Nutritive Evaluation of Mechanically-Pressed Canola (*Brassica napus* L.) Meal for Broiler Chickens

By

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ABSTRACT

Mechanically pressed *Brassica napus* L. meals' digestibility nutritional compositions were evaluated with digestibility studies. Two growth trials were conducted identifying the meals' maximum dietary inclusion level in broiler chicken diets. Heat reduced nutrient digestibility of mechanically pressed black canola meal (MPBCM). Meals with higher residual oil had higher AMEn. Enzyme addition did not affect MPBCM AMEn but increased standardized ileal amino acid digestibility (SIAAD) of meal with high residual oil. Carbohydrase increased AMEn of mechanically pressed yellow canola meal (MPYCM) while lipase generally improved SIAAD. MPBCM with 12 and 17% residual oil can be fed up 15% in the starter and grower diets but at 10% in finisher diet. Mechanically pressed black canola meal (MPYCM) with 12% residual oil should be fed in the starter and finisher diets at 10% and 15% in grower diet. Meal with 17% residual oil should be fed only at 10% in finisher diet.

Key words: Yellow and Black canola, Enzymes, Mechanically-pressed, Amino acid, Metabolizable energy, Feed consumption

LIST OF ABBREVIATIONS USED

ALA	Alanine
AID	Apparent ileal digestibility
AME	Apparent metabolizable energy
AMEn	Apparent metabolizable energy (nitrogen corrected)
ANOVA	Analysis of variance
ANR	Apparent nitrogen retained
ARG	Arginine
ASP	Aspartic acid
CP	Crude protein
CYS	Cysteine
DM	Dry matter
DMI	Dry matter index
EDTA	Ethylenediaminetetraacetic acid
FCR	Feed conversion ratio
g	Gram
g kg^{-1}	Gram per kilogram
$g \cdot b^{-1}$	Gram per bird
$g \cdot b^{-1} \cdot d^{-1}$	Gram per bird per day
GE	Gross energy
GLU	Glutamic acid
GLY	Glycine
HIS	Histidine
HOM	14% residual oil meal
HOM-H	Heated 14% residual oil meal
ILE	Isoleucine
IU kg^{-1}	International Unit per kilogram
$kcal \cdot kg^{-1}$	Kilocalorie per kilogram
LEU	Leucine
LOM	10% residual oil meal
LOM-H	Heated 10% residual oil meal
LYS	Lysine
MPBCM	Mechanically pressed black canola meal
MPYCM	Mechanically pressed yellow canola meal
MET	Methionine
$mg \cdot kg^{-1}$	Milligram per kilogram

N	Nitrogen
NaOH	Sodium hydroxide
NDF	Neutral detergent fiber
NRC	National Research Council
NSP	Non-starch polysaccharides
PHE	Phenylalanine
PRO	Proline
SER	Serine
SIAAD	Standardized ileal amino acid digestibility
SID	Standardized ileal digestibility
THR	Threonine
TRP	Tryptophan
TYR	Tyrosine
VAL	Valine

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CHAPTER 1: INTRODUCTION

The growing demand for poultry products worldwide (FAPRI Database 2010) calls for efficient means of production to meet future demands without compromising product quality. The quantity and quality of poultry meat is influenced by the bird's diet which is often formulated with corn or wheat as the energy source supplemented with soybean and/or canola meal as the protein source (Leeson and Summers 2005). There is an anticipated rise in the cost of grains like corn due to demands from the energy sector (Daynard and Daynard 2011) and there is competition for protein sources among feed companies. All these factors will affect poultry farmers' ability to effectively produce least cost rations.

There is interest to convert extracted oil from oilseeds, such as soybeans and canola into biofuels (Hill et al. 2006). The oil is extracted by mechanical pressing (Unger 1990) creating by-products with different nutritional compositions than regular solvent extracted canola. Mechanical-pressing of oilseeds produces meals with higher residual oil. This provides cost-effective alternatives to the current pre-press solvent extraction procedure, especially for smaller oilseed plants that often use oilseeds currently available in small quantities like yellow seed canola (Hill et al. 2006).

Canola is a term developed in Canada to delineate rapeseed *Brassica* species having less than 2% erucic acid in the seeds and 30 $\mu mol \cdot g^{-1}$ glucosinolates or less in the meal after oil extraction (Canola Council of Canada 2009). The use of the term canola has been embraced worldwide since its development and canola is a very important oil-seed crop in many countries. *Brassica* rape species, of canola quality, produce tiny oil-rich seeds and produce high quality protein meal for animals (Canola Council of Canada

2009). Emerging interest to create biodiesel from oilseeds like black and yellow canola (Thacker and Petri 2009a) could see these seeds providing feedstock for on farm biofuel production. If the seeds are pressed using an expeller or cold oil press equipment as least cost process during production, new meals with potential to become protein and energy sources for animal feeds (Leming and Lember 2005) may become available. Efficient use of new meal ingredients for poultry requires knowledge of their feeding value.

The development of yellow-seeded *Brassica napus* lines may lead to improvements in the feeding value of canola for poultry. Yellow-seeded canola meals have lower fibre content and higher true metabolizable energy values than brown-seeded canola meal (Slominski et al. 1999). Feeding full fat canola seeds to broilers provide substantial modification of the fatty acid composition of the carcass (Ajuyah et al. 1991) and the fatty acid profile of canola may provide health benefits to humans.

Canola press cake is known to have high feeding value in pigs with no effect on palatability, but it may contain more glucosinolates than commercial canola meal (Keith and Bell 1991). Canola seeds contain relatively low levels of glucosinolates and deactivation of myrosinase enzyme is usually accomplished during the cooking phase of the solvent extraction process (Unger 1990). Heat treating of mechanically pressed canola meals may be needed to inactivate the enzyme myrosinase to reduce any anti-nutritional effects of glucosinolates. The residual oil present in the mechanically pressed meals could be a source of digestible energy (Keith and Bell 1991).

Adding enzymes to poultry diets can increase nutrient utilization from oilseed ingredients (Leeson and Summers 2005, Khajali and Slominski 2012). Several enzyme cocktails containing carbohydrases, proteases and lipases are available commercially

(Khajali and Slominski 2012). These enzymes have the potential to improve the utilization of protein and energy-containing components of diets (Meng et al. 2006). Currently, multi-carbohydrases are typically used in poultry diets containing wheat, barley and rye (Khajali and Slominski 2012). Lipase enzymes may improve digestibility of fat-containing ingredients (Leeson and Summers 2005). Recent research, testing the effects of carbohydrases and lipase in poultry diets formulated with 6% solvent extracted canola meals in a wheat-based diet showed promising results for carbohydrase (Meng et al. 2004). There is no data available showing the effects of these enzymes in diets formulated with mechanically pressed canola.

During processing of oilseed meals on farms, alteration can be made to provide heat treatment if needed. Enzymes can be added to diets for poultry containing those meals to make more effective use of all potential nutrients in the meals. Significant gaps in our knowledge exist for effective inclusion of mechanically pressed canola meals in diets for poultry due to its absence from the National Research Council (1994) nutrient requirements. This lack of information results from limited scientific assessment of these meals in the years leading up to the National Research Council (1994) publication. Since that publication, there has been significant progress in our knowledge of nutrient assessment techniques and genetic improvements to the modern commercial broiler. This research focused on evaluation of mechanically pressed black and yellow canola meals using the most recent nutrient assessment techniques and a modern strain of broiler chickens. Steps were taken to improve the nutritive value of mechanically pressed meals through the use of enzymes and heat treatments and the nutritive values of the meals developed for broilers were verified using production performance studies.

CHAPTER 2: LITERATURE REVIEW

2.1 Development of canola

Canola meal usage continues to increase as significant genetic breakthroughs by Canadian plant breeders occurs. Breeders have focused on reduction of erucic acid, with historical values from 24-45% to now less than 2%. Additionally glucosinolates have been reduced from 50-100 $\mu mol \cdot g^{-1}$ to 30 $\mu mol \cdot g^{-1}$ or less in the meal (Bell 1993). *Brassica napus* continues to be a very important oil seed plant today and is grown extensively in North America, Europe and Australia (Rahman and McVetty 2011). The main cause of this may be due to the fact that the glucosonolate level of canola has consistently been reduced to about 10 $\mu mol \cdot g^{-1}$ today (Khajali and Slominski 2012). *Brassica napus* black seed is the most commonly grown canola in Canada and is the main source of regular solvent extracted canola meal (Canola Council of Canada 2009). Another reason for the dramatic rise in the oil seed popularity is the consistent development of new genetic lines. *Brassica napus* Line YNO1-429 is one new germplasm with yellow seeds (Rakow and Relf-Eckstein 2005).

Traditionally there were no naturally occurring yellow seed *Brassica napus* but other *Brassica* species started to show mutants in their populations after they were bred (Rahman and McVetty 2011). It is known that seed with yellow or brown color has reduced hull and fiber content in comparison to the traditional black coated seeds (Simbaya et al. 1995). The nutritional advantages of the change in canola seed color led to the investigation and development of yellow seed lines of *Brassica napus* (Rahman and McVetty 2011). The yellow seed canola lines have higher oil and protein content and lower fiber. These differences are believed to result in improved broiler chicken

performance (Slominski et al. 1999). These nutritional advantages make yellow seed canola more attractive for feeding of poultry than black seed *Brassica napus* (Khajali and Slominski 2012).

2.2 Processing of canola meal

Processing of traditional canola meal varies slightly from country to country but there are specific parts of the procedure (Fig 2.0) which are similar (Unger 1990). In Canada, the process (Fig 2.0) includes seed cleaning, seed preconditioning, flaking, cooking, pressing, solvent extraction, desolventization and meal toasting (Canola Council of Canada 2009). The cleaning step removes foreign materials like dust and leaves through size screening and aspiration (Unger 1990). Before the seeds are rolled into 0.3-0.38 mm thickness flakes (Canola Council of Canada 2009), they are preconditioned by heated to around 30ºC (Unger 1990). The general purpose of cooking is to inactivate myrosinase enzyme in the flakes. This is achieved by heating the flakes to 80-105ºC for 15-20 minutes (Canola Council of Canada 2009) followed by oil extraction.

2.2.1 Solvent extraction method

Before solvent extraction, the flakes are screw pressed or expelled to remove 60-70% of the oil after the above steps in section 2.1. Hexane is added and the mixture is heated to 50 or 60ºC to remove residual oil by dissolving the oil in the hexane (Unger 1990). The cake with 25-35% residual hexane moves to the desolventizing and toasting stage where it is heated to around 95 to 115ºC to remove hexane residues. Soap stock is added to the cake which moves to the mills to be milled into meal (Unger 1990, Canola Council of Canada 2009). This meal tends to have a lipid content ranging from 1.5 to 3% which is much lower than other extraction procedures (Spragg and Mailer 2007).

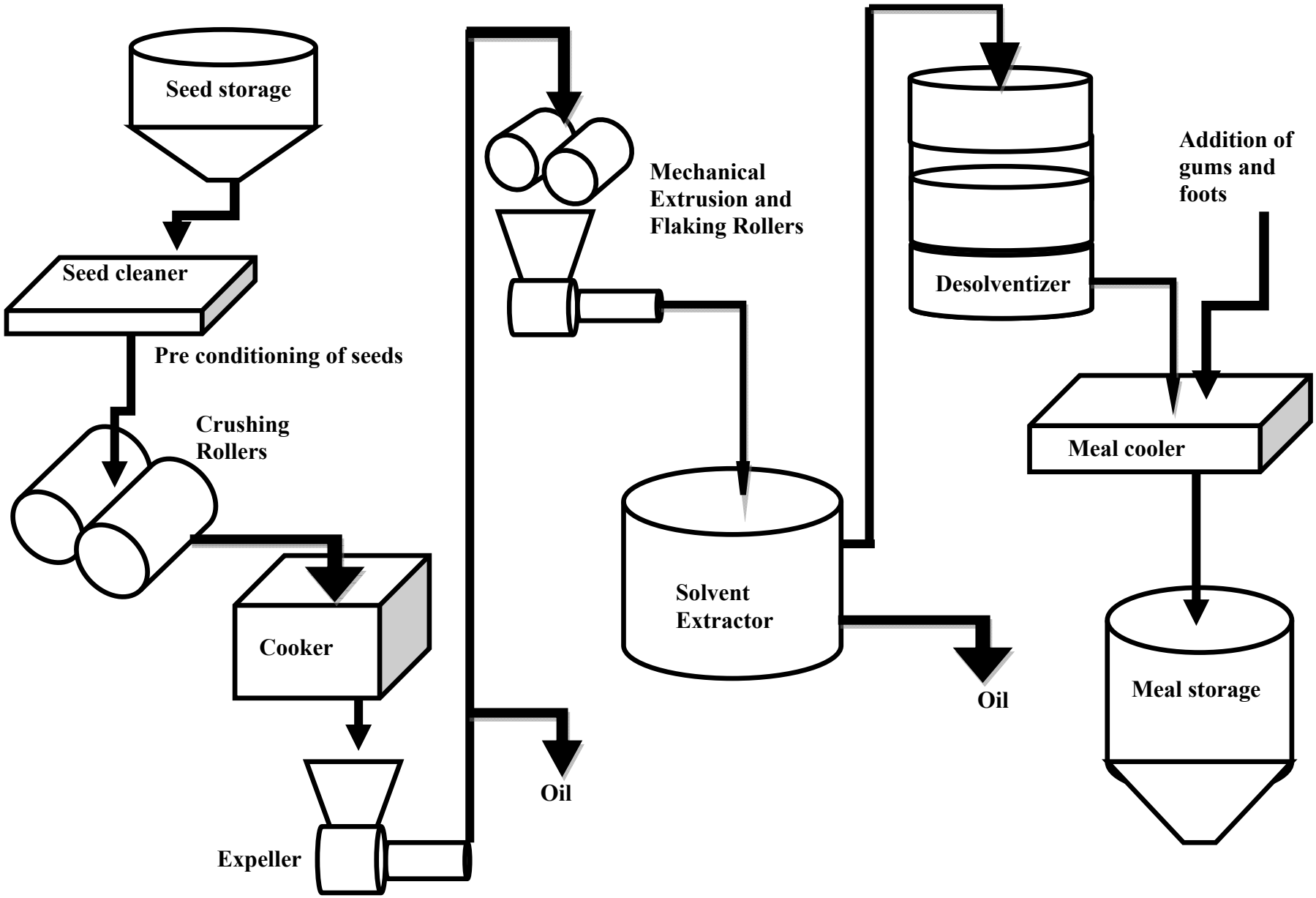

Fig.2.0 General steps in solvent extracted canola meal production adopted and modified from (Canola Council of Canada 2009).

2.2.2 Mechanical extraction method

The most common method for oil extraction from oilseeds is pre-press solvent extraction. However, approximately 300,000 tons of canola seed is mechanically pressed annually in Canada (Canola Council of Canada 2009). Various methods of mechanical pressing are used based on the intended use of the oil. The two most common methods of mechanical extraction in Canada are cold pressing and expelling (Seneviratne 2009). An expanding global interest in biodiesel production has resulted in an increased tonnage of meals without solvent extraction. Majority of biodiesel in Canada comes from micro-scale biofuel industry where expelling is used to obtain oil from off graded commercial canola (Thacker and Petri 2009a). Canola oil produced for markets such as cosmetic and pharmaceutical industry is produced using the more gentle process of cold pressing.

During mechanical extraction (Fig. 2.1), seeds may or may not be preconditioned before screw pressed or expelled (Spragg and Mailer 2007). If seeds are preconditioned and pressed using an expeller, the cake may reach up to 160ºC for a very short period of time due to friction in the expeller (Canola Council of Canada 2009). This temperature difference marks the distinction between cold pressed and expelling. During cold pressing the temperature of the meals is kept below 60ºC during the process and the seeds undergo a very gentle pressing to maximize the oil quality (Leming and Lember 2005). The oil is forced from the cells of the seeds under pressure as they pass through the press. The cake may be double pressed or the speed of the screw altered to increase the level of efficiency of oil extraction (Seneviratne et al. 2011). After pressing, the cake does not undergo solvent addition either desolventizing or toasting. It is milled into meal or made into pellets which have a higher residual oil (Canola Council of Canada 2009).

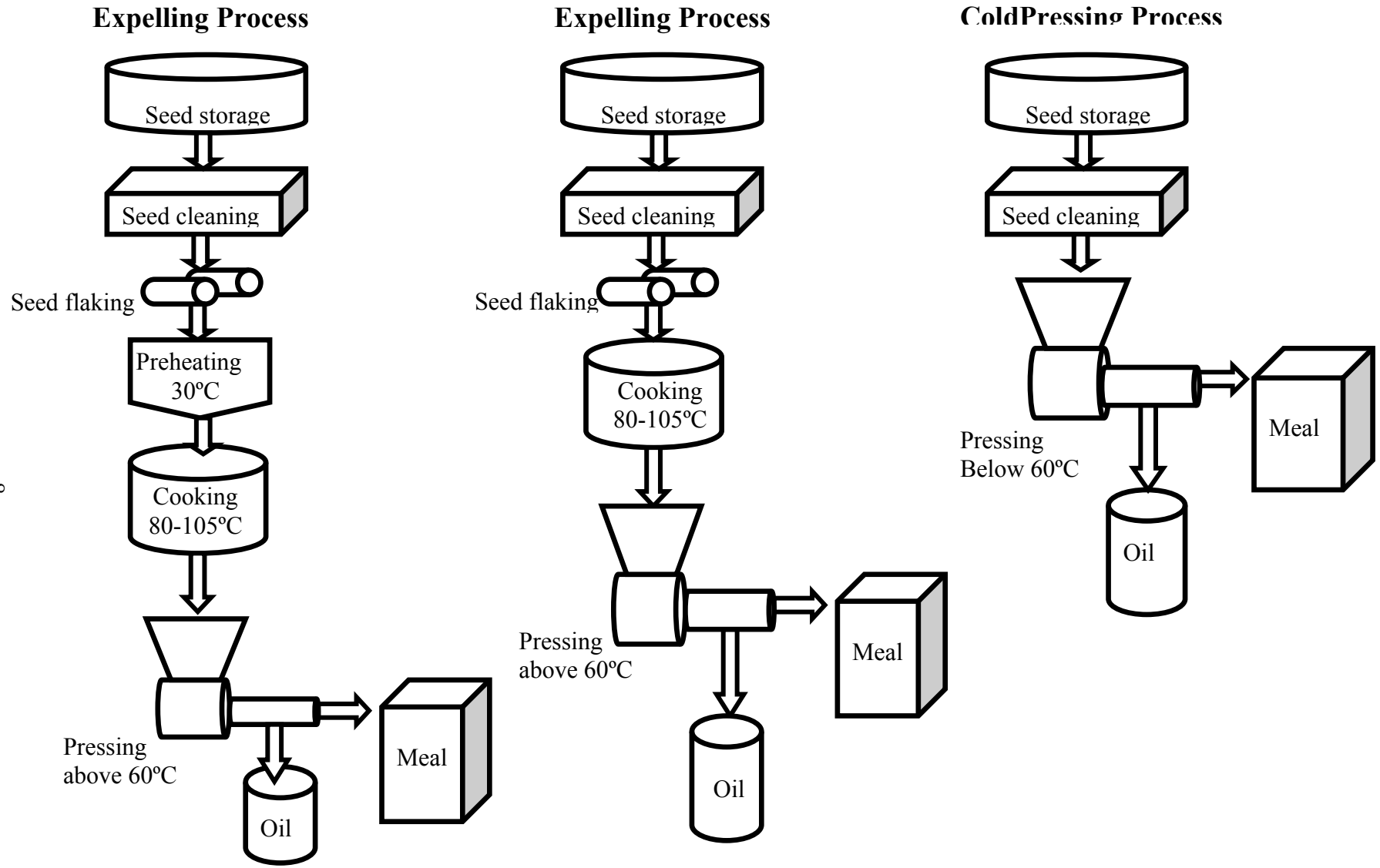

Fig. 2.1 General steps found in expelling and cold pressing of canola seeds adopted and modified from (Leming and Lember 2009)

2.2.3 Processing effects on protein and amino acid composition of canola meals

Newkirk et al. (2003a) and Mustafa et al. (2000) investigated the effects of processing on the protein and amino acid composition of canola meal (Fig.2.2). For canola processed by traditional solvent extraction the crude protein content steadily increases from cleaning until cooking. There is a rapid increase in crude protein after cooking which peaks and levels out at the solvent extraction stage to the final meal. There is a relative reduction in crude protein digestibility of canola meal for poultry after the application of heat during cooking and toasting. The amino acid content of the meal is relatively high until the desolventization and toasting stage (Newkirk et al. 2003a). Amino acids like lysine (LYS) are lost when they undergo the Maillard reaction with carbohydrate sources. This results in a color change of the meal after toasting (Newkirk et al. 2003a). Spragg and Mailer (2007) reported similar reductions in crude protein and amino acid content in solvent extracted canola meal compared to expelled meal. Newkirk et al. (2003b) reported that the amino acid digestibility of non-toasted canola meal was higher than toasted canola meal by broilers.

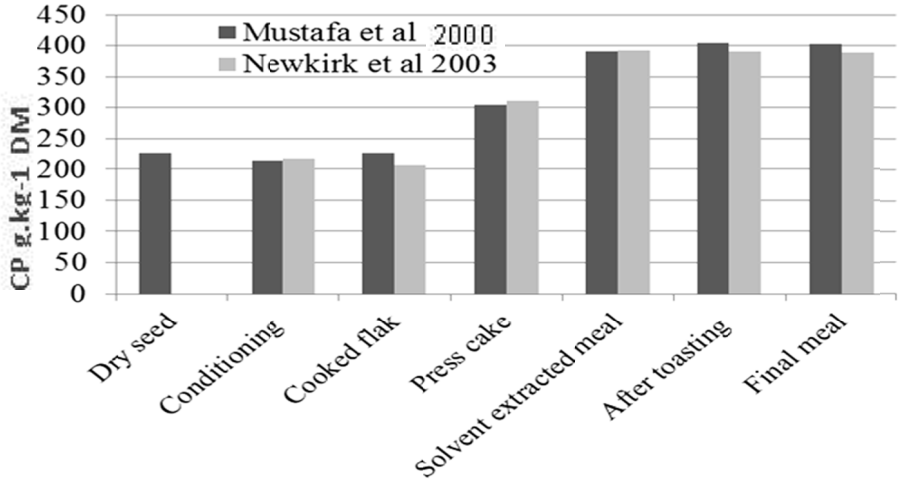

Fig .2.2 Effects of processing on canola crude protein (dry matter basis) adopted from Newkirk et al. (2003a) and Mustafa et al. (2000).

Various stages of canola meal processing may influence the nutrient characteristics of the meal and affect the meal quality for poultry diets (Classen et al. 2004). The effects of those changes on the general quality and characteristics of mechanically pressed canola meal should be determined in terms of nutrient availability for poultry. It is only through digestibility assays that the true effects processing has on the meal nutrient availability to the animals. However it is not practical to test each batch of meal using the assays in everyday commercial feed production.

2.3 Nutritional characteristics of mechanically pressed canola meal for broilers

Canola meal is considered to be a good protein source for animal production but it may contain additional nutrients which may be of importance to poultry production. *Brassica napus* accounts for 95% of the canola meal production in Canada, while *Brassica rapa and Brassica juncea* contribute 5% (Canola Council of Canada 2009). The nutrient composition of canola meal is influenced by factors including cultivar, length of growing season and method of processing (Newkirk et al. 2003a, Canola Council of Canada 2009, Seneviratne et al. 2011). The meal is composed of protein, oil and low levels of carbohydrates and minerals. Some vitamins and anti-nutritional compounds such as glucosinolates, fiber and sinapine may be present (Canola Council of Canada 2009).

2.3.1 Mechanically pressed black and yellow canola meal general composition

The protein content of mechanically pressed meals ranges from 31% to 38% and usually contains more available amino acids than solvent extracted meal (Newkirk et al. 2003a, Spragg and Mailer 2007, Thacker and Petri 2009a). Seneviratne et al. (2010) noticed that the crude protein of mechanically pressed black canola meal (MPBCM) was higher at 36%-38% than yellow seeded cold pressed meal 34%-35%. The residual oil levels in

mechanically pressed canola meal range from 8% to 26%, with yellow seed canola meals having higher oil levels than black seed meals (Schöne et al. 1996, Jayaraman 2010, Woyengo et al. 2010b).

Carbohydrates account for a small proportion of canola meal compared to other components in canola seed (Slominski et al. 1994). Naczk and Shahidi (1990) evaluated the meals of several cultivars of *Brassica napus* and found that they contained 16% to 18% carbohydrate on a dry weight basis. These carbohydrates include sucrose, raffinose, stachyose, galactinol,n digalactosyl glycerol, arabans, arabogalacturonic acids, hemicelluloses, cellulose and starch. Defatted yellow canola meals have more crude carbohydrate than defatted black canola meal (Naczk and Shahidi 1990). Schöne et al. (1996) reported that the expeller cake of canola contained 9.8% free sugar while the non-starch polysaccharides accounted for 13.7% of the meal. The crude fiber content of canola meal may range from 9% to 30% in cold press meals (Spragg and Mailer 2007). Thacker and Petri (2009b) reported that the neutral detergent fiber (NDF) of regular press cake of canola was 26%. Jayaraman (2010) reported that the neutral detergent fiber in yellow mechanically pressed canola meal was less, at 9% to 12% compared to black canola at 15% to 19%.

2.3.2 Yellow and black canola meal fatty acid profile

Canola seeds contain 42% to 43% oil (Thacker and Petri 2009a); the quantity left in the meal depends on the processing technique (Newkirk and Classen 2002). The oil levels of cold press canola meal range from 8% to 28% but this variability depends on processing (Canola Council of Canada 2009). The fatty acid profile of mechanically pressed canola meal is similar in portion for each fatty acid as solvent extracted canola meal, but it has

proportionally higher quantities of each fatty acid (Spragg and Mailer 2007, Seneviratne et al. 2010, Seneviratne et al. 2011). The major fatty acids in the meal are oleic acid C18:1, linoleic acid C18:2, alpha linolenic acid C18:3, palmitic acid C16:0 and stearic acid C18:0. Other fatty acids may be found at various levels below one percent in the meal (Spragg and Mailer 2007).

2.3.3 Digestibility of yellow and black canola meal fatty acids by broilers

The digestibility values of individual fatty acids in canola oil for broilers are deficient in the literature. When pigs were given 8l%-86% of the total dietary fat as oil from black and yellow samples of canola, the digestibility coefficients of the added oils were 75% for black and 81% for yellow (Spragg and Mailer 2007). Bell and Shires (1982) evaluated canola press cake with 18% oil in the diet of pigs with mean live weights of 86 kg and the crude fat apparent digestibility was 78%. When regular canola press cake with 27.5% ether-extract was fed at 150 $g \cdot kg^{-1}$ in the diets of broilers at 20 days old, the ether extract digestibility was 83.7% (Atteh et al. 1989).

Seneviratne et al. (2011) evaluated the effects of mechanically processed meals in the diet of 66 day old pigs. The meals were included in the diet at 44% and were of the following treatment groups based on pressing speed of the expeller (fast or slow) and applying or not applying heat to the pressing cylinder head of the expeller (heated or non-heated). The slow with non-heated settings gave 9.6% residual oil, the fast with non-heated gave 16.6% residual oil, the slow with heated gave 24.2% residual oil and the fast with heated gave 14.3% residual oil. The apparent ileal digestibility (AID) values of the residual oil extracted from the meals were 78.3% for the slow non-heated treatment, 92.5% for the fast non-heated, 94.2% for the slow heated and 93.8% for the fast heated

treatment settings of the expeller. Heating the meal and/or increasing the processing speed improved digestibility of the meals. Representative data on ether-extract digestibility for mechanically pressed canola meals fed to poultry are lacking in the literature. However, there is evidence (Thacker and Petri 2009a, Seneviratne et al. 2011) that ether extracts from mechanically pressed meals are well digested by other monogastric animals. It should be noted that processing conditions may influence apparent ileal and total track digestibility in poultry (Atteh et al. 1989).

2.3.4 Amino acid profile and digestibility of black and yellow canola meal

Canola contains both essential and non-essential amino acids which become concentrated in the meal after oil extraction (Thacker and Petri 2009b). The amino acid profile of canola is excellent for animal feeding since it contains a wide range of essential amino acids (Canola Council of Canada 2009). Most of the amino acid data for canola meal presented in the literature over the years was evaluated using the true amino acid and the ileal digestibility techniques. The main difference between these techniques is based on the site of sample collection and correction for endogenous source of the nutrient assessed. In the true digestibility assay, samples are collected from the excreta and are corrected for endogenous source of that nutrient. While in the ileal assay, samples are collected from the ileum and if corrected for endogenous source it is considered as standardized but if not then apparent (Ravindran et al. 1999, Ravindran and Bryden 1999). The main amino acids present in mechanically pressed canola are presented in Table 2.0. On average, the amino acids proportions of expelled canola meal are relatively equal in portion in both studies (Newkirk et al. 2003a, Keith and Bell 1991).

TABLE 2.0 Amino acid composition of expelled canola meal as a % in sample on a DM basis and there corresponding digestibility coefficients (%)

	Processing method		digestibility %			
	Expelled		Excreta[w]	Ileal		
	%		True	Apparent		Standardized
Amino acid						
Alanine	1.77[y]	1.89[x]	---	84.3[z]	78.1[y]	79.7[y]
Arginine	2.43	2.61	92	89.8	82.9	83.7
Aspartic acid	2.73	3.34	---	98.4	75.5	77.5
Cystine	0.88	1.24	79	78.8	73.8	74.2
Glutamic acid	7.65	8.26	---	98.3	84.8	86.5
Glycine	2.02	2.22	---	81.1	81.5	82.7
Histidine	1.14	1.54	80	86.6	83.5	84.9
Isoleucine	1.67	1.81	84	83.0	81.0	83.3
Leucine	2.83	3.03	89	82.1	78.2	79.5
Lysine	2.31	2.59	85	85.9	77.5	78.7
Methionine	0.68	0.86	84	89.5	82.3	83.7
Phenylalanine	1.59	1.74	90	85.7	79.4	80.4
Proline	2.66	2.56	---	77.2	71.2	72.6
Serine	1.39	1.99	---	76.1	77.9	82.8
Threonine	1.56	1.91	---	77.5	79.7	83.3
Tyrosine	0.96	1.29	81	78.2	78.0	79.5
Valine	2.18	2.33	85	82.1	82.0	83.6

(Newkirk et al. 2003a[z]; Woyengo et al. 2010a[y]; Keith and Bell 1991[x]; Anderson-Hafemannet al.1993[w])
Expelled canola[v]

Expelled and press cake canola meal contains higher levels of glutamic acid (GLU) than any other amino acid present in the meal. Indispensable amino acids such as LYS are well represented and balanced in mechanically pressed canola meals. There is not a lot of information on the digestibility of amino acid in mechanically pressed canola meals for broilers. Anderson-Hafemann et al. (1993) reported true digestibility values for canola meal obtained after the expelling process but just before the solvent extraction step during regular processing of canola seeds (Table 2.0). The coefficients for some of those amino acid digestibility values reported by Anderson-Hafemann et al. (1993) were similar to Newkirk et al. (2003a) while others show very large variability. However the values were consistently higher than the apparent and standardized ileal coefficients

reported by Wayengo et al. (2010a). This variation in amino acid digestibility values may be due to differences in age of the birds, meal preparation and the metabolism assay used in each study. The apparent ileal amino acid digestibility values reported by Woyengo et al. (2010a) (Table 2.0) were similar to the standardized ileal amino acids digestibility values. After standardizing, it was revealed that the apparent ileal amino acid digestibility values were underestimated when they were not standardized. There is variability in the apparent ileal amino acid digestibility among the two studies but most amino acids seem to have relatively high digestibility by broilers. Not all mechanically pressed canola amino acid digestibility values are the same (Table 2.0). The values may be influenced by the digestibility assay technique and the method of processing the meal which included the pre-treatments before mechanical pressing (Ravindran et al. 1999).

2.3.5 Metabolizable energy of mechanically pressed canola meal for broilers

Leming and Lember (2005) evaluated the chemical composition of cold pressed canola and expelled canola. They reported the metabolizable energy to be 3463 kcal·kg^{-1} and 3392 kcal·kg^{-1} respectively on a dry matter basis. After cold pressing or expelling, the oil content of the meal were 17.8% and 11.6%, respectively. Woyengo et al. (2010a) fed double press expelled canola meal with 12% residual oil at 30% substitution in the diet of three week old broilers. The apparent metabolizable energy (AME) was 3039 kcal·kg^{-1} on a dry matter basis and the nitrogen corrected (AMEn) 2694 kcal·kg^{-1}. In the same study, expelled canola meal had superior AMEn over solvent extracted canola meal 1801 kcal·kg^{-1} on a dry matter basis (Woyengo et al. 2010a). When Jayaraman (2010) fed single expelled yellow and black canola meal at 30% to 21 day old broilers, the AMEn were 3507 and 2902 kcal·kg^{-1} on a dry matter basis respectively. After the expelling

process, yellow canola meal oil content ranged from 23.3 to 26.4% and black was 18%. Mechanically pressed canola contains relatively high AMEn which can contribute to broiler performance but AMEn determined on more samples is needed for the ingredient to gain popularity in ration formulation.

2.4 Anti-nutritional factors in canola meal

Anti-nutritional factors are secondary plant metabolites and structural components present in plant based feed ingredients which interfere with the normal metabolic activities of animals who consume these ingredients (Bones and Rossiter 1996). Some of these compounds are used to protect the plant from insects and animal damage and have evolved into defense mechanisms (Chen and Andreasson 2001). Some plants store these compounds as sources of various minerals and important molecules used during various stages of development (Bones and Rossiter 1996).

2.4.1 Glucosinolate

Glucosinolates are secondary plant metabolites used for defense when plants are attacked by insects, herbivores or diseases (Chen and Andreasson 2001). Plants in the *Brassica* group contain glucosinolates which are converted to thiohydroximate-O-sulphonate by myrosinase hydrolysis following tissue damage. Thiohydroximate-O-sulphonate is very unstable and is converted to isothiocyanates, nitriles, and thiocyanates (Bones and Rossiter 1996). The glucosinolate content in mechanically pressed canola meal ranges from 5.26 $\mu mol \cdot g^{-1}$ to 30 $\mu mol \cdot g^{-1}$ dry matter depending on the variety of canola used (Schöne et al. 1996, Spragg and Mailer 2007, Thacker and Petri 2009b). Feeding rapeseed meal high in glucosinolates may reduce feed intake and weight gain and increase incidence of haemorrhagic livers in broiler (Bones and Rossiter 1996).

Glucosinolates present in the varieties of canola grown in Canada are relatively low at 30 $\mu mol \cdot g^{-1}$ or less and are not present in high level in meals (Spragg and Mailer 2007, Thacker and Petri 2009b, Canola Council of Canada 2009). Adequate heat during the processing of canola may inactivate the myrosinase enzyme which causes the release of glucosinolates (Unger 1990).

2.4.2 Sinapine

Sinapine a choline ester of 3, 5-dimethoxy-4-hydroxyinnamic acid is a phenolic compound found in many plants. It is the main source of choline and sinapic acid in growing plants (Campbell and Smith 1979). Canola contains sinapine not only in the embryo but also in the hull and levels may be as high as 0.24 % (Canola Council of Canada 2009). Expelled canola contains about 8.2-11 $g \cdot kg^{-1}$ sinapine which shows a tendency to decline with heat processing (Bell and Shires 1982). Sinapine can react with amino acids and other compounds contributing to the color and bitter taste of rapeseed meal (Kozlowaka et al. 1990). Kozlowaka et al. (1990) reported that sinapine bisulphate and sinapine ethanol extract had no effects on feed intake and performance of broiler. Protein digestibility and AME was increased with sinapine bisulphate and sinapine ethanol extract in the diet and the ceca was the major metabolic site for the compounds.

2.4.3 Fiber

Canola meal may contain up to 30% hull and this provides high levels of fiber as a single component in the meal (Spragg and Mailer 2007). The fiber content of expelled canola is about 11.5% (Spragg and Mailer 2007) and it contains polyphenols, non-starch polysaccharides (NSP) and lignin (Kozlowaka et al. 1990). Bell and Shires (1982) indicated yellow varieties of canola had less fiber with less neutral detergent fiber (NDF),

lignin and phenols and more neutral-detergent-soluble polysaccharides and non-starch polysaccharides than black lines. The lower levels of lignin and NDF may positively contribute to digestibility of yellow canola meal in poultry (Khajali and Slominski 2012).

2.5 Effects of mechanically pressed canola meal on broiler performance

Thacker and Petri (2009a) investigated the effect of 50, 100 and 150 $g \cdot kg^{-1}$ high oil canola press cake in a wheat based broiler diet. Birds fed the canola press cake had the same body weight gain and feed intake as those fed the regular canola meal. The feed conversion ratio (FCR) of birds on canola press cake was slightly improved in relation to those on canola meal. There was a significant linear improvement in the FCR as the level of canola press cake increased in the diets. Woyengo et al (2010a) found no effects of expelled canola on average daily gain, average daily fed intake and FCR despite the high level of apparent metabolizable energy in the meal. It was concluded that the time period of 7 days for the experiment was too short to observe the real effects of the meal on production performance. In a second study Woyengo and Nyachoti (2011) noticed that as the level of expeller canola meal increased from 0-40% in the diets, there was a linear decrease in feed consumption and broiler body weights. There was a linear increase in FCR as the meal level increased. The inclusion of mechanically pressed canola cake at levels of 150 $g \cdot kg^{-1}$ in the diets of other monogastics did not have any adverse effects on performance (Schöne et al. 1996) but up to levels of 40% showed signs of linear increase in liver weight relative to body weight (Woyengo and Nyachoti 2011). It should be noted that there were no effects of treatment on the blood serum triiodothyronine concentrations, hemoglobin and hematocrit content in the study (Woyengo and Nyachoti 2011).

2.6 Use of enzymes in poultry diets

There are little performance and digestibility data in the literature on the use of enzymes in broiler diets containing mechanically pressed canola meals. This area of research was not exploited in the past possibly due to the availability of cheap alternative protein sources other than mechanically pressed canola meals. It was not until recently that the level of mechanically pressed canola meal production started to increase in Canada. This might have contributed to the lack of research on the meal by Canadian researchers. The nutritional characteristics of mechanically pressed canola make it a good candidate for use in poultry (Woyengo et al. 2010a).

The use of enzymes in mechanically pressed canola diets could improve the nutrient availability of the meal since past attempts with solvent and full fat canola seeds have been successful (Khajali and Slominski 2012). Meng et al. (2006) reported an increase in total metabolizable energy in full-fat canola when poultry diets were supplemented with multicarbohydrase enzymes. The multicarbohydrase enzymes improved the feeding value of canola meal for broiler chickens by degrading the non-starch polysaccharides, improving fat digestibility and energy utilization. Jayaraman (2010) however, reported no effects of dietary addition of a multicarbohydrase enzyme on apparent energy digestibility of mechanically pressed black and yellow canola meal when fed to broiler chickens.

The use of lipase in diets is controversial since lipases may hydrolyze triglycerides in mixed feeds prior to consumption. Kermanshahi 1998) observed that lipase had a negative effect on growth performance and fat digestibility in broiler

chickens fed various levels of lipase in a corn, soybean meal and tallow diet. Protection of the lipase with encapsulation may overcome this problem (Kermanshahi 1998).

Information on the use of proteases to enhance protein digestibility in mechanically pressed canola meals is very limited in the literature. Simbaya et al. (1996) evaluated the effects of protease supplementation on the nutritive value of canola meal *in vitro* and *in vivo*. The study indicated that the use of protease enzyme in the presence of pancreatin or pronase yielded significant levels of protein hydrolysis which improved broiler chick growth performance. Enzyme addition to broiler diets with mechanically pressed canola meal provides the opportunity to release amino acids from complexes and break down complex carbohydrate and lipids. This provides nutrients in a form which is easily digested in the ileum of birds (Khajali and Slominski 2012)**.**

2.7 Standardized ileal amino acid digestibility concept in broilers

One of the most expensive ingredients in animal rations is protein. The efficient use of this ingredient by animals must be a priority in diet formulation. To better predict animal performance, precision in diet formulation must be achieved. Protein recommendations should be based on amino acid digestibility instead of total amino acid of diets (Lemme et al. 2004). There is a need for consensus on the most appropriate method for determining amino acid digestibility in broilers (Parsons 2006). Lemme et al. (2004) defined digestibility as the amount of nutrient absorbed by the animal from feed consumed. There has been a general consensus among nutritionists around the world with the use of ileal and excreta digestibility assays, as the two basic techniques for evaluating nutrient digestibility (Parsons 2006).

In the excreta collection methodology, feed may be administered as precision feeding to cecectomized adult roosters or *ad libitum* feeding to growing birds (Lemme et al. 2004). Cecetomized birds require surgery and are force fed which makes it difficult to get animal care approval (Parsons 2006). After the birds are given test diets, excreta samples are collected a few days later and analyzed for nutrients. This method does not correct for nutrients from microbial, urine, enzymatic and epithelial sources in the excreta. Those facts have led to its criticism and the need to correct for endogenous amino acid loss (Lemme et al. 2004).

Apparent ileal amino acid assays account for endogenous amino acid sources in the lower part of the tract but not for those originating before the end of the ileum (Lemme et al. 2004). In apparent ileal assays, birds of any age can be used and they are not force fed (Parsons 2006). It is well known that there are differences in the bird's ability to digest some nutrients as they age (Huang et al. 2005). The samples are taken from the ileum after euthanasia which better estimates available nutrients present in the feed (Parsons 2006).

Ravindran and Bryden (1999) highlighted the importance of standardizing apparent ileal digestibility assays which often underestimates available nutrients if not converted by standardization. This assay is a promising method that can be used by nutritionists and researchers. The apparent values must be standardized to reduce the effects of endogenous nutrient sources before the ileum which leads to underestimation of the available nutrients at the ileum (Ravindran and Bryden 1999, Lemme et al. 2004). When evaluating amino acid digestibility, the use of regression method values, nitrogen free or highly digestible protein diets ileal flow values for standardizing ileal amino acid

is still up for debate. Ravindran and Bryden (1999) proved that standardization of apparent ileal amino acid values using a nitrogen free diet gave similar standardized ileal amino acid digestibility in various plant protein sources. Adedokun et al. (2008) looked at using a nitrogen free diet versus highly digestible protein diets for standardization. They noticed that at 5 day of age there were no differences between the diets used to standardize the apparent ileal amino acids in broiler chicks. At day 21 there was a significant difference in ileal amino acid digestibility. The high protein diet standardized values were consistently higher than the nitrogen free diets. Golian et al. (2008) evaluated the use of all three methods of standardizing amino acids and noticed that apparent values standardized with a nitrogen free diet and those standardized with the regression method gave similar results which were different from the highly digestible protein diets.

2.8 Current area of research interest

If the current trend of on farm biodiesel production and the development of new canola lines continues, more and more by-product material in the form of mechanically pressed canola meal will be generated. Research related to these of canola by-product has not been investigated fully. There is little data in the literature related to their use in poultry. The lack of research in this area has resulted in the by-products absence from the recommendations for poultry (National Research Council 1994).

Since the introduction of the term canola to the feed industry, most of the amino acid digestibility values published for canola meals over the past decades are based on solvent extracted canola meal. The methodology used to generate those amino acid and crude protein digestibility values were based on the excreta method. Likewise the diets used to generate poultry performance data for solvent extracted canola meal during that

time were formulated on those apparent digestible amino acid and AMEn digestibility recommendations generated using adult birds. The literature is lacking growth performance data on the use of mechanically pressed canola *Brassica napus,* both black and yellow seeds. To our knowledge, there are just a few studies from the early 1990's to present which involved the use of MPBCM for evaluation of broiler growth performance. It is well known from the literature that mechanically pressed canola meals residual oil may vary and this could influence the total energy of the meal. Previous studies did not investigate the effects of the variability of MPBCM residual oil on broiler performance (Woyengo and Nyachoti 2011). To date no data could be found on the use of mechanically pressed yellow canola in assessing broiler chicken growth performance.

There have been considerable improvements in current enzyme technology and the refinement of the standardized ileal digestibility methodology since the publication of the National Research Council (1994) nutrient requirements. Data from the literature suggest that digestibility values of adult birds seemed to be different from younger birds and those values are being improved by gains in genetics of the birds (Parsons 2006). The past nutrient recommendation might not represent the true nutrient usage by modern poultry lines.

While the concept of ileal digestibility is well accepted and is the method of choice for research in other monogastic species like pigs, the transition in poultry nutrition is slow. There is need to generate new AMEn and standardized ileal amino acid digestibility values not just for solvent extracted canola meal but also for mechanically pressed canola meal ingredients. The new digestibility values must be developed using the ileal digestibility assay methodology and current enzyme technology. This will help

nutritionists to formulate diets based on amino acid availability when using the by-products.

There is evidence from past research that formulating diets based on digestible amino acids leads to better prediction of bird performance and gain in efficiency of protein usage. Parsons (2006) concluded that there is virtue in the formulation of broiler diets using digestible amino acid values as it relates to feed efficiency, bird performance and meat yields. The problem that nutritionist face when formulating diets based on digestible amino acids is the lack of amino acid digestibility values for the ingredients being used. Mechanically pressed canola is one such ingredient which needs digestibility estimates and the generation of growth performance data. Since there is limited research conducted using mechanically pressed black *Brassica napus* meal and its yellow counterpart in the literature, this research will evaluate the nutritive value of mechanically pressed yellow and black canola meals. The ileal digestibility concept will be used to assay the ingredients. During the assessment of the meals nutritive values, enzymes will be used to increase the available nutrients as suggested by Khajali and Slominski (2012). The effect of the absence of various heating processes normally observed with regular solvent extraction from mechanically pressing of the seeds will also be evaluated. The digestibility data gathered will be confirmed using a growth performance trial with diets that are formulated on digestible amino acids.

CHAPTER 3: THESIS MAIN OBJECTIVES AND HYPOTHESES

The research took the form of three digestibility assays to determine the digestibility of mechanically pressed yellow and black seeded *Brassica napus*. Two growth studies were conducted evaluating the effects of mechanically pressed yellow and black meals on the bird production performance.

3.1 Objectives

To assess the nutritive value of the meals the following objectives were investigated:

1. To determine the apparent metabolizable energy (AMEn), standardized ileal amino acid digestibility (SIAAD) for mechanically pressed yellow and black canola meals with 10% and 14% retained oil, using broiler chickens assays.
2. To determine the effects heat and meal residual oil content has on AMEn and SIAAD values of MPBCM.
3. To determine the effect of dietary enzyme addition on AMEn and SIAAD values of MPBCM.
4. To determine the effect of heat, enzyme and meal residual oil content on AMEn and SIAAD values of (MPYCM).
5. To determine the best level at which mechanically pressed black and yellow meals with 12% and 17% residual oil should be included in the diets of broiler chickens using the broiler chicken's growth performance as the criteria.

3.2 Hypotheses

1. The AME_n and SIAAD values for MPBCM with 14% residual oil will be higher than the AME_n and SIAAD for black canola meals with 10% residual oil.
2. Heating MPBCM with 14% and 10% residual oil will increase the meals AME_n but decrease the SIAAD.
3. The addition of enzymes to MPBCM with 14% and 10% residual oil will increase the meals AME_n and SIAAD.
4. Enzyme addition and heating MPYCM with 10 and 14 % residual oil will increase the AMEn and SIAAD values of the meal.
5. Birds fed mechanically pressed canola meal with higher residual oil will have better performance than those fed canola with lower residual oil.
6. Birds fed 0, 5, 10, and 15% mechanically pressed canola meal should have similar growth performance.

CHAPTER 4: EFFECTS OF HEAT AND OIL LEVELS ON THE NUTRITIVE VALUE OF MECHANICALLY PRESSED BLACK CANOLA MEAL (*BRASSICA NAPUS*) IN 21 DAY OLD BROILER CHICKENS

4.1 Abstract

Canola is important to the biofuel industries due to its high oil content. Mechanical-pressing of oilseeds is more cost-effective over solvent extraction procedures for small biofuel processors. Meals produced have variable residual oil and they are not toasted. Limited data exists regarding digestibility in poultry. This study assessed apparent ileal digestible nutrients and metabolizable energy (AMEn) of black canola meal with 14% and 10% residual oil by the substitution method using broiler chicks. Half of each meal was heat treated at 115ºC for 25 minutes. Diets were a corn-soybean meal basal diet substituted with 30% of one of the canola meals [14% residual oil heated or non-heated meal and 10% residual oil heated or non-heated meal]. One hundred and eighty day old Ross-308 male chicks were assigned to the five dietary treatments (6 birds per cage, 5 replicate cages per treatment) in a completely randomized design with a 2x2 factorial arrangement (residual oil levels x heat treatments) from day 15 to 21. Heat treatments and oil levels affected the AMEn but their interactions were not significant ($P > 0.05$). Meals with 14% residual oil had greater ($P < 0.05$) AMEn 2457.1±59 kcal·kg^{-1} than 10% residual oil meals 2170±59 kcal·kg^{-1}. Heat reduced ($P<0.0001$) the AMEn of the meals 2541±59 kcal·kg^{-1} to 2086±59 kcal·kg^{-1} regardless of the oil levels. Heat lowered ($P=0.0002$) the digestibility of all amino acids. Oil levels did not affect ($P>0.05$) methionine (MET), lysine (LYS), cysteine (CYS), isoleucine (ILE), arginine (ARG), valine (VAL) only threonine (THR), glycine (GLY), aspartic acid (ASP), serine (SER) and tryptophan (TRP) digestibility. The interaction of heat treatment and residual oil levels did not significantly ($P>0.05$) affect the digestibility of MET, CYS and TRP. Application of heat reduced the AMEn, standardized ileal crude protein (CP) and digestible amino acids in mechanically pressed canola black meal (MPBCM).

Keywords: High oil residue canola meal, Digestible nutrients, Broilers, Amino acids

4.2 Introduction

There is global pressure to actively develop more renewable fuel sources. Even with its large resource of fossil fuels, Canada is no exception to the drive for more renewable sources of fuel (Natural Resource Canada 2010). Some countries are more proactive in their efforts and have passed constitutional regulations to subsidize their current fuel consumption with various levels of renewable fuels like ethanol and biodiesel (Natural Resources Canada 2010, Daynard and Daynard 2011). In 2008, the Canadian government launched a program called the National Renewable Diesel Demonstration Initiative. The idea of encouraging the use of renewable fuels is an attempt to increase productivity in rural communities and reduce Canada's greenhouse gas emissions (Natural Resource Canada 2010).

The interest in biodiesel production in Canada resulted in off graded canola seed being used as one of the feedstocks mainly due to its high oil content (Canola Council of Canada 2009, Thacker and Petri 2009a). The meal produced from the use of canola as a biodiesel feedstock tends to have a nutritional quality different from traditional solvent extracted canola meal (Newkirk et al. 2003a, Thacker and Petri 2009a and 2009b). The level of oil in meal of biodiesel origin is higher than regular canola meal (Thacker and Petri 2009b). The level of residual oil in the meal depends on the process used to extract the oil which may or may not be similar to that used during the first stage of solvent extracted canola meal production (Leming and Lember 2005).

Recent work has been performed on the nutritive value of canola meal of biodiesel origin (Leming and Lember 2005, Thacker and Petri 2009a), supporting the idea that birds gain higher energy from meals from the biodiesel industry as a result of

the oil content. The authors did not look at the influence of a potential interaction between the levels of oil in the meal with the presence or absence of heat treatment during processing of these meals. Nutrient digestibility of canola meals is influenced by method of oil extraction and heat exposure during processing (Newkirk et al. 2003a and 2003b). Since the preheating and toasting stage are missing during mechanical pressing of oil seed during biodiesel production it is predicted that heating those meals after oil extraction will increase the meal AMEn but reduce the digestible amino acids. Therefore the objective of this study was to evaluate the effects of heating low and high residual oil *Brassica napus* meals produced from a simple expelling process on the nutritive value in broiler chicken diets.

4.3 Materials and Methods

4.3.1 Preparation of ingredients

Black canola seeds were cleaned, then expelled with an (model KEK-P0500, Egon Keller GmbH & Co. KG Germany) to produce a meal with 12% residual oil along with crude unfiltered oil. To prepare low and high oil meals, a batch of meal was mixed with petroleum ether at a ratio of 1:3 by weight with intermittent stirring. The meal was stirred one minute every fifteen minute, for one hour. After mixing the meal, the ether was poured off and the meal placed on an absorbent pad (Universal pad model no S-17293 Uline, Brampton, Canada) then gently squeezed by hand to remove excess ether. The meal was then transferred to a new absorbent and placed in a fume hood over night to dry. This mixing sequence and time produced a meal with 3% residual oil after air drying at room temperature in a fume hood.

The 3% oil meal was used to reduce the oil level of the 12% residual oil meal from the expeller process by mixing both meals to produce a meal with 10% residual oil (LOM). The untreated oil from the expelling process was added to the 12% oil meal to produce a meal with 14% residual oil (HOM). Both sets of meal were divided in half. One half of each meal was heat treated at 115±3ºC for 25 minutes using an industrial drying oven (Model ST33ATUL208V9KW, JPW Design Manufacturing, Trout Run, USA) to create heated 10% oil meal and heated 14% oil meal. During the heating process the meal was place on 88.9cm x 88.9cm x 2.54cm stainless steel trays and then evenly spread over the area of the tray. The trays were place in the oven and the meal allowed to reach up to 115º C then held at that temperature for 25 minutes. All the trays were then removed from the oven and the meals pooled from each tray then mix.

4.3.2 Diet preparation

In this experiment six diets were prepared in mash form using a Hobart mixer after all grains were milled. The starter diet (Table 4.0) was formulated to contain 3050 kcal·kg^{-1} metabolizable energy and 23% CP while the basal grower diets were formulated to contain 3150 kcal·kg^{-1} metabolizable energy and 20 percent crude protein. The starter and grower diets were corn-soybean meal based diets. The test diets were 70% basal diet substituted with either 30% heated mechanically pressed black canola meal (MPBCM) having 10 or 14% residual oil or 30% non-heated MPBCM having 10 or 14 % residual oil. This created four different grower test diets all of which along with the grower basal were in mash form and contained 0.5% chromic oxide as an indigestible marker. A nitrogen free diet was prepared from corn starch and dextrose with 0.5% chromic oxide (Table 4.0) to measure ileal CP and amino acid flow.

TABLE 4.0 Diet formulations used to test the effects of heat and oil levels on the nutritive value of mechanically pressed black canola meals

		Grower Diets		
	Starter Diet	Basal	Test Diets	Nitrogen Free
Ingredients as fed basis (%)				
Corn	44.5	65.8	41.8	-----
Corn starch	-----	-----	-----	20
Soybean meal	38.7	30.2	24.3	-----
MPBCM [z]	-----	-----	30.0	-----
Wheat	10.0	-----	-----	-----
Dextrose[y]	-----	-----	-----	64
Cellulose	-----	-----	-----	5.0
Soybean oil	-----	-----	-----	5.0
Tallow-grease blend	3.2	-----	-----	-----
Limestone ground	1.7	1.6	1.6	1.3
Mono-Dicalcium phosphate	0.6	0.8	0.8	1.9
Chromic oxide	-----	0.5	0.5	0.5
Vitamin/mineral premix[X]	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	-----
Methionine premix[W]	0.4	0.2	0.1	-----
Sodium hydrogen carbonate	-----	-----	-----	0.8
Potassium chloride	-----	-----	-----	0.3
Potassium carbonate	-----	-----	-----	0.3
Magnesium oxide	-----	-----	-----	0.2
Choline chloride	-----	-----	-----	0.3
Total	100	100	100	100
Calculated analysis				
Metabolizable Energy (AMEn)kcal·kg^{-1}	3050	3150	-----	-----
Protein%	23	20	-----	-----
Standardized ileal dig Lysine %	1.4	1.1		
Standardized ileal dig Methionine %	0.6	0.4		
Calcium%	1	0.9	-----	-----
Phosphorus %	0.5	0.4	-----	-----
Determined Analysis				
AMEn(kcal·kg^{-1})		3244	3074	-----
Protein%	23.5	21.7	23.1	-----
Calcium %	1.17	-----	------	-----
Phosphorus %	0.6	-----	------	-----

[z]MPBCM is black canola meal with 10 or 14% residual oil treated with or without heat

[Y]Atlantic Superstore Truro NS.

[X]Starter premix (amount per tonne), vitamin A (650×106IU kg^{-1}),15g, vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}), 50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000 mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g; Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g; Wheat middlings 1432g; Ground limestone (38%), 500g. or

[W]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

4.3.3 Animal husbandry

One hundred and eighty male Ross 308 day old broiler chicks were obtained from a local hatchery on January 11, 2012. Upon arrival, the birds were weighed (six bird per group) and distributed randomly to 30 battery cages (6 birds per cage) in one environmentally controlled room. The temperature and lighting of the rooms when the birds arrived were 32ºC and 20 lux. The temperature was reduced by 1ºC every 2 days until a temperature of 21ºC was reached. The lighting was reduced by 5 lux every 4 days until 5 lux was reached then maintained at 5 lux until the end of the trial. All the experiments were conducted at the Atlantic Poultry Research Center located in Bible Hill, NS. From the day of arrival at the research facilities to 14 days post-hatch, all birds were fed a common broiler starter diet in mash form (Table 4.0). On day 14, the birds were batch weighed per cage and assigned to the basal mash grower diets, test or nitrogen-free diets with five replicate cages per dietary treatment. The five cages, given the nitrogen-free diet were placed in a battery having a buffer zone of five feet from the other test birds to prevent any cross contamination of the nitrogen free diet. All the birds were hand fed daily as the feed given each day was weighed in and weighed back on day 14 and 21. The birds had free access to feed and water via trough and nipple drinkers from start to the end of the experiment. Mortalities were recorded throughout the trial and when it happened the birds were weighed and feed weighed back from the trough. All birds that died were examined via postmortem by a veterinary pathologist. All broilers were managed under the supervision of the Dalhousie Faculty of Agriculture Animal Care and Use Committee using guidelines provided by the Canadian Council on Animal Care (2009).

4.3.4 Performance data collection

Production performance was measured as body weight gain, feed consumption, Feed conversion ratio (FCR) and mortality. Body weights were taken on the first day, 14 and 21 days after arrival. Feed consumed was recorded throughout the trial which was divided into two periods: day 0-14 and 15-21 days. Both sets of data were used to calculate FCR. The mortalities were recorded throughout the trial and were expressed as a percentage of the total birds entering each phase of growth.

4.4.5 Sample collection and analysis

Clean excreta samples were collected in individual containers from day 20 to 21 from beneath all cages after which the birds were batch weighted per cage on day 21. Following excreta collection on day 21, six birds per cage were killed by cervical dislocation, dissected and the intestinal contents from the Meckel's diverticulum to about 1 cm above the ileal-cecal junction gently flushed with distilled water and collected in containers. The ileal digesta samples per cage were pooled in individual containers and sealed. Feed samples were taken from the starter, grower and nitrogen free diets. All samples were stored at -20ºC until analyzed.

Dry matter of the ileal digesta, excreta, feed and canola meal samples was determined as follows. The feed and ingredient samples were weighed out in 40 g duplicate and placed in a hot air oven at 60ºC for 24 hours then dry matter calculated as described by the method 935.29 (Association of Official Analytical Chemists (AOAC) 2000). Ileal digesta and excreta samples were weighed out in 35 g duplicates then frozen (-20°C) and freeze-dried. Freeze drying of digesta samples was performed without supplemental heat. After freeze-drying the excreta samples were weighed and dry matter

calculated using method 935.29 (AOAC 2000). All dried samples were ground using a coffee grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT).

Crude protein (CP) of all samples (% N x 6.25) was determined by the combustion method 990.03 (AOAC 2000) with a Leco Nitrogen Determinator (Leco Corporation, St. Joseph, MI), using EDTA as the calibration standard. Gross energies of the samples were determined using a Parr adiabatic bomb calorimeter (Parr Instrument Company, Moline, Illinois). The concentration of chromic oxide in feed, digesta and excreta was determined by the method of Fenton and Fenton (1979) using a Bausch and Lamb Spectronic 501 model 33.51.03 9 Milton Ray company USA). Amino acid profiles of the test ingredients, diets and ileal digesta samples were analyzed by HPLC (Sykam, Eresing, Germany) according to the method described by Woyengo et al. (2010a). For the amino acid sample preparation method 994.2 (AOAC 200), 100 mg of sample was digested in 4 mL of 6 N HCl at 110°C for 24 hours then neutralized with 4 mL of 25% NaOH (wt/vol). After cooling to room temperature the mixture was equalized to 50 mL volume with sodium citrate buffer (pH 2.2) then analyzed. The sulfur containing amino acids underwent performic acid oxidation before acid hydrolysis method 985.28 (AOAC 200). During tryptophan (TRP) analysis method 988.15 (AOAC 200), 50mg of sample was place in a plastic tube with 0.25ml distilled water and 1.00ml 25% sodium hydroxide. Tubes were flush with nitrogen to displace oxygen then caps were tightly screwed and tubes autoclave at 120º C overnight. After cooling the tubes 1 ml of 6N hydrochloric acid was used to neutralize the solution then made up to 25ml volume with sodium citrate buffer ph 4.25.

4.4.6 Calculations

Digestibility of test diets, ingredients and nutrients were calculated using the following calculation adapted from Lloyd et al. (1978).

Digestibility of Diets = 100-[100 x {(% Cr_2O_3 in diets/% Cr_2O_3 in excreta) x (% nutrient in diets/% nutrient in excreta)}].

Digestible nutrient in ingredient = [100x (nutrient in test diets- nutrient in ingredient in basal diet)/ proportion of test ingredient in test diet (30%)].

The ileal nutrient flow, apparent ileal digestibility and standard ileal digestibility were calculated using the following formula from Moughan et al. (1992):

Ileal nutrient flow mg/kg of dry matter = [nutrient in ileal digesta x (Cr_2O_3 in diets/ Cr_2O_3 in ileal digesta).

Apparent ileal digestibility = [1-{(% Cr_2O_3 in diets/% Cr_2O_3 in ileal digesta) x (% nutrient in ileal digesta/% nutrient in diet).

Standard ileal digestibility = Apparent ileal digestibility + {(Ileal nutrient flow mg/kg of dry matter/ nutrient content in test ingredient) x 100}.

The AME content and AMEn (kcal·kg^{-1}) of the test ingredients were calculated using the formulas described by Woyengo et al. (2010a).

The AME content = [(Gross energy retention for ingredients, %) x (Gross energy in ingredients, kcal·kg^{-1})]/100.

AMEn (kcal·kg^{-1}) content of ingredients = The AME content − (8.22 x ANR), where ANR is apparent nitrogen retained (g·kg^{-1}) of feed intake calculated as described by Jayaraman (2010).

4.4.7 Statistical analysis

The apparent digestibility of crude protein, dry matter, standardized ileal digestible crude protein, standard ileal digestible amino acids, apparent metabolizable energy (AMEn), digestibility values and production performance data were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3, (SAS Institute Inc., Cary, NC). The experimental design was completely randomized design (CRD) with 2 x 2 factorial (meal process type x residual oil level) where processing methods x meal residual oil content was (heated meal and non-heated meal) x (10% and14% residual oil).

Experiment model for nutritional data: $Y_{ijk}= \mu + A_{i\ (1-2)} + B_{j\ (1-2)}\ AB_{ij}+ \varepsilon_{ijk(5)}$

The statistical model of the experiment as shown above where Y is the response variable and μ is the overall mean response for that factor.

$A_{i\ (1-2)}$ is the effect of meal at the i^{th} process type (1 = heated process and 2 = non-heated process).

$B_{j\ (1-2)}$ is the effect of residual oil at the j^{th} level (1 = 10% and 2 = 14%).

AB_{ij} is the effect of the interaction at the ij^{th} processing type and level.

ε_{ijk} is the residual error of the model with $_k$ replication of five.

Experiment model growth data: $Y_{ij}= \mu + \alpha_i + \varepsilon_{ij}$

Where, Y is the response variable (body weight, feed consumption and FCR),

μ is overall mean, α_i is the effect of diets and ε_{ij} is the residual error

If significant main effects or interactions were found ($P \leq 0.05$) for the apparent digestibility of crude protein, dry matter, standardized ileal digestible crude protein,

standard ileal digestible amino acids, apparent metabolizable energy (AMEn), and digestibility values, Tukey Kramer test (Littell et al. 1996) was used to compare differences among the least square means at ($\alpha = 0.05$). Orthogonal contrasts were done for production performance data comparing basal and treatment group.

4.4 Results and Discussion

4.4.1 Analyzed composition of diets and ingredients

The analyzed nutrient contents of the test ingredients and the diets used in this experiment are given in Tables 4.1 and 4.2. The analyzed apparent metabolizable energy (AMEn) of the basal diet was higher at 3,244 kcal·kg^{-1} than its calculated value of 3,150 kcal·kg^{-1}. All the diets, except for the basal had lower AMEn than (National Research Council (NRC) 1994)) recommendation of 3200 kcal·kg^{-1} for growing broilers age 0-3 weeks old. The diets AMEn were similar to the expeller extracted canola meal substituted diets reported by Woyengo et al. (2010a). The CP content of all the diets except the basal were higher or in the same range as the 23% recommended by NRC (1994) for 0-3 week old broilers. The basal diet actually had 1% more crude protein than the calculated value of 20%. The diet was in the range of 20% recommended by NRC (1994) for broiler 3 to 6 weeks old. All the diets had more LYS than the calculated estimate of 1.1%. The analyzed methionine (MET) content of all the diets were in the range of the 0.4% of the calculated estimate. The analyzed LYS values of all the diets were above the NRC (1994) recommendation for broilers 0 to 6 weeks old. The MET of all diets was above the recommendation for 3-6 weeks old birds, but the basal and HOM diets MET were lower than the recommendation for 0-3 week's old birds.

The dry matter contents (DM) of the meals used in this study (Table 4.2) were similar to the 92% reported by Woyengo et al. (2010a) but higher than the 90% reported by Schöne et al. (1996). Only the DM for LOM was similar to the 91-89.8% reported for single cold press black canola by Jayaraman (2010). The DM of LOM-H was marginally higher than LOM. The DM in this study and that reported by the above studies were similar to the 89.6 to 98.2 reported by (Leming and Lember 2005).

TABLE 4.1. Analyzed nutrient composition of diets used to test the effects of heat and meal residual oil levels of mechanically pressed black *Brassica napus* nutrient digestibility in 21 day old broilers (as fed basis).

	Diets				
	Basal	LOM	LOM-H	HOM	HOM-H
Analyzed Nutrients					
Gross energy (kcal·kg^{-1})	4,177	4,302	4,317	4,351	4,349
AME_n (kcal·kg^{-1})	3,244	2,972	2,872	3,095	2,921
Crude Protein %	21.3	25.0	23.9	23.2	23.5
Methionine (%)	0.41	0.46	0.50	0.44	0.45
Lysine (%)	1.28	1.44	1.41	1.39	1.35
Cysteine (%)	0.32	0.43	0.43	0.43	0.41
Threonine (%)	0.90	1.06	1.05	1.02	1.00
Tryptophan (%)	0.18	0.26	0.26	0.23	0.22
Isoleucine (%)	0.83	0.83	0.85	0.85	0.86
Arginine (%)	1.58	1.68	1.64	1.66	1.60
Valine (%)	0.97	1.04	1.07	1.06	1.08
Leucine (%)	1.88	1.89	1.89	1.86	1.87
Phenylalanine (%)	1.07	1.07	1.06	1.07	1.05
Serine (%)	1.24	1.32	1.29	1.27	1.23
Glycine (%)	0.94	1.16	1.15	1.09	1.08
Histidine (%)	0.74	0.81	0.80	0.78	0.76
Aspartic acid (%)	2.38	2.38	2.31	2.33	2.23
Glutamic acid (%)	4.23	4.57	4.47	4.40	4.33
Proline (%)	1.51	1.69	1.66	1.62	1.60
Alanine (%)	1.05	1.14	1.15	1.08	1.10
Tyrosine (%)	0.70	0.74	0.71	0.72	0.71
NH3 (%)	0.51	0.57	0.55	0.54	0.55

LOM = 30% black canola with 10% residual oil+70 Basal diet, LOM-H= 30% heated black canola with 10% residual oil, HOM = 30% black canola with 14% residual oil+70 Basal diet, HOM-H= 30% heated black canola with 14% residual oil

TABLE 4.2. Analyzed nutrient composition (DM basis) of meals used to test the effects of heat and meal residual oil level on the nutrient digestibility of black *Brassica napus* in 21 day old broilers.

	LOM-H	LOM	HOM-H	HOM
Analyzed Nutrients				
Dry matter (%)	93	91	92	92
Gross energy (kcal·kg^{-1})	4,540	4,796	4,740	5,016
Crude Protein %	31.3	33.2	29.4	30.2
Fat (%)	10.2	10.3	14.1	14.0
Calcium %	0.56	0.56	0.52	0.52
Phosphorus %	1.26	1.26	1.23	1.23
Methionine (%)	0.54	0.58	0.53	0.54
Lysine (%)	1.85	1.78	1.71	1.70
Cysteine (%)	0.62	0.66	0.56	0.64
Threonine (%)	1.45	1.47	1.34	1.41
Tryptophan (%)	0.29	0.35	0.29	0.29
Isoleucine (%)	1.09	1.05	0.97	1.06
Arginine (%)	2.07	2.10	1.95	1.99
Valine (%)	1.50	1.46	1.36	1.44
Leucine (%)	2.14	2.18	1.96	2.08
Phenylalanine (%)	1.23	1.24	1.13	1.19
Serine (%)	1.50	1.56	1.39	1.45
Glycine (%)	1.65	1.71	1.53	1.61
Histidine (%)	0.99	1.05	0.94	0.97
Aspartic acid (%)	2.44	2.52	2.27	2.37
Glutamic acid (%)	5.42	5.59	5.04	5.23
Proline (%)	2.02	2.13	1.92	2.01
Alanine (%)	1.39	1.43	1.26	1.35
Tyrosine (%)	0.90	0.90	0.82	0.86
NH3 (%)	0.70	0.71	0.66	0.67

LOM = black canola with 10% residual oil, LOM-H= heated black canola with 10% residual oil, HOM = black canola with 14% residual oil, HOM-H= heated black canola with 14% residual oil

The gross energy (GE) of the meals on a DM basis range from 4540 to 5016 kcal·kg^{-1} and oil levels did not have a huge impact in varying the GE. The GE values in this study were slightly different from those reported for expeller extracted canola meal 5199 kcal·kg^{-1} (Woyengo et al. 2010a), 5134 kcal·kg^{-1} (Leming and Lember 2005) and 5570 to 5402 kcal·kg^{-1}for single cold press black canola reported by Jayaraman (2010). The oil content of canola meal is known to influence its GE content (Newkirk et al.

2003a). The difference seen in this study may be due to differences in the oil content of the meal compared to the other studies. The CP of the meals ranged from 29 to 33% and were less than the 41% reported by Woyengo et al. (2010a). Except for heated 14% residual oil meal at 29%, the CP of the meals were similar to the 32% and 31% reported by Schöne et al. (1996) and Newkirk et al. (2003a). The canola seeds used in this study did not undergo flaking and cooking before they were expelled. Other researchers have shown that the protein quality is affected by processing (Classen et al. 2004). The LYS and MET levels in the meals ranged from 1.58 to 1.70% and 0.58 to 0.53% respectively. Both amino acid concentrations were less than the 2.31% for LYS and 0.68% for MET reported for expeller extracted canola meal (Woyengo et al. 2010a). Anderson-Hafemann et al. (1993) evaluated the nutritive value of canola and noticed that processing may affect the amino acid content of the meals. The chemical composition of nutrients present in mechanically pressed canola meal is known to be influenced by heat during processing (Classen et al. 2004, Newkirk et al. 2003a). The variability in meal nutrient content of this study in relation to what others have found is largely due to the mechanically pressing technique of the seeds and processing temperature used. Seed pressing technique and processing temperature can influence the meal nutritive value from batch to batch (Classen et al. 2004, Leming and Lember 2005).

4.4.2 Animal performance

In this study, the birds' production performance (Table 4.3) was in the normal range based on the research facility averages (Jayaraman 2010). There was no effect of treatment on body weight, feed consumption, FCR or mortalities over the 7 day period of this experiment. It is known that this time period may not be long enough to see any real

change in performance attributed by dietary treatments (Woyengo et al. 2010a). The mortality was 0.5% which occurred primarily in the grower phase. Autopsy revealed that the mortalities were not treatment related. There was no effect of treatment on body weight gain and feed consumption from day 15 to 21.

TABLE 4.3.Growth performance of birds testing the effects of meal residual oil and heat of black *Brassica napus* meals in broilers 15 to 21 days of age

Performance parameters	Basal diets	Treatment diets
Body weight gain ($g \cdot b^{-1} \cdot d^{-1}$)	41±2	46±1
Feed consumption ($g \cdot b^{-1} \cdot d^{-1}$)	71±2	74±1
FCR	1.7±0.05	1.6±0.03

ANOVA *P*-Values

Contrast	Body weight gain	Feed consumption	FCR
Basal VS Treatments	0.0583	0.2127	0.1261

Mean ± SEM

4.4.3 Apparent digestible nutrients

The digestible DM coefficients of MPBCM used in this study in (Table 4.4) ranged from 73 to 81% and were affected by heat treatment and oil levels of the meal. Meals with 14% residual oil had significantly higher digestible DM coefficients than those with 10% residual oil. The application of heat to the meals reduced the digestible DM coefficients as observed with high to low residual oil levels. Thacker and Petri (2009a) investigated the nutritive value of regular and off grade green seed canola pressed cake in broilers and found the digestible DM to be 67.2 and 68.1% for the meals, respectively. The regular canola pressed cake had 27% residual oil while the green seed cake had 16%. In another study by Thacker and Petri (2009b) using Brassica *napus* press cake with 27.5% residual oil had a DM digestibility of 67.6%. Unfortunately, the effects of the residual oil level of

the meals on DM digestibility of those meals were not tested by Thacker and Petri (2009a, 2009b). DM values in this study were much higher than those reported by Thacker and Petri (2009a). One possible reason could be differences in the level of meal inclusion in the test diets of each study. Thacker and Petri (2009a) observed significant linear and quadratic effects of increasing level of canola meal in the test diets on DM digestibility. This study had 30% inclusion while that of Thacker and Petri (2009a) had a maximum inclusion of 15%. Another reason for the difference in DM digestibility might be related to the residual oil level of the meals used in this study compared to Thacker and Petri (2009a, 2009b).

TABLE 4.4. Effects of heat and oil level on mechanically pressed black canola meal apparent DM digestibility coefficients in 21 day old broilers

	Digestibility Coefficients (%)		
	No heat	Heat	Oil levels coefficients
Black Canola meals			
10% Oil meal	77±1	73±1	75±1b
14% Oil meal	81±1	77±1	79±1a
Effects of heat	79±1a	75±1b	
Source of variation		P>F	
Oil level		0.0066	
Heat treatment		0.0066	
Oil level*Heat		0.9602	

[a-b] Mean± SEM with no common letters in the same group: (oil level), (heat treatment) are different at $\alpha = 0.05$

The AMEn digestibility coefficients (Table 4.5) of MPBCM were influenced by the interaction between the residual oil levels of the meals and the heat treatment applied to the meals. Heated meals had higher AMEn digestibility coefficients than the no heat 14% residual oil meal but not the 10%. The non-heated meal with 14% residual oil had lower AMEn digestibility coefficients than the heated meals but was similar to the 10% residual oil meal with no heat coefficients. The AMEn coefficients seen in this study were higher than the 60.8% for cold pressed *Brassica napus* with 18% residual oil

previously reported by Jayaraman (2010); reasons are unknown. Newkirk et al. (2003a) noted that as canola meal moved through the prepress solvent extraction process, meal from the expelling stage had significantly higher AMEn than meals from the toasting stage.

TABLE.4.5. Effects of heat and oil level on mechanically pressed black canola meal AMEn digestibility coefficients in 21 day old broilers

	Digestibility Coefficients (%)		
	No heat	Heat	Oil levels coefficients
Black Canola meals			
10% Oil meal	89±1ab	92±1a	91±0
14% Oil meal	88±1b	92±1a	90±0
Effects of heat	85±1	92±1	
Source of variation		P>F	
Oil level		0.3567	
Heat treatment		<.0001	
Oil level*Heat		0.0295	

[a-c] Means± SEM with no common letters in oil*heat interaction are different at $\alpha = 0.05$

The AMEn of the meals (Table 4.6) was influenced by the level of residual oil in the meal and the heat treatment. The AMEn of mechanically pressed canola meals with 10% residual oil meal had significantly lower AMEn than the 14% residual oil meal. The correlation of higher AMEn with increased residual oil content of mechanically pressed canola meal was also reported by others (Jayaraman 2010 and Wayengo et al. 2010a). As heat was applied to the meals, the AMEn was significantly reduced. The AMEn of canola meal is known to be reduced by the application of heat during toasting stage and by the reduction of oil in the meal (Newkirk et al. 2003a). Application of heat to cold pressed canola is known to reduce the nutrient content, including digestible energy, in other monogastric species (Seneviratne et al. 2011). Some researchers attribute this reduction

in AMEn to carbohydrates and amino acids undergoing Maillard reaction at elevated temperature (Newkirk et al. 2003a). The AMEn content of the meals was well within the range for mechanically pressed canola in relation to residual oil content, as reported by others (Newkirk et al. 2003a, Jayaraman 2010, Wayengo et al. 2010a).

TABLE 4.6. Effects of heat and oil level on mechanically pressed black canola meal AMEn in 21 day old broilers

	Meal AMEn (kcal·kg^{-1} as fed)		
	No heat	Heat	Effects of oil levels means
Black Canola meals			
10% Oil meal	2336±84	2004±84	2170±59b
14% Oil meal	2747±84	2168±84	2457±59a
Effects of heat	2541±59a	2086±59b	
Source of variation		P>F	
Oil level		0.0036	
Heat treatment		<.0001	
Oil level*Heat		0.1613	

[a-b] Mean± SEM with no common letters in the same group: (oil level), (heat treatment) are different α = 0.05

Table (4.7) shows the apparent CP digestibility coefficients of the meals used in this study. Only the heat treatment of the meals had significant effects on the apparent CP digestibility coefficients. There was a significant reduction in the CP digestibility after the application of heat. Newkirk et al. (2003a) investigated the effects of each stage of processing during regular canola meal production and noticed that meal sampled after expelling had higher CP digestibility (80.6%) than meals samples after desolventizing /toasting (73.8%). The same brownish color change was observed in meals heat treated Newkirk et al. (2003a), signifying that some level of Maillard reaction had taken place.

TABLE 4.7. Effects of heat and oil level on mechanically pressed black canola meal apparent CP digestibility coefficients in 21 day old broilers

	Digestibility Coefficients (%)		
	No heat	Heat	Oil levels coefficients
Black Canola meals			
10% Oil meal	72±2	60±2	67±2
14% Oil meal	72±2	63±2	66±2
Effects of heat	72±2a	61±2b	
Source of variation		P>F	
Oil level		0.7407	
Heat treatment		0.0004	
Oil level*Heat		0.5174	

[a-b] Mean ± SEM with no common letters in the heat treatment group are different $\alpha = 0.05$

4.4.4 Ileal digestible nutrient

The ileal amino acid and crude protein flows presented in Table 4.8 represent the flow of those nutrients in the birds that were used in this study. It is known from previous reviews (Lemme et al. 2004) that ileal amino acid flow and possibly CP is influenced by bird age, choice of digestibility marker and test diet used in the assay. In this study the ileal CP and amino acid flow were evaluated using birds that were subjected to the same environmental conditions as the digestibility assay birds. The diet (Table 4.0) was nitrogen free with chromic oxide as the inert marker.

The CP endogenous flow was 7177 $mg \cdot kg^{-1}$ of dry matter intake ($mg \cdot kg^{-1}$ DMI) and would represent 1148 $mg \cdot kg^{-1}$ DMI of nitrogen similar to the 1234 $mg \cdot kg^{-1}$ DMI reported by Woyengo et al. (2010a). They fed a 10% casein-starch assay diet with titanium oxide indigestible marker to 21 day old Ross 308 birds. Parsons (2006) mentioned a trend for increased level of endogenous ileal amino acid flow when assay diets were used which had increasing levels of casein. The same author noticed that when

birds were given a nitrogen free diet in different labs, the endogenous flows were not affected by location. The endogenous ileal amino acid flows in this study ranged from 812 to 59 mg·kg^{-1} DMI with (TRP) at the lower end and histidine (HIS) at the higher end. Parsons (2006) reported 44, 138 and 182 mg·kg^{-1} DMI endogenous flows for MET, cysteine (CYS) and LYS respectively using nitrogen free assay diet.

TABLE 4.8. Ileal amino acids and crude protein flow of 21 day old Ross 308 broilers birds fed a nitrogen free diet from day 15 to 21

Amino Acids	Flow mg·kg^{-1} of DMI*
Methionine	95
Lysine	307
Cysteine	174
Threonine	454
Tryptophan	59
Isoleucine	239
Arginine	353
Valine	361
Leucine	409
Phenylalanine	236
Serine	453
Glycine	318
Histidine	812
Aspartic acid	540
Glutamic acid	722
Proline	406
Alanine	263
Tyrosine	189
NH3	222
Crude protein	7177

*Data represent means of 5 pens of broilers with 6 broilers per pen.

As observed with the apparent CP digestibility coefficients, the standardized ileal CP digestibility coefficients (Table 4.9) were affected only by the heat treatment. The heat treatment reduced the standardized ileal CP digestibility. The standardized ileal CP values were higher than the apparent values. This might be an indication that some of the

CP or nitrogen present in the ileum is of endogenous origin. This would lead to an under estimation of the bird's use of the protein from the ingredients (Lemme et al. 2004). Since the cost of protein rich feed ingredients is high, using standardized CP might be a better digestibility estimate in diet formulations.

TABLE 4.9. Effects of heat and oil level on mechanically pressed black canola meal standardized ileal CP digestibility coefficients in 21 day old broilers

	Digestibility Coefficients (%)		
	No heat	Heat	Oil levels coefficients
Black Canola meals			
10% Oil meal	75±2	62±2	69±2
14% Oil meal	74±2	65±2	69±2
Effects of heat	74±2a	64±2b	
Source of variation		P>F	
Oil level		0.7047	
Heat treatment		0.0003	
Oil level*Heat		0.5393	

[a-b] Mean ± SEM with no common letters in the heat treatment group are different $\alpha = 0.05$

Table (4.10) and (4.11) shows the standardized ileal amino acid digestibility of MPBCM used in this study. The heat treatment had significant effects on the digestibility coefficients of all amino acids reported. The effects of the meal oil levels were only significant for some amino acids as were the effects of the interactions between the application of the heat treatments and oil levels of the meals.

The standardized ileal digestibility coefficients that were affected by the individual treatments were MET, HIS, CYS, TRP and tyrosine (TYR). The application of heat significantly (P=0.0012) reduced the standardized ileal digestibility coefficients of MET, HIS and TRP. CYS and TYR were also reduced by the application of heat to the meals. The differences in the oil levels of the meals did not affect MET, HIS, CYS and

TABLE 4.10. Effects of heat and oil level on the standardized ileal amino acid digestibility coefficients of mechanically pressed black canola meal in 21 day old male broilers

	Essential Amino Acids Digestibility Coefficients (%)									
Effects of Treatment	LYS	MET	TRP	THR	ARG	LEU	HIS	PHE	VAL	GLY
Effects of oil										
Low Oil meal	77	86	85a	69	83	74	56	75	66.3	75.8
High Oil meal	74	83	75b	61	81	71	54	72	64.3	69.0
Effects of heat										
No heat	83	89a	90a	76	87	81	64a	82	74	82
Heat	68	80b	70b	54	77	65	45b	65	57	63
Effects of oil x heat										
LOM	87a	91	97	84a	91a	86a	66	87a	79a	89a
LOM-H	67b	81	72	68b	74b	63c	46	63c	54c	63c
HOM	79a	86	82	54c	83ab	76b	63	78ab	69ab	75b
HOM-H	68b	79	67	54c	80ab	67b	44	67b	60bc	63c
Source of variation	P>F									
Oil level	0.1082	0.1014	0.0014	0.0046	0.6888	0.2121	0.6582	0.3573	0.4886	0.0117
Heat treatment	<.0001	0.0002	<.0001	<.0001	0.0026	<.0001	0.0012	<.0001	<.0001	<.0001
Oil level*Heat	0.0376	0.3916	0.0767	0.0030	0.0418	0.0132	0.8821	0.0221	0.0115	0.0082

[a-c] Mean in the same column in the same effect group with no common letters are different $\alpha = 0.05$

Methionine =MET, Lysine=LYS, Threonine =THR, Tryptophan =TRP, Arginine =ARG, Valine =VAL, Leucine = LEU, Phenylalanine =PHE, Glycine =GLY, Histidine =HIS

TABLE 4.11. Effects of heat and oil level on the standardized ileal amino acid digestibility of mechanically pressed black canola meal in 21 day old male broilers

	Non-Essential Amino Acids Digestibility Coefficients (%)								
Effects of Treatment	ILE	CYS	ALA	ASP	PRO	SER	GLU	TYR	NH3
Effects of oil									
Low Oil meal	67	72	79	74	70	69	83	75	75
High Oil meal	66	68	73	69	64	62	80	74	69
Effects of heat									
No heat	75	79a	83	82	76	76	88	82a	82
Heat	57	61b	69	61	58	55	75	67b	63
Effects of Oil x Heat									
LOM	80a	75	89a	87a	83a	83a	92a	86	88a
LOM-H	54c	60	68c	60c	57c	54c	74b	64	63c
HOM	71ab	82	77b	76b	70b	69b	85a	79	75b
HOM-H	61bc	62	69bc	62c	59c	56c	76b	69	64c
Source of variation					P>F				
Oil level	0.7603	0.3089	0.1080	0.0277	0.1790	0.0272	0.1424	0.7741	0.0072
Heat treatment	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	<.0001
Oil level*Heat	0.0153	0.5690	0.0269	0.0036	0.0789	0.0081	0.0247	0.0779	0.0020

[a-c] Mean in the same column in the same effect group with no common letters are different $\alpha = 0.05$

Cysteine = CYS, Isoleucine = ILE, Serine =SER, Aspartic acid =ASP, Glutamic acid =GLU, Proline =PRO, Alanine =ALA, Tyrosine =TYR, Ammonia =NH3

TYR standardized ileal digestibility coefficients. This trend in the reduction of the digestibility coefficients of amino acids after the application of heat was also reported by other researchers (Anderson-Hafemann et al. 1993, Newkirk et al. 2003a). Newkirk et al. (2003a) saw the standardized ileal digestibility coefficients of MET, HIS, CYS and TYR reduce from 89.5, 86.6, 78.8 and 78.2 in expelled canola meal to 85.9, 81.8, 65.4 and 77.4% in toasted canola meal respectively for each amino acid. Anderson-Hafermann et al. (1993) reported the effects of different autoclaving times of canola meal on the true digestibility of amino acids. As the autoclaving time increased, the amino acids digestibility was reduced. The same reducing trend was observed for CYS and MET true digestibility of canola meals tested after expelling and after toasting, but HIS did not change (Anderson-Hafermann et al. 1993).

Amino acids such as LYS, threonine (THR), arginine (ARG), leucine (LEU), phenylalanine (PHE), valine (VAL), glycine (GLY), isoleucine (ILE), alanine (ALA), aspartic acid (ASP), proline (PRO), serine (SER) and glutamic acid (GLU) standardized ileal digestibility coefficients were all significantly ($P \leq 0.05$) affected by the interaction effects of oil levels and the heat treatment. The standardized ileal digestibility coefficients for THR, LEU, GLY, ALA, ASP, PRO and SER were higher in the meal of the 10% residual oil meal diets than the other diets. Those amino acids were better utilized when the oil level of the meal was at the lower end 10% and no heat was applied to that meal. There was no significant difference between 10% residual oil meal and 14% residual oil meal standardized ileal digestibility coefficients for LYS, PHE, VAL, ILE and GLU. Of those amino acids, LYS and GLU coefficients from the meals that were not heat treated digestibility coefficients were significantly higher than the coefficients from

meals that were heat treated. It seems that oil levels may not have a large impact on those amino acids digestibility but heat may. Newkirk et al. (2003a) and Spragg and Mailer (2007) suggested that some amino acids, especially LYS, tend to participate more in Maillard reaction due to the very reactive amino group leaving less LYS in the meal after heat treatment. When looking at ARG standardized ileal digestibility coefficients, only the heated 10% residual oil meal was significantly different from none heated 10% residual oil meal. The data from this study demonstrated that processing environment and meal residual oil may induce variation in the standardized ileal amino acids digestibility coefficients for mechanically pressed canola meal. Other researchers (Newkirk et al. 2003a, Anderson-Hafermann et al. 1993) also found the effects variation in processing have on the digestible coefficients but they did not look at the effects of meal residual oil or the interaction of processing and meal residual oil.

4.5 Conclusion

This study shows that dry air oven heating of MPBCM at 115º C for 25 minutes significantly reduced the available nutrients to broilers. The effects of variable residual oil levels found in mechanically pressed canola meal influenced some nutrient availability. The application of heat reduced the AMEn and the standardized ileal amino acid digestibility in MPBCM. The 14% residual oil meals gave higher AMEn values than the 10% residual oil meal. There was no specific influence of residual oil levels in the meals on the standardized ileal amino acid digestibility of mechanically pressed meals black canola meal.

CHAPTER 5: THE EFFECTS OF OIL LEVELS AND ENZYME ON THE NUTRITIVE VALUE OF MECHANICALLY PRESSED BLACK CANOLA MEAL (*BRASSICA NAPUS*) IN 21 DAY OLD BROILER CHICKENS

5.1 Abstract

Mechanical-pressing of oilseeds from small biofuel processors produces meal with variable residual oil. The nutritive value of *Brassica napus* black canola meal is influenced by the type of oil extraction process. Black canola meal nutritive value may be improved by the use of exogenous dietary enzymes. Limited digestibility data is available for poultry on the effects of enzymes on the digestibility of mechanically pressed black canola from small biofuel processors. This study assessed apparent ileal digestible nutrients and metabolizable energy (AMEn) of black canola (*Brassica napus)* meals high oil meal (HOM) and low oil meal (LOM) by the substitution method using broiler chicks. Two hundred and forty, day old Ross-308 male chicks were assigned to the eight dietary treatments (6 birds per cage, 5 replicate cages per treatment) in a completely randomized design with a 2 x 4 factorial arrangement (residual oil levels x enzyme treatments) from day 15 to 21. Diets were corn-soybean meal basal diets substituted with 30% canola meal [heated or non heated] fed with no enzyme, a carbohydrase, a protease or a lipase. The AMEn digestibility coefficient was depended on oil level. The AMEn values from this study ranged from 2890 to 3984 kcal·kg^{-1}. The addition of protease or lipase reduced the AMEn of the 10% residual oil meal. The 14% residual oil meal with no enzyme had the lowest AMEn but the addition of enzyme had no significant improvement in the AMEn. The ileal crude protein (CP) digestibility values were significantly (P=0.0363) affected by the interaction of the enzyme and residual oil levels of the meals. Additions of protease to meals with 14% residual oil significantly increased the ileal CP digestibility. The addition of lipase reduced the ileal CP in the meal with 10% residual oil. There was a trend for higher digestibility of amino acids in the LOM than the HOM. Tryptophan (TRP) and histidine (HIS) digestibility were significantly (P<0.05) affected by meal oil levels. The meals with higher residual oils had lower TRP and HIS digestibility. All standardized ileal amino acid digestibilities except for TRP and HIS were significantly (P<0.05) affected by the oil by enzyme interaction.

Keywords: High oil residue canola meal, Digestible nutrients, Broilers, Dietary enzymes

5.2 Introduction

The use of enzyme technology to improve the nutritive value of solvent extracted canola for poultry is well documented (Khajali and Slominski 2012). There has been some improvement in the selection and development of specific enzymes for improving canola nutrient digestion in poultry (Simbaya et al. 1996, Kermanshahi 1998, Meng et al. 2004). All of those studies involved the use of the most available form of black canola meal, solvent extracted *Brassica napus*. It is well known that the chemical compositions of mechanically extracted canola *Brassica napus* black canola meal is influenced by the type of oil extraction process (Leming and Lember 2005). There are differences between solvent extracted and mechanically extracted canola meal chemical compositions, especially for the oil content (Spragg and Mailer 2007).

Kermanshahi (1998) evaluated the use of exogenous lipase enzyme and its interaction with different dietary fat types. It is known that the ability of young chickens to use dietary fat is limited mainly due to the low level of lipase activity in the digestive tract (Kermanshahi 1998). Exogenous lipase supplementation of poultry diets by Kermanshahi (1998) did not show improvement in growth performance due to hydrolysis of the dietary fat by the lipase before the diets were consumed. Since mechanically pressed canola contains more residual oil than solvent extract meal, its use in diet with lipase might increase the risk of hydrolysis. Encapsulation of the enzyme might be possible to prevent hydrolysis of the meal before it is consumed. Dietary carbohydrates and proteases are also known to improve nutrient utilization from solvent extracted canola meal (Simbaya et al. 1996) and are routinely used in animal diets with solvent extracted canola.

Mechanically pressed canola meals often have different residual oil levels which may interact with enzyme which can affect nutrient availability of the meals. The interaction effects between the residual oil levels of mechanically extracted canola meal *Brassica napus* with different enzymes have not been tested to date. It is hypothesized that the interaction of exogenous enzymes with mechanically pressed canola will increase the AMEn and amino acid digestibility despite variability in the residual oil of the meal. The objective of this study was to identify the effects each enzyme has on the nutrients present in mechanically extracted canola meal with variable residual oil levels.

5.3 Materials and Methods

5.3.1 Preparation of ingredients

Black canola seeds were cleaned then expelled as described in chapter 4 section 4.3.1 to produce a meal with 12% residual oil and crude unfiltered oil. A sample of meal with 3% residual oil was prepared as described in section 4.31 of chapter 4. In brief a batch of meal was mixed with petroleum ether in a 1:3 ratio by weight then stirred for one minute in fifteen minute intervals for one hour. After mixing the meal, the ether was poured off and the meal dried at room temperature in a fume hood. The meal had 3% residual oil after drying. This meal with 3% oil was used to reduce the oil level of the 12% oil meal from the expelled process by mixing both meals to produce a meal with 10% residual oil as described in chapter 4 section 4.3.1. The unfiltered oil from the expelling process was used to raise the oil content of the 12% oil meal to 14%.

5.3.2 Diet preparation

For this experiment, ten diets in mash form were prepared from milled grains by mixing in a Hobart mixer. The starter and grower diets were corn soybean meal based diets. The starter diet was the same as that used in chapter 4 (Table 4.0) and it was formulated to have 3050 kcal·kg^{-1} metabolizable energy with 23% crude protein. The basal grower diet (Table 5.0) was formulated to have 3150 kcal·kg^{-1} metabolizable energy with 20% protein. The test diets were 70% basal diet substituted with either 30% MPBCM with 10 or 14% residual oil supplemented with 100g·tonne^{-1} of prioritized (protease 5000 μ·kg^{-1} feed), (carbohydrase: xylanase 2400 μ·kg^{-1} feed and amylase 240 μ·kg^{-1} feed) or (lipase 3300 μ·kg^{-1} feed). Enzymes were source from Genencor a Danisco division Denmark. The combinations created eight different grower test diets which were all in mash form like the grower basal. All the grower diets used in this study contained 0.5% chromic oxide as an indigestible marker.

5.3.3 Animal husbandry

Two hundred and forty male, day-old Ross 308 broiler chicks were obtained from a local hatchery. When the birds arrived they were randomly selected and placed in groups of six then the group was weighed and distributed randomly to 45 battery cages in an environmentally controlled room at the Atlantic Poultry Research Center. The temperature and lighting of the rooms when the birds arrived were 32ºC and 20 lux. The temperature was reduced by 1ºC every 2 days until a temperature of 21ºC was reached. The light intensity was reduced by 5 lux every 4 days until 5 lux was reached. All the birds were given a common broiler starter diet in mash form (Table 4.0) from the day of arrival at the research facility to 14 days post-hatch. On day 14 all the birds in each cage

were weighed as a group and assigned to the basal or test diets with each treatment diet fed to five replicate cages of six birds.

TABLE 5.0 Diet formulations used to test the effects of enzyme and oil levels on the nutritive value of mechanically pressed black canola meal by 21 day old broiler birds

	Grower test diets		
Ingredient (%) as fed basis	Basal	without enzyme	with enzyme
Corn	65.8	41.8	41.7
Soybean meal	30.2	24.3	24.3
Mechanically pressed meal[z]	---	30	30
Limestone	1.6	1.6	1.6
Mono-dicalcium phosphate	0.8	0.8	0.8
Iodized Salt	0.4	0.4	0.4
Methionine premix[y]	0.2	0.1	0.1
Vitamin/mineral premix[x]	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5
Enzyme[w]	---	---	0.05
Total	100	100	100
Calculated Analyses			
Metabolizable energy (kcal·kg-1)	3150	---	---
Crude protein (%)	20	---	---
Standardized ileal dig lysine %	1.1	---	---
Standardized ileal dig methionine %	0.4	---	---
Calcium (%)	0.9	---	---
Available phosphorus (%)	0.4	---	---

[z]Mechanically pressed meal is black canola meal with 10 or 14% residual oil

[y]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

[x]Premix, vitamin A (650×106 IU kg^{-1}), 15g,vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}),50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g; Wheat middlings 1532g; Ground limestone (38%),500g.

[w]Enzyme 100g·$tonne^{-1}$ protease 5000 μ·kg^{-1} feed, (carbohydrase: xylanase 2400 μ·kg^{-1} feed and amylase 240 μ·kg^{-1} feed) or lipase 3300 μ·kg^{-1} feed (Genencor A Danisco Division, Denmark)

Daily feeding was done by hand, with feed weighed in each day and weighed back at day 14, 21 and when mortality occurred. The birds were fed *ad libitum* and had free access to water throughout the experiment. The dead birds were weighed and feed weighed back from the trough of that cage and the mortalities recorded. All birds that died were necropsied by a veterinary pathologist. All broilers were managed based on the local Animal Care and Use Committee of Dalhousie University using the guidelines provided by the Canadian Council on Animal Care (2009).

5.3.4 Performance data collection

Production performance was measured as feed consumption, body weight gain, feed conversion ratio (FCR) and mortality. At day of arrival, day 14 and 21, body weight were recorded and feed consumed recorded on day 14 and 21. The mortalities were recorded as they occurred throughout the trial. The body weight gain and feed consumption data were used to determine FCR. The mortalities recorded were expressed as percentage of total birds entering each growth phase.

5.3.5 Sample collection and analysis

Sample collection and analysis were done as outlined in chapter 4 section 4.4.5. In brief excreta samples were collected from day 20 to 21 from beneath all cages and birds were group weighed by cage on day 21. All the birds were killed by cervical dislocation, dissected for the removal of gastrointestinal tracts. Gastrointestinal contents from Meckel's diverticulum to approximately 1 cm anterior to the ileal-cecal junction were collected by flushing the selected intestine sample with distilled water into containers. Digesta from birds in one cage were pooled in individual containers. Feed samples were collected from all diets. All samples were stored at -20ºC until analyzed.

Dry matter of the ileal digesta, excreta, feeds and canola meal samples was determined as outlined in chapter 4 section 4.5.5. Crude protein of all samples were determined by combustion method 990.03 (Association of official Analytical Chemists (AOAC) 2000) with a Leco Nitrogen Determinator (Leco Corporation, St. Joseph, MI), using EDTA as the calibration standard.

The gross energy of samples was analyzed using a parr adiabatic bomb calorimeter (Parr Instrument Company, Moline, Illinois). The concentration of chromic oxide in feed, digesta and excreta were determined by the method of Fenton and Fenton (1979) using a Bausch and Lomb Spectronic 501 model 33.51.039 (Milton Ray Company USA).

Amino acid profile of test ingredients, diets and ileal digesta samples were analyzed using an amino acid analyzer (Sykam, Eresing, Germany) using method 985.28, 994.2 and 988.15 AOAC (2000) according to the modifications described by Woyengo et al. (2010a) as described in chapter 4, section 4.45.

5.3.6 Calculations

All digestibility calculations were done using the methods of Lloyd et al. (1978), Moughan et al. (1992), Woyengo et al. (2010a) and Jayaraman (2010) as described in chapter 4 section 4.5.6. Ileal CP and amino acid flows as described in section 4.4.6 were incorporated in the calculations.

5.3.7 Statistical analysis

The standardized ileal digestible crude protein, standardized ileal digestible amino acids, apparent digestibility of crude protein, dry matter and apparent metabolizable energy (AMEn) and its digestibility values were subjected to analysis of variance using the Proc

Mixed procedure of SAS 9.3, (SAS Institute Inc., Cary, NC). The experimental design was completely randomized in a 2 x 4 factorial with meal residual oil level and enzyme addition as the factors. Meal residual oil level was 10% or 14% and enzyme treatments were no-enzyme or protease or carbohydrase or lipase.

Experiment model: $Y_{ijk}= \mu + A_{i\ (1-2)} + B_{j\ (1-4)}\ AB_{ij}+ \varepsilon_{ijk(5)}$

The statistical model of the experiment as shown above where Y is the response variable and μ is the overall mean response of that factor.

$A_{i\ (1-2)}$ is the effect of meal at the i^{th} residual oil level (1= 10% and 2 = 14%).

$B_{j\ (1-4)}$ is the effect of enzyme at the j^{th} treatments (1= non-enzyme, 2 = protease, 3 = carbohydrase and 4 = lipase).

AB_{ij} is the effects of the interaction at the ij^{th} oil level and treatments.

ε_{ijk} is the residual error of the model with $_k$ replication of five.

Experiment model growth data: $Y_{ij}= \mu +\alpha_i+ \varepsilon_{ij}$

Where, Y is the response variable (body weight, feed consumption and FCR),

μ is overall mean, α_i is the effect of diets and ε_{ij} is the residual error

If significant main effects or interactions were found ($P \leq 0.05$) for the apparent digestibility of crude protein, dry matter, standardized ileal digestible crude protein, standard ileal digestible amino acids, apparent metabolizable energy (AMEn), and digestibility values, Tukey Kramer test (Littell et al. 1996) was used to compare differences among the least square means at ($\alpha = 0.05$). Orthogonal contrasts were done for production performance data comparing basal and treatment group.

5.4 Results and Discussion

5.4.1 Analyzed composition of diets and test ingredients

The analyzed nutrient content of the test ingredients and the diets used in this experiment are given in Tables 5.1 and 5.2. The analyzed apparent metabolizable energy (AMEn) of the basal diet was lower at 2,751 kcal·kg^{-1} than its calculated value of 3,150 kcal·kg^{-1}. All grower test diets (Table 5.1.) used in this study had lower AMEn than the NRC (1994) recommendations 3200 kcal·kg^{-1} for growing broilers age 0-3 week old which is expected since they are digestibility substitution diets not recommended diets. The AMEn of 10% residual oil meal no-enzyme diet (3,001 kcal·kg^{-1}), 14% residual oil meal carbohydrase diet (2,933 kcal·kg^{-1}) and 14% residual oil meal lipase diet (2,934 kcal·kg^{-1}) diets were similar to the AMEn of 2,917 kcal·kg^{-1} for expeller extracted canola meal substituted diets reported by Woyengo et al. (2010a). All the other test diets AMEn were slightly lower than Woyengo et al. (2010a) diets but higher than 2520 kcal·kg^{-1} for expeller extracted *Brassica campestris* reported by Bayley et al. (1974). The variation seen in the diets AMEn in relation to Wayengo et al. (2010a) and Bayley et al. (1974) from this study could be related to the level of residual oil in the meals that were substituted in the basal diets.

The CP contents of all the test diets (25.9 to 27.2%) Table 5.1 was higher than the 23% recommended by NRC (1994) for broilers 0-3 weeks old, but the basal diet was lower at 21.6%. The basal diet had 1.56% more crude protein when compared to the calculated value of 20% in Table 5.0. However, all the diets were similar to the 20% recommended by NRC (1994) for broilers 3 to 6 weeks old. The LYS content of all diets (1.81 to 1.39%) exceeded that of the calculated estimate (1.1%) in Table 5.0.

TABLE 5.1. Analyzed nutrient composition of diets used to test the effects of enzyme and meal residual oil levels of mechanically pressed black *Brassica napus* nutrient digestibility in 21 day old broilers (as fed basis).

	Diets								
		LOM				HOM			
	Basal	No-E	C	P	L	No-E	C	P	L
Analyzed Nutrients									
Gross energy (kcal·kg^{-1})	4,040	4,331	4,316	4,324	4,287	4,385	4,413	4,367	4,388
AME$_n$ (kcal·kg^{-1})	2,751	3,001	2,883	2,865	2,793	2,820	2,933	2,872	2,934
Crude Protein %	21.6	27.2	26.4	27.1	27.2	26.5	25.9	26.3	26.1
Methionine (%)	0.37	0.55	0.53	0.51	0.51	0.49	0.52	0.52	0.51
Lysine (%)	1.81	1.53	1.69	1.62	1.60	1.39	1.55	1.53	1.51
Cysteine (%)	0.26	0.47	0.49	0.48	0.47	0.45	0.47	0.45	0.45
Threonine (%)	0.97	1.13	1.23	1.16	1.16	1.02	1.12	1.13	1.10
Tryptophan (%)	0.20	0.27	0.29	0.29	0.29	0.28	0.28	0.28	0.25
Isoleucine (%)	0.89	0.99	1.05	1.02	1.01	0.88	1.02	0.89	0.94
Arginine (%)	1.64	1.82	1.95	1.87	1.82	1.61	1.80	1.77	1.77
Valine (%)	1.00	1.24	1.26	1.22	1.23	1.04	1.23	1.11	1.14
Leucine (%)	1.97	2.04	2.18	2.13	2.12	1.87	2.07	1.96	2.02
Phenylalanine (%)	1.15	1.21	1.30	1.23	1.30	1.08	1.23	1.19	1.23
Serine (%)	1.31	1.38	1.49	1.42	1.42	1.27	1.35	1.41	1.36
Glycine (%)	1.01	1.23	1.32	1.27	1.26	1.13	1.21	1.21	1.19
Histidine (%)	0.75	0.83	0.90	0.86	0.87	0.89	0.81	0.82	0.82
Aspartic acid (%)	2.50	2.50	2.73	2.61	2.59	2.29	2.49	2.58	2.47
Glutamic acid (%)	4.48	4.80	5.08	4.94	4.93	4.34	4.75	4.85	4.71
Proline (%)	1.58	1.76	1.82	1.81	1.81	1.61	1.74	1.82	1.73
Alanine (%)	1.10	1.21	1.27	1.23	1.23	1.11	1.20	1.19	1.18
Tyrosine (%)	0.76	0.83	0.90	0.83	0.85	0.72	0.83	0.82	0.82
NH3 (%)	0.50	0.54	0.55	0.53	0.53	0.52	0.54	0.53	0.53

LOM = 30% black canola with 10% residual oil+70 Basal diet, HOM = 30% black canola with 14% residual oil+70 Basal diet
L=Lipase, P=Protease, C=Carbohydrase, No-E=No-Enzyme

TABLE 5.2. Analyzed nutrient composition (DM basis) of meals used to test the effects of enzyme and meal residual oil level on the nutrient digestibility of black *Brassica napus* in 21 day old broilers.

Analyzed Nutrients	LOM	HOM
Dry matter (%)	93	92
Gross energy (kcal·kg^{-1})	4,877	5,120
Crude Protein %	34.9	34.4
Fat (%)	10.2	14.0
Calcium %	0.56	0.52
Phosphorus %	1.26	1.23
Methionine (%)	0.60	0.58
Lysine (%)	1.47	1.81
Cysteine (%)	0.71	0.67
Threonine (%)	1.09	1.42
Tryptophan (%)	0.37	0.35
Isoleucine (%)	0.95	1.06
Arginine (%)	1.74	2.09
Valine (%)	1.25	1.46
Leucine (%)	1.95	2.10
Phenylalanine (%)	1.16	1.23
Serine (%)	1.32	1.46
Glycine (%)	1.19	1.61
Histidine (%)	0.80	0.99
Aspartic acid (%)	2.35	2.39
Glutamic acid (%)	4.61	5.28
Proline (%)	1.71	2.01
Alanine (%)	1.18	1.32
Tyrosine (%)	0.78	0.86
NH3 (%)	0.53	0.69

LOM = black canola with 10% residual oil, HOM = black canola with 14% residual oil

The analyzed methionine (MET) content (0.55 to 0.49%) of all the diets was above the value of the 0.4% of the calculated estimate except for the basal diet which was lower at 0.37%. The analyzed LYS values of all the diets in this study were above the NRC (1994) recommendation for broiler 0 to 6 weeks old. The analyzed MET contents of all diets except the basal diet were similar to the recommendation for 0-3 week old birds.

The dry matter (DM) content of the meals used in this study (Table 5.2.) was similar to the 92% reported by Woyengo et al. (2010a). The DM of 10% residual oil meal

was 1% higher than 14% residual oil meal. The DM of the meals in this study also fell in the range of the 89.6 to 98.2% reported by Leming and Lember (2005).

The gross energy (GE) of the meals (Table 5.2) ranged from 4877 to 5120 kcal·kg^{-1} and the meal with the higher oil levels was associated with the higher GE. The GE values for 14% residual oil meal in this study were similar to the 5199 kcal·kg^{-1} reported by Woyengo et al. (2010a) for expeller extracted canola meal. While 10% residual oil meal GE was lower than the 5570 to 5402 kcal·kg^{-1} range reported by Jayaraman (2010) and the 6019 kcal·kg^{-1} reported by Smulikowska et al. (2006) for single cold press black canola. Smulikowska et al. (2006) meal residual oil was 17.4% and that of Jayaraman (2010) was 18%. The oil content of canola meal is known to influence its GE content (Newkirk et al. 2003a) and the difference observed between this study and that of Smulikowska et al. (2006) and Jayaraman (2010) may be due to variation in the residual oil content of the meal.

The CP of the meals (Table 5.2) were 35 and 34% respectively for 10% residual oil meal and 14% residual oil meal and were less than the 41% reported by Woyengo et al. (2010a) but higher than the 31.1% to 32% reported by Smulikowska et al. (2006) and Schöne et al. (1996) and Newkirk et al. (2003a). The canola seeds used in this study were expelled without any pretreatment except cleaning as reported in Fig 2.1, chapter 1 so the CP might be less prone to damage (Newkirk et al. 2003a). The LYS content reported in this study were similar to the 1.78 and 1.61% reported for regular press cake canola and green seeded press cake canola by Thacker and Petri (2009a) but their MET levels were much higher which ranged from 1.42 to 1.82%. The cause of this large difference in LYS content of the meals used in this study to what Thacker and Petri (2009a) have found is

not known. One possibility could be due to the maturity of the seeds that were used in this study compared to the off grade seed used by Thacker and Petri (2009a). Off grade canola seeds may have a large proportion of immature seed and as such cannot be used for quality cooking oil production (Canola Council of Canada 2009).

5.4.2 Animal performance

In this study the birds production performance (Table 5.3.) were in the normal range based on our research facility averages (Jayaraman 2010). There was no effect of dietary treatment on body weight, feed consumption, FCR and mortalities over the 7 day period of this experiment. Mortality was 9.6% which would be considered high, however there were no differences between treatments for mortality. After careful evaluation of the autopsy reports it was revealed that 21 of the birds were culled due to bad legs and the other 5 were due to chick a combination of omphalitis, ascites and coliform septicemia.

TABLE 5.3. Growth performance of birds testing the effects of meal residual oil and enzymes on the digestible nutrients of black *Brassica napus* in 21day old broilers

Performance parameters	Basal diets	Treatment diets
Body weight gain ($g \cdot b^{-1} \cdot d^{-1}$)	30±2.8	35±1.0
Feed consumption ($g \cdot b^{-1} \cdot d^{-1}$)	53±4.7	57±1.7
FCR	1.8±0.19	1.7±0.07

ANOVA *P*-Values

Contrast	Body weight gain	Feed consumption	FCR
Basal VS Treatments	0.1049	0.3556	0.5323

Mean ± SEM

5.4.3 Apparent digestible nutrients

There were no effects of treatments on the DM digestibility coefficients of MPBCM in this study (Table 5.4.). In chapter 4 Table 4.4. oil levels influenced the DM digestibility coefficients of the meal. The DM digestibility coefficients in this study were higher than the range reported in chapter 4 and those reported for regular (67 to 68%) and green seeded (67 to 68%) canola pressed cake (Thacker and Petri 2009a). Those authors reported significant linear and quadratic effects of increasing level of both meals in the diet which increased the DM digestibility. Their lower DM digestibility compared to this study could be due to meal quality after processing of the seeds having more than 20% green seeds. The seeds used in this study were high quality that would normally use for quality oil production.

TABLE 5.4. Effects of enzyme and oil level on mechanically pressed black canola meal DM digestibility coefficients

	Digestibility Coefficients (%)		
	10% Oil meal	14% Oil meal	Effects of Enzyme
Enzyme treatments			
No-Enzyme	85±2	81±2	83±1
Carbohydrase	84±2	82±2	83±1
Protease	82±2	82±2	82±1
Lipase	84±2	82±2	83±1
Effects of Oil	83±2	82±2	
Source of variation		P>F	
Oil		0.2297	
Enzyme		0.8881	
Oil *Enzyme		0.6344	

Mean ± SEM

The AMEn digestibility coefficients (Table 5.5.) were influenced by the interaction between the oil levels and the enzymes. The oil levels made a difference in the diets without enzyme but oil level did not make a difference in the enzyme diets. The lipase in the 14% residual oil meal diets, the protease in the 10% residual oil meal diet and the 14% residual oil meal diets with no enzyme had significantly higher AMEn digestibility coefficients than the 10% residual oil meal diet with no enzyme. There was no difference between the 10% residual oil meal diet with no enzyme and the rest of diets. On average the AMEn digestibility coefficients from this study were lower than those reported in the chapter 4 by 11-16%. A similar difference was seen between the no enzyme group and the non heated group AMEn digestibility coefficients chapter 4.

TABLE 5.5. Effects of enzyme and oil level on mechanically pressed black canola meal AMEn digestibility coefficients in 21 day old broilers

	Digestibility Coefficients (%)		
	10% Oil meal	14% Oil meal	Effects of Enzyme
Enzyme treatments			
No-Enzyme	73±1b	77±1a	75±1
Carbohydrase	74±1ab	76±1ab	75±1
Protease	77±1a	76±1ab	76±1
Lipase	74±1ab	77±1a	75±1
	75±1	77±1	
Effects of Oil			
Source of variation		P>F	
Oil level		0.0006	
Enzyme treatment		0.3149	
Oil level x Enzyme treatment		0.0091	

[a-b] Means ± SEM with no common letters in the oil*enzyme interaction are significantly different at $\alpha = 0.05$

The digestible AMEn values of the meals used in this study (Table 5.6) were influenced by the interaction between the meal residual oil levels and enzyme. The AMEn values were much higher than those reported in chapter 4. The correlation of higher AMEn with

increased residual oil content of mechanically pressed canola reported by Jayaraman (2010) and Wayengo et al. (2010a) and as reported in chapter 4 was not observed in this study. This may be due to the interaction effects of the enzyme and oil levels of the meals. When the lipase or protease enzyme was added to the 10% residual oil meal diet AMEn was reduced. The diet with 10% residual oil meal with no enzyme had the highest AMEn 3584 kcal·kg^{-1}. Careful evaluation of the concentration of chromic oxide in excreta samples for that treatment shows that the chromic oxide was relatively higher for all replicated cages compared to the other treatments in the trial. The 3584 kcal·kg^{-1} was not due to error since the standard deviation between the chromic oxide concentrations for the replicate cages of that treatment was very low and no outliers were detected for that treatment during statistical analysis.

TABLE 5.6. Effects of enzyme and oil level on mechanically pressed black canola meal AMEn in 21day old broilers

	Meal AMEn (kcal·kg^{-1} as fed)		
	10% Oil meal	14% Oil meal	Effects of Enzyme
Enzyme treatments			
No enzyme	3584±87a	2981±87bc	3282±62
Carbohydrase	3193±87abc	3358±87ab	3276±62
Protease	3133±87bc	3155±87bc	3144±62
Lipase	2890±87c	3362±87ab	3126±62
Effects of Oil	3200±44	3214±44	
Source of variation		P>F	
Oil		0.8219	
Enzyme		0.1607	
Oil*Enzyme		<.0001	

[a-c] Means ±SEM with no common letters in oil*enzyme interaction are significantly different at $\alpha = 0.05$

The addition of enzymes gave no improvements in the AMEn of the 14% residual oil meal diet or 10% residual oil meal diet. Meng et al. (2004) identified an interaction

effects between fat type in the diet and carbohydrase supplementation which increased the AMEn of the meals. In their study they attributed this increase in AMEn with the ability of the carbohydrase enzyme to release the non-starch polysaccharide components of the diets thereby making the oil cell exposed to endogenous digestive enzymes. Simbaya et al. (1996) investigated the effectiveness of carbohydrase supplementation on improving the nutritive value of canola meal in vitro and noticed that the mode of action of the enzyme was through the solubilization of the cell wall polysaccharide of the meal. Since the un-supplemented 14% residual oil meal or un-supplemented 10% residual oil meal AMEn was not different from the supplemented meal, the mode of action described by Meng et al. (2004) and Simbaya et al. (1996) must have not taken place which resulted in no effect of carbohydrase on the meals AMEn in this study.

The lipase enzyme was just as ineffective as the carbohydrase in increasing the AMEn of the meals. In the study conducted by Meng et al. (2004) there was no effect of lipase on the digestibility of fat, starch, nitrogen or non-starch polysaccharide components of the diets used. Unfortunately none of those components were tested in this study so the reason for which the AMEn in this study was not improved was not revealed. However, it is known that pancreatic lipase in poultry prefers etherification of fatty acids to glycerol by leaving the 2-monoglycerides intact which have a greater solubility for micelle formation and are absorbed in this form (Leeson and Summers 2005). The supplemental lipase in this study did not act like pancreatic lipase enzyme even though they are encapsulated and would have passed the upper digestive tract intact. If the supplemented lipase had increased micelle formation and absorption, then less fat would be available for excretion thereby increasing the AMEn from lipase addition.

There were no effects of enzyme on the apparent CP digestibility coefficients in this study (Table 5.7). The apparent CP digestibility for mechanically pressed black canola range from 48% to 73 and was less than previously reported for the meal in chapter 4. The effects of oil levels were significant for the apparent CP digestibility. The 10% residual oil meal had significantly higher CP digestibility than the 14% residual oil meal. These digestibility values were lower than those reported for expelled canola meal by other authors (Newkirk et al. 2003a, Peter and Danicke 2003, Smulikowska et al. 2006). This CP digestibility was a measure of N x 6.25 so the lower CP digestibility was an indication that the birds were retaining less nitrogen. The CP digestibility measurement was taken using the excreta sample which could have had a higher microbial load at the time of sampling which would result in higher nitrogen content of those samples than there would normally be.

TABLE 5.7. Effects of enzyme and oil level on mechanically pressed black canola meal apparent crude protein digestibility coefficients

	Digestibility Coefficients (%)		
	10% Oil meal	14% Oil meal	Effects of Enzyme
Enzyme treatments			
No-Enzyme	73±5	48±5	61±4
Carbohydrase	66±5	54±5	60±4
Protease	63±5	52±5	57±4
Lipase	62±5	59±5	61±4
Effects of Oil	65±3a	53±3b	
Source of variation		P>F	
Oil level		0.0010	
Enzyme treatment		0.8759	
Oil level x Enzyme treatment		0.1837	

[a-b] Mean ± SEM with no common letters in the oil effects group are significantly different at $\alpha = 0.05$

5.4.4 Ileal digestible nutrients

The standardized ileal CP digestibility values presented in Table 5.8 range from 73 to 80% and were not different from those reported for the meals that were not heat treated in chapter 4. The ileal CP digestibility values were affected by the interaction of the enzyme and residual oil levels of the meals. The supplementation of lipase significantly ($P \leq 0.05$) reduced the meal ileal CP in the 10% residual oil meal diet. This reduction in ileal CP was also significantly ($P \leq 0.05$) lower than that of birds fed the protease supplemented 14 % residual oil meal diet. The protease enzyme significantly improved the ileal CP digestibility of the meal in the 14 % residual oil meal diet. The data presented in Table 5.8 suggest that the supplementation of protease on CP digestibility was more beneficial in the meal with higher residual oil. The effects of protease on CP digestibility were only detected using the standardized ileal CP digestibility approach since no effects of protease was observed in the apparent CP digestibility (Table 5.7). The data support the concept of sampling from ileum instead of excreta as reported by Lemme et al. (2004).

TABLE 5.8. Effects of enzyme and oil level on mechanically pressed black canola meal standardized ileal crude protein digestibility coefficients

	Digestibility Coefficients (%)		
	10% Oil meal	14% Oil meal	Effects of Enzyme
Enzyme treatments			
No-Enzyme	79±1ab	74±1bc	77±1
Carbohydrase	76±1abc	77±1abc	76±1
Protease	78±1abc	80±1a	79±1
Lipase	73±1c	74±1bc	74±1
Effects of Oil	76±1	77±1	
Source of variation		P>F	
Oil level		0.8220	
Enzyme treatment		0.0010	
Oil level x Enzyme		0.0363	

[a-c] Mean ± SEM with no common letters in the oil*enzyme interaction are significantly different at $\alpha = 0.05$

Table (5.9) and (5.10) show the standardized ileal amino acid digestibility of MPBCM used in this study. The enzyme by oil interaction significantly ($P \leq 0.05$) affected all amino acids except TRP and histidine (HIS). Only the effects of oil level significantly ($P \leq 0.05$) affected the standardized ileal digestibility of TRP and HIS. The digestibility of TRP was significantly ($P=0.0457$) higher in the meals fed in the 10% residual oil meal diets than the 14% residual oil meal diets. The TRP digestibility values for each oil effects in this study were similar to those reported in chapter 4. The same reduction in digestibility with increased level of residual oil was true for HIS digestibility as well. HIS digestibility was significantly higher in the 10% residual oil meal diets than the 14% residual oil meal diets. The values reported for HIS were higher than those reported in chapter 4, but it should be noted that the effects of oil levels were not significant for HIS in chapter 4. The data from this study suggested that HIS and TRP digestibility were influenced mostly by the canola meal residual oil levels if the meals do not undergo heat treatment during processing.

All standardized ileal amino acid digestibility except for TRP and HIS were significantly affected by the interaction between the enzymes and the oil levels. The application of enzyme to the diets with 10% residual oil meal had no effect on the meal amino acids digestibility. Adding enzymes to the 14% residual oil meal diets improved the amino acids digestibility of the meal.

The enzymes by oil interactions for digestibility of LYS, threonine (THR), ILE, ARG, VAL, LEU, phenylanine (PHE), serine (SER), glycine (GLY), aspartic acid (ASP), glutamic acid (GLU), proline (PRO), alanine (ALA) and tyrosine (TYR) was only significantly different ($P \leq 0.05$) for the meals fed in the 14% residual oil meal diets with

no enzyme compared to the meals fed in the 14% residual oil meal diets supplemented with enzymes and the meals in the 10% residual oil meal diets. Each enzyme was able to (P<0.05) increase the amino acid digestibility for the meals fed in the 14% residual oil meal diets but no change was observed for 10% residual oil meal diets. Data from this study suggested that the effectiveness of those enzymes in improving the digestible amino acids of mechanically pressed canola meal is more useful for meal with higher residual oil.

The MPBCM standardized ileal amino acid digestibility values that were not supplemented with enzyme ranged from 63 to 89%. The average standardized ileal amino acid digestibility values were lower than the apparent values reported for expeller extracted by Newkirk et al. (2003a). Most of the amino acid digestibility coefficients of this study were in the range with the standardized ileal values reported by Woyengo et al. (2010a) for expelled canola meal. The differences seen between in the amino acid digestibility values of this study and that of Newkirk et al. (2003a) could be due to processing. The meals used in by Newkirk et al. (2003a) came after the pressing stage during regular canola oil production and as such would have underwent pre heating and conditioning before the seeds was pressed. The meals used in this study on the other hand had no pre heating stage before seed pressing and this could have caused the differences seen between the amino acid digestibility values in both study.

TABLE 5.9. Effects of oil levels and enzymes on the standardized ileal amino acid digestibility of mechanically pressed black canola meal in 21 day old broiler chickens

	Essential Amino Acid Digestibility Coefficient (%)									
Effects of Treatment	LYS	MET	TRP	THR	ARG	LEU	HIS	PHE	VAL	GLY
Oil										
LOM	75	93	90a	77	85	80	73a	81	79	81
HOM	71	81	87b	68	82	76	64b	76	72	75
Enzyme										
NO-E	68	89	87	64	79	73	63	73	70	73
C	75	91	91	75	85	79	73	80	77	80
P	76	90	90	75	85	79	65	80	76	80
L	75	94	87	75	84	80	73	81	78	80
Oil x Enzyme										
LOM x NO- E	74a	93a	88	74a	86a	81a	63	81a	81a	81a
LOM x C	76a	90ab	93	80a	86a	79a	84	81a	77a	82a
LOM x P	76a	91a	91	76a	85a	79a	72	80a	77a	82a
LOM x L	75a	96a	89	76a	84a	80a	72	83a	80a	81a
HOM x NO- E	61b	85b	85	54b	73b	65b	63	66b	59b	65b
HOM x C	73a	90ab	90	71a	84a	79a	62	79a	77a	78a
HOM x P	76a	90ab	89	74a	86a	79a	58	81a	76a	79a
HOM x L	75a	92a	84	74a	84a	80a	74	79a	75a	79a
Source of variation	P>F									
Oil	0.0007	0.0009	0.0457	<.0001	0.0021	0.0006	0.0242	0.0005	<.0001	<.0001
Enzyme	<.0001	0.0101	0.0791	<.0001	0.0002	0.0001	0.1102	0.0002	0.0024	0.0002
Oil*Enzyme	0.0010	0.0287	0.9766	0.0007	<.0001	<.0001	0.0588	<.0001	<.0001	0.0006

LOM = 30% black canola with 10% residual oil+70 basal diet, HOM = 30% black canola with 14% residual oil+70 Basal diet
L=Lipase, P=Protease, C=Carbohydrase, No-E=No-Enzyme, Methionine =MET, Lysine=LYS, Threonine =THR, Tryptophan =TRP, Arginine =ARG, Valine =VAL, Leucine = LEU, Phenylalanine =PHE, Glycine =GLY, Histidine =HIS
[a-b] Mean (n=5) with no common letters in an effect group: (oil*enzyme), (enzyme) or (oil) are significantly different at $\alpha = 0.05$

TABLE 5.10. Effects of oil levels and enzymes on the standardized ileal amino acid digestibility of mechanically pressed black canola meal in 21 day old broiler chickens

	Non-Essential Amino Acid Digestibility Coefficient (%)							
Effects of Treatment	ILE	ALA	ASP	PRO	SER	GLU	TYR	NH3
Oil								
LOM	77	83	77	80	74	86	78	74
HOM	71	78	71	75	67	83	73	71
Enzyme								
No-Enzyme	69	76	68	72	64	81	69	72
Carbohydrase	76	81	76	79	73	85	78	72
Protease	75	82	77	81	74	86	77	73
Lipase	76	83	75	79	73	86	76	74
Oil x Enzyme								
LOM x NO- E	78a	83a	76a	80a	72a	87a	79a	79a
LOM x C	77a	82a	78a	79a	76a	85a	80a	72ab
LOM x P	76a	82a	76a	81a	75a	86a	75a	73ab
LOM x L	78a	83a	74a	79a	73a	86a	78a	74ab
HOM x NO- E	61b	69b	60b	64b	55b	74b	60b	66b
HOM x C	76a	79a	74a	79a	69a	85a	75a	71ab
HOM x P	74a	82a	77a	81a	74a	87a	80a	72ab
HOM x L	74a	82a	74a	78a	72a	85a	75a	75ab
Source of variation	P>F							
Oil	0.0004	0.0002	0.0002	0.0012	<.0001	0.0012	0.0013	0.0181
Enzyme	0.0100	0.0005	<.0001	0.0002	<.0001	0.0001	0.0014	0.6274
Oil*Enzyme	0.0012	<.0001	<.0001	0.0001	0.0007	<.0001	0.0002	0.0093

LOM = 30% black canola with 10% residual oil+70 basal diet, HOM = 30% black canola with 14% residual oil+70 Basal diet
L=Lipase, P=Protease, C=Carbohydrase, No-E=No-Enzyme, Isoleucine = ILE, Serine =SER, Aspartic acid =ASP, Glutamic acid =GLU, Proline =PRO, Alanine =ALA, Tyrosine =TYR, Ammonia =NH3
[a-b] Means (n=5)* with no common letters in a oil*enzyme interaction group are significantly different at $\alpha = 0.05$

5.5 Conclusion

The dynamics between mechanically pressed *Brassica napus* black canola meal residual oil levels and enzyme supplementation must be taken into consideration when meals are used in broiler diets. The exogenous supplementation of enzymes had their greatest positive effects on the standardized ileal amino acids in diets containing meal with higher residual oil but no benefits to meal AME_n. Protease, lipase and carbohydrase increased all ammo acid digestibility coefficients in the HOM but no effects on ammo acid digestibility coefficients in the LOM. Protease and lipase reduced the AME_n of LOM but no effects in the HOM. Practical benefits can be gained by adding those enzymes to increase amino acid digestibility in diets having mechanically pressed canola meals with high residual oil.

CHAPTER 6: THE EFFECTS OF HEAT, OIL LEVELS AND ENZYMES ON MECHANICALLY PRESSED YELLOW CANOLA MEAL (*BRASSICA NAPUS*) NUTRITIVE VALUE IN 21 DAY OLD BROILER CHICKENS

6.1 Abstract

The mechanical-pressing of *Brassica napus* oilseeds appears to be an option for small biofuel processors in Canada. Recent developments in *Brassica* seed breeding have resulted in a new yellow line of *Brassica napus.* The yellow seed napus seemed to have higher oil content and better quality meal for monogastric animals. Limited research has been done in poultry on the digestibility of yellow Brassica napus meals from mechanical pressing. This study measured apparent ileal digestible nutrients and metabolizable energy (AMEn) of yellow *Brassica napus* meal with 14% and 10% residual oil, by the substitution method using broiler chicks. Half of both meals were heat treated at 115ºC for 25 minutes. Test diets were corn-soybean meal basal diet substituted with 30% of one of these canola meals [14% residual oil heated or no heated meal and 10% residual oil heated or no heated meal] fed in a protease, carbohydrase, lipase or no enzyme diet. Five hundred and ten, day old, Ross-308, male chicks were assigned to the seventeen dietary treatments (6 birds per cage, 5 replicate cages per treatment) in a completely randomized design in a 2x2x4 factorial arrangement with (residual oil levels x heat treatments x enzyme supplementations) from day 15 to 21. Heat treatments, oil levels and enzymes supplementation three-way interaction affected the AME_n significantly. Meals with 14% residual oil that were not heat treated but fed in a carbohydrase supplemented diet had higher (P<0.05) AME_n 3451±121 kcal·kg^{-1} than the same meal fed in a un-supplemented diet 2823±121 kcal·kg^{-1}. The amino acids digestibility ranged from a high of 97% to a low of 46% depending on the amino acid and the kind of treatment interactions that was significant. There was no effect of treatment on methionine digestibility. The addition of lipase generally improved the standardized ileal amino acid digestibility of mechanically pressed yellow *Brassica napus.*

Keywords: Yellow *Brassica napus* pressed meal, Digestible nutrients, Broilers, Dietary enzyme

6.2 Introduction

There has been consistent development of canola varieties through plant breeding to improve the nutritional quality of the meals for monogastric animals since the development of canola from rape seed (Bell 1993, Jia et al. 2012). It was observed early on in the breeding of canola that seed with yellow color had nutritional qualities of interest that were not seen in the black seed lines (Bell and Shires 1982, Slominski 1997). The relationship on how seed coat color influences the nutritional quality has been the subject of reviews (Rahman and McVetty 2011). Since yellow seed had better nutritional values in terms of protein content (Slominski 1997) plant breeders have focused their attention on the development of yellow lines of canola (Somers et al. 2001).

Currently, plant breeders in Canada were able to develop a new line of yellow seeded *Brassica napus* having a stabilized seed color and agronomic characteristics with the potential to become a commercial line (Somers et al. 2001). Development in breeding geared towards improving yellow *Brassica napus* is also been conducted in other countries as well (Bartkowiak-Broda et al. 2011, Slominski et al. 2012).

Studies (Bell and Shires 1982, Slominski et al. 1999, Jia et al. 2012) have assessed the nutritive value of solvent extracted yellow line *Brassica napus* meals in monogastric animals. To date, limited research has been done on the digestibility of yellow *Brassica napus* meals from mechanical pressed seeds in poultry diets. Meals of mechanically pressed yellow *Brassica napus* may have better nutritional quality than its solvent extracted counterpart. The objective of this study was to determine the influence of meal residual oil level, heat and dietary enzyme supplementation on the digestible nutrient content of mechanically pressed yellow *Brassica napus* meals.

6.3 Materials and Methods

6.3.1 Preparation of ingredients

Yellow canola (*Brassica napus*) seeds were cleaned then expelled to produce a meal with 16% residual oil. The oil obtained from the process was unfiltered or treated. To prepare a low and high residual oil meal for this experiment, the meal was prepared as described in chapter 4 section 4.3.1 with the following modifications. Mechanically pressed yellow canola meal (MPYCM) was mixed with petroleum ether at a ratio of 1:3 by weight. The mixture was stirred for two minutes at fifteen minute intervals for one hour. After mixing with the meal, the ether was poured off and the meal placed on an absorbent pad then firmly squeezed by hand to remove excess ether. The meal was then transferred to a new absorbent pad, then firmly squeezed by hand again, then transferred to a new absorbent pad and placed in a fume hood over night to dry. This mixing sequence and time produced a meal with 3% residual oil after air drying at room temperature in a fume hood.

The 3% oil meal was used to reduce the oil level of the 16% oil meal from the expeller process. Both meals were mixed to produce two meals, one with 10% residual oil and the other with 14% residual oil. Both sets of meals that were created were divided into halves, of which one half of each meal was heat treated as described in section 4.3.1.

6.3.2 Diet preparation

Eighteen diets were prepared in mash form using a Hobart mixer as described in chapter 4 section 4.3.2. The starter diet was the same as the one used in chapter 4 (Table 4.). It was formulated to have 3050 kcal·kg^{-1} metabolizable energy and 23% CP while the basal grower diets were formulated to have 3150 kcal·kg^{-1} metabolizable energy and 20%

crude protein. The starter and basal grower diets were corn soybean meal based diets. The grower test diets (Table 6.0) were 70% basal diet substituted with either 30% heated MPYCM having 10 or 14% residual oil in protease, lipase, carbohydrase or no enzyme diet. The enzyme was source from Genencor a Danisco division Denmark and supplemented at 100g·tonne^{-1} of prioritized (protease 5000 μ·kg^{-1} feed), (carbohydrase: xylanase 2400 μ·kg^{-1} feed and amylase 240 μ·kg^{-1} feed) or (lipase 3300 μ·kg^{-1} feed). This created 16 different grower test diets in mash form, all of which along with the grower basal diet contained 0.5% chromic oxide as an indigestible marker.

6.3.3 Animal husbandry

Five hundred and ten male Ross 308 day old broiler chicks were obtained from a local hatchery. Upon arrival the birds were weighed and distributed randomly to 85 battery cages (6 birds per cage) in a controlled environment room at the Atlantic Poultry Research Center. The temperature and lighting of the rooms when the birds arrived were 32ºC and 20 lux. The temperature was reduced by 1ºC every 2 days until a temperature of 21ºC was reached. The lighting was reduced by 5 lux every 4 days until 5lux was reached then held until the end of the trial. From the day of arrival at the research facility to 14 days post-hatch, all the birds were given a common broiler starter diet in mash form (Table 4.0) chapter 4. On day 14, the birds were batch weighed per cage and assigned to grower diets of the basal or test treatments in five replicate cages per dietary treatment. All the birds were hand fed daily as the feed given each day was weighed. The feed was weighed back when mortality occurred and at day 14 and 21. Throughout the experiment, birds had unrestricted access to feed via trough and water via nipple drinkers. Mortalities were recorded throughout the trial and necropised by a veterinary pathologist. Causes and

timing of the mortalities were analyzed. All broilers were managed under the supervision of the Dalhousie University local Animal Care and Use Committee using guidelines provided by the Canadian Council on Animal Care (2009).

TABLE 6.0 Diet formulations used to test the effects of enzyme, heat and oil levels on the nutritive value of mechanically pressed yellow canola meal in 21 day old broiler chickens

	Grower test diets		
	Basal	without enzyme	with enzyme
Ingredient as fed basis (%)			
Corn	65.8	41.8	41.7
Soybean meal	30.2	24.3	24.3
Mechanically pressed meal[z]	---	30	30
Limestone	1.6	1.6	1.6
Mono-dicalcium phosphate	0.8	0.8	0.8
Iodized salt	0.4	0.4	0.4
Methionine premix[y]	0.2	0.1	0.1
Vitamin/mineral premix[x]	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5
Enzyme[w]	---	---	0.05
Total	100	100	100
Calculated Analyses			
Metabolizable energy (kcal /kg)	3150	---	---
Crude protein (%)	20	---	---
Standardized ileal dig lysine %	1.1	---	---
Standardized ileal dig methionine %	0.4	---	---
Calcium (%)	0.9	---	---
Available phosphorus (%)	0.4	---	---

[z]Mechanically pressed meal: yellow canola meal with 10 or 14% residual oil treated with or without heat
[y]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings
[x]Premix, vitamin A (650×106 IU kg^{-1}), 15g vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}),50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g; Wheat middlings 1532g; Ground limestone (38%), 500g.
[w]Enzyme ·$tonne^{-1}$ protease 5000 μ·kg^{-1} feed, (carbohydrase: xylanase 2400 μ·kg^{-1} feed and amylase 240 μ·kg^{-1} feed) or lipase 3300 μ·kg^{-1} feed (Genencor A Danisco Division, Denmark)

6.3.4 Performance data collection

Production performance was measured as body weight gain, feed consumption, feed conversion ratio (FCR) and mortality during the trial. On day 0, 14 and 21 of the trial, the birds were weighed and feed consumed was recorded at day 14 and 21. Both sets of data were used to calculate FCR. The mortalities recorded as described in section 4.3.4.

6.3.5 Sample collection and analysis

Sample collection and analysis were done as outlined in chapter 4 section 4.4.5. In brief excreta samples were collected from day 20 to 21 from beneath all cages and birds were group weighed by cage on day 21. All the birds were then killed by cervical dislocation, dissected and the gastrointestinal contents from the Meckel's diverticulum to 1 cm above the ileal-cecal junction was collected with distilled water in containers. The digesta from the birds of one cage was pooled in individual containers. Feed samples were collected from all diets. All samples were stored at -20ºC until analyzed.

Dry matter of the ileal digesta, excreta, feed and meal samples was determined and prepared as outlined in chapter 4 section 4.5.5. Crude protein of all samples was determined by combustion method 990.03 AOAC (2000) with a Leco Nitrogen Determinator (Leco Corporation, St. Joseph, MI) using EDTA as the calibration standard. The gross energy of the samples was analyzed using a parr adiabatic bomb calorimeter (Parr Instrument Company, Moline, Illinois). The concentration of chromic oxide in feed, digesta and excreta were determined by the method of Fenton and Fenton (1979) using a Bausch and Lomb Spectronic 501 (model 33.51.039, Milton Ray Company, USA). Amino acid profiles of the test ingredients, diets and ileal digesta samples were analyzed

by HPLC using method 985.28, 994.2 and 988.15 AOAC (2000) with the modifications as described in section 4.3.5 using ion exchange chromatographic methodology..

6.3.6 Calculations

All digestibility calculations were done using the methods of Lloyd et al. (1978), Moughan et al. (1992), Woyengo et al. (2010a) and Jayaraman. (2010) as described in chapter 4 section (4.5.6) using the ileal flows developed in chapter 4.

6.3.7 Statistical analysis

The apparent digestibility of crude protein, dry matter, standard ileal digestible crude protein, standard ileal digestible amino acids and apparent metabolizable energy (AMEn) and its digestibility values were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3, (SAS Institute Inc., Cary, NC). The experimental design was completely randomized in a 2 x 2 x 4 factorial arrangement with meal process type x residual oil level x enzyme addition where:

Processing methods = (heated meal and non-heated meal),

Meal residual oil content was = (10% and 14% residual oil)

Enzyme addition = (no-enzyme or protease or carbohydrase or lipase).

Experiment model for nutritional data:

$$Y_{ijkl}=\mu + A_{i(1-2)} + B_{j(1-2)} + AB_{ij} + C_{k(1-4)} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijk(5)}$$

The statistical model of the experiment as shown above where:

Y_{ijkl} = The response variable.

μ = The overall mean response for that factor.

$A_{i\,(1-2)}$ = Effect of meal at the i^{th} process type (1= heated and 2 = non-heat).

$B_{j\,(1\text{-}2)}$ = Effect of residual oil at the j^{th} level (1= 10% and 2 = 14%).

AB_{ij} = Effect of two way interactions at the ij^{th} level of both effects.

$C_{k\,(1\text{-}4)}$ = Effect of enzyme at the k^{th} treatments (1= non-enzyme, 2 = protease, 3 = carbohydrase and 4 = lipase).

AC_{ik} = Effect of two way interactions at the ik^{th} level of both effects.

BC_{jk} = Effect of two way interactions at the jk^{th} level of both effects.

ABC_{ijk} = Effect of three way interactions at the ijk^{th} level of all effects

ε_{ijk} = The residual error of the model with $_k$ replication of five

Experiment model for growth data: $Y_{ij}= \mu +\alpha_i+ \varepsilon_{ij}$

Where, Y is the response variable (body weight, feed consumption and FCR), μ is overall mean, α_i is the effect of diets and ε_{ij} is the residual error

If significant main effects or interactions were found ($P \leq 0.05$) for the apparent digestibility of crude protein, dry matter, standardized ileal digestible crude protein, standard ileal digestible amino acids, apparent metabolizable energy (AMEn), and digestibility values, Tukey Kramer test (Littell et al. 1996) was used to compare differences among the least square means at ($\alpha = 0.05$). Orthogonal contrasts were done for production performance data comparing basal and treatment group.

6.4 Results and Discussion

6.4.1 Analyzed compositions of diets and ingredients

The analyzed nutrient content of the LOM test diets (Table 6.1) and the HOM test diets (table 6.2.) used in this experiment show the analyzed apparent metabolizable energy (AMEn) of the basal diet was 3,121 kcal·kg^{-1}. All the test diets, except for Y-HOM-C AMEn were lower than the 3200 kcal·kg^{-1} recommendation for broiler chick age 0-3 weeks old. The AMEn of Y-LOM-H-L, Y-LOM-H-P, Y-HOM-C, Y-HOM-L, Y-HOM-P and Y-HOM-H-C diets were higher than the basal diet AMEn.

The CP of all the diets except the basal 22% were higher than the 23% recommended by NRC (1994) for 0-3 weeks old broilers but the basal exceeded the CP recommendation for broilers 3-6 week old. The LYS contents of all the diets ranged from 1.29 to 1.64 and were more than the 1.1% recommendation for broilers at that age. The analyzed methionine (MET) content of all the test diets exceeded the 0.4% of the calculated estimate except for the basal diet which was 0.36%. The MET of all substituted test diets were above the 0.38% NRC (1994) recommendation for 3-6 week old birds and in the recommended range for 0-3 week old birds.

The dry matter contents (DM) of the meals used in this study (Table 6.3.) ranged from 94 to 98%.The gross energy (GE) of the yellow *Brassica napus* meals used in this study ranged from 4761 to 4911 kcal·kg^{-1}. There was no noticeable difference between the low and high oil meals or between the heated and non heated meals. The 4% difference in oil levels between the LOM and the HOM did not translate into large difference in GE. The GE values of yellow *Brassica napus* meal in this study were lower than those reported for single cold press yellow *Brassica napus* 5846 to 5570 kcal·kg^{-1}

TABLE 6.1. Analyzed nutrient composition of diets used to test the effects of enzyme, heat and residual oil levels of mechanically pressed yellow *Brassica napus* nutrient digestibility in 21 day old broilers (as fed basis).

	Diets								
		Y-LOM				Y-LOM-H			
	Basal	No-E	C	L	P	No-E	C	L	P
Analyzed Nutrients									
Gross energy (kcal·kg^{-1})	4,077	4,298	4,289	4,259	4,228	4,284	4,318	4,250	4,290
AME_n (kcal·kg^{-1})	3,121	2,977	2,967	3,050	2,907	2,965	2,971	3,141	3139
Crude Protein %	22.4	26.3	26.4	26.1	25.4	27.1	26.3	26.1	26.2
Methionine (%)	0.36	0.49	0.50	0.51	0.50	0.51	0.47	0.49	0.48
Lysine (%)	1.29	1.56	1.57	1.59	1.60	1.48	1.54	1.54	1.64
Cysteine (%)	0.28	0.48	0.47	0.48	0.48	0.48	0.46	0.47	0.47
Threonine (%)	0.89	1.14	1.18	1.17	1.18	1.10	1.14	1.15	1.21
Tryptophan (%)	0.19	0.26	0.26	0.24	0.22	0.22	0.22	0.25	0.28
Isoleucine (%)	0.89	0.98	1.02	0.98	0.97	0.94	0.97	0.96	0.98
Arginine (%)	1.58	1.72	1.78	1.81	1.83	1.69	1.77	1.75	1.87
Valine (%)	1.07	1.24	1.33	1.28	1.26	1.24	1.26	1.26	1.27
Leucine (%)	1.92	2.09	2.10	2.06	2.07	2.00	2.07	2.06	2.14
Phenylalanine (%)	1.13	1.17	1.24	1.21	1.21	1.15	1.19	1.19	1.26
Serine (%)	1.21	1.37	1.39	1.39	1.41	1.31	1.36	1.35	1.46
Glycine (%)	0.92	1.20	1.25	1.23	1.23	1.17	1.21	1.20	1.28
Histidine (%)	0.74	0.87	0.87	0.87	0.87	0.82	0.84	0.84	0.89
Aspartic acid (%)	2.37	2.52	2.58	2.56	2.59	2.40	2.50	2.48	2.69
Glutamic acid (%)	4.24	4.77	4.86	4.80	4.85	4.62	4.75	4.72	5.03
Proline (%)	2.37	1.80	2.58	2.56	2.59	2.40	2.50	2.48	2.69
Alanine (%)	1.01	1.15	1.27	1.20	1.18	1.17	1.24	1.21	1.30
Tyrosine (%)	0.73	0.85	0.84	0.82	0.82	0.77	0.81	0.80	0.85

Y-LOM diets = 30%Yellow canola with 10% residual oil+70 Basal diet, Y-HOM diet = 30% Yellow canola with 14% residual oil+70 Basal diet, Y-LOM-H diet =30% Heated Yellow canola with 10% residual oil+70 Basal diet, Y-HOM-H diet = 30% Heated Yellow canola with 14% residual oil+70 Basal diet

L=Lipase, P=Protease, C=Carbohydrase, No-E=No-Enzyme

TABLE 6.2. Analyzed nutrient composition of high oil meal diets used to test the effects of enzyme, heat and residual oil levels of mechanically pressed yellow *Brassica napus* nutrient digestibility in 21 day old broilers (as fed basis).

	Diets							
	Y-HOM				Y-HOM-H			
	No-E	C	L	P	No-E	C	L	P
Analyzed Nutrients								
Gross energy (kcal·kg^{-1})	4,295	4,360	4,263	4,327	4,298	4,326	4,296	4,306
AME_n (kcal·kg^{-1})	3,032	3,220	3,156	3,160	3,060	3,178	2,902	2,988
Crude Protein %	26.2	26.4	27.1	25.4	26.3	26.2	26.4	26.1
Methionine (%)	0.49	0.46	0.48	0.49	0.46	0.48	0.43	0.46
Lysine (%)	1.56	1.59	1.40	1.49	1.55	1.52	1.54	1.58
Cysteine (%)	0.46	0.44	0.46	0.48	0.45	0.45	0.44	0.43
Threonine (%)	1.15	1.18	1.05	1.10	1.14	1.13	1.14	1.16
Tryptophan (%)	0.27	0.27	0.21	0.27	0.27	0.25	0.28	0.27
Isoleucine (%)	0.98	0.94	0.82	0.95	1.01	0.93	0.98	0.99
Arginine (%)	1.76	1.80	1.60	1.72	1.79	1.75	1.78	1.79
Valine (%)	1.26	1.23	1.09	1.26	1.31	1.22	1.27	1.28
Leucine (%)	2.07	2.10	1.90	2.01	2.08	2.02	2.08	2.08
Phenylalanine (%)	1.21	1.23	1.08	1.15	1.29	1.18	1.21	1.23
Serine (%)	1.38	1.43	1.28	1.30	1.36	1.36	1.37	1.41
Glycine (%)	1.20	1.25	1.11	1.15	1.19	1.20	1.20	1.22
Histidine (%)	0.84	0.87	0.79	0.81	0.83	0.85	0.82	0.87
Aspartic acid (%)	2.55	2.62	2.32	2.41	2.55	2.52	2.55	2.63
Glutamic acid (%)	4.79	4.92	4.43	4.62	4.80	4.74	4.81	4.91
Proline (%)	2.55	2.62	2.32	2.41	2.55	2.52	2.55	2.63
Alanine (%)	1.19	1.26	1.12	1.17	1.16	1.19	1.20	1.17
Tyrosine (%)	0.81	0.82	0.73	0.78	0.84	0.79	0.82	0.83

Y-LOM diets = 30%Yellow canola with 10% residual oil+70 Basal diet, Y-HOM diet = 30% Yellow canola with 14% residual oil+70 Basal diet, Y-LOM-H diet =30% Heated Yellow canola with 10% residual oil+70 Basal diet, Y-HOM-H diet = 30% Heated Yellow canola with 14% residual oil+70 Basal diet, L=Lipase, P=Protease, C=Carbohydrase, No-E=No-Enzyme

TABLE 6.3. Analyzed nutrient composition (DM basis) of meals used to test the effects of enzyme, heat and meal residual oil levels on the nutrient digestibility of yellow *Brassica napus* in 21 day old broilers.

	Y-LOM	Y-LOM-H	Y-HOM	Y-HOM-H
Analyzed Nutrients				
Dry matter (%)	95	97	94	98
Gross energy (kcal·kg^{-1})	4,761	4,788	4,911	4,902
Crude Protein %	33.5	32.8	33.8	33.5
Fat (%)	10.2	10.4	14.1	14.3
Calcium (%)	0.58	------	0.58	------
Phosphorus (%)	1.86	------	1.32	------
Methionine (%)	0.64	0.65	0.63	0.61
Lysine (%)	2.11	2.06	2.14	1.96
Cysteine (%)	0.76	0.76	0.75	0.73
Threonine (%)	1.66	1.64	1.61	1.61
Tryptophan (%)	0.29	0.32	0.32	0.30
Isoleucine (%)	1.24	1.18	1.23	1.13
Arginine (%)	2.19	2.18	2.15	2.10
Valine (%)	1.78	1.70	1.77	1.64
Leucine (%)	2.39	2.32	2.31	2.25
Phenylalanine (%)	1.37	1.34	1.34	1.33
Serine (%)	1.64	1.63	1.58	1.60
Glycine (%)	1.79	1.76	1.72	1.71
Histidine (%)	1.12	1.10	1.09	1.10
Aspartic acid (%)	2.77	2.73	2.68	2.66
Glutamic acid (%)	5.72	5.64	5.55	5.50
Proline (%)	2.77	2.73	2.68	2.66
Alanine (%)	1.56	1.52	1.46	1.49
Tyrosine (%)	1.00	0.98	0.98	0.97
NH3 (%)	0.74	0.73	0.65	0.69

Y-LOM meal = Yellow canola with 10% residual oil+ no heat, Y-HOM meal = Yellow canola with 14% residual oil+ no heat, Y-LOM-H diet = Heated Yellow canola with 10% residual oil, Y-HOM-H = Yellow canola with 14% residual oil

(Jayaraman 2010) and yellow *Brassica napus* pressed cake 5963 to 6133 kcal·kg^{-1} (Czerwinski et al. 2012). The oil content of the meals in this study (14 and 10%) were almost half that of the meal used by the above authors. This difference in meals oil levels

translated into differences in the GE content (Newkirk et al. 2003a, Leming and Lember 2005).

The mechanically pressed yellow *Brassica napus* meals CP ranged from 32.8 to 33.8% and were higher than the 31.7 to 30.6 % reported by others (Czerwinski et al. 2012, Smulikowska et al. 1998). This difference in CP may be due to the diluting effects of higher residual oil on the CP of the meal in the other studies. The yellow *Brassica napus* seed used in this study did not undergo flaking and cooking before they were expelled but one batch from each set of meal were heat treated. Other researchers have shown that the protein quality of *Brassica* napus meal can be affected by heat during processing (Classen et al. 2004).

The MET and LYS levels in the yellow *Brassica napus* meals range from 0.61 to 0.65% and 1.96 to 2.14 %, respectively. Yellow *Brassica napus* cake protein is known to have both amino acids in concentrations of 5.98 g^{-1}·16 g nitrogen for LYS and 2.04 g^{-1}·16g nitrogen for MET (Smulikowska et al. 1998). The MET level was in similar to its black counterpart 0.63% but the LYS level was much higher than the expelled meal 1.32% reported by Woyengo et al. (2010a).

6.4.2 Animal performance

The bird's production performance data reported in (Table 6.4.) in this study were well within the normal range of our research facility averages (Jayaraman 2010). The starting weights of the birds used in this study were 2-3 g lower than the normal receiving weights of birds from the local hatchery but were similar to the average receiving weight of birds used in chapter 5. There was no effect of treatment on body weight, feed consumption, FCR and mortalities over the 7 day assay period. Mortality was 5% over

the 14days with 3% occurring in the starter phase and 2% in the grower. The body weights, FCR and feed consumption of the birds in this study were similar to birds fed yellow *Brassica napus* cake substituted diet reported by Smulikowska et al. (1998). This suggested that the birds performance in this study were normal.

TABLE 6.4. Growth performance of birds testing the effects of meal residual oil level, heat and enzyme on digestible nutrients of yellow meal in 21day old broilers

Performance parameters	Basal diets	Treatment diets
Body weight gain ($g \cdot b^{-1} \cdot d^{-1}$)	40±0	42±1
Feed consumption ($g \cdot b^{-1} \cdot d^{-1}$)	65±2	62±1
FCR	1.6±0.1	1.5±0.0

ANOVA *P*-Values

Contrast	Body weight gain	Feed consumption	FCR
Basal VS Treatments	0.4529	0.2632	0.0617

Mean ± SEM

6.4.3 Apparent digestible nutrients

There was a significant three-way interaction effect of treatments on the DM digestibility coefficients of mechanically pressed yellow *Brassica napus* meal in this study (Table 6.5.). The meals fed in the 14% oil meal + lipase and the heated 10% oil meal + lipase diets had significantly (P=0.05) higher DM digestibility than the meals fed in heated 14% oil meal + lipase, heated 10% oil meal + no enzyme, heated 10% oil meal + carbohydrase, 10% oil meal + no enzyme, 10% oil meal + carbohydrase and 10% oil meal + protease diets. All of the other treatments had DM digestibility that were intermediate and not significantly different from any of the treatments. Adding lipase to diets formulated with heated mechanically pressed yellow *Brassica napus* meals with

10% residual oil level improved that meal DM digestibility. There were no effects of adding the protease or carbohydrase on DM digestibility.

TABLE 6.5. Effects of oil level, heat and enzyme treatment on the apparent DM digestibility coefficient of mechanically pressed yellow canola meal in 21day old broilers.

	Heat x Enzyme x Oil				
	14% oil meal		10% oil meal		
	Heated	No Heat	Heated	No Heat	Enzymes effects
Enzymes treatments					
No-Enzyme	88±1ab	87±1ab	84±1b	85±1b	86±1
Carbohydrase	87±1ab	88±1ab	84±1b	84±1b	86±1
Protease	85±1ab	88±1ab	88±1ab	84±1b	86±1
Lipase	84±1b	90±1a	90±1a	86±1ab	88±1
Oil x Heat	86±1	88±1	86±1	85±1	
Oil x Enzyme					
No-Enzyme		88±1		85±1	
Carbohydrase		88±1		83±1	
Protease		86±1		85±1	
Lipase		87±1		88±1	
Oil		87±0.4		85±0.4	
Source of variation		P>F			
Oil		0.0003			
Heat		0.5375			
Oil x Heat		0.0021			
Enzyme		0.0183			
Oil x Enzyme		0.0048			
Heat x Enzyme		0.8602			
Oil x Heat x Enzyme		0.0016			

[a-b] Means ±SE in the het*enzyme*oil interaction with no common letters are significantly different at α =0.05

The AMEn digestibility coefficients (Table 6.6.) were significantly (*P*=0.0007) affected by the three-way interaction between the oil levels, enzymes and heat treatments. The coefficients ranged from a high of 83% to a low of 91. The meals fed in the heated 14% oil meal + lipase diet had significantly higher ($P \leq 0.05$) AMEn digestibility coefficients then those fed in 14% oil meal + carbohyrase, 14% oil meal + lipase, 10% oil meal +

protease and 10% oil meal + lipase diets. All the other treatments were intermediate and not significantly different. The addition of lipase to the diet with heated 10% residual oil meal significantly reduced the AMEn digestibility coefficients of that meal; the reason for this is not known.

TABLE 6.6. Effects of oil level, heat and enzyme treatment on the AMEn digestibility coefficients of mechanically pressed yellow canola meal in 21day old broilers.

	Enzyme x Oil x Heat			
	14% oil meal		10% oil meal	
	Heated	No Heat	Heated	No Heat
Enzymes treatments				
No-Enzyme	86±1abcd	87±1abcd	88±1abc	89±1ab
Carbohydrase	86±1abcd	86±1bcd	90±1ab	89±1ab
Protease	89±1ab	87±1abcd	86±1bcd	88±1abc
Lipase	91±1a	84±1cd	83±1d	86±1abcd
Oil x Heat	88±1	86±1	87±1	88±1
Oil x Enzyme				
No-Enzyme	87±1		89±1	
Carbohydrase	86±1		89±1	
Protease	88±1		87±1	
Lipase	87±1		85±1	
Source of variation	P>F			
Oil	0.5327			
Heat	0.5950			
Oil x Heat	0.0005			
Enzyme	0.0531			
Oil x Enzyme	<.0001			
Heat x Enzyme	0.2263			
Oil x Heat x Enzyme	0.0007			

[a-d] Means ± SE in het*enzyme*oil interaction with no common letters are significantly different at α = 0.05

The AMEn values of the meals used in this study (Table 6.7.) were significantly affected by the three way interaction between the meal residual oil levels, enzyme and heat treatment. The meals AMEn values in this study ranged from a low of 3451 kcal·kg^{-1} to a high of 2389 kcal·kg^{-1} and some treatments were in the range of 3520 kcal·kg-1 to 3238 kcal reported by others (Czerwinski et al. 2012, Smulikowska et al. 1998). The

carbohydrase supplemented in non heated 14% oil meal diet significantly ($P \leq 0.05$) improved the meals digestible AMEn compared to 14% oil meal + no enzyme. The 10% heated and non heated meals did not benefit from enzyme addition. Carbohydrase enzymes are known to act upon the non-starch polysaccharides component of canola meal which helps to yield more energy from that meal (Khajali and Slominski. 2012). The carbohydrase supplementation gave an extra 628 $kcal \cdot kg^{-1}$ of AMEn from the non heat 14% oil meal. This may be due to the non-starch polysaccharides component and fiber fractions of yellow *Brassica napus* are known to be less than black Brassica napus (Slominski et al. 2012). With less hull to embryo ratio in the yellow *Brassica napus* (Slominski et al. 2012) less substrate might be available for the carbohydrase to act upon.

The apparent CP digestibility coefficients (Table 6.8.) were significantly affected by the three-way interaction of meals residual oil, heat treatment and enzyme supplementation. The apparent CP digestibility of yellow *Brassica napus* ranged from 72 to 28% and was less than 80.2 to 79.6% previously reported for yellow *Brassica napus* cake (Czerwinski et al. 2012). Enzymes made no improvement in the apparent CP digestibility. The lipase enzyme had no effect on apparent CP digestibility regardless of meal oil levels and heat treatments. When canola meal is heated amino acids might react with reducing sugars creating Maillard reaction products (Newkirk et al. 2003b). The Maillard reaction could be using the amino acid in the proteins present in the meal. The Maillard reaction product and presence of the exogenous enzyme might be blocking the ability of the endogenous protease to effectively catalyze the breakdown of the remaining CP in the meal. More CP would be present in the excreta of the birds which would give a lower apparent CP digestibility value.

TABLE 6.7. Effects of oil level, heat and enzyme treatment on the AMEn of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Enzyme x Oil x Heat effects				
	14% oil meal		10% oil meal		
	Heated	No Heat	Heated	No Heat	Enzyme effects
Enzyme treatments					
No-Enzyme	2918±121abcdef	2823±121bcdef	2599±121ef	2640±121def	2745±61
Carbohydrase	3311±121ab	3451±121a	2622±121ef	2607±121ef	2998±61
Protease	2676±121cdef	3251±121abc	3181±121abcde	2407±121f	2879±61
Lipase	2389±121f	3237±121bcd	3188±121abcde	2883±121abcdef	2924±61
Oil	3007±43		2766±43		
Oil x Enzyme					
No-Enzyme	2870±86		2619±86		
Carbohydrase	3381±86		2614±86		
Protease	2964±86		2794±86		
Lipase	2813±86		3035±86		
Oil x Heat	2823±61	3190±61	2897±61	2634±61	
Source of variation	P>F				
Oil	0.0002				
Heat	0.3944				
Oil x Heat	<0.0001				
Enzyme	0.0334				
Oil x Enzyme	<0.0001				
Heat x Enzyme	0.1643				
Oil x Heat x Enzyme	<0.0001				

[a-f] Means ±SE in the het*enzyme*oil interaction with no common letters are significantly different at $\alpha = 0.05$

TABLE 6.8. Effects of oil level heat and enzyme treatment on the apparent crude protein digestibility coefficient of mechanically pressed yellow canola meal in 21day old broilers.

	Heat x Enzyme x Oil					
	14% oil meal		10% oil meal		Heat x Enzyme	
	Heated	No Heat	Heated	No Heat	Heated	No Heat
Enzyme treatments						
No-Enzyme	50±6abcd	51±6abcd	37±6bcd	36±6bcd	43±4	44±4
Carbohydrase	61±6ab	58±6abc	40±7abcd	36±6bcd	50±5	47±4
Protease	49±6abcd	44±6abcd	57±6abcd	31±6cd	53±4	38±4
Lipase	28±6d	72±6a	61±6ab	48±6abcd	45±4	60±4
Oil x Heat	47±3	56±3	49±3	38±3		
Oil x Enzyme						
No-Enzyme	51±4		37±4			
Carbohydrase	59±4		38±4			
Protease	47±4		44±4			
Lipase	50±4		54±4			
Oil	52±2		43±2			
Source of variation	P>F					
Oil	0.0060					
Heat	0.7755					
Oil x Heat	0.0008					
Enzyme	0.1676					
Oil x Enzyme	0.0155					
Heat x Enzyme	0.0052					
Oil x Heat x Enzyme	0.0038					

[a-e] Means ±SE in the het*enzyme*oil interaction with no common letters are significantly different at $\alpha = 0.05$

6.4.4 Ileal digestible nutrients

The standardized ileal CP digestibility values presented in (Table 6.9.) range from 87 to 93% and were different from the apparent values reported for the meals. The ileal CP digestibility values in this experiment were significantly affected by the residual oil levels and heat two-way interaction and by the enzymes and heat two-way interaction. In the two-way interactions with heat and residual oil levels, the ileal CP digestibility of meals fed in the 10% oil meal + no heat diets was significantly ($P \leq 0.05$) higher than the ileal CP from meals in the no heat 14% oil meal diet. All the other treatments were intermediate and not significantly different from the others. MPYCM that are lower in oil level if not heated will provide more digestible CP at the ileum of the birds than meals with higher residual oil. The reason for that effect occurring only in the no heat treatments is still unknown and warrants further investigation. There was no effect of enzyme in the 14% heated meals but protease significantly improved the ileal CP digestibility of the no heat 14% oil meal. Exogenous protease addition to the diet of broilers having high levels of canola meal is known to improve the meals available CP through protein hydrolysis (Simbaya et al. 1996). The exogenous enzyme may interact with the endogenous protease in a synergistic way to make more of the protein fraction of the meal become hydrolyzed to amino acid than if only the endogenous enzymes were present. The effects protease has in this study could be relation to improvement gain in the efficiency of amino acid extraction from CP fraction of the meal. Heating the meals could be making the protein faction insoluble thus not available for hydrolysis by the protease enzymes (Simbaya et al. 1996, Mustafa et al. 2000).

TABLE 6.9. Effects of oil level, heat and enzyme treatments on the standardized ileal crude protein digestibility in mechanically pressed yellow canola meals by 21day old broilers.

	14% oil meal		10% oil meal	
Treatment effects	Heated	No Heat	Heated	No Heat
Oil x Heat	90±1ab	87±1b	89±1ab	92±1a
Oil	88±1		91±1	
Enzymes	Heat x Enzyme			
No-Enzyme	90±1ab	87±1b		
Carbohydrase	90±1ab	89±1ab		
Protease	89±1ab	93±1a		
Lipase	89±1ab	90±1ab		
Source of variation	P>F			
Oil	0.0033			
Heat	0.9235			
Oil x Heat	0.0006			
Enzyme	0.1651			
Oil x Enzyme	0.5325			
Heat x Enzyme	0.0148			
Oil x Heat x Enzyme	0.7433			

[a-b] Means ± SE in the same interaction group: (heat*enzyme) and (oil*heat) with no common letters are significantly different at $\alpha = 0.05$

The digestibility of LYS (Table 6.10) was significantly affected only by the two-way interaction between heat and enzyme. There was no significant ($P \leq 0.05$) difference detected by Tukey-Kramer test between any of those treatments least square means in that two-way interaction. The coefficients of LYS range from 97 to 93% and was higher than the 89.2% and 80.8% reported for solvent extracted yellow *Brassica napus* (Slominski et al. 1999, Jia et al. 2012). There were no effects of treatment on MET digestibility coefficients (Table 6.11.) but the coefficients of MET range from 84 to 86% and was lower than the 98.9% and 88.8% reported for solvent extracted yellow *Brassica napus* (Slominski et al. 1999, Jia et al. 2012). The difference in MET digestibility of this

study with Slominski et al. (1999) and Jia et al. (2012) could be related to kind of heating and processing technique used to obtain the meals.

TABLE 6.10. Oil level, heat and enzyme effects on lysine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Heat x Enzyme	
	Heated	No Heat
Enzyme treatments		
No-Enzyme	97±1	94±1
Carbohydrase	97±1	93±1
Protease	94±1	96±1
Lipase	96±1	97±1
Source of variation		P>F
Oil		0.3376
Heat		0.2187
Oil x Heat		0.7834
Enzyme		0.2329
Oil x Enzyme		0.2703
Heat x Enzyme		0.0061
Oil x Heat x Enzyme		0.4470

Means ±SE

TABLE 6.11. Oil level, heat and enzyme effects on methionine digestibility in mechanically pressed yellow *Brassica napus* meal by 21day old broilers.

	14% oil meal		10% oil meal
Main effects	Heated	No Heat	
Oil	85±1		85±1
Heat	86±1	85±1	
Enzyme			
No-Enzyme	84 ±1		
Carbohydrase	85 ±1		
Protease	85 ±1		
Lipase	86 ±1		
Source of variation		P>F	
Oil		0.8244	
Heat		0.1687	
Oil x Heat		0.0708	
Enzyme		0.7542	
Oil x Enzyme		0.8785	
Heat x Enzyme		0.7667	
Oil x Heat x Enzyme		0.6576	

*Means ±SE

TRP digestibility (Table 6.12.) was significantly affected by the three-way interaction of meal residual oil, heat and enzyme. The digestibility of TRP was similar to the 70.1% reported for solvent extracted yellow *Brassica napus* (Jia et al. 2012). Enzymes did not improve TRP digestibility coefficient for the heated meals. For the no heat meals, lipase improved TRP digestibility for the 14% and the 10 oil meal. Protease improved the TRP digestibility for the no heat 10% oil meal but not for the no heat 14% oil meal. The highest TRP digestibility were the no heat 14% oil + lipase, no heat 10% oil +lipase and the no heat 10% oil + protease meals there were not significantly different from the heated 10% oil meal with no enzyme. The complex interaction seen with TRP digestibility in the yellow *napus* show that TRP digestibility was sensitive to the three way synergistic effects meal oil level, each enzyme and heating of the meal.

THR digestibility of the meals (Table 6.13.) was significantly affected by the two-way interaction of enzyme plus heat and by the two-way interaction of enzyme plus oil. The digestibility coefficients of THR were high and ranged from 81 to 90%, although both meals are not comparable the values were in range with the 84.4% and 80.3% reported for solvent extracted yellow *Brassica napus* (Slominski et al. 1999, Jia et al. 2012). In the oil plus enzyme two-way interaction lipase improved the THR digestibility of the 14% oil meal but not the 10% oil meal. There was no effect of protease or carbohydrase on THR digestibility in the 14% or 10% oil meal. In the two-way interaction of heat and enzyme lipase improved THR digestibility of the no heat meals but not the heated meals. Protease reduced the THR digestibility of the heated but not the no heat meals. It is clear that lipase enzyme had beneficial effect on the THR digestibility of yellow *Brassica napus*

TABLE 6.12. Oil level, heat and enzyme effects on tryptophan digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Oil x Heat x Enzyme				Heat x Enzyme		
	14% oil meal		10% oil meal				
	Heated	No Heat	Heated	No Heat	Heated	No Heat	Enzymes
Enzyme treatments							
No-Enzyme	76±2cd	70±2d	89±2ab	78±2bcd	83±2	74±2	78±2
Carbohydrase	77±2cd	72±2d	87±2abc	81±2abcd	82±2	76±2	79±2
Protease	72±2d	73±2d	73±2d	90±2a	72±2	81±2	77±2
Lipase	75±2d	89±2ab	78±2bcd	90±2a	51±2	90±2	83±2
Oil x Enzyme							
No-Enzyme		73±2		83±2			
Carbohydrase		74±2		84±2			
Protease		72±2		81±2			
Lipase		82±2		84±2			
Oil		76±1		83±1			
Source of variation			P>F				
Oil			<.0001				
Heat			0.0964				
Oil x Heat			0.3901				
Enzyme			0.0036				
Oil x Enzyme			0.0424				
Heat x Enzyme			<.0001				
Oil x Heat x Enzyme			0.0058				

[a-d] Means ± SE in the oil*heat*enzyme interaction with no common letters are significantly different at α =0.05

TABLE 6.13 Oil level, heat and enzyme effects on threonine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Enzyme x Oil		Heat x Enzyme		
	14% oil meal	10% oil meal	Heated	No Heat	Enzymes
Enzyme treatments					
No-Enzyme	83±1b	87±1ab	87±1ab	83±1bc	85±1
Carbohydrase	82±1b	86±1ab	86±1abc	82±1bc	88±1
Protease	85±1ab	84±1ab	81±1c	87±1ab	84±1
Lipase	88±1a	88±1a	86±1abc	90±1a	84±1

Source of variation	P>F
Oil	0.0571
Heat	0.6824
Oil x Heat	0.6401
Enzyme	0.0075
Oil x Enzyme	0.0490
Heat x Enzyme	<.0001
Oil x Heat x Enzyme	0.5798

[a-c] Means ±SE in the same interaction group: (heat*enzyme), (oil*enzyme) with no common letters are significantly different at $\alpha = 0.05$

when the meal residual oil was high. It could be that this was as a result of the lipase actions on increasing overall fat digestibility which increase the concentration of THR substrate for endogenous digestion. Another possibility is that the lipase enzyme hydrolysis products are aiding the transfer THR to the intestinal cells or helping breakdown of larger peptides which are then readily absorbed by the intestinal cells. As for the reduction in THR caused by the protease heating the meal may have created folds in the proteins which are not soluble. When the protease breaks these proteins down in to peptides they may have sections which are still insoluble and cannot enter the intestinal

cell, but instead block absorption sites for THR on the intestinal cells. The precise mechanism for THR behavior under the influence of lipase and protease are not known.

ARG digestibility (Table 6.14.) was significantly affected by the two-way interaction of heat and enzyme. The digestibility coefficients of ARG were high and were higher than the 89% and 91.2% reported for solvent extracted yellow *Brassica napus* (Slominski et al. 1999, Jia et al. 2012). The addition of protease to the heated meals had significantly less ARG digestibility than the no heat treatments + lipase. All the other treatments had ARG digestibility values which were intermediate and were not significantly different from those treatments. There was no improvement in ARG digestibility from adding enzyme to either the no heat or heated meal

Leucine (LEU) digestibility (Table 6.15.) in the yellow *Brassica* meals using this study was significantly affected by the oil levels of the meals and the two-way interaction between heat and enzyme. LEU digestibility coefficients of the meals using this study were similar to the 88% and 89.5% reported for solvent extracted yellow *Brassica napus* (Slominski et al. 1999, Jia et al. 2012). When the meals that were not heat treated were fed with lipase the LEU digestibility was significantly higher compared to the non-heat + carbohydrase, the non-heat treated with no enzyme and the heat treated + protease meals the addition. Enzyme only improved the LEU digestibility in the no heat meals there were effects on enzyme in the heated meals. The meals used in this study seemed to be mostly affected by lipases and residual oil levels. Lipase had the highest positive effects on LEU digestibility and the lower oil level were birds were better the able to use LEU.

TABLE 6.14. Oil level, heat and enzyme effects on arginine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Heat x Enzyme	
	Heated	No Heat
Enzyme treatments		
No-Enzyme	96±1ab	94±1ab
Carbohydrase	95±1ab	94±1ab
Protease	93±1b	96±1ab
Lipase	95±1ab	97±1a

Source of variation	P>F
Oil	0.3509
Heat	0.6546
Oil x Heat	0.7143
Enzyme	0.1158
Oil x Enzyme	0.3255
Heat x Enzyme	0.0046
Oil x Heat x Enzyme	0.3498

[a-b] Means ±SE in the heat* enzyme interaction with no common letters are significantly different at α =0.05

TABLE 6.15. Oil level, heat and enzyme effects on leucine digestibility coefficient of mechanically pressed yellow canola meal by 21day old broilers.

	Heat x Enzyme		
	Heated	No Heat	Enzymes
Enzyme treatments			
No-Enzyme	94±1ab	92±1b	93±1
Carbohydrase	94±1ab	92±1b	93±1
Protease	92±1b	96±1ab	94±1
Lipase	94±1ab	98±1a	96±1
Oil	14% oil		10%oil
	93±1b		95±1a

Source of variation	P>F
Oil	0.0320
Heat	0.5268
Oil x Heat	1.0000
Enzyme	0.0160
Oil x Enzyme	0.6825
Heat x Enzyme	0.0006
Oil x Heat x Enzyme	0.9293

a-b Means ±SE in the same heat*enzyme interaction with no common letters are significantly different at α = 0.05

Histidine (HIS) digestibility (Table 6.16.) of the meal used in the study was only significantly affected by the two-way interaction of the heat and enzyme treatments. The HIS digestibility coefficients similar to the 72.3% reported for solvent extracted yellow *Brassica napus* by Slominski et al. (1999) but lower than the and 98% reported by Jia et al. (2012). The HIS digestibility in the heated no enzyme meal was significantly higher than that of the non-heated + carbohydrase meal. All the remaining treatments HIS digestibility was intermediate and not significantly different from those two. There was no effect of enzyme addition in either the heat or no heat meals.

Phenylanine (PHE) digestibility coefficients of mechanically pressed yellow *Brassica napus* meal (Table 6.17.) used in this study were significantly affected by heat and enzyme. PHE digestibility coefficients values were similar to the 86.8% reported for solvent extracted yellow *Brassica napus* (Jia et al. 2012). Meals that were heated had significantly lower PHE digestibility than then no-heated meals. The addition of carbohydrase enzyme to the meals significantly reduced the digestible PHE compared to the lipase but there were no significant differences between the reductions caused by the enzymes and the other treatments. PHE is the only amino acid which was not influenced by an interaction effect and is the only one that was significantly influenced by two main effects. This suggested that PHE is very sensitive to the effect of heat. The difference between the lipase and carbohydrase additions could be related to the effects of the products of those to enzyme on the absorption of PHE.

TABLE 6.16. Oil level, heat and enzyme effects on histidine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Heat x Enzyme		
	Heated	No Heat	Enzymes
Enzyme treatments			
No-Enzyme	77±3a	68±3ab	72±2
Carbohydrase	71±3ab	65±3b	67±2
Protease	66±3ab	75±3ab	70±2
Lipase	74±3ab	76±3ab	75±2
Source of variation		P>F	
Oil		0.8819	
Heat		0.6955	
Oil x Heat		0.5803	
Enzyme		0.0624	
Oil x Enzyme		0.1313	
Heat x Enzyme		0.0037	
Oil x Heat x Enzyme		0.2539	

a-b Means ±SE in the heat*enzyme interaction group with no common letters are significantly different at $\alpha = 0.05$

TABLE 6.17. Oil level, heat and enzyme effects on phenylalanine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

		Heat	
	Enzymes	Heated	No Heat
Enzyme treatments			
No-Enzyme	92±1ab	92±1b	94±1a
Carbohydrase	91±1b		
Protease	92±1ab		
Lipase	95±1a		
Source of variation		P>F	
Oil		0.2719	
Heat		0.0152	
Oil x Heat		0.5443	
Enzyme		0.0446	
Oil x Enzyme		0.6468	
Heat x Enzyme		0.0971	
Oil x Heat x Enzyme		0.7694	

[a-b] Means ±SE in the heat*enzyme group with no common letters are significantly different at $\alpha = 0.05$

Glycine (GLY) digestibility coefficients (Table 6.18.) of the meal were significantly influenced by the two-way interaction of heat and enzyme. When lipase was added to the non-heated meal diets, GLY digestibility was significantly improved in the lipase + no heat meal comparison to the none heated meal + no enzyme and the no heat + carbohydrase meal. There were no effects of enzyme addition on the GLY digestibility in the heated meals. The products from the lipase hydrolysis might be aiding the digestion and absorption of GLY in the intestinal cells there by promoting the higher digestibility than there would normally be without their presence.

The two-way interaction of heat treatment and enzyme seen with some indispensible amino acids was also seen in the dispensable amino acid except for cysteine (CYS). Published data on most of the dispensable amino acid for yellow *Brassica napus* is limited in the literature weather solvent extracted for mechanically extracted. CYS digestibility coefficients (Table 6.19.) were significantly influenced by the two-way interaction of meal oil levels and heat treatment. The digestibility of CYS in this study were lower than any other amino acids and the coefficients values ranged from 44 to 52% and were not in range with the 81.1% reported for solvent extracted yellow *Brassica napus* (Jia et al. 2012). Meals with 14% residual oil that were heated had significantly higher CYS digestibility than the non-heated 14% oil meals which was not significantly different 10% oil meal whether heat treated or not. The reason for the low CYS digestibility in this study is unknown and requires more investigations.

TABLE 6.18. Oil level, heat and enzyme effects on glycine digestibility of mechanically pressed yellow meal by 21day old broilers.

	Heat x Enzyme		
	Heated	No Heat	Enzymes
Enzyme treatments			
No-Enzyme	83±1ab	79±1bc	81±1
Carbohydrase	82±1abc	78±1c	81±1
Protease	79±1bc	84±1ab	80±1
Lipase	82±1abc	86±1a	84±1
Source of variation		P>F	
Oil		0.5101	
Heat		0.8859	
Oil x Heat		0.8859	
Enzyme		0.0111	
Oil x Enzyme		0.1241	
Heat x Enzyme		0.0001	
Oil x Heat x Enzyme		0.8447	

[a-c] Means ±SE in the heat*enzyme interaction with no common letters are significantly different at α = 0.05

TABLE 6.19. Oil level, heat and enzyme effects on cysteine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	14% oil meal		10% oil meal	
Treatment effects	Heated	No Heat	Heated	No Heat
Oil x Heat	52±2a	44±2b	47±2ab	48±2ab
Oil	48±1		47±1	
Heat	49±1	46±1		
Enzyme				
No-Enzyme	46±2			
Carbohydrase	47±2			
Protease	47±2			
Lipase	50±2			
Source of variation			P>F	
Oil			0.8339	
Heat			0.0586	
Oil x Heat			0.0053	
Enzyme			0.5809	
Oil x Enzyme			0.7416	
Heat x Enzyme			0.2715	
Oil x Heat x Enzyme			0.7406	

[a-b] Means ±SE in the oil*enzyme interaction group with no common letters are significantly different at α = 0.05

Alanine (ALA) digestibility (Table 6.20.) range from 90 to 83% and was significantly affected by the two-way interaction of heat and enzyme. The application of lipase to the non-heated meals gave significantly higher digestibility of ALA when compared to the no enzyme no heat meal, carbohydrase + no heat meal and the heated meals + protease. The addition of lipase improved the no heat meal ALA digestibility. There was no improvement of ALA digestibility with the addition of enzyme in the heated meal. The exact effect of lipase seen with ALA was also seen with GLY which suggested that both amino acids might be benefiting from the same mode of action of lipase.

TABLE 6.20. Oil level, heat and enzyme effects on alanine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	Heat x Enzyme		
	Heated	No Heat	Enzymes
Enzyme treatments			
No-Enzyme	88±1ab	85±1bc	87±1
Carbohydrase	87±1abc	83±1c	85±1
Protease	84±1bc	89±1ab	86±1
Lipase	87±1abc	90±1a	89±1
Source of variation		P>F	
Oil		0.2897	
Heat		0.9743	
Oil x Heat		0.3197	
Enzyme		0.0098	
Oil x Enzyme		0.4062	
Heat x Enzyme		0.0001	
Oil x Heat x Enzyme		0.4808	

[a-c] Means ±SE in the heat*enzyme interaction with no common letters are significantly different at $\alpha = 0.05$

Aspartic acid (ASP) digestibility in the yellow *Brassica napus* meals was also significantly affected by the two-way interaction of heat and enzyme. The coefficients of ASP (Table 6.21.) ranged from 95 to 86% in this study. The supplementation of lipase to the non-heated meals gave significantly higher digestibility of ASP when compared to the no enzyme no heated meal, carbohydrase + no heated meals and the heated meal + protease. Protease addition significantly reduced the ASP digestibility in the heated meals the reason for this is unknown and need more investigation.

TABLE 6.21. Oil level, heat and enzyme effects on aspartic acid digestibility coefficient of mechanically pressed yellow meal in 21day old broilers.

	Heat x Enzyme		
	No Heated	Heated	Enzymes
Enzyme treatments			
No-Enzyme	88±1bc	93±1ab	91±1
Carbohydrase	88±1bc	91±1ab	90±1
Protease	92±1ab	86±1c	89±1
Lipase	95±1a	91±1ab	93±1

Source of variation	P>F
Oil	0.0943
Heat	0.4531
Oil x Heat	0.8022
Enzyme	0.0176
Oil x Enzyme	0.1897
Heat x Enzyme	<.0001
Oil x Heat x Enzyme	0.7584

[a-c] Means ±SE in the heat*enzyme interaction with no common letters are significantly different at α = 0.05

The digestibility of serine (SER) (Table 6.22.) was significantly changed by the two-way interaction of heat and enzyme and ranged from 96 to 87%. The non-heated meals fed with lipase had significantly higher digestible SER than the non-heated meal with no enzyme, non-heated with carbohydrase and the heated meals with protease. The addition of protease significantly reduced the SER digestibility in the heated meal. The same effect of protease was also seen with ASP.

TABLE 6.22. Oil level, heat and enzyme effects on serine digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broiler.

Treatment effects	14% oil meal		10% oil meal
	Heated	No Heat	
Oil	91±1b		93±1a
Enzymes	Heat x Enzyme		Enzymes
No-Enzyme	95±1ab	90±1bcd	92±1
Carbohydrase	93±1abc	89±1cd	91±1
Protease	87±1d	94±1ab	91±1
Lipase	92±1abcd	96±1a	95±1

Source of variation	P>F
Oil	0.0252
Heat	0.8397
Oil x Heat	0.8851
Enzyme	0.0252
Oil x Enzyme	0.0984
Heat x Enzyme	<.0001
Oil x Heat x Enzyme	0.8176

[a-d] Means ±SE in the same group: (oil), (heat*enzyme) with no common letters are significantly different at $\alpha = 0.05$

Glutamic acid (GLU) digestibility (Table 6.23.) was significantly modified by the interaction of heat and enzyme and the coefficients range from89 to 94%. The application of lipase to the no heated meals gave significantly higher digestibility of GLU when compared to the no enzyme non-heated meal, no heated meals + carbohydrase and the heated meals + protease. The same effects of the lipase were seen with the digestibility of ALA, SER and ASP. Tyrosine (TYR) digestibility (Table 6.24.) of the yellow meals was significantly influence by the two-way interaction of enzyme and heat. The two-way interaction of meal oil levels and heat was also significant but no differences were observed among the least square means of the treatment by the Tukey-Kramer test. TYR responded to the two-way interaction of heat and enzyme exactly like the GLU above. The application of lipase to the non-heated meals gave significantly higher digestibility of

TYR when compared to the no enzyme no heat meal, carbohydrase + no heat meal and the heated meal + protease.

TABLE 6.23. Oil level, heat and enzyme effects on glutamic acid digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers

	Heat x Enzyme		
	Heated	No Heat	Enzymes
Enzyme treatments			
No-Enzyme	93±1ab	90±1b	92±1
Carbohydrase	93±1ab	90±1b	92±1
Protease	89±1b	93±1ab	91±1
Lipase	92±1ab	94±1a	93±1
Heat	92±0	92±0	
Source of variation		P>F	
Oil		0.2005	
Heat		0.6874	
Oil x Heat		0.7475	
Enzyme		0.1311	
Oil x Enzyme		0.5143	
Heat x Enzyme		0.0004	
Oil x Heat x Enzyme		0.8684	

[a-b] Means± SE in heat*enzyme interaction with no common letters are significantly different at α = 0.05

TABLE 6.24. Oil level, heat and enzyme effects on tyrosine digestibility coefficient of mechanically pressed yellow meal in 21day old broilers

	Heat x Enzyme		
	Heated	No Heat	Enzymes
Enzyme treatments			
No-Enzyme	95±1ab	92±1b	94±1
Carbohydrase	96±1ab	93±1b	95±1
Protease	92±1b	96±1ab	94±1
Lipase	95±1ab	98±1a	97±1
Source of variation		P>F	
Oil		0.6115	
Heat		0.3981	
Oil x Heat		0.0232	
Enzyme		0.0169	
Oil x Enzyme		0.8212	
Heat x Enzyme		0.0016	
Oil x Heat x Enzyme		0.9367	

[a-b] Means ±SE in the same interaction group: (oil*heat), (heat*enzyme) with no common letters are significantly different at α = 0.05

The NH_3 digestibility (Table 6.25.) was influence by two-way interaction of oil and heat. Meals that were from the no heat treatment group that had 10% residual oil had digestibility values that were higher than those with 14% residual oil that were not heat treated. All the other treatments were intermediate and significantly different. The lipase enzyme improved the NH3 digestibility when compared to the no enzyme with the meal and the meal with carbohydrase.

TABLE 6.25. Oil level, heat and enzyme effects on NH3 digestibility coefficient of mechanically pressed yellow *Brassica napus* meal in 21day old broilers.

	14% oil meal		10% oil meal	
Treatment effects	Heated	No Heat	Heated	No Heat
Oil x Heat	91±1ab	89±1b	91±1ab	94±1a
Oil	90±1		92±1	
Enzymes	Enzymes			
No-Enzyme	89±1b			
Carbohydrase	91±1ab			
Protease	90±1b			
Lipase	95±1a			
Source of variation		P>F		
Oil		0.0313		
Heat		0.2915		
Oil x Heat		0.0219		
Enzyme		0.0017		
Oil x Enzyme		0.9422		
Heat x Enzyme		0.3361		
Oil x Heat x Enzyme		0.9482		

[a-b] Means ±SE in the same group: (oil*heat), (enzyme) with no common letters are significantly different at $\alpha = 0.05$

6.5 Conclusion

Heat plus lipase addition increased the AMEn digestibility coefficients of mechanically pressed yellow *Brassica napus* meal. However by not heating the meals plus the addition of carbohydrase increased the AMEn values. The addition of lipase generally improved the standardized ileal amino acid digestibility of MPYCM. Heat did not provide any noticeable improvement in the standardized ileal amino acid digestibility of the meals. Protease did not affect the AMEn or standardized ileal amino acid digestibility of MPYCM. To maintain or improve the AMEn and standardized ileal amino acid digestibility of MPYCM the addition of lipase or carbohydrase plus not heating the meal is recommended.

CHAPTER 7: GROWTH PERFORMANCE OF BROILER CHICKENS FED GRADED LEVELS OF MECHANICALLY PRESSED BLACK CANOLA MEAL (*BRASSICA NAPUS*) FROM 0-35 DAYS

7.1 Abstract

Mechanical-pressing of oil seeds is a cheaper process than solvent extraction procedures and is the method of choice for small biofuel processors. The material left after pressing has high oil content which varies and has not been heat treated like conventional canola meal. There is little poultry growth performance study conducted on the use of these by-products. The meal is an attractive ingredient for use in the poultry feed industry with its relatively high protein and energy content. There are no recommended levels of inclusion for its use in the starter, grower and finisher diets for broilers. To answer those questions a full growth study was conducted to generate body weight, feed consumption and feed conversion ratio (FCR) data using male Ross 308 broilers. Black canola (*Brassica Napus*) seeds were pressed to have 12% and 17% residual oil. Test diets used in the starter, grower and finisher phase were formulated to have 0, 5, 10 and 15% of the two meals creating 8 test diets in each phase. Nineteen hundred and twenty day old Ross 308 birds were placed in forty eight 2.13m x 1.40m floor pens with 40 birds per pen and five pens per treatment diet. Birds had free access to water and feed over the 35 days. Birds were weighed on day 0, 14, 24 and 35 and feed consumption calculated for each period. The experimental design was completely randomized with a 2x4 factorial arrangement. All data were analyzed using Mixed Model procedure of SAS 9.3. No difference ($P \geq 0.05$) occurred among the birds fed the 0% meal diet and the substituted diets for feed consumption in the starter, grower and finisher phases. During the starter and grower period all groups of birds had the same body weight ($P \geq 0.05$) regardless of diets. The 15% meal birds were lighter ($P \leq 0.05$) in weight than the 0% and 5% meal fed birds during the finisher phase. Birds given the substituted diets had the same FCR ratio ($P \geq 0.05$) as the 0% meal diets in all phases of production and oil levels of the meal did not influence ($P \geq 0.05$) FCR. Mechanically pressed *Brassica napus* canola meal with 12 and 17% residual oil can be fed up to 15% in the starter and grower diets without any significant effects on body weight and feed conversion and up to 10% in the finisher.

Keywords: Black canola meal, Broilers, Body weight gain, Feed consumption

7.2 Introduction

Mechanical-pressed canola meal is a by-product from the biofuel industries. This meal may have been expelled or pressed during the oil extraction process (Smulikowska et al. 2006). Mechanical-pressing tends to be cheaper than solvent extraction procedures and is the method of choice for small biofuel processors. The material left after pressing has high oil content which varies. It has not been heat treated like conventional canola meal (Thacker and Petri 2009a). The main area of concern in the animal feed industry with the used of this by-product in monogastric feed is related to its ability to act as an effective feeding ingredient like its solvent counterpart. There is relatively little broiler growth performance study conducted on the use of these by-products. Their increasing abundance makes them more attractive to the feed industry. This high residual oil meal is very attractive for use in the feed industry since it is characterized by a relatively high protein and increased energy content compared to prepress solvent extracted canola meal (Thacker and Petri 2009a). Most studies to date that have evaluated mechanically pressed canola were for a short period of time 21days (Thacker and Petri 2009a and 2009b, Woyengo et al. 2011). There are no recommended levels of inclusion set for its use in the starter, grower and finisher diets for broilers. Studies (Petri 2009a and 2009b, Woyengo et al. 2011) evaluate the meals based on the residual oil content which is known to influence the metabolizable energy in the feed ingredient (Smulikowska et al. 2006). A full cycle growth study was conducted to generate body weight, feed consumption and feed conversion ratio (FCR) data using Ross 308 broilers. The objective of the study was to determine the effects of graded levels (0, 5, 10 and 15%) of mechanically pressed black canola with 17 and 12% residual oil on the performance of Ross 308 broilers.

7.3 Materials and Methods

7.3.1 Preparation of ingredients

Black canola seeds were cleaned then expelled to produce a meal with 12% residual oil along with crude unfiltered oil. To prepare a low and a high oil level meal, the crude unfiltered oil was added to the 12% meal to produce a meal with 17% residual oil. The 17% residual oil meal was mixed at the Dalhousie Faculty of Agriculture feed mill using a horizontal Marion mixer and sampled for nutrient analysis (Appendix A).

7.3.2 Diet preparation

In this experiment twenty four corn based diets were formulated on a digestible amino acid basis. Black canola meals from section 7.3.1 were substituted in the formulations using AMEn and digestible amino acid values reported in chapter 4. The starters and growers diets were fed in mash form while the finisher diets were pelleted. Diets were formulated to be isonitrogenous and isocaloric within each period. All diets were formulated on a digestible amino acid basis which met or exceeded (NRC 1994) nutrient requirements for broilers at each stage of growth.

There were eight diets per growth phase formulated with 0, 5, 10 and 15% black canola meal with 12% residual oil and 0, 5, 10 and 15% black canola meal with 17% residual oil. Diet 1 and 5 had 0% meal representing the control diets in each residual oil meal for starter, grower and finisher birds. All the starter diets (Table 7.0) had 3050 $kcal \cdot kg^{-1}$ metabolizable energy and 23 CP. The eight grower diets (Table 7.1.) were formulated to have the same meal inclusion levels as the starter diets and they all had 3150 $kcal \cdot kg^{-1}$ metabolizable energy and 20% CP. The eight finisher diets (Table 7.2.)

were formulated to have the same meal inclusion levels as the starter diets and they all had 3200 kcal·kg^{-1} metabolizable energy and 18 % CP.

TABLE 7.0 Ingredient, calculated analyses and analyzed composition for starter broiler diets composed of mechanically pressed black canola meal (% as fed).

	Control	12% oil meal			17% oil meal		
	Diet 1&5	Diet 2	Diet 3	Diet 4	Diet 6	Diet 7	Diet 8
Ingredients as fed							
Corn	44.4	41.7	39.1	36.4	41.7	39.1	36.5
Soybean meal	38.8	35.9	33.1	30.3	36.1	33.5	30.9
Wheat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Meal[z]	-----	5.0	10.0	15.0	5.0	10.0	15.0
Tallow-grease blend	3.3	3.8	4.3	4.9	3.6	3.9	4.2
Limestone ground	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Mono-Dicalcium phosphate	0.6	0.5	0.5	0.4	0.5	0.5	0.4
Vitamin mineral premix[y]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine premix[x]	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Coban[w]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stafac 44[v]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated Analysis							
MEn kcal·kg-1	3050	3050	3050	3050	3050	3050	3050
Protein %	23	23	23	23	23	23	23
Calcium %	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Phosphorus %	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine %	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Methionine %	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Met+Cys %	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Analyzed Values (as fed)							
Dry matter %	87.9	88.3	88.6	88.3	88.7	88.4	88.6
Protein %	21.8	22.7	22.6	22.5	23.1	22.3	22.9
Calcium %	0.86	0.93	1.05	0.94	1.00	0.90	0.81
Phosphorus %	0.52	0.57	0.58	0.59	0.55	0.56	0.59
Sodium %	0.17	0.19	0.20	0.19	0.14	0.18	0.17
Potassium%	0.97	1.02	1.00	0.99	1.00	1.00	1.03
Magnesium %	0.20	0.19	0.20	0.18	0.19	0.20	0.20
Fat %	5.73	6.53	8.98	8.79	6.48	7.37	8.19

[z]Mechanically pressed meal is black canola with 12 or 17% residual oil

[y]Starter premix (amount per tonne), vitamin A (650×106IU kg^{-1}),15g, vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}), 50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca-pentothenate (45%), 30g; vitamin B12 (l000 mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g; Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g;Wheat middlings 1432g;Ground limestone (38%),500g.

[x]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

[w]Coban: Coccidiostat-Pfizer Animal Health, London, ON, Canada

[v]Sufac 44: Antibiotic- Elanco Animal Health, Guelph, ON, Canada

TABLE 7.1. Ingredient, calculated analyses and analyzed composition for grower broiler diets composed of mechanically pressed black canola meal (% as fed).

	Control	12% oil meal			17% oil meal		
	Diet 1&5	Diet 2	Diet 3	Diet 4	Diet 6	Diet 7	Diet 8
Ingredients as fed							
Corn	52.0	49.3	47.0	44.0	49.3	46.7	44.1
Soybean meal	31.0	28.2	25.3	22.5	28.4	25.7	23.1
Wheat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Meal[z]	-----	5.0	10.0	15.0	5.0	10.0	15.0
Tallow-grease blend	3.8	4.4	4.9	5.4	4.1	4.5	4.8
Limestone ground	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Mono-Dicalcium phosphate	0.5	0.4	0.3	0.3	0.4	0.3	0.3
Vitamin mineral premix[y]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine premix[x]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Coban[w]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stafac 44[v]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated Analysis							
MEn kcal·kg-1	3150	3150	3150	3150	3150	3150	3150
Protein %	20	20	20	20	20	20	20
Calcium %	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Non-Phytate Phosphorus %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine %	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionine %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Met+Cys %	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Analyzed Values (as fed)							
Dry matter %	87.9	88.1	88.3	88.9	88.6	88.3	88.8
Protein %	20.2	19.6	19.3	20.3	20.2	19.7	20.2
Calcium %	0.88	0.85	0.98	0.97	0.88	0.99	0.93
Phosphorus %	0.48	0.49	0.51	0.54	0.50	0.53	0.56
Sodium %	0.18	0.14	0.19	0.19	0.18	0.21	0.18
Potassium%	0.89	0.88	0.86	0.89	0.87	0.84	0.87
Magnesium %	0.16	0.17	0.18	0.20	0.17	0.18	0.20
Fat %	6.07	6.31	8.21	9.07	6.80	8.42	9.24

[z]Mechanically pressed meal is black canola with 12 or 17% residual oil

[y]grower premix, vitamin A (650×106 IU kg^{-1}), 15g,vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}),50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g Pyridoxine (990,000 mg kg-1), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g;Wheat middlings 1532g;Ground limestone (38%),500g.

[x]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

[w]Coban: Coccidiostat-Pfizer Animal Health, London, ON, Canada

[v]Sufac 44: Antibiotic- Elanco Animal Health, Guelph, ON, Canada

TABLE 7.2. Ingredient, calculated analyses and analyzed composition for finisher broiler diets composed of mechanically pressed black canola meal (% as fed).

	Control	12% oil meal			17% oil meal		
	Diet 1&5	Diet 2	Diet 3	Diet 4	Diet 6	Diet 7	Diet 8
Ingredients as fed							
Corn	57.0	54.4	52.0	49.3	54.4	52.0	49.2
Soybean meal	26.0	23.0	20.0	17.3	23.2	20.5	18.0
Wheat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Meal[z]	-----	5.0	10.0	15.0	5.0	10.0	15.0
Tallow-grease blend	3.6	4.1	4.5	5.0	4.0	4.2	4.5
Limestone ground	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Pel-Stik[y]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mono-Dicalcium phosphate	0.5	0.4	0.3	0.2	0.4	0.3	0.3
Vitamin mineral premix[x]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine premix[w]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated Analysis							
MEn kcal·kg-1	3200	3200	3200	3200	3200	3200	3200
Protein %	18	18	18	18	18	18	18
Calcium %	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Non-Phytate Phosphorus %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine %	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Methionine %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Met+Cys %	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Analyzed Values (as fed)							
Dry matter %	87.4	87.8	87.8	87.5	87.5	87.2	87.7
Protein %	18.0	18.9	17.4	19.2	18.3	17.3	18.0
Calcium %	0.91	0.76	0.85	0.87	0.82	0.90	1.01
Phosphorus %	0.45	0.48	0.52	0.53	0.48	0.50	0.54
Sodium %	0.18	0.18	0.19	0.19	0.17	0.18	0.15
Potassium%	0.80	0.80	0.81	0.81	0.79	0.80	0.79
Magnesium %	0.16	0.17	0.18	0.19	0.17	0.18	0.19
Fat %	5.92	7.91	8.02	8.57	7.33	8.04	8.97

[z]Mechanically pressed meal is black canola with 12 or 17% residual oil

[y]Pel-Stik

[x]grower premix, vitamin A (650×106 IU kg^{-1}), 15g,vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}),50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg-1), 220g; Ethoxyquin (50%), l00g;Wheat middlings 1532g;Ground limestone (38%),500g.

[w]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

7.3.3 Animal husbandry

Nineteen hundred and twenty, Ross 308, male, day-old broiler chicks were obtained from a local hatchery. Upon arrival, each bird was randomly selected and placed in groups of forty which were weighed and distributed randomly to 48 floor pens. The pens at the Atlantic Poultry Research Center had 3 to 4 cm of litter made of pine shavings. Before the birds arrived the room was preheated to 30 ºC and each pen measuring 2.13m x 1.40m had one tube feeders and a watering pan with nipple drinkers. Each pen had a 51 x 43x 2.5cm cardboard box on the floor embedded in the litter with feed in it for one week plus a the tube feeder was filled as well. When the birds arrived at the research facility they were introduced to water and feed by dipping the beaks of the chicks in the watering pan and placing the birds on the cardboard box with feed. From the day of arrival at the research facilities to 35 days post-hatch, all the birds had *ad libitum* access to the diets in replicates of six pens per treatment. All the birds were hand fed daily. The feed given each day was weighed in and weighed back when mortality occurred and at days 14 and 24 and 35. On days 14, 24 and 35 the birds were batch weighed per pen and weights recorded. Mortalities were recorded throughout the trial and when it happened, the dead birds were weighed and feed weighed back from the trough. All birds that died were necropsied by a veterinary pathologist to determine cause and timing of mortalities. The lighting and temperature schedule used in this trial (Appendix B) was measured using a data logger and temperature manually by hand twice daily using a Raytek Mini temp gun. All broilers were managed under the supervision of the Animal Care and Use Committee of Dalhousie University using guidelines provided by the Canadian Council on Animal Care (2009).

7.3.4 Performance data collection

Production performance was measured by body weight gain, feed consumption, FCR and mortality. At each weigh day body weight and feed consumed were recorded to obtain data for body weight gain and feed consumption. Both sets of data were used to calculate FCR. The mortalities recorded were express as percentage of the birds on each treatment.

7.3.5 Statistical analysis

The performance data collected were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3, (SAS Institute Inc., Cary, NC) (Littell et al. 1996) with day as a repeated factor. The experimental design was completely randomized with a 2 x 4 factorial arrangement with (residual oil level x meal substitution) where meal residual oil content was 12% and17% residual oil and meal substitution was 0, 5, 10 and15%. Model:

Y_{ijk}= μ+ meal residual oil $level_i$ + meal $substitution_j$ + meal residual oil level*meal $substitution_{ij}$ + day_k + meal residual oil level*day_{ik} + meal substitution*day_{jk} + meal residual oil level*meal substitution*day_{ijkl} +ε_{ijkl}

The statistical model of the experiment as shown above where Y is the response variable and μ is the overall mean response for that factor. Meal residual oil $level_{i\,(1\text{-}2)}$ is the effect of meal residual oil at the i^{th} level (1= 12% and 2 =17%). Meal $substitution_{j\,(1\text{-}4)}$ is the effect of meal substitution in diets at the j^{th} level (1= 0%, 2 = 5%, 3= 10% and 4= 15%). Day is the effects of age (day 14, 24 and 35). Meal residual oil level*meal $substitution_{ij}$ is the effects of the interaction at the ij^{th} oil level and substitution. ε_{ijkl} is the residual error of the model with $_l$ replication of six.

If significant main effects or interactions were found ($P \leq 0.05$), Tukey Kramer test (Littell et al, 1996) was used to compare differences among the least square means at ($\alpha \leq 0.05$).

7.4 Results and Discussion

7.4.1 Feed consumption by birds.

The ANOVA data given in (Table 7.3.) starter, (Table 7.4.) grower and (Table 7.5.) finisher on the feed consumption of birds fed mechanically pressed black canola from 0 to 35 days shows no treatment effects. This means that birds given diets containing 15% mechanically pressed canola meal consumed the same level of feed as those given no mechanically pressed canola meal. From 0 to 14 days of age the feed consumption per bird, per day ranged from 34 to 38 g. Between days 15 to 24 each bird was consuming 92 to 102 g a day, while from 25 to 35 days 158 to 174 g were consumed. Woyengo et al. (2011) fed 10% expeller extracted canola meals to broilers which consumed 46g a day at 21 days old. The feed intake was reduced linearly as the level of expeller extracted meal in the diets increased from 0 to 40% at 10% intervals; this linear reduction was not seen in this study. Woyengo et al. (2011) attributed their reduction in feed intake to the cumulative increase in ant-nutritional compounds in the diets as the meal level increased. Feeding rapeseed meal high in compounds like glucosinolates is known to reduce feed intake in broilers (Bell 1993, Bones and Rossiter 1996). Though not tested in this study the level of anti-nutritional compounds in the meals used in this study may have been too low to result in any obvious effects on the feed consumption of the birds. The higher residual oil may have diluted the glucosinolates or they were present at levels below the threshold that causes changes in fee consumption. Based on current industry measures of glucosinolates in meal (8-12$\mu g \cdot g^{-1}$ of meal) the levels in our diets would have been below 2 $\mu g \cdot g^{-1}$ of diet.

TABLE 7.3. Effects of substitution level of mechanically pressed black canola meal on starter feed consumption ($g \cdot b^{-1} \cdot d^{-1}$) for 14 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	37±1	36±1	38±1
5%	38±1	38±1	36±1
10%	35±1	37±1	35±1
15%	34±1	37±1	35±1
Effects of Oil	36±1	37±1	

Source of variation	P>F
Oil	0.3830
Substitution	0.1871
Oil x Substitution	0.2373

Mean ± SEM

TABLE 7.4. Effects of substitution level of mechanically pressed black canola meal on grower feed consumption ($g \cdot b^{-1} \cdot d^{-1}$) for 24 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	102±2	97±2	99±2
5%	99±2	96±2	97±2
10%	92±2	99±2	96±2
15%	93±2	93±2	93±2
Effects of Oil	96±1	96±1	

Source of variation	P>F
Oil	1.0000
Substitution	0.0527
Oil x Substitution	0.0821

Mean ± SEM

TABLE 7.5. Effects of substitution level of mechanically pressed black canola meal on finisher feed consumption ($g \cdot b^{-1} \cdot d^{-1}$) from 25-35 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	167±4	167±4	167±3
5%	168±4	174±4	171±3
10%	162±4	163±4	163±3
15%	158±4	162±4	160±3
Effects of Oil	164±2	166±2	
Source of variation		P>F	
Oil		0.4133	
Substitution		0.0722	
Oil x Substitution		0.8540	

Mean ± SEM

7.4.2 Body weight of birds.

There were no effects of treatments on the body weight gain of birds fed the MPBCM during the starter phase (Table 7.6.) and grower phase (Table 7.7.). However at the finisher phase (Table 7.8.) the levels of meal substituted in the diets significantly (P=0.0153) influenced the body weight gain of the birds. Birds given diets with 5% meal had statistically higher ($P \leq 0.05$) body weight gain than those given the diets with 15% meal. Both diets were not significantly different from the other test diet with 10% meal and the control diet without meal. The same reduction in body weight gain with increased level of expelled canola meals seen in the finisher phase was reported by Woyengo et al. (2011) in birds at 21 days of age. The same author found a linear increase in liver weight relative to body weights as the level of meal increased in the diets. This was an indication of increased liver activity due to the presence of the meal in the diets which may have lead to more metabolic energy being diverted to liver metabolism rather than for growth

(Woyengo et al. 2011). Myrosinase hydrolyses glucosinolates and the production of degradation products like thiocyanates and 1-cyano-2-hydroxy-3-butene which have an adverse effect on poultry livers and are likely to occur during the crushing of canola seed (Smulikowska et al. 2006). Thacker and Petri (2009a, b) fed canola press cake to broilers at the same inclusion levels as the present study and found no effects of level of meal inclusion on body weight gains at 21 days of age. There were no effects of treatments on the body weight of birds fed MPBCM during the starter phase 0-14 days (Table 7.9.) and grower phase 15-24 days (Table 7.10.). During the finisher phase 25-35 days (Table 7.11.) the meal substitution level in the diets significantly influenced the body weight of the birds. Birds given diets with 0 and 5% meal had significantly higher ($P \leq 0.05$) body weight than those given the diets with 15% meal. The 15% meal diets were not significantly different from the test diets with 10% meal.

TABLE 7.6. Effects of substitution level of mechanically pressed black canola meal on body weight gain ($g \cdot b^{-1} \cdot d^{-1}$) from 0-14 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	23±1	24±1	24±0
5%	23±1	24±1	23±0
10%	23±1	23±1	23±0
15%	23±1	24±1	23±0
Effects of Oil	23±0	24±0	
Source of variation		P>F	
Oil		0.0516	
Substitution		0.9086	
Oil x Substitution		0.2543	

Mean ± SEM

TABLE 7.7. Effects of substitution level of mechanically pressed black canola meal on body weight gain ($g \cdot b^{-1} \cdot d^{-1}$) for 15-24 day old broilers.

Meal level of Substitution	Meal Oil level 12%	Meal Oil level 17%	Effects of Substitutions
0%	61±2	56±2	59±2
5%	57±2	60±2	58±2
10%	59±2	59±2	59±2
15%	57±2	59±2	58±2
Effects of Oil	59±1	58±1	

Source of variation	P>F
Oil	0.3830
Substitution	0.1871
Oil x Substitution	0.2373

Mean ± SEM

TABLE 7.8. Effects of substitution level of mechanically pressed black canola meal on body weight gain ($g \cdot b^{-1} \cdot d^{-1}$) for 25-35 day old broilers.

Meal level of Substitution	Meal Oil level 12%	Meal Oil level 17%	Effects of Substitutions
0%	88±3	91±3	89±2ab
5%	92±3	91±3	92±2a
10%	91±3	84±3	87±2ab
15%	83±3	79±3	81±2b
Effects of Oil	89±2	86±2	

Source of variation	P>F
Oil	0.3109
Substitution	0.0153
Oil x Substitution	0.4486

[a-b] Mean ± SEM with no common letters in the substitution effects group are significantly different at $\alpha = 0.05$

TABLE 7.9. Effects of substitution level of mechanically pressed black canola meal on 0-14 day broiler body weight ($g \cdot b^{-1}$).

Meal level of Substitution	Meal Oil level 12%	Meal Oil level 17%	Effects of Substitutions
0%	373±9	386±9	379±6
5%	364±9	378±9	371±6
10%	375±9	365±9	370±6
15%	358±9	386±9	372±6
Effects of Oil	367±4	379±4	

Source of variation	P>F
Oil	0.0650
Substitution	0.6656
Oil x Substitution	0.1784

Mean ± SEM

TABLE 7.10. Effects of substitution level of mechanically pressed black canola meal on 15-24 day broiler body weight ($g \cdot b^{-1}$).

Meal level of Substitution	Meal Oil level 12%	Meal Oil level 17%	Effects of Substitutions
0%	985±19	947±19	966±13
5%	933±19	975±19	954±13
10%	963±19	951±19	957±13
15%	925±19	978±19	952±13
Effects of Oil	952±9	963±9	

Source of variation	P>F
Oil	0.4029
Substitution	0.8822
Oil x Substitution	0.0599

Mean ± SEM

TABLE 7.11. Effects of substitution level of mechanically pressed black canola meal on 25-35 day broiler body weight ($g \cdot b^{-1}$).

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	1954±34	1947±34	1951±24a
5%	1944±34	1976±34	1960±24a
10%	1962±34	1872±34	1917±24ab
15%	1839±34	1844±34	1841±24b
Effects of Oil	1926±17	1910±17	
Source of variation		P>F	
Oil		0.5308	
Substitution		0.0042	
Oil x Substitution		0.3173	

[a-b] Mean ± SEM with no common letters in the substitution effects group are significantly different at $\alpha = 0.05$

7.4.3 Feed conversion ratio of birds.

The ANOVA data from Table 7.12., Table 7.13. and Table 7.14. show that there was no effect of treatments on the birds FCR. Woyengo et al. (2011) observed a linear increase in the FCR of broilers fed 0, 10, 20, 30 and 40% expeller extracted canola meal at 21 days of age. The FCR during the starter period (0-14 days) of this study ranged from 1.48 to 1.69, the grower period (15 -24 days) was 1.57 to 1.79 and the finisher period (25-35 days) was 1.79 to 2.11. At 21 days Woyengo et al. (2011) FCR of the test diets ranged from 1.25 in the control diet which then increased linearly to 1.41 in the 40% meal diets. The level of meals substituted in the diets did not statistically influence the feed consumption of the birds during the 35 days but it had an effect on the body weights of the birds during the finisher stage.

TABLE 7.12. Effects of substitution level of mechanically pressed black canola meal on FCR from 0-14 day old broilers.

Meal of level Substitution	Meal Oil level Means		
	12% Oil meal	17% Oil meal	Effects of Substitutions
0% meal	1.59±0.07	1.48±0.07	1.53±0.05
5% meal	1.69±0.07	1.61±0.07	1.65±0.05
10% meal	1.50±0.07	1.58±0.07	1.53±0.05
15% meal	1.52±0.07	1.54±0.07	1.53±0.05
Effects of Oil	1.57±0.03	1.55±0.03	
Source of variation		P>F	
Oil		0.6333	
Substitution		0.1839	
Oil x Substitution		0.3808	

Mean ± SEM

TABLE 7.13. Effects of substitution level of mechanically pressed black canola meal on FCR from 15-24 day old broilers.

Meal of level Substitution	Meal Oil level Means		
	12% Oil meal	17% Oil meal	Effects of Substitutions
0% meal	1.66±0.08	1.79±0.08	1.73±0.05
5% meal	1.73±0.08	1.61±0.08	1.67±0.05
10% meal	1.57±0.08	1.74±0.08	1.65±0.05
15% meal	1.64±0.08	1.57±0.08	1.60±0.05
Effects of Oil	1.65±0.04	1.68±0.04	
Source of variation		P>F	
Oil		0.6075	
Substitution		0.4376	
Oil x Substitution		0.1439	

Mean ± SEM

TABLE 7.14. Effects of substitution level of mechanically pressed black canola meal on FCR from 25-35 day old broilers.

Meal of level Substitution	Meal Oil level Means		
	12% Oil meal	17% Oil meal	Effects of Substitutions
0% meal	1.90±0.10	1.84±0.10	1.87±0.07
5% meal	1.83±0.10	1.91±0.10	1.87±0.07
10% meal	1.79±0.10	1.97±0.10	1.88±0.07
15% meal	1.93±0.10	2.11±0.10	2.02±0.07
Effects of Oil	1.86±0.05	1.96±0.05	
Source of variation		P>F	
Oil		0.1828	
Substitution		0.3361	
Oil x Substitution		0.5937	

Mean ± SEM

Birds given the diets with 15% meal did not have significantly lower feed efficiency than other treatments. Thacker and Peti (2009a, b) fed canola press cake to broilers at 0, 5, 10 and 15% in the diets and found a linear effect of level of meal inclusion on feed conversion at 21 days of age. As the level of meal increased in the diets the birds were not able to become more efficient in the conversion of feed to weight gain. Woyengo et al. (2011) also MCPCM at graded level of the diets to broilers and found the same linear effects of meal on feed conversion at 21 days of age. Thacker and Peti (2009a, b) and Woyengo et al. (2011) had the same result even though the level at which the meals were included in the diets were different. In the study conducted by Woyengo et al. (2011) the meal levels of inclusion where higher at 0, 10, 20, 30 and 40% and the diet was corn based while in the studies conducted by Thacker and Peti (2009a, b) the levels of inclusion were 0, 5, 10 and 15 % while the diets were wheat based. The current study did not use regression analysis to evaluate the data so this might be why that relationship between meal inclusion levels and feed conversion was not seen.

7.4.4 Bird mortality

The total mortality for the trial was 3.7% most of which was in the starter period. The postmortems revealed that mortalities occurring in the starter period were mostly related to a combination of omphalitis, ascites and coliform septicemia. Those that died during the grower and finisher period were related mostly to ascites. There was no effect of treatments (Table 7.15.) on the mortalities which occurred during the starter, grower or finisher stage. There were also no effects of treatment on the mortalities during the overall growth period of the study. Thacker and Peti (2009a, b.) did not observe any effects of treatment on the mortality of the birds during the 21 days of their experiment.

TABLE 7.15. Mortality ANOVA for black canola meal growth trial.

	Starter	Grower	Finisher	From 0-35 days
Effect	P-Value	P-Value	P-Value	P-Value
Oil	0.3474	0.1329	0.0679	0.1923
Level	0.7109	0.7593	0.3498	0.4547
Oil*Level	0.3803	0.3308	0.2985	0.3892

7.5 Conclusion

Birds given mechanically pressed black *Brassica napus* canola meal performed equally well to those given no canola meal based on feed consumption and feed conversion to meat. Consideration must be given when using 15% MPBCM in finisher period as it gave lower body weights than the 0% diets. The oil levels of the meal did not influence the bird's production performance. MPBCM with 12 and 17% residual oil can be feed up 15% in the starter and grower diets without any significant effects on body weight gain, feed conversion and final body weight, but a maximum of 10% is recommended for the finisher period.

CHAPTER 8: GROWTH PERFORMANCE OF BROILER CHICKENS FED GRADED LEVELS OF MECHANICALLY PRESSED YELLOW CANOLA MEAL (*BRASSICA NAPUS*) FROM 0-35 DAYS

8.1 Abstract

A new line of yellow seeded *Brassica napus* was developed by Canadian plant breeders. There is no growth study testing the use of mechanically pressed meal from the seed. To study the production performance of chicks fed 0, 5, 10 and 15% mechanically pressed yellow canola (*Brassica napus*) meal a total of 1920, Ross 308, male day old broiler chickens. The birds were reared in environmentally controlled rooms at the Atlantic Poultry Research Center. The chickens were randomly allocated to eight dietary treatment having six replicates of 40 birds per rep. The experimental diets contained meals with two level of residual oil 12 and 17%. The experimental design was completely randomized with a 2x4 factorial arrangement. Experimental diets and fresh water were offered *ad libitum* during all three growth phases (starter 0-14 days, grower 15-24 days and finisher 25-35 days). Birds fed meals with lower residual oil consumed more feed ($P \leq 0.05$) during the starter and finisher period but not in the grower period. Having 15% meals in the diet reduced ($P \leq 0.05$) the feed consumed during the starter and finisher period but not in the grower period. The highest residual oil and the 15% inclusion level of the meal both reduced ($P \leq 0.05$) the body weight gain during the starter and grower period but not the finisher period. This resulted in lower ($P \leq 0.05$) final body weight of the 15% meal diet birds during all three growth phases. The residual oil level of the meal did not ($P \geq 0.05$) influence the birds' 35 day final body weight. There were no effects ($P \geq 0.05$) of meal inclusion levels or residual oil on the feed conversion ratio of the birds. Both the level of residual oil and the rate of inclusion of mechanically pressed yellow *Brassica napus* meal in the diets of broiler chicken influenced production performance during the various phases of production. Mechanically pressed yellow meal (MPYCM) can be included in the broiler diets up to 10% without any detrimental effect on production performance. It is recommenced that MPYCM with 17% or greater be fed only in the finisher phase at 10% in the diet of broiler chickens.

Keywords: Yellow canola meal, Broilers, Body weight gain, Feed consumption

8.2 Introduction

There have been consistent efforts through plant breeding to improve the nutritional value of canola meals for monogastric animal (Bell. 1993, Jia et al. 2012). During the improvements of older canola varieties it was observed that seed with yellow color possessed nutritional qualities superior to their black seeded counterpart (Slominski 1997, Rahman and McVetty 2011). Rahman and McVetty (2011) presented a review with details on how seed coat color influences the nutritional quality of canola seeds. To benefit from the improved nutritional values of yellow seed canola, plant breeders at Agriculture and AgriFood Canada have focused their attention on selecting yellow lines of *Brassica napus* canola (Somers et al. 2001). A new line of yellow seeded *Brassica napus* was developed by Canadian plant breeders. This yellow seeded *Brassica napus* has stable seed color and good agronomic characteristics which may see it becoming a future commercial line (Somers et al. 2001). If this seed line becomes commercial it will be available to the oil pressing industry which, in turn, will provide its meal to the feed industry as a feed ingredient.

Other researchers (Czerwinski et al. 2012) have evaluated the growth of broiler chickens comparing various lines of cold pressed yellow canola cake with the effects of phytase supplementation. To our knowledge no full performance trial has been conducted testing various inclusion levels of mechanically pressed yellow *Brassica napus* meals while evaluating the effects of the meals residual oil on performance. The objective of this study was to determine the influence of meal residual oil level and dietary inclusion level of mechanically pressed yellow *Brassica napus* meals on the growth performance of broiler chicken over a 35 days period.

8.3 Materials and Methods

8.3.1 Preparation of ingredients

Yellow canola seeds were cleaned then expelled to produce a meal with 17% residual oil along with crude unfiltered oil. To prepare a low and a high oil level meal, the 17% percent residual oil meal was passed through a vegetable oil expeller (Antonfries Vegetable oil press P500R, Maschinenbau, Meitin-herbertshofen, Germany). After expelling, the meal had 12% residual oil. Meals were sampled for analysis (Appendix A).

8.3.2 Diet preparation

Twenty four corn based diets were formulated on a digestible amino acid basis. Yellow canola meals from section 8.3.1 were substituted in the formulations using metabolizable energy and amino acid content determined in chapter 6. The starter and grower diets were in mash form while the finisher diets were pelleted. Diets were formulated to be isonitrogenous and isocaloric for each period and on a digestible amino acid basis and met or exceeded (NRC 1994) nutrient requirements for broilers at each growth stage. Each growth phase had eight diets formulated with 0, 5, 10 or 15% yellow canola meal with 12% residual oil and 0, 5, 10 or 15% yellow canola meal with 17% residual oil. Diet 1 and 5 had 0% meal and represented the control diets in each residual oil meal for starter, grower and finisher. All the starter diets (Table 8.0) had 3050 $kcal \cdot kg^{-1}$ metabolizable energy and 23% CP. The eight grower diets (Table 8.1.) were formulated to have the same meal inclusion levels as the starter diets but contained 3150 $kcal \cdot kg^{-1}$ metabolizable energy and 20 % CP. The eight finisher diets (Table 8.2.) were formulated to have the same meal inclusion levels as the starter diets with 3200 $kcal \cdot kg^{-1}$ metabolizable energy and 18%t CP.

TABLE 8.0 Ingredient, calculated analyses and analyzed composition for starter broiler diets composed of mechanically pressed yellow canola meal (% as fed).

	Control	12% oil meal			17% oil meal		
	Diet 1&5	Diet 2	Diet 3	Diet 4	Diet 6	Diet 7	Diet 8
Ingredients as fed							
Corn	44.4	42.3	40.3	38.3	42.2	40.1	38.0
Soybean meal	38.8	35.8	32.8	29.8	36.1	33.4	30.6
Wheat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Meal[z]	-----	5.0	10.0	15.0	5.0	10.0	15.0
Tallow-grease blend	3.3	3.3	3.4	3.5	3.2	3.1	3.0
Limestone ground	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Mono-Dicalcium phosphate	0.6	0.5	0.5	0.4	0.5	0.4	0.4
Vitamin mineral premix[y]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine premix[x]	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Coban[w]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stafac 44[v]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Lysine 98%	----	----	----	0.01	----	----	----
Calculated Analysis							
MEn kcal·kg-1	3050	3050	3050	3050	3050	3050	3050
Protein %	23	23	23	23	23	23	23
Calcium %	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Non-Phytate Phosphorus %	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine %	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Methionine %	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Met+Cys %	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Analyzed Values (as fed)							
Dry matter %	90.3	91.2	89.7	90.0	90.2	90.4	90.2
Protein %	23.6	24.2	22.3	23.1	23.5	22.7	23.3
Calcium %	0.95	0.76	1.01	0.96	0.87	0.97	1.03
Phosphorus %	0.55	0.64	0.58	0.60	0.58	0.56	0.61
Sodium %	0.19	0.10	0.21	0.18	0.17	0.18	0.20
Potassium%	1.09	1.13	0.99	1.04	1.07	1.04	1.06
Magnesium %	0.19	0.25	0.20	0.23	0.21	0.21	0.21
Fat %	5.87	7.73	7.21	7.60	6.08	6.65	6.33

[z]Mechanically pressed meal is yellow canola with 12 or 17% residual oil

[y]Starter premix (amount per tonne), vitamin A (650×106IU kg^{-1}),15g, vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}), 50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca-pentothenate (45%), 30g; vitamin B12 (l000 mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g; Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g;Wheat middlings 1432g;Ground limestone (38%),500g.

[x]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

[w]Coban: Coccidiostat-Pfizer Animal Health, London, ON, Canada

[v]Sufac 44: Antibiotic- Elanco Animal Health, Guelph, ON, Canada

TABLE 8.1. Ingredient, calculated analyses and analyzed composition for grower broiler diets composed of mechanically pressed yellow canola meal (% as fed).

	Control	12% oil meal			17% oil meal		
	Diet 1&5	Diet 2	Diet 3	Diet 4	Diet 6	Diet 7	Diet 8
Ingredients as fed							
Corn	52.0	50.0	47.9	46.0	50.0	47.7	45.6
Soybean meal	31.0	28.0	25.0	22.0	28.3	25.6	22.9
Wheat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Meal[z]	-----	5.0	10.0	15.0	5.0	10.0	15.0
Tallow-grease blend	3.8	4.0	4.0	4.0	3.7	3.6	3.5
Limestone ground	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Mono-Dicalcium phosphate	0.5	0.4	0.3	0.2	0.4	0.3	0.2
Vitamin mineral premix[y]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine premix[x]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Coban[w]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stafac 44[v]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated Analysis							
MEn kcal·kg-1	3150	3150	3150	3150	3150	3150	3150
Protein %	20	20	20	20	20	20	20
Calcium %	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Non-Phytate Phosphorus %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine %	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionine %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Met+Cys %	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Analyzed Values (as fed)							
Dry matter %	89.0	88.8	89.3	89.8	88.6	88.7	89.0
Protein %	19.4	20.6	19.5	19.6	20.1	19.6	20.2
Calcium %	0.99	0.89	0.88	0.96	0.82	0.90	0.91
Phosphorus %	0.51	0.51	0.52	0.57	0.51	0.53	0.54
Sodium %	0.20	0.17	0.18	0.20	0.15	0.19	0.20
Potassium%	0.85	0.89	0.99	0.89	0.88	0.84	0.84
Magnesium %	0.17	0.19	0.20	0.22	0.19	0.20	0.20
Fat %	7.40	7.24	7.61	8.76	6.82	7.24	7.86

[z]Mechanically pressed meal is yellow canola with 12 or 17% residual oil

[y]grower premix, vitamin A (650×106 IU kg^{-1}), 15g,vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}),50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g;Wheat middlings 1532g;Ground limestone (38%),500g.

[x]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

[w]Coban: Coccidiostat-Pfizer Animal Health, London, ON, Canada

[v]Sufac 44: Antibiotic- Elanco Animal Health, Guelph, ON, Canada

TABLE 8.2. Ingredient, calculated analyses and analyzed composition for finisher broiler diets composed of mechanically pressed yellow canola meal (% as fed).

	Control	12% oil meal			17% oil meal		
	Diet 1&5	Diet 2	Diet 3	Diet 4	Diet 6	Diet 7	Diet 8
Ingredients as fed				(%)			
Corn	57.0	55.0	53.0	51.0	54.8	52.7	50.5
Soybean meal	26.0	23.0	20.0	17.0	23.1	20.4	18.0
Wheat	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Meal[z]	-----	5.0	10.0	15.0	5.0	10.0	15.0
Tallow-grease blend	3.6	3.7	3.8	3.8	4.0	3.5	3.4
Limestone ground	1.6	1.6	1.6	1.6	1.6	1.6	1.7
Pel-Stik[y]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mono-Dicalcium phosphate	0.5	0.4	0.3	0.2	0.4	0.3	0.2
Vitamin mineral premix[x]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Methionine premix[w]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated Analysis							
MEn kcal·kg-1	3200	3200	3200	3200	3200	3200	3200
Protein %	18	18	18	18	18	18	18
Calcium %	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Non-Phytate Phosphorus %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine %	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Methionine %	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Met+Cys %	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Analyzed Values (as fed)							
Dry matter %	86.7	86.9	87.1	87.4	86.5	87.1	87.4
Protein %	18.2	18.6	17.8	18.3	17.6	18.4	17.6
Calcium %	0.91	0.91	0.93	0.93	0.91	0.90	0.90
Phosphorus %	0.43	0.46	0.48	0.50	0.46	0.47	0.50
Sodium %	0.18	0.17	0.18	0.19	0.19	0.19	0.19
Potassium%	0.76	0.75	0.71	0.74	0.76	0.73	0.75
Magnesium %	0.16	0.17	0.18	0.19	0.16	0.18	0.19
Fat %	6.02	6.52	7.31	7.83	6.75	7.23	7.96

[z]Mechanically pressed meal is yellow canola with 12 or 17% residual oil

[y]Pel-Stik

[x]grower premix, vitamin A (650×106 IU kg^{-1}), 15g,vitamin D3 permix (50×106 IU kg^{-1}), 40g; vitamin E (500,000 IU kg^{-1}),50g; vitamin K (33%), 9g; Riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg^{-1}), 23g; Niacin (99%), 30; Folic acid (3%), 133g; Choline chloride (60%), 1335g; Biotin (0.04%), 750g Pyridoxine (990,000 mg kg^{-1}), 5g; Thiamin (970,000 mg kg^{-1}), 3g; Manganous oxide (60%), 117g; Zinc oxide (80%), l00g; Copper sulphate (25%), l00g; Selenium premix (675 mg kg^{-1}), 220g; Ethoxyquin (50%), l00g;Wheat middlings 1532g;Ground limestone (38%),500g.

[w]Methionine premix contained 500g kg^{-1} DL- Methionine and 500g kg^{-1} wheat middlings

8.3.3 Animal husbandry

Nineteen hundred and twenty Ross 308 male day-old broiler chicks were obtained from a local hatchery. On the day of arrival, individual birds were randomly selected and placed in groups of forty. Each group was weighed and distributed randomly to one of 48 floor pens. The pens at the Atlantic Poultry Research Center had 3 to 4 cm of litter made of pine shavings. Before the birds arrived, the room was preheated to 35^{o}C then allowed to settle at 30ºC. Each pen measured 2.13 m x 1.40 m and had one tube feeder and a watering pan with nipple drinkers. Each pen had a 51 x 43x 2.5 cm cardboard box on the floor embedded in the litter with feed in it for one week plus the tube feeder was filled form day 1. All the birds had *ad libitum* accesses to the diets in replicates of six pens per treatment from the day of arrival to 35 days post-hatch. Birds were hand fed daily. The feed given each day was weighed in and weighed back when mortality occurred and at days 14 and 24 and 35. On days 14, 24 and 35, the birds were batch weighed per pen and weights recorded. Mortalities were recorded throughout the trial and, when it happened, the dead birds were weighed and feed weighed back from the trough. All birds that died were examined via postmortem by a veterinary pathologist. The lighting and temperature schedule used in this trial (Appendix B) was measured using a data logger and temperature manually by hand twice daily using a Raytek Mini temp gun. All broilers were managed under the supervision of the Animal Care and Use Committee of Dalhousie University using guidelines provided by the Canadian Council on Animal Care (2009).

8.3.4 Performance data collection

Production performance was measured as body weight gain, feed consumption, feed conversion ratio (FCR) and mortality. At each weigh day, body weight and feed consumed were recorded to obtain data for body weight gain and feed consumption. Both set of data were used to calculate FCR. The mortalities recorded were expressed as percentage of birds entering each growth phase.

8.3.5 Statistical analysis

The performance data collected were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3, (SAS Institute Inc., Cary, NC) (Littell et al. 1996) with day as a repeated factor. The experimental design was completely randomized with a 2 x 4 factorial arrangement with (residual oil level x meal substitution) where meal residual oil content was 12% and17% residual oil and meal substitution was 0, 5, 10 and15%. Model:

Y_{ijk}= μ+ meal residual oil $level_i$ + meal $substitution_j$ + meal residual oil level*meal $substitution_{ij}$ + day_k + meal residual oil level*day_{ik} + meal substitution*day_{jk} + meal residual oil level*meal substitution*day_{ijkl} +ε_{ijkl}

The statistical model of the experiment as shown above where Y is the response variable and μ is the overall mean response for that factor. Meal residual oil $level_{i\ (1\text{-}2)}$ is the effect of meal residual oil at the i^{th} level (1= 12% and 2 =17%). Meal $substitution_{j\ (1\text{-}4)}$ is the effect of meal substitution in diets at the j^{th} level (1= 0%, 2 = 5%, 3= 10% and 4= 15%). Day is the effects of age (day 14, 24 and 35). Meal residual oil level*meal $substitution_{ij}$ is the effects of the interaction at the ij^{th} oil level and substitution. ε_{ijkl} is the residual error of the model with $_l$ replication of six.

If significant main effects or interactions were found ($P \leq 0.05$), Tukey Kramer test (Littell et al, 1996) was used to compare differences among the least square means at ($\alpha \leq 0.05$).

8.4 Results and Discussion

Careful evaluation of the literature revealed a lack of research related to the use of MPYCM in broiler chickens. To the author's knowledge only one such paper was published (Czerwinski et al. 2012) which evaluated the used of cold pressed expelled yellow canola using Ross 308 broilers. This paper evaluated the use of phytase and three lines of yellow seeded canola all at 30% inclusion in a grower diet fed from 0 to 35 days in cages. The birds were feed deprived prior to body weight measurement were taken. Differences in experimental design, methodology and diet formulation prevented the use of this paper as a source of comparison for this study. As such the performance data presented in this study from Czerwinski et al. (2012) is for a point of note and not comparison.

8.4.1 Feed consumption by birds.

Feed consumption of the birds during the starter period (day 0 to14) ranged from 32 to 43 $g \cdot b^{-1} \cdot d^{-1}$. The ANOVA data from Table 8.3 indicated that there were treatment effects of oil and meal substitution level on the feed consumption of the birds from the first day to day 14 of the trial. Birds fed diets with meals containing 17% residual oil consumed significantly less feed than those fed diets with meal having 12% residual oil. As the level of meals in the diets increased, birds fed diets with 15% meal consumed less feed than those given the control and 5% meal starter diets.

TABLE 8.3. Effects of substitution level of mechanically pressed yellow canola meal on feed consumption ($g \cdot b^{-1} \cdot d^{-1}$) for 0-14 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	38±2	36±2	37±1a
5%	43±2	35±2	39±1a
10%	37±2	34±2	36±1ab
15%	33±2	32±2	32±1b
Effects of Oil	38±1a	34±1b	

Source of variation	P>F
Oil	0.0101
Substitution	0.0015
Oil x Substitution	0.1331

[a-b] Mean ± SEM in the same effect group (oil or substitution) with no common letters are significantly different at $\alpha = 0.05$

The feed consumption of the birds during the grower stage (Table 8.4.) ranged from 84 to 93 $g \cdot b^{-1} \cdot d^{-1}$. There were no effects of treatment on the feed consumption of the birds during the grower stage of the trial. However as the birds moved to the finisher stage (Table 8.5.) the feed consumption ranged from 133 to 168 $g \cdot b^{-1} \cdot d^{-1}$. The same treatment effects observed in the starter phase with oil and substitution level occurred in the finisher phase. Table 8.5 indicated that there were treatment effects of oil and meal substitution level on the feed consumption of the birds from day 25 to 35 of the trial. Birds fed diets with meals containing 17% residual oil consumed significantly less feed than those fed diets with meal having 12% residual oil. As the level of meals in the diets increased from 0 to 15%, birds consumed considerably less ($P \leq 0.05$) feed than those given the other three finisher diets. Czerwinski et al. (2012) fed broiler 30% yellow or black canola from day 8 to 35 and the birds consumed 93 to 101 $g \cdot b^{-1} \cdot d^{-1}$ depending on the lines of yellow canola fed.

TABLE 8.4. Effects of substitution level of mechanically pressed yellow canola meal on feed consumption ($g \cdot b^{-1} \cdot d^{-1}$) for 15-24 day old broilers.

Meal level of Substitution	Meal Oil level 12%	17%	Effects of Substitutions
0%	87±5	85±5	86±3
5%	97±5	93±5	95±3
10%	91±5	86±4	89±3
15%	88±5	84±5	86±3
Effects of Oil	91±2	87±2	

Source of variation	P>F
Oil	0.2925
Substitution	0.1960
Oil x Substitution	0.9967

Mean ± SEM

TABLE 8.5. Effects of substitution level of mechanically pressed yellow canola meal on feed consumption ($g \cdot b^{-1} \cdot d^{-1}$) for 25-35 day old broilers.

Meal level of Substitution	Meal Oil level 12%	17%	Effects of Substitutions
0%	163±5	151±6	157±4a
5%	168±5	154±5	161±4a
10%	161±5	142±5	152±4a
15%	141±5	133±5	137±4b
Effects of Oil	158±3a	145±3b	

Source of variation	P>F
Oil	0.0011
Substitution	0.0003
Oil x Substitution	0.7710

[a-b] Mean± SEM in the same effect group (oil or substitution) with no common letters are significantly different at $\alpha = 0.05$

The birds fed yellow canola meal had significantly better feed consumption than those fed black canola. In their study, alkenyl glucosinolate content was the main limiting factor on the feed consumption of the birds fed the black canola. The total glucosinolate content of the meals used in this study is expected to be lower than the 17.1 $\mu mol \cdot g^{-1}$ DM reported for the meal solvent counterpart (Slominski et al. 2012). The total glucosinolates levels in meal should be too low base on the (8-12$\mu g \cdot g^{-1}$ of meal) presently reported as industry measures. It is expected that the levels in our diets would have been below 2 $\mu g \cdot g^{-1}$ of diet which should not result in any obvious effects on the feed consumption of the birds. The higher residual oil should have diluted the glucosinolates levels below the threshold that causes changes in fee consumption. Since the birds consuming diets with 15% meal had lower feed consumption it would be interesting to evaluate the alkenyl glucosinolate content of the diets used as to rule out any correlation of feed intake with the alkenyl glucosinolate fraction of the diets.

There are other anti-nutritional factors such as sinapine which may reduce feed intake in broilers fed mechanically pressed canola meal since the meal used in this study were not exposed to any heat other than those generated from the friction during expelling. Expelled canola contains about 8.2 to 11 $g^{-1} \cdot kg^{-1}$ sinapine which shows a tendency to decline with heat processing (Bell and Shires 1982). Sinapine can react with amino acids and other compounds contributing to a bitter taste of rapeseed meal (Kozlowaka et al. 1990). Evaluation of the sinapine content of the diet would be one way to identify if this was a liming factor to the birds feed consumption.

8.4.2 Body weight of birds.

The body weight gain of the birds (Table 8.6.) during the starter period day 0 to14 ranged from 19 to 21 $g \cdot b^{-1} \cdot d^{-1}$. Table 8.6 indicated that there were treatment effects of oil and meal substitution level on the body weight gain of the birds from 0 to 14 days of the trial. Birds fed diets with meals containing 17% residual oil gained significantly less weight on a daily basis than those fed diets with meal having 12% residual oil. Birds consuming diets with 15% meal gained significantly less weight daily than those given the 0, 5 and 10% meal starter diets.

TABLE 8.6. Effects of substitution level of mechanically pressed yellow canola meal on body weight gain ($g \cdot b^{-1} \cdot d^{-1}$) for 0-14day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	21±1	21±1	21±0a
5%	21±1	19±1	20±0a
10%	22±1	19±1	20±0a
15%	19±1	19±1	19±0b
Effects of Oil	20±0a	19±0b	

Source of variation	P>F
Oil	0.0211
Substitution	0.0009
Oil x Substitution	0.1452

[a-b] Mean± SEM in the same effect group (oil or substitution) with no common letters are significantly different at $\alpha = 0.05$

The body weight gain of the birds during the grower stage (Table 8.7.) ranged from 47 to 57 $g \cdot b^{-1} \cdot d^{-1}$. During the grower stage, birds fed diets with meals containing 17% residual oil gain significantly less weight on a daily basis than those fed diets with meal having 12% residual oil. Birds given diets with 15% meal gained significantly less weight on a daily basis than those given the 0, 5 and 10% meal grower diets. During the finisher stage (Table 8.8) the body weight gains of the birds ranged from 76 to 97$g \cdot b^{-1} \cdot d^{-1}$ and there were no effects of treatment on the body weight gains of the birds.

TABLE 8.7. Effects of substitution level of mechanically pressed yellow canola meal on body weight gain ($g \cdot b^{-1} \cdot d^{-1}$) for 15-24 day old broilers.

Meal level of Substitution	Meal Oil level 12%	Meal Oil level 17%	Effects of Substitutions
0%	52±1	55±1	55±1a
5%	57±1	53±1	55±1a
10%	55±1	52±1	53±1a
15%	49±1	47±1	48±1b
Effects of Oil	53±1a	52±1b	

Source of variation	P>F
Oil	0.0430
Substitution	<.0001
Oil x Substitution	0.0532

[a-b] Mean± SEM in the same effect group (oil or substitution) with no common letters are significantly different at $\alpha = 0.05$

TABLE 8.8. Effects of substitution level of mechanically pressed yellow canola meal on body weight gain ($g \cdot b^{-1} \cdot d^{-1}$) for 25-35 day old broilers.

Meal level of Substitution	Meal Oil level 12%	Meal Oil level 17%	Effects of Substitutions
0%	91±6	94±7	92±4
5%	89±6	91±6	90±4
10%	91±6	86±6	89±4
15%	85±6	76±6	81±4
Effects of Oil	89±3	97±3	

Source of variation	P>F
Oil	0.6314
Substitution	0.2527
Oil x Substitution	0.7092

Mean ± SEM

The 14 and 24 day body weight of the birds (Table 8.9 and Table 8.10., respectively) were influenced by the effects of meal residual oil and meal substitution in the starter and grower diets. The level of meal residual oil and substitution significantly influenced the 14 day body weights which ranged from 310 to 352 $g \cdot b^{-1}$. Birds fed starter diets with meals containing 17% residual oil had significantly lower 14 day body weight than those fed diets with meal having 12% residual oil. As the level of meals in the diets increased, birds consuming diets with 15% meal had the same 14 day body weight as those given 5% meal diets, but significantly lower 14 day body weight than those given the 0 and 10% meal starter diets. The level of meal residual oil and substitution significantly influenced the 24 day body weights which ranged from 775 to 939 $g \cdot b^{-1}$. Birds fed grower diets with meals containing 17% residual oil had significantly lower 24 day body weight than those fed grower diets with meal having 12% residual oil. Birds consuming diets with 15% meal had significantly lower 24 day body weight than those given the 0, 5 and 10% meal grower. Only the effects of meal level (Table 8.11.) affected the 35 days body weight of the bird which ranged from 1616 to 1926g per bird. Birds given diets with 15% meal had significantly lower 35day body weight than those given the 0 and 5% meal grower diets but it was not significantly different from the 10% diet. The effect of meal levels on the body weights of the bird might be an indirect result of the lower feed intake associated with the inclusion level of the meals. Another possibility could be related to the energy to protein ratio of the diets. The calculated analysis of the diets used shows that the CP levels of all the diets were within the required levels for the birds. The energy levels however were not evaluated so it is not know if they were higher than what was formulated for in the 15% meal diets. If this was the case then the birds

would have just consumed enough feed to meet their energy need which would lead to less units of CP been consumed than is needed.

TABLE 8.9. Effects of substitution level of mechanically pressed yellow canola meal on 14 day old broilers' body weight ($g \cdot b^{-1}$)

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	343±7	337±8	340±5a
5%	336±7	322±7	329±5ab
10%	352±7	320±7	336±5a
15%	311±7	310±7	311±5b
Effects of Oil	335±4a	322±4b	

Source of variation	P>F
Oil	0.0148
Substitution	0.0011
Oil x Substitution	0.1874

[a-b] Mean ± SEM in the same effect group (oil or substitution) with no common letters are significantly different at α= 0.05

TABLE 8.10. Effects of substitution level of mechanically pressed yellow canola meal on 24 day old broilers' body weight ($g \cdot b^{-1}$)

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	868±17	889±19	879±13a
5%	907±17	851±17	879±12a
10%	899±17	939±16	869±12a
15%	800±17	775±17	787±12b
Effects of Oil	869±9a	838±9b	

Source of variation	P>F
Oil	0.0166
Substitution	<.0001
Oil x Substitution	0.0895

[a-b] Mean ± SEM in the same effect group (oil or substitution) with no common letters are significantly different at α= 0.05

TABLE 8.11. Effects of substitution level of mechanically pressed yellow canola meal on 35 day old broilers' body weight ($g \cdot b^{-1}$)

Meal level of Substitution	Meal Oil level		
	12%	17%	Effects of Substitutions
0%	1866±66	1926±72	1896±49a
5%	1886±66	1849±66	1868±47a
10%	1901±66	1789±61	1845±45ab
15%	1732±66	1616±66	1674±47b
Effects of Oil	1847±33	1795±33	

Source of variation	P>F
Oil	0.2779
Substitution	0.0080
Oil x Substitution	0.5269

[a-b] Mean ± SEM in the substitution effect group with no common letters are significantly different at α= 0.05

8.4.3 Feed conversion ratio of birds.

The feed conversion ratio (FCR) ANOVA results for the starter (Table 8.12.), grower (Table 8.13.) and finisher (Table 8.14.) period shows that there were no effects of treatment. During the starter period the FCR ranged from 2.1 to 1.7, the grower 1.80 to 1.54 and the finisher period 1.92 to 1.66. The FCR in all treatments including the control seemed relatively high during the start of the experiment. There are many factors which may reduce broiler chicken feed efficiency during production. One possibility may be due to the birds being subjected to lower than normal room temperature (Appendix Y) due to loss of central heat during the first few days in the barn. The birds were grouping together during this period of lower heat which suggested that they were trying to maintain their body temperature so more energy from the food would go into heat production rather than body mass. The lower FCR observed in the grower and finisher

stages compared to the starter stage suggest birds recovered from the diversion of metabolic energy away from growth caused by the low temperature period.

TABLE 8.12. Effects of substitution level of mechanically pressed yellow canola meal on FCR (per bird) for 0-14 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	1.8±0.12	1.8±0.13	1.8±0.09
5%	2.1±0.12	1.8±0.12	2.0±0.08
10%	1.7±0.12	1.8±0.11	1.8±0.08
15%	1.8±0.12	1.8±0.12	1.8±0.09
Effects of Oil	1.8±0.06	1.8±0.06	

Source of variation	P>F
Oil	0.5820
Substitution	0.2370
Oil x Substitution	0.4530

Mean ± SEM

TABLE 8.13. Effects of substitution level of mechanically pressed yellow canola meal on FCR (per bird) for 14-24 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	1.65±0.08	1.54±0.09	1.60±0.06
5%	1.70±0.08	1.75±0.08	1.72±0.06
10%	1.67±0.08	1.70±0.08	1.68±0.06
15%	1.78±0.08	1.80±0.08	1.79±0.06
Effects of Oil	1.70±0.04	1.70±0.04	

Source of variation	P>F
Oil	0.9326
Substitution	0.1280
Oil x Substitution	0.7814

Mean ± SEM

TABLE 8.14. Effects of substitution level of mechanically pressed yellow canola meal on FCR (per bird) for 25-35 day old broilers.

Meal level of Substitution	Meal Oil level		Effects of Substitutions
	12%	17%	
0%	1.82±0.11	1.66±0.12	1.74±0.08
5%	1.92±0.11	1.73±0.11	1.83±0.08
10%	1.80±0.11	1.69±0.10	1.74±0.08
15%	1.70±0.11	1.73±0.11	1.72±0.08
Effects of Oil	1.81±0.06	1.70±0.06	
Source of variation		P>F	
Oil		0.1673	
Substitution		0.5081	
Oil x Substitution		0.7549	

*Mean ± SEM

8.4.4 Bird mortality

The mortality (Table 8.15) for the trial was 2% starter phase, 3% grower phase and 2.8% finisher phase. The ANOVA result suggested that there were effects of treatments on mortality in the starter and grower phase but not the finisher phase. During the starter phase the highest mortalities were seen in the 17% residual oil meal fed at 10% of the diet. If the mortalities were treatment related birds fed the 15% meal would have higher mortalities than lower inclusion levels. In this case the 17% residual oil meal when fed at 10% in the diets had significantly higher mortalities than at 5% inclusion. All the other treatments including those given no meal and 15% of the 17% residual oil meal were not significantly different from the 17% residual oil meal fed at 10%. This is a clear indication that the effects of treatment reported by the ANOVA were not treatment related.

During the grower stage the ANOVA indicated that meal oil levels had an effect on mortality. The birds fed 17% oil meals had significantly higher mortalities than those fed 12% oil meal birds. The ANOVA did not detect any effects of treatment on mortalities in the finisher period. The ANOVA also suggested that from day 0 to 35, meal oil levels and meal inclusion levels significantly affected the mortalities of the birds.

The ANOVA is only capable of tell if a main effect influences a response base on the location of a response in relation to a treatment. It cannot tell what caused that main effect to affect the response variable and as such could not tell what caused the mortalities within the grower and starter phase. Production records indicated that of the 157 mortalities 78 were culled as a result of acute leg problems during the trial. Those 78 culled birds represented 50% of the total mortalities during the trial. During the starter phase 25 of the birds were culled because of leg problem, 42 in the grower phase and 11 in the finisher phase. The ANOVA was not able to give this information.

Postmortems reveled that the leg problems were related to rickets disease. The calcium and phosphorous analysis of the diets suggested that calcium and phosphorous were adequate in the diets and they were in the correct ratio. The postmortems of the other mortalities revealed that during the starter period birds died as a result of a combination of omphalitis, bacterial pericarditis and coliform septicemia. During the grower period other mortalities occurring were mostly related to ascites and septicemia, while during the finisher period they were mostly related to flip over's and ascites. Using the production records and the postmortem reports data it is safe to say that the effects o treatment seen in the mortality ANOVA were not treatment related.

TABLE 8.15. Effects of substitution level of mechanically pressed yellow canola meal on starter, grower and overall % mortality broilers.

0-14 days % mortality		
Effects of Oil*level	**12% Oil meal**	**17% Oil meal**
0% meal	0.03±0.01ab	0.03±0.01ab
5% meal	0.04±0.01ab	0.01±0.01b
10% meal	0.02±0.01b	0.11±0.01a
15% meal	0.08±0.01ab	0.04±0.01ab
15-24 days % mortality		
Effects of Oil	0.05±0.01b	0.09±0.01a
0-35 days % mortality		
Effects of level		
0% meal	0.06±0.01ab	
5% meal	0.03±0.01b	
10% meal	0.06±0.01ab	
15% meal	0.08±0.01a	
Effects of Oil	0.04±0.01b	0.07±0.01a

ANOVA P-Value

Effect	Starter	Grower	Finisher	From 0-35 days
Oil	0.6916	0.0128	0.4656	0.0374
Level	0.1365	0.0794	0.2114	0.0089
Oil*Level	0.0090	0.0614	0.1173	0.6250

Mean ± SEM in the same effects group with common letters are not significantly different at α =0.05

8.5 Conclusion

The level of residual oil and the rate of inclusion of mechanically pressed yellow *Brassica napus* in the diets influenced the growth, feed consumption and but not the FCR of broiler chickens. It is recommended that MPYCM with 12% residual oil should be included in broiler chickens diets at 10% during the starter and finisher periods. MPYCM with 12% residual oil can be included at levels up to 15% of the diet during the grower period. MPYCM with 17% residual should only be included up to 10% in the grower and finisher diet.

CHAPTER 9: INTEGRATED SUMMARY AND CONCLUSION

9.1 Summaries

The data from this study shows that dry air oven heating of mechanically pressed canola meal significantly reduced the available nutrients to broilers. The effects of variable residual oil levels found in mechanically pressed canola meal may influence some nutrient availability. There is a positive link between higher canola meal residual oil and increased energy from those meals. Standardizing crude protein and amino acid digestibility values provides a better estimation of the use of these nutrients by broilers. The dynamics between residual oil and heat treatment of canola meal need to be taken into consideration during meal production because it may influence the nutrient availability of those meals. The application of heat reduced the AMEn and the SIAAD in MPBCM. It was evident that black meals with higher oil level gave higher AMEn values (chapter 4). There was no specific influence of meal oil levels on the SIAAD of MPBCM (chapter 4).

There were no added benefits in the AMEn of mechanically pressed black canola from using any of the enzymes with the meals (chapter 5). Each amino acid may react differently when influenced by different dietary factors such as enzymes and meal residual oil (chapter 5). A reduction in TRP and HIS digestibility with respect to increasing level of meal residual oil levels was observed (chapter4) but only for TRP in chapter 4. The low digestibility at higher meal residual oil level is an indication that there might be a limitation in the young bird's ability to effectively digest and absorb amino acids when the canola meal residual oil level is high. One possible explanation could be the limited lipase activity in young chick's digestive tracts (Kermanshahi 1998), since the addition of exogenous enzyme alleviated this problem in meals with high residual oil.

The mode of action through which the exogenous enzymes improved the amino acid digestion in the high oil meal was not established. The exogenous enzymes may have stimulated the secretion of bile products in the jejunum and the release of more co-protease enzyme in the duodenum which increased the rate of fat micelle formation which consequently increased amino acid absorption (Simbaya et al. 1996, Kermanshahi 1998). The exogenous supplementation of enzymes had their greatest positive effects in diets containing meal with higher residual oil. The practical benefits of those enzymes may only be applicable to diets having meals with poor nutrient digestibility due to high residual oil. It would be interesting to see the relationship of fat digestibility of the high residual oil meal compared to the low residual oil meal. It would also be interesting to see what effects exogenous enzymes would have on that relationship. Future research should focus on identifying the relationship between fat digestibility in low and high residual oil meals since mechanically pressed meals are known to vary in residual oil content. The role that fat might have on the digestibility of other nutrients in mechanically pressed meals should also be examined. Perhaps combination of enzymes maybe synergistic a factor not tested in this study.

The dry air oven heating of mechanically pressed yellow *Brassica napus* meals (chapter 6), the level of the residual oil after pressing and the use of exogenous dietary enzymes three-way and two-way interactions plus just the main effects affected the digestibility of nutrient in the mechanically pressed yellow meals. The complexity of the interactions on the nutrient digestibility varies from nutrient to nutrient. It was difficult to identify any specific trends in the interactions that could fit all the nutrients evaluated in this study. Each amino acid responded differently to the treatments and there were no

specific trends across all amino acid digestibility coefficients. It was clear from the data that most amino acids were influenced by the two-way interaction between the heat treatments and enzymes while the AMEn was influence by three-way interactions.

The addition of carbohydrase to the 14% residual oil meal that was not heat treated improved the AMEn of that meal. The added benefits gained from the use of the carbohydrase are of practical importance when feeding MPYCM to chickens. The effect of the carbohydrase was not seen in chapter 5 probably due to the level of substrate available from the meals. Yellow *Brassica napus* is known to have more xylose, sucrose, arabinose and galactose than black seeded *Brassica napus* which may have provided more substrate for the xylanase amylase enzyme mix.

There were no effects of treatment on MET digestibility. HIS, ARG, PHE, GLY, ALA, ASP, SER and GLU digestibility were all influenced by the two-way interaction of the heat treatment of the meals and the enzymes addition in the diets. THR digestibility on the other hand was influence by the two-way interaction of the heat and enzyme treatment and the two-way interaction of the enzyme and oil treatments. LEU digestibility was influenced by the oil level of the meals and the two-way interaction between the heat and enzyme treatments. CYS digestibility was only influenced by the two-way interaction of oil levels of the meal and enzyme supplementation. TYR digestibility was affected by the two-way interaction of oil and heat and by the two-way interaction of enzyme and heat. Only TRP digestibility was significantly affected by the three-way interaction of meal residual oil, heat and enzyme. Since there were unique reactions among the amino acids to the treatments, actual differences due to the factors tested should be taken into account when similar processing and dietary enzyme

supplementation for mechanically pressed yellow seed meals is used in broiler chicken diets.

The birds were given MPBCM (chapter 7) with 12 and 17% residual oil at graded levels of 0, 5, 10 and 15% of the diets. There was no significant difference in feed consumption among the 0% meal diet and substituted diets at the starter, grower and finisher phase. It should be noted however, that there was a marginal effects of meal substitution on feed consumption during the grower phase. During the starter and grower period there was no statistically significant difference between any of the groups' body weight regardless of diets however a marginal effect of oil was seen during the starter period.

The 15% meal birds produced less weight than the 0% and 5% meal birds during the finisher phase. Birds given the substituted diets had the same FCR ratio as the 0% meal diets in all phase of production. The oil levels of the meal did not influence the bird's production performance. Mechanically pressed *Brassica Napus* canola meal with 12 and 17% residual oil can be feed up 15% in the starter and grower diets without any adverse effects on body weight gain, feed conversion and final body weight. Consideration must be given when using 15% MPBCM in finisher period which would base on the desired 35 days body weights needed.

The level of residual oil and the level of inclusion of mechanically pressed yellow *Brassica napus* (chapter 8) in the diets influenced the growth, feed consumption and mortalities of broiler chickens. Birds fed meals with lower residual oil generally consumed more feed during the starter and finisher period but not in the grower period. Having 15% meals in the diet reduced the feed consumed during the starter and finisher

period but not in the grower period. The highest residual oil and the 15% inclusion level of the meal both reduced the body weight gain during the starter and grower period but not the finisher period. This resulted in lower final body weight of the 15% inclusion diet birds in all three growth phases but the residual oil level of the meal did not influence the bird 35 day final body weights. There were no effects on meal inclusion levels or residual oil on the FCR of the birds. Both the level of residual oil and the rate of inclusion of mechanically pressed yellow *Brassica napus* in the diets of broiler should be taken into consideration during each phase of production since the effects were not all the same.

9.2 Conclusions

The data from this study shows that dry air oven heating of mechanically pressed canola meal significantly reduced the AMEn and the SIAAD in MPBCM. The meals with 14% residual oil gave higher AMEn than 10% residual oil meal and variations in oil levels did not influence the standardized ileal amino acid digestibility of mechanically pressed meals black canola meal. When enzymes were added to the black meal the greatest positive effects on the SIAAD were observed in meal with higher residual oil but no benefits to AMEn. The enzymes should be fed in diets with high residual oil meals since they were able to increase the amino acid digestibility of those meals.

Not heating the meals plus the addition of carbohydrase increased the AMEn values of mechanically pressed yellow *Brassica napus.* The addition of lipase generally improved the standardized ileal amino acid digestibility of yellow *Brassica napus.* To maintain or improve the AMEn and SIAAD of MPYCM the addition of lipase and carbohydrase without heating the meal is recommended

Birds given mechanically pressed black *Brassica napus* canola meal performed equally well as those given no canola meal as it related to feed consumption and FCR. Feeding 15% MPBCM in finisher period gave the lowest body weights. Meal residual oil cont did not influence performance. MPBCM with 12 and 17% residual oil can be feed up 15% in the starter and grower diets but a maximum of 10% is recommended for the finisher period. The level of residual oil and the rate of inclusion of MPYCM in the diets influenced the growth, feed consumption and mortalities of broiler chickens. It is recommended that meals with 12% residual oil should be included in broiler chickens diets at 10% during the starter and finisher periods but 15% can be used in the grower period. MPYCM with 17% residual should only be included up to 10% in the finisher diet and grower period.

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APPENDIX A. NUTRIENT COMPOSITION OF YELLOW AND BLACK CANOLA MEAL USED IN GROWTH STUDIES CHAPTER 7 AND 8.

Ingredient composition as fed	12% Oil black canola meal	17% Oil black canola meal	12% Oil yellow canola meal	17% Oil yellow canola meal
Dry matter (%)	90.00	90.00	91.00	93.00
Crude Protein (%)	31.00	29.00	31.00	30.00
Calcium (%)	0.50	0.48	0.53	0.55
Phosphorus (%)	1.13	1.07	1.20	1.20
Sodium (%)	0.02	0.02	0.01	0.01
Potassium (%)	1.43	1.33	1.50	1.47
Magnesium (%)	0.44	0.42	0.47	0.47
Crude Fat (%)	12.29	17.00	12.53	17.14

APPENDIX B. LIGHTING AND TEMPERATURE SCHEDULES FOR BROILER CHICKENS DURING THE MECHANICALLY PRESSED CANOLA GROWTH TRIALS.

Days post hatch	Temperature (°C)		Light Hours	Light Intensity (lux)
	Black meal trial	Yellow meal trial		
1-2	31	27	24	20
3-4	29	27	23	20
5-6	28	27	16	20
7-8	28	29	16	15
9	27	29	16	15
10-11	27	28	16	10
12-13	27	28	16	10
14-16	26	27	16	10
17-18	25	26	16	10
19-20	24	25	16	10
21-23	23	24	16	10
24-27	23	23	16	10
28	22	22	16	10
29	22	22	16	10
30-32	22	22	16	10
33	22	22	17	10
34-35	22	22	18	10