Chapter 1

General Introduction

In intensive poultry production systems, feed is the most important input and accounts for 60-70 % of total variable production costs. In this vein, protein is one of the main components of poultry feed and is one of the major contributors to the finished feed cost. The protein fraction of diet is mainly supplied by meals from oil seeds such as soybean and canola. Canola meal (CM) is a co-product of the canola oil crushing industry, which is considered as a possible cost-effective protein substitute for protein sources in broiler chicken diet and trades around 60-70 % of soybean meal (SBM) price. The global production of rapeseed, including canola varieties, ranks second among oilseed crops worldwide (USDA 2011). Canola is now the third-largest crop (after wheat and barley) produced in Australia; its production has grown from 1.9 to 3.6 million metric tons per year over the past 5 years, making the country one of the world's largest exporters of canola seed (Seberry et al., 2013). Canola meal has lower protein content than SBM but the meal compares favourably with SBM with regard to amino acid profile. Canola meal contains more methionine and cysteine but less lysine and arginine than SBM (Khajali and Slominski, 2012). Canola meal is also a good source of available calcium, iron, manganese, selenium, choline and many of the B vitamins (Newkirk, 2009).

Despite the good balance of essential amino acids in CM, the lower and more variable digestibility of energy and amino acids compared to SBM, and the presence of some anti-nutritional factors limit the inclusion rate of CM in monogastric animals (Khajali and Slominski, 2012). The major anti-nutritional factors (ANF) found in CM include fibre (non-starch polysaccharides (NSP), lignin-oligosaccharides, with associated polyphenols, glycoproteins), phenolic compounds, phytic

acid and glucosinolates (Bell, 1993). The high fibre content of CM has been reported to lower both apparent metabolisable energy (AME) and crude protein (CP) digestibility of the meal (Newkirk et al., 2003). The variation in canola meal quality has been attributed to agronomic conditions, oil extraction methods, temperature and moisture during processing (Spragg and Mailer, 2007). Although the initial physical extraction of oil from the meal is common to all crushing plants, there are alternatives to processing to obtain the final product, including cold press, expeller press and solvent extraction methods (Spragg and Mailer, 2007). The method of extraction and the heat and mechanical input during each stage of processing result in different quality characteristics and residual oil level in the meal. The temperature applied to condition the seeds, and also in the desolventising and toasting stages of solvent extraction procedures, can help to deactivate the myrosinase enzyme in the seed, to prevent the break down of glucosinolates into toxic metabolites. However, the temperature could also have a negative impact on protein quality and digestibility due to the occurrence of Maillard reaction involving amino acids, especially lysine (Newkirk and Classen, 1999).

Exogenous enzyme supplements have routinely been used to improve the uniformity of diets when ingredient variability is perceived to be a factor limiting predictability of broiler performance. Enzyme supplementation can improve nutrient digestibility and consequently allow for a higher inclusion level of ingredients that contain ANF. Enzymes such as carbohydrases can help weaken cell wall integrity and release encapsulated nutrients such as starch and protein bodies, and consequently increase the availability of these nutrients for further digestion and absorption. Poor quality ingredients have been shown to be more responsive to dietary enzyme supplements than higher quality ingredients (Bedford et al., 1998). The high phytic acid, fibre and NSP content of CM, make the meal a vulnerable ingredient to exogenous carbohydrases and phytase supplements. Several studies have targeted the use of dietary enzymes to improve protein, carbohydrate and

phosphorus digestibility in CM, and CM based diets (Slominski and Campbell, 1990; Simbaya et al., 1996; Ravindran et al., 1999; Kocher et al., 2000; Meng and Slominski, 2005; Woyengo et al., 2010a; Jia et al. 2012). However, the use of cellulase and NSP degrading enzymes to improve canola meal nutritional value has not always been consistent and successful to show significant improvements. Improved metabolizable energy (ME) and performance parameters of broiler chicks fed a CM based diet have been reported in response to a multi-carbohydrase (Woyengo et al., 2010a; Jia et al. 2012). In an *in vitro* study Meng and Slominski (2005) also reported that combination of multiple carbohydrase enzymes improve the digestibility of NSP in CM based diets, but this improvement in digestibility was not reflected in performance parameters. In a later study Meng et al. (2006) found enzyme supplementation of canola meal diets improved FCR, NSP digestion and ME content of the diet.

Metabolizable energy has long been used to express energy utilization in poultry. The effect of enzyme application in broiler diets, particularly carbohydrases, on performance indices and ME has been inconsistent. Some studies have reported improved productivity by carbohydrase supplementation of the diet through improved ME (Bedford and Schulze, 1998; Meng et al., 2005). However, various reports on carbohydrase application have also shown improved growth performance without any changes in the ME value of the diet (Hong et al., 2002; Wu et al., 2004). The ME system in poultry only accounts for the energy loss being measured in excreta, but the energy loss through heat production (HP) is not considered (Noblet et al., 2015). In this regard, accounting for net energy (NE) might be a more sensitive measure of energy utilisation than ME in response to dietary enzyme supplementation of poultry feed because it takes into account the efficiency of utilization of ME for growth (Olukosi et al., 2008). Fibre-rich feedstuffs have been reported to affect energy partitioning of the diet through increased HP and consequently decreased NE of a feed (Noblet et al., 1994; Barekatain et al., 2014). The nutritive value and ME content of

CM, as a high fibre protein meal, for broiler chickens have been well investigated (Perez-Maldonado, 2002; Classen et al., 2004; Woyengo et al., 2010b) but the information concerning the possible impact of CM particularly at a high level of inclusion, on NE value of broiler diets is sparse.

Currently the reports on the inclusion rate of canola meal in broiler diets are inconsistent. A great number of reports and research are based on previous studies having used strains of broilers that are different from today's commercial birds for meat production. Modern strains of broiler chickens have genetically been improved to have higher feed efficiency and to reach market weight in a shorter time compared to one or two decades ago. It seems that these genetically improved strains of broiler chicks are likely to respond differently in utilization of CM. Some of the earlier reports suggest that CM could fully replace SBM in broiler diets without any detrimental effects on broiler performance (Lesson et al., 1987; Borcea et al., 1996; Kocher et al., 2000, 2001; Ramesh et al., 2006). However, recent studies have shown that dietary inclusion levels of CM beyond 20 % negatively affect productive traits (Mushtaq et al., 2007; Woyengo et al. 2011; Khajali et al., 2011; Gopinger et al., 2014). The depressed growth rate of birds offered CM-based diets in almost all the studies mentioned above has been associated with reduced feed consumption. However it is not been investigated and verified whether this reduced feed consumption is the cause or the effect of the depressed growth rate observed in birds fed a CM-based diets.

Considering the dearth of data in the literature, the hypotheses tested in this thesis were:

1. Energy and amino acid digestibility of expeller-extracted CM would differ depending on the processing conditions to which the meal is subjected during oil extraction procedures.

2. Having an expeller-extracted CM with lower heat damaged protein, and with its ME content, crude protein and amino acid digestibility already being determined through bio-assays will allow

its higher inclusion rate in substitution of SBM in broiler chickens diet, and exogenous dietary enzymes will improve growth performance of birds fed CM-based diets. It was also hypothesized that CM as a rich fibre protein meal can influence partitioning of energy in broiler chicken diets.

3. The depressed feed consumption of broiler chickens fed CM-based diet *per se* accounts for the lower growth rate compared to birds offered SBM-based diets, and increasing dietary digestible amino acids in the diet would attenuate the deterioration in growth rate.

Accordingly, the broad objectives of the work reported within this thesis were to determine and compare the impact of processing conditions including conditioning temperature (90, 95 or 100^{o} C) and screw torque (high or low) on chemical composition, AME and standardized ileal amino acid digestibility (SID AA) of ECM for broiler chickens. The relationship between quality parameters (fibre composition, neutral detergent insoluble nitrogen, reactive lysine, glucosinolate) with AME and SID AA content of ECM samples will also be investigated. In a subsequent experiment, based on digestibility values obtained for energy and AA in previous trials, the effect of high inclusion rate of ECM and exogenous supplemental enzymes (multi-component carbohydrase and mono-component protease) on performance, nutrient digestibility, and partitioning of energy in broiler chickens will be investigated. The last experiment is designed to determine to what extent growth depression with high CM diets observed in broiler chickens could be attributed to the decrease in feed intake in a pair-feeding experiment. The effect of higher levels of digestible amino acids in CM-based diets on performance, meat yield, blood serum metabolites and ceca microbiota composition will also be evaluated.

Chapter 2

Literature Review

2.1 Canola Seed

Canola is the term applied to new cultivars of rapeseed (*Brassica napus,* Argentinan canola, or *Brassica campestris*/*rapa*, Polish canola), which have genetically been bred and selected to have low levels of erucic acid (less than 2%) and glucosinolates (less than 30 μmol g^{-1}) and higher levels of crude protein in the final meal (38-40%) (Canola Council of Canada, 2009). Canola seed is crushed to yield 40 to 48 % oil, with the remainder being used as canola meal (CM). As the oil from canola seed is accepted as a healthy oil by consumers, canola seed production has been boosted in recent years worldwide and canola is considered to be the second most available source of edible oil in the world (Aider and Barbana, 2011).

Table 2.1 Average oil content and major fatty acid composition of Australian canola seed

Quality Parameter	Australian Mean
Oil content (%)	43.4
Oleic acid concentration (C18:1), % in oil	63.0
Linoleic acid concentration (C18:2), % in oil	18.2
Linolenic acid concentration (C18:3), % in oil	9.8
Erucic acid concentration (C22:1), % in oil	< 0.1
Saturated fatty acid concentration, % in oil	7.3
Iodine value	112.5

Data adapted from Seberry et al. (2013)

Australia is recognized as one of the major canola seed producers in the world, with an estimated canola seed production of around 1.6 million tonnes per year. Canola seed is 1-2 mm in diameter and contains approximately 43-48 % oil and 20% crude protein (Canola Council of Canada, 2009). The ώ-3 fatty acid content (alpha-linolenic acid C18:3) of canola oil is higher compared to the other vegetable oil, and thus the oil is regarded as one of the most heart-healthy oils and has been

reported to reduce cholesterol levels and lower serum concentration of triglyceride when consumed by human (Davis and Melina, 2000). Table 2.1 shows the major fatty acid contents of Australian canola seed.

Table 2.2 Chemical composition of Australian canola seed (as is basis, N = 12, 2015 harvest)

Composition %	Max	Min	Mean	CV %
DM	95.0	94.1	94.3	0.38
Crude protein	24.1	17.2	21.5	9.19
Crude fat	47.9	40.8	42.9	5.86
Crude fibre	19.3	15.2	16.9	7.24
Neutral detergent fibre	34.4	24.8	29.2	12.63
Acid detergent fibre	27.1	18.7	22.1	11.40
Tannins	0.42	0.36	0.39	4.95
Calcium	0.45	0.30	0.37	12.50
Phosphorus	0.80	0.55	0.65	12.13
Sulphur	0.44	0.30	0.38	10.23
Ash	4.26	3.63	4.04	7.53
Glucosinolate (μmole g^{-1})	13.1	7.0	10.7	17.66
Gross energy (kcal kg^{-1})	7126	6941	7016	0.94

Data adapted from Australian Poultry CRC report, Barekatain et al. (2016)

Intact canola seed is considered as an economic feed ingredient, which has a well-balanced source of protein (19 to 22%) and oil (approx. 45%) for poultry (Meng et al., 2004, 2006). Tables 2.2 and 2.3 highlight some of the chemical composition and amino acid profile of Australian canola seeds, respectively. Recently, the use of canola seed in poultry diets has become common following an increase in price of fat and oil for use in broiler feed due to increased demand from biofuels and human foods. However, the level of inclusion of full fat canola seed compared to canola meal is typically below (8%) the amount for complete removal of supplemental oil content of the diet as there are concerns over glucosinolate and isothiocyanate content of the finished feed.

Table 2.3 Analyzed amino acids composition of Australian canola seed (as is basis, N = 12, 2015 harvest)

AA profile	Max	Min	Mean	CV %
Indispensable amino acids				
Arginine	1.46	1.01	1.28	9.80
Histidine	0.73	0.53	0.67	7.84
Isoleucine	0.99	0.73	0.90	8.17
Leucine	1.7	1.21	1.52	9.10
Lysine	1.56	1.14	1.42	8.41
Available Lysine[1]	1.54	1.12	1.40	8.49
Methionine	0.52	0.37	0.46	8.79
Phenylalanine	0.99	0.72	0.89	8.78
Threonine	1.01	0.78	0.94	7.27
Dispensable amino acids				
Alanine	1.01	0.74	0.92	8.61
Aspartic acid	1.65	1.24	1.48	8.28
Cysteine	0.62	0.41	0.53	10.46
Glutamine	4.01	2.61	3.48	10.68
Glycine	1.15	0.83	1.03	8.72
Proline	1.49	0.96	1.25	11.73
Serine	0.94	0.70	0.86	7.82
Tryptophan	0.33	0.22	0.27	13.02
Tyrosine	0.73	0.55	0.67	7.31
Valine	1.24	0.91	1.13	8.44
Total amino acid	22.39	15.97	20.00	8.91

[1]Available Lysine, determined via carpenter assay: fluoro dinitro benzene reaction with epsilon amino group of lysine, Data adapted from Australian Poultry CRC report, Barekatain et al. (2016)

2.2 Canola Meal

Canola seed is crushed to yield canola oil with the residue being used as canola meal (CM). Canola oil, besides being used as a healthy oil for human consumption, is also used for producing biofuel, which should increase the availability of CM for animal feed (Thacker and Petri, 2007). Canola meal, the co-product of oilseed crushing industry, contains approximately 36-40% crude protein on as-fed basis, making it a good protein source in poultry diets. Canola meal has a good balance of essential amino acids and is higher in methionine and cysteine on a protein basis than SBM (Canola Council of Canada, 2009). However, CM energy and digestibility of amino acids is typically lower than SBM. In a general comparison between CM as a protein meal in poultry diets

and soybean meal (SBM) as the most common plant protein source in poultry diets, CM contains less protein (36-40 vs. 42-48 %), more crude fibre (10-12 vs. 2-3.5%) and less metabolizable energy (2,230 kcal kg^{-1} vs. 2,440 kcal kg^{-1}) (Table 2.4).

Table 2.4 Average chemical composition of canola meal compared to dehulled soybean meal

Chemical composition	Canola meal	Soybean meal
Dry matter, %	90.0	90.0
Crude protein, %	36.5	45.6
Ether extract, %	3.6	1.3
Gross energy, kcal/kg	4,450	4,850
Carbohydrates, %		
Starch	2.5	0.7
Sucrose	6.0	6.2
Sugar	7.7	6.9
Oligosaccharide	2.5	5.3
Fibre, %		
Crude fibre	11.6	5.4
Non-starch polysaccharide	18.0	17.8
Neutral detergent fibre	26.0	12.0
Acid detergent fibre	18.2	7.5
Total dietary fibre	31.7	21.8
Amino acids, %		
Arginine	2.04	3.23
Lysine	2.00	2.86
Threonine	1.57	1.74
Methionine	0.74	0.65
Cysteine	0.85	0.67
Tryptophan	0.48	0.64
Minerals, %		
Calcium	0.7	0.3
Phosphorus	1.2	0.7
Magnesium	0.6	0.3
Sodium	0.08	0.01
Potassium	1.29	2.0
Vitamins, mg/kg		
Biotin	1.0	0.3
Folic acid	2.3	1.3
Niacin	169.5	29.0
Pantothenic acid	9.5	16.0
Riboflavin	3.7	2.9
Thiamine	5.2	4.5

Data adapted from Bell, (1993); Simbaya, (1996); Khajali and Slominski, (2012)

The good balance of CM essential amino acids and its fairly high protein efficiency ratio make the meal a high quality protein ingredient for poultry diets (Sarwar et al., 1984). The contents of essential minerals such as potassium, sulphur, calcium and iron in canola meal are relatively high and it is considered a good source of selenium and phosphorus as well (Bell et al., 1999). However, the presence of phytic acid and high fibre in the meal reduces the bioavailability of most of the minerals (Canola Council of Canada, 2009). Table 2.5 shows the mineral content of 26 Australian CM samples.

Table 2.5 Mineral content of Australian canola meal (as is basis)

Mineral	Mean	Min	Max	SD
Calcium (%)	0.56	0.45	0.67	0.056
Phosphorus (%)	0.96	0.79	1.19	0.116
Phytate-P (%)	0.83	0.63	1.01	1.084
Phytate-P in total P (%)	85.9	67.0	95.0	-
Chlorine (%)	0.10	0.06	0.13	0.019
Potassium (%)	1.26	1.05	1.44	1.010
Sulphur (%)	0.62	0.50	0.70	0.057
Magnesium (%)	0.47	0.38	0.55	0.052
Copper (mg/kg)	3.9	3.0	4.7	0.39
Iron (mg/kg)	138	78	457	78.4
Manganese (mg/kg)	52	40	61	6.5
Zinc (mg/kg)	45	36	54	4.9

Data adapted from Spragg and Mailer, (2007)

A highly negative correlation between protein and dietary fibre contents in CM derived from black seeded canola (*Brassica napus*), has been reported by Jiang et al. (1999). Various approaches have been taken to reduce the fibre content, increase the protein content, and improve the nutritive value of canola meal. The greatest attempt has been made towards the development of new varieties of the seed to minimize the presence of ANF through breeding techniques (Slominski et al., 2012). *Brassica juncea* (a new cultivar of canola) has slightly higher protein, but lower ADF and NDF compared to rapeseed (Newkirk et al., 1997). Yellow seeded *B. napus* (another new canola cultivar) has been reported to have reduced fibre and increased protein content (Simbaya et al., 1995; Slominski, 1997). Earlier studies with *Brassica rapa* (*campestris*) canola have demonstrated

that the black and yellow seeds differed significantly in oil, protein, and fibre contents, with yellow seeds containing more oil and protein and less fibre (Slominski, 1997).

Table 2.6 Chemical and nutrient composition of canola meal derived from different varieties of seed (% of DM)

Component	Black-seeded (*B. napus*)	Yellow-seeded (*B. napus*)	Yellow-seeded (*B. juncea*)
Crude protein	43.8	47.4	49.8
Crude fibre	1.8	1.7	1.6
None starch polysaccharide	20.2	18.7	20.0
Total fibre	30.1	24.1	25.8
ADF	20.6	12.6	-
NDF	25.7	21.1	-
Starch	0.4	0.4	0.3
Ash	7.3	7.0	7.2
Total phosphorus	1.30	1.24	1.23
Phytate phosphorus	0.78	0.80	0.78
Nonphytate phosphorus	0.52	0.44	0.45
Minerals	0.7	0.3	0.3
Glucosinolate (µmol g^{-1})	30.7	18.8	20.0

Data adapted from Newkirk, (1997); Slominski et al. (1999) and (2012)

Slominski et al. (2012) compared the chemical and nutritive composition of CM derived from black and yellow canola seed (*Brassica napus*) and yellow-seeded *Brassica juncea*. Both yellow-seeded *B. napus* and *B. juncea* meals had higher protein (49.8 and 47.4 % vs. 43.8 %), lower crude fibre (24.1 and 25.8 % vs. 30.1 %) and glucosinolate content (17.1 and 71.2 µmol g^{-1} vs. 27.1 µmol g^{-1}) compared to black-seeded *B. napus* meal. The authors concluded that, the new yellow-seeded *B. napus* canola had superior quality characteristics compared with that of its black-seeded counterpart as manifested by its higher protein and sucrose (energy) contents and lower dietary fibre content. Table 2.6 summarizes the chemical composition of canola meals derived from different varieties.

2.3 Anti-nutritional Factors of Canola Meal

The presence of several ANF limits high inclusion rate of CM in poultry diets. The main ANF found in CM include high dietary fibre (including NSP, lignin with associated polyphenols, glycoproteins), glucosinolates, phytate and phenolic components such as tannins and sinnapine.

2.3.1 Fibre

Edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the small intestine of monogastric animals, with partial or complete fermentation in the large intestine, are defined as dietary fibre. It includes polysaccharides, oligosaccharides, lignin, and associated substances (Van Der Kamp 2004). The polysaccharide content of feed ingredients include starch and NSP. Different feedstuffs have different amounts and structures of NSP (Bach Knudsen 1997; McNab and Boorman, 2002). The soluble NSP concentrations in SBM and CM are broadly similar (63 in SBM and 55 g kg^{-1} in CM) but differ in that SBM contains elevated concentrations of soluble galactose whereas CM contains higher proportions of soluble arabinose and xylose (Meng et al., 2005; Cowieson et al., 2016). However, the insoluble NSP concentration of CM is higher than that of SBM (123 vs 92 g kg^{-1} in SBM) and this is particularly true for arabinose and uronic acids (Bach Knudsen, 1997). The total dietary fibre in CM is considerably higher (up to 3 times) than that of SBM (Bell, 1993). The small seed size (2 mm diameter) and greater oil content are possibly the main reasons contributing to the high percentage of fibre in the meal portion (Bell, 1993). Canola meal contains cellulose (4-6%), non-cellulosic polysaccharide (13-16%), lignin and polyphenols (5-8%) and proteins and minerals associated with fibre fraction as the major fibre components (Simbaya, 1996). Previous studies demonstrated that yellow-seeded meals have a lower amount of fibre than black-seeded meals. For instance, the ADF and NDF contents of *B. juncea* (12.7% and 21.1%) are lower than those (20.1% and 25.7%) of *B. napus* (Newkirk et al., 1997). The high fibre content of CM lowers the metabolizable energy content,

which is referred to as one of the main factors restricting high inclusion rate of CM in broiler diets (Khajali and Slominski, 2012). The high fibre content of the diets increases the transition rate of nutrients in the digestive tract and consequently reduces nutrient digestibility (Imbeah and Sauer, 1991).

Table 2.7 Carbohydrate components of Australian canola meal (as is basis)

Component (%)	Average
Starch	5.1
Sugars	6.7
Sucrose	6.2
Fructose +glucose	0.5
Cellulose	4.5
Oligosaccharides	2.2
Non-starch polysaccharides	15.7
Soluble NSP's	1.4
Insoluble NSP'S	14.4
Crude fibre	11.7
Acid detergent fibre	16.8
Acid detergent lignin	5.1
Neutral detergent fibre	20.7
Total dietary fibre	32.3

Data adapted from Australian Oilseeds Federation, (2015)

The high NSP content of CM, particularly the water-soluble fraction, may affect nutrient digestibility, both directly due to physical hindrance and indirectly due to physiological changes in the gut, such as increased digesta viscosity, as high viscosity interferes with enzymatic digestion of the nutrients and their subsequent absorption (Huismann et al., 1998; Meng et al., 2005). Furthermore, NSP can cause fermentation in the GIT of broilers and be depolymerized to soluble NSP and resulting in significant losses of energy in the form of heat and volatile fatty acids (VFA), which affects the availability of energy to animals (McNab and Boorman, 2002). However, part of the energy in form of VFA can be reabsorbed in ceca and serve as a source of energy for the bird. Another problem associated with dietary fibre is the binding activities which are associated with some components of fibre such as pectin which can interact with dietary cations and decrease their digestibility (Kirk and Oram, 1981). The pectin content of CM is around 33.0 % higher than

that of SBM, being around 9% of the meal (Huismann et al., 1998). The pectin remains largely intact during the digestion process in the small intestine, increasing digesta viscosity and reducing digestion and absorption of the nutrients (Langhout and Schütte, 1996).

2.3.2 Glucosinolates

A large group of sulphur-containing secondary plant metabolites, known as glucosinolates (Gls) can be found in all the economically important varieties of Brassica. The main structural feature found in different classes of glucosinolates is β-D-thioglucose group (Figure 2.1). Aliphatic (85%) and indolyl (15%) are the most common types of Gls found in CM (Newkirik et al., 2003). Gluconapin, glucobrassicanapin, progoitrin and napoleiferin are the major aliphatic Gls present in CM of which progoitrin is the major Gls responsible for the anti-nutritional effects of the meal (Simbaya, 1996). Various environmental conditions during the growing phase of canola such as dryness, humidity or hot conditions can influence the Gls content of canola seed and thereby the meal. The Gls content is generally higher in CM varieties grown under tropical environment than those in temperate regions. In drought conditions, lack of water increases the synthesis of amino acids and sugars, as precursors of Gls, resulting in an increase content of Gls (Tripathi and Mishra, 2007). The ingestion of considerable amounts of Gls has been reported to cause some negative effects on animal health (Tripathi and Mishra, 2007). The adverse effects are greater in monogastric animals than in ruminants; also, young animals are more sensitive to Gls than older animals (Tripathi and Mishra, 2007).

Biologically, Gls are inactive molecules and intact Gls do not cause any harmful effects to animals, but their degradation products are biologically active. The break down products of Gls either by enzyme myrosinase or by non-enzymatic factors such as heat, low pH, anatomical and physiological structure of the gastrointestinal tract (GIT), digesta transit time and microbial activity cause harmful effects to animals (Bell 1993).

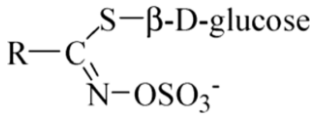

Figure 2.1 General structure of glucosinolate

Figure adapted from Tripathi and Mishra, 2007

Depending on the nature of Gls, reaction condition and concentration, the break down products-thiocyanate, isothiocyanate, oxazolidinethione (goitrin) and nitriles may be formed and impair not only feed intake (due to their bitter taste) and growth performance, but also affect thyroid function thereby inhibiting thyroid hormone production and impairing liver and kidney function (Bell 1993; Campbell and Schone, 1998; Mullan et al., 2000). Glucosinolate derivatives (higher than 30 μmole g^{-1} of the meal), can lead to hypothyroidism in poultry; this can cause a reduction in thyroid hormones and changes the ratio between triiodothyronine (T3) and thyroxin (T4) in the blood (Adibmoradi and Pedram, 2007). Increased thyroid stimulating hormones resulting in thyroid enlargement in broilers fed CM diets containing more than 30 μmole g^{-1} Gls has been reported by Schöne et al. (1993). Although new varieties of canola have lower Gls content, they still have enough goitrogenic activity resulting in significant increases in thyroid weight. Adibmoradi and Pedram (2007) showed that, feeding modern CM with low levels of Gls (less than 30 μmole g^{-1}) at between 5 and 20% of diet could affect morphology of the thyroid gland. They reported increased thyroid gland weight at more than 5 % CM in broiler diets without negative effects on bird performance.

Another toxic effect of Gls on animals is an increased incidence of leg problems with feeding diets containing high levels of glucosinolates. Summers et al. (1992) indicated that the sulphur level of Gls can cause leg problems in broilers as sulphur interferes with calcium absorption (Canola Council of Canada, 2009). However, various breeding techniques have been employed to breed canola seeds with low Gls content and currently the meals derived from new varieties of rapeseed

known as 00-rapeseed or canola contain less than 30 µmol g^{-1} Gls (Tripathi and Mishra, 2007). Table 2.8 shows the Gls content and composition of different varieties of canola meal. The average Gls content for Australian canola seed has been reported to be around 10 µmol g^{-1} (McFadden 2006; Seberry et al., 2013; Figure 2.2). Previous research has indicated that the maximum level of glucosinolate in young broiler chickens should not exceed 4 µmol g^{-1} of feed (Mawson et al., 1994).

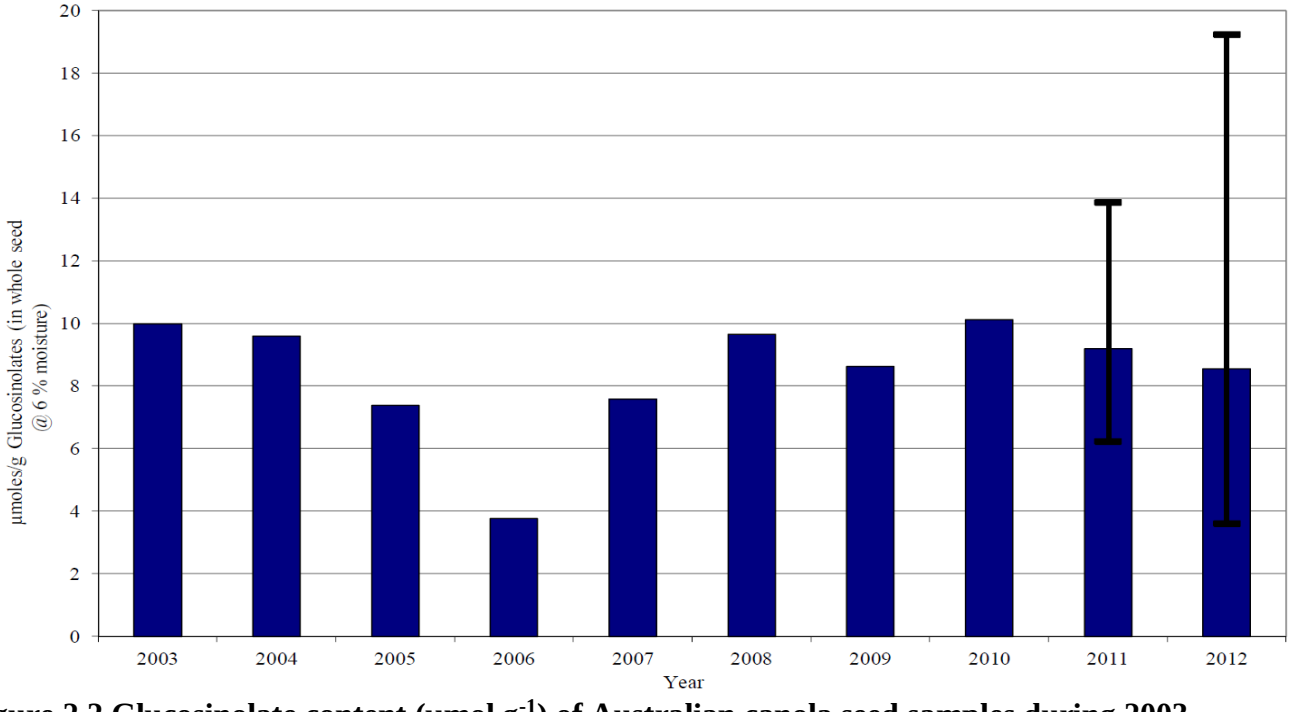

Figure 2.2 Glucosinolate content (µmol g^{-1}) of Australian canola seed samples during 2003 to 2012 harvest.

Figure adapted from Seberry et al., 2013

Various treatment methods have been applied to remove or reduce Gls content and to minimize Gls-associated deleterious effects on animal health and production. Most of these methodologies include hydrolysis or decomposition of glucosinolate before supplementation of the meal in animal feeds (Tripathi and Mishra, 2007). Some of the methods include microwaving, micronization and extrusion, treatment with water and metal solutions, solid state fermentation, heat and water treatment. Each treatment may result in different degrees of Gls reduction and decomposition.

Water soaking seems a promising method in removal of Gls because of its economic feasibility and ease (Tripathi and Mishra, 2007).

Table 2.8 Glucosinolates content and composition of different varieties of canola meal (µmol g^{-1} dry matter).

Item	Black *B. napus*	Yellow *B. juncea*	Yellow *B. napus*
Total glucosinolate	11.4	21.7	11.4
Allyl (Sinigrin)	-	1.2	-
3-Butenyl (Gluconapin)	2.3	16.1	1.8
4-Pentenyl (Glucobrassicanapin)	0.5	1.2	0.5
2-OH-3 butenyl (Progoitrin)	4.6	1.8	4.6
2-OH-3-pentenyl (Napoleiferin)	0.1	0.1	0.2
3-Indolylmethyl (Glucobrassicin)	0.3	-	0.5
4-OH-indolylmethyl ((Hydroxyglucobrassicin)	3.5	1.3	3.8

Data adapted from Simbaya, (1996)

2.3.3 Phytate

Phytic acid (*Myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate*) is the major storage form of phosphorus in plants. Phytate phosphorus consists of 60-80% of the total phosphorus in grains and their by-products (Ravindran et al., 1995). Phytate is considered an ANF because it forms insoluble complexes with proteins and several minerals such as Ca, Fe, Zn, Mn and Mg, which consequently reduce their bioavailability (Cabahug et al., 1999). The proportion of phytate phosphorus to the total phosphorus content of Brassica meals ranges from 36 (Broz and Ward, 2007) to over 70% (Summers et al., 1983). The higher concentration of phytic acid in CM (26.24 in CM vs. 16.67 g kg^{-1}in SBM) is of great concern with respect to the nutritional availability of most minerals, particularly calcium and phosphorus (Ravindran et al., 1999). Nwokolo and Bragg (1997) reported Zn, Ca and Mg deficiency syndromes in chickens due to the phytate present in rapeseed. Also, as a result of high phytate content of CM the bioavailability of phosphorus has been estimated to be around 30 to 50% of the total phosphorus in CM (Enami, 2011). Summers et al. (1990) demonstrated a depressing effect of phytic acid on the availability of calcium, phosphorus, magnesium and zinc from CM in poultry. It is recognised that phytate phosphorus in plant

materials is normally positively correlated with total phosphorus content, the same trend has also been found in canola seed and subsequently CM (Spragg and Mailer 2007, Figure 2.3).

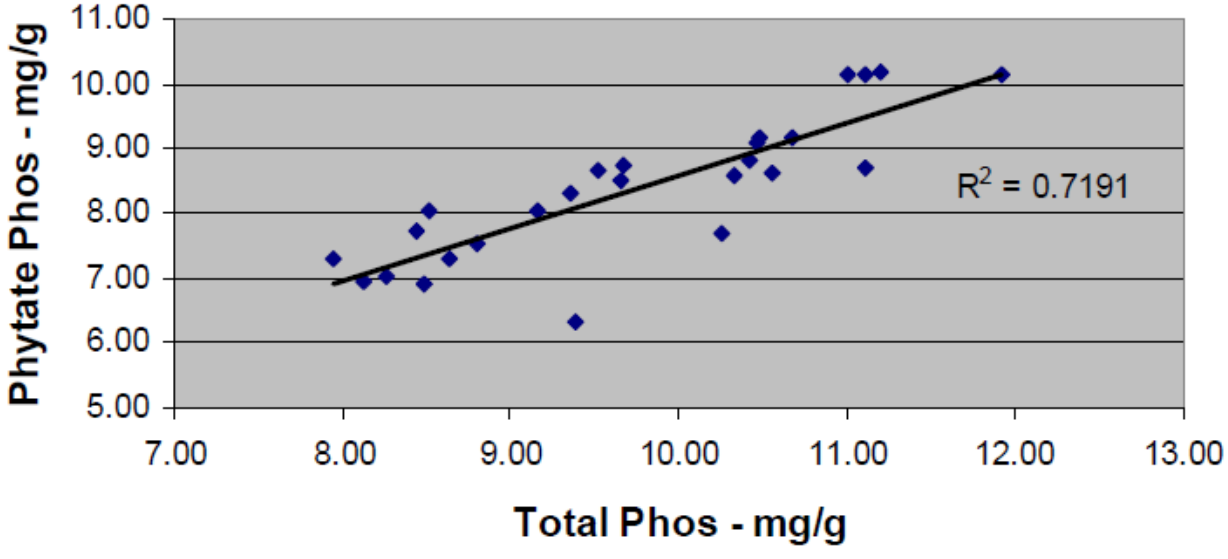

Figure 2.3 Relationship between total and phytate phosphorus in canola meal.

Figure adapted from Spragg and Mailer, 2007

Myo-inositol phosphate esters are bound to magnesium and potassium in mineral-phytate complexes (Lott et al., 2000). Mineral analyses of CM samples has shown a trend for positive correlations between phytate phosphorus and calcium (R^2 = 0.404), potassium (R^2 = 0.292) and magnesium (R^2 = 0.354). There was no correlation found between phytate phosphorus and meal protein, crude fibre, ADF or NDF (Spragg and Mailer, 2007).

It is generally accepted that endogenous secretion of phytase and phytase activity occurring in the crop, stomach and small intestine is negligible (Nahashon et al., 1994). Dietary exogenous phytase is added to poultry feed to both release the phosphorus and to reduce the negative impact of phytate on bioavailability of other nutrients. Several microbial phytase products are now commercially available, which are usually derived from *Aspergillus niger*, which is a 3-phytase and *Peniophora lycii* and *Escherichia coli*, which are 6-phytases (Butani and Parnerkar, 2015).

2.3.4 Sinapine

The choline ester of sinapic acid is known as sinapine. The sinapine content of CM is approximately 0.6 to 1% on dry matter basis, which may result in a fishy odour in eggs from some brown-shell layers (Khajali and Slominski, 2012). Sinapine can be hydrolysed to trimethylamine, and when deposited in the egg produces a fishy odour (Butler et al., 1982). Sinapine is also responsible for the bitterness of CM, which makes the meal less palatable for animals; nevertheless no major negative effects of sinapine have been reported in broiler performance. The bitter taste of sinapine may not affect the feed intake and growth rate in broiler chickens; interestingly, when purified sinapine extract was added to broiler chicken diets an improvement in metabolizable energy content and protein digestibility was observed (Qiao and Classen, 2003).

2.3.5 Tannins

Tannins are complex polyphenolic compounds that are anti-nutritional in canola meal. Tannins are usually subdivided into hydrolysable and condensed portions mostly found in the seed coat (Theander et al., 1977). The total amount of tannins in canola hulls has been determined to range from 1.9 to 6.2% of oil-free hulls (Naczk et al., 2000). Higher levels of condensed tannins have been reported in brown-seeded canola compared to yellow-seeded varieties (Theander et al., 1977; Canola Council of Canada 2009). Insoluble tannins predominate in canola/rapeseed hulls and comprise from 70 to 96% of the total tannins present (Khajali and Slominski, 2012). As reviewed by Khajali and Slominski (2012), tannins have the potential to bind protein and proteolytic enzymes in GIT tract, thereby reducing the protein digestibility. Mansoori and Acamovic (2007) illustrated that tannic acid added to broiler chicken diet was responsible for the negative effect on growth performance as it affected the bioavailability of amino acids in the diets, especially methionine, histidine and lysine. They concluded that the poor digestibility of amino acids could

be due to an increased excretion of inactivated enzyme and glycoproteins from gastrointestinal mucosa of broiler chickens. Nevertheless, since a great portion of tannins in canola cell walls are water insoluble, the ANF of tannin in CM would be small (Khajali and Slominski, 2012).

2.3.6 Erucic Acid

Rapeseed oil contains a large amount of erucic acid (C22:1n-9 monounsaturated fatty acid). Consumption of large amounts of erucic acid oil has been found to cause some health risk for humans (Ecke et al., 1995). Diets high in erucic acid (more than 10%) have been shown to result in some adverse effects on rats, such as, accumulation of erucic acid in tissue lipids, myocardial lesions and reduction in growth rate (Green and Innis, 2000). New cultivars of canola seeds have been bred to contain less than 2% erucic acid. Inclusion of low erucic acid canola meal has been found to have no significant negative effect on growth performance and productive traits of broiler chickens (Khajali and Slominski, 2012).

2.3.7 Electrolyte Balance

Optimal dietary electrolyte balance is crucial for optimum broiler performance and livability of the birds since inappropriate dietary ratios of minerals can affect the acid-base balance in the body. In relation to acid-base homeostasis, the physiological importance of the combined intakes by broiler chickens of Na, K, and Cl, or the dietary electrolyte balance (**DEB**), has been recognized for some time (Nesheim et al., 1964; Mongin, 1981). As discussed by Mongin (1981), DEB may be defined by the following formula: DEB (mEq kg^{-1}) = $Na^+ + K^+ - Cl^-$, and a value in the order of 250 mEq kg^{-1} is deemed satisfactory for optimal broiler growth and litter quality. Canola meal has a lower electrolyte balance (307 meq kg^{-1}) than SBM (504 meq kg^{-1}) because of its lower potassium content (11.4 vs. 19.6 g kg^{-1}) (Khajali and Slominski, 2012). There is a positive correlation between feed intake and cation-anion balance of the diet for broiler chicks (Summers

1995). Decreased feed intake by broilers offered diets high in CM could to some extent be related to dietary cation-anion balance of the diet; accordingly, increasing dietary cations such as Ca^{++} or K^{+} in the diet may alleviate this effect (Canola Council of Canada, 2009). The high sulphur content of CM compared to SBM (0.65 vs. 0.44%) can cause leg abnormalities in broilers because it interferes with calcium absorption (Summers et al., 1990; Khajali and Slominski 2012). The sodium content of CM is also lower than that of SBM (Bell and Keith, 1991). Increasing dietary NaCl from 2.5 to 5.0 g kg^{-1} of diet has been reported to alleviate the negative impact of CM-based diets on retarded growth rate of broiler chickens (March ,1984). Also Mushtaq et al. (2007) showed that correcting the DEB of CM-based diets can improve growth performance of broiler chickens. As suggested by Newkirk (2009) and Khajali et al. (2011) supplementation of potassium carbonate to CM-based diets can correct the DEB of diets and consequently overcome the problems associated with high CM in diet such as reduced feed intake.

2.4 Canola Meal Processing Conditions

Processing conditions during oil extraction are key factors that influence CM nutritional quality, particularly for mono-gastric animals. There are three main methods commercially practiced to extract the canola oil content. These methods include solvent extraction, expeller and cold pressed extractions (Mailer, 2004). Although the initial physical extraction of oil from the meal is common to all crushing plants, as mentioned earlier, there are alternatives to processing to reach the final product. These include:

2.4.1 Cold Press Oil Extraction

In this method of extraction, canola seed is not pre-conditioned prior to oil extraction. Although there is no added heat in this method, temperatures of around 65°C can be generated within the expeller due to frictional forces.

2.4.2 Expeller Press Oil Extraction

In this method, canola seed is heat conditioned prior to the expeller press and the expeller is operated to optimise oil extraction. This can generate meal temperatures of up to 135°C for the brief period when seed cake is passing through the press. Some crushing plants operate double pass systems where seed cake is reprocessed, to increase oil recovery.

2.4.3 Solvent Extraction

This method of oil extraction involves a two-stage process, starting with an initial expeller extraction operating at 100-120°C, resulting in the production of a seed cake with approximately 20% oil. This then undergoes solvent oil extraction using hexane, and then a final desolventising and toasting process at temperatures of 100-115°C to remove the solvent residues of the meal.

The key steps in oil extraction procedures involve:

Conditioning and flaking: initially the seed is preheated and then passed through roller mills at about 35^{o}C to break the seed. This "flaking" operation ruptures cell walls and reduces the seed to flakes without damage to the oil. The flakes are cooked at 80-105^{o}C for 15-20 minutes to complete cell breakdown and to reduce the viscosity of the oil. This also provides the opportunity for endogenous myrosinase enzyme to be hydrolysed and thus prevent breakdown of glucosinolates in the meal to undesirable products which would affect the quality of the oil and meal. The moisture content of around 10% is critical to the hydrolysis of this enzyme and the temperature must be raised quickly to around 90^{o}C.

Physical extraction: flaked canola is passed through a continuous screw press to remove around 60% of the oil. Excessive pressure and temperature need to be avoided to prevent damage to the product. Generally the temperature does not exceed 105^{o}C although in some cases it may reach 130^{o}C for a short period of time.

Solvent Extraction: solvent extraction is used to remove the residue of oil which constitutes around 20% of the cake. The solvent is generally hexane and is recovered for re-use following separation of the oil from the meal. The meal at this stage is virtually free of oil and is referred to as "marc".

Desolventising and toasting: in the desolventizer-toaster, the residue of the solvent is removed by the use of steam and the meal is finally dried for about 20 minutes at around 105^{o}C. The final product is solvent-free and contains about 10% moisture and less than 1% oil.

Additives: after the desolventizer-toaster operation, some of the by-products of solvent extraction, including gums and soap stocks, may be remixed with the meal, theoretically increasing the meal quality and energy value. This also has the effect of increasing the oil content and darkening the colour of the meal.

Both expeller and solvent oil extraction processes apply significant levels of heat to canola seed as it is being processed. The level of heat damage is influenced by the temperatures in operation, whether meal is double processed from expeller plant operation and the degree of heat and time applied during desolventising and toasting. Generally, during the process of solvent extraction, CM is subjected to higher moisture (15-18 %) and moderate temperature (95-115^{o}C), whereas in the expeller press method, the meal is subjected to less than 12% moisture and higher temperature up to 140^{o}C (Canola Council of Canada, 2009). One of the key factors that influence CM feeding quality for broiler chickens is the processing conditions and temperatures during oil extraction. The solvent extraction method is common and the most efficient method of extracting oil from seed, resulting in a meal with less than 3% residual oil, while the oil content in expeller- and cold-pressed meals can be up to 10 and 15 %, respectively. The residual oil level has the greatest impact on energy content of canola meals. Practically, ME values of around 2,000, 2,400 and 2650 kcal kg^{-1} DM are standards for solvent, expeller and cold-pressed canola meals, respectively.

The current techniques applied in oilseed crushing plants have been reported to cause the least impact on the nutritive value of CM (Beach and Hickling, 2010). While the temperature during processing can be helpful in deactivating myrosinase enzyme in the seed to avoid break down of glucosinolates into toxic metabolites, there are some adverse effects of temperature during processing. Spragg and Mailer (2007) showed that there was a decline in glucosinolate content with increasing levels of heat during processing, with both expeller and solvent extracted meals showing reduced glucosinolate levels compared to cold pressed meals (Figure 2.4). They also showed a decreasing trend toward sinapine with heat processing and reported the greatest reduction to occur at the desolventising and toasting stage. Amino acids, especially lysine, which are sensitive to heat, might be damaged during processing. This affects the quality of the protein in the meal (Newkirk and Classen, 1999). A decrease in protein quality and digestibility has been reported due to the Maillard reaction involving amino acids, especially lysine (Newkirk and Classen, 1999). Newkirk (2009) indicated that high temperatures during oil extraction process can reduce lysine content from 6.03 to 5.50 % of crude protein, and decrease lysine and crude protein digestibility from 88 to 79% and 81% to 76%, respectively.

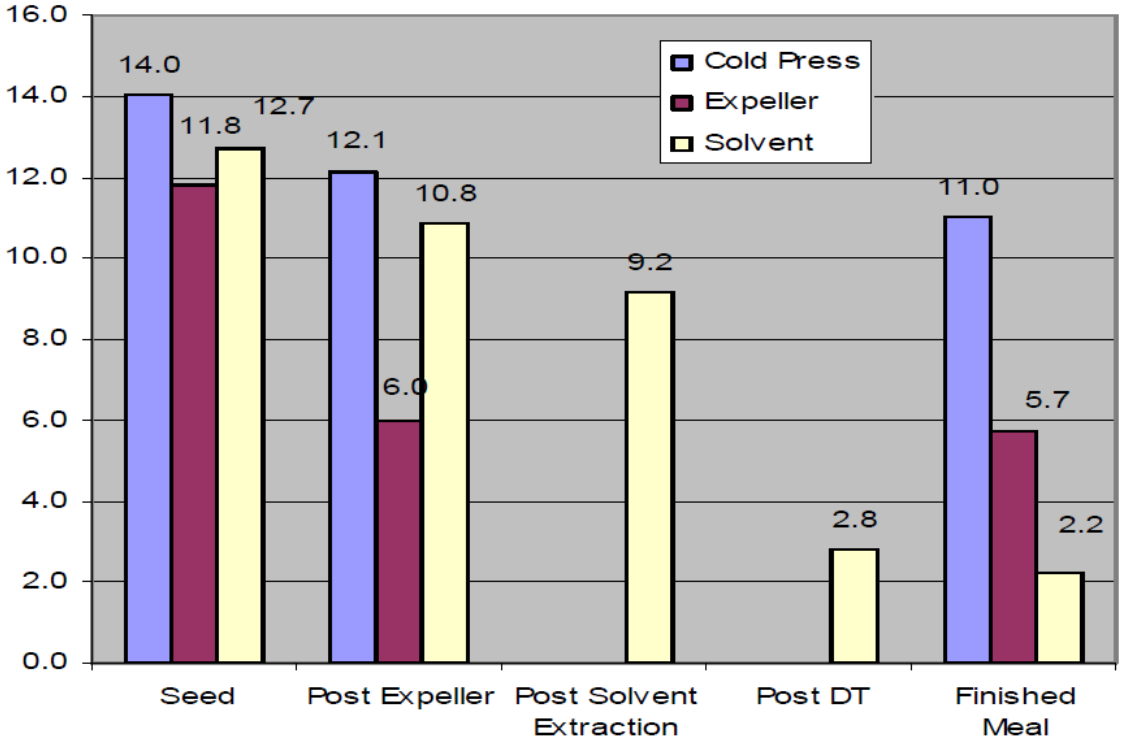

Figure 2.4 Glucosinolate content (µmol g^{-1}) of canola seed, in process and finished canola meal samples (as-is basis)

Figure adapted from Spragg and Mailer, (2007)

Higher total lysine contents in crude protein have been observed with cold pressed meals, compared to both expeller and solvent extracted meals with lower total lysine levels in finished meal (Spragg and Mailer, 2007).

Table 2.9 Total, reactive and revert lysine content of cold pressed, expeller and solvent extracted canola meals (as is basis)

	Cold-pressed	Expeller-extracted	Solvent-extracted
Total Lysine (g kg^{-1})	20.68	19.58	20.17
Reactive Lysine (g kg^{-1})	17.80	15.46	15.42
Non-reactive (% of total)	13.9	21.0	23.50

Data adapted from Spragg and Mailer, (2007)

Processing temperature has also been shown to modify the composition of phytate in finished meal. Cold pressed canola meal was shown to have less IP_5 phytate bound phosphorus than expeller or solvent meals (Spragg and Mailer, 2007; Figure 2.5).

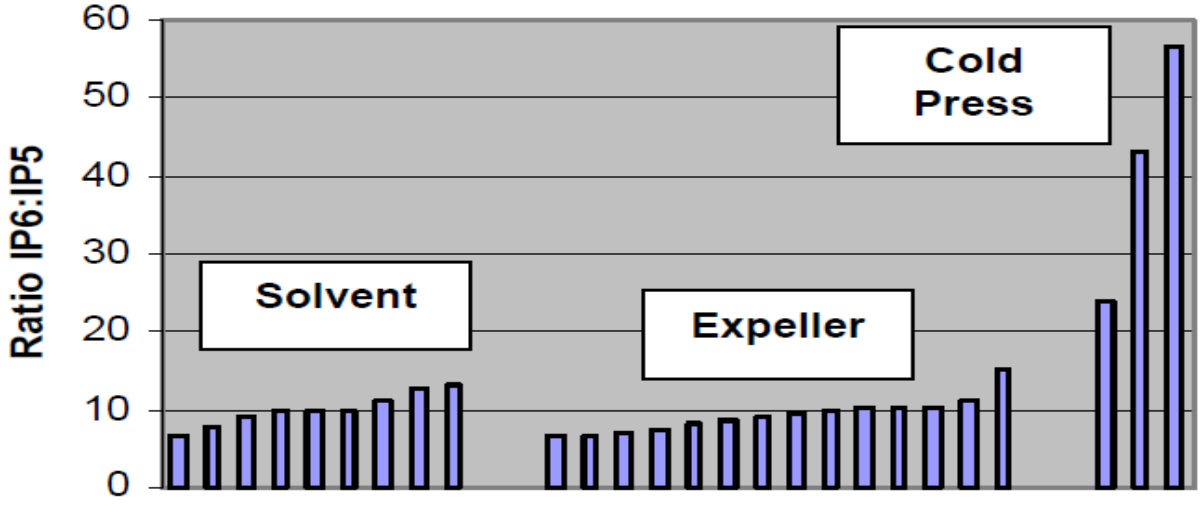

Figure 2.5 Ratio IP_6: IP_5 phytate phosphorus for canola meal samples from solvent, expeller or cold press processing

Figure adapted from Spragg and Mailer, (2007)

Spragg and Mailer (2007) did not report any significant effect of processing condition on total protein and fibre contents and composition of canola meal. Nonetheless, they showed that crude protein in the finished meal is positively correlated with canola seed protein content (R^2 = 0.68) and negatively correlated with canola seed oil content (R^2 = -0.66). Recent data from our group indicated that different oil extraction methods may not significantly affect the lysine content of the finished meal but the heat damage index and consequently lysine digestibility is markedly different between cold-pressed, expeller and solvent-extracted CM samples (Swick and Wu, 2015). Cold-pressed canola meal was found to have the lowest heat damage index and the greatest lysine digestibility compared to expeller and solvent-extracted meal (Table 2.10). Also as indicated in Table 2.11, the concentrations of other amino acids were not markedly different between cold-pressed and expeller-extracted meals, but the digestibility values were considerably higher in cold-pressed meal samples.

Table 2.10 Range of dry matter, protein, lysine, lysine digestibility and heat damage index (HDI) in cold-press (CCM), expeller (ECM) and solvent (SCM) extracted canola meal samples obtained commercially in Australia

Meal type	Dry matter, g/kg	Crude protein g/kg, (as is)	Crude protein, g/kg (DM)	Lysine, g/kg, (as is)	Lysine digestibility %	HDI
ECM						
Average	923	356	386	19.4	83	98
Min	903	327	357	18.3	81	96
Max	967	423	437	23.1	85	99
N	20	20	20	20	20	20
SCM						
Average	905	378	418	21.2	86	95
Min	895	365	403	20.3	85	95
Max	909	387	426	21.8	87	96
N	6	6	6	6	6	6
CCM						
	913	335	364	20.2	92	90

Data adapted from Swick and Wu (2015)

As illustrated in Figure 2.4 the heat applied during processing can deactivate the enzyme, myrosinase, and reduce the glucosinolate content of the meal, but excessive heating during processing can result in reduced digestibility of some amino acids. Work by Newkirk et al. (2003) revealed that canola meal is a uniform and high-quality product until it enters the desolventizer-toaster phase. During this stage crude protein and lysine digestibility and lysine content were significantly reduced. This research by Newkirk suggests that the commonly used temperatures in the desolventizer-toaster stage of 107°C cause some protein damage. Processing with a maximum temperature of 100°C in the desolventizer-toaster significantly increases lysine digestibility to similar levels found in SBM. Swick and Wu (2015) showed that measuring the available lysine content of canola meal can be a worthwhile indicator of meal nutritional quality. They reported that body weight gain and feed conversion ratio of birds fed diets with CM samples of different quality were positively correlated with available:total lysine ratio of meal samples ($R^2 = 0.74$ and $R^2 = 0.58$, respectively).

Table 2.11 Level and variation of methionine, cysteine and threonine content and digestibility[1] in cold-pressed canola meal vs. expeller-pressed canola meal

Item	Cold-pressed	Expeller-pressed
Total Methionine g/kg		
Avg	7.37	7.48
CV, %	2.7	4.5
N	12	5
Met digestibility, %		
Avg	93.9	83.6
CV, %	0.9	0.8
N	12	5
Total cysteine		
Avg	9.74	9.20
CV, %	3.2	6.6
N	12	5
Cys digestibility, %		
Avg	90.0	81.0
CV, %	1.1	0.9
N	12	5
Total threonine g/kg		
Avg	15.6	16.1
CV, %	2.0	3.0
N	12	5
Thr digestibility, %		
Avg	83.6	76.6
CV, %	1.0	0.8
N	12	5

[1]Values were determined using NIR, Evonik Amino-prox
Data adapted from Swick and Wu (2015)

2.5 Dietary Enzymes

Dietary enzymes have long been used to improve poultry productive traits, particularly when ingredient variability is perceived to be a factor limiting predictability of broiler performance. Feed enzymes are mainly dominated by carbohydrases and phytase (Adeola and Cowieson, 2011). The mode of action of exogenous enzymes is described by a couple of possible mechanisms: breakdown of ANF present in feed ingredients, elimination of nutrient encapsulation effect thus increasing availability, breakdown of specific chemical bonds in raw materials not cleaved by endogenous enzymes, releasing more nutrients, and complementation of the enzymes produced by

young animals (Bedford and Schulze, 1998). Therefore, the principal reason to use exogenous enzymes is that they improve digestibility of a number of nutrients, specific to the enzyme employed, and as a result enable the use of less digestible, cheaper and most frequently more sustainable ingredients in the ration. The anticipated improvement in productive traits from exogenous enzyme application has been reported to be closely associated with an improvement in nutrient and energy utilization (Olukosi et al., 2008). As an example, NSPases are principally designed to act on NSP and spare more energy from the diet energy, however, there is some evidence suggesting that amino acids are also spared by using exogenous carbohydrases, particularly in viscous grain diets (Bedford 2014).

The most common fibre-degrading enzymes used in pig and poultry diets are β-glucanase, xylanase, and cellulase. Generally, a wide range of positive effects of supplementing carbohydrase enzyme has been shown in poultry. Different feedstuffs have different amounts and structures of fibre; as a result, the selection of enzyme for each feed ingredient for improving the nutritional value of feed is really important (McNab and Boorman, 2002). Xylanase and β-glucanase are considered to be effective by increasing digestibility and improving birds' performance in cereal based diets (Pettersson et al., 1990). It has been shown that the use of a xylanase in both pig and poultry rations leads to a constant 16% improvement in digestibility of the undigested fraction of each amino acid (Cowieson and Bedford, 2009).

Bedford et al. (1998) reported that enzymes had stronger impact on poor quality ingredients than on higher quality ingredients. Several studies have targeted the use of dietary enzymes to improve protein, carbohydrate and phosphorus digestibility in CM, and CM-based diets (Slominski and Campbell, 1990; Simbaya et al., 1996; Ravindran et al., 1999; Kocher et al., 2000; Meng and Slominski, 2005; Woyengo et al., 2010a; Jia et al., 2012). Ravindran et al. (1999) reported an average increase of 2% in amino acid digestibility in canola meal diets supplemented with 1,200

unit kg^{-1} of phytase. However, the uses of carbohydrases (cellulase or NSPases) to improve CM nutritional value have not always been consistent and successful to show significant improvements. Meng and Slominski (2005) examined the effects of adding a multi-enzyme complex (xylanase, glucanase, pectinase, cellulase, mannanase and galactonase) to broiler diets. The enzyme combination increased total tract NSP digestibility of canola meal but no improvements were observed in nutrient digestibilities or animal performance. In a later study Meng et al. (2006) found that enzyme supplementation of CM diets improved FCR, NSP digestion and ME content of the diet. Some studies have reported improved productivity by carbohydrase supplementation of the diet though improved ME (Jeroch et al., 1995; Bedford and Schulze, 1998). However, there are also reports of improved growth performance by carbohydrase application without any changes in the ME value of the diet (Hong et al., 2002; Wu et al., 2004). In purified diets containing 350 g kg^{-1} CM, enzyme products containing cellulase, hemicellulase, β-glucanase, xylanase and pectinase were not effective in enhancing growth, FCR or ME but were effective in reducing visocity of digesta contents (Kocher et al., 2000). Many nutritional and environmental factors may influence the response of birds to enzyme supplementation of diet including: cereal type, breed, growing environment, cereal inclusion rate, age of animal, pelleting of diet, age at first exposure to the enzyme and fat type and inclusion rate (Bedford 2014).

2.6 Inclusion Level of Canola Meal in Broiler Chickens Diet

In intensive poultry production systems, feed is the most important input and accounts for 60 to 70 % of total variable production costs. In this vein, protein is one of the main components of poultry feed and is one of the major contributors to the finished feed cost. Canola meal has always been regarded as a cost-effective substitute to soybean meal in broiler chickens diets. During the past few decades considerable research has been directed towards the determination of CM replacement value in poultry diets (Canola Council of Canada 2009). Nonetheless, the optimum

dietary inclusion rate of CM without negatively affecting productive performance of birds has not been well established thus far. The earlier reports suggest that CM could fully replace SBM in broiler diets without any detrimental effects on broiler performance (Lesson et al., 1987; Borcea et al., 1996; Kocher et al., 2001). Later on, Newkirk and Classen (2002) and Ramesh et al. (2006) also reported that canola meal can be effectively used in broiler diets up to 30% without negatively affecting performance as long as the diets are formulated on a digestible amino acid basis. However, recent studies have shown that inclusion levels of CM beyond 20 % in broiler chickens diet can negatively affect productive traits (Mushtaq et al., 2007; Khajali et al., 2011; Woyengo et al., 2011; Gopinger et al., 2014).

The quality characteristics of CM namely energy and amino acid digestibility and the content of ANF such as glucosinolate and high and complex dietary fibre are very variable (Khajali and Slominski, 2012). Variation in canola meal quality is related to agronomic conditions, oil extraction methods, temperature and moisture during processing (Spragg and Mailer, 2007). Apparently, the discrepancy in literature could be due to the differences in CM quality and processing conditions and also the basal diets used in different studies. In addition there is the possibility that modern strains of broiler chickens with higher genetic potential and different nutritional requirements are more susceptible and responsive to diet manipulation. However, according to Canola Council of Canada (2009), the maximum recommended levels of low glucosinolate CM for starter and grower phase in broiler production are 10 and 20 %, respectively due to low energy levels of the meal.

Although CM is rich in sulphur-containing amino acids (Met + Cys), the meal has lower Arg and Lys contents and generally lower essential amino acid digestibility than SBM (Khajali and Slominski, 2012). When high CM is included in the diet, such diets should be formulated based on digestible amino acids to meet the required levels of the essential amino acids. Another concern

about formulating broilers diets with high levels of CM is decreasing feed intake (Summers and Bedford, 1994). All the ANF present in CM such as high dietary fibre, glucosinolate, tannins, and the lower cation-anion balance of CM have been reported to account for the decreased feed intake of broiler chickens fed CM-based diets (Woyengo et al., 2011; Khajali and Slominski, 2012). Almost all the studies failing to demonstrate identical growth rate of birds fed CM-based diets compared to SBM-based diets have reported reduced feed consumption of birds by feeding high canola meal diets (Mushtaq et al., 2007; Woyengo et al., 2011; Khajali et al., 2011; Gopinger et al., 2014). Yet, no study has indicated whether this reduced feed consumption is the cause or the effect of depressed growth rate observed in broiler chickens fed CM-based diets.

2.7 Conclusion

Canola meal has long been considered as a cost-effective protein source in poultry diets, particularly in the countries where the meal is locally produced such as Canada and Australia. From the literature, it is evident that the level of glucosinolates and their breakdown products, heat treatment during processing and the residual oil content in the meal are the three main factors affecting the quality of CM. The impact of heat during processing on CM quality characteristics, particularly in solvent oil extraction procedures, has been well investigated. However, the possible impact of processing conditions on meal nutritional quality in expeller extraction operations still remains unclear, and needs further investigations. Processing conditions can to a great extent change the ANF, particularly glucosinolate content of CM, and also can significantly affect the content and availability of most of the amino acids of the meal. The ME and amino acid digestibility of canola meal is highly variable, and part of the growth depression observed by feeding high canola meal diets could be related to an inaccurate estimate of available nutrients in CM for the birds. Determination of ME and amino acid digestibility of differently processed canola

meal is essential in order to formulate diets efficiently, thereby helping to achieve predictable growth performance in broilers.

The optimum inclusion rate of CM and enzyme supplementation in CM-based diets for broiler chickens has yielded inconsistent results. Part of this inconsistency could be due to variability in CM nutritional composition and differences in processing conditions during oil extraction. Furthermore, it has been proposed that NE might be a more sensitive measure of energy utilisation than ME in response to dietary enzyme supplementation of poultry feed as it takes into account the efficiency of utilization of ME for growth. The ME system overestimates the energy value of fibrous and high-protein feedstuffs and underestimates the energy value of starch- or fat-rich ingredients. Canola meal as a fibrous protein meal may affect the NE value of a diet particularly at high inclusion rate.

The recent studies designed to increase inclusion rate of CM in broiler chickens diet have reported depressed feed intake and growth rate by inclusion levels higher than 20 %. Though the lower growth performance has been in part attributed to the lower feed consumption, but it is not clear whether this depressed feed consumption is the cause or the effect of lower growth rate observed in birds fed high CM diets.

The feeding studies outlined in this thesis were designed and conducted to address the dearth of research in the literature. The first two experiments (Chapters 3 and 4) addressed the impact of processing conditions of expeller canola meal on metabolizable energy, protein and amino acid digestibility values of the meal. The feeding study outlined in Chapter 5, aimed to determine the effect of high inclusion level of expeller canola meal in diet with exogenous enzyme supplementation on growth performance, digestibility of nutrients and partitioning of energy in male broiler chickens. The last Chapter (6), further determined to what extent feed intake reduction of broiler chickens offered CM based diets accounts for the depressed growth performance and if

this growth depression can be compensated for, by supplementing higher digestible of levels of essential amino acids in the CM based diets.

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Chapter 3

Apparent metabolizable energy value of expeller-extracted canola meal subjected to different processing conditions for growing broiler chickens

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This chapter has been published as:

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

Chapter 4

Standardized ileal amino acid digestibility of expeller-extracted canola meal subjected to different processing conditions for starter and grower broiler chickens

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Chapter 5

Performance, nutrient utilisation and energy partitioning in broiler chickens offered high canola meal diets supplemented with multicomponent carbohydrase and monocomponent protease

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This chapter has been submitted for publication in Poultry Science, and is currently under review. Submission date 05/05/2016

Chapter 6

Effect of elevated dietary amino acid levels in high canola meal diets on productive traits and cecal microbiota population of broiler chickens in a pair-feeding study

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Chapter 7

General Conclusion

Processing conditions of expeller canola meal [conditioning temperatures (90, 95 or 100 °C), screw torque (high or low)], had some marked effects on chemical composition of the meal and consequently metabolisable energy and amino acid digestibility values for broiler chickens (Chapters 3 and 4). Increasing temperature at high screw torque resulted in reduced glucosinolate content of the meal. Also, a decreasing trend in lysine levels was detected with increasing temperature at high screw torque, suggesting that there could have been some advanced Maillard reactions occurring possibly due to the interaction of high conditioning temperature and heat generated during the expelling process. The meal sample processed at low temperature and high screw torque appeared to be of the highest quality based on chemical analysis, with the highest reactive lysine (1.99 %) and lowest neutral detergent insoluble nitrogen (NDIN = 0.78 %). While the sample processed at low temperature and low screw torque had the highest NDIN and NDF, lowest crude protein, total lysine and reactive lysine (1.21, 26.48, 35.5, 1.79, and 1.62 %, respectively). With regard to energy digestibility meal samples subjected to medium processing temperature (95°C) and low screw torque during oil extraction had higher energy utilization values. The combined effect of high conditioning temperature and heat generated at high screw torque led to occurrence of some advanced Maillard reactions. Consequently, lower total and available Lys and decreased CP and TAA digestibility were observed for this sample. The significant negative correlations detected between AMEn and SID Lys values of the meal samples with NDF ($r = -0.93$, $r = -0.79$; $P = 0.001$, respectively) and NDIN content of the meals ($r = -0.87$, $r = -0.76$; $P = 0.001$, respectively) magnify the marked effect of fibre complexes of CM on its availability of nutrients.

Feeding high canola meals either from expeller- or cold-pressed sources in diets reduced the feed consumption and growth rate of the birds compared to control birds fed SBM-based diets (Chapters 5 and 6). However, the high expeller CM in the diet also impaired digestibility of DM, crude protein and energy of the diet, and consequently feed efficiency. However, diets with a high level of cold-pressed CM did not have any negative effect on nutrient utilisation and feed efficiency. The better nutrient utilisation of birds fed cold-pressed CM-based diets compared to expeller-pressed CM-based diets could most likely be related to the higher nutritional quality of coldpressed meals. As the cold-pressed CM is not subjected to any heat treatment during oil extraction, the availability of nutrients (protein and energy) is better preserved.

The findings of Chapter 6 indicate that the growth depression of birds fed CM-based diets is primarily mediated through reduced feed consumption of the birds. The reduced feed intake could be attributed to the augmentative effects of all the ANF present in CM such as high dietary fibre, glucosinolate content, phenolic compounds and low dietary electrolyte balance of CM. However, the magnitude and sole roles of each ANF of CM in reducing feed consumption still remains unclear and is worthy of further investigation.

Based on the findings of Chapter 5, both microbial multi-carbohydrase and mono-component protease supplementation improved ileal energy and protein digestibility, with the effect of carbohydrase being more pronounced on energy digestibility and protease on protein, when compared to non-supplemented diets. The disruption of cell wall integrity and release of encapsulated nutrients such as starch and protein bodies most likely contributed to the overall improvements with the multi-carbohydrase enzyme supplementation. The higher nutrient digestibility was translated into higher weight gain and improved feed conversion ratio with dietary enzyme supplementation. There was no specific advantage of the enzymes alone or in combination for CM diets over SBM-based diets. Despite the improvement in energy digestibility observed

with supplementation of carbohydrase, the ME of the diets was not affected. But the carbohydrase effectively increased the NE of the diets, particularly in CM-based diets. The higher HP and lower NE in birds fed CM-based diet could to some extent be attributed to the higher dietary fibre content of diets based on CM.

Higher supplemental essential amino acids in CM based diets did not have any marked effect on feed consumption of birds. However, birds fed the CM-based diets with 6 and 9 % higher essential AA levels had improved FCR. Moreover, the higher AA levels, particularly 6 and 9 %, reduced fat deposition and increased carcass and breast meat yield compared to non-supplemented CM- and SBM-based diets. As all the CM diet with higher AA, had the same digestible AA ratio to lysine, an imbalance in AA was not likely to occur. The higher essential AA content and digestibility observed with 6 and 9 % extra AA should have increased protein accretion, resulting in improved FCR and higher breast meat yield.

In conclusion, the results reported in this thesis show that nutritional quality and value of CM for broiler chickens are highly variable. In this regard, CM source and differences in processing conditions can to a large extent account for this variability. Considering the high correlation of energy and protein digestibility values with concentrations of NDF and NDIN, these constituents can be used as the most relevant quality parameters of CM.

Inclusion of high CM (beyond 20%) in broiler chickens diet either from an expeller or cold-pressed source can reduce feed consumption and consequently growth rate. The depressed growth rate is primarily mediated through reduced feed intake. However, high expeller-pressed CM can also compromise nutrient digestibility and energy partitioning of the diet for the birds. Supplemental microbial enzymes (carbohydrase and protease) are effective in eliminating the negative impact of CM-based diets on nutrient digestibility and restoring feed efficiency but not on reduced feed intake. Although birds offered high CM diets benefited from 6 and 9 % higher essential amino

acid density of the diet in terms of improved FCR and higher meat yield, the lower feed intake and consequently lower growth rate was not completely compensated for. Replacing SBM with CM in broiler chickens diet did not change the abundance and composition of microbiota population in cecal content of broiler chickens.

Poultry producers and nutritionists should be fully aware of the source of the meal and the data for energy and amino acid digestibility values of the meal when including CM in poultry diets. Also, the desired growth performance and the cost of CM should be considered, to determine the optimum inclusion level of CM in broiler chicken diets.

Future research could be directed towards determining the individual role and effect of each ANF present in CM on depressing feed consumption of broiler chickens. This could be achieved by adding purified fibre, glocusinolate, tannins or sinapine to an iso-caloric and iso-nitrogenous SBM diet separately or in combination. More investigation into correction of dietary electrolyte balance of CM-based diet by increasing levels of dietary cations would be worthwhile. More experiments need to be carried out to determine the replacement value and optimum inclusion level of different sources of CM in broiler chicken diet. It would be of interest to examine phytase at normal and super doses and more fully investigate microbial carbohydrase and/or protease on nutritional value of CM.