted to determine the apparent metabolisable energy (AME) and ileal AA digestibility of these feed ingredients for broilers (Blair et al., 1986). Therefore, the objective of the present study was to assess the chemical composition, nitrogen corrcted AME (AMEn) and standardised ileal AA digestibility of CM and CS in broiler chickens.

The present work includes a general introduction (Chapter 1), a review of literature (Chapter 2) and an experiment (Chapter 3). The review is focused to address the nutritional composition of CM/CS, processing of CM, presence of anti-nutritional factors, the effect of feeding CM/CS on performance parameters of broiler chickens, the optimum inclusion level and its potential as an alternative protein source in poultry diet. The experiment evaluates the nutritional composition, AMEn and standardised ileal AA digestibility of CM/CS for broilers using laboratory analysis and animal studies.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Rapeseed is one of the most important oilseed crops in many countries. With the advancements in agronomic techniques and breeding programme, the world rapeseed production is increasing over the years. The seed contains about 40% oil and post-extraction yields a protein supplement known as rapeseed meal, consisting about 38% protein (Canola Council of Canada, 2015). Despite the appreciable level of protein content, the use of rapeseed meal is not fully evaluated as a protein source in poultry diets and lacks a clear understanding.

The presence of some anti-nutritional factors in the meal such as glucosinolates, erucic acid and fibre limit the use of rapeseed meal in animal feed formulations (Bell, 1984). Tripathi and Mishra (2007) observed that during processing or ingestion, the glucosinolates in the rapeseed undergoes enzymatic degradation by myrosinase enzyme to form hydrolysis products known as aglucones, which are unstable and undergo further reactions to form isothiocynates, nitriles, thiocyanates or oxazolidithione. Elwinger and Säterby (1986) reported that the end products of glucosinolates hydrolysis cause goitre and problems associated with thyroid, liver and kidney and subsequently reduces growth and health of animals. Likewise, Karunajeewa et al. (1990) found that the performance of the broiler chickens is affected by the level of rapeseed meal in the diet. Weight gain and feed intake decreased with increasing levels of dietary rapeseed meal while mortality increased. The exact reason is not well understood. However, Butler et al. (1982) revealed that not one specific constituent of rapeseed meal but several substances in the rapeseed

meal cause liver haemorrhages, deposition of fat in the animal tissue and myocardial lesion in laying hens.

In 1979, Canola Council of Canada (2015) introduced the new variety of rapeseed and named it canola (Canadian oil). The new variety of rapeseed consisting of comparatively low levels of erucic acid and glucosinolates was developed by collaboratively implementing genetic manipulation and plant breeding (Canola Council of Canada, 2015). Characteristically, the ameliorated variety of canola seed (CS) contains less than 2% erucic acid and less than 30 μmoles glucosinolates in its meal. This not only improved the chemical and nutritional quality of canola meal (CM) but also enabled nutritionists to increase the inclusion level of CM/CS in animal feed. Newkirk (2009) suggested that CM can be recommended up to 20% in broiler diet. However, conflicting reports exist regarding nutritional value of CM/CS and its optimum inclusion level in poultry diets.

The following review will help in gaining better understanding about the transition of rapeseed to canola, the various processing techniques involved in oil extraction of CS, the chemical composition and nutritional value of CM/CS. Anti-nutritional factors present in CM/CS and their negative effects on the health and performance of the bird will be discussed. Optimum inclusion level of CM/CS in poultry diets reported in different studies will be covered here.

2.1.1 Brief history of the development of rapeseed

Rapeseed has been cultivated over thousands years ago. Records indicate that rapeseed was grown in India over 3,000 years ago (Prakash, 1980) and then in China (Li, 1980). Later, it extended across Europe and was grown for its oil as a source of illumination and as cooking oil. However, it is not clear when the oil was used for human consumption. In

the 20th century, the rapeseed production escalated as its oil was widely used as a marine engine lubricant. Today, most of the rapeseed production is processed for the extraction of its oil which is used for various purposes such as cooking, in preparation of different food items, biofuels, lubrication (Gunstone, 2004). The remaining product left after the extraction of oil is known as rapeseed meal which is believed to possess great potential as a protein source in animal feed.

The word "rape" originates from a Latin word *rapum* which means turnip. Turnip, cabbage, mustard, brussel sprouts and other vegetables are closely related to rapeseed. In the 18th century, a Swedish botanist named Carolus Linnaeus studied the *Brassicaceae* family and found that the turnip and oilseed producing variant were two species of a crop and named them as *B. rapa* and *B. campestris* (Canola Council of Canada, 2015). However, in the 20th century, it was learnt that the two crops belonged to the same species and were cross-fertile. Since the turnip was first named as *Brassica rapa* by Carolus Linnaeus, the name was adopted permanently as *Brassica rapa* (Canola Council of Canada, 2015).

In Canada, in 1936, forage rape (*B. napus*) was already grown as an annual pasture crop. A Polish immigrant, Fred Solvonik, introduced *B. rapa* in Canada (Bell, 1982). He received an envelope of seeds from a contact in Poland and started cultivating it. This material was subsequently used by Canada Department of Agriculture. As seeds from the cultivar *B. napus* and *B. rapa* were obtained from Argentina and Poland, they are also known as Argentine and Polish seeds, respectively. The two types and their varieties possessed agronomic features that suited them to different geographic and climatic conditions (Bell, 1982). Nowadays, the term rapeseed is commonly used for the oilseeds of several species of the genus *Brassica*. Table 2.1 enlists the most important *Brassica* species, their common name and the major oilseed producing countries of the world.

Table 2. 1 *Brassica* species with their common and generic names and the major producing countries

Common name	Generic name	Country	Other names
Rape	*B. napus*	Canada	Rapeseed
		Europe	Swede Rape
			Oil Rape
			Oilseed Rape
			Argentine Rape
			Winter Rape
Turnip Rape	*B. campestris*	Canada	Rapeseed
		India	Polish Rape
		Europe	Oil Turnip
		China	
Leaf Mustard	*B. junceae*	India	Rapeseed
		China	Indian Mustard
			Brown Mustard
			Oriental Mustard

Source: Shahidi (1990).

2.1.2 Modification of rapeseed to canola

During the 20th century, the improvements in plant breeding, agronomic techniques, processing methods and advancement in technology escalated the demand for rapeseed oil. However, high levels of erucic acid in the oil and meal, and the perception that it caused heart diseases in animals was a major concern that impacted its use in animal feed. In the late 20th century, efforts were made by plant breeders to improve the quality of the rapeseed oil and meal. By the 1960s, breeding program in Canada produced the first low erucic acid rapeseed variety which was used for production of rapeseed oil for human consumption. The by-product/meal left after extraction of oil was used as a source of protein in animal feed. But it could only be fed in limited quantities due to the presence of sulfur compounds known as glucosinolates (Bell, 1984). It was observed that high intake of rapeseed meal was goitrogenic and caused abnormalities associated with thyroid, liver, kidney and fertility problems in livestock (Butler et al., 1982; Elwinger and Säterby, 1986).

Further development in breeding techniques led to invention of a new variety of rapeseed with low in both erucic acid and glucosinolates levels. To differentiate it from rapeseed, the term "canola" (Canadian oil) was coined by the Canola Council of Canada. Canola is an offspring of rapeseed (*Brassica napus* and *Brassica campestris/rapa*) characterised by less than 2% erucic acid in the oil and less than 30 μmol/g glucosinolates in the meal (Canola Council of Canada, 2015). The term is accepted in English speaking countries such as USA and Australia, but in the UK, rapeseed is used to refer to all quality types rapeseed. In Europe, the term "double-zero rapeseed" is used to identify seed, oil and meal pertaining to "canola quality" i.e. low erucic acid and low glucosinolates (Canola Council of Canada, 2015).

It has been a remarkable achievement to convert rapeseed into canola which is highly acceptable nutritionally and commercially. Still new breeding program and genetic modifications are underway to modify traits and introduce new quality characteristics in rapeseed. Further developments in *Brassica* species are being facilitated by new techniques in biotechnology such as genetic fingerprinting.

2.1.3 Uses of canola meal

Canola is crushed to produce canola oil which is primarily used for human consumption. Canola oil is widely used in salad oils, salad dressings, mayonnaise and margarine. Non-food uses include anti-stick cooking sprays and vacuum packed canned foods (Gunstone, 2004). Some part of canola oil is sold to biofuel market where it is converted into biodiesel. The canola by-product is used in livestock industry as a protein source and as fertiliser in agriculture industry (Bonnardeaux, 2007).

Among all the industries, livestock industry is the main market for CM. It is used as a protein source for different animal species with markedly different digestive

capacities and nutrient requirements (Bonnardeaux, 2007). About 10-15% of CM is mixed with a full ration for cattle and sheep. In pigs and poultry diets about 15% inclusion of CM can be supplemented (Roth-Maier, 1999). Full protein supplementation of CM is not accepted due to the presence of anti-nutritional factors, low energy and protein and high fibre content (Newkirk, 2009). A new potential market is the aquaculture (Enami, 2011) and pet industry wherein CM is used in protein concentrates and dog biscuits (Fig. 2.1; Bonnardeaux, 2007).

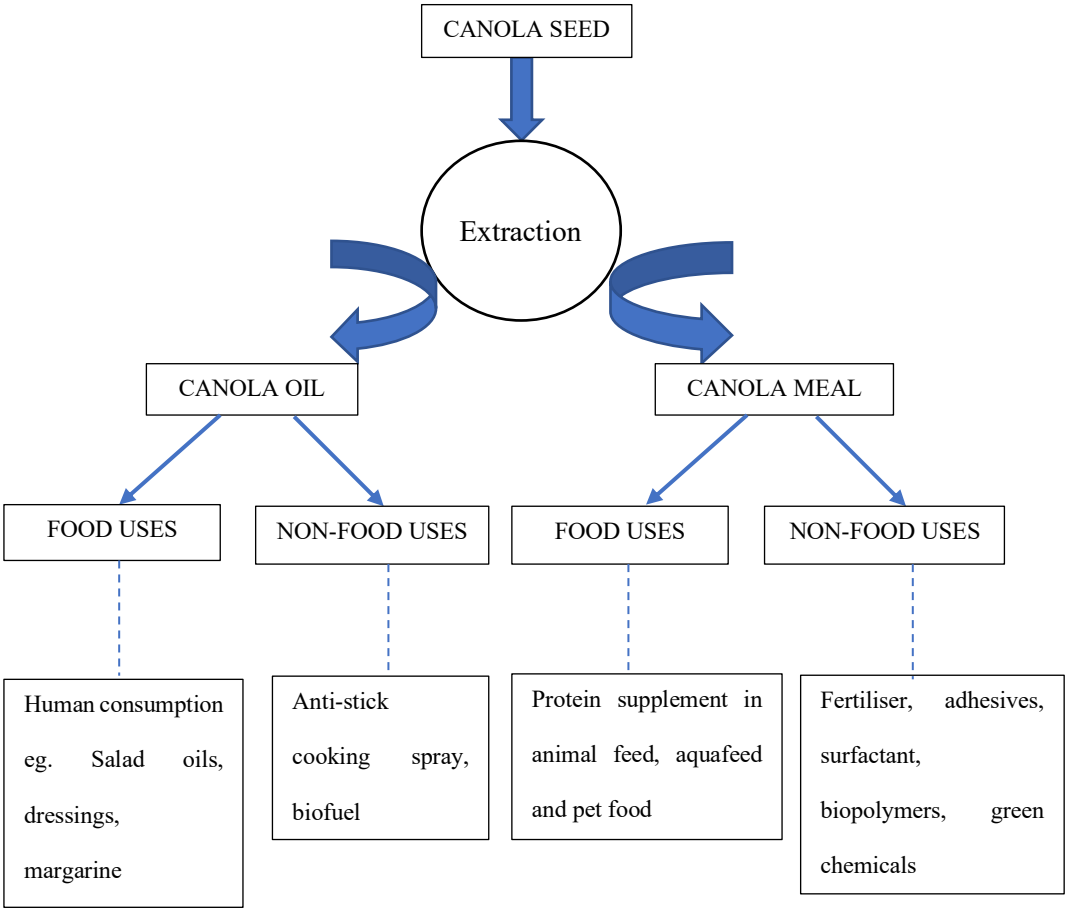


Figure 2. 1 Uses of canola oil and meal

Source: Bonnardeaux (2007)

Canola meal is also a good organic fertiliser and soil amendment agent due to presence of appreciable amounts of nitrogen, phosphorous and sulphur. Non-feed uses from CM include adhesives, surfactants, green chemical and biopolymers (Bonnardeaux,

2007). However, there is great scope for exploring the potential of CM for further utilisation in different industries.

2.1.4 Global production of canola meal

Over many years, oilseeds and their by-products have been the most valuable agricultural crop in the global trade. Over the past 10 years, the production of oilseeds has increased by approximately 30% due to the growth and development in agriculture sector. Among oilseeds, rapeseed is the second most produced oilseed behind soybean (Carré and Pouzet, 2014). A survey in 2007 showed that Canada, China, India, France and Australia are the top five leading rapeseed producing countries in the world (Fig. 2.2; Sawe, 2019). The production of canola in Canada has been steadily increasing and targets an increase to 26 million tonnes per year by 2025. About half of Canada's canola seed is processed for extraction of canola oil which is used for various purposes while the other half is exported to other countries (Canola Council of Canada, 2015). Japan is a consistent importer of CS mainly for the oil

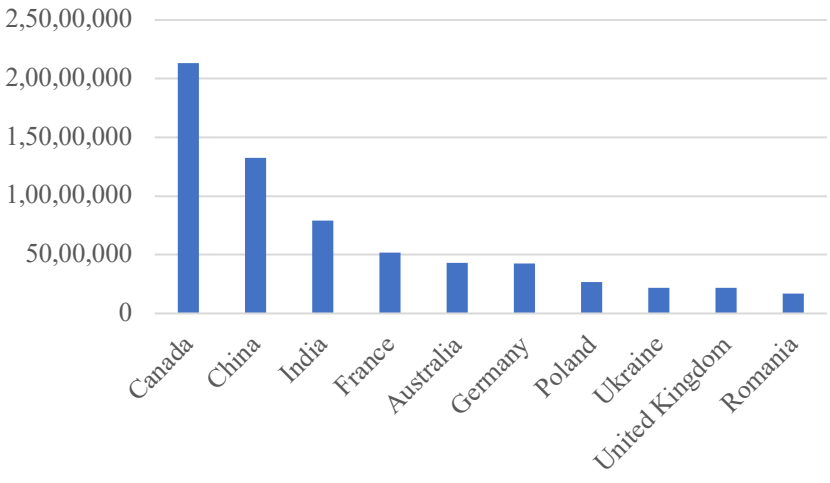


Figure 2. 2 World rapeseed production (in tonnes; Source: Sawe, 2019)

In parallel, a remarkable evolution was noticed in the rapeseed/canola crushing industry. The major producers of CM are Australia, Canada, China, European Union and India (Canola Council of Canada, 2015). Rapeseed meals and CM are widely available and traded, usually in bulk form, mash or pellet to be used in animal feeds around the world. Canada and India are the main exporters of CM (Carré and Pouzet, 2014). In the USA, CM is primarily used in dairy feed as a protein supplement. European Union, China and other countries have also had an increase in consumption of CM for feeding pigs, poultry and fish. However, knowing the drawbacks of CM compared to soybean meal (SBM), it is likely to remain a secondary meal in animal feed (Khajali and Slominski, 2012).

2.2 Processing of canola meal

There are a range of scales and methods of processing which aim at obtaining higher oil yields, minimal damage to oil and meal and possessing minimum concentration of impurities in both. Commercially large-scale processing of CM involves numerous steps including seed cleaning, tempering, dehulling, flaking, conditioning, extraction by mechanical pressing or by use of solvent, desolventising, toasting and cooling of the meal. The following section outlines the steps involved in processing of CM.

2.2.1 Pre-treatment of canola meal

2.2.1.1 Seed cleaning

Prior to processing, seed cleaning is important as it contains dockage material such as plant, weed seeds, stems, pods, other grains, dust or soil material (Booth, 2004). The removal of over and undersized foreign particles is done by using aspirators, graders and sieves individually or in combination to improve the purification (Matthäus, 2012).

Matthäus (2012) noted that seed cleaning also helps in decreasing the seed temperature and drying of the seed surface which in turn delays metabolic processes and reduces chances of fungal infestation (Fig. 2.3).

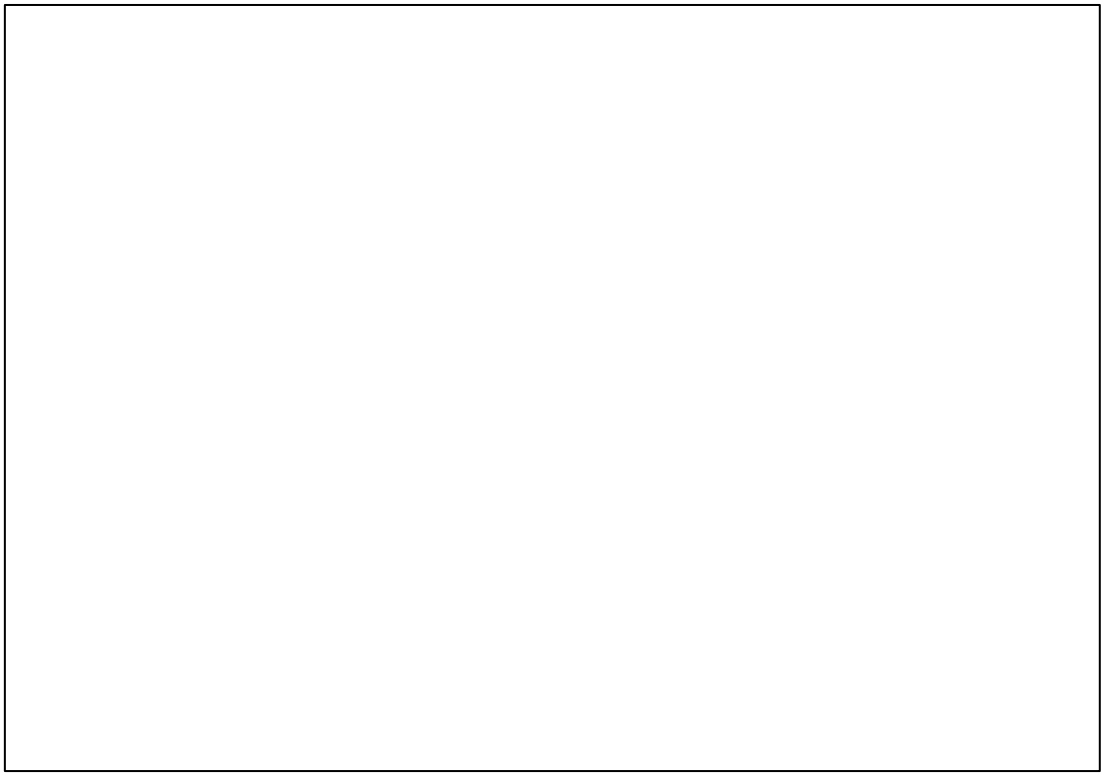


Figure 2. 3 Commercial processing of canola. Source: Xu and Diosady (2012)

2.2.1.2 Tempering

In cold climatic areas and in extraction plants wherein cold seeds from storage are used, the seeds are preheated with grain dryers (Unger, 1990). The cleaned seeds are heated to 30-40 °C for 30-45 min by direct or indirect application of heat in a rotary kiln with steam-heated tubes (Booth, 2004). This prevents shattering of cold seeds during flaking process, which can lead to irregular flake size and reduce extraction efficiency (Booth, 2004). This also improves cake formation, increase extractability and hexane recovery from the extracted oilseed flakes (Unger, 1990).

2.2.1.3 Dehulling

Rapeseed contains about 16-19% hull, majorly composed of fibre, sometimes waxes and pigments which impair the oil quality (Matthäus, 2012). The process of removal of the hull of the seed is called dehulling. Further, the hulls are separated from the dehulled material mechanically by sieving (Matthäus, 2012) or by pneumatic impact separation and air floating and/ or fluidised air sorter to remove the hulls (Booth, 2004).

Nutritionally, dehulled rapeseed meal contains less amount of fibre and phenolic compounds making it favourable for its use in animal feeding (Matthäus, 2012). It also reduces the transfer of impurities such as hull pigments in the crushing process (Booth, 2004). However, economical point of view, dehulling of rapeseed is an expensive process due to the small size of the seeds (Booth, 2004). Matthäus (2012) stated that hulls are responsible for the porosity of the extracted material. Therefore, dehulling will reduce the percolation of solvent through the cake and also increase the proportion of anti-nutritive compounds such as glucosinolates, sinapine, and inositol phosphates in the dehulled material.

2.2.1.4 Flaking

The oil seed is covered by seed coat, cell wall and cell membranes which prevents the rupture of oil from the oil cells. The objective of the flaking is to rupture the cell wall and expose the lipid material without damaging the quality of the oil. During the flaking process, the oil seeds are passed through two smooth cast-iron rollers with diameter 500-800 and 1000-1500 mm long having an appropriate gap to control the thickness of the flakes. Vibrating feeders are used to evenly distribute the seeds on the roller while scrapers on the roller prevent sticking of flakes on the roller surface (Booth, 2004). This results in the destruction of the seed coat and ruptures the cell membrane to facilitate the

release of oil from the seed. After the flaking process, about 80% of these membranes are destroyed, surface area of the seed is enlarged which results in easier migration of lipid particles from the seed material (Matthäus, 2012). In addition, during the extraction process, it eases out the penetration of solvent in the seed, to liquify and dilute the lipid material so that it can efflux out the lipid particles from the seed to the outer surface of the flake (Booth, 2004).

The efficiency of oil extraction depends on the thickness of the flakes. The optimum thickness of the flake should be of 0.3-0.38 mm. The distance of diffusion between the solvent and oil is less in thin flakes, therefore oil extraction is better (Carr, 1995). However, flakes thinner than 0.2 mm are very small and fragile, thereby complicating its removal during oil filtration, while thicker flakes with more than 0.4 mm have low extractability (Canola Council of Canada, 2015). Post flaking process, the processing of oil needs to be carried out without any delay, as the destruction of seed coat exposes the lipid material to attendant microorganisms and results in initiation of enzymatic activity (Matthäus, 2012).

2.2.1.5 Cooking

The flaked seeds are cooked or conditioned to breakdown the oil content within the seed. The flakes are heated between temperature range of 80-105 °C with an optimum of about 88 °C for about 15-20 min (Canola Council of Canada, 2015). This process helps in rupturing the oilseed that survived flaking, reduce viscosity to promote coalescing of smaller lipid particles to form larger oil droplets and allow the oil to segregate from the solid residue. This imparts an oily appearance to the cooked seeds (Booth, 2004). In addition, during this stage the moisture content of the seed can be adjusted before the actual extraction process is initiated. The optimum moisture content scales between 3-6%

as it helps in having correct elasticity for the use of screw press in extraction process (Matthäus, 2012).

Moreover, the moisture content and temperature play a crucial role in hydrolysis or denaturation of enzymes within the seeds. In the presence of moisture, myrosinase enzymes present in the seed breakdown glucosinolates into compounds such as isothiocyanates and nitriles which are harmful when fed to animals (Booth, 2004). Also, glucosinolates breakdown can lead to release of sulphur in the oil. Sulphur interferes with nickel catalyst which is used during hydrogenation and therefore influence its activity (Matthäus, 2012). Other enzymes such as lipases which are responsible for the breakdown of triacylglycerols and phospholipids are also denatured during cooking.

Drum or stack-type conditioner consisting of 4-8 vertical, cylindrical, steam heated steel kettles are used for the cooking process. Initially the flaked seeds are rapidly heated to 80-90 °C in the top container for the denaturation of myrosinase at optimum moisture level of 6-10% and then gradually maintained at 80-105 °C in the successive kettles by maintaining uniform depth of the cake on each tray (Carr, 1995). Friedman (1996) observed that excessive heating of seeds at high temperatures for prolong period leads to protein damage and negatively affect the meal quality and protein availability. Similar findings were observed by Clandinin et al. (1959), wherein he observed that the lysine content and protein quality of the meal is affected due to higher temperature used during cooking/conditioning.

In conventional rapeseed processing, cell wall degrading enzymes such as cellulases, hemicellulases and pectinases are used to increase permeability of the cell wall and facilitate the release of oil from the seed. Derksen et al. (1994) suggested that enzyme application helps in increasing mechanical pressing efficiency by 90% and decreases the

solvent extraction time by half. However, implementation of this strategy in large-scale processing plant requires high input cost.

2.2.2 Extraction

There are different methods of processing for extraction of oil and separation of meal such as cold pressed, expeller and solvent extracted. In the cold press method, oil and meal is physically separated without application of heat while in expeller method, heated is applied for extraction. Solvent extraction involves use of combination of physical expulsion and extraction of oil and meal using solvent. The following sections describe the various extraction methods.

2.2.2.1 Mechanical extraction

In the past, extraction of oil from the oilseed was solely done by use of mechanical methods such as pressing. But nowadays this method is used particularly to obtain speciality oils such as virgin oils or extra virgin oils. In this process, horizontal barrel with rotating screw shafts are used for extraction. The resultant press cake contains about 15-20% oil (Matthäus, 2012). High oil content in the meal is undesirable as it suppresses the value of meal if it is solely used as a protein source in animal feed. However, few believe that this adds value to the meal and additional energy content due to the residual oil (Barbour and Sim, 1991).

A special variant of press cake is double press cake wherein, the seed is expelled twice to extract the oil from the oilseed (Canola Council of Canada, 2015). The resultant meal is high in metabolisable, digestible and net energy content due to about 8-10% oil content. The protein quality of the meal is preserved due to low moisture content and

shorter processing time. However, heat generated due to friction during expelling can slightly hamper the meal quality.

In the recent times, mechanical extraction is done prior to solvent extraction. Unger (1990) stated that pre-pressing improves removal of oil from the flaked seeds and produces small size cakes to allow good solvent percolation in the extractor. Pre-pressed cakes prove advantageous as the cake becomes spongy, permeable, resistant to disintegration, have correct consistency and optimum thickness for solvent extraction. Carr (1995) recommended that the thickness of the pre-press cake should be between 3.2-4.8 mm as the diffusion of solvent through the cake is greatly influenced by the cake thickness. Over the last 15 years, many processing plants have introduced an additional step post mechanical pressing which is known as extrusion (Buhr, 1990). Extrusion involves addition of steam into the cake to restructure the cake, increase bulk density, improve extractability, reduce oil content in the residue and inactivate enzymes (Booth, 2004). It is observed that the percolation of solvent through the cake post extrusion is better as compared to pre-press cake derived from conventional processing (Booth, 2004).

2.2.2.2 Solvent extraction

In this type of extraction, solvents are used to remove oil from the pre-pressed cakes containing about 18-20% oil. Solvents are agents which are pure, stable, non-reactive and non-toxic which can remove triacylglycerols from the oil and meal (Derksen et al., 1994). Commonly, n-hexane is used for extraction as it is readily available and has higher oil solubility. Other solvents such as isopropanol and supercritical CO_2 (fluid state of CO_2 under critical pressure and temperature) are also gaining attention (Booth, 2004).

Basket- based extractors and continuous loop-type extractors are used in this process. The pre-pressed cakes are placed in the extractor and drenched with pure solvent

or miscella (a mixture of solvent plus oil) in 5-8 stages (Canola Council of Canada, 2015). With each successive stage the ratio of solvent in proportion to the oil increases. Due to the gravitational force, the solvent percolates through the cake, diffuses and saturates the cake fragments. Through the length of the extractor, the cake is conveyed on steel belts and sprayed with solvent and miscella (Booth, 2004). Gradually, the miscella becomes richer in oil as it moves through the solvent extractor and the cake is left with less than 1% of oil content. Finally, the hexane-saturated cake known as marc is washed with pure solvent before leaving the solvent extractor (Canola Council of Canada, 2015).

On comparison, the solvent extraction is more efficient than mechanical pressing as it extracts more oil, has less oil residue in the cake and low concentration of glucosinolates and their volatile by-products. While in mechanical pressing, the amino acid (AA) losses and protein damage is lower as less heat is generated during the process. Therefore, the characteristics of the meal greatly depends on the method used for extraction.

2.2.3 Desolventising-toasting, meal cooler and storage

The solvent loaded meal known as marc is moved to a desolventiser-toaster for removal of solvent from the meal. The meal contains about 30-35% solvent which needs to be removed before feeding to the animals. Desolventiser-toaster is an enclosed vessel consisting of series compartments which are steam heated from the base. Meal enters at 57 °C and gradually heated to 105 °C and drops down by gravity into the successive compartment. Lastly, live steam is injected through the meal by a process known as toasting (Booth, 2004). The solvent absorbed by the meal is displaced, vaporised and recovered for further use. The time taken for completion of the process is approximately 50 to 90 min (Canola Council of Canada, 2015). Friedman (1996) observed that excessive

heating of meal leads to protein damage and loss of AAs. During thermal processing, Maillard reaction products are produced due to formation of covalent bond between a free re-active NH2 group of AA (especially lysine and argenine) and the carbonyl group of a reducing sugar. This reduces the total and reactive lysine content of the meal. Therefore, high temperatures in desolventiser are found to deteriorate the overall meal quality,

Post desolventisation-toasting, the meal becomes dry and crisp (Booth, 2004). Also, the use of high temperature and live steam degrade the anti-nutritive compounds such as glucosinolates and inactivate the enzymes like myrosinase (Matthäus, 2012). However, to improve the protein digestibility and reduce AA degradation proper adjustments are needed to control temperature, moisture and retention time in the desolventiser-toaster.

Finally, the meal is shifted to a drier cooler at a temperature of approximately 100 °C and moisture content of 10-12%. The meal is cooled and dried by blowing air through it (Canola Council of Canada, 2015). The end product is composed of 8-10% moisture with a lipid content of 1% and is virtually solvent free (Booth, 2004). Eventually, the meal is ground to uniform consistency using a hammer mill, or either pelleted or directly stored in mash form and milled for delivery to feed manufacturers.

2.3 Chemical composition and nutritional value of canola meal and seed

The nutrient composition of CM may be influenced by environmental conditions during growing of the crop, harvesting conditions, type of cultivar and processing of the seed and meal. Further, this affects the growth performance in broiler and layer birds.

2.3.1 Metabolisable energy (ME)

Energy is a property of nutrients which is released when nutrients are oxidised during metabolism in the form of heat. The energy levels vary as nutrient composition varies especially protein, oil and fibre. Most nutrient values for CS can be calculated from the nutrient values in CM and oil, considering that approximately 56% of the seed is meal and 44% is oil (Canola Council of Canada, 2015). However, the energy value of the canola seed cannot be reliably estimated from the addition of the energy values for canola oil and meal as the seed is not processed in the same manner as meal and oil, therefore, not as well digested (Canola Council of Canada, 2015). The gross energy (GE) value of CS has been reported to be higher compared to CM (27.2 vs 18.4 MJ/kg; Table 2.2). Barbour and Sim (1991) suggested that high GE of CS can be attributed to the high oil content present in the seed. Adequate processing of CS liberates the oil trapped within the cell walls and thereby increase energy value. Processing methods such as heat treatment or particle size reduction increase the energy digestibility of CS (Canola Council of Canada, 2015). Montoya and Leterme (2010) proposed that the high energy content of CS could fortify the energy deficiency of CM.

Table 2. 2 Chemical and nutritive composition of canola seed (CS)[1], solvent-extracted canola meal (CMS)[2], expeller canola meal (CME)[2] and soybean meal (SBM)[3]

Content	CS	CMS	CME	SBM
Moisture (%)	6.8	12.0	5.0	10.0
Crude protein (N x 6.25; %)	18.4	38.7	37.6	45.6
Ether extract (%)	40.5	3.3	10.2	1.3
Linoleic acid (%)	8.3	0.7	2.1	-
Linolenic acid (%)	4.1	0.3	1.0	-
Ash (%)	3.8	6.7	6.7	6.4
Calcium (%)	0.4	0.7	0.6	0.3
Phosphorous (%)	0.6	1.0	0.9	0.7
Crude fibre (%)	8.9	11.2	12.4	5.4
Acid detergent fibre (%)	12.7	16.2	18.0	7.5
Neutral detergent fibre (%)	17.9	25.4	25.7	12.0
Total dietary fibre (%)	-	32.4	-	21.8
Sinapine (%)	-	1.0	-	NA[4]
Phytic acid (%)	-	2.3	-	-
Glucosinolate (µmol/g)[5]	-	4.2	10.3	NA
Metabolisable energy (MJ/kg)	-	8.4	-	9.3
Gross energy (MJ/kg)[6]	27.2	18.4	-	-

[1]Feedipedia (2015).
[2] Slominski (2015); Broderick (2015).
[3]NRC (1994); Newkirk (2011).
[4]NA= not applicable.
[5]Includes gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin and 4-hydroxyglucobrassicinin.
[6]Barbour and Sim (1991).

Compared to many other plant protein sources, CM has a low metabolisable energy (ME) value (8.4 MJ/kg) which limits its use in high density diets (National Research Council; NRC, 1994). The energy content is low because majority of the oil is removed from the meal during the extraction process. To compensate the energy levels, other by-products formed during refining processing are used. McCuaig and Bell (1981) reported that addition of gums (glycolipids and phospholipids) that are removed from the

oil while refining, aid in improving the energy content of the meal. They observed that the inclusion of up to 6% gums in the meal did not show any detrimental effect on the feeding value of CM for swine. In addition, March et al. (1978) indicated that the addition of gums increased the ME of CM by approximately 0.63 MJ/kg.

Some oilseed processing plants use press expeller rather than solvent extraction to extract oil from oilseeds. Since the oil is extracted by mechanical means, the resultant meal contains more oil (15-18%) than the standard solvent-extracted CM (1%). It is believed that the amount of energy supply from CM is directly related to the residual oil in the meal (Barbour and Sim, 1991). However, the fat content of the expeller cake varies widely, so it is important to analyse the cake for fat and adjust the energy value accordingly.

2.3.2 Protein and amino acids (AAs)

Protein is the most important nutrient in the poultry diet. Apart from crude protein (CP) content of feed ingredients, the total and digestible AA contents and digestibility coefficient are also important. To achieve a desired growth rate and feed conversion efficiency, broilers require each AA at precise levels during each phase of growth. (Ravindran and Bryden, 1999). Therefore, digestibility assays are found to be most favoured technique for measuring nutrient availability, as it calculates the amount the AAs that are released by digestion, absorption and utilisation by animals.

Canola seeds are a rich source of protein. They contain about 21% of protein (Table 2.3; Feedipedia. 2015). Protein with a well-balanced AA composition is the most valuable component of CM. The actual CP content (Tables 2.2 and 2.3) of CM ranges from 34-38% (Slominski, 2015). Canola meal has a good AA profile and has high level

of sulphur containing AA, methionine and cysteine (Newkirk, 2011). But like most other protein sources, it is limiting in lysine.

Table 2. 3 Comparison between total and digestible protein and amino acid (AA) and digestibility coefficient (DC) of canola seed (CS), canola meal (CM) and soybean meal (SBM).

	CS[1]	CM (38% CP)[2]			SBM (48% CP)		
Nutrients	**Total**	**Total**	**Digestible**	**DC**	**Total**	**Digestible**	**DC**
Crude protein	20.9	37.97	29.62	78.1	48.1	43.96	91.4
Lys	6.3	2.0	1.7	85.4	2.9	2.7	92.5
Met	2.0	0.8	0.7	90.0	0.7	0.6	92.5
Met + Cys[4]	2.75	1.6	1.5	90.1	1.4	1.2	89.8
Thr	4.8	1.6	1.3	83.0	1.9	1.7	88.7
Trp	1.3	0.5	0.4	86.0	0.7	0.6	90.9
Arg	6.2	2.3	2.1	90.4	3.5	3.3	93.8
Gly + Ser[5]	5.0	3.4	2.9	85.0	4.5	4.2	89.2
Val	5.5	1.8	1.6	86.2	2.3	2.1	90.1
Iso	4.3	1.6	1.2	79.8	2.3	2.1	90.8
Leu	7.3	2.7	2.2	82.9	3.7	3.4	92.9
His	2.9	1.0	0.9	89.3	1.3	1.1	91.2
Phe	4.3	1.5	1.3	87.8	2.5	2.3	93.8
Phe + Tyr[6]	3.7	2.4	2.0	85.7	4.2	3.9	91.9

Lys, Lysine; Met, Methionine; Cys, Cysteine; Thr, Threonine; Trp, Tryptophan; Arg, Arginine; Gly, Glycine; Ser, Serine; Val, Valine; Iso, Isoleucine; Leu, Leucine; His, Histidine; Phe, Phenylalanine; Tyr, Tyrosine.

[1]Feedipedia (2015).

[2, 3]Rostagno et al. (2011).

[4, 5, 6]Average value of the two AAs.

The bioavailability of the AAs in CM is low because of the high processing temperature. It was found that overheating of the CM leads to losses in the content and digestibility of AAs (Friedman, 1996). A study done by Anderson-Hafermann et al. (1993) observed that the apparent digestibility of lysine in canola decreased by 5% during desolventisation/toasting of the meal in solvent-extraction process. Hence, this supports the finding that high temperatures during processing negatively impacts the protein

quality of the meal. Therefore, it is prudent to monitor AA bioavailability as a part of quality control in canola processing plants. Two rapid *in vitro* test namely, potassium hydroxide (KOH) protein solubility test (Anderson-Hafermann et al., 1993) and the neutral detergent insoluble nitrogen (NDIN; Newkirk et al., 2000) test are used to correlate AA digestibility in CM. Anderson-Hafermann et al. (1993) stated that protein solubility in KOH may be a useful index of over processing of CM and 0.2% KOH values of 35% or less or 0.5% KOH values of 45% or less are suggestive of over processed CM. However, NDIN method appears to offer greater prediction accuracy than KOH test. Newkirk et al. (2000) found that NDIN values below 10% indicate a CM with greater than 85% lysine availability.

2.3.3 Fibre

The fibre content of CS is relatively low (8.9%; Table 2.2) compared to other oilseeds except soybean. This can be attributed to the small size of canola seed (approx. 2 mm diameter) and therefore has a higher surface area and more hull. The hull contributes about 10.5-17% of the canola seed. In addition, it is difficult to dehull the seed due to smaller seed size (Canola Council of Canada, 2015). Liu et al. (1995) reported that higher oil content in seed concentrates the hull and other fibre components during extraction, making it practically difficult to separate out. Therefore, compared to CS, CM has a higher fraction of fibre content.

The percentage of dietary fibre fraction of CM reported by Slominski (2015) are presented in Table 2.4. Acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF) in CM were 16.2, 25.4 and 32.4%, respectively. Montoya and Leterme (2010) observed a strong negative correlation between the digestibility values of energy and the fibre content, higher fibre content lowers the digestibility of energy.

Table 2. 4 The percentage of dietary fibre fraction of canola meal

Item	%
Crude fibre (CF)	11.2
Acid detergent fibre (ADF)	16.2
Neutral detergent fibre (NDF)	25.4
Total dietary fibre (TDF)	32.4
Non-starch polysaccharides (NSP)	18.9
Cellulose	7.9
Non-cellulosic polysaccharides	11.0
Glycoprotein	4.6
Lignin and polyphenols	8.9
Lignin	5.8

Source: Slominski (2015).

Various approaches have been undertaken in an attempt to reduce the fibre content, increase protein content, and to overall improve the nutritive value of CM. Campbell et al. (1995) reported that dehulling helps in increasing the energy and protein content and decreasing the total dietary fibre content but had no effect on total available AA content. This further proves that the nutrient quality of dehulled CM is superior as compared to conventional commercial CM. In addition, development in plant breeding techniques have successfully developed low-fibre canola variety. A study by Slominski et al. (2012) showed that the meal derived from yellow-seeded *B. napus* canola contains more protein, more sucrose and less dietary fibre as compared to black-seeded *B. napus* and *B. juncea*. The low fibre content of *B. napus* was attributed to bigger seed size, lower contribution of hull fraction to the seed, and lower lignin content with associated polyphenols of the hull fraction. They further suggested that the meal derived from yellow-seeded *B. napus* has superior meal quality characteristics. Another approach to improve energy utilisation from full fat oilseed is by application of dietary enzymes such

as cellulase, mannanase, protease, phytase and carbohydrases. Meng et al. (2006) reported that multiactivity carbohydrase enzyme supplementation may be used as a means to improve energy and protein utilisation from oilseeds and, thus, enhance its feeding value for poultry.

2.4 Nutritional comparisons between soybean and canola meal/seed

2.4.1 Metabolisable energy

Soybean meal is a stable protein source, sometimes referred to as the "gold standard" as it is rich in highly digestible protein and superior blend of AAs. On comparison, CM is unstable and there is more variance within the production of CM. Soybean meal provides more energy from its nutrients when compared to CM and CS (Table 2.2). Khajali and Slominski et al. (2012) speculated that the differences between the ME content of SBM and CM are due to the variation in the oligosaccharides (5.6 vs 2.0%, respectively) and fibre content (5.4 vs 11.2%, respectively). They also found that high fibre content in CM accelerates the digesta passage rate, which in turn, may reduce digestion time and thus reduce nutrient absorption. However, the significantly higher fat content of CM can minimise the difference in ME content of the two meals (Khajali and Slominski 2012). Also, addition of gums or other refining by-products back into the meal can also have significant effect in ME value of CM (March et al., 1978).

2.4.2 Amino acids (AAs)

As mentioned above, SBM is considered as a standard protein source in poultry nutrition. Compared to SBM, CS and CM contain low protein content (Table 2.3; 48 vs 21 and 37%, respectively; Feedipedia, 2015) low digestibility coefficients (Table 2.5; Kim et al., 2012) and less consistent AAs (Khajali and Slominski, 2012). Digestible protein content

of SBM is also higher than CM (Table 2.3; 44 vs 30%, respectively). Kim et al. (2012) suggested that the standardised ileal AA digestibility coefficient of CM is lower than SBM due to desolventisation and toasting stage during the prepress solvent extraction of CS. These results agree with the findings observed by Anderson-Hafermann et al. (1993) and Friedman (1996). In general, methionine and lysine are the first two limiting AAs in poultry diets. On comparison, CM has less lysine but more methionine and cysteine while SBM is rich in lysine (Newkirk, 2011). Khajali and Slominski (2012) suggested that when both SBM and CM are used together in rations for poultry, they tend to complement each other.

Table 2. 5 Standardised ileal digestibility (SID, %) of amino acids (AA) in canola meal (CM) and soybean meal (SBM)

Item	CM	SBM
Indispensable AA		
Arg	84.6	91.7
His	82.0	89.4
Ile	76.8	87.0
Leu	78.6	87.5
Lys	76.9	89.1
Met	81.9	90.4
Phe	80.2	88.5
Thr	73.4	85.1
Val	75.7	85.8
Dispensable AA		
Ala	78.1	87.3
Asp	76.8	86.3
Cys	77.0	83.0
Glu	85.3	90.3
Pro	78.8	88.4
Ser	77.9	88.9
Tyr	77.5	88.9

Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, Leucine; Lysine, Lysine; Met, Methionine; Phe, Phenylalanine; Thr, Threonine; Val, Valine; Ala, Alanine; Asp, Aspartic acid; Cys, Cysteine; Glu, Glutamic acid; Pro, Proline; Ser, Serine; Tyr, Tyrosine
Source: Kim et al. (2012).

2.5 Anti-nutritional content in canola

2.5.1 Glucosinolates

Glucosinolates (GLS), commonly known as goitrogens, is a large group of sulphur-containing secondary metabolites found in Cruciferae family. A diverse range of GLS exists out of which more than 120 have been identified (Chen and Andreasson, 2001). All GLS are composed of a basic structure (Fig. 2.4) modified at the side chain, which is responsible for the difference in the chemical nature and end products of hydrolysis between different GLS.

Figure 2. 4 Chemical structure of glucosinolates R = Side chain

Source: Chen and Andreasson (2001)

Up to 27 GLS have been identified in CM, but only 6 are present in significant quantities namely gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin and neoglucobrassicin (Bell, 1984).

Glucosinolate compounds are biologically inactive and non-toxic in intact form. During processing of seed or when chewed by animals, the GLS are released due to disruption of cell wall and cell membranes of the plant cell (Underhill, 1980). Myrosinase enzyme is found in all tissue of plants containing GLS which is responsible for enzymatic degradation of GLS molecule to yield D-glucose, a sulphate and an aglycone in the presence of moisture (Tripathi and Mishra, 2007). The aglycone is an unstable compound

which further decomposes to isothiocyanates, thiocyanates and nitriles. Different end products such as isothiocyanates, thiocyanates, nitriles goitrin, oxazolidine-thione, epithionitrile are formed depending upon the reaction condition and the structure of the individual GLS. For instance, at low pH conditions, aglycone is decomposed to nitriles with liberation of sulphur while in neutral pH, it rearranges to an isothiocyanates (Underhill, 1980).

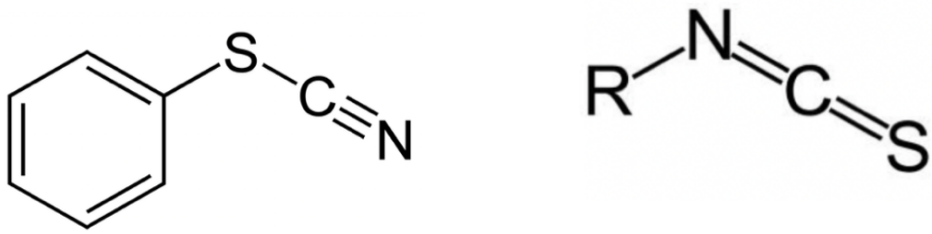
Figure 2. 5 Hydrolysis of glucosinolates by the myrosinase enzyme
Source: Tripathi and Mishra (2007)

It is believed that the presence of GLS in rapeseed meal hinders its use on more extensive level. Even though GLS possess antibacterial, antifungal, antiviral and cancer preventing ability, the end products formed during hydrolysis minimises its use in animal feed (Szydłowska-Czerniak et al., 2011). Slominski et al. (1988) found that the microbes present in ceca are also responsible for major hydrolytic degradation of intact GLS in the gastrointestinal tract of poultry. Elwinger and Säterby (1986) observed that feeding large quantities of rapeseed meal or meal containing high level of glucosinolates cause goiters, hemorrhagic liver syndrome and suppress growth. Likewise, Smith and Campbell (1976) noticed lower egg productivity and off-flavour in brown eggs in layer birds. Fortunately,

the development of new varieties of CM obtained by genetic manipulation and plant breeding contain low levels of GLS (Canola Council of Canada, 2015). These have shown to have fewer toxic effects but still further reduction in the levels of GLS content would be beneficial.

2.5.2 Thiocyanates and isothiocyanates

Thiocyanates (Fig. 2.6a) and isothiocyanates (Fig. 2.6b) are the end products of indolylglucosinolates hydrolysis which are found in significant quantities in rapeseed meal (Bell, 1984). Both the compounds are potent contributors to goitre and exhibit powerful anti-thyroid effects. These compounds depress the iodine uptake, reduce the iodination of tyrosine, decrease the production of thyroid hormone and disturb the T3:T4 ratios (Bell, 1984). It was found that heat promotes the conversion of isothiocyanate to L-5-vinyl-2-thio-oxazolidone and increase the goitrogenicity of rapeseed meal (Clandinin et al., 1959). However, these compounds are formed occasionally, and the exact mode of mechanism is unclear.



(a) (b)

Figure 2. 6 Chemical structure of phenyl-thiocyanates (a) and isothiocyanates (b)

Source: Pubchem (2013)

(https://pubchem.ncbi.nlm.nih.gov/compound/Phenyl-thiocyanate.)

2.5.3 Erucic acid

Erucic acid (Fig. 2.7) is a monosaturated omega-9 fatty acid found in plants of *Brassicaceae* family. The conventional variety of rapeseed contains high level of erucic acid (54%) which is responsible for deleterious effects on health if consumed in large quantities. A study done by Ratanasethkul et al. (1976) showed that 25% inclusion of rapeseed containing about 32% erucic acid caused growth depression, increased anaemia and feed conversion ratio and resulted in death of some birds due to hydropericardium and ascites. They also observed that young birds showed severe fatty acid changes in heart, spleen and kidney when fed on the same rapeseed. Also, they noticed lower feed intake as the feed seemed to be less palatable due to its bitter taste. Therefore, it is speculated that the rapeseed oil and meal extracted from high erucic acid rapeseed is not suitable for consumption both by humans and animals. However, genetic modification has successfully led to development of a new variety of rapeseed containing low concentration of erucic acid or low erucic acid rapeseed (LEAR) less than 2% and low glucosinolates concentration (Canola Council of Canada, 2015). Vogt (1981) recommended that higher levels of inclusion of LEAR can be allowed than those recommended for high erucic acid rapeseed (HEAR) in poultry diets.

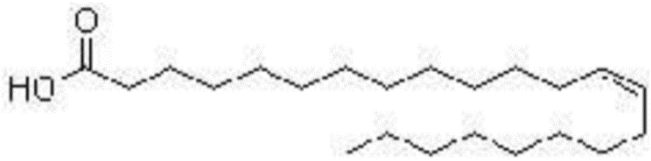


Figure 2. 7 Chemical structure of erucic acid

Source: Lookchem (2013)
(http://www.lookchem.com/Product_2107/CasNo_112-86-7/Erucic-acid.html.)

2.5.4 Phytic acid

Phytic acid also known as phytate is typically found in hulls of grains, nuts, beans and seeds. Phytate (myo-inositol-1,2,3,4,5,6 - hexakis-dihydrogen phosphate) serves as the main storage form of phosphorous and myo-inositol in plant seeds (Maga, 1982). At neutral pH, the phosphate group has two negatively charged oxygen atom, hence it shows a strong propensity towards protein and many essential minerals (Ca, Fe, Zn, Mn, Mg; Khajali and Slominski, 2012). The protein-phytate or mineral-phytate complex is poorly digested and thereby reduce the availability of some AAs and minerals. Compared to SBM, CM has a high phytate content (Bell and Keith, 1991).

In poultry, phytate is not bioavailable because of the lack of enzyme phytase which breaks it down. The excess undigested phytate passes through the excreta and degrades water quality and subsequently raises environmental concern (Ravindran et al., 1999). Wickramasuriya et al. (2015) observed that feeding of excessive phytate in poultry diet caused hypertrophy of thyroid glands and lowered growth performance. Ravindran et al. (1999) suggested that addition of commercial phytase enzymes hydrolyses phytic acid to inositol and inorganic phosphorous, which improves phosphorous utilisation and growth performance, lowers phosphorous supplementation and reduces its concentration in the excreta. These results were further confirmed by a study done by Kong and Adeola (2011). They showed that supplementation of phytase with CM significantly improved body weight and overall performance of the bird. However, phytase supplementation did not affect the true ileal digestibility of AAs.

2.5.5 Sinapine

Sinapine is the choline ester of sinapic acid (3,5- dimethyl- 4 hydroxycinnamic acid). Among all phenolics, sinapine is the most abundant phenolic compound in CM

(approximately 1%). The dark colour, bitter taste and astringency drastically reduce palatability and feed intake of CM (Kozlowska et al., 1990). Intestinal bacteria in chickens hydrolyses sinapine into trimethylamine (TMA) which is absorbed and later degraded by the enzyme trimethylamine oxidase in the liver. Fenwick et al. (1979) observed that feeding large amounts of rapeseed meal to layers produced fishy or crabby taint in some strains of brown shelled eggs and produced liver hemorrhages in flocks. These observations were further explained by Hobson-Frohock et al. (1973). They reported that few strains of layers producing brown shelled eggs lack trimethylamine oxidase which resulted in deposition of TMA in the eggs and hence produced fishy odour in eggs. However, development in breeding program has successfully corrected the genetic defect and eggs are no longer affected by sinapine in certain breeds of laying hens. No taint or off- flavour has been detected in broiler carcass.

2.5.6 Fibre

Canola meal contains high fibre content approximately one-third of the meal. Dietary fibre contains cellulose (4-6%), non-cellulosic polysaccharides such as arabinose, xylose, galactose, glucose, mannose, rhamnose, fucose and uronic acids (13-16%), lignin and polyphenols (5-8%) and small fraction of protein associated with fibre (Wickramasuriy et al., 2015). The hull of the seed is mostly concentrated with fibre. Another insoluble complex polyphenolic compound known as tannin imparts a dark unattractive colour to the meal and forms complexes with protein and enzymes, thereby affecting protein digestion (Khajali and Slominski, 2012). The effect of tannin on energy content of rapeseed meal was studied by Yapar and Clandinin (1972). They found that the ME of the rapeseed meal increased after extraction of tannins from the meal. This was probably due to increased activities of endogenous enzymes. Similarly, Leslie et al. (1976)

observed that addition of tannic acid in broiler diets reduced growth performance. To rectify the deleterious effect of different constituents of fibre new variety of canola was developed. Advancement in breeding program successfully developed yellow seeded *Brassica napus* canola variety with having lower fibre content in the seed. The low fibre content was attributed to larger seed size, lower ratio of hull fraction to seed mass and low lignin content in the new CS (Slominski et al., 2012). It is believed that dehulling can also possibly help in reducing the negative effect of the fibre (Campbell et al., 1995). However, it is practically difficult to dehull canola seed on a larger scale as it increases the cost of production.

2.6 Effect of processing on anti-nutrient content of rapeseed

The concentration of various anti-nutritive factors in the canola can be reduced by certain processing techniques such as lime treatment, ammoniation, micronisation, dry extrusion etc. These methods improve the nutritive quality of CM. Keith and Bell (1982) reported that treating CM with ammonia or steam or both during desolventisation, reduces glucosinolates concentration, increases the CP concentration (39 to 42%), decreases proportion of alkaline soluble nitrogen and increases the available lysine level. On the contrary, they noticed that the energy digestibility of the meal was reduced when treated in combination with steam. In another study, Goh et al. (1984) found that sparging CM with ammonia in the absence or presence of steam lowers the sinapine content of meal. Further, this lowered the TMA content of eggs and reduced the fishy odour. Similarly, Fenwick et al. (1979) found that lime treatment lowers the sinapine content of CM by more than 90%. It is believed that sinapine readily hydrolyses under alkaline conditions to produce choline and sinapic acid.

Fenwick et al. (1986) studied the effect of dry extrusion on CM nutritive value. Dry extrusion is a type of heat treatment applied at lower moisture levels wherein it effectively inactivates myrosinase at 150 °C. However, it has less effects on GLS content unless chemicals are added before extrusion. In another study, Mustafa et al. (2003) studied another processing technique known as micronisation, wherein infrared gas generators heat the feedstuffs (such as CS and flaxseed) to approximately 110-115 °C to denature the protein matrix surrounding the fat droplets and thus increase the supply of polysaturated fatty acids to the small intestine in ruminants. It also protects the seed from ruminal degradation without compromising the quality of AAs available for post-ruminal digestion. The most recent method used by Lacki and Duvnjak (1999) is based on addition of enzyme preparation to upgrade the quality of CM by decreasing its phenolic content. However, the mechanism involved with the different processing techniques is not known.

2.7 *In vivo* evaluation of canola meal and seed for poultry

The most important factor in efficient utilisation of feed for growth, maintenance, and production of domestic animals is bioavailability of nutrients (Ravindran and Bryden, 1999). The bioavailability of nutrients may be defined as nutrients which can be released by digestion, absorbed and utilised by animals. Digestibility assays is a method of *in vivo* evaluation of feed ingredients, wherein the difference between input and output is used as a valid indicator of bioavailability (Ravindran and Bryden, 1999).

2.7.1 Apparent metabolisable energy

Studies have shown that the apparent metabolisable energy (AME) content of different CS/CM vary in each batch. These differences may be due to variation in the chemical composition. Such as variation in fat, protein sucrose, NDF and ADF contents (Adewole

et al., 2017b). Khajali and Slominski (2012) stated that the AME content of CS is higher than CM due to the higher amount of fat and lower fibre content. Further, Adewole et al. (2017b) suggested that high fibre content (NDF and ADF) in CM accelerates digesta transit time and limits the capacity of gut microbiota to use complex carbohydrates, which leads to higher excreta output and ultimately lowers AME content. In another study Woyengo et al. (2010) studied the effect of processing of CM on the AME value. They observed variation in AME content of CM depended on type of processing involved. Expeller extracted CM (CME) had higher AME (12.72 vs 8.39 MJ/g, respectively) and AMEn (11.28 vs 7.54 MJ/g) compared to solvent extracted CM (CMS). Hence, based on the findings it can be assumed that CME can be a better source of energy for broilers diets.

2.7.2 Standardised ileal digestible amino acid content

Feed formulations are based on digestible AA contents to precisely formulate feed and predict animal performance (Lemme et al., 2004). A preferred approach to estimate AA availability in feed ingredients for poultry is by determining the standardised ileal digestible AA content wherein the endogenous AA losses are taken into account (Lemme et al., 2004; Kim et al., 2012). The differences in SID of AA reflect differences in the total content of AA and standardised ileal digestibility coefficients (Newkirk et al., 2003). Park et al. (2019) compared the standardised ileal digestibility of canola seed, CMS and SME (Table 2.6). However, there is a very limited data on SID values of AA in canola seed in poultry.

Several studies have shown that there are differences in the CP and standardised ileal AA content of CM (Bell and Keith, 1991; Adewole et al., 2017a). These differences could be due to the differences in CS processing conditions, total dietary fibre content

and changes in weather or soil conditions that affect CS composition during the growing season (Bell and Keith, 1991). Woyengo et al. (2010) reported that CMS had lower apparent ileal digestiblility (AID) and SID of AA than CME. Newkirk et al. (2003) observed that the AID of AA for non-toasted CM was higher than toasted CM. These observations were attributed to overheating of CM during processing which led to AA losses and lowered AA digestibility. Friedman (1996) found that the lysine content was significantly reduced in desolventised and toasted CM due to the formation of Maillard reaction products. During the first stage of Maillard reaction, AA are detectable by chemical analysis, but are no longer bioavailable. Adrian et al. (1966) observed that the destruction of lysine content is 5-15 times more than other AAs. Therefore, changes in lysine content and its bioavailability indicate the occurrence and intensity of Maillard reaction.

Adewole et al. (2016) noted a positive relationship between lysine and heat sensitive glucosinolates contents, as well as a negative relationship between lysine and NDF and TDF contents. They proposed that ADF can be used as a simple measure for predicting the SID of AA content in CM. This is probably because advanced glycation products of the late stage Maillard reaction are concentrated in the ADF fraction (Adrian et al., 1966). This supports the finding that heat damage of feed ingredients is associated with an increase in ADF value, which results in a decrease in nitrogen digestibility in cattle and sheep (Broesder et al., 1992).

Table 2. 6 Standardised ileal digestibility (%) of crude protein (CP) and amino acids (AA) in full fat canola seed (FFCS), solvent extracted canola meal (CMS) and expeller extracted canola meal (CME)

Item	FFCS	CMS	CME
Indispensable AA			
Arg	89.2	87.5	88.4
His	87.7	85.2	86.5
Ile	81.5	79.0	79.8
Leu	84.8	82.4	83.2
Lys	84.6	82.2	83.3
Met	88.0	86.9	87.8
Phe	84.2	82.9	83.6
Thr	78.5	75.3	76.7
Trp	96.4	87.4	89.9
Val	79.3	76.8	77.4
Dispensable AA			
Ala	84.6	82.4	83.0
Asp	82.8	80.1	81.4
Cys	83.6	78.3	79.8
Glu	90.2	88.7	89.5
Gly	83.3	79.1	80.7
Pro	81.9	79.6	80.2
Ser	80.7	77.1	80.1
Tyr	82.9	79.6	80.9

Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, Leucine; Lys, Lysine; Met, Methionine; Phe, Phenylalanine; Thr, Threonine; Trp, Tryptophan; Val, Valine; Ala, Alanine; Asp, Aspartic acid; Cys, Cysteine; Glu, Glutamic acid; Gly, Glycine; Pro, Proline; Ser, Serine; Tyr, Tyrosine

Source: Park et al. (2019)

2.8 Uses of canola meal in poultry diets

2.8.1 Effect of inclusion of canola meal and seed in broilers

Full fat CS, after heat treatment and particle size reduction, is a mainstay protein and energy ingredients in broiler feeds in some countries like Denmark (Newkirk, 2009). But generally, it has not been extensively investigated as a nutrient source in broilers. Nwokolo and Sim (1989) observed that supplementation of raw full fat CS in barley-based diet significantly lowered weight gain and elevated levels of linoleic and linolenic acids in tissue lipids of chick. Likewise, Summers et al. (1982) observed that CS at dietary

level of 17.5% or higher resulted in reduced weight gain and feed intake in broiler chickens. These findings suggest that inclusion of full fat CS in broiler diets negatively impact the health and performance of the birds. These findings were similar to those observed by Roth-Maier (1999) who reported that the performance reduced continuously with increasing CS level in the diet. However, still there is a need to extensively explore the potential of full fat CS as a feed ingredient in poultry diets.

On the other hand, CM has become a popular feed ingredient in animal nutrition. Previously, CM was found to impart deleterious effect on the health and performance of the birds (Elwinger and Säterby, 1986; Karunajeewa et al., 1990; Table 2. 7). However, most recent studies have proved that current varieties of CM contain low levels of glucosinolates and therefore do not have any negative effects on broiler mortality or feed intake. Naseem et al. (2006) found that broilers fed diet containing 25% CM had higher weight gain and lower FCR compared to broilers fed diets containing 5% CM. The exact reason for decreased feed intake is not known but it may be due to its taste and high fibre content. Another study done by Ramesh et al. (2006) indicated that CM can be included up to 300 g/kg (30%) in broiler diets without any adverse effect on health and performance. However, the lower energy values compared with other protein sources such as SBM has limited its use in broiler feeds.

The level of glucosinolates present in the CM, is the primary driving factor affecting growth performance of broiler chickens. McNeill et al. (2004) observed that feeding CM with high level of dietary glucosinolates resulted in reduced feed intake and growth rate and increased mortality. In another study, Tripathi and Mishra (2007) reported that glucosinolates content above 8.0 μmol/g of diet would result in a growth depression in broilers. Therefore, evaluation of CM for glucosinolates level is important consideration while formulating broiler diets. Khajali and Slominski (2012) proposed that

considering a conservative 4 μmol/g as the maximum inclusion level of glucosinolates, will allow higher supplementation of CM in the diet than the currently recommended 20% in broiler ration, without producing any adverse effect in broilers.

2.8.2 Effect of inclusion of canola meal and seed in layers

Very little data is available on CS optimum inclusion level in the layer diets. It was reported that the inclusion of 5 and 10% of CS significantly reduced the performance of layers (Roth-Maier,1999). However, compared to CS, CM is a commonly fed and economically effective feed ingredient in commercial layer diets

Several studies have investigated effects of feeding CM on egg production parameters (Nassar et al., 1985; Badshah et al., 2001; Perez-Maldonado and Barram, 2004). These researchers have found that feeding 15-20% CM had no negative effect on egg productivity, feed intake or egg size when fed to layer birds. Perez-Maldonado and Barram (2004) fed two strains of layer birds, ISA brown and Inghams White Supertint with 10, 15, and 20% CM and found that the production performance and egg quality remained unaffected by feeding birds with CM and no mortalities were noted. However, fresh eggs from ISA brown had fishy taint, but odour reduced substantially when eggs were stored at 10 °C for 2-5 weeks. Contrary to these results, Ibrahim and Hill (1980) observed lower egg production and high mortality in layers fed with high-glucosinolates CM.

Table 2. 7 Effects of dietary inclusion of canola meal (CM) on performance parameters in poultry

Bird type	Inclusion rates of CM[1] (%)	Observations	Recommended inclusion level	Reference
Broilers	0, 25, 50, 75, 100	No adverse effect on BW gain, FCR and feed intake, only slight decrease in BW gain	38%	Leeson et al. (1987)
Layers	0, 25, 50, 100	Increased BW gain	25%	
Broilers	0, 10, 15, 20, 25, 30	Slight reduction in BW gain, feed intake, good for fattening chickens	15%	Roth-Maier (1999)
Layers	0. 6.7, 10, 13.3, 16.7, 20	Significant decrease in feed intake, egg mass. No adverse effect on egg productivity, odour or flavour	17%	
Broilers	0, 4, 8, 16 (RSM)	Decrease in BW gain, feed intake, and FCR	8%	Montazer-Sadegh et al. (2008)
Layers	0, 15, 20 (RSM)	Exceeding 15% RSM inclusion depress layer performance	15%	Ciurescu (2009)
Broilers	0, 5, 10, 15 (fermented RSM)	Poor BW gain and FCR at 15% inclusion	10%	Xu et al. (2012)
Turkey	0, 6, 12, 18 (RSM)	No adverse effect on BW, carcass traits, but significantly increase in FCR	18%	Mikulski et al. (2012)
Broilers	0, 10, 20, 30, 40	Inclusion level can be increased with advancing age without any adverse effects	7-14d, 16.4% 14-21d, 22.9% 21-28d, 30%	Gopinger et al. (2014)

[1]BW, Body weight; FCR, Feed conversion ratio; d, day

Traditionally, inclusion of CM in layer diets was limited to a maximum of 10%, as it was noted that higher inclusion level of CM caused liver haemorrhages, thyroid

related abnormalities and mortality (Clandinin et al., 1959; Bell, 1984). Plant breeding has steadily reduced glucosinolates levels to almost one-third of those found in the first canola. Most recent studies have shown that the glucosinolates content differs in different varieties of canola. Ibrahim and Hill (1980) showed that brown hybrid layers fed with the British variety of *B. napus* had lower egg production, with fishy taints eggs, and showed liver haemorrhages and enlarged thyroid gland. While the layers fed on Canadian variety (Tower), showed no depression in egg production, lesser degree of thyroid glands enlargement and fewer deaths due to liver haemorrhages. However, most recent studies with current low-glucosinolates meal varieties of CM failed to observe incidence of liver haemorrhages even at 20% inclusion level (Oryschak and Beltranena, 2013).

2.9 Optimum inclusion rate of canola meal in poultry diets

The recommended maximum inclusion level of CM in different bird type is presented in Table 2.8. In broiler chicken diets, 10 and 20% CM could be included during starter and grower phases, respectively, without any negative effect. However, in laying hens and breeders the inclusion levels are 10 and 5%, respectively (Newkirk, 2009).

Table 2. 8 Recommended maximum inclusion levels (%) of canola meal in poultry diets

Animal diet type	Maximum inclusion level	Reason
Chick starter	10	-
Broiler grower	20	Energy level
Egg layer	10	Potential effect on mortality
Breeder	5	Small egg size and chick weight

Source: Newkirk (2009).

2.10 Conclusion

Based on the available literature, it can be concluded that CS and CM are not nutritionally comparable to SBM as the standard protein sources due to their low energy and protein content. However, CS and CM are attractive feed ingredients for poultry and with careful considerations CS and CM can be used as a partial replacement for SBM in poultry diets. Although, the presence of anti-nutritional factors such as high fibre, glucosinolates content and sinapine limit their use in poultry diets, but various processing techniques (especially heat treatments) have shown to successfully reduce the concentration of these anti-nutritional factors. In near future, CS and CM is expected to show a great potential in poultry nutrition. However, very little is known about nutrient digestibility and optimum inclusion of CM and CS in poultry diets. More research is needed to define the optimum inclusion levels of both CS and CM in poultry in different stage of life.

CHAPTER 3

NUTRIENT ANALYSIS, METABOLISABLE ENERGY AND ILEAL AMINO ACID DIGESTIBILITY OF CANOLA MEAL AND CANOLA SEED IN BROILER CHICKENS

3.1 Introduction

Poultry industry is emerging globally due to the increased demand for animal protein sources. This increased demand for poultry-derived protein can be achieved by inclusion of highly digestible protein sources such as soybean meal (SBM) in poultry diets. However, the increasing price of soybean has become a concern for the economic sustainability of the poultry industry. There is a need to explore potential alternative protein sources which can be used as feed ingredient in poultry diets (Ravindran and Blair, 1992).

Canola seed (CS) is one of the alternative protein sources available and potentially inexpensive feedstuff containing well-balanced protein and high oil content (Meng et al., 2006). Another alternative feed ingredient is canola meal (CM). It is a co-product of the canola oil industry, produced by extracting the canola oil from CS using mechanical or solvent extraction (Canada Council of Canada, 2015). However, CM inclusion in poultry diets is limited due to the presence of anti-nutritional factors such as glucosinolates, erucic acid, sinapine, and high fibre content (Bell, 1984). Moreover, lower crude protein (CP) content (Feedipedia, 2015) and lower apparent metabolisable energy (NRC, 1994) compared to SBM makes CM less desirable feed ingredient in poultry diets.

The nutritional component and protein quality of CS and CM varies with the variety, harvesting conditions, and processing of the seed and meal (Bell and Keith, 1991; Khajali and Slominski, 2012; Adewole et al., 2017a). Moreover, the extraction process requires various steps, each involving a wide range of temperature, moisture and time which also contribute to chemical and nutritional variations (Clandinin et al., 1959).

Continuous increasing cost of conventional ingredients has motivated poultry nutritionists to evaluate the use of locally available feed ingredients such as CS and CM. Despite this interest, only few studies have been conducted to determine the ileal amino acid (AA) digestibility and AMEn of CS and CM for broilers. The objective of the present study was to assess the chemical composition, AMEn and standardised ileal digestibility (SID) of AAs in CS and CM in broilers.

3.2 Materials and methods

3.2.1 Ethical consideration

The experimental procedures employed were approved and in accordance with the guidelines of Massey University Animal Ethics Committee and conducted at the Massey University poultry unit farm.

3.2.2 Diets

Two CM and one CS samples, originated from Australia, were obtained from Ridley, Australia. Both CMs (CM1 and CM2) were processed by solvent extraction with slight differences (Fig. 3.1). First, the canola seed was cleaned and heated to soften the seed coat. The CM1 was heated to 70-80 ^{o}C before flaking. The CM2 was heated to 60 ^{o}C for 30 min pre-flaking and then cooked at 105 ^{o}C for 25-30 min post-flaking. Both the samples were pressed mechanically to extract about 2/3rd of the oil and the resultant cake

was washed with hexane to extract the residual oil. Later, both CM were exposed to heat to remove the remaining hexane. Lastly, both meals were hammer milled to obtain a consistent particle size. The CS was crushed to disrupt the seed coat and to release oil from the seed. The nutritional evaluation of both CM and CS were assessed in three phases namely, (i) proximate and nutrient composition, (ii) metabolisable energy evaluation and (iii) ileal AA digestibility assay.

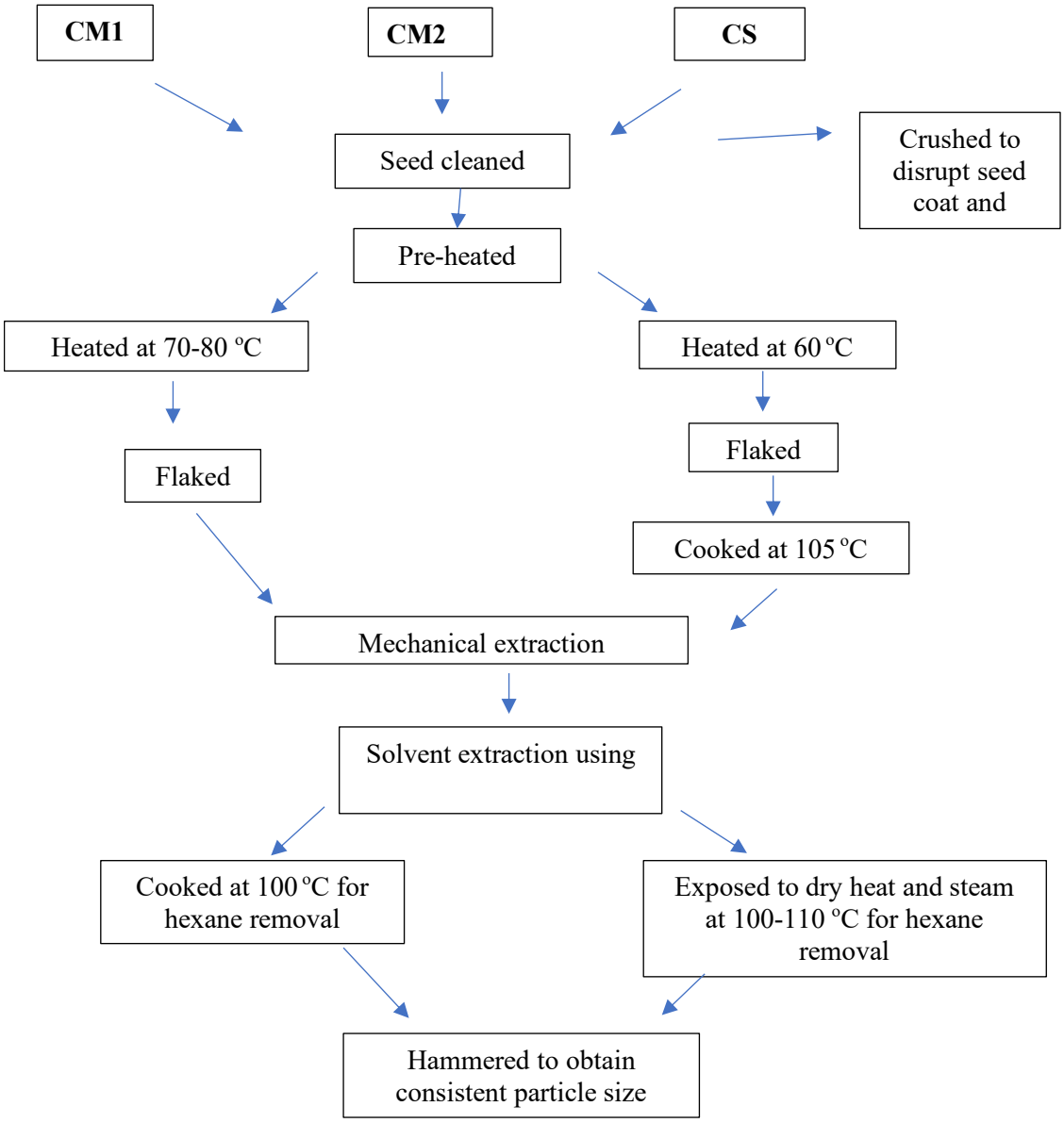


Figure 3. 1 Different processing steps of canola meals (CM) and canola seed (CS) used in current study

The proximate and nutrient composition analysis of CM and CS were conducted in an ISO17025 accredited laboratory (Nutrition Laboratory, Massey University). Representative samples of CM and CS were analysed, in duplicate, for dry matter (DM), gross energy (GE), nitrogen (N), crude fat, neural detergent fibre (NDF), insoluble dietary fibre (IDF), soluble dietary fibre (SDF), total dietary fibre (TDF), AA, calcium (Ca), phosphorous (P) and other minerals.

Table 3. 1 Composition (g/kg) of the basal diet used in the apparent metabolisable energy (AME) assay

Item	Inclusion (g/kg)
Maize	604.4
Soybean meal	338.1
Soybean oil	14.2
Dicalcium phosphate	15.8
Limestone	10.4
Sodium chloride	1.0
Sodium bicarbonate	3.9
DL-Methionine	3.1
L- Lysine HCl	3.7
L-Threonine	2.0
L-Valine	0.7
Vitamin premix	1.0
Mineral premix	1.0
Choline chloride 60%	0.7

[1] Test diet was developed by replacing 30% of the basal diet by CM/CS.
[2] Supplied per kg diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; trans-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamin, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium pantothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg.

The AME of CM and CS was determined by difference method (Nalle et al., 2011) In this method, a maize-soy basal diet was formulated (Table 3.1) and three test diets, containing CMs and CS were developed by replacing (w/w) 30% of the basal diet with CM or CS. Thus, a total of 4 diets were assayed. All diets were steam-conditioned at 75

ºC for 30 seconds and pelleted through a pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3 mm apertures and a depth of 35 mm.

The coefficient of apparent ileal digestibility (CAID) of DM, N and AA of CMs and CS was determined using direct method. Total of 3 assay diets based on maize starch and CM or CS as the only source of protein were formulated to supply 16% crude protein in the diet (Table 3.2). A N-free diet was also prepared to determine the endogenous N/AA losses for the calculation of standardised digestibility values. Titanium dioxide (TiO_2) was added to all diets as an indigestible marker to calculate the digestibility of N/AAs. All diets except N-free diet were steam conditioned at 75 ºC for 30 seconds and pelleted with a die ring with 3 mm apertures and depth of 35 mm.

Table 3. 2 Composition (g/kg, as fed basis) of the test and N-free diets used in the ileal amino acid (AA) digestibility assay

Ingredient	Canola meal 1	Canola meal 2	Canola seed	N-free diet
Test ingredient	410.0	410.0	680.0	-
Soybean oil	30.0	30.0	-	50.0
Titanium dioxide	5.0	5.0	5.0	5.0
Dicalcium phosphate	19.0	19.0	19.0	19.0
Limestone	10.0	10.0	10.0	13.0
Salt	2.0	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0	2.0
Trace mineral premix	1.0	1.0	1.0	2.0
Vitamin premix	1.0	1.0	1.0	2.0
Maize starch	520.0	520.0	280.0	840.3
Solkafloc (Cellulose)	-	-	-	50.0
Dipotassium hydrogen	-	-	-	12.0

3.2.3 Birds and housing

Day-old male broilers (Ross 308), obtained from a commercial hatchery, were raised in floor pens and fed a commercial broiler starter diet till day 18. Feed and water were available at all times. The temperature was maintained at 32 ºC during the first week and gradually decreased to approximately 23 ºC by the end of the third week. Ventilation was controlled by a central ceiling extraction fan and wall inlet ducts. On day 18, 144 birds of uniform initial body weight were selected and randomly assigned to 24 experimental cages (6 birds per cage) and six replicate cages were randomly assigned to each of the 4 assay diets. The nipple drinkers and feed troughs were provided in the cages. Fresh and clean water readily available, and feed was provided ad libitum throughout the experimental period.

3.2.4 Determination of nitrogen-corrected apparent metabolisable energy (AMEn)

The AME assay was conducted by the classical total excreta collection method. The experimental diets were fed for 7 days (d 18-24), with the first 3 days serving as an adaptation period. During the last 4 days, feed intake was monitored, and the excreta were collected daily, weighed and pooled within a cage. Pooled excreta were mixed well in a blender and, representative samples were obtained and freeze-dried. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 ºC for laboratory analyses. The DM, GE and N of the diet and excreta samples were determined.

3.2.5 Determination of standardised ileal protein and amino acid digestibility

On day 25, 144 birds of uniform body weight were selected and randomly assigned to 24 cages (6 birds per cage). Each diet was fed to six replicate cages for four days (d 25-29), were offered *ad libitum* and water was available at all times.

On day 29 post-hatch, all birds in each cage were euthanised by an intravenous injection (1 ml per 2 kg live weight) of sodium pentobarbitone solution (Provet NZ Pty Ltd., Auckland, New Zealand) and eviscerated. The contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. Digesta samples were pooled within a cage. The ileum was defined as the portion of the small intestine extending from vitelline diverticulum to a point 40 mm proximal to the ileo-caecal junction. The ileum was then divided into two halves, the digesta was collected from the lower half towards the ileo-caecal junction by gently flushing with distilled water, as described by Ravindran et al. (2005). Digesta from birds within a cage were pooled, lyophilised, ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 ^{o}C until laboratory analysis. The diet and digesta samples were then analysed for DM, titanium (Ti) and N and AAs.

Basal endogenous AA flow was determined in a cohort assay by offering a N-free diet to six cages of six birds each from day 25-29, according to the procedures described by Ravindran et al. (2008).

3.2.6 Chemical analysis

The DM was determined using standard procedures (Methods 930.10, 930.15; AOAC. 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a CNS-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). The crude protein (CP) content was calculated as N x 6.25. Fat was determined by gravimetric

extraction using acid (Method 922.06; AOAC, 2016) for CM and cold extraction for CS. The NDF (Method 2002.04; AOAC, 2016) was determined using Fibertec™ (FOSS Analytical AB, Höganäs, Sweden). The TDF, SDF and IDF were determined using assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase, protease and amyloglucosidase. The adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardised with benzoic acid was used for the determination of GE.

For mineral analysis, the samples were wet digested in a nitric and perchloric acid mixture, and concentrations of phosphorus (P), calcium (Ca), potassium (K), sodium (Na), and chloride (Cl) were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument.

Amino acids content of diets and digesta were determined as described by Ravindran et al. (2008). Briefly, the samples were hydrolysed with 6N HCl (containing phenol) for 24 h at 110 ± 2 °C in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion-exchange HPLC system, and the chromatograms were integrated using dedicated software (Millennium, Version 3.05.01, Waters, Millipore, Milford, MA), with the AA identified and quantified using a standard AA mixture (Product no. A2908, Sigma, St. Louis, MO). The HPLC system consisted of an ion-exchange column, two 510 pumps, Waters 715 ultra WISP sample processor, a column heater, a post-column reaction coil heater, a ninhydrin pump and a dual wavelength detector. Amino acids were eluted by a gradient of pH 3.3 sodium citrate eluent to pH 9.8 sodium borate eluent at a flow rate of 0.4 ml/min and a column temperature of 60 °C. Cysteine and methionine were analysed as cysteic acid and methionine sulphone, respectively, by oxidation with performic acid for 16 h at 0 °C and neutralisation with hydrobromic acid prior to hydrolysis.

3.2.7 Calculations

The AME value of the diets were calculated using the following formulas:

$$AME_{diet}\ (MJ/kg) = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

$$AME_{CS/CM}\ (MJ/kg) = \frac{\text{AME of test diet} - (\text{AME of basal diet} \times 0.70)}{0.30}$$

Nitrogen-corrected AME (AMEn) was determined by correction for zero nitrogen retention by using a factor of 36.54 kJ per gram nitrogen retained in the body as described by Hill and Anderson (1958).

The apparent ileal amino acid digestibility coefficients (AIDC) of AA were calculated from the dietary ratio of amino acid to Ti relative to the corresponding ratio in the ileal digesta.

$$\text{AIDC of CM/CS} = \frac{(AA / Ti)_{diet} - (AA / Ti)_{ileal}}{(AA / Ti)_{diet}}$$

where, $(AA / Ti)_{diet}$ = ratio of amino acid to titanium in diet; and $(AA / Ti)_{ileal}$ = ratio of amino acid to titanium in ileal digesta.

The apparent digestibility data were converted to standardised ileal digestibility coefficient (SIDC), using basal endogenous AA values from birds fed N-free diet method.

$$SIDC\ (\%) = AIDC\ (\%) + \frac{[\text{Basal EAA (g/kg DM intake)}]}{\text{Ing. AA (g/kg DM)}} \times 100$$

where, AIDC = % apparent ileal digestibility of the amino acid; Basal EAA = Basal endogenous of the amino acid; Ing. AA = Concentration of the amino acid in the ingredient

3.2.8 Statistical analysis

Means of SIDC of protein and AA were separated using independent (unpaired) samples t-test for unequal variances. Significant differences between means were separated by Least Significant Difference (LSD) test. Differences were considered to be significant at $P < 0.05$.

3.3 Results

The results are presented in 'as received' basis. The analysed chemical composition of CM and CS samples are summarised in Table 3.3. The CP content of CS was lower compared to CM samples. Between CM samples, CM1 had higher CP content compared to CM2, (411 and 393 g/kg, respectively). Canola seed had the highest NDF and lowest TDF value compared to CM samples. The GE content were 17.8, 17.7 and 24.7 MJ/kg for CM1, CM2 and CS, respectively. Calcium was exceptionally higher (17.7%) in CS, compared to CM1 (5.8%) and CM2 (6.2%). Potassium was the major mineral in CM samples (13.6 and 13% in CM1 and CM2, respectively).

Amino acid concentration of CM and CS samples are summarised in Table 3.3. Overall, the AA concentration was low in CS sample compared to both CM samples. Among indispensable AA, leucine was the major AA, whereas methionine and histidine were the AA with lowest content in all the samples. Glutamic acid was the major dispensable AA followed by aspartic acid in all the three samples.

The N retention, AME and AMEn of CM and CS samples are summarised in Table 3.4. Canola seed had higher ($P < 0.05$) N retention compared to both CM samples. There were tendency on AME ($P = 0.085$) and AMEn ($P = 0.059$) for CM and canola seed samples. Canola seed tended to have higher AME and AMEn values compared to CM1 and CM2 (11.17 and 10.29 MJ/kg DM vs. 7.82 and 6.78 MJ/kg DM, respectively).

The SIDC of CP and AA determined by the direct method are summarised in Table 3.5. A tendency ($P = 0.052$) was observed for effect of dietary treatments on SIDC of CP. Birds fed canola seed tended to have higher coefficient than those birds fed CM1 or CM2. The SIDC of all AA was significantly ($P < 0.05$) influenced by the CM or CS, except for methionine and phenylalanine ($P > 0.05$). The SIDC of all AA were higher in CS compared to CM1 and CM2. There were no significant differences ($P > 0.05$) in SIDC of all AA between CM1 and CM2 samples.

The standardised ileal digestible AA content of the CM and CS samples are summarised in Table 3.6. There was a significant difference ($P < 0.05$) in the standardised ileal digestible CP and AA content between all the three samples. The standardised ileal digestible CP content was highest ($P < 0.05$) in CM1 (293 g/kg), followed by CM2 (279 g/kg) and CS (176 g/kg) samples. The standardised ileal digestible AA content for all AA was higher in both CM samples compared to CS sample. For most indispensable AA, CM1 had the highest and CS had the lowest ileal digestible AA values($P < 0.05$), while for most dispensable AA, birds fed CM1 and CM2 showed higher ($P < 0.05$) ileal digestible AA content compared to birds fed CS ($P < 0.05$).

Table 3. 3 Proximate, carbohydrate, mineral and amino acid composition (g/kg), and gross energy (GE; MJ/kg) of canola meal 1 (CM1), canola meal 2 (CM2), and canola seed (CS) (as received basis)

	CM1	CM2	CS		CM1	CM2	CS
Proximate and carbohydrate composition				**Amino acid concentration**			
Dry matter	906	912	942	**Indispensable amino acids**			
Nitrogen (N)	66.0	63.0	38.0	Arginine	21.1	21.4	13.3
Crude protein (N × 6.25)	411	393	235	Histidine	9.4	9.4	5.7
Crude fat	46.0	53.0	347	Isoleucine	14.3	13.6	8.8
NDF[a]	264	290	336	Leucine	24.9	24.0	15.1
IDF[a]	282	282	188	Lysine	19.8	18.6	13.3
SDF[a]	29.0	27.0	14.0	Methionine	8.4	7.5	4.9
TDF[a]	311	309	202	Phenylalanine	14.4	13.8	8.5
GE (MJ/kg)	17.8	17.7	24.7	Threonine	15.2	15.2	9.3
				Valine	18.8	18.4	11.6
Minerals composition				**Dispensable amino acids**			
Calcium	5.8	6.2	17.7	Alanine	15.6	15.4	9.0
Potassium	13.6	13.0	8.4	Aspartic acid	25.2	25.2	15.5
Sodium	0.21	0.68	<0.05	Cysteine[b]	10.0	8.6	5.6
Phosphorus	10.6	10.5	7.1	Glycine[b]	19.0	18.4	11.1
Chloride	0.60	0.66	0.29	Glutamic acid	61.8	61.2	36.9
				Proline	22.7	22.2	13.0
				Serine	14.8	14.9	8.8
				Tyrosine	10.2	10.3	6.5

[1]NDF, neutral detergent fibre; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.
[2]Semi-essential amino acids for poultry.
[3]Each value represents the mean of six replicates (six birds per replicate), measured from d 21 to 24 post-hatch.

Table 3. 4 Total tract retention of nitrogen (N), apparent metabolisable energy (AME, MJ/kg DM) and N-corrected apparent metabolisable energy (AMEn, MJ/kg DM) of canola meal 1 (CM1), canola meal 2 (CM2), and canola seed (CS) in broiler starters[1]

	N retention	AME	AMEn
Canola meal 1	52.2b	8.08ab	7.22ab
Canola meal 2	53.8b	7.82b	6.78b
Canola seed	60.9a	11.17a	10.29a
Pooled SEM	1.49	1.09	1.028
Probabilities, P ≤	0.002	0.085	0.059

Means in a column not sharing a common letter (a-b) are significantly different ($P < 0.05$).
[1]Each value represents the mean of six replicates (six birds per replicate), measured from d 21 to 24 post-hatch.

Table 3. 5 Standardised ileal protein and amino acid (AA) digestibility coefficients of canola meal 1 (CM1), canola meal 2 (CM2), and canola seed (CS) in broiler starters[1,2]

Component	CM1	CM2	CS	SEM	P-value
Crude protein	0.711[b]	0.711[b]	0.749[a]	0.012	0.052
Indispensable AA					
Arginine	0.775[b]	0.791[b]	0.831[a]	0.009	0.002
Histidine	0.756[b]	0.766[b]	0.815[a]	0.011	0.004
Isoleucine	0.701[b]	0.694[b]	0.753[a]	0.012	0.007
Leucine	0.741[b]	0.732[b]	0.781[a]	0.011	0.018
Lysine	0.663[b]	0.666[b]	0.761[a]	0.013	0.001
Methionine	0.827[a]	0.816[a]	0.835[a]	0.010	0.397
Phenylalanine	0.752[ab]	0.747[b]	0.782[a]	0.012	0.102
Threonine	0.615[b]	0.626[b]	0.707[a]	0.017	0.003
Valine	0.672[b]	0.677[b]	0.733[a]	0.012	0.006
Dispensable AA					
Alanine	0.715[b]	0.714[b]	0.758[a]	0.013	0.045
Aspartic acid	0.622[b]	0.633[b]	0.739[a]	0.015	0.001
Cysteine[b]	0.652[b]	0.647[b]	0.791[a]	0.015	0.001
Glycine[b]	0.693[b]	0.692[b]	0.775[a]	0.012	0.001
Glutamic acid	0.810[b]	0.812[b]	0.836[a]	0.007	0.024
Proline	0.639[b]	0.642[b]	0.732[a]	0.017	0.002
Serine	0.663[b]	0.667[b]	0.720[a]	0.015	0.026
Tyrosine	0.650[b]	0.672[ab]	0.707[a]	0.013	0.018

Means in a row not sharing a common letter (a-b) are significantly different ($P < 0.05$).
[1]Each value represents the mean of six replicates (six birds per replicate). Ileal digestibility measurements were made on d 29 post-hatch.
[2]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by feeding protein-free diet: crude protein, 12.05; Arg, 0.44; His, 0.23; Ile, 0.41; Leu, 0.63; Lys, 0.46; Met, 0.14; Phe, 0.40; Thr, 0.79; Val, 0.61; Ala, 0.43; Asp, 1.03; Cys, 0.32; Gly, 0.51; Glu, 1.03; Pro, 0.68; Ser, 0.65; and Tyr, 0.39.
[3]Semi-indispensable amino acids for poultry.

Table 3. 6 Standardised ileal protein and digestible amino acid (AA) contents (g/kg) of canola meal 1 (CM1), canola meal 2 (CM2), and canola seed (CS) in broiler starters[1,2] (as received)

Component	CM1	CM2	CS	SEM	P-value
Crude protein	293[a]	279[b]	176[c]	4.240	0.001
Indispensable AA					
Arginine	16.39[a]	16.89[a]	11.08[b]	0.189	0.001
Histidine	7.14[a]	7.16[a]	4.67[b]	0.095	0.001
Isoleucine	10.06[a]	9.43[b]	6.60[c]	0.164	0.001
Leucine	18.44[a]	17.57[b]	11.79[c]	0.262	0.001
Lysine	13.17[a]	12.37[b]	10.14[c]	0.253	0.001
Methionine	6.93[a]	6.14[b]	4.05[c]	0.073	0.001
Phenylalanine	10.81[a]	10.28[b]	6.64[c]	0.153	0.001
Threonine	9.34[a]	9.50[a]	6.58[b]	0.237	0.001
Valine	12.60[a]	12.47[a]	8.49[b]	0.220	0.001
Dispensable AA					
Alanine	11.12[a]	11.02[a]	6.85[b]	0.189	0.001
Aspartic acid	15.67[a]	15.96[a]	11.47[b]	0.366	0.001
Cysteine[c]	6.55[a]	5.56[b]	4.41[c]	0.145	0.001
Glycine[c]	13.14[a]	12.74[a]	8.58[b]	0.210	0.001
Glutamic acid	50.03[a]	49.69[a]	30.88[b]	0.385	0.001
Proline	14.49[a]	14.25[a]	9.50[b]	0.352	0.001
Serine	9.82[a]	9.91[a]	6.37[b]	0.204	0.001
Tyrosine	6.61[a]	6.90[a]	4.63[b]	0.122	0.001

Means in a row not sharing a common letter (a-c) are significantly different ($P < 0.05$).
[1]Each value represents the mean of six replicates (six birds per replicate). Ileal digestibility measurements were made on d 29 post-hatch.
[2]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg DM intake), determined by feeding protein-free diet: crude protein, 12.05; Arg, 0.44; His, 0.23; Ile, 0.41; Leu, 0.63; Lys, 0.46; Met, 0.14; Phe, 0.40; Thr, 0.79; Val, 0.61; Ala, 0.43; Asp, 1.03; Cys, 0.32; Gly, 0.51; Glu, 1.03; Pro, 0.68; Ser, 0.65; and Tyr, 0.39.
[3]Semi-indispensable amino acids for poultry.

3.4 Discussion

Soybean meal is generally considered as the standard protein source in poultry diets. However, due to its low productivity and high demand, SBM is becoming an expensive protein source. Many possible potential protein sources are being explored to replace SBM to meet the animal's dietary nutrient requirements with proper formulation. This has motivated nutritionists to evaluate economically viable alternative protein sources such as CS and CM. Therefore, it is essential to determine the nutrient composition, ME value and SIDC of CP and AA of these alternative ingredients to formulate a proper diet and meet animal requirements. The present study was conducted to evaluate the nutrient composition, AME, SIDC and standardised ileal digestible amino acid (AA) content of two CM samples (CM1 and CM2) and CS in broiler chickens.

Protein is considered as the most valuable component in oilseed meals. Canola meal is reported to have lower CP content than that of SBM (Khajali and Slominski, 2012). The CM samples used in the present study had CP content of 41 and 39%, respectively, which is significantly lower than the range reported for SBM (44-48%; NRC, 1994). However, the proximate analysis of both CM samples used in the current study were within the range reported in the previous literatures (Blair et al., 1986; NRC, 1994). Bell and Keith (1991) reported similar contents of CP (37.9 to 43.5%), but lower NDF (22.6 to 24.5%) and higher GE content (20.02 to 20.53 MJ/kg) in seven different CM crushing plants, compared to samples used in current study. Adewole et al. (2016) studied the variation in chemical composition of CM from eleven CM and reported CP content of 40.5 to 43.2% and NDF content of 26.3 to 33.5%. Spragg and Mailer (2007) also reported the CP content of 37.3 to 47.6% and NDF content of 24.3 to 30.5% in 8 CM samples from Australian crushing plants.

The lysine content observed in current study were lower compared to those reported by Bell and Keith (1991) and Spragg and Mailer (2007). Bell and Keith (1991) reported 2.4 to 2.51% and Spragg and Mailer (2007) reported 2.18 to 2.40% lysin content. In Adewole et al. (2016) study lysine content of CM samples were 2.0 to 2.9%. These variations in the chemical composition of CM were attributed to the differences in the agronomic characteristics, genetic factors, geographical locations and environmental factors during crop development, harvesting conditions, and processing of the seed and meal (Bell and Keith, 1991). Chen et al. (2015) and Gorski (2015) evaluated nutritional composition of CM from different varieties of CS and found higher CP content in CM derived from CS containing less fibre and more CP and AAs. Azam et al. (2019) compared CM samples from two different locations (Multan and Sukkar) and reported that variability exists among sample of same protein source from different locations. Woyengo et al. (2010) reported differences in chemical composition of CMS and CME due to method used for extraction of oil. Although, processing facility uses similar equipment, but processing conditions may not be consistent among processors and this may cause some variability in the nutritive composition of CM (Spragg and Mailer, 2007).

The proximate and nutrient composition of CS in current study were in the range with previous studies. Toghyani et al. (2017) reported CP value of 17.2-24.1% and NDF content of 24.8-34.4% in CS samples. In contrast, Lee et al. (1995) observed lower CP content in CS (20.4%) than those reported in the present study. However, the analysed nutrient composition differed markedly between CM and CS sample.

In current study, protein was the main component followed by energy in all three samples. The protein content in both the CM1 and CM2 sample was almost 40% more compared to CS sample. The energy content of CS compared to CM samples was higher

due to the high oil content in CS, which was in agreement with the finding reported by Barbour and Sim (1991). The TDF content was high in both the CM samples with almost 90% of TDF being in the form of IDF. Liu e al. (1995) attributed high TDF content in CM to the small size of CS. Smaller size of oilseed makes the seed difficult to dehull and nearly impossible to separate it out during extraction process.

Potassium was the major mineral in the CM samples followed by P, while Ca was high in CS sample. The reported K concentration for CM was in range of the values reported earlier by Bell and Keith (1991). The P content of CM fell within the range of previous studies as well. Chen et al. (2015) and Adewole et al. (2016) stated the P content of 1.30-1.50% and 1.10-1.28% for CM samples. However, Ca content in CS in present study was above the range of values reported by Assadi et al. (2011) and Toghyani et al. (2017). Calcium concentration in CS samples in their studies were 0.9% and 0.30-0.45%, respectively. These differences in Ca content of seeds were attributed to the agronomic and climatological conditions and mineral content of the soil.

In avian species, AME is corrected for zero nitrogen retention by subtracting 36.54 KJ per gram nitrogen retained in the body. In current study, the AMEn of CM1, CM2 and CS were 12, 15 and 8.5% lower than their AME values, respectively. Like chemical composition, variations were observed in the AME and AMEn values of CM and CS samples. The AME and AMEn were highest in CS, followed by CM1 and CM2. The AMEn value of the CS was almost 47% greater than the mean AMEn of both the CM samples, which may be associated to high oil content present in the CS. The AMEn value of CS in present study was lower than the AMEn value reported by Assadi et al. (2011) and Toghani et al. (2017). They reported an AMEn of 18.55 and 19.53 MJ/kg, respectively, for CS. This variation may be explained, in part, by the high fat content of 429 g/kg and 487 g/kg of their CS samples, respectively. In addition, Kiiskinen and Huida

(1984) compared the AMEn content of ground and whole CS and found improvement in crude fat digestibility and higher AMEn content in ground CS. The AMEn content of CM samples were in range with the values reported by Woyengo et al. (2010) for CMS. Adewole et al. (2017b) also reported the AMEn value of 7.07-8.5 MJ/kg for CM. However, the determined range of AMEn in current study were lower than the values reported by Rostagno et al. (2011), Gorski (2015) and Azam et al. (2019). Rostagno et al. (2011), Gorski (2015) and Azam et al. (2019), reported an AMEn of 9.2, 7.29-8.08 and 9.90-10.02 MJ/kg, respectively, for CM samples.

Moreover, CM1 had higher AMEn compared to CM2, which may be related to the lower NDF and slightly higher CP content in CM1. In agreement to the present finding, Jia et al. (2012) and Chen et al. (2015) observed that CM containing high protein and low fibre content yielded higher AMEn values in broilers. Similarly, Zhang and Adeola (2017) found that high concentration of fibre and anti-nutrients, lower the AMEn content of CM. In addition, Adewole et al. (2017b) observed variations in the AMEn content among CM samples with 6 different canola processing plants and related these differences to the differences in the chemical composition such as fat, sucrose, NDF and ADF content.

The differences in the CP content were reflected in AA contents, with CM1 having the highest total AA content (325.6 g/kg) followed by CM2 and CS sample, 318.1 and 196.9 g/kg, respectively. In all the three samples, among indispensable AA, leucine content was highest and methionine content was lowest while, among dispensable AA, glutamic acid was highest. Both the CM samples contained highest concentration of AA content compared to CS samples. This clearly indicates that the CM is a better source of AA, compared to CS. Amino acid concentrations of the tested CM and CS sample were within the range reported in the literature (Adewole et al., 2016; Azam et al., 2019).

Adewole et al. (2016) observed variation in the AA content of CM from different processing facilities, with methionine having the lowest concentration ranging from 6.40 to 7.20 g/kg while, glutamic acid with highest concentration ranging from 64.8 to 70.7 g/kg. However, higher values of AA content in CM were reported by Bell and Keith (1991) and Chen et al. (2015), which highlighted the variability that exists between canola cultivars grown in different geographical locations and exposed to variable environmental factors during seed development. In addition, Adewole et al. (2017a) stated that variation in AA content of CM may be expected due to the difference in canola crushing conditions and variation in the dietary fibre fraction between the CM samples. Moreover, the current study showed that vast variations exists between the AA content of CM and CS. It was observed that the mean AA content in CS was 39% less than the mean AA content of CM samples. The AA content in CS in current study were within the range reported by Toghyani et al. (2017), who analysed the AA composition of eleven CS samples, with methionine having lowest (3.7-5.2 g/kg) and glutamic acid having the highest (26.1-40.1 g/kg) concentration.

A significant difference was observed in the SIDC of protein and AA in all the dietary treatments. Between CM1, CM2 and CS sample, the CS sample had highest SIDC for protein and AA. Among all the AAs, SIDC of threonine was lowest, while that of glutamic acid was highest in all the three samples. However, despite some variations for individual AA, the determined SIDC of AA in CM were lower than those reported by Woyengo et al. (2010), Adewole et al. (2017b) and Azam et al. (2019). To the best of our knowledge, the study by Park et al. (2019) is the only one reporting the SIDC of AA in CS in broilers, and the values were higher than those reported in the present study. In general, CS showed the higher SIDC of CP and AA compared to both CM samples. Khajali and Slominski (2012) stated that the reduced digestibility in CM is, at least in

part, related to processing conditions. During thermal processing of oilseed meal, Maillard reactions products are produced due to formation of covalent bond between a free re-active NH2 group of amino acid (especially lysine and arginine) and the carbonyl group of a reducing sugar, which affect the total protein and AA digestibility (Friedman, 1996). Anderson-Hafermann et al. (1993) reported that the apparent digestibility of lysine in CM decreased by 5% (from 0.85 to 0.80) during desolventisation and toasting of CM. Likewise, Newkirk et al. (2003) found similar results, wherein the apparent lysine digestibility coefficient reduced significantly from 0.87 to 0.79 in desolventised and toasted CM. Adequate heat treatment of CM is essential for destruction of anti-nutritional factors, but excessive heating of meal can influence digestibility of nutrients, especially protein and AAs (Friedman, 1996). Wiseman (2013) stated that heat processing mainly influences nutrient digestibility and shows minimal effect on the content of nutrients present in the meal. Adewole et al. (2016) observed a negative relationship between NDF and digestibility of nutrients in CM, higher crude fibre and lignin content in CM decreased the nutrient digestibility of CM. In addition, Huang et al. (2005) suggested that the digestibility of CM is also influenced by age of broiler chickens. Higher AA digestibility value was observed for broiler chickens at 42 d of age than those for 14 d of age, which means a linear relationship exists between AA digestibility and age of the bird. Therefore, in practical feed formulation, these differences in AA digestibility can produce a significant effect when high inclusion levels of CM are used.

The determined standardised ileal digestible protein and AA content of both the CM samples were within the range reported by Adewole et al. (2017b). However, the standardised ileal digestible AA content of both the CM was lower compared to the values reported for CMS by Woyengo et al. (2010). Differences in the standardised ileal digestible AA content among different CM samples can be reflected through the

differences in their total AA content and SIDC of AA (Adewole et al. 2017b). Adewole et al. (2017b) observed that the variations in the digestible AA content between CM samples might be due to early stages of Maillard reactions which resulted in the formation of aldose derivative of AA by Amadori rearrangement. These products are not effectively digested but yield AA after acid hydrolysis during AA analysis (Mauron, 1981).

To the best of our knowledge, there are apparently no studies which evaluated standardised ileal digestible AA content of CS in broiler. In the present study, there was a significant difference in digestible AA content of CM and CS. The standardised ileal digestible content of protein and AA were lower in CS compared to both the CM samples. A similar finding in soybean samples was observed by Ravindran et al. (2014), who reported that the standardised ileal digestible protein and AA content were lower in full fat soybean than those for SBM due to the diluting effect of high fat content. The standardised digestible AA content values of CM and CS can be used when formulating broiler diets as the nutritive value of feed ingredients is more precisely evaluated and match closely with the bird's requirements than those based on total AA concentrations.

3.5 Conclusion

In conclusion, the present evaluation showed that both CM and CS are attractive feed ingredients for poultry. The data indicates that the nutrient composition, AMEn, SIDC of protein and AA and standardised ileal digestible AA content varies between CM and CS samples. CM has greater digestible AA content, and hence, it is a better source of protein than CS. However, CS contains more fat and AMEn, and therefore offers as a potential energy source to poultry diets. The standardised ileal digestible AA content and AMEn values of CM and CS can be used to precisely formulate feed and predict animal performance.

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