Nutritive Evaluation of Mechanically-Pressed Camelina (*Camelina sativa*), Carinata (*Brassica carinata*) and Soybean (*Glycine max*) Meals for Broiler Chickens

By

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ABSTRACT

Three digestibility experiments were conducted to evaluate the nutritive value of mechanically-pressed camelina (MPCM), carinata (MPCARIM) and soybean (MPSBM) meals. Growth trials were conducted to determine effects of camelina and soybean meals on broiler performances. The 11.5% residual oil MPCM had higher nitrogen-corrected apparent metabolizable energy (AME_n) than 14.5% oil MPCM. Carbohydrase improved AME_n of MPCM. Heated MPCM showed a lower AME_n than non-heated MPCM. Lipase and no-heat gave highest AME_n for 7% oil MPSBM. Carbohydrase and heat had highest AME_n in 11% oil MPSBM. Carbohydrase and wet-heat gave highest AME_n for 12.5% oil MPCARIM. Lipase and wet-heat showed highest AME_n for 16.5% oil MPCARIM. Heat did not alter standardized ileal amino acid digestibility (SIAAD) of MPCM, improved SIAAD in MPSBM. Oil levels affected some SIAAD and enzymes improved SIAAD of MPCM and MPSBM. MPCM and MPSBM can be incorporated up to 10% in starter, grower and finisher broiler diets.

Key words: amino acids, camelina, carinata, enzyme, growth, heat, mechanically-pressed meals, apparent metabolizable energy, soybean, standardized ileal available amino acids

LIST OF ABBREVIATIONS USED

AIAAD	Apparent ileal amino acid digestibility
AICC	Corrected akaike information criterion
AME_n	Nitrogen-corrected apparent metabolizable energy
ANOVA	Analysis of variance
atm	Standard atomosphere
BIC	Bayesian information criterion
BW	Body weight
BWG	Body weight gain
°C	Celsius degrees
cm	Centimeters
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EAAD	Excreta amino acid digestibility
EE-SBM	Expeller-extruded
FC	Feed consumption
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
g	Gram
$g \cdot bird^{-1} \cdot day^{-1}$	Gram per bird per day
$g \cdot kg^{-1}$	Gram per kilogram
$g \cdot tonne^{-1}$	Gram per tonne

h	Hour
IAAD	Ileal amino acid digestibility
IDAA	Ileal digestible amino acids
IEAAF	Ileal endogenous amino acid flow
$IU \cdot kg^{-1}$	International Unit per kilogram
J	Joule
kg	Kilogram
$kcal \cdot kg^{-1}$	Kilocalorie per kilogram
Lux	International System of Unit of illuminance
mg	Milligram
$mg \cdot g^{-1}$	Milligram per gram
$mg \cdot kg^{-1}$	Milligram per kilogram
min	Minutes
mL	Milliliters
MPCARIM	Mechanically-pressed carinata meal
MPCM	Mechanically-pressed camelina meal
MPSBM	Mechanically-pressed soybean meal
N	Nitrogen
Na	Sodium
NC	Nitrogen correction
NH_3	Ammonia
NSP	Non-starch polysaccharide
psi	Pounds per square inch

S	Second
SAS	Statistical analysis system
SBE	Extruded full-fat soybeans
SBR	Roasted soybeans
SE	Standard error
SEM	Standard error mean
SE-SBM	Solvent-extracted soybean meal
SIAAD	Standardized ileal amino acid digestibility
$TIU \cdot g^{-1}$	Trypsin inhibitor units per gram
$TIU \cdot mg^{-1}$	Trypsin inhibitor units per milligram
TME_n	Nitrogen-corrected true metabolizable energy
W/V	Weight/volume

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CHAPTER 1: INTRODUCTION

The global bio-fuel production in 2000 was 16 billion litres and it increased to more than 100 billion litres in 2010 (International Energy Agency 2011). In 2010, the global demand for bio-fuel was around 2.5 x 10^{18} J and it has been projected to reach 32 x 10^{18} J by 2050 (International Energy Agency 2011). As demand for bio-fuel is rapidly expanding throughout the world (International Energy Agency 2011), there is a high demand for corn and oilseed crops (soybean, canola, palm and sunflower) (International Energy Agency 2011) for biofuel production. Therefore, there is a growing interest to find alternative oilseed crops in order to meet the energy demand in the world. When there is high oil content in the seed, it can be considered as a potential source of bio-fuel. *Camelina sativa* (Zubr 1997) and *Brassica carinata* (Getinet et al. 1995) oilseeds were found to contain high oil content when compared to soybean seeds (Nelson et al. 1987). Therefore, *Camelina sativa* and *Brassica carinata* oilseeds can be considered as alternative sources of biofuels. Currently, there is a renewed interest in *Camelina sativa* now as a source of bio-fuel (Bernardo et al. 2003, Moser and Vaughn 2010). *Brassica carinata* is grown for biofuels. When biofuel production is carried-out on a small-scale, the oil is extracted from camelina, carinata and soybean seeds by mechanical pressing. The resultant by-products of mechanical oil extraction process are mechanically-pressed camelina, carinata and soybean meals. Nowadays, there is a consumer demand for mechanically-pressed soybean and camelina oils. However, carinata oil is not popular yet. The resultant by-products of this process are the mechanically-pressed camelina and soybean meals.

The high residual oil left in mechanically-pressed camelina (Ryhanen et al. 2007 and Almeida et al. 2013) and soybean (Powell et al. 2011 and Opapeju et al. 2006) meals,

suggests that these meals can be used as energy supplements for broiler chickens. Moreover, the high CP content in mechanically-pressed camelina (Ryhanen et al. 2007, Pekel et al. 2009 and Almeida et al. 2013), soybean (Powell et al. 2011 and Opapeju et al. 2006) and carinata (Getinet et al. 1995) meals showed that these mechanically-pressed meals can also be used as protein supplements for broiler chickens.

However, when new meal ingredients are introduced to the broiler feed industry it is necessary to look at the anti-nutritional factors that hinder the use of these meals in diets. The anti-nutritional factors, trypsin inhibitors (Birk and Gertler 1961, Leiner and Tomlinson 1981) and lectins (Maenz et al. 1999, Fasina et al. 2003) present in soybean meal can be inactivated by heat treatment (Nelson et al. 1987, Fasina et al. 2003). Camelina and carinata meals were found to contain glucosinolates as anti-nutritional factors which can be degraded by heat treatment (Jensen et al. 1995) or water treatment (Tyagi 2002). However, during water or heat treatment the nutritive value of meals may change. Therefore, it will be useful to determine the effect of heat treatment on the nutritive value of mechanically-pressed meals. According to the literature, the residual oil content in a particular mechanically-pressed meal varied from one research report to another. This might be due to the differences in the processing conditions which were used to extract oil from seeds. For example, the residual oil content of mechanically-pressed camelina meal was found to be 13.6% (Pekel et al. 2009), 17% (Ryhanen et al. 2007) or 11 % (Almeida et al. 2013) on an as-fed basis. Moreover, the residual oil content of mechanically-pressed soybean meal was 8.1% (Powell et al. 2011) or 9.5% (Opapeju et al. 2006) on an as-fed basis. The nutritive value of mechanically-pressed meal may be affected due to the varying residual oil content in the meal. The effect of residual oil level on the nutritive value of

mechanically-pressed meals has not been described. Meng and Slominski (2005) and Zanella et al. (1999) found that enzyme supplementation improved the nutrient digestibility of diets. The effect of different enzymes like carbohydrase, lipase or protease on the nutritive value of mechanically-pressed meals have not been identified.

Although mechanically-pressed camelina, carinata and soybean meals were found to be good sources of CP and energy, it is not known how well these meals are utilized by broiler chickens. Therefore, the nutritive value of these meals must be determined by means of nitrogen-corrected apparent metabolizable energy (AME_n) and standardized ileal amino acid digestibility (SIAAD). This research investigated the effects of residual oil levels, heat and enzyme treatments on nutritive value of mechanically-pressed camelina, carinata and soybean meals, using broiler chickens. Subsequently, the production performances of broiler chickens fed graded levels of mechanically-pressed camelina and soybean meals were determined using digestible nutrient information derived from the digestibility studies.

CHAPTER 2: LITERATURE REVIEW

2.1 *Camelina sativa* (L.) Crantz.

Camelina sativa (L.) Crantz known as "false flax" or "gold of pleasure" is an oilseed crop which belongs to the family Brassicaceae, commonly known as the mustard family. According to Zubr (1997), camelina seeds from winter and summer varieties contained 40% - 43% and 44% - 47% of oil on a DM basis, respectively. As a result of this high oil content, *Camelina sativa* has been considered as a crop potentially used as a source of biofuel. The increased interest in *Camelina sativa* is further extended as the camelina crop is recognized as a minimal input crop where the demand for N is moderate to low (Zubr 1997). In growing *Camelina sativa*, the use of insecticides and pesticides is very limited as the camelina plant is not usually attacked by common insects and pests (Zubr 1997). Camelina is considered as an environmentally friendly crop that causes low environmental impact under extensive cultivation. The renewed interest in *Camelina sativa* is further extended because of the promising characteristics of the oil in the seed, mainly due to the essential fatty acids, especially the omega-3 fatty acid (alpha-linolenic acid) present in camelina oil (Vollmann et al. 2007). Out of the total fatty acids in camelina oil, the alpha-linolenic acid content was found to be in the range of 29 - 35%. The oleic, linoleic and eicosenoic fatty acids contribution to the total fatty acids were 16.5%, 17.7% and 15.6%, respectively (Vollmann et al. 2007). After extracting oil from camelina seeds, the resultant by-product is the camelina meal which has different CP and residual oil levels. The residual oil, CP and essential ω-3 and ω-6 fatty acid contents of camelina meal suggest that the meal can be used as an alternative source of protein and energy supplement in poultry diets (Cherian et al. 2009, Aziza et al. 2010). By considering the potential food and non-food

applications of *Camelina sativa*, there may be increasing interest in this crop in the future, making *Camelina sativa* the next important oilseed crop in the world.

2.1.1 Processing of camelina seeds.

The oil is extracted from oilseeds by solvent extraction or through mechanical pressing (Giampietro et al. 1997) or a combination of these processes. After harvesting camelina plants, they are threshed. Then, the seeds are cleaned and dried (Rode 2002). In *Camelina sativa*, mechanical pressing is the main method of processing the seeds which can be conducted in different ways. One way is by pressing camelina seeds as a toasted paste in a mechanical-press (Rode 2002). In this method, the seeds are mixed with an equal volume of water which will give the mixture a pasty appearance. Then, the paste is roasted at 60 - 90 °C. After roasting, the mixture becomes "sandy" in nature. Then, the roasted paste is placed in a mechanical-press and the oil is extracted. Finally, the oil is filtered to clarify (Rode 2002). Alternatively, the oil from camelina seeds can be extracted in an expeller-press without adding water. After cleaning the seeds, they are directly pressed in an expeller-press. The pressure and heat generated inside the expeller-press, easily expels the oil from the seeds. Either of these processes produces camelina oil and oilseed cake. Finally, camelina oilseed cake is hammer-milled to make camelina meal which is of interest as an animal feed. The camelina meal for the current experiments was produced by pressing camelina seeds in a single expeller-press. When the oil from camelina seeds is solvent extracted, it can be processed as described by Mustakas et al. (1981) and Serrato (1981).

2.1.2 Anti-nutritional factors of camelina meal (Glucosinolates).

Glucosinolates are considered as secondary plant metabolites present in *Camelina sativa* seeds. Schuster and Friedt (1998), conducted an experiment to determine the total glucosinolate content of camelina seeds, using 278 camelina genotypes. The three types of glucosinolates found in camelina seeds were 10-methyl-sulfinyl-decyl-glucosinolate (glucocamelinin), 11-methyl-sulfinylundecyl-glucosinolate and 9-methyl-sulfinylnonyl-glucosinolate (Schuster and Friedt 1998). The total glucosinolate content in camelina seed was found to be in the range of 13.2 to 36.2 µmol/g on a DM basis (Schuster and Friedt 1998). After extracting oil from camelina seeds, the residue is camelina expeller cake. The camelina cake contains glucosinolates. According to Matthaus and Zubr (2000), total glusosinolate content of camelina cake ranged from 14.5 to 23.4 µmol/g, with 10-methyl-sulfinyl-decyl-glucosinolate constituting the major component (62 - 72%). However, according to Almeida et al. (2013), 10-methy-lsulfinyl-decyl-glucosinolate constituted 61% of total glucosinolates. The other quantitatively important glucosinolates of camelina expeller cake were 11-methyl-sulfinylundecyl-glucosinolate and 9-methylsulfinylnonyl-glucosinolate (Almeida et al. 2013). According to Ryhanen et al. (2007) and Almeida et al. (2013), the glucosinolate content of camelina expeller cake was 22.9 and 42.3 µmol/g, respectively. Glucosinolates themselves do not cause any negative effects on animals. There is an enzyme called thioglucosidase, which is commonly known as myrosinase, in plant parts which contain glucosinolates (Bones 1990). Myrosinases hydrolyze glucosinolates and produce toxic compounds such as nitriles, thiocyanates and isothiocyanates (Bones and Rossiter 1996). However, this reaction takes place in a damaged plant or during ingestion of plant parts by animals. When camelina seeds are

mechanically-pressed, the seeds are ruptured. Hence, myrosinases can become activated and produce toxic compounds, which remain in the meal. Upon the ingestion of meal by birds, these toxic compounds can further be produced. Myrosinases can also be produced by gut bacteria (Tani et al. 1974). Therefore, when the meal is passing through the digestive tract, the glucosinolates can be hydrolyzed into toxic compounds. These toxic compounds may exert negative effects on the growth performance of birds. Pearson et al. (1983) observed significant reduction in body weight ($P = <0.001$), increase in liver weight ($P = <0.001$) and thyroid hypertrophy in 8-week-old broiler chickens fed diets containing rapeseed meal (500 g/kg of diet) at high concentration of glucosinolates (33 g/kg of rapeseed meal). McNeill et al. (2004) observed a reduction ($P<0.05$) in total feed intake and body weight gain in broiler chickens (7 weeks of age) fed rapeseed meal (100 g/kg of diet) with low glucosinolate content. However, FCE was not influenced ($P>0.05$). The results of McNeill et al. (2004) illustrate that even low glucosinolate levels are high enough to cause significant negative impact on feed intake and weight gain of birds. However, equal FCE suggests that glucosinolates might not have significantly altered the nutrient digestion and absorption in the birds.

2.1.3 Methods to reduce glucosinolate content of camelina meal.

According to Jensen et al. (1995), heat treatment reduced the total glucosinolate content of rapeseed meal. Even though the glucosinolate content was reduced by 95% by heating the meal at 100 °C for 120 min, the initial protein solubility and initial lysine content of the meal decreased from 85% to 40% and 5.93 to 4.91 g per 16 g N, respectively. It was found that there was a 46% reduction in glucosinolate content when the meal was heated at 100 °C for 30 min. Moreover, at this time-temperature combination, the initial protein solubility

and initial lysine content of the meal decreased from 85% to 61% and 5.93 to 5.72 g per 16 g N, respectively. By looking at the reduction in protein solubility, lysine content, true digestibility of protein, biological value of protein and net protein utilization values with increasing heating time, it was recommended to heat the meal at 100 °C for 30 min. This is the optimum processing condition that reduces the glucosinolate content of rapeseed meal by approximately 50%, with a minimum compromise in protein solubility, lysine content, true-digestibility of protein, biological value of protein and net protein utilization values. As rapeseed and camelina crops belong to the same family, Brassicaceace, it may be possible to heat camelina meal at 100 °C for 30 min to reduce the glucosinolates before feeding to poultry. Another method of reducing glucosinolates in Brassica seed meals is water soaking (Tyagi 2002). Therefore, water soaking can be used to reduce the glucosinolates in camelina meal.

2.1.4 Chemical composition of mechanically-pressed camelina meal.

According to Ryhanen et al. (2007) camelina expeller cake contained 35.6% of CP on DM basis. However, Pekel et al. (2009) and Almeida et al. (2013) found that the CP content of MPCM was 38% on a DM basis. Camelina meal contains essential and non-essential amino acids (Table 2.1). Among the essential amino acids, arginine was found to be the highest (Pekel et al. 2009, Almeida et al. 2013) in MPCM whereas methionine (Pekel et al. 2009) and tryptophan (Almeida et al. 2013) were the lowest. Among the non-essential amino acids (Table 2.1), glutamine content was considerably higher when compared to other non-essential amino acids. All the amino acid contents determined by Pekel et al. (2009) and Almeida et al. (2013), were quite similar.

Table 2.1 Amino acid composition of mechanically-pressed camelina meal (as-fed basis).

Amino acid	% (*)	% (**)
Essential amino acids		
Methionine	0.59	0.63
Cysteine	0.74	0.83
Lysine	1.59	1.64
Threonine	1.34	1.40
Leucine	2.20	2.25
Isoleucine	1.25	1.26
Phenylalanine	1.44	1.43
Valine	1.75	1.74
Histidine	0.83	0.84
Arginine	2.86	2.91
Tryptophan	-	0.43
Nonessential amino acids		
Glutamine	5.74	5.82
Proline	1.77	1.87
Serine	1.51	1.58
Glycine	1.77	1.81
Asparagine	2.83	2.79
Alanine	1.52	1.61

Source: *Pekel et al. (2009); **Almeida et al. (2013), camelina crop variety: Blane Creek

When oil was extracted from camelina seeds by mechanical means, a considerable amount of oil was left in the meal. The residual oil content of MPCM was found to be 13.6% on an as-fed basis (Pekel et al. 2009). According to Ryhanen et al. (2007) and Almeida et al. (2013), the residual oil content was 17% and 11% respectively, on an as-fed basis. Therefore, the oil content in the meal can range from 11% to 17% on an as-fed basis, under practical oil extraction conditions. Camelina meal was found to be a good source of essential fatty acids (Cherian et al. 2009). According to Cherian et al. (2009), alpha-linolenic, eicosenoic, oleic and linoleic fatty acids. Out of the total fatty acid content in camelina meal, alpha-linolenic and linoleic acid contents were found to be 29.6 and 23.4%, respectively. According to Aziza et al. (2010), camelina meal contained 29.4% alpha-linolenic acid and 24.4% linoleic acid. Both Cherian et al. (2009) and Aziza et al. (2010)

observed similar values for alpha-linolenic acid and linoleic acid for camelina meal. These two essential fatty acids constituted more than 50% of total fatty acids in camelina meal. By looking at the CP content, residual oil content and fatty acid profile of camelina meal, camelina meal can potentially be used as a good protein and energy source for poultry.

2.1.5 Standardized ileal amino acid digestibility coefficients of camelina meal

The reported high values for CP (35.6 - 38%) (Ryhanen et al. 2007, Pekel et al. 2009 and Almeida et al. 2013) in MPCM, suggest the potential use of the meal as a protein supplement in broiler diets. However, it is important to elucidate the digestibility of meal protein and amino acids for broiler chickens. This will indicate to what extent, the meal protein and amino acids are digestible at the distal ileum of broiler chickens. As camelina meal is a new ingredient being introduced to the poultry feed industry, there was no information in the literature on SIAAD and true-amino acid digestibility coefficients determined using broiler chickens. However, camelina meal has been incorporated in broiler diets (Ryhanen et al. 2007, Pekel et al. 2009 and Aziza et al. 2010) in the past, to evaluate the production performance of broiler chickens. Although there is no information on SIAAD of MPCM in broiler chickens, research conducted by Almeida et al. (2013) using growing pigs, to determine the SIAAD coefficients (Table 2.2), indicated a range in SIAAD from 62 - 87% among the essential amino acids.

Among the essential amino acids, the highest SIAAD was recorded in arginine whereas the lowest was in cysteine (Table 2.2). The reported high values for SIAAD for methionine (84%) and lysine (72%) illustrated that these amino acids are substantially digestible in growing pigs (Almeida et al. 2013). When the non-essential amino acids were considered, the highest SIAAD was found to be for glutamine whereas the lowest was noted for serine.

When SIAAD for both essential and non-essential amino acids were considered, the camelina expeller produced meal could be considered as a good blend of digestible amino acids for growing pigs. To be applicable to broilers, this needs to be verified by determining the digestibility in broiler chickens.

Table 2.2 Standardized ileal amino acid digestibility of camellina* expeller cake in growing pigs.

Amino acid	% (*)
Essential amino acids	
Methionine	84
Cysteine	62
Lysine	72
Threonine	64
Leucine	77
Isoleucine	73
Phenylalanine	76
Valine	74
Histidine	80
Arginine	87
Tryptophan	67
Non-essential amino acids	
Glutamine	81
Proline	79
Serine	64
Glycine	68
Asparagine	73
Alanine	71

Source: Almeida et al. 2013, *camelina crop variety: Blane Creek

2.1.6 Nitrogen-corrected apparent metabolizable energy (AME_n) or true metabolizable energy (TME_n) of camelina meal in broiler chickens.

There were no values for growing chickens for AME_n or TME_n of either solvent-extracted or mechanically-pressed camelina meal found in the literature. However, Acamovic et al. (1999) conducted an experiment to determine the AME_n of camelina meal using adult broiler chickens weighing 3.2 kg. The ether-extract value of camelina meal was 14.5% on a DM basis. The birds were fed 50 g of camelina meal by the precision method (Sibbald

1976). The AME_n was determined using excreta and it was found to be 1836 $kcal \cdot kg^{-1}$, on a DM basis. Acamovic et al. (1999) have suggested that birds could not utilize the meal well because of the negative effects of non-starch polysaccharides and glucosinolates found in the camelina meal. The presence of glucosinolates could be verified by authors from the strong smell of isothiocyanate, experienced during excreta collection (Acamovic et al. 1999).

2.1.7 Effect of heat treatment and enzyme supplementation on nutritive value of camelina meal

The effect of heat treatment and enzyme supplementation on AME_n, TME_n, SIAAD and true-amino acid digestibility of mechanically-pressed or solvent-extracted *Camelina sativa* meal in broiler chickens, have not been elucidated previously. Moreover, production performance trials conducted in the past, did not incorporate enzymes in camelina meal containing broiler diets (Ryhanen et al. 2007, Pekel et al. 2009).

2.1.8 Effect of feeding camelina meal on production performance of broilers

Experiments have been conducted in the past to determine the effect of inclusion of graded levels of MPCM on production performance of broiler chickens. In an experiment, Pekel et al. (2009) incorporated camelina meal, which contained 13.6% crude fat (as-fed basis) at 10% inclusion, in a corn-soybean meal based diet. The diet was isonitrogenous and isocaloric and formulated to meet or exceed the NRC (1994) nutrient requirements for broiler chickens from 0 - 3 weeks of age. Ten percent inclusion of camelina meal reduced ($P<0.05$) the body weight (BW) at Day 21 and feed intake during the first 21 days in broiler chickens (Cobb x Avian-48) compared to the control birds. However, there was no difference ($P>0.05$) between the FCR of birds fed camelina meal and control diets. With

equal FCR, camelina meal can be incorporated into broiler diets at 10% inclusion, with no additional feed needed to grow birds to market weight. In the past, feeding the camelina meal impaired the growth performance of birds. Ryhanen et al. (2007) conducted an experiment to determine the effect of feeding camelina expeller cake on growth performance of Ross male broiler chickens. The ether-extract value of camelina expeller cake used in their experiment was 16.9% on an as-fed basis. Diets were isocaloric and were formulated to meet the NRC (1994) nutrient requirements of growing broiler chickens. During the starter phase (Day 1 - Day 14), 5% ($P=0.01$) and 10% ($P<0.001$), inclusion of camelina expeller cake significantly reduced the feed intake of birds when compared to control birds. The birds fed diets at 5% ($P = 0.002$) and 10% ($P <0.001$) camelina expeller cake inclusions had significantly increased FCR when compared to control birds. However, during the grower phase (Day 15 - Day 37), the feed intake was not affected by either 5% ($P = 0.29$) or 10% ($P = 0.10$) inclusion levels. During that period, only 10% inclusion of camelina expeller cake significantly increased ($P=0.01$) the FCR of birds when compared to control birds. Therefore, during the grower phase, camelina meal could be incorporated at 5%. When the entire 37 day period was considered, the FCR was significantly increased in birds fed diets at 5% ($P=0.04$) and 10% ($P=0.001$) inclusions when compared to control birds.

2.2 *Glycine max*.

Soybean (*Glycine max*) is a legume which is considered as an oilseed crop. The crop belongs to the family Fabaceae. Among the oilseed crops in the world, soybeans occupied 56% of the world's oilseed production in 2013 (American Soybean Association 2014). The world's annual soybean production, during 1961 - 1965, was 28.6 million metric tons

increasing to 217.6 million metric tons during 2005 - 2007 (Masuda and Goldsmith 2009). During the past 50 years, soybean production increased 7.6 times (Masuda and Goldsmith 2009). The global soybean production has been predicted to increase by 2.2%, annually (Masuda and Goldsmith 2009). By 2030, soybean production in the world has been projected to reach 371.3 million tons (Masuda and Goldsmith 2009). United States, Argentina, Brazil, China and India, the five leading countries combined produce more than 90% of the global soybeans (American Soybean Association 2014). The increasing trend in soybean production is due to the demand in soybean oil for human consumption and biofuel production. The meal by-product left after extracting oil, is a good protein and energy supplement for farm animals. Ground whole soybeans contain 20.3% oil on a DM basis (Nelson et al. 1987). Soybean oil contains lauric, palmitic, myristic, palmitoleic, stearic, oleic, linoleic and linolenic fatty acids (Wiseman and Salvador 1991). The contents of quantitatively important fatty acids, linoleic acid and linolenic acid in soybean oil were found to be 45.2 and 6.6%, respectively (Wiseman and Salvador 1991). As soybean oil contains essential fatty acids, the oil is good for human consumption. Even though the oil content in soybeans is comparatively lower when compared to camelina seeds, there is a high demand for soybeans as a source of biofuel, being one of the leading biofuels sources in the world (International Energy Agency 2011). In extracting oil, by solvent extraction (Mustakas et al. 1981), screw-pressing or extruded-expelling (Nelson et al. 1987), the resultant by-product is soybean meal with varying nutritional compositions. When compared to screw-pressing and extruded-expelling, the solvent extraction process removes significantly more oil from the soybean seeds leaving a low residual oil content (1.2% as-fed basis) in the meal (Wang and Johnson 2001). The greater oil content left in

the meal, after extruded-expelling (7.2% as-fed basis) and screw-pressing (6.3% as-fed basis) (Wang and Johnson 2001), would make a soybean meal with a higher AME_n content to supplement in poultry diets. The solvent-extracted soybean meal which is considered as the "gold standard", is the most popular protein supplement, currently used in poultry feed formulations. The high CP content (48.8% as-fed basis) (Wang and Johnson 2001) and highly digestible ileal amino acids present in the meal (Adedokun et al. 2008) make it attractive. Extruded-expelled and screw-pressed meals containing CP content of 42.5% and 43.2% respectively, on an as-fed basis (Wang and Johnson 2001), can also be considered as protein supplements in poultry diets. The demand for soybean meal from the feed industry, continues to exert pressure on soybean producers to increase global soybean production. Soybean production is further extended because of the trend in building mini-mills which employ mechanical-extraction, in order to produce edible oil without residual solvents and to produce oil for the special target industry groups. The beneficial food and non-food applications of *Glycine max*, ensure that the demand for soybeans is high all-around the world.

2.2.1 Processing of soybean seeds.

The oil in soybean seeds can be extracted by screw-pressing, extruding-expelling or solvent extraction. Screw-pressing and extruding-expelling are mechanical extraction methods. However, the most common processing method is solvent extraction. With this method, soybean seeds are cleaned to remove unwanted materials before the oil is extracted. The beans are cracked into small pieces by passing them through a cracking mill with corrugated rollers **(**Mustakas et al. 1981). The hulls are removed by aspiration (Mustakas et al. 1981). The dehulled soybeans are known as meats (Mustakas et al. 1981, Serrato

1981). The cracked soybean meats are then conditioned to about 65 - 70 °C (Serrato 1981) or 73.9 °C (Mustakas et al. 1981) temperature and 11±0.5% moisture (Serrato 1981). The heated cracked meats are passed through a smooth roller mill (Mustakas et al. 1981, Serrato 1981) in order to produce flakes to a 0.025 cm thickness (Mustakas et al. 1981). The flakes are then conveyed directly to the solvent extractor where the oil is extracted for around 1 h with preheated hexane (60 °C) in a 2: 1 hexane/flake weight ratio (Mustakas et al. 1981). The oil extracted flakes are desolventized with steam to remove hexane (Mustakas et al. 1981). Then, the flakes are toasted at around 100 °C for varying time intervals (20 - 50 min), (Mustakas et al. 1981), followed by rapid cooling. Finally, the flakes are air-dried until the final moisture content reaches 10.7% (Mustakas et al. 1981). The final solvent-extracted soybean meal can contain 1.2% (Wang and Johnson 2001) or 2.3% (Powell et al. 2011) of crude fat and about 55.5% (Wang and Johnson 2001) or 53.7% (Powell et al. 2011) CP (on a DM basis).

When soybeans are mechanically processed using screw presses (Nelson et al. 1987), the oil is mechanically-squeezed from heated soybeans. The anti-nutritional factors in raw soybeans are destroyed due to the heat generated by the friction of the screw press. In another mechanical oil extraction method, soybeans can be passed through an extruding-expelling process. In the extruder, coarsely ground soybeans (10 - 14% moisture) are cooked at 135 °C temperature for less than 30 s (Nelson et al. 1987). Finally, the extrudate is pressed in a continuous screw-press. The meal, produced after extruding-expelling process, contained a residual oil content of 6.5% (Nelson et al. 1987) and 8.7% (Powell et al. 2011) (on a DM basis). Therefore, mechanically-pressed soybean meal (MPSBM) contains a higher residual oil content when compared to solvent-extracted soybean meal.

2.2.2 Anti-nutritional factors of soybean meal.

Soybean seeds and soybean meal contain trypsin inhibitors (Birk and Gertler 1961, Liener and Tomlinson 1981), lectins (Maenz et al. 1999, Fasina et al. 2003) and oligosaccharides (Coon et al. 1990, Graham et al. 2002) as major anti-nutritional factors. Other less significant anti-nutritional factors in soybean meal are tannins, phytoestrogens, phytic acid and saponins (Canadian International Grains Institute 2010).

2.2.2.1 Trypsin inhibitors

Trypsin inhibitors inhibit the pancreatic protease enzyme activity in the intestine which reduces the protein breakdown in the intestine of chickens (Alumot and Nitsan 1961). Continuous production of trypsinogen by the pancreas may result in a larger pancreas and impaired proteolysis may reduce the growth of the birds. Hence, a reduction in body weight and an increase in pancreas weight to the percent of total body weight have been observed (Alumot and Nitsan 1961).

2.2.2.2 Lectins

Raw soybean meal contains carbohydrate binding and agglutinating lectin proteins (Maenz et al. 1999, Fasina et al. 2003). These soybean lectins bind with the brush border membrane of the small intestine (Pusztai et al. 1990) and agglutinate the brush border membrane cells (Maenz et al. 1999). The ultimate effect is the impairment of the nutrient absorption due to the disrupted brush border membrane.

2.2.2.3 Oligosaccharides

The problematic oligosaccharides in soybean meal consist of two α-galactosides, stachyose and raffinose. These oligosaccharides are known to increase the intestinal feed passage rate and reduce the transit time, fiber digestion, TME_n (Coon et al. 1990) as well as AME_n

(Perryman and Dozier 2012) in broiler chickens. However, these oligosaccharides in soybean meal can either be removed by hydrolyzing the raffinose and stachyose with α-galactosidase enzyme (Graham et al. 2002) or by ethanol extraction (Coon et al. 1990).

2.2.3 Methods to reduce trypsin inhibitors and lectins in soybean meal

Trypsin inhibitors and lectins in soybean meal can be destroyed by heat treatment (Nelson et al. 1987, Fasina et al. 2003). There are numerous heat treatment methods that can be carried out to inactivate these anti-nutritional factors in soybean meal. During the desolventization stage of solvent extraction, the extracted soybean flakes are toasted at around 100 °C (Mustakas et al. 1981) to remove the residual hexane. During toasting, the trypsin inhibitors in the soybean meal are inactivated (Mustakas et al. 1981). Mustakas et al. (1981) observed trypsin inhibitor activity of 5.42, 3.38 and 3.02 $mg \cdot g^{-1}$ of meal, when defatted flakes (trypsin inhibitor activity, 23 $mg \cdot g^{-1}$ of meal) were heated for 20, 35 and 50 min respectively, at around 100 °C. Therefore, a reduction in trypsin inhibitor activity could be observed after toasting. The urease activity (unit rise in pH) for different time periods were 0.97 (20 min), 0.36 (35 min) and 0.37 (50 min). It should be noted, when the duration of heating was increased, the trypsin inhibitor and urease activities have been decreased.

During expeller-pressing (screw-pressing), the soybean seeds are forced by a screw through an orifice. The heat produced due to the pressure and the friction in the expeller barrel, inactivates the trypsin inhibitors (Nelson et al. 1987). Nelson et al. (1987), reported a 94% destruction of trypsin inhibitor activity in soybean press cake, when expeller-pressed.

In the expeller-extruded method, during the extrusion, the ground soybeans are heated for a short time period at a high temperature (Nelson et al. 1987). Nelson et al. (1987), observed 91% inactivation of trypsin inhibitors in extruded soybean meal, when the ground soybeans were extruded at 135 °C for 30 s. Fasina et al. (2003) found that soybean meal trypsin inhibitors and lectins could be inactivated when the defatted raw soybean meal was steam-heated at 100 °C for 5 min. At this heat treatment, the total carbohydrate-binding lectins were reduced from 2.68 to 0.13 $mg \cdot g^{-1}$ of meal whereas the agglutinating lectins dropped to 0 from 1.35 mg/g of meal. The trypsin inhibitors (46.59 $mg \cdot g^{-1}$ of meal) and the urease activity (2.37 unit rise in pH) were reduced to 4.88 $mg \cdot g^{-1}$ of meal and 0.09 respectively. According to Fasina et al. (2003), for this heat treatment, around 95, 100 and 90 and 96% reductions were observed in total carbohydrate-binding lectins, agglutinating lectins, trypsin inhibitors and urease activity, respectively. Fasina et al. (2003) recommended heating soybean meal at 100 °C for 5 min, as all the anti-nutritional factors were inactivated by more than 90%. Friedman et al. (1991), noticed a reduction in trypsin inhibitor activity in raw soybean meal (7136 $TIU \cdot g^{-1}$) to 3933, 1058 and 1030 $TIU \cdot g^{-1}$when defatted soybean meal was autoclaved at 121 °C for 10, 20 and 30 min, respectively. After 30 min, the reduction in trypsin inhibitors in defatted raw soybean meal was approximately 87% whereas at 10 min and 20 min, the reduction was 31 and 81%, respectively. Different time-temperature combinations and heat-processing methods have been used in the past in order to eliminate the soybean trypsin inhibitors and lectins. However, after heat processing, the meal quality can be evaluated to confirm that it is either free of trypsin inhibitors and lectins.

2.2.4 Assessing the soybean meal quality

Soybean meal is known to contain urease enzyme (Mustakas et al. 1981, Fasina et al. 2003). According to Fasina et al. (2003), the urease activity of the meal was inactivated in a similar manner as soybean meal lectins because both ureases and lectins were inactivated at 100 °C for 5 min. At 100 °C, the rate of inactivation of total carbohydrate-binding lectins (r^2 = 0.998) and trypsin inhibitor activity (r^2 = 0.996) were positively correlated to the rate of denaturation of urease activity. Interestingly, the correlation coefficients were similar. Therefore, urease activity can be used as a measure of the trypsin inhibitors and lectins in under-processed soybean meals. Determination of the urease activity is much easier than determining the trypsin inhibitors and lectins in the soybean meal. Interestingly, there is no minimum permissible level for trypsin inhibitors that ensures safe feeding of soybean meal in poultry nutrition. However, there are acceptable ranges for urease activity which indicate adequate processing of soybean meal. According to the American Feed Manufacturers Association (1979), an increase in pH of 0.05 to 0.20, was considered as a standard for the urease activity in a well processed soybean meal. The urease activity (0.37) determined in the soybean meal which was steam-toasted at 100 °C for 50 min (Mustakas et al. 1981), was not in the range of 0.05 - 0.20. However, the soybean meal which was steam-heated using an autoclave, at 100 °C (0.2-16.5 psi) of steam for 5 min (Fasina et al. 2003), showed a desirable urease activity (0.09) which was in the pH range of 0.05 - 0.20, indicating that the meal was properly processed. Therefore, the heat processing method should be taken into consideration in determining the proper time-temperature combination, in order to have acceptable urease activity.

2.2.5 Chemical composition of expeller-extruded soybean meal

The expeller-extruded soybean meal is known to be a good source of CP. The CP content of MPSBM was 44.9% (Powell et al. 2011) and 42.6% (Opapeju et al. 2006) on an as-fed basis. The amino acid composition of expeller-extruded and solvent-extracted soybean meals determined after acid hydrolysis, is given in Table 2.3 (Opapeju et al. 2006). The amino acid found in greatest quantity was glutamine, whereas the lowest was methionine (Opapeju et al. 2006). According to NAS (1971), the lowest concentration was found to be tryptophan. However, Opapeju et al. (2006) did not determine the tryptophan content in expeller-extruded soybean meal. All the amino acids in expeller-extruded soybean meal except methionine and cysteine, were lower than that of solvent-extracted soybean meal (Opapeju et al. 2006). According to NAS (1971), all amino acids in mechanically-extracted soybean meal except isoleucine, methionine and tryptophan were lower than that of solvent-extracted soybean meal. The amino acids except serine and cysteine reported by NAS (1971) for mechanically-extracted soybean meal were greater than those for expeller-extruded soybean meal reported by Opapeju et al. (2006). However, proline and alanine amino acid contents were not reported by NAS (1971). As expeller-extruded soybean meal contains both methionine and lysine, which are considered as the major limiting amino acids for poultry, the meal can be used as a good source of protein supplement in poultry diets. The high residual oil content of 8.1% (Powell et al. 2011), 9.5% (Opapeju et al. 2006) and 4.7% (NAS 1971) on an as-fed basis, emphasizes the variable oil content among samples of these meals. The potential use of expeller-extruded soybean meal as an energy supplement in poultry diet formulations requires knowledge of the AME_n and SIAAD in high oil residue soybean meals.

Table 2.3 Amino acid composition (%) of expeller-extruded and solvent-extracted soybean meal (as-fed basis).

Item	Solvent-extracted soybean meal*	Expeller-extruded soybean meal*	Solvent-extracted soybean meal**	Mechanically-extracted soybean meal**
Fat %	2.4	9.5	1.2	4.7
Crude protein %	46.8	42.6	46.7	42.4
Amino acid %				
Serine	2.32	2.09	2.59	2.02
Threonine	1.82	1.66	1.98	1.72
Proline	2.56	1.98	2.92	-
Glutamine	7.16	6.66	9.31	7.59
Cysteine	0.64	0.66	0.70	0.63
Valine	1.86	1.74	2.46	2.23
Alanine	1.86	1.74	2.46	-
Glycine	1.82	1.71	2.44	2.41
Isoleucine	1.86	1.59	2.47	2.83
Methionine	0.52	0.53	0.60	0.71
Leucine	3.20	2.86	3.84	3.64
Tyrosine	1.60	1.30	1.54	1.42
Phenylalanine	2.19	1.90	2.50	2.12
Lysine	2.77	2.61	3.05	2.81
Histidine	1.10	1.02	1.29	1.11
Arginine	3.21	2.79	3.39	2.90
Tryptophan	-	-	0.58	0.59

Source: *Opapeju et al. (2006), **NAS (1971)

2.2.6 Standardized ileal amino acid digestibility of soybean meal in broilers

In the literature, there is no information on the SIAAD of MPSBM for broiler chickens. However, the SIAAD of solvent-extracted soybean meal (Table 2.4) in 21 days old broiler chickens, have been determined by Adedokun et al. (2008).

Table 2.4. Standardized ileal amino acid digestibility of solvent-extracted soybean meal in 21 days old broiler chickens.

Amino acid	SIAAD (%)
Essential amino acids	
Methionine	91.5
Lysine	89.5
Leucine	87.0
Isoleucine	87.8
Phenylalanine	87.5
Hisitidine	89.2
Tryptophan	-
Valine	87.0
Arginine	91.5
Threonine	83.7
Non-essential amino acids	
Serine	85.7
Proline	87.5
Alanine	86.8
Cysteine	81.8
Aspartic acid	85.8
Glutamic acid	89.5
Tyrosine	88.0
Glycine	85.0

Source: Adedokun et al. 2008

Among the essential amino acids, the highest SIAAD was recorded for methionine and arginine while the lowest was observed for threonine with an approximately 8 percentage unit difference. For the non-essential amino acids, the greatest digestibility was reported for glutamic acid with cysteine being the lowest. All the amino acids had a higher than 80% SIAAD in 21 days old broiler chickens. The amino acids present in solvent-extracted soybean meal are highly digestible by broiler chickens. This is one of the reasons solvent-extracted soybean meal is considered as the main protein supplement in commercial poultry diets. The determination of SIAAD of MPSBM will be interesting to compare with the well-established solvent-extracted soybean meal.

2.2.7 True-amino acid digestibility of solvent-extracted soybean meal, extruded-expelled soybean meal and toasted soybeans in broilers

The true-amino acid digestibility values of solvent-extracted soybean meal, extruded-expelled soybean meal (Opapeju et al. 2006) and toasted full-fat soybeans (Anderson-Hafermann et al. 1992) (Table 2.5) show an important variation.

Table 2.5 True-amino acid digestibility (%) of soybean products

Item	* Solvent-extracted soybean meal	*Extruded-expelled soybean meal	**Toasted full-fat soybeans
Fat %	2.41	9.51	-
Crude protein %	46.81	42.63	-
Amino acid			
Methionine	89.0	89.4	83
Lysine	93.3	93.2	87
Cysteine	90.7	82.3	83
Threonine	90.5	88.4	82
Leucine	97.1	93.5	87
Isoleucine	97.9	91.8	85
Valine	94.3	90.1	83
Histidine	94.9	89.4	86
Tryptophan	-	-	-
Phenylalanine	96.7	93.3	85
Arginine	98.9	96.2	91
Serine	95.5	90.8	-
Aspartate	92.4	91.3	-
Glutamate	96.6	95.1	-
Proline	100.2	92.6	-
Alanine	95.1	92.9	-
Tyrosine	100.2	92.9	-

Source: *Opapeju et al. 2006; ** Anderson-Hafermann et al. 1992

The true-amino acid digestibilities for isoleucine, leucine, cysteine, glycine, proline, tyrosine and serine in solvent-extracted soybean meal were greater ($P \leq 0.05$) than extruded-expelled soybean meal (Opapeju et al. 2006). However, in solvent-extracted soybean meal, except for methionine, the true-amino acid digestibilities of all the other amino acids, were numerically higher than that of extruded-expelled soybean meal. The amino acid

digestibility determined for toasted full-fat soybeans (Anderson-Hafermann et al. 1992), was comparatively lower for all the reported amino acids when compared to solvent-extracted and extruded-expelled soybean meals.

2.2.8 Nitrogen-corrected apparent metabolizable energy (AME_n) of soybean meal

The AME_n of solvent-extracted soybean meal (1.7% of fat and 47.6% of CP a as-is basis) has been determined by Perryman and Dozier (2012) using 29 day old broiler chickens. The reported AME_n was 2241 $kcal \cdot kg^{-1}$. According to NRC (1994), the AME_n of solvent-extracted soybean meal was 2440 $kcal \cdot kg^{-1}$ (CP = 48.5%). Sauvant et al. (2004) found the AME_n of solvent-extracted soybean meal (CP = 48%) to be 2223 $kcal \cdot kg^{-1}$ in broiler chickens. Therefore, the AME_n of solvent-extracted soybean meal is in the range of 2223 - 2440 $kcal \cdot kg^{-1}$. According to Sauvant et al. (2004), the AME_n of pelleted full-fat toasted soybean was 3274 $kcal \cdot kg^{-1}$ in broiler chickens compared to NRC (1994) with an AME_n of 3300 kcal/kg for heat processed soybeans. Theoretically, the AME_n of MPSBM might be within the range of 2223 - 3300 $kcal \cdot kg^{-1}$ which defines solvent-extracted soybean meal at the low end and full-fat soybeans at the high end. An equation (Poultry AME_n = 2208 + 63.4*fat) has been given by the Canadian International Grain Institute (2010) to predict the AME_n of MPSBM. However, under evaluation, where live birds are used, the AME_n values may not be achieved as predicted. Tables provided in Sauvant et al. (2004) and NRC (1994) did not mention the age of the birds or the method of AME_n determination when reporting AME_n values. Therefore, the accuracy of comparison of AME_n values reported in the literature is questionable.

2.2.9 Effect of heat treatment on amino acid concentrations in soybean meal

When soybean meal is heated in order to destroy trypsin inhibitors and lectins, the protein in the meal can be denatured. Therefore, the effect of toasting temperature and time on amino acid content of soybean meal, must be taken into consideration. Parsons et al. (1992) determined the effect of autoclaving time on the amino acid concentrations of dehulled solvent-extracted soybean meal (Table 2.6).

Table 2.6 Effect of autoclaving time on the amino acid concentrations (%)* of dehulled solvent-extracted soybean meal.

	Autoclaving time (min)			
	0	20	40	60
Amino acid				
Methionine	0.68	0.70	0.67	0.70
Cysteine	0.73	0.72	0.67	0.64
Leucine	3.76	3.82	3.63	3.60
Isoleucine	2.26	2.28	2.19	2.17
Threonine	1.88	1.99	1.79	1.78
Arginine	3.50	3.52	3.21	3.15
Histidine	1.28	1.28	1.20	1.18
Valine	2.31	2.33	2.24	2.23
Proline	2.44	2.47	2.35	2.32
Glycine	2.04	2.06	1.95	1.95
Tyrosine	1.52	1.55	1.34	1.45
Glutamate	8.77	8.87	8.41	8.35
Aspartate	5.53	5.57	5.27	5.23
Alanine	2.12	2.14	2.03	2.03
Serine	2.42	2.44	2.30	2.27
Phenylalanine	2.45	2.47	2.35	2.33
Lysine	3.15	2.95	2.65	2.47

*Amino acid concentrations are expressed on a 90% DM basis in quadruplicate
Source: Parsons et al. (1992)

All the amino acids except lysine and cysteine, were increased when the solvent-extracted soybean meal was autoclaved at 121 °C for 20 min. However, all the amino acids were reduced when the unautoclaved solvent-extracted soybean meal was heated for 40 min. Except for the methionine, all the other amino acids were decreased at 60 min. Interestingly, the lysine and cysteine amino acid concentrations in unautoclaved solvent-

extracted soybean meal were reduced when the autoclaving time was increased. Between these two amino acids, the highest reduction was observed in lysine. The lysine content in the unautoclaved meal was reduced by 6, 16 and 22%, when the meal was autoclaved at 121 °C for 20, 40 and 60 min, respectively. The reduction for cysteine was 1.4, 8 and 12% for these 3 temperatures, respectively. Among all the amino acids, lysine and cysteine were found to be most heat sensitive. Therefore, in soybean processing, attention should be given to these particular amino acids.

2.2.10 Effect of heat treatment on nutritive value of soybean products

The effect of heat treatment and enzyme supplementation on AME_n, TME_n, SIAAD and true-amino acid digestibility of MPSBM, in broiler chickens, have not been elucidated. However, the heat effect on TME_n of solvent-extracted soybean meal (Parson et al. 1992) and the heat effect on true-amino acid digestibility of full-fat soybeans (Anderson-Hafermann et al. 1992) and solvent-extracted soybean meal (Parson et al. 1992) have previously been determined.

2.2.10.1 Effect of heat treatment on true-amino acid digestibility of conventional full-fat soybeans

According to Anderson-Hafermann et al. (1992), the true-amino acid digestibility of conventional full-fat soybeans fed to cecectomized birds, increased with longer autoclaving time. The true-amino acid digestibility coefficients of the full-fat soybeans, autoclaved at 121 °C for 18 min were higher ($P<0.05$) than that of full-fat soybeans autoclaved at 0 and 9 min (Table 2.7). This was due to the denaturation of trypsin inhibitors with the increasing autoclaving time.

Table 2.7 Effect of autoclaving time on true-amino acid digestibility* (%) of conventional full-fat soybeans.

	Autoclaving time (min)		
	0	9	18
Amino acid			
Methionine	65	69	83
Lysine	73	74	87
Threonine	64	66	82
Valine	65	67	83
Leucine	68	68	87
Phenylalanine	68	69	85
Arginine	78	78	91
Histidine	72	73	86
Isoleucine	64	67	85
Cysteine	67	68	83

Source: Anderson-Hafermann et al. (1992)

*Values from 4 cecectomized cockerels

2.2.10.2 Effect of heat treatment on true-amino acid digestibility of dehulled solvent extracted soybean meal

According to Parson et al. (1992), the true-amino acid digestibilities of threonine, alanine, serine, methionine, valine, leucine, isoleucine, phenylalanine and tyrosine determined using cecectomized birds, were increased when the unautoclaved solvent-extracted meal was heated at 121 °C for 20 min (Table 2.8). However, except for valine, isoleucine and tyrosine, the true-amino acid digestibility of other amino acids was reduced when the unautoclaved solvent-extracted soybean meal was heated for 40 min (Parson et al. 1992). The true-amino acid digestibility of lysine ($P<0.001$), histidine, cysteine and aspartate ($P<0.05$) were reduced with increasing autoclaving temperature (Parson et al. 1992). Approximately a 22, 15, 20 and 16% reduction in true-lysine, histidine, cysteine and aspartate digestibility was observed at 40 min (Parson et al. 1992). Therefore, when true-amino acid digestibility was considered, lysine and cysteine attracted much attention over the other amino acids.

Table 2.8 Effect of autoclaving time on true-amino acid digestibility* (%) of dehulled solvent-extracted soybean meal.

	Autoclaving time (min)		
	0	20	40
Amino acid			
Methionine	85.6	86.3	83.3
Lysine	90.8	77.9	69.2
Cysteine	81.6	69.1	61.5
Threonine	84.3	85.7	80.1
Leucine	90.1	92.4	89.3
Isoleucine	90.5	91.6	91.2
Serine	87.6	89.2	83.0
Valine	87.4	92.7	89.0
Phenylalanine	92.8	95.5	92.7
Histidine	88.1	80.3	73.3
Arginine	92.9	91.9	85.8
Aspartate	88.6	81.1	72.4
Glutamate	93.0	91.0	86.2
Proline	90.8	90.7	86.7
Alanine	84.7	88.3	83.8

Source: Parson et al. (1992) *Mean of 3 birds

2.2.10.3 Effect of heat treatment on nitrogen-corrected true metabolizable energy content of dehulled solvent-extracted soybean meal

Parson et al. (1992) (Table 2.9), found that there was no effect ($P>0.10$) of autoclaving time on nitrogen-corrected true metabolizable energy content of dehulled solvent-extracted soybean meal in caecectomized birds.

Table 2.9 Effect of autoclaving time on TME_n* of dehulled solvent-extracted soybean meal

Autoclaving time (min)	TME_n (kcal / g of DM)
0	2.314±0.09
20	2.483±0.09
40	2.350±0.09

Source: Parson et al. (1992) *Mean of 3 birds

The TME_n of unautoclaved solvent-extracted soybean meal was increased when the meal was heated. However, the greatest TME_n was recorded when the meal was autoclaved at 121 °C for 20 min (Parson et al. 1992).

2.2.11 Effect of feeding expeller-extruded soybean meal on growth performance of broiler chickens

Research on feeding MPSBM to broiler chickens is very limited in the literature since extracting oil from soybeans through mechanical means is less popular than using solvent-extraction. However, Powell et al. (2011) conducted an experiment to evaluate the effect of feeding expeller-extruded soybean meal (EE-SBM) and solvent extracted soybean meal (SE-SBM) on production performance of broiler chickens. The CP and crude fat content of EE-SBM were 44.9% and 8.1% (on as-fed basis), respectively. The SE-SBM contained 2% crude fat and 47.2% CP. During the starter phase (Day 0 to Day 14), EE-SBM reduced ($P<0.001$) the average daily gain and average daily feed intake of birds when compared to birds fed the SE-SBM. The gain:feed intake ratio of birds was approaching significance ($P=0.059$) in that the gain: feed intake was greater for EE-SBM. This suggested that the birds fed EE-SBM were able to use EE-SBM more efficiently. There was no difference in average daily feed intake ($P=0.480$), average daily gain ($P=0.892$) and gain:feed intake ratio ($P=0.25$) during the grower phase (Day 15 to Day 35) between the birds fed SE-SBM and EE-SBMs. Between the two treatment groups, there was no difference in average daily feed intake ($P=0.635$), average daily gain ($P=0.568$) and gain:feed intake ratio ($P=0.825$) during the finisher phase (Day 36 to day 49). Over the entire 49 days of the experiment, no significant differences in body weight ($P=0.763$), average daily gain ($P=0.865$), average daily feed intake ($P=0.541$) and gain:feed intake ratio ($P=0.391$) occurred between the two groups of birds fed either SE-SBM or EE-SBMs. The growth performance of birds fed SE-SBM and EE-SBM was similar and EE-SBM could be incorporated into broiler diets without having significant negative effects, when added at up to 30%.

2.3 *Brassica carinata*

Brassica carinata A. Braun is an oilseed crop which is known as "Ethiopian mustard". It belongs to the family Brassicaceae. The crop is native to Ethiopia and widely grown there as an oilseed crop. The oil content of brown-seeded carinata seeds was in the range of 30.5 - 34.8% (Getinet et al. 1995). Because of the high oil content in seeds, *Brassica carinata* has potential to compete with soybeans, canola, palm and sunflower, as sources of biofuels. However, the erucic acid content of carinata oil ranged from 37.8 - 44.2% as a per cent of total fatty acids (Getinet et al. 1995). Therefore, low erucic acid lines of *Brassica carinata* needed to be developed to produce oil with zero erucic acid to make high quality edible oil. Getinet et al. (1994) were able to develop *Brassica carinata* with zero erucic acid by crossing *Brassica juncea* species with *Brassica carinata*. The other concern with *Brassica carinata* is the high glucosinolate content in the seeds which is estimated to result in more than 130 $\mu mol \cdot g^{-1}$ in the seed meal (Getinet et al. 1995). Therefore, low glucosinolate *Brassica carinata* lines should be developed in the future. Compared to *Brassica napus*, *Brassica carinata* showed a 2 to 3 weeks later maturity when grown in Saskatchewan in Western Canada (Getinet et al. 1994). The seed weight of *Brassica carinata* was greater than that of *Brassica napus* and *Brassica juncea* (Getinet et al. 1994). Increased seed weight is considered a favorable characteristic over other *Brassica* species. There is potential to make *Brassica carinata* popular, as an oilseed crop in Canada, if plant breeders develop early-maturing carinata lines in the future (Getinet et al. 1995). As the demand for biofuel is rapidly expanding throughout the world (International Energy Agency 2011), there is a high demand for alternative oilseed crops in order to meet the energy demand in

the world. Therefore, the high oil content in carinata seeds (Getinet et al. 1995) suggests its' possible use as a biofuel.

2.3.1 Processing of carinata seeds

The oil from carinata seeds can be extracted by hexane (Simbaya et al. 1995). Under laboratory conditions, Simbaya et al. (1995) crushed brown-seeded carinata seeds and extracted oil with hexane for 2 h using a soxhlet apparatus. After drying the meal in a fumehood, the meal was ground and extracted again with hexane for 8 h. This process produced a solvent-extracted carinata meal with 2.9% fat on a DM basis (Simbaya et al. 1995). When the solvent extraction is done on a large scale, it can be carried out as described by Mustakas et al. (1981) and Serrato, (1981) (Section 2.2.1). However, like camelina seeds, the carinata seeds can be mechanically-pressed using an expeller-press.

2.3.2 Anti-nutritional factors in carinata meal (Glucosinolates)

Getinet et al. (1995) found that oil-free *Brassica carinata* seed meal contained 134 - 188 $\mu mol \cdot g^{-1}$ of glucosinolates. The major type of glucosinolates found in the meal was allyl glucosinolate (Getinet et al. (1995). The adverse effects of glucosinolates on broiler chickens have been previously discussed in Section 2.1.2.

2.3.3 Methods to reduce glucosinolates

2.3.3.1 Heat treatment

Jensen et al. (1995) found glucosinolate content of rapeseed meal was reduced by approximately 50%, with a minimum compromise in protein solubility, lysine content, true-digestibility of protein, biological value of protein and net protein utilization values when the extracted meal was heated at 100 °C for 30 min. As rapeseed and carinata crops

belong to same family (Brassicaceace), it is possible to heat carinata meal at 100 °C for 30 min to reduce the glucosinolate content in the meal.

2.3.3.2 Water treatment

Another method of reducing the meal glucosinolates in *Brassica* species is soaking the meal in water. Tyagi (2002) conducted an experiment to determine the effect of water treatment of Indian mustard cake on glucosinolate hydrolysis. The mustard cake was treated with water at 1:5 meal to water ratio for 2, 4, 6 and 8 h. Treating the meal with water for 8 h, significantly reduced ($P<0.01$) the glucosinolate content by 76%. Therefore, when compared to heat treatment, soaking the meal with water, may reduce the glucosinolates effectively, without altering the meal protein quality.

2.3.4 Chemical composition of *Brassica carinata* meal

The CP content of brown-seeded *Brassica carinata* meal was in the range of 44.3 - 50% (Getinet et al. 1995). Because of the high CP content, the meal can be considered as a good source of protein for poultry. The linoleic and linolenic acid contents as a per cent of total fatty acids in *Brassica carinata* oil was found to be in the range of 16.1 - 20.4 and 11.7 - 17.3%, respectively (Getinet et al. 1995). When the oil is extracted from carinata seeds by mechanical-pressing, a significant amount of oil remains in the meal. Therefore, the meal can be used as an energy supplement in poultry diets. As the oil contains linoleic and linolenic fatty acids, residual oil in the meal can be considered as a good source of essential fatty acids. No value was found in the literature for residual oil level in carinata meal.

2.3.5 Standardized ileal amino acid digestibility of carinata meal

As carinata meal is a novel meal ingredient being introduced to the poultry feed industry, there was no previous work with broiler chickens on elucidating the SIAAD of either

mechanically-pressed or solvent-extracted cariata meal. No information on true-amino acid digestibility of carinata meal was found in the literature.

2.3.6 Nitrogen-corrected apparent metabolizable energy or true metabolizable energy of carinata meal in broiler chickens

No AME_n or TME_n of either solvent-extracted or mechanically-pressed carinata meal for broiler chickens, was found.

2.3.7 Effect of heat treatment and enzyme supplementation on nutritive value of *Brassica carinata* meal

The effect of enzyme supplementation and heat treatment on AME_n, TME_n, SIAAD and true-amino acid digestibility of either solvent-extracted or mechanically-pressed carinata meal has not been evaluated in the past. There are no studies with incorporated enzymes in production performance experiments, evaluating the supplementation of *Brassica carinata* meal in broiler diets.

2.3.8 Effect of feeding *Brassica carinata* meal on production performance of broilers

The research on evaluation of feeding carinata meal on production performance of broiler chickens is very limited. However, Tadelle et al. (2003) conducted a study using Hubbard broiler chicks to determine the effect of inclusion of graded levels of *Brassica carinata* meal on production performance of broiler chickens. In a seven-week trial, carinata meal was included at levels of 0, 7, 14, 21, 28, and 35% in broiler rations. When the total experimental period (48 days) was considered, no differences ($P>0.05$) in daily weight gain, daily feed intake and FCR occurred among the treatment groups. However, Tadelle et al. (2003) recommended to include carinata meal up to 28% in broiler diets.

2.4 Effect of enzyme supplementation and processing type on nutrient digestibility of diets

2.4.1 Effect of enzyme supplementation on nutrient digestibility of diets

Meng and Slominski (2005), evaluated the effect of supplementation of a multicarbohydrase enzyme mixture on nutritive value of a corn-soybean meal based diet. The enzyme mixture contained cell wall degrading enzymes; xylanase, pectinase, glucanase, mannanase, cellulase, and galactanase. The corn-soybean meal diet was formulated to contain 5% lower AME_n and CP than the NRC (1994) requirements in order to improve the sensitivity of diets to enzymes. However, the per cent of lysine, methionine, methionine + cysteine, calcium and available phosphorous met the NRC (1994) requirements for broilers 0 to 3 weeks of age. The AME_n of the diet ($kcal \cdot kg^{-1}$ as-fed basis) and total tract non-starch polysaccharide digestibility were determined using excreta containing chromic oxide on Day 18. The AME_n ($P<0.003$) and apparent total tract non-starch polysaccharide digestibility of the diet ($P<0.001$) were improved when the corn-soybean meal diet was supplemented with enzymes. The apparent ileal protein and ileal non-starch polysaccharide contents of diets were determined using the contents of the ileum collected on Day 19 and 20. The apparent ileal protein digestibility ($P=0.016$) and the ileal water-soluble non-starch polysaccharides (NSP) were higher ($P=0.001$) in birds fed the diet supplemented with enzymes than that of birds fed no enzymes. The water-insoluble NSP content in the ileum was lower ($P=0.022$) in birds fed the enzyme supplemented diet when compared to ileal water-insoluble NSP in birds fed no enzymes. In this study, although the ileal CP and ileal non-starch polysaccharide digestibilities were improved ($P<0.05$) with enzyme supplementation, the growth performance of birds was

not improved (P<0.05). Since the AME_n and CP of the corn-soybean meal diet did not meet the NRC (1994) specifications, a difference in growth performance can not be expected. However, there was a trend to improve the BWG (P = 0.07) and FCR (P = 0.08) with the enzyme supplementation.

2.4.2 Effect of processing type and enzyme supplementation on nutrient digestibility of corn-soybean diets in broiler chickens

Zanella et al. (1999), conducted an experiment to determine the effect of enzyme supplementation and soybean processing type on nutrient digestibility of broiler chickens. The diets were formulated using solvent-extracted soybean meal, roasted full-fat soybeans (100 °C, 40 min) and extruded full-fat soybeans (120 °C - 130 °C, 10 - 20 s, 70 atm). Each of the diets was evaluated without enzymes and supplemented with 0.1% of Avizyme®1500 enzyme mixture which contained protease, xylanase and amylase as main activities.

2.4.2.1 Effect of enzyme supplementation on nutrient digestibility of corn-soybean diets in broiler chickens

The ileal CP, fat, starch and amino acid digestibility of diets were determined using diets with 0.5% chromic oxide (Zanella et al. 1999). Enzyme supplementation improved (P<0.05) the ileal protein, starch and fat digestibility of diets regardless of the soybean processing type (Zanella et al. 1999). Enzyme supplementation improved (P<0.05) the ileal threonine, serine, glycine, valine and tyrosine digestibility of diets. In the case of methionine, with enzymes, there was no improvement in ileal digestibility (Zanella et al. 1999). It was noted that the digestibility of both ileal lysine and methionine, which are considered major limiting amino acids in poultry, was not improved (P>0.05) with enzyme supplementation.

2.4.2.2 Effect of processing type on nutrient digestibility of corn-soybean diets in broiler chickens

When the main effect processing type was evaluated, the highest ileal protein, fat and starch digestibility of corn-soybean diets occurred in extruded full-fat soybeans (Zanella et al. 1999). Incorporation of roasted full-fat soybeans reduced ($P<0.05$) the ileal crude protein and fat digestibilities of the diet compared to diets with extruded full-fat soybeans (Zanella et al. 1999). The ileal starch digestibility of diets supplemented with extruded full-fat soybeans and roasted full-fat soybeans were not different ($P>0.05$) (Zanella et al. 1999). Extruded full-fat soybean diets resulted in greater ($P<0.05$) arginine, asparagine, glutamine, glycine, alanine, valine, leucine, tyrosine, phenyl alanine, lysine and histidine digestibility of diets when compared to solvent-extracted soybean meal and roasted full-fat soybean diets. The amino acid digestibilities of all amino acids except for isoleucine, were highest in diets incorporated with extruded full-fat soybeans (Zanella et al. 1999). These findings suggest that the processing type of an ingredient exert an impact on ileal CP, fat, starch and amino acid digestibility of diets in broiler chickens.

2.5 Effect of enzyme supplementation on production performance of broilers

Previous research determined the effect of supplementation of enzymes on production performance of broiler chickens. Abudabos (2010) conducted an experiment from 1 to 49 days of age to evaluate the performance of broiler chickens (Cobb) fed a corn-soybean meal diet supplemented with a commercial enzyme which contained β-pentosanase, amylase, β-glucanase and galactomannases. The enzyme supplementation at 250 and 500 $g \cdot tonne^{-1}$ of feed increased ($P<0.001$) the body weight of birds when compared to control group fed no enzyme (Abudabos 2010). Enzyme supplementation did not affect ($P>0.05$)

the feed intake. Interestingly, when compared to control birds, enzyme supplementation improved ($P<0.05$) the FCR (Abudabos 2010). As the feed intake of the birds fed the control and enzyme supplemented diets was not different ($P>0.05$), the birds fed enzyme supplemented diets better utilized the diets compared to the control birds (Abudabos 2010). The improvement in the body weight of the birds fed enzyme supplemented diets, without increase in feed intake was the reason for the improved FCR in the groups fed enzymes.

Kidd et al. (2001) conducted an experiment to determine the effect of enzyme supplementation on production performance of broiler chickens. The liquid enzyme used in this experiment was KEMZYME C/S (112 $g \cdot tonne^{-1}$); a mixture of α-galactosidase, xylanase, β-glucanase, α-amylase, cellulase and protease. At Day 28, enzyme treatment did not affect the body weight ($P=0.374$) and FCR ($P=0.349$) of birds (Kidd et al. 2001). At Day 49, the body weight was not affected ($P=0.125$) with enzyme supplementation (Kidd et al. 2001), however, the birds fed the enzyme treated corn-soybean meal diet showed a better FCR ($P=0.003$) from Day 1 to 49 compared to the control birds (Kidd et al. 2001). The increased energy digestibility was due to the functions of α-galactosidase, α-amylase, β-glucanase, cellulase and xylanase and the improved amino acid availability was due to the function of protease and these benefits may have contributed to the observed improvements in FCR ($P=0.003$) over the whole experiment.

Zanella et al. (1999), conducted a production performance trial using Hubbard day-old male broiler chickens. The birds were fed with one of six diets which contained solvent extracted soybean meal (SE-SBM), SBM + 0.1% enzyme, extruded full-fat soybeans (SBE), SBE + 0.1% enzyme, roasted soybeans (SBR) and SBR + 0.1% enzyme. The

enzyme mixture, Avizyme®1500 contained protease, xylanase and amylase. The main effect of enzyme supplementation improved ($P<0.05$) the body weight gain of birds at 45 days when compared to body weight gain of birds fed no enzymes (Zanella et al. 1999). The FCR of birds at 45 days, was lower ($P<0.05$) when the birds were fed diets with enzymes compared to FCR of birds fed diets with no enzymes (Zanella et al. 1999). Hence, enzyme supplementation improved the nutrient utilization and production performance of birds. When the poultry diets were supplemented with enzyme mixtures, the production performance was improved. Improved nutrient digestibilities of the diets with enzyme supplementation (Meng and Slominski 2005 and Zanella et al. 1999) may be the reason for the better production performance of broiler chickens.

2.6 Concept of standardized ileal amino acid digestibility in broiler chickens

By definition, the amino acid digestibility is the ratio of an amino acid that is absorbed by the animal to the amino acid that is ingested by the animal (Lemme et al. 2004). Therefore, the amino acid digestibility can be determined by considering the amino acid that was consumed and excreted. The amino acid digestibility in a feed ingredient can be determined using two techniques. They are excreta method and ileal digestibility method (Ravindran et al. 1999). In a review paper, Lemme et al. (2004) mentioned that the majority of amino acid digestibility data have been determined using the excreta method. The excreta amino acid digestibility can either be determined by the total collection of excreta from growing birds (Ravindran et al. 1999, Zanella et al. 1999) or by the precision method (Sibbald 1976, Sibbald 1979, Zanella et al. 1999). As Lemme et al. (2004) mentioned, the majority of excreta amino acid digestibility data have been determined in the past, using the precision feeding method. In the precision feeding method, the cockerels are fasted for 21 h then

force-fed a known amount of test feed ingredient into the crop directly (Sibbald 1976). Finally excreta materials are collected for 24 h. This assay is simple and a large number of feed ingredients can be tested in a shorter period of time with a small number of cockerels (Siriwan et al. 1993). However, in both total collection and precision methods, there are disadvantages. One of them is that the excreta materials contain amino acids originating from both excreta and urine. The other drawback is, the excreta methods do not correct for amino acids, originating from microorganisms present in hindgut. However, this can be prevented when caecectomised cockerels are used (Parson 1986). As far as animal welfare is concerned, the precision feeding method may not be considered a normal feeding method because the birds are fasted for 21 h and then are force-fed. Furthermore, feeding only the test ingredient may influence the digestive process creating problems in enzyme secretion. Therefore, resulting amino acid digestibility values may be inaccurate. Moreover, the excreta amino acid digestibility values determined using the precision method with adult cockerels, cannot be applied to growing birds because the birds had different physiological states. Unlike the total excreta collection method, precision feeding corrects for amino acids from an endogenous origin. However, this is criticized and may be inaccurate, as during fasting, the experience is an abnormal physiological condition. To overcome the drawbacks in precision feeding and total excreta collection methods, a new approach is to use the ileal digestibility assay because it evaluates amino acid disappearance at a location where the bird's ability to absorb is greatest. Ileal digesta can either be collected by euthanizing birds and flushing with distilled water (Siriwan et al. 1993, Ravindran et al. 1999) or through an intestinal cannula (Siriwan et al. 1993). When intestinal cannula is used the same bird can be used to test several test diets (Siriwan et al. 1993). However, this

method needs surgical skills to insert the fistula. Moreover, it is required to continue the collection of ileal contents for several hours in order to obtain a sufficient amount of ileal contents. However, when the birds are euthanized, digesta can easily be removed by flushing with distilled water and researchers can collect enough digesta for lab analysis by pooling the digesta samples from several birds which are kept on the same treatment (Siriwan et al. 1993, Ravindran et al. 1999). In an ileal digestibility assay, it is difficult to collect the digesta quantitatively. Therefore, in this approach, an indigestible marker which has a 100% recovery, has been used in digestibility experiments (Siriwan et al. 1993, Ravindran et al. 1999, Zanella et al. 1999, Almeida et al. 2013). To determine the ileal amino acid digestibility, the amino acid content in digesta and diet must be compared with the indigestible marker concentrations in digesta and diet (Ravindran et al. 1999, Zanella et al. 1999). Chromic oxide is often used as an indigestible marker (Zanella et al. 1999, Almeida et al. 2013). In the ileal amino acid digestibility assay, the endogenous amino acid losses are taken into consideration (Siriwan et al. 1993, Almeida et al. 2013). The basal endogenous amino acid losses can either be determined by feeding protein-free diets (Perez et al. 1993, Fernandez-Figares et al. 2002, Adedokun et al. 2008) or feeding totally digestible casein (Adedokun et al. 2008). The standardized ileal amino acid digestibility is calculated using apparent ileal amino acid digestibility, basal endogenous amino acid losses and amino acid content of the raw material (Lemme et al. 2004). The standardized ileal digestible amino acid content of raw material is calculated using standardized ileal amino acid digestibility and amino acid content in raw material (Lemme et al. 2004). The standardized ileal digestible amino acid content of a feed ingredient indicates the amount of each amino acid remaining at the end of ileum. Therefore, through this analysis, the

available amino acid content of a particular feed ingredient is evaluated. To formulate feed for poultry, the standardized ileal digestible amino acids content of feed ingredients should be used rather than the total amino acid contents (Lemme et al. 2004). The standardized ileal digestibility concept is more precise and accurate, as it determines the ileal amino acid digestibility of feed ingredients using growing chickens, with a normal feeding behavior. The method is not significantly affected by the amino acids of caecal, endogenous and urinary origins.

Ravindran et al. (1999) have conducted an experiment using broiler chickens to compare the ileal and excreta amino acid digestibility of feed ingredients. The birds were fed test diets from Day 35 to day 42. The total excreta materials were collected during final 4 days of the experiment. Finally, the birds were killed at Day 42 and ileal contents were collected. The excreta and ileal amino acid digestibilities were determined using the acid-insoluble ash marker. The mean excreta and ileal amino acid digestibility of corn and sorghum samples were not different ($P>0.05$) (Table 2.9). However, in wheat, the mean ileal amino acid digestibility was higher ($P<0.05$) than the mean excreta amino acid digestibility (Table 2.9). In one of the soybean meal samples, the overall mean of ileal amino digestibility was higher ($P<0.05$) than the mean of excreta amino acid digestibility (Table 2.9). There was no difference ($P>0.05$) in overall mean of ileal amino digestibility and excreta amino acid digestibility in canola meal (Table 2.10).

In these meals, there were differences ($P<0.001$, $P<0.01$ and $P<0.05$) in some of the ileal and excreta individual amino acids. For example, in wheat, ileal amino acid digestibility (ID) of threonine, alanine and tyrosine ($P<0.01$); valine, methionine, lysine, arginine, serine and glutamic acid ($P<0.05$) were higher than that of excreta assay (Table 2.11).

Table 2.10 Mean apparent ileal and excreta amino acid digestibilities (%) of feed ingredients for broiler chickens.

Feedstuff	IAAD	EAAD	SEM	Probability
Wheat	81	68	0.012	P<0.05
Maize	82	81	0.011	NS
Sorghum	79	74	0.021	NS
Soybean meal 1	85	84	0.001	P<0.05
Soybean meal 2	83	84	0.006	NS
Soybean meal 3	83	83	0.003	NS
Canola meal	77	76	0.033	NS

Source: Ravindran et al. 1999
IAAD = Ileal amino acid digestibility; EAAD = Excreta amino acid digestibility
SEM = Standard error mean
NS = Not significant

In corn, ID of valine (P<0.01), isoleucine (P<0.05), glutamic acid (P<0.05) and alanine (P<0.001) were higher than the corresponding excreta amino acid digestibility (ED) (Table 2.12). When the soybean meal sample was considered; ID of threonine (P<0.05), valine (P<0.05), leucine (P<0.05), phenylalanine (P<0.05), aspartic acid (P<0.01); isoleucine (P<0.01), alanine (P<0.01) and serine (P<0.001) were higher than amino acid digestibility determined using excreta analysis (Table 2.13). There was no difference in ID and ED of canola meal (Table 2.14). Since excreta materials contain amino acids originating from both excreta and urine and the excreta method does not correct for amino acids, originating from microorganisms present in hindgut, the amino acid digestibility values will not be accurate. It should be noted that the differences in ileal and excreta amino acid digestibility would vary with the individual amino acid and the type of the feed ingredient being evaluated.

Table 2.11 Apparent ileal and excreta amino acid digestibilities (%) of wheat for broiler chickens.

Amino acid	IAAD	EAAD	SEM	Probability
Threonine	69	49	0.003	P<0.01
Valine	81	66	0.011	P<0.05
Methionine	85	75	0.002	P<0.05
Isoleucine	84	70	0.015	P = 0.064
Leucine	86	75	0.013	P = 0.068
Phenylalanine	87	77	0.010	P = 0.059
Histidine	83	65	0.027	P = 0.088
Lysine	77	60	0.014	P<0.05
Arginine	81	74	0.005	P<0.05
Aspartic acid	75	57	0.018	P = 0.055
Serine	81	70	0.007	P<0.05
Glutamine	94	88	0.004	P<0.05
Alanine	79	54	0.035	P<0.01
Tyrosine	73	68	0.001	P<0.01

Source: Ravindran et al. 1999
IAAD = Ileal amino acid digestibility; EAAD = Excreta amino acid digestibility
SEM = Standard error mean

Table 2.12 Apparent ileal and excreta amino acid digestibilities (%) of corn for broiler chickens.

Amino acid	IAAD	EAAD	SEM	Probability
Threonine	62	69	0.027	NS
Valine	82	76	0.006	P<0.01
Methionine	88	89	0.022	NS
Isoleucine	84	76	0.012	P<0.05
Leucine	91	90	0.005	NS
Phenylalanine	87	84	0.011	NS
Histidine	-	-	-	-
Lysine	74	75	0.004	P = 0.094
Arginine	86	85	0.009	NS
Aspartic acid	76	76	0.012	NS
Serine	75	80	0.022	NS
Glutamine	90	88	0.004	P<0.05
Alanine	88	83	0.001	P<0.001
Tyrosine	78	79	0.019	NS

Source: Ravindran et al. 1999
IAAD = Ileal amino acid digestibility; EAAD = Excreta amino acid digestibility
SEM = Standard error mean

Table 2.13 Apparent ileal and excreta amino acid digestibilities (%) of soybean meal for broiler chickens.

Amino acid	IAAD	EAAD	SEM	Probability
Threonine	77	79	0.005	P<0.05
Valine	84	79	0.011	P<0.05
Methionine	89	88	0.005	P = 0.094
Isoleucine	86	81	0.003	P<0.01
Leucine	85	84	0.002	P<0.05
Phenylalanine	86	85	0.002	P<0.05
Histidine	-	-	-	-
Lysine	86	86	0.007	NS
Arginine	89	91	0.006	NS
Aspartic acid	81	85	0.002	P<0.01
Serine	81	85	0.003	P<0.001
Glutamine	87	87	0.006	NS
Alanine	83	74	0.006	P<0.01
Tyrosine	86	85	0.003	P = 0.059

Source: Ravindran et al. 1999
IAAD = Ileal amino acid digestibility; EAAD = Excreta amino acid digestibility
SEM = Standard error mean

Table 2.14 Apparent ileal and excreta amino acid digestibilities (%) of canola meal for broiler chickens.

Amino acid	IAAD	EAAD	SEM	Probability
Threonine	65	66	0.045	NS
Valine	73	71	0.038	NS
Methionine	91	90	0.018	NS
Isoleucine	75	73	0.033	NS
Leucine	78	77	0.034	NS
Phenylalanine	79	77	0.030	NS
Histidine	77	76	0.038	NS
Lysine	76	75	0.035	NS
Arginine	83	85	0.028	NS
Aspartic acid	70	72	0.046	NS
Serine	67	70	0.047	NS
Glutamine	84	83	0.028	NS
Alanine	78	74	0.011	NS
Tyrosine	75	75	0.023	NS

Source: Ravindran et al. 1999
IAAD = Ileal amino acid digestibility; EAAD = Excreta amino acid digestibility
SEM = Standard error mean

2.7 Focus of the literature

Since demand for bio-fuel is rapidly expanding throughout the world (International Energy Agency 2011), there is an interest to find alternative oilseed crops to extract oil. Currently, there is a renewed interest in *Camelina sativa* (Bernardo et al. 2003, Moser and Vaughn 2010) and *Brassica carinata* as sources of biofuel, since camelina (Zubr 1997) and carinata (Getinet et al. 1995) seeds contain high amounts of oil. Although the oil content (Nelson et al. 1987) of soybean seeds is comparatively lower than camelina (Zubr 1997) and carinata (Getinet et al. 1995) seeds, soybeans occupied 56% of the world oilseed production in 2013 (American Soybean Association 2014).

When bio-fuel is produced on a small-scale, camelina, carinata and soybean seeds can be mechanically-pressed. Today, people demand for mechanically-pressed oils for their consumption. Among camelina, carinata and soybean oil, carinata oil is not popular. Currently consumer demand is mainly for soybean and camelina oil. Mechanical pressing to extract oil produces mechanically-pressed meals. When there is a growing pressure on oil producers to produce oil for bio-fuels and human consumption, there may be mechanically-pressed meals in significant amounts. The high residual oil contents in mechanically-pressed camelina (Ryhanen et al. 2007 and Almeida et al. 2013) and soybean (Opapeju et al. 2006 and Powell et al. 2011) meals, suggest that these meals can be used as energy supplements for broiler chickens but will have different AME_n content. The high CP contents in mechanically-pressed camelina (Ryhanen et al. 2007, Pekel et al. 2009 and Almeida et al. 2013), soybean (Opapeju et al. 2006 and Powell et al. 2011) and carinata (Getinet et al. 1995) meals showed that these mechanically-pressed meals meet the requirements for protein supplements for broiler chickens. However, when these meals are

introduced, it is necessary to look at the status of the anti-nutritional factors present in the meals. Soybean meal contains trypsin inhibitors (Birk and Gertler 1961, Liener and Tomlinson 1981) and lectins (Maenz et al. 1999, Fasina et al. 2003) while camelina (Matthaus and Zubr 2000, Ryhanen et al. 2007 and Almeida et al. 2013) and carinata (Getinet et al. 1995) meals contain glucosinolates as anti-nutritional factors. However, trypsin inhibitors and lectins can be inactivated by heat treatment (Nelson et al. 1987, Fasina et al. 2003), whereas glucosinolates can be degraded by heat treatment (Jensen et al. 1995) or water treatment (Tyagi 2002). Research on full-fat soybeans (Zanella et al. 1999) and solvent-extracted soybean meal (Parson et al. 1992) showed that heat affected the nutrient digestibility of diets and nutritive value of solvent-extracted soybean meal, respectively. Therefore, the nutritve value of mechanically-pressed camelina, carinata and soybean meals may change during heat treatment. Therefore, evaluation of the effect of heat treatment on the nutritive value of these meals is critical. Varying residual oil contents were observed in mechanically-pressed camelina (Ryhanen et al. 2007, Pekel et al. 2009 and Almeida et al. 2013) and soybean (Opapeju et al. 2006 and Powell et al. 2011) meals. Therefore, the nutritive value of mechanically-pressed meal may be affected due to the different residual oil content in the meals. The effect of residual oil level on the nutritive value of mechanically-pressed meals has not been described. Meng and Slominski (2005) and Zanella et al. (1999) found that enzyme supplementation improved the nutrient digestibility of diets. The effects of different enzymes like carbohydrase, lipase or protease on the nutritive value of mechanically-pressed camelina, carinata and soybean meals have not been identified. Although mechanically-pressed camelina, carinata and soybean meals were found to be good sources of protein and energy, it is not known how well these

nutients in high oil residue meals are utilized by broiler chickens. The nutritive value of these meals must be determined by means of AME_n and SIAAD for ration formulations.The amino acid digestibility in a feed ingredient can be determined either by the excreta or ileal digestibility methods (Ravindran et al. 1999). In a review, Lemme et al. (2004) pointed out that the majority of amino acid digestibility have been determined using the excreta method. Since excreta materials contain amino acids originating from both excreta and urine and the excreta method does not correct for amino acids, originating from microorganisms present in hindgut, the amino acid digestibility values may not be accurate. A new apparoach is to use the standardized ileal amino acid digestibility assay where the endogenous amino acid losses are taken into consideration (Siriwan et al. 1993, Almeida et al. 2013). The standardized ileal digestible amino acid content of a feed ingredient indicates the amount of each amino acid in the meal that disappears at the end of the ileum. Therefore, to formulate feed for poultry, the standardized ileal digestible amino acid contents of feed ingredients should be used rather than the total amino acid contents in the feed ingredient (Lemme et al. 2004) or the total tract digestibility estimate (Ravindran et al. 1999).

In this research, the nutritive value of mechanically-pressed camelina, carinata and soybean meals will be determined from AME_n and SIAAD determinations that include evaluating the effects of heat and enzyme treaments, using broiler chickens.

The effects of residual oil levels of mechanically-pressed carinata and soybean meals on production performance of broiler chickens have not been determined in the past. There are no officially recommended inclusion levels of mechanically-pressed soybean and carinata meals, established for starter, grower and finisher broiler diets. In this research,

the production performance of broiler chickens fed graded levels of mechanically-pressed camelina and soybean meals will be determined using diets formulated based on determined AME_n and SIAAD for the feed ingredients used in formulation.

CHAPTER 3: THE EFFECTS OF OIL LEVELS, HEATING AND ENZYMES ON THE NUTRITIVE VALUE OF MECHANICALLY-PRESSED CAMELINA (*CAMELINA SATIVA*) MEAL IN 21 DAY OLD BROILER CHICKENS

3.1 Abstract

This trial determined the effects of oil levels, heat treatment and enzymes on nitrogen-corrected apparent metabolizable energy (AME_n) and standardized ileal amino acid digestibility (SIAAD) of mechanically-pressed camelina meal (MPCM). The trial was a completely randomized design with a factorial arrangement of treatments: 2 oil levels (11.5 or 14.5%) x 2 heat treatments (heat or no-heat) x 4 enzyme treatments (carbohydrase, protease, lipase or no-enzyme), using 510 Ross-308 broilers (6 birds/cage and 5 replicates/treatment). AME_n was determined using excreta and diets. SIAAD were calculated using amino acid contents of diets and digesta. AME_n and SIAAD data were analyzed using Proc Mixed procedure of SAS. AME_n of 11.5% MPCM was greater ($P<0.05$) than 14.5% MPCM. Heated MPCM showed a lower ($P<0.05$) AME_n than non-heated meal. Carbohydrase improved ($P<0.05$) AME_n but AME_n was not improved by protease and lipase. Heat did not alter ($P>0.05$) SIAAD. The oil levels affected ($P<0.05$) some SIAAD but generally, enzymes improved SIAAD of MPCM. The AME_n of 1157 and 1004 $kcal \cdot kg^{-1}$ for 11.5 and 14.5% MPCM respectively (on an as-fed basis), would be used in practical broiler ration formulations.

Keywords: amino acid digestibility, broilers, camelina meal, energy, enzyme, heat, mechanical pressing

3.2 Introduction

Camelina seeds from winter and summer varieties contain 40 - 43% and 44% - 47% oil on a DM basis, respectively (Zubr 1997). Currently, there is renewed interest in *Camelina sativa* seed as a source of biofuel (Bernardo et al. 2003, Moser and Vaughn 2010) because of its high oil content. For *Camelina sativa*, mechanical pressing is the main method of extracting oil from the seeds. Oil from camelina seeds is mechanically-extracted using an expeller-press. The resultant by-product of this process is mechanically-pressed camelina meal (MPCM), with varying nutritional compositions. When compared to the solvent-extraction method, mechanical extraction leaves a greater oil content in the meal. The residual oil content of MPCM was found to be 17% (Ryhanen et al. 2007), 13.6% (Pekel

et al. 2009) or 11% (Almeida et al. 2013), on an as-fed basis. The high residual oil content left in the meal may be a good source of energy for broiler chickens. According to Ryhanen et al. (2007), Pekel et al. (2009) and Almeida et al. (2013), MPCM contained 35.6, 38 and 38% of CP on a DM basis, respectively. Although MPCM has the potential to be a protein and energy supplement in broiler diets, the nutritive value of MPCM still needs to be investigated in terms of AME_n and SIAAD content, which reflect how broiler chickens utilize MPCM. As heat treatment reduced the total glucosinolate content of rapeseed meal (Jensen et al. 1995), the same approach can be used to eliminate the glucosinolates present in MPCM. However, heat treatment may affect the nutritive value of camelina meal. Under practical oil extraction conditions, the oil content in MPCM can range from 11 to 17% on an as-fed basis (Ryhanen et al. 2007, Pekel et al. 2009 and Almeida et al. 2013). The different oil levels present in the meal may affect the nutritive value of MPCM. As enzyme supplementation improved the nutrient digestibility of corn-soybean diets in broiler chickens (Zanella et al. 1999, Meng and Slominski 2005), the same approach could be used to improve the nutritive value of MPCM. Therefore, it would be useful to determine how heat and enzyme treatments affect the nutritive value of MPCM with different residual oil levels in broiler chickens.

3.3 Objectives

1. To determine the effect of residual oil level (11.5 or 14.5%) in MPCM on AME_n, for broiler chickens
2. To determine the effect of residual oil level (11.5 or 14.5%) in MPCM on SIAAD, for broiler chickens

3. To determine the effect of heat on AME_n of MPCM with 11.5 or 14.5% residual oil, for broiler chickens
4. To determine the effect of heat on SIAAD of MPCM with 11.5 or 14.5% residual oil, for broiler chickens
5. To determine the effect of dietary enzyme supplementation (carbohydrase, protease, lipase or no-enzyme) on AME_n of MPCM with 11.5 or 14.5% residual oil, for broiler chickens
6. To determine the effect of dietary enzyme supplementation on SIAAD of MPCM with 11.5 or 14.5% residual oil, for broiler chickens

3.4 Hypotheses

1. AME_n of MPCM with 14.5% residual oil will be higher than MPCM with 11.5% residual oil.
2. SIAAD of MPCM with 11.5% residual oil will be higher than MPCM with 14.5% residual oil.
3. Heat will increase AME_n of MPCM with 11.5 or 14.5% residual oil.
4. Heat will decrease SIAAD of MPCM with 11.5 or 14.5% residual oil.
5. Enzyme supplementation will increase AME_n of MPCM with 11.5 or 14.5% residual oil.
6. Enzyme supplementation will increase SIAAD of MPCM with 11.5 or 14.5% residual oil.

3.5 Materials and methods

3.5.1 Preparation of 11.5% and 14.5% residual oil camelina meals

Camelina seeds grown in Atlantic Canada were cleaned to remove unwanted materials. Then, the seeds were pressed using an expeller-press (model KEK-500, Egon Keller GmbH & Co. KG, Germany) in Prince Edward Island.This process produced camelina oilseed cake. Finally, camelina oilseed cake was hammer-milled to produce MPCM with 13.1% residual oil. The crude oil which was expelled during the oil extraction process was collected and stored in a cool environment. The objective was to produce two oil meals with 11.5% and 14.5% residual oil levels, using 13.1% residual oil MPCM. One half of 13.1% residual oil meal was further pressed using a micro-scale oil press (Anton-fries vegetable oil press P500R, Maschinenbau GmbH, Meitingen-Herbertshofen, Germany) in order to produce 11.5% residual oil MPCM. Camelina crude oil was added to the other half of the 13.1% camelina meal and mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, USA) to produce 14.5% residual oil MPCM.

3.5.2 Preparation of heat-treated 11.5% and 14.5% residual oil camelina meals

Each 11.5% and 14.5% residual oil meal was divided equally with one half subjected to heat treatment at 100 °C for 30 min using a drying oven (model ST33ATUL208V9KW, JPW Design and Manufacturing, Trout Run, Pennsylvania, USA). The meal was placed and uniformly spread on stainless steel trays (87.9 cm x 87.9 cm x 2.55 cm) which were placed in the drying oven. Then, the oven was heated to 100 °C and maintained at that temperature for 30 min at which time the trays were removed and allowed to cool. After cooling to room temperature, the meal from the trays was transferred into Rubbermaid

containers. Finally, each heated oil meal was mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, USA) to blend the meal from all trays.

3.5.3 Preparation of grower diets

Four test meal ingredients (heated camelina meal with 11.5% residual oil, non-heated camelina meal with 11.5% residual oil, heated camelina meal with 14.5% residual oil, non-heated camelina meal with 14.5% residual oil) were each made into diets supplemented with four enzyme treatments; carbohydrase, protease, lipase and no enzyme to prepare sixteen test diets. A basal grower diet (Table 3.1) was prepared. Therefore, 17 treatments were tested. Test diets consisted of the basal diet at 69.5% and one of four test meal ingredients at 30% inclusion level. Each treatment diet contained 0.5% chromic oxide as an inert marker. All diets were mixed in a Hobart bowl type mixer (model L.800, The Hobart Manufacturing Co. Ltd, Don Mills, Ontario, Canada). The basal grower diet contained 20% crude protein and 2964 $kcal \cdot kg^{-1}$ AME_n (Table 3.1). The starter diet was formulated to contain 23% CP and 3050 $kcal \cdot kg^{-1}$ AME_n (Table 3.1). Both the starter and basal grower diets were formulated using MIXIT-WIN a professional feed formulation program (version 6.22, Agricultural Software Consultants, Inc.). The carbohydrase, protease and lipase enzymes were obtained from Genencor, Danisco Division, Denmark. Carbohydrase is a mixture of amylase and xylanase, while protease and lipase were pure enzymes.

Table 3.1 Composition of diets formulated to determine the nitrogen-corrected apparent metabolizable energy and standardized ileal amino acid digestibility of mechanically-pressed camelina meal (as fed basis).

		Grower Diets		
	Starter Diet	Basal	Test Diet (No enzyme)	Test diet (With enzyme)
Ingredients as fed basis (%)				
Corn	44.5	65.8	41.8	41.7
Soybean meal	38.7	30.2	24.3	24.3
Mechanically-pressed camelina[Z] meal	-	-	30.0	30.0
Wheat	10.0	-	-	-
Tallow-grease blend	3.2	-	-	-
Ground limestone	1.7	1.6	1.6	1.6
Mono-dicalcium phosphate	0.6	0.8	0.8	0.8
Chromic oxide	-	0.5	0.5	0.5
Enzyme[Y]	-	-	-	0.05
Vitamin/mineral premix[X]	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4
Methionine premix[W]	0.4	0.2	0.1	0.1
Total	100	100	100	100
Calculated Analysis				
AME_n (kcal/kg)	3050	2964	-	-
Protein %	23	20	-	-
Lysine %	1.4	1.1	-	-
Methionine %	0.6	0.4	-	-
Calcium %	1	0.9	-	-
Available Phosphorus %	0.5	0.4	-	-

[Z]Mechanically-pressed camelina meal: 11.5 or 14.5% residual oil

[Y]Enzyme (1000 g $tonne^{-1}$ feed): Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed (Genencor, A Danisco Division, Denmark)

[X]Starter premix (Amount per kilogram): Vitamin A ($1.00x10^9$ IU kg^{-1}), 1.56 g; Vitamin D3 premix ($3.00x10^7$ IU kg^{-1}), 16 g; Vitamin E ($5x10^5$ IU kg^{-1}), 10 g; Vitamin K (33%), 1.8 g; Riboflavin (80%), 1.9 g; DL Ca-pantothenate (45%), 6 g; Vitamin B12 (1000 mg kg^{-1}), 4.6 g; Niacin (98%), 6 g; Folic acid (3%), 26.6 g; Choline chloride (60%), 267 g; Biotin (400 ppm), 60 g; Pyridoxine (990000 mg kg^{-1}), 1 g; Thiamine (970000 mg kg^{-1}), 0.6 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 20.78 g; Copper sulfate (25%), 20 g; Selenium premix (1000 mg kg^{-1}), 14.85 g; Ethoxyquin (60%), 16.6 g; Ground corn, 401.31 g; Ground limestone, 100 g

[W]Methionine premix: DL-Methionine (50%), Ground corn (50%)

3.5.4 Animal husbandry

Five hundred and ten, Ross-308 male day old broiler chickens were obtained from Clark's Chick Hatchery Ltd, Burtts Corner, New Brunswick, Canada. The experiment was conducted in a controlled environment room at the Atlantic Poultry Research Center at Bible Hill, Nova Scotia, Canada. Upon the arrival of the chicks, light intensity and the temperature inside the room were maintained at 20 lux and 30 °C, respectively. When the birds were five days old, the light intensity was reduced from 20 lux to 15 lux. Then, the light intensity was reduced from 15 lux to 5 lux by 5 lux every 2 days until the birds reached 9 days old. Five lux was maintained until the end of the experiment (21 days). On the first day, twenty four hours of light was provided. From 4 days of age, 16 h of light and 8 h of darkness were provided until chicks were 21 days of age.

The temperature inside the room was reduced from 30 °C to 28 °C when the chicks were 4 days old and maintained at 28 °C until the birds were 13 days old. When the birds were 13 days old, the temperature was reduced to 26 °C and maintained at that temperature until the birds reached 15 days old. The temperature was reduced to 25 °C until the birds became 18 days old. At this stage, the temperature was reduced to 24 °C. When the birds were 20 days old, the temperature was reduced to 23 °C until the end of the experiment (21 days).

The birds were weighed and randomly assigned to 85 cages with six birds per cage at Day 1. A standard starter diet was fed to all birds from Day 1 to Day 14. From Day 15 to 21 days of age, the cages were randomly assigned to one of the control diet or sixteen test diets with five replicate cages per treatment. Feed was provided *ad libitum* from feed troughs and was measured into the feeders as needed. Remaining feed in the feeders was weighed on Days 15 and 21. At 0, 14, and 21 days of age, the birds from each cage were

weighed as a group. The feed consumption was measured to make sure that birds consumed meals during the experiment. The body weights were measured to make sure that no growth depression occurred due to feeding new meal ingredients. Water was provided *ad libitum* from a nipple system. Birds were monitored for physical and behavioral changes daily, following the principles established by the Canadian Council on Animal Care (2009) under the guidance of the Dalhousie Animal Care and Use Committee.

3.5.5 Sample collection

At 19, 20 and 21 days of age, representative samples of clean excreta were collected from the cleaned trays underneath each cage. The excreta samples were frozen in a -20 °C freezer then freeze-dried (Section 3.5.6). At 21 days of age, all birds per cage were euthanized by cervical dislocation. The contents of the whole ileum (part of the small intestine from Meckel's diverticulum to 1 cm proximal to the ileal-cecal junction) (Adedokun et al. 2008) were gently flushed with distilled water into sample jars. The ileal contents per cage were pooled in a sample jar and frozen at -20 °C and then freeze-dried.

3.5.6 Chemical analysis

Two grams of test diet and camelina meal ingredient samples were separately weighed into aluminium dishes in triplicate using an analytical balance (Mettler-Toledo GmbH, Laboratory & Weighing Technologies, Greifensee, Switzerland). To determine the dry matter content, the samples were dried to constant weight in a drying oven (Iso temp 300 series, Fisher Scientific Company, Ottawa, Ontario, Canada) at 100 °C, for approximately 3 h. After 3 h, the dry matter content was calculated as described by the method 934.01 (AOAC 2005). Forty grams of excreta and ileal digesta content samples were separately weighed into aluminium dishes using a top loading balance (Denver Instrument Company,

Ltd, Arvada, Colorado, USA) and placed in the freezer (model MDF-U51VA, Sanyo Electric Biomedical Co., Ltd, Moriguchi, Osaka, Japan) at -20 °C. After freezing, the samples were freeze-dried using a freeze-dryer (MODULYOD-115, Thermo Fisher Scientific Company, Waltham, USA). All freeze-dried samples were ground using a coffee grinder (model 43-1964-8, Lancaster^{TM}, China). Nitrogen content (%) of test diets, test meal ingredients, excreta and ileal contents was determined by combusting the samples with pure oxygen as described by the method 990.03 (AOAC 2005) with a Leco Nitrogen Determinator (Leco FP-528, Leco Corporation, St. Joseph, Michigan, USA). EDTA was used for the calibration. The crude protein content (%) was calculated (N% x 6.25). Gross energy content of the dried excreta, test diet samples and test meal ingredients was determined using a Parr 6300 adiabatic bomb calorimeter (model IL 61265, Parr Instrument Company, Moline, Illinois, USA). Crude fat content of test meal ingredients and test diets was determined by extracting the samples with anhydrous ether as described by the method 920.39 (AOAC, 2005) with an ANKOM^{XT15} Extractor (model XT, Ankom Technology, Macedon, New York, USA). Chromic oxide content of test diets, dried ileal contents and excreta samples was determined with a perchloric acid digestion followed by a spectrophotometric measurement (Fenton and Fenton 1979), using a Bausch and Lomb Spectronic (model 501, Milton Roy Company, Ivyland, USA).

Amino acid concentrations in the dried ileal samples, test meal ingredients and test diets were determined as described by the method 994.12 (AOAC 2005) using high-performance liquid chromatography (Sykam Gmbh, Eresing, Germany). Amino acids; asparagine, threonine, serine, glutamine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine were analyzed using a regular acid hydrolysis

procedure (AOAC 2005). In this regular acid hydrolysis procedure, 100 mg of fine ground sample was weighed into a regular hydrolysis tube. Then, 4 mL of 6N phenolic hydrochloric acid was added to the hydrolysis tubes. The hydrolysis tubes containing samples were placed in a heating block and samples were digested at 110 °C for 24 h. After 24 h of digestion, tubes were cooled immediately and 4 mL of 25% w/v sodium hydroxide solution was added. When the tubes reached room temperature, all the neutralized solutions were separately transferred into 50 mL volumetric flasks. The tubes were rinsed with sodium citrate buffer (pH = 2.2) three times and flasks were made up to 50 mL volume with citrate buffer. Finally, about 10 mL of each solution was filtered into scintillation vials. The scintillation vials were labeled and kept in a freezer until the analysis was conducted.

Amino acids; methionine and cysteine were analyzed using an oxidized acid hydrolysis procedure (AOAC 2005). In this procedure, 2 mL of performic acid was added into hydrolysis tubes, each containing 100 mg of fine ground sample. The samples were digested at 110 °C for 16 h. The procedure after the digestion stage was the same as in the regular acid hydrolysis procedure.

Procedure used to analyze tryptophan (AOAC 2005), 50 mg of sample was weighed into each plastic tube. 0.25 mL of distilled water and 1 mL of 25% sodium hydroxide solution were added into each plastic tube containing a sample. The tubes were flushed with nitrogen and screw caps were tightened. Then, the tubes were placed in an autoclave at 120 °C overnight. After removing the tubes from the autoclave, 1 mL of 6N hydrochloric acid was added to each sample. Then, the mixture in the tubes was separately transferred into 25 mL volumetric flasks and the tubes were rinsed with sodium citrate buffer (pH = 4.25)

at least three times. The flasks were made up to 25 mL volume with sodium citrate buffer. Finally, 10 mL of each solution was filtered in a scintillation vial. The scintillation vials were labeled and kept in a freezer until the analysis was completed.

The glucosinolate content of heated and non-heated 11.5 and 14.5% MPCM was determined using high-performance liquid chromatography, according to the protocol of Dann and McGregor (1981), using benzyl glucosinolate as the internal standard**.**

3.5.7 Calculations

3.5.7.1 AME_n in diets and ingredients

AME_n of test diets, basal diets and MPCM ingredients were calculated using the equations from Leeson and Summers (2001) as follows.

AME_n of diets (Basal and test)

$$E\text{xcreta energy/g diet} = \text{Gross energy in excreta} \times \frac{\text{Chromic oxide diet}}{\text{Chromic oxide excreta}}$$

$$\text{N retained/g diet} = \text{Nitrogen g/g diet} - \left(\text{Nitrogen g/g excreta} \times \frac{\text{Chromic oxide diet}}{\text{Chromic oxide excreta}}\right)$$

$$\text{Nitrogen correction (N.C)} = \text{Nitrogen retained/g diet} \times 8.22$$

$$M\text{etabolizable energy content of diet} = \text{Gross energy in diet} - \left(\frac{\text{Excreta energy}}{\text{g diet}} + \text{N.C}\right)$$

$$A\text{MEn of test ingredients} = \text{AMEn of basal diet} - \frac{(\text{AMEn of basal diet} - \text{AMEn of test diet})}{0.3}$$

The content of chromic oxide in the diet and excreta was measured in $mg \cdot g^{-1}$ while gross energy in diets and excreta measured in $kcal \cdot g^{-1}$.

3.5.7.2 Ileal endogenous amino acid flow (IEAAF)

The IEAAF values were determined in a previous study (Bryan 2013), from birds fed a nitrogen-free diet. However, IEAAF can be calculated using the equations from Moughan et al. (1992).

$$\text{IEAAF of DMI (mg/kg)} = \text{(Amino acid in ileal digesta)} \times \frac{\text{Chromic oxide diet}}{\text{Chromic oxide ileal}}$$

Chromic oxide and amino acids in the diet and ileal digesta were measured in mg/kg.

3.5.7.3 Apparent ileal amino acid digestibility (AIAAD) and standardized ileal amino acid digestibility (SIAAD)

The AIAAD and SIAAD values were calculated using the equations (Lemme et al. 2004) as described below.

$$AIAAD, \% = 1 - \left(\frac{Chromic\ oxide\ in\ diet}{Chromic\ oxide\ in\ ileal\ digesta} \times \frac{Amino\ acid\ in\ digesta}{Amino\ acid\ in\ diet}\right)$$

$$SIAAD, \% = AIAAD\ \% + \left(\frac{IEAAF}{Amino\ acid\ content\ of\ the\ raw\ material, g/kg\ of\ DM} \times 100\right)$$

3.5.8 Statistical analysis

The experimental design was a completely randomized design with 2 x 2 x 4 factorial arrangement with two residual oil levels (11.5 or 14.5%), two heat treatments (heat or no-heat) and four enzyme treatments (no enzyme, carbohydrase, protease or lipase). The statistical model of the experiment was as follows.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} was the response variable (apparent DM digestibility, apparent CP digestibility, standardized ileal CP digestibility, AME_n and SIAAD).

μ was the overall mean of the response variable (apparent DM digestibility, apparent CP digestibility, standardized ileal CP digestibility, AME_n and SIAAD).

α_i was the effect of i^{th} level of residual oil in meal (11.5 or 14.5%).

β_j was the effect of j^{th} heat treatment (heat or no-heat).

γ_k was the effect of k^{th} enzyme (no enzyme, carbohydrase, protease or lipase).

$(\alpha\beta)_{ij}$ was the two-way interaction effect of i^{th} level of meal residual oil and j^{th} heat treatment

$(\alpha\gamma)_{ik}$ was the two-way interaction effect of i^{th} level of meal residual oil and k^{th} enzyme.

$(\beta\gamma)_{jk}$ was the two-way interaction effect of j^{th} heat treatment and k^{th} enzyme.

$(\alpha\beta\gamma)_{ijk}$ was the three-way interaction effect of i^{th} level of meal residual oil, j^{th} heat treatment and k^{th} enzyme.

ε_{ijkl} was the residual error.

The apparent DM digestibility, apparent CP digestibility, standardized ileal CP digestibility, AME_n and SIAAD data were subjected to analysis of variance using the Proc

Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). If main effects or interaction effects were significant ($P<0.05$), the least square means were compared ($\alpha = 0.05$) using the Tukey-Kramer option (Gbur et al. 2012).

3.6 Results and Discussion

3.6.1 Analyzed nutrient compositions of camelina test meal and diets

The analyzed CP content of the basal grower diet (20%) was similar to the calculated CP content (Table 3.1). The analyzed AME_n of basal grower diet was 131 $kcal \cdot kg^{-1}$ less than the calculated value (Table 3.1). The analyzed lysine and methionine contents of the basal diet were lower than the calculated analysis (Table 3.1) by 0.1 and 0.06%, respectively. In this experiment, the test diets containing 11.5% MPCM (Table 3.2) and 14.5% MPCM (Table 3.3) were not balanced for energy and CP to meet NRC (1994) recommendations. However, the basal diet was balanced for CP according to NRC (1994) recommendations (Table 3.1).

According to Ryhanen et al. (2007), camelina expeller cake contained 35.6% of CP on a DM basis. However, Pekel et al. (2009) and Almeida et al. (2013) found that the CP content of MPCM was 38% on a DM basis. Therefore, the CP content of MPCM ranged from 35.6 to 38%. When the CP content of MPCM reported in Table 3.3 was expressed on a DM basis, non-heated 11.5 and 14.5% MPCM contained 38 and 37% CP, respectively, which were in the range of previously reported values. Heat treatment was not sufficient to reduce the glucosinolates in 11.5 and 14.5% MPCM. Although the initial glucosinolate content in 11.5% MPCM (42.5 $\mu mol \cdot g^{-1}$) was reduced by 0.8 $\mu mol \cdot g^{-1}$ of meal with heating, it was

Table 3.2 Analyzed nutrient composition of diets (as fed basis) with 11.5% low oil camelina meal (LOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of meal using 21 day old broiler chickens.

	Basal	Diets with heated LOM				Diets with non-heated LOM			
		C	P	L	NE	C	P	L	NE
Analyzed nutrients									
DM (%)	89	91	91	91	91	90	90	91	91
CP (%)	20	25	25	26	26	26	27	25	26
AME_n ($kcal \cdot kg^{-1}$)	2833	2300	2256	2274	2229	2269	2237	2319	2312
Gross energy ($kcal \cdot kg^{-1}$)	3719	4051	4010	4052	4041	3984	3960	3955	3965
Methionine (%)	0.34	0.40	0.40	0.40	0.40	0.38	0.40	0.43	0.40
Lysine (%)	1.00	1.25	1.26	1.21	1.28	1.18	1.17	1.18	1.19
Threonine (%)	0.64	0.84	0.83	0.83	0.85	0.81	0.79	0.80	0.80
Valine (%)	0.73	0.93	0.92	0.88	0.93	0.94	0.85	0.84	0.98
Isoleucine (%)	0.61	0.71	0.72	0.69	0.72	0.72	0.65	0.63	0.73
Leucine (%)	1.53	1.71	1.70	1.67	1.74	1.67	1.63	1.63	1.68
Phenyl alanine (%)	0.89	1.04	1.03	1.01	1.05	1.00	0.97	0.97	1.01
Tryptophan (%)	0.21	0.23	0.23	0.24	0.23	0.23	0.24	0.24	0.23
Arginine (%)	1.20	1.70	1.70	1.67	1.69	1.65	1.57	1.64	1.68
Histidine (%)	0.58	0.71	0.71	0.71	0.73	0.67	0.69	0.70	0.67
Serine (%)	1.04	1.31	1.30	1.27	1.32	1.22	1.23	1.27	1.24
Glycine (%)	0.66	0.96	0.94	0.95	0.99	0.91	0.90	0.92	0.90
Asparagine (%)	1.89	2.31	2.29	2.25	2.35	2.16	2.18	2.22	2.17
Glutamine (%)	3.50	4.28	4.24	4.17	4.35	4.05	4.05	4.13	4.10
Proline (%)	1.52	1.72	1.74	1.69	1.74	1.71	1.73	1.73	1.72
Alanine (%)	0.93	1.15	1.12	1.09	1.13	1.09	1.08	1.12	1.15
Cysteine (%)	0.30	0.46	0.48	0.48	0.46	0.48	0.49	0.48	0.47
Tyrosine (%)	0.58	0.67	0.67	0.64	0.67	0.64	0.63	0.61	0.62
NH_3 (%)	0.34	0.44	0.43	0.42	0.45	0.42	0.43	0.42	0.43

C = carbohydrase, P = protease, L = lipase, NE = no-enzyme

Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed

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Table 3.3 Analyzed nutrient composition of diets (as fed basis) with 14.5% high oil camelina meal (HOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of camelina meal using 21 day old broilers.

	Diets with heated HOM				Diets with non-heated HOM			
	C	P	L	NE	C	P	L	NE
Analyzed nutrients								
DM (%)	90	90	90	90	89	90	90	89
CP (%)	26	26	25	26	25	25	26	25
AME_n ($kcal \cdot kg^{-1}$)	2310	2290	2295	2281	2311	2268	2312	2282
Gross energy ($kcal \cdot kg^{-1}$)	4008	4039	4032	4025	3934	3997	4007	3985
Methionine (%)	0.39	0.38	0.39	0.39	0.40	0.38	0.39	0.40
Lysine (%)	1.15	1.14	1.19	1.14	1.11	1.27	1.43	1.45
Threonine (%)	0.70	0.70	0.72	0.68	0.85	0.79	0.88	0.88
Valine (%)	0.94	0.96	0.97	0.91	0.94	1.05	1.26	1.07
Isoleucine (%)	0.69	0.70	0.70	0.67	0.85	0.78	0.95	0.81
Leucine (%)	1.56	1.58	1.66	1.55	1.88	1.78	2.01	1.98
Phenyl alanine (%)	0.94	0.95	0.98	0.93	1.00	1.07	1.23	1.19
Tryptophan (%)	0.25	0.25	0.25	0.28	0.25	0.26	0.26	0.23
Arginine (%)	1.62	1.62	1.66	1.59	1.69	1.79	1.99	1.95
Histidine (%)	0.60	0.59	0.61	0.59	0.49	0.68	0.73	0.77
Serine (%)	1.15	1.16	1.21	1.14	0.94	1.30	1.42	1.51
Glycine (%)	0.82	0.82	0.83	0.79	0.64	0.92	1.03	1.00
Asparagine (%)	2.00	2.00	2.09	1.97	1.61	2.27	2.55	2.62
Glutamine (%)	3.67	3.70	3.85	3.63	3.98	4.18	4.7	4.74
Proline (%)	1.36	1.36	1.46	1.36	1.13	1.53	1.70	1.77
Alanine (%)	1.02	1.05	1.08	1.02	1.02	1.16	1.30	1.30
Cysteine (%)	0.43	0.42	0.42	0.43	0.41	0.42	0.42	0.43
Tyrosine (%)	0.58	0.58	0.60	0.57	0.56	0.66	0.75	0.75
NH_3 (%)	0.46	0.46	0.46	0.44	0.49	0.49	0.53	0.51

C = carbohydrase, P = protease, L = lipase, NE = no-enzyme

Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed

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not a significant reduction (Table 3.4). However, the glucosinolate content in 14.5% MPCM (39.5 μmol·g^{-1}) was not reduced at all (Table 3.4). Although the oven temperature was set at 100 °C, the measured temperature in the meal was 80 °C, after 30 min. This processing condition might not be enough to destroy glucosinolates present in MPCM.

Table 3.4 Analyzed nutrient composition (as fed basis) of heated and non-heated LOM and HOM used to determine the effects of oil levels, heat and enzyme treatment on nutritive value camelina meal using 21 day old broilers.

	Heated LOM	Non-heated LOM	Heated HOM	Non-heated HOM
Analyzed nutrients				
DM (%)	97	94	97	93
CP (%)	38	38	37	37
Fat (%)	11.5	11.5	15.0	14.6
Glucosinolates (μmol·g^{-1})	41.7	42.5	39.5	39.5
Gross energy (kcal·kg^{-1})	4900	4659	4840	4696
Methionine (%)	0.54	0.48	0.46	0.48
Lysine (%)	1.72	1.73	1.71	1.60
Threonine (%)	1.27	1.25	1.20	1.14
Valine (%)	1.44	1.58	1.54	1.36
Isoleucine (%)	0.93	1.02	0.99	0.86
Leucine (%)	2.24	2.29	2.24	2.07
Phenyl alanine (%)	1.39	1.42	1.39	1.27
Tryptophan (%)	0.36	0.33	0.34	0.34
Arginine (%)	2.97	3.03	2.93	2.77
Histidine (%)	1.03	0.99	0.97	0.93
Serine (%)	2.00	1.96	1.91	1.85
Glycine (%)	1.62	1.58	1.52	1.44
Asparagine (%)	3.13	3.09	2.99	2.84
Glutamine (%)	6.08	6.04	5.86	5.59
Proline (%)	2.23	2.20	2.18	2.09
Alanine (%)	1.76	1.78	1.74	1.67
Cysteine (%)	0.68	0.72	0.70	0.62
Tyrosine (%)	0.85	0.86	0.77	0.77
NH_3 (%)	0.69	0.67	0.68	0.65

LOM = 11.5% low oil camelina meal, HOM = 14.5% high oil camelina meal

3.6.2 Apparent digestible nutrients

3.6.2.1 Apparent dry matter digestibility (%)

The apparent DM digestibility of MPCM (Table 3.5) was affected ($P<0.05$) by the three-way interaction among oil levels, heat treatment and enzyme supplementation (Table 3.5). The apparent DM digestibility of heated 11.5% MPCM was not affected ($P>0.05$) by any of the four enzyme treatments. When compared to carbohydrase enzyme, the protease enzyme improved ($P<0.05$) the digestible dry matter content of non-heated 11.5% MPCM. However, when compared to the no-enzyme treatment, none of the enzymes improved ($P>0.05$) the DM digestibility of non-heated 11.5% MPCM. For 14.5% MPCM, the heat + no-enzyme treatment combination gave the highest DM digestibility, which was greater ($P<0.05$) than that given by the no-heat + carbohydrase and no-heat + protease treatment combinations. For 11.5% MPCM, the heat + lipase combination showed the greatest DM digestibility, which was higher ($P<0.05$) than that in the no-heat + carbohydrase treatment combination.

Table 3.5 Effects of oil level, heat and enzymes on the apparent dry matter digestibility (%) of mechanically- pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	72±0.8 ab	71±0.8 bc	76±0.8 a	71±0.8 abc	74±0.6	71±0.6	73±0.4
Carbohydrase	72±0.8 abc	68±0.8 c	72±0.8 ab	73±0.8 bc	72±0.6	70±0.6	71±0.4
Protease	73±0.8 ab	72±0.8 ab	74±0.8 ab	71±0.8 bc	73±0.6	72±0.6	73±0.4
Lipase	74±0.8 ab	72±0.8 abc	75±0.8 ab	74±0.8 ab	75±0.6	73±0.6	74±0.4
Oil x Enzyme							
No Enzyme	72±0.6		74±0.6				
Carbohydrase	70±0.6		73±0.6				
Protease	72±0.6		73±0.6				
Lipase	73±0.6		75±0.6				
Oil	72±0.3		73±0.3				
Heat	74±0.3	72±0.3					
Oil x Heat	73±0.4	71±0.4	74±0.4	72±0.3			
Source of variation	Pr>F						
Oil	0.0002						
Heat	<.0001						
Oil x Heat	0.9052						
Enzyme	0.0012						
Oil x Enzyme	0.1700						
Heat x Enzyme	0.7807						
Oil x Heat x Enzyme	0.0034						

[a-d]Means ± SE in the oil x heat x enzyme interaction effect with no common letters are significantly different ($\alpha = 0.05$).

3.6.2.2 Apparent crude protein digestibility (%)

The apparent CP digestibility of MPCM was influenced ($P<0.05$) by the main effect, enzymes (Table 3.6). The heat treatment and meal residual oil level did not affect ($P>0.05$) the apparent CP digestibility of MPCM. When compared to the no-enzyme treatment, supplementation of carbohydrase and lipase enzymes ($P<0.05$) enhanced the apparent CP digestibility. However, the digestible CP contents reported for no-enzyme and protease supplemented meals, were not different ($P>0.05$). Moreover, similar ($P>0.05$) apparent CP digestibilities occurred in meals supplemented with either carbohydrase or lipase.

3.6.2.3 Nitrogen-corrected apparent metabolizable energy

The AME_n content of MPCM (Table 3.7) was ($P<0.05$) influenced by all three main effects; oil level, heat and enzyme treatments. None of the two-way or three-way interactions were found to be significant ($P>0.05$). Heat treatment negatively affected ($P<0.05$) the AME_n of MPCM. The 11.5% MPCM was higher ($P<0.05$) in AME_n content than 14.5% MPCM, fro which the reason was unclear. When compared to the no-enzyme treatment, the carbohydrase enzyme ($P<0.05$) improved the AME_n of MPCM. The addition of protease enzyme gave a lower ($P<0.05$) AME_n value, when compared to carbohydrase enzyme supplementation. However, the addition of lipase resulted in a similar ($P>0.05$) AME_n, when compared to both protease and carbohydrase supplementation.

Table 3.6 Effects of oil level, heat and enzymes on the apparent crude protein digestibility (%) of mechanically- pressed *Camelina sativa* meal in 21day old broilers (on a DM basis).

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	45±1	44±1	43±1	43±1	44±0.9	44±0.9	44±0.6 c
Carbohydrase	46±1	48±1	47±1	49±1	47±0.9	49±0.9	48±0.6 a
Protease	45±1	45±1	46±1	46±1	45±0.9	45±0.9	45±0.6 bc
Lipase	48±1	46±1	45±1	46±1	47±0.9	46±0.9	46±0.6 ab
Oil x Enzyme							
No Enzyme	45±0.9		43±0.9				
Carbohydrase	47±0.9		48±0.9				
Protease	45±0.9		46±0.9				
Lipase	47±0.9		45±0.9				
Oil	46±0.4		45±0.4				
Heat	45±0.4	46±0.4					
Oil x Heat	46±0.6	46±0.6	45±0.6	46±0.6			
Source of variation	Pr>F						
Oil	0.3542						
Heat	0.7162						
Oil x Heat	0.4436						
Enzyme	0.0003						
Oil x Enzyme	0.2234						
Heat x Enzyme	0.3726						
Oil x Heat x Enzyme	0.8566						

[a-e]Means ± SE in enzyme effect with no common letters are significantly different (α = 0.05).

Table 3.7 Effects of oil level, heat and enzymes on the nitrogen-corrected apparent metabolizable energy (kcal·kg^{-1}) content of mechanically- pressed *Camelina sativa* meal in 21day old broilers (on a DM basis).

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	1084±59	1289±59	1006±59	1094±59	1045±42	1191±42	1118±30b
Carbohydrase	1321±59	1307±59	1108±59	1209±59	1215±42	1258±42	1236±30a
Protease	1176±59	1180±59	1053±59	1021±59	1115±42	1101±42	1108±30b
Lipase	1194±59	1328±59	1070±59	1123±59	1132±42	1126±42	1179±30ab
Oil x Enzyme							
No Enzyme	1186±42		1050±42				
Carbohydrase	1314±42		1158±42				
Protease	1178±42		1037±42				
Lipase	1261±42		1097±42				
Oil	1235±21 a		1085±21 b				
Heat	1127±21b	1193±21 a					
Oil x Heat	1194±30	1276±30	1059±30	1112±30			
Source of variation	Pr>F						
Oil	<.0001						
Heat	0.0269						
Oil x Heat	0.6186						
Enzyme	0.0109						
Oil x Enzyme	0.9861						
Heat x Enzyme	0.2730						
Oil x Heat x Enzyme	0.5332						

[a-c]Means ± SE in the same main effect oil, heat or enzyme with no common letters are significantly different (α = 0.05).

A thorough search of the literature failed to identify any previous determination of AME_n for MPCM using growing broilers. However, Acamovic et al. (1999) conducted an experiment to determine the AME_n of MPCM (88% on a DM basis), using chickens weighing 3.2 kg. The ether-extract content of their camelina meal was 14.5% on a DM basis. The AME_n determined using excreta was found to be 1836 $kcal \cdot kg^{-1}$, on a DM basis. When this AME_n was expressed on an as-fed basis, it was 1616 $kcal \cdot kg^{-1}$ for the meal with a 12.8% ether-extract value (on as-fed). The AME_n of 11.5 and 14.5% MPCM reported in the current study were 1157 and 1004 $kcal \cdot kg^{-1}$ (on an as-fed basis) respectively, which were fairly low. This suggested that birds in this study were unable to utilize high residual oil meals efficiently at the age examined. This was supported by a low AME_n (1836 $kcal \cdot kg^{-1}$) of MPCM, determined using the precision method by Acamovic et al. (1999) using adult broiler type chickens.

The lower AME_n of MPCM might be due to the presence of condensed tannins in the meal. Longstaff and McNab (1991) found that apparent starch and lipid digestibility were reduced dramatically in young chicks fed a control diet substituted by field bean (*Vicia faba* L.) hulls rich in condensed tannins. The reduction in starch and lipid digestion was due to the inactivation of amylase and lipase, respectively by tannins forming tannin-enzyme complexes (Longstaff and McNab (1991). As carbohydrates and lipids are considered to be main energy sources, reduction in starch and lipid digestibility would cause poor AME_n in camelina meal. However, the condensed tannins content of camelina meal was not determined in the current study.

The lower AME_n in MPCM might be due to the presence of mucilage. Camelina seeds were found to contain 6.7% (on a DM basis) mucilage, which is considered to be a fine

water soluble fibre fraction (Zubr 2010). Since mucilage is water soluble, during the oil extraction process, it is concentrated in the meal. Camelina meal contains mucilage, which is a gummy material that increases the viscosity of the digesta, thereby potentially reducing the nutrient digestion and absorption. The energy containing nutrients will not be absorbed from the chick intestinal epithilum, resulting in lowered AME_n.

3.6.3 Ileal digestible nutrients

3.6.3.1 Standardized ileal crude protein digestibility (%)

The standardized ileal CP digestibility of MPCM was influenced ($P<0.05$) by three types of two-way interactions between oil levels and heat treatment, oil levels and enzyme treatment and heat and enzyme treatment (Table 3.8). Although the interaction between oil levels and heat treatment was significant ($P<0.05$), the Tukey-Kramer option did not differentiate among the interaction means. When the oil level and enzyme two-way interaction was considered, for 11.5% MPCM, both carbohydrase and protease enzymes improved ($P<0.05$) the standardized ileal digestible CP, when compared to no-enzyme and lipase treatments. However, there was no difference ($P>0.05$) in standardized ileal CP digestibility between the two 11.5% residual oil camelina meals, supplemented with either carbohydrase or protease. Moreover, addition of lipase did not improve ($P>0.05$) the standardized ileal digestible CP content, when compared to the no-enzyme treatment. For 14.5% MPCM, none of the enzyme treatments improved ($P>0.05$) the standardized ileal digestible CP content. When the heat and enzyme, two-way interaction was considered, in both heated meals, the standardized ileal CP digestibility improved ($P<0.05$) with carbohydrase supplementation, compared to the no-enzyme treatment, while no difference ($P>0.05$) was observed among carbohydrase, protease and lipase enzyme treatments. For non-heated meals, carbohydrase and protease were superior ($P<0.05$) to lipase. Moreover, in these non-heated meals, when compared to the no-enzyme treatment, the addition of enzymes did not improve ($P>0.05$) the standardized ileal digestible CP content. In this study, the standardized ileal CP digestibility in MPCM reported were greater than the apparent CP digestibility values (Table 3.6).

Table 3.8 Effects of oil level, heat and enzymes on the standardized ileal crude protein digestibility (%) of mechanically-pressed *Camelina sativa* meal in 21 day old broilers (on a DM basis).

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	53±1	55±1	57±1	57±1	55±0.8 b	56±0.8 ab	56±0.5
Carbohydrase	60±1	61±1	57±1	56±1	59±0.8 a	59±0.8 a	59±0.5
Protease	57±1	62±1	57±1	57±1	57±0.8 ab	60±0.8 a	58±0.5
Lipase	57±1	55±1	60±1	55±1	58±0.8 ab	55±0.8 b	57±0.5
Oil x Enzyme							
No Enzyme	54±0.8 d		57±0.8 bcd				
Carbohydrase	61±0.8 a		57±0.8 bcd				
Protease	60±0.8 ab		57±0.8bcd				
Lipase	55±0.8 cd		58±0.8 abc				
Oil	57±0.3		57±0.3				
Heat	57±0.3	57±0.3					
Oil x Heat	57±0.5	58±0.5	58±0.5	56±0.5			
Source of variation	Pr>F						
Oil	0.6426						
Heat	0.7568						
Oil x Heat	0.0025*						
Enzyme	0.0011						
Oil x Enzyme	<.0001						
Heat x Enzyme	0.0041						
Oil x Heat x Enzyme	0.5270						

[a-b]Means ± SE in the same group: oil x heat, oil x enzyme, heat x enzyme effects with no common letters are significantly different ($\alpha = 0.05$), *Tukey Kramer option did not differentiate among these means.

A similar trend was reported for amino acids by Ravindran et al. (1999), where for wheat and one of two soybean samples, the overall mean ileal amino acid digestibility was greater ($P<0.05$) than the mean of excreta amino acid digestibility. The standardized ileal CP digestibility concept is more precise, as it determines the ileal CP digestibility of feed ingredients by excluding the CP of caecal fermentation and endogenous and urinary origins.

3.6.3.2 Standardized ileal essential amino acid digestibility (%)

The standardized phenylalanine digestibility was affected (P<0.05) by enzyme and by the two-way interaction between oil levels and heat treatment (Table 3.9). Supplementation of carbohydrase improved (P<0.05) the ileal phenylalanine digestibility, when compared to no-enzyme treatment. However, no difference (P>0.05) was seen among carbohydrase, protease and lipase enzyme treatments. When the oil and heat two-way interaction was considered, the ileal phenylalanine digestibility for heated 11.5% MPCM was greater (P<0.05) than heated 14.5% MPCM. The ileal digestible phenylalanine content of heated and non-heated 11.5% MPCM was similar (P>0.05). A comparable trend was observed in heated and non-heated 14.5% MPCM.

The standardized ileal isoleucine digestibility of 11.5 and 14.5% MPCM was affected (P<0.05) by enzyme treatment (Table 3.10). The addition of lipase enhanced (P<0.05) the ileal digestible isoleucine content of meals, when compared to no-enzyme and protease treatment. No difference (P>0.05) was oberved between carbohydrase and lipase additions.

The standardized ileal arginine digestibility was affected (P<0.05) by oil and enzyme main effects (Table 3.11). Heat treatment did not influence (P>0.05) the arginine digestibility. The ileal digestible arginine content in 11.5% MPCM was greater (P<0.05) than in 14.5% MPCM. When compared to the no-enzyme treatment, the addition of carbohydrase upgraded (P<0.05) the standardized ileal arginine digestibility, while no difference was found (P>0.05) among carbohydrase, protease and lipase enzyme treatments. However, there was a strong trend (P=0.052) in heat x enzyme, two-way interaction on standardized ileal arginine digestibility.

Table 3.9 Effects of oil levels, heat and enzymes on standardized ileal phenylalanine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	63±2	64±2	60±2	63±2	62±1	64±1	63±1 b
Carbohydrase	71±2	66±2	68±2	64±2	70±1	65±1	67±1 a
Protease	69±2	66±2	64±2	67±2	66±1	67±1	66±1 ab
Lipase	70±2	65±2	62±2	67±2	66±1	66±1	66±1 ab
Oil x Enzyme							
No Enzyme	64±1		62±1				
Carbohydrase	69±1		66±1				
Protease	67±1		66±1				
Lipase	68±1		65±1				
Oil	67±0.7		64±0.7				
Heat	66±0.7	65±0.7					
Oil x Heat	68±1 a	65±1 ab	64±1 b	65±1 ab			
Source of variation	Pr>F						
Oil	0.0180						
Heat	0.4777						
Oil x Heat	0.0219						
Enzyme	0.0182						
Oil x Enzyme	0.9529						
Heat x Enzyme	0.1500						
Oil x Heat x Enzyme	0.3181						

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

Table 3.10 Effects of oil levels, heat and enzymes on standardized ileal isoleucine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	70±3	68±3	70±3	70±3	70±2	69±2	70±2 b
Carbohydrase	73±3	73±3	74±3	71±3	74±2	72±2	73±2 ab
Protease	73±3	72±3	71±3	72±3	72±2	72±2	72±2 b
Lipase	76±3	75±3	78±3	79±3	77±2	77±2	77±2 a
Oil x Enzyme							
No Enzyme	69±2		70±2				
Carbohydrase	73±2		73±2				
Protease	72±2		71±2				
Lipase	76±2		79±2				
Oil	73±1		73±1				
Heat	73±1	73±1					
Oil x Heat	73±2	72±2	73±2	73±2			
Source of variation	Pr>F						
Oil	0.6928						
Heat	0.7348						
Oil x Heat	0.8654						
Enzyme	0.0058						
Oil x Enzyme	0.7730						
Heat x Enzyme	0.9812						
Oil x Heat x Enzyme	0.8224						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

Table 3.11 Effects of oil levels, heat and enzymes on standardized ileal arginine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	61±2	70±2	71±2	69±2	71±2	70±2	70±1 b
Carbohydrase	75±2	78±2	72±2	74±2	74±2	76±2	75±1 a
Protease	76±2	70±2	71±2	70±2	74±2	70±2	72±1 ab
Lipase	77±2	63±2	74±2	68±2	75±2	70±2	73±1 ab
Oil x Enzyme							
No Enzyme	71±2		70±2				
Carbohydrase	77±2		73±2				
Protease	73±2		71±2				
Lipase	74±2		71±2				
Oil	74±0.7 a		71±0.7 b				
Heat	73±0.7	71±0.7					
Oil x Heat	75±1	73±1	72±1	70±1			
Source of variation	Pr>F						
Oil	0.0305						
Heat	0.0711						
Oil x Heat	0.8439						
Enzyme	0.0231						
Oil x Enzyme	0.8099						
Heat x Enzyme	0.0517						
Oil x Heat x Enzyme	0.7121						

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

The standardized ileal leucine digestibility was affected (P<0.05) by oil and two-way interaction effect between heat and enzymes (Table 3.12). The 11.5% MPCM had a better (P<0.05) ileal digestible leucine content than 14.5% MPCM. When the interaction between heat and enzymes was considered, the heated meals supplemented with protease showed an improvement (P<0.05) in leucine digestibility, when compared to heated meals supplemented with either no-enzyme or lipase. However, there was no difference (P>0.05) in leucine digestibility between heated meals supplemented with either carbohydrase or protease. Addition of carbohydrase enhanced (P<0.05) the ileal digestible leucine content in non-heated meals when compared to those meals with no-enzyme, while no difference (P>0.05) was seen among carbohydrase, protease and lipase treatments.

The standardized ileal digestible lysine (Table 3.13), valine (Table 3.14) and histidine (Table 3.15) contents of 11.5 and 14.5% MPCM were affected (P<0.05) by enzyme treatment. The heat treatment did not (P>0.05) alter the ileal lysine, valine and hisitidine digestibility. When compared to the no-enzyme treatment, the addition of carbohydrase gave higher (P<0.05) lysine, valine and histidine digestibilities. However, for each amino acid, the digestibility was similar (P>0.05) among carbohydrase, protease and lipase treatments.

The standardized ileal methionine digestibility was influenced (P<0.05) by the main effect, enzyme treatment (Table 3.16). There were no (P>0.05) oil and heat main effects on methionine digestibility. Supplementation of protease and lipase enriched (P<0.05) the methionine digestibility at the distal ileum when compared to meal with no-enzyme. Carbohydrase supplementation did not improve (P>0.05) methionine digestibility when

Table 3.12 Effects of oil levels, heat and enzymes on standardized ileal leucine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	63±2	66±2	63±2	62±2	63±1 b	64±1 b	64±1
Carbohydrase	66±2	68±2	66±2	70±2	66±1 ab	69±1 a	68±1
Protease	72±2	67±2	66±2	64±2	69±1 a	66±1 ab	67±1
Lipase	65±2	69±2	63±2	66±2	64±1 b	68±1 ab	66±1
Oil x Enzyme							
No Enzyme	65±1		62±1				
Carbohydrase	67±1		68±1				
Protease	70±1		65±1				
Lipase	67±1		65±1				
Oil	67±0.5 a		65±0.5 b				
Heat	66±0.5	67±0.5					
Oil x Heat	67±0.7	68±0.7	65±0.7	66±0.7			
Source of variation	Pr>F						
Oil	0.0096						
Heat	0.1782						
Oil x Heat	0.9094						
Enzyme	0.0013*						
Oil x Enzyme	0.1156						
Heat x Enzyme	0.0134						
Oil x Heat x Enzyme	0.4113						

[a-b]Means ± SE in the same group: oil and heat x enzyme effects with no common letters are significantly different (α = 0.05).
*Tukey Kramer option did not differentiate among these means.

Table 3.13 Effects of oil levels, heat and enzymes on standardized ileal lysine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	63±2	63±2	62±2	63±2	63±2	63±2	63±1 b
Carbohydrase	69±2	68±2	62±2	70±2	66±2	69±2	67±1 a
Protease	65±2	65±2	64±2	63±2	65±2	64±2	64±1 ab
Lipase	67±2	63±2	65±2	63±2	66±2	63±2	64±1 ab
Oil x Enzyme							
No Enzyme	63±2		62±2				
Carbohydrase	69±2		66±2				
Protease	65±2		64±2				
Lipase	65±2		64±2				
Oil	65±0.8		64±0.8				
Heat	65±0.8	65±0.8					
Oil x Heat	66±1	65±1	63±1	64±1			
Source of variation	Pr>F						
Oil	0.2531						
Heat	0.8852						
Oil x Heat	0.2252						
Enzyme	0.0496						
Oil x Enzyme	0.9621						
Heat x Enzyme	0.2711						
Oil x Heat x Enzyme	0.4814						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different ($\alpha = 0.05$).

Table 3.14 Effects of oil levels, heat and enzymes on standardized ileal valine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	59±3	60±3	61±3	62±3	60±2	61±2	61±1 b
Carbohydrase	62±3	68±3	69±3	66±3	66±2	67±2	66±1 a
Protease	63±2	68±3	65±2	65±3	64±2	66±2	65±1 ab
Lipase	67±3	63±3	65±3	64±3	66±2	64±2	65±1 ab
Oil x Enzyme							
No Enzyme	60±2		62±2				
Carbohydrase	65±2		67±2				
Protease	66±2		65±2				
Lipase	65±2		64±2				
Oil	64±1		64±1				
Heat	64±1	64±1					
Oil x Heat	63±1	65±1	65±1	64±1			
Source of variation	Pr>F						
Oil	0.6300						
Heat	0.7177						
Oil x Heat	0.3686						
Enzyme	0.0365						
Oil x Enzyme	0.7771						
Heat x Enzyme	0.6881						
Oil x Heat x Enzyme	0.5165						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

Table 3.15 Effects of oil levels, heat and enzymes on standardized ileal histidine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	59±2	59±2	61±2	62±2	60±2	60±2	60±1 b
Carbohydrase	66±2	65±2	64±2	66±2	65±2	66±2	65±1 a
Protease	62±2	62±2	65±2	65±2	63±2	64±2	63±1 ab
Lipase	63±2	63±2	65±2	64±2	64±2	63±2	64±1 ab
Oil x Enzyme							
No Enzyme	59±2		62±2				
Carbohydrase	66±2		65±2				
Protease	62±2		65±2				
Lipase	63±2		64±2				
Oil	62±0.8		64±0.8				
Heat	63±0.8	63±0.8					
Oil x Heat	62±0.8	62±0.8	64±0.8	64±0.8			
Source of variation	Pr>F						
Oil	0.1704						
Heat	0.8772						
Oil x Heat	0.8167						
Enzyme	0.0163						
Oil x Enzyme	0.7085						
Heat x Enzyme	0.9678						
Oil x Heat x Enzyme	0.9688						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

Table 3.16 Effects of oil levels, heat and enzymes on standardized ileal methionine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	70±2	69±2	69±2	69±2	70±2	69±2	69±1 b
Carbohydrase	76±2	74±2	72±2	70±2	74±2	72±2	73±1 ab
Protease	71±2	75±2	71±2	78±2	71±2	77±2	74±1 a
Lipase	74±2	77±2	73±2	71±2	74±2	74±2	74±1 a
Oil x Enzyme							
No Enzyme	69±2		69±2				
Carbohydrase	75±2		71±2				
Protease	73±2		74±2				
Lipase	76±2		72±2				
Oil	73±0.8		72±0.8				
Heat	72±0.8	73±0.8					
Oil x Heat	73±1	74±1	71±1	72±1			
Source of variation	Pr>F						
Oil	0.1249						
Heat	0.3833						
Oil x Heat	0.9089						
Enzyme	0.0173						
Oil x Enzyme	0.2150						
Heat x Enzyme	0.0971						
Oil x Heat x Enzyme	0.7187						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

compared to no-enzyme treatment. The methionine digestibility observed for either carbohydrase, protease or lipase did not differ ($P>0.05$).

The standardized ileal threonine (Table 3.17) digestibility was affected by oil and enzyme main effects. The lower residual oil meal (11.5%) gave a higher ($P<0.05$) threonine digestibility. The addition of either lipase or carbohydrase outperformed ($P<0.05$) the threonine digestibility when the meal was supplemented with no-enzyme. However, there was no change ($P>0.05$) in threonine digestibility among the carbohydrase, protease and lipase enzyme treatments.

The standardized ileal tryptophan digestibility was not affected ($P>0.05$) by any of the oil, heat and enzyme main effect or interaction effect (Table 3.18).

Table 3.17 Effects of oil levels, heat and enzymes on standardized ileal threonine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	61±2	63±2	58±2	61±2	59±2	62±2	61±1 b
Carbohydrase	65±2	69±2	61±2	64±2	63±2	66±2	65±1 a
Protease	63±2	66±2	60±2	62±2	62±2	64±2	63±1 ab
Lipase	68±2	66±2	62±2	64±2	65±2	65±2	65±1 a
Oil x Enzyme							
No Enzyme	62±2		59±2				
Carbohydrase	67±2		63±2				
Protease	65±2		61±2				
Lipase	67±2		63±2				
Oil	65±0.7 a		61±0.7 b				
Heat	62±0.7	64±0.7					
Oil x Heat	64±1	66±1	60±1	63±1			
Source of variation	Pr>F						
Oil	0.0018						
Heat	0.0512						
Oil x Heat	0.6301						
Enzyme	0.0183						
Oil x Enzyme	0.9066						
Heat x Enzyme	0.7108						
Oil x Heat x Enzyme	0.7335						

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

Table 3.18 Effects of oil levels, heat and enzymes on standardized ileal tryptophan digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	52±3	55±3	53±3	52±3	53±2	54±2	53±2
Carbohydrase	56±3	56±3	56±3	55±3	56±2	56±2	56±2
Protease	50±3	54±3	52±3	52±3	51±2	53±2	52±2
Lipase	50±3	57±3	51±3	53±3	50±2	55±2	53±2
Oil x Enzyme							
No Enzyme	53±2		53±2				
Carbohydrase	56±2		56±2				
Protease	52±2		52±2				
Lipase	53±2		52±2				
Oil	54±1		53±1				
Heat	53±1	54±1					
Oil x Heat	52±1	56±1	53±1	53±1			
Source of variation	Pr>F						
Oil	0.7682						
Heat	0.2839						
Oil x Heat	0.2839						
Enzyme	0.3985						
Oil x Enzyme	0.9901						
Heat x Enzyme	0.7396						
Oil x Heat x Enzyme	0.9754						

3.6.3.3 Standardized ileal non-essential amino acid digestibility (%)

The addition of either carbohydrase, protease or lipase improved ($P<0.05$) the ileal serine (Table 3.19) and asparagine (Table 3.20) digestibility, when compared to the no-enzyme treatment. Moreover, for each amino acid, no difference was observed ($P>0.05$) among carbohydrase, protease and lipase treatments. Neither oil level nor heat treatment influenced ($P>0.05$) the ileal serine and asparagine digestibility.

According to the analysis of variance, the enzyme main effect for cysteine digestibility (Table 3.21) was found to be significant ($P<0.05$). However, the Tukey-Kramer option did not differentiate the means. The ileal cysteine digestibility was not influenced by heat or oil effect.

The ileal glutamine digestibility of MPCM was affected ($P<0.05$) by the enzyme treatment, (Table 3.22). Although no difference among carbohydrase, protease and lipase enzyme treatments was seen, the lipase showed a better ($P<0.05$) ileal glutamine digestibility, when compared to no-enzyme treatment.

The glycine (Table 3.23), proline (Table 3.24) and tyrosine (Table 3.25) ileal digestibilities were not influenced ($P>0.05$) by the main effects or interaction effects. The standardized ileal NH_3 digestibility of 11.5% MPCM was greater than 14.5% MPCM (Table 3.26).

The ileal digestible alanine content of MPCM was influenced ($P<0.05$) by the enzyme main effect and the two-way interaction between oil and heat effects (Table 3.27). However, the Tukey-Kramer option did not differentiate between the two-way interactions means. The protease enzyme enhanced ($P<0.05$) the ileal alanine digestibility, when compared to no-enzyme treatment. However, the ileal alanine digestibility among carbohydrase, protease and lipase treatments did not differ ($P>0.05$).

Table 3.19 Effects of oil levels, heat and enzymes on standardized ileal serine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	67±2	69±2	68±2	69±2	69±0.9	70±0.9	69±0.6 b
Carbohydrase	71±2	72±2	72±2	70±2	74±0.9	71±0.9	72±0.6 a
Protease	71±2	69±2	68±2	67±2	73±0.9	70±0.9	72±0.6 a
Lipase	69±2	70±2	71±2	71±2	72±0.9	73±0.9	73±0.6 a
Oil x Enzyme							
No Enzyme	70±0.9		69±0.9				
Carbohydrase	73±0.9		71±0.9				
Protease	71±0.9		73±0.9				
Lipase	72±0.9		74±0.9				
Oil	71±0.5		72±0.5				
Heat	72±0.5	71±0.5					
Oil x Heat	72±0.6	71±0.6	72±0.6	71±0.6			
Source of variation	Pr>F						
Oil	0.5629						
Heat	0.1142						
Oil x Heat	0.9486						
Enzyme	0.0031						
Oil x Enzyme	0.0596						
Heat x Enzyme	0.0819						
Oil x Heat x Enzyme	0.3069						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

Table 3.20 Effects of oil levels, heat and enzymes on standardized ileal asparagine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	56±2	60±2	59±2	59±2	58±1	60±1	59±1 b
Carbohydrase	66±2	64±2	65±2	61±2	66±1	62±1	64±1 a
Protease	62±2	62±2	62±2	65±2	62±1	63±1	63±1 a
Lipase	68±2	64±2	64±2	62±2	66±1	63±1	65±1 a
Oil x Enzyme							
No Enzyme	58±1		59±1				
Carbohydrase	65±1		63±1				
Protease	62±1		64±1				
Lipase	66±1		63±1				
Oil	63±0.6		62±0.6				
Heat	63±0.6	62±0.6					
Oil x Heat	63±1	63±1	63±1	62±1			
Source of variation	Pr>F						
Oil	0.5916						
Heat	0.4085						
Oil x Heat	0.7325						
Enzyme	0.0001						
Oil x Enzyme	0.2224						
Heat x Enzyme	0.0603						
Oil x Heat x Enzyme	0.4324						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

Table 3.21 Effects of oil levels, heat and enzymes on standardized ileal cysteine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	60±2	61±2	58±2	62±2	59±1	62±1	60±1
Carbohydrase	66±2	64±2	62±2	64±2	64±1	64±1	64±1
Protease	66±2	65±2	63±2	61±2	65±1	63±1	64±1
Lipase	61±2	62±2	63±2	61±2	62±1	61±1	62±1
Oil x Enzyme							
No Enzyme	61±1		60±1				
Carbohydrase	65±1		63±1				
Protease	66±1		62±1				
Lipase	62±1		62±1				
Oil	63±0.7		62±0.7				
Heat	62±0.7	62±0.7					
Oil x Heat	63±1	63±1	61±1	62±1			
Source of variation	Pr>F						
Oil	0.1241						
Heat	0.9343						
Oil x Heat	0.6214						
Enzyme	0.0307*						
Oil x Enzyme	0.5135						
Heat x Enzyme	0.3810						
Oil x Heat x Enzyme	0.5753						

*Tukey Kramer option did not differentiate the enzyme treatments that were different in the ANOVA.

Table 3.22 Effects of oil levels, heat and enzymes on standardized ileal glutamine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	61±2	62±2	62±2	60±2	62±1	61±1	61±1 b
Carbohydrase	67±2	65±2	63±2	62±2	65±1	64±1	64±1 ab
Protease	65±2	62±2	62±2	66±2	64±1	64±1	64±1 ab
Lipase	67±2	64±2	63±2	67±2	65±1	66±1	65±1 a
Oil x Enzyme							
No Enzyme	62±1		61±1				
Carbohydrase	66±1		63±1				
Protease	64±1		64±1				
Lipase	66±1		65±1				
Oil	64±0.6		63±0.6				
Heat	64±0.6	64±0.6					
Oil x Heat	65±1	64±1	63±1	64±1			
Source of variation	Pr>F						
Oil	0.2589						
Heat	0.9636						
Oil x Heat	0.1174						
Enzyme	0.0281						
Oil x Enzyme	0.5649						
Heat x Enzyme	0.8204						
Oil x Heat x Enzyme	0.1869						

[a-b]Means ± SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

Table 3.23 Effects of oil levels, heat and enzymes on standardized ileal glycine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	64±2	63±2	63±2	64±2	64±1	64±1	64±1
Carbohydrase	65±2	65±2	62±2	63±2	63±1	64±1	64±1
Protease	64±2	62±2	63±2	65±2	63±1	63±1	63±1
Lipase	63±2	65±2	65±2	64±2	64±1	64±1	64±1
Oil x Enzyme							
No Enzyme	64±1		64±1				
Carbohydrase	65±1		63±1				
Protease	63±1		64±1				
Lipase	64±1		64±1				
Oil	64±0.6		64±0.6				
Heat	63±0.6	64±0.6					
Oil x Heat	64±1	64±1	63±1	64±1			
Source of variation	Pr>F						
Oil	0.6690						
Heat	0.8121						
Oil x Heat	0.5375						
Enzyme	0.9253						
Oil x Enzyme	0.5372						
Heat x Enzyme	0.9693						
Oil x Heat x Enzyme	0.5134						

Table 3.24 Effects of oil levels, heat and enzymes on standardized ileal proline digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	67±2	69±2	68±2	69±2	68±1	69±1	68±1
Carbohydrase	71±2	72±2	72±2	70±2	72±1	71±1	71±1
Protease	71±2	69±2	68±2	67±2	69±1	68±1	69±1
Lipase	69±2	70±2	71±2	71±2	70±1	71±1	70±1
Oil x Enzyme							
No Enzyme	68±1		68±1				
Carbohydrase	72±1		71±1				
Protease	70±1		68±1				
Lipase	70±1		71±1				
Oil	70±0.6		69±0.6				
Heat	70±0.6	70±0.6					
Oil x Heat	70±1	70±1	69±1	69±1			
Source of variation	Pr>F						
Oil	0.5186						
Heat	0.9263						
Oil x Heat	0.7116						
Enzyme	0.0834						
Oil x Enzyme	0.4832						
Heat x Enzyme	0.7560						
Oil x Heat x Enzyme	0.8365						

Table 3.25 Effects of oil levels, heat and enzymes on standardized ileal tyrosine digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	71±2	72±2	70±3	71±2	70±2	71±2	71±1
Carbohydrase	74±2	75±2	73±2	72±2	73±2	74±2	74±1
Protease	70±2	72±2	72±2	71±2	71±2	72±2	71±1
Lipase	72±3	73±2	72±2	73±2	72±2	73±2	73±1
Oil x Enzyme							
No Enzyme	71±2		70±2				
Carbohydrase	75±2		73±2				
Protease	71±2		71±2				
Lipase	72±2		73±2				
Oil	72±0.8		72±0.8				
Heat	71±0.8	72±0.8					
Oil x Heat	72±1	73±1	72±1	72±1			
Source of variation	Pr>F						
Oil	0.6111						
Heat	0.5138						
Oil x Heat	0.5138						
Enzyme	0.2969						
Oil x Enzyme	0.8712						
Heat x Enzyme	0.9962						
Oil x Heat x Enzyme	0.9203						

Table 3.26 Effects of oil levels, heat and enzymes on standardized ileal NH_3 digestibility coefficients of mechanically-pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	70±2	71±2	65±2	67±2	68±1	69±1	68±0.8
Carbohydrase	71±2	72±2	69±2	69±2	70±1	71±1	70±0.8
Protease	71±2	72±2	70±2	67±2	71±1	69±1	70±0.8
Lipase	68±2	73±2	67±2	65±2	68±1	69±1	68±0.8
Oil x Enzyme							
No Enzyme	71±1		66±1				
Carbohydrase	72±1		69±1				
Protease	71±1		68±1				
Lipase	71±1		65±1				
Oil	71±0.5 a		67±0.5 b				
Heat	69±0.5	70±0.5					
Oil x Heat	70±0.8	72±0.8	68±0.8	67±0.8			
Source of variation	Pr>F						
Oil	<.0001						
Heat	0.4565						
Oil x Heat	0.0946						
Enzyme	0.1705						
Oil x Enzyme	0.6228						
Heat x Enzyme	0.5327						
Oil x Heat x Enzyme	0.3262						

[a-b]Means ± SE in the oil main effect with no common letters are significantly different ($\alpha = 0.05$).

Table 3.27 Effects of oil levels, heat and enzymes on standardized ileal alanine digestibility coefficients of mechanically pressed *Camelina sativa* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	11.5% oil meal		14.5% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	61±2	63±2	61±2	62±2	60±1	62±1	61±1 b
Carbohydrase	64±2	64±2	66±2	63±2	65±1	64±1	64±1 ab
Protease	64±2	65±2	66±2	63±2	65±1	64±1	65±1 a
Lipase	61±2	63±2	64±2	61±2	63±1	62±1	62±2 ab
Oil x Enzyme							
No Enzyme	61±1		61±1				
Carbohydrase	64±1		65±1				
Protease	65±1		65±1				
Lipase	62±1		63±1				
Oil	63±1		63±1				
Heat	63±1	63±1					
Oil x Heat	62±1	64±1	64±1	62±1			
Source of variation	Pr>F						
Oil	0.7629						
Heat	0.5472						
Oil x Heat	0.0328*						
Enzyme	0.0192						
Oil x Enzyme	0.9674						
Heat x Enzyme	0.4205						
Oil x Heat x Enzyme	0.8775						

[a-b]Means ± SE in the enzyme with no common letters are significantly different (α = 0.05).

*Tukey Kramer option did not differentiate the oil x heat interaction effect that was different in the ANOVA.

In general, heating 11.5 and 14.5% MPCM at 100 °C for 30 min, did not affect SIAAD. The set temperature of 100 °C could not be achieved after 30 min and the processing conditions were not high enough to reduce or increase SIAAD of MPCM.

As MPCM is a new meal ingredient being introduced to the broiler feed industry, there is no information about SIAAD in broilers in the literature. However, Almeida et al. (2013) conducted research to determine the SIAAD of camelina expeller cake with 11.3% ether-extract content (as fed basis), using growing pigs. The SIAAD calculated by Almeida et al. (2013) (Chapter 2, Section 2.1.5, Table 2.2) for phenylalanine, arginine, leucine, lysine, methionine, tryptophan, valine, histidine, alanine, asparagine, glutamine, glycine and proline were greater than SIAAD of MPCM (11.5 or 14.5%) reported in the current study with broiler chickens. The serine SIAAD of 11.5 and 14.5% MPCM were higher than SIAAD reported by Almeida et al. (2013). The isoleucine and cysteine SIAAD observed in the two studies were similar. The threonine SIAAD for 11.5% MPCM was greater than SIAAD (Almeida et al. 2013), while in 14.5% MPCM, threonine digestibility was lower than SIAAD reported by Almeida et al. (2013). In both studies, methionine and arginine SIAAD were comparatively greater than other SIAAD. This suggests that for growing broiler chickens, MPCM is a good source of methionine, which is generally considered as the first limiting amino acid.

Another oil seed meal which is being fed to broiler chickens is mechanically-pressed canola meal, which is a by-product produced during the mechanical extraction of oil from canola (*Brassica napus*) seeds. *Brassica napus* and *Camelina sativa* belong to a common family, Brassicaceae. The SIAAD reported for expeller-extracted canola meal with an ether-extract value of 11.1% (on as-fed basis) (Woyengo et al. 2010), ranged from 73 to 87%. In the

current study, SIAAD for 11.5 and 14.5% MPCM ranged from 54 to 74% and 53 - 73%, respectively. Compared to canola, the SIAAD for arginine, methionine, valine, isoleucine, threonine, histidine, lysine, phenylalanine, serine, asparagine, glycine, alanine, cysteine, proline and tyrosine (Woyengo et al. 2010) were greater than those found in MPCM. Therefore, when compared to mechanically-pressed canola meal, for broiler chickens, MPCM is not as good a source of ileal digestible amino acids.

Camelina meal contained 1.0 to 2.4 $mg \cdot g^{-1}$ of condensed tannins (Matthaus and Zubr, 2000). The condensed tannins (12 $mg \cdot g^{-1}$) in field bean (*Vicia faba* L.) hulls were found to lower considerably the apparent amino acid digestibility in poultry diets (Longstaff and McNab 1991). Analysis of trypsin in digesta revealed that tannins inactivated trypsin enzymes which caused a reduction in apparent amino acid digestibility in young chicks, as a result of the formation of tannin-trypsin enzyme complexes (Longstaff and McNab 1991). As camelina meal was found to contain condensed tannins (1.0 - 2.4 $mg \cdot g^{-1}$) (Matthaus and Zubr 2000), lower SIAAD values might be expected for camelina meal in the current study. However, when compared to the level of condensed tannins in field bean hulls, the amount of tannins in camelina meal (Matthaus and Zubr, 2000) was very low. The condensed tannin content of MPCM was not determined for current experiment.

Mucilage present in linseed (75 g/kg DM basis) impaired the protein efficiency ratio, net protein ratio, nitrogen and amino acid retention in broiler chicks (Trevino et al. 2000). Therefore, the mucilage present in camelina meal might be a reason for lower SIAAD for MPCM in the current study, though the mucilage content was not determined for 11.5 and 14.5% MPCM.

Camelina expellers with 11.3% residual oil level (on as-is basis), were known to contain 18.4 TIU·mg^{-1} (on as-is basis) of trypsin inhibitor activity (Almeida et al. 2013). Trypsin inhibitors were found to suppress the pancreatic protease enzyme activity in the intestine and reduce the protein digestibility in the chicken intestine (Alumot and Nitsan 1961). Therefore, lower SIAAD of MPCM might be expected in the current study if the meal contained trypsin inhibitors. However, trypsin inhibitor activity was not determined for 11.5 and 14.5% MPCM in the current study.

For this experiment, the SIAAD for both essential and non-essential amino acids of MPCM ranged from 53 - 73%, regardless of the heat and enzyme treatments. This is in accordance with SIAAD for flaxseed (*Linum usitatissimum*), reported by Bandegan et al. (2011). According to Bandegan et al. (2011), SIAAD ranged from 55 - 68%, which were determined using broiler chickens at 21 day of age, using chromic oxide as an inert marker. MPCM behaved similar to flaxseed meal in terms of a source of SIAAD amino acids.

3.7 Conclusions

The AME_n of 11.5% MPCM was 1235 kcal·kg^{-1} while AME_n of 14.5% MPCM was 1085 kcal·kg^{-1} on a DM basis. Heated MPCM showed a lower AME_n (1127 kcal·kg^{-1} on a DM basis) than non-heated meal (1193 kcal·kg^{-1} on a DM basis). Carbohydrase improved AME_n (1236 kcal·kg^{-1}), compared to no-enzyme (1118 kcal·kg^{-1}) on a DM basis. Heat treatment did not affect SIAAD. Except for tryptophan, glycine, proline and tyrosine digestibility, the addition of enzymes generally improved SIAAD of MPCM. There was no effect of oil level on SIAAD, except for arginine, threonine and leucine, irrespective of heat and enzyme treatments. AME_n of MPCM improved with carbohydrase addition and heat reduced AME_n. However, SIAAD was enhanced with either carbohydrase, protease or

lipase addition. Since carbohydrase improved AME_n and SIAAD was enriched with either carbohydrase, protease or lipase addition, a mixture of carbohydrase, protease and lipase enzymes can be used to improve the nutritive value of MPCM with 11.5 and 14.5% residual oil. The combination of enzyme treatment may provide a synergistic interaction. The AME_n of 1157 and 1004 $kcal \cdot kg^{-1}$ (on an as-fed basis) for 11.5 and 14.5% MPSBM respectively, could be used in practical broiler ration formulations. The AME_n and IDAA of MPCM were summarized in Table 3.28 and Table 3.29.

Table 3.28 AME_n and IDAA of mechanically-pressed *Camelina sativa* meal (on a DM basis).

Oil level (%)	Heat treatment	Enzyme	AME_n	DM (%)	IDAA (%)				
					Methionine	Threonine	Leucine	Isoleucine	Tryptophan
11.5	Yes	NE	1084	97	0.40	0.79	1.46	0.67	0.19
		C	1321	97	0.44	0.85	1.53	0.70	0.21
		P	1176	97	0.41	0.82	1.66	0.70	0.19
		L	1194	97	0.43	0.89	1.49	0.74	0.18
	No	NE	1289	94	0.33	0.78	1.52	0.70	0.18
		C	1307	94	0.36	0.86	1.55	0.75	0.19
		P	1180	94	0.36	0.83	1.55	0.73	0.18
		L	1328	94	0.37	0.82	1.57	0.77	0.19
14.5	Yes	NE	1006	97	0.34	0.73	1.49	0.73	0.19
		C	1108	97	0.35	0.78	1.56	0.78	0.20
		P	1053	97	0.35	0.76	1.56	0.74	0.19
		L	1070	97	0.35	0.78	1.50	0.82	0.18
	No	NE	1094	93	0.36	0.76	1.39	0.66	0.20
		C	1209	93	0.37	0.79	1.57	0.66	0.21
		P	1021	93	0.41	0.78	1.45	0.67	0.20
		L	1123	93	0.37	0.80	1.50	0.74	0.20

NE = No-enzyme, C = carbohydrase, P = protease, L = Lipase
AME_n = Nitrogen-corrected apparent metabolizable energy
SIAAD = Standardized ileal amino acid digestibility
IDAA = Ileal digestible amino acid
DM = Dry matter

Table 3.29 IDAA of mechanically-pressed *Camelina sativa* meal (on a DM basis).

Oil level (%)	Heat treatment	Enzyme	DM (%)	IDAA (%)					
				Arginine	Valine	Histidine	Lysine	Phenylalanine	Cysteine
11.5	Yes	NE	97	2.18	0.89	0.62	1.12	0.91	0.42
		C	97	2.30	0.93	0.69	1.23	1.02	0.46
		P	97	2.33	0.94	0.65	1.16	0.99	0.46
		L	97	2.37	1.00	0.67	1.18	1.00	0.43
	No	NE	94	2.14	0.95	0.59	1.10	0.91	0.44
		C	94	2.38	1.07	0.65	1.18	0.94	0.46
		P	94	2.13	1.07	0.62	1.12	0.93	0.46
		L	94	2.15	1.00	0.62	1.09	0.93	0.44
14.5	Yes	NE	97	2.20	1.00	0.63	1.12	0.89	0.42
		C	97	2.24	1.12	0.66	1.12	1.00	0.46
		P	97	2.22	1.06	0.66	1.15	0.93	0.47
		L	97	2.28	1.06	0.63	1.17	0.89	0.42
14.5	No	NE	93	2.06	0.92	0.63	1.09	0.89	0.42
		C	93	2.24	0.98	0.67	1.23	0.90	0.43
		P	93	2.11	0.96	0.66	1.10	0.96	0.42
		L	93	2.05	0.95	0.65	1.10	0.95	0.41

NE = No-enzyme, C = carbohydrase, P = protease, L = Lipase
AME_n = Nitrogen-corrected apparent metabolizable energy
SIAAD = Standardized ileal amino acid digestibility
IDAA = Ileal digestible amino acid
DM = Dry matter

CHAPTER 4: THE EFFECTS OF OIL LEVELS, HEATING AND ENZYMES ON THE NUTRITIVE VALUE OF MECHANICALLY-PRESSED SOYBEAN (*GLYCINE MAX*) MEAL IN 21 DAY OLD BROILER CHICKENS

4.1 Abstract

This experiment determined the effects of oil levels, heat treatment and enzymes on nitrogen-corrected apparent metabolizable energy (AME_n) and standardized ileal amino acid digestibility (SIAAD) of mechanically-pressed soybean meal (MPSBM). The trial was a completely randomized design with a factorial arrangement of treatments: 2 oil levels (7 or 11%) x 2 heat treatments (heat or no-heat) x 4 enzyme treatments (carbohydrase, protease, lipase or no-enzyme), using 510 Ross-308 broilers (6 birds/cage and 5 replicates/treatment). AME_n was determined using excreta and diets. SIAAD were calculated using amino acid contents of diets and digesta. AME_n and SIAAD were analyzed using Proc Mixed procedure of SAS. The highest AME_n for 7% MPSBM occurred when the meal was supplemented with lipase, without heat treatment. The heat and carbohydrase enzyme treatment gave the highest AME_n for 11% MPSBM. AME_n of 1469 and 1283 $kcal \cdot kg^{-1}$ for 7 and 11% MPSBM respectively (on an as-fed basis), could be used in practical broiler ration formulations, regardless of the heat and enzyme treatments. Heating improved the SIAAD of 7 and 11% MPSBM. Addition of either carbohydrase, protease or lipase enhanced the SIAAD, except for serine and asparagine. The majority of SIAAD were improved with protease supplementation. Oil levels affected SIAAD of histidine, tryptophan, NH_3 and alanine.

Keywords: amino acid digestibility, broilers, energy, enzyme, heat**,** mechanical pressing, soybean meal

4.2 Introduction

Mechanical extraction of soybean oil is becoming popular in the oil industry for two main reasons. One of these is the increased consumer demand for mechanically-pressed soybean oil and the other reason is to produce oil for small-scale biofuel industries. These options exert pressure on soybean oil producers to have a continuous supply of mechanically-extracted soybean oil. The resultant by-product of mechanically extracting oil from soybean seeds is the MPSBM which lacks consistency in nutritional composition. Soybean meals from extruded-expelled and screw-pressed processing contained 7.2 and 6.3% of residual oil (as-fed basis), respectively (Wang and Johnson 2001). Higher residual oil

makes these soybean meals attractive energy supplements in broiler diets. Moreover, extruded-expelled and screw-pressed soybean meals contained CP contents of 42.5 and 43.2%, respectively, (as-fed basis) (Wang and Johnson 2001) thus maintaining soybean meal an attractive protein supplement for broiler chickens. However, the greater residual oil and protein content does not fully explain the nutritive value of MPSBM. MPSBM should be evaluated in terms of AME_n and SIAAD which reflects the extent MPSBM can be utilized by broiler chickens. Before feeding MPSBM to broiler chickens, the anti-nutritional factors present in the meal must be eliminated. Soybean meal was known to contain trypsin inhibitors (Birk and Gertler 1961, Liener and Tomlinson 1981), lectins (Maenz et al. 1999, Fasina et al. 2003) and oligosaccharides (Coon et al. 1990, Graham et al. 2002) as major anti-nutritional factors. Among these, the most important anti-nutritional factors are trypsin inhibitors and lectins. These trypsin inhibitors and lectins can be destroyed by heat treatment (Nelson et al. 1987, Fasina et al. 2003). However, applying heat may affect the nutritive value of MPSBM. According to Wang and Johnson (2001), different mechanical oil extraction procedures resulted in varying residual oil contents in the meal. The different residual oil contents in the meal may affect its nutritive value, as birds may utilize those meals differently. The nutrient digestibilities of diets containing solvent-extracted soybean meal, roasted full-fat soybeans and extruded full-fat soybeans (Zanella et al. 1999) were improved with an enzyme mixture which contained protease, xylanase and amylase. Moreover, supplementation of a multicarbohydrase enzyme mixture (xylanase, pectinase, glucanase, mannanase, cellulase and galactanase) enhanced the nutrient digestibility of a corn-soybean meal diet (Meng and Slominski 2005). Hence, the same approach can be used to improve the nutritive value of MPSBM. Therefore,

determining the effects of oil levels, heat and enzymes on the nutritive value of MPSBM would be worthwhile to ensure efficient use of MPSBM in broiler diets. Since AME_n and SIAAD are used in formulating diets for broilers, accurate knowledge of these in mechanically-pressed soybean meal is required before MPSBM will be readiliy accepted as a feedstuff for broilers.

4.3 Objectives

1. To determine the effect of residual oil level (7 or 11%) in MPSBM on AME_n, for broiler chickens.
2. To determine the effect of residual oil level (7 or 11%) in MPSBM on SIAAD, for broiler chickens.
3. To determine the effect of heat on AME_n of MPSBM with 7 or 11% residual oil, for broiler chickens.
4. To determine the effect of heat on SIAAD of MPSBM with 7 or 11% residual oil, for broiler chickens.
5. To determine the effect of dietary enzyme supplementation (carbohydrase, protease, lipase or no-enzyme) on AME_n of MPSBM with 7 or 11% residual oil, for broiler chickens.
6. To determine the effect of dietary enzyme supplementation on SIAAD of MPSBM with 7 or 11% residual oil, for broiler chickens.

4.4 Hypotheses

1. AME_n of MPSBM with 11% residual oil will be higher than MPSBM with 7% residual oil.

2. SIAAD of MPSBM with 7% residual oil will be higher than MPSBM with 11% residual oil.
3. Heat will increase AME_n in both 7 and 11% MPSBM.
4. Heat will decrease SIAAD in both 7 and 11% MPSBM.
5. Enzyme supplementation will increase AME_n in both 7 and 11% MPSBM.
6. Enzyme supplementation will increase SIAAD in both 7 and 11% MPSBM.

4.5 Materials and methods

4.5.1 Preparation of 7 and 11% residual oil soybean meals

Soybean seeds were pressed using an expeller-press (YZS-120 Mamoth Oilpress, Anyang Gemco, Henan, China) from Agri Bio Fuel Ltd, Truro, Nova Scotia to produce soybean oilseed cake. Soybean oilseed cake was hammer-milled to produce soybean meal with 9.3% residual oil. The crude soybean oil produced from the oil extraction process was collected and stored in a cool environment. However, the objective was to produce two different oil meals containing 7% or 11% residual oil levels from the 9.3% residual oil meal. This 9.3% residual oil meal could not be further pressed using a micro-scale oil press (Anton-fries vegetable oil press P500R, Maschinenbau GmbH, Meitingen-Herbertshofen, Germany). No additional oil could be extracted with this equipment to produce the 7% residual oil meal. Therefore, a portion of 9% residual oil soybean meal was solvent extracted. The solvent-extracted soybean meal was mixed with 9% residual oil soybean meal in a Marion mixer (Rapids Machinery Company, Marion, Iowa, United States) to reduce the residual oil content to 7% in the soybean meal blend. The solvent extraction process involved 0.8 kg of soybean meal placed in a 4 L beaker, then, petroleum ether (2.4 L) was added into the beaker at a 3:1 ratio (ether: soybean meal) in a fume hood and stirred

with a glass rod. The mixture was placed for 1 h in the fume hood and stirred every 15 min. After 1 h, the ether was poured off into a bottle in the fume hood. The wet pre-pressed soybean meal was placed on an absorbent pad on a tray in the fume hood, then, patted with another absorbent pad to remove excess ether. This was continued until the soybean meal appeared dry. The solvent-extracted soybean meal was placed on an absorbent pad in the fume hood to drain off the remaining solvent and left out overnight to air dry.

Crude soybean oil was added to 9% residual oil soybean meal and mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, United States) to produce 11% residual oil soybean meal.

4.5.2 Preparation of heat-treated 7 and 11% residual oil soybean meals

4.5.2.1 Urease assay

Urease assay (method Ba 9-58, American Oil Chemists Society, AOCS 1980) was conducted to determine a suitable time-temperature combination for the heat-treatment in order to eliminate the trypsin inhibitor in the soybean meal. A small quantity (0.200 ± 0.001 g) of finely ground soybean meal was placed into a test tube and 10 mL of buffered urea solution was added. The test tube was stoppered and swirled gently. Six replicates of each test sample and blank sample were prepared. All the test and blank sample test tubes were placed in a shaking water bath (model SW22, Julabo Inc, Allentown, Pennsylvania, USA) at 30 °C for 30 min. Both test and blank sample test tubes were swirled approximately every 5 min for 30 min. At 30 min, all sample test tubes were removed from the water bath and contents in the test tubes were swirled one last time. Then, all tubes were allowed to stand for a few minutes. Approximately 5 mL of the supernatant liquid from each test tube was transferred into a small labelled beaker. The pH of the supernatant liquid from the test

sample and blank sample was read using a pH meter (pH tester 20, Fisher Scientific Company, Ottawa, Ontario, Canada). The difference between the pH of the test sample and that of the blank sample was calculated as an index of urease activity (American Oil Chemists Society, AOCS 1980).

4.5.2.2 Determination of time-temperature combination for the heat treatment

Urease assay was conducted for the non-heat treated 7% and 11% residual oil soybean meal samples. The pH difference of test and blank sample in both non-heat treated 7% and 11% residual oil soybean meal samples was not in the acceptable range; 0.05 - 0.20 (American Feed Manufacturers Association 1979). Therefore, both soybean meals needed to undergo a heat process before being incorporated into the test diets. The 7 and 11% residual oil soybean meal samples were subjected to a series of time-temperature tests to select the best time-temperature combination, by placing in a drying oven (model ST33ATUL208V9KW, JPW Design Manufacturing, Trout Run, Pennsylvania, USA). After each heat treatment, the urease assay was conducted for both meals. This procedure was repeated until the pH difference was between 0.05 - 0.20. After a series of time-temperature combinations, 130 °C temperature for 30 min, was selected as the optimal heat process for the soybean meal to achieve inactivation of the trypsin inhibitors. The pH differences of 0.12 (for 11% residual oil meal) and 0.11 (for 7% residual oil meal) were the reason for selecting this time temperature combination.

4.5.2.3 Heat processing of 7% and 11% residual oil soybean meal ingredients

Each batch of 7 and 11% residual oil meals were divided into two halves, where one half was heat treated at 130 °C for 30 min using a drying oven (model ST33ATUL208V9KW, JPW Design Manufacturing, Trout Run, Pennsylvania, USA). The meal was uniformly

spread on stainless steel trays (87.9 cm x 87.9 cm x 2.55 cm) then placed in the drying oven. The oven was allowed to reach 130 °C and maintained at that temperature. The meal was heated at 130 °C for 30 min. After 30 min, the trays were removed from the drying oven and allowed to cool. The meal from the trays was transferred into Rubbermaid containers. Finally, the two heated oil meals were separately mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, United States).

4.5.3 Preparation of grower test diets

Heated and non-heated soybean meals with 7 and 11% residual oil were supplemented with one of four enzyme treatments; carbohydrase, protease, lipase or no enzyme to prepare sixteen test diets. A basal diet (Table 4.1) was prepared. Therefore, 17 diets were tested. The 16 test diets consisted of 69.5% basal diet and one of four test meal ingredients at 30% inclusion level. All grower diets contained 0.5% chromic oxide as an inert marker. All diets were mixed in a Hobart, bowl type, mixer (model L.800, The Hobart manufacturing Co. Ltd, Don Mills, Ontario, Canada). The basal grower diet was formulated to contain 20% CP and 2964 $kcal \cdot kg^{-1}$ AME_n energy (Table 4.1). The starter diet was formulated to 23% CP and 3050 $kcal \cdot kg^{-1}$ AME_n (Table 4.1). The starter and basal grower diets were formulated using the MIXIT-WIN professional feed formulation program (version 6.22, Agricultural Software Consultants, Inc.). The carbohydrase, protease and lipase enzymes were obtained from Genencor, Danisco Division, Denmark. Carbohydrase is a mixture of amylase and xylanase, while protease and lipase were pure enzymes.

Table 4.1 Composition of diets formulated to determine the nitrogen-corrected apparent metabolizable energy and standardized ileal amino acid digestibility of mechanically-pressed soybean meal (as-fed basis).

		Grower Diets		
	Starter Diet	Basal	Test Diet (No enzyme)	Test diet (With enzyme)
Ingredients as fed basis (%)				
Corn	44.5	65.8	41.8	41.7
Soybean meal	38.7	30.2	24.3	24.3
Mechanically-pressed soybean[Z] meal	-	-	30.0	30.0
Wheat	10.0	-	-	-
Tallow-grease blend	3.2	-	-	-
Ground limestone	1.7	1.6	1.6	1.6
Mono-dicalcium phosphate	0.6	0.8	0.8	0.8
Chromic oxide	-	0.5	0.5	0.5
Enzyme[Y]	-	-	-	0.05
Vitamin/mineral premix[X]	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4
Methionine premix[W]	0.4	0.2	0.1	0.1
Total	100	100	100	100
Calculated Analysis				
AME_n ($kcal \cdot kg^{-1}$)	3050	2964	-	-
Protein %	23	20	-	-
Lysine %	1.4	1.1	-	-
Methionine %	0.6	0.4	-	-
Calcium %	1	0.9	-	-
Phosphorus %	0.5	0.4	-	-

[Z]Mechanically-pressed soybean meal: 7 or 11% residual oil

[Y]Enzyme (1000 g $tonne^{-1}$ feed): Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed (Genencor, A Danisco Division, Denmark)

[X]Vitamin/mineral premix (Amount per kilogram): Vitamin A ($1.00x10^9$ IU kg^{-1}), 1.56 g; Vitamin D3 premix ($3.00x10^7$ IU kg^{-1}), 16 g; Vitamin E ($5x10^5$ IU kg^{-1}), 10 g; Vitamin K (33%), 1.8 g; Riboflavin (80%), 1.9 g; DL Ca-pantothenate (45%), 6 g; Vitamin B12 (1000 mg kg^{-1}), 4.6 g; Niacin (98%), 6 g; Folic acid (3%), 26.6 g; Choline chloride (60%), 267 g; Biotin (400 ppm), 60 g; Pyridoxine (990000 mg kg^{-1}), 1 g; Thiamine (970000 mg kg^{-1}), 0.6 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 20.78 g; Copper sulfate (25%), 20 g; Selenium premix (1000 mg kg^{-1}), 14.85 g; Ethoxyquin (60%), 16.6 g; Ground corn, 401.31 g; Ground limestone, 100 g

[W]Methionine premix: DL-Methionine (50%), Ground corn (50%)

4.5.4 Animal husbandry

Five hundred and ten, Ross-308 male, day-old, broiler chickens were obtained from Clark's Chick Hatchery Ltd, Burtts Corner, New Brunswick, Canada. The experiment was conducted in a controlled environment room at the Atlantic Poultry Research Center in Bible Hill, Nova Scotia. The temperature, light intensity and lighting schedule (lights hr on/off) inside the controlled environment room were previously described in Chapter 3, Section 3.5.4.

The birds were weighed and randomly assigned to 85 cages with six birds per cage at Day 1. A standard starter diet was fed to all birds from Day 1 to Day 14. From Day 15 to 21 days of age, the cages were randomly assigned to one of the basal diet or sixteen test diets with five replicate cages per treatment. Feed was provided *ad libitum* from feed troughs and was measured into the feeders as needed. Remaining feed in the feeders was weighed on Days 15 and 21. At 0, 14, and 21 days of age, the birds from each cage were weighed as a group. Water was provided *ad libitum* from a nipple system. Birds were monitored for physical and behavioral changes daily, following the principles established by the Canadian Council on Animal Care (2009) under the guidance of the Dalhousie Animal Care and Use Committee.

4.5.5 Sample collection

At 19, 20 and 21 days of age, representative samples of clean excreta were collected from the cleaned trays underneath each cage. The excreta samples were stored in a -20 °C freezer then freeze-dried (Chapter 3, Section 3.5.6). At 21 days of age, all birds per cage were euthanized by cervical dislocation. The contents in the whole ileum (part of the small intestine from Meckel's diverticulum to 1 cm proximal to the ileal-cecal junction)

(Adedokun et al. 2008) were gently flushed with distilled water into sample jars. The ileal contents per cage were pooled in a sample jar and frozen at -20 °C, then freeze-dried as described in (Chapter 3, Section 3.5.6).

4.5.6 Chemical analysis

Dry matter content of test diet and soybean meal ingredient samples were determined (method 934.01, AOAC 2005) as described in Chapter 3 Section 3.5.6. Excreta samples and ileal digesta contents were freeze-dried as described in Chapter 3 Section 3.5.6. All freeze-dried samples were ground using a coffee grinder (model 43-1964-8, Lancaster™, China). The CP content in test diets, test meal ingredients, excreta and ileal contents was determined as described in Chapter 3 Section 3.5.6. Gross energy content of the dried excreta, test diet samples and test meal ingredients was determined using a Parr 6300 adiabatic bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA). Chromic oxide content of test diets, dried ileal contents and excreta samples was determined with a perchloric acid digestion (Fenton and Fenton 1979) followed by a spectrophotometric measurement using a Bausch and Lomb Spectronic (model 501, Milton Roy Company, Ivyland, USA).

Amino acid concentrations in the dried ileal samples, soybean meal ingredients and test diets were determined using a high-performance liquid chromatography system (Sykam Gmbh, Eresing, Germany) (method 994.12, AOAC 2005) as described in Chapter 3 Section 3.5.6.

4.5.7 Calculations

AME_n of test diets, basal diets and soybean meal ingredients were calculated (Leeson and Summers, 2001) as described in Chapter 3 Section 3.5.7. AIAAD, SIAAD and IDAA values were calculated (Lemme et al. 2004) as described in Chapter 3 Section 3.5.7.

4.5.8 Statistical Analysis

The experimental design was a completely randomized design with 2 x 2 x 4 factorial arrangement with 2 residual oil levels (7 or 11%), 2 heat treatments (heat or no-heat) and 4 enzyme treatments (no enzyme, carbohydrase, protease or lipase). The statistical model of the experiment was as follows.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} was the response variable (apparent DM digestibility, apparent CP digestibility, standardized ileal CP digestibility, AME_n and SIAAD).

μ was the overall mean of the response variable (apparent DM digestibility, apparent CP digestibility, standardized ileal CP digestibility, AME_n and SIAAD).

α_i was the effect of i^{th} level of residual oil in meal (7 or 11%).

β_j was the effect of j^{th} heat treatment (heated or non-heated).

γ_k was the effect of k^{th} enzyme (no enzyme, carbohydrase, protease or lipase).

$(\alpha\beta)_{ij}$ was the two-way interaction effect of i^{th} level of meal residual oil and j^{th} heat treatment.

$(\alpha\gamma)_{ik}$ was the two-way interaction effect of i^{th} level of meal residual oil and k^{th} enzyme.

$(\beta\gamma)_{jk}$ was the two-way interaction effect of j^{th} heat treatment and k^{th} enzyme.

$(\alpha\beta\gamma)_{ijk}$ was the three-way interaction effect of i^{th} level of meal residual oil, j^{th} heat treatment and k^{th} enzyme.

ε_{ijkl} is the residual error.

The apparent DM digestibility, apparent CP digestibility, standardized ileal CP digestibility, AME_n and SIAAD data were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). If main effects or interaction effects were significant ($P<0.05$), the least square means were compared ($\alpha = 0.05$) using the Tukey-Kramer option (Gbur et al. 2012).

4.6 Results and Discussion

4.6.1 Analyzed nutrient compositions of soybean test meal and diets

The CP contents of 7 and 11% MPSBM ingredients ranged from 34 to 40%, on an as-fed basis (Table 4.2). The heated MPSBM showed greater CP contents than non-heated MPSBM, as with heat, CP got concentrated in the meals. The non-heated 7% MPSBM showed a higher CP content than non-heated 11% MPSBM, as with oil, CP got diluted in the meal. There was a 5% difference in CP contents between heated and non-heated 11% MPSBM, for which the reason was unknown. According to Powell et al. (2011) the CP content of MPSBM with 8.1% of residual oil level was 44.9%, on an as-fed basis. Opapeju et al. (2006) reported a CP content of 42.6% in MPSBM (9.5% residual oil), on an as-fed basis. Therefore, the CP contents of MPSBM reported in the current study were less than those reported by Powell et al. (2011) and Opapeju et al. (2006). The urease acitivities of non-heated 7 and 11% MPSBM were 0.56 and 0.58 (rise in unit pH), respectively (Table 4.2). When the two meals were heated at 130 °C for 30 min, the urease activities of 7 and 11% MPSBM dropped to 0.11 and 0.12 respectively, a reduction of 80 and 82%,

respectively. According to the American Feed Manufacturers Association (1979), the rise in unit pH of 0.05 to 0.20, was considered as a standard for the urease activity in a well processed soybean meal. This proved that the trypsin inhibitors and lectins in MPSBM were inactivated to a greater extent. The analyzed nutrient compositions of test diets containing 7% residual oil soybean meal and 11% residual oil soybean meal were shown in Table 4.3 and Table 4.4, respectively. The test diets containing MPSBM were not balanced for CP and energy. However, the basal diet was balanced for CP (Table 4.1). The analyzed CP content (Table 4.3) of the basal diet (21%) fed from 14 - 21 days, was greater than the calculated analysis (20%) (Table 4.1) while the analyzed AME_n (2858 $kcal \cdot kg^{-1}$) of the basal diet (Table 4.3) was less than the calculated analysis (2964 $kcal \cdot kg^{-1}$) (Table 4.1).

Table 4.2 Analyzed nutrient composition (on as-fed basis) of 7 and 11% mechanically-pressed soybean meals used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of soybean meal using 21 day old broilers.

	Heated LOM	Non-heated LOM	Heated HOM	Non-heated HOM
Analyzed nutrients				
DM (%)	98	91	94	90
CP (%)	40	38	39	34
Fat (%)	7.1	7.1	11.1	11.0
Urease activity (pH units)	0.11	0.56	0.12	0.58
Methionine (%)	0.51	0.48	0.45	0.43
Lysine (%)	2.37	2.28	2.34	2.13
Threonine (%)	1.53	1.41	1.41	1.27
Valine (%)	1.57	1.45	1.58	1.45
Isoleucine (%)	1.42	1.32	1.41	1.30
Leucine (%)	2.88	2.63	2.70	2.47
Phenylalanine (%)	1.83	1.75	1.73	1.57
Tryptophan (%)	0.42	0.39	0.34	0.35
Arginine (%)	2.87	2.67	2.72	2.47
Histidine (%)	1.15	1.09	1.11	0.99
Serine (%)	2.17	1.98	1.41	1.27
Glycine (%)	1.6	1.47	1.47	1.33
Asparagine (%)	4.37	3.95	4.05	3.69
Glutamine (%)	7.15	6.53	6.65	6.03
Proline (%)	2.13	2.10	2.03	1.96
Alanine (%)	1.62	1.47	1.50	1.38
Cysteine (%)	0.58	0.53	0.51	0.49
Tyrosine (%)	1.23	1.14	1.15	1.05
NH_3 (%)	0.73	0.69	0.69	0.65

LOM = 7% low oil soybean meal, HOM = 11% high oil soybean meal.

Table 4.3 Analyzed nutrient composition of diets (as fed basis) with 7% low oil soybean meal (LOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of meal using 21 day old broiler chickens.

	Basal	Diets with heated LOM				Diets with non-heated LOM			
		C	P	L	NE	C	P	L	NE
Analyzed nutrients									
DM (%)	89	92	92	93	92	90	90	91	89
CP (%)	21	27	26	27	28	25	26	26	25
AME_n (kcal/kg)	2858	2465	2369	2462	2446	2459	2395	2462	2329
Gross energy ($kcal \cdot kg^{-1}$)	3766	4043	4065	4107	4074	3923	3937	3998	3890
Methionine (%)	0.36	0.37	0.33	0.37	0.43	0.41	0.44	0.44	0.43
Lysine (%)	1.05	1.40	1.41	1.44	1.40	1.41	1.44	1.41	1.38
Threonine (%)	0.73	0.96	0.97	1.01	0.96	0.96	0.97	0.96	0.94
Valine (%)	0.78	1.01	1.01	1.02	1.03	0.98	1.03	1.00	0.97
Isoleucine (%)	0.67	0.89	0.89	0.91	0.92	0.88	0.92	0.90	0.86
Leucine (%)	1.55	1.90	1.90	1.99	1.89	1.87	1.91	1.90	1.84
Phenylalanine (%)	0.89	1.16	1.17	1.22	1.15	1.14	1.17	1.15	1.12
Tryptophan (%)	0.19	0.28	0.27	0.29	0.30	0.28	0.28	0.29	0.29
Arginine (%)	1.26	1.67	1.72	1.75	1.66	1.65	1.70	1.66	1.64
Histidine (%)	0.56	0.72	0.72	0.76	0.71	0.72	0.71	0.71	0.70
Serine (%)	0.98	1.31	1.31	1.37	1.28	1.30	1.30	1.29	1.26
Glycine (%)	0.75	1.01	1.01	1.06	1.00	1.01	1.00	1.01	0.98
Asparagine (%)	1.88	2.58	2.56	2.69	2.54	2.60	2.60	2.59	2.50
Glutamine (%)	3.37	4.41	4.39	4.59	4.36	4.40	4.41	4.38	4.26
Proline (%)	1.23	1.59	1.51	1.57	1.46	1.46	1.48	1.51	1.45
Alanine (%)	0.85	1.04	1.04	1.08	1.03	1.03	1.03	1.03	1.01
Cysteine (%)	0.27	0.33	0.34	0.34	0.36	0.34	0.37	0.37	0.36
Tyrosine (%)	0.59	0.77	0.78	0.81	0.77	0.77	0.80	0.77	0.75
NH_3 (%)	0.38	0.44	0.43	0.45	0.45	0.45	0.44	0.44	0.43

C = carbohydrase, P = protease, L = lipase, NE = no-enzyme

Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed

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Table 4.4 Analyzed nutrient composition of diets (as fed basis) with 11% high oil soybean meal (HOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of meal using 21 day old broilers.

	Diets with heated HOM				Diets with non-heated HOM			
	C	P	L	NE	C	P	L	NE
Analyzed nutrients								
DM (%)	92	91	91	92	89	90	89	89
CP (%)	29	27	28	26	27	27	26	26
AME_n (kcal/kg)	2563	2422	2377	2382	2456	2453	2358	2367
Gross energy ($kcal \cdot kg^{-1}$)	4106	4075	4072	4119	3981	4046	3990	3985
Methionine (%)	0.41	0.40	0.39	0.43	0.43	0.39	0.41	0.40
Lysine (%)	1.46	1.40	1.42	1.32	1.26	1.36	1.31	1.30
Threonine (%)	0.99	0.96	0.97	0.92	0.87	0.92	0.92	0.92
Valine (%)	1.02	1.03	1.04	0.95	0.91	0.92	0.91	0.94
Isoleucine (%)	0.91	0.92	0.93	0.84	0.81	0.82	0.81	0.85
Leucine (%)	1.95	1.92	1.93	1.85	1.74	1.79	1.80	1.85
Phenylalanine (%)	1.17	1.16	1.17	1.11	1.04	1.09	1.08	1.11
Tryptophan (%)	0.22	0.22	0.22	0.25	0.25	0.24	0.23	0.25
Arginine (%)	1.71	1.66	1.68	1.57	1.51	1.61	1.56	1.57
Histidine (%)	0.73	0.71	0.71	0.69	0.65	0.68	0.69	0.68
Serine (%)	1.33	1.29	1.30	1.25	1.18	1.25	1.25	1.25
Glycine (%)	1.03	0.99	1.01	0.96	0.91	0.95	0.95	0.94
Asparagine (%)	2.65	2.60	2.61	2.43	2.32	2.47	2.46	2.45
Glutamine (%)	4.52	4.39	4.43	4.23	4.00	4.17	4.20	4.24
Proline (%)	1.53	1.49	1.50	1.46	1.37	1.41	1.43	1.43
Alanine (%)	1.06	1.04	1.04	1.00	0.95	0.99	0.99	1.00
Cysteine (%)	0.33	0.34	0.35	0.35	0.36	0.34	0.33	0.33
Tyrosine (%)	0.79	0.77	0.79	0.75	0.71	0.74	0.75	0.75
NH_3 (%)	0.45	0.45	0.44	0.43	0.41	0.41	0.42	0.42

C = carbohydrase, P = protease, L = lipase, NE = no-enzyme

Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed

4.6.2 Apparent digestible nutrients

4.6.2.1 Apparent dry matter digestibility (%)

The DM digestibility of MPSBM was influenced ($P<0.05$) by oil x enzyme and heat x enzyme, two-way interactions (Table 4.5). For the oil x enzyme interaction, when compared to no-enzyme treatment, carbohydrase, protease and lipase enzyme treatments did not improve ($P>0.05$) the DM digestibility of 7% MPSBM. Within 11% MPSBM, supplementation carbohydrase improved ($P<0.05$) the DM digestibility, when compared to no enzyme and lipase. For the heat x enzyme interaction, the heated meals with carbohydrase or protease showed greater ($P<0.05$) DM digestibilities than the heated meals with lipase or no-enzyme. When compared to carbohydrase, the DM digestibility in non-heated meal supplemented with protease, was lower ($P<0.05$). The DM digestibilities of non-heated meals supplemented with no-enzyme or protease were not different ($P>0.05$). Moreover, the DM digestibilities observed with protease or lipase treatments did not differ ($P>0.05$).

Table 4.5 Effects of oil level, heat and enzymes on the apparent dry matter digestibility (%) of mechanically- pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	79±1	83±1	72±1	81±1	74±1 d	82±1 abc	77±0.7
Carbohydrase	79±1	85±1	82±1	87±1	81±1 c	86±1 a	83±0.7
Protease	81±1	83±1	80±1	81±1	81±1 c	82±1 bc	81±0.7
Lipase	77±1	88±1	71±1	84±1	74±1 d	86±1 ab	80±0.7
Oil x Enzyme							
No Enzyme	79±1 bcd		76±1 d				
Carbohydrase	82±1 abc		85±1 a				
Protease	82±1 abc		81±1 abcd				
Lipase	83±1 ab		78±1 cd				
Oil	81±0.5		80±0.5				
Heat	77±0.5	84±0.5					
Oil x Heat	78±0.7	85±0.7	76±0.7	83±0.7			
Source of variation	Pr>F						
Oil	0.0363						
Heat	<.0001						
Oil x Heat	0.8330						
Enzyme	<.0001						
Oil x Enzyme	0.0019						
Heat x Enzyme	<.0001						
Oil x Heat x Enzyme	0.7825						

[a-d]Mean±SE in the same group: oil x enzyme and heat x enzyme interactions with no common letters are significantly different ($\alpha = 0.05$).

4.6.2.2 Apparent crude protein digestibility (%)

The apparent CP digestibility was influenced ($P<0.05$) by the oil, heat and enzyme main effects (Table 4.6). Heat treatment improved ($P<0.05$) the apparent CP digestibility. The 7% MPSBM gave a greater ($P<0.05$) apparent CP digestibility, than the 11% MPSBM. When compared to no-enzyme treatment, either carbohydrase, protease or lipase enhanced ($P<0.05$) the apparent CP digestibility of the meal irrespective of the two oil levels. There were no values for apparent crude protein digestibilities of MPSBM found in the literature.

4.6.2.3 Nitrogen-corrected apparent metabolizable energy

The AME_n of MPSBM was affected by three-way interaction among oil level, heat and enzyme treatments (Table 4.7). Addition of carbohydrase improved ($P<0.05$) AME_n of heated 11% MPSBM, compared to protease, lipase and no-enzyme treatment. However, in non-heated 11% MPSBM, carbohydrase enhanced ($P<0.05$) the AME_n compared to lipase and no-enzyme treatments which were similar ($P>0.05$) to carbohydrase and protease additions. In both heated and non-heated 11% MPSBM, carbohydrase enzyme improved ($P<0.05$) the AME_n when compared to no-enzyme treatment. The highest AME_n for 11% MPSBM was observed when the meal was heated and supplemented with carbohydrase enzyme which was significantly greater ($P<0.05$) than AME_n for the heat + protease, heat + lipase, heat + no-enzyme, no-heat + lipase and no-heat + no-enzyme treatment combinations. When compared to no-enzyme treatment, there was no significant advantage ($P>0.05$) in adding enzymes on AME_n in heated 7% MPSBM. Among carbohydrase, protease and lipase enzyme treatments, there was no difference ($P>0.05$) in AME_n of heated 7% MPSBM supplemented with carbohydrase or protease and carbohydrase or lipase.

Table 4.6 Effects of oil level, heat and enzymes on the apparent crude protein digestibility (%) of mechanically- pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	55±1	49±1	51±1	47±1	53±0.6	48±0.6	51±0.4 c
Carbohydrase	58±1	53±1	57±1	53±1	58±0.6	53±0.6	55±0.4 a
Protease	62±1	54±1	57±1	53±1	59±0.6	53±0.6	56±0.4 a
Lipase	59±1	51±1	55±1	49±1	57±0.6	50±0.6	54±0.4 b
Oil x Enzyme							
No Enzyme	52±0.6		49±0.6				
Carbohydrase	56±0.6		55±0.6				
Protease	58±0.6		55±0.6				
Lipase	55±0.6		52±0.6				
Oil	55±0.3 a		53±0.3 b				
Heat	57±0.3 a	52±0.3 b					
Oil x Heat	58±0.4	52±0.4	55±0.4	50±0.4			
Source of variation	Pr>F						
Oil	<.0001						
Heat	<.0001						
Oil x Heat	0.1098						
Enzyme	<.0001						
Oil x Enzyme	0.1569						
Heat x Enzyme	0.2138						
Oil x Heat x Enzyme	0.3339						

[a-c]Mean±SE in the same group: oil, enzyme, heat main effects with no common letters are significantly different (α = 0.05).

Table 4.7 Effects of oil level, heat and enzymes on the nitrogen-corrected apparent metabolizable energy content (kcal·kg^{-1}) of mechanically- pressed *Glycine max* meal in 21day old broilers (on a DM basis).

	Oil x Heat x Enzyme				
	7% oil meal		11% oil meal		
	Heat	No heat	Heat	No heat	Enzyme
Enzyme treatments					
No-enzyme	1515±56 bcdef	1587±65 bcdef	1120±50 h	1357±50 efgh	1395±28
Carbohydrase	1671±65 abcd	1657±50 abc	1775±50 ab	1687±50 abc	1697±27
Protease	1766±65 ab	1378±50 defg	1364±50 efgh	1577±50 bcde	1521±27
Lipase	1430±56cdefg	1904±56 a	1198±50 gh	1318±50 fgh	1463±27
Oil	1614±21		1425±18		
Heat	1480±20	1558±19			
Oil x Heat	1595±30	1632±28	1365±25	1485±25	
Oil x Enzyme					
No-enzyme	1551±43		1239±35		
Carbohydrase	1664±41		1731±35		
Protease	1572±41		1471±35		
Lipase	1667±40		1258±35		
Heat x Enzyme					
No-enzyme	1318±38	1472±41			
Carbohydrase	1723±41	1672±35			
Protease	1565±41	1477±35			
Lipase	1314±38	1611±38			
Source of variation	Pr>F				
Oil	<.0001				
Heat	0.0055				
Oil x Heat	0.1268				
Enzyme	<.0001				
Oil x Enzyme	<.0001				
Heat x Enzyme	<.0001				
Oil x Heat x Enzyme	<.0001				

[a-h]Mean±SE in the oil x heat x enzyme interaction with no common letters are significantly different (α = 0.05).

Addition of protease into heated 7% MPSBM, a greater ($P<0.05$) AME_n existed compared to lipase. Addition of lipase into non-heated 7% MPSBM gave a better ($P<0.05$) AME_n than protease or no-enzyme. However, AME_n reported for non-heated 7% MPSBM with carbohydrase or lipase was not different ($P>0.05$). The greatest AME_n for 7% MPSBM was observed when the meal was not heated and supplemented with lipase enzyme. However, this was similar ($P>0.05$) to AME_n for heat + carbohydrase, heat + protease and no-heat + carbohydrase treatment combinations. In general, carbohydrase enzyme supplementation improved the AME_n of heated and non-heated both 7 and 11% MPSBM.

The 7 and 11% MPSBM gave AME_n of 1614 and 1425 kcal·kg^{-1}, respectively on a DM basis regardless of the heat and enzyme treatments. When these values were expressed on an as-fed basis, the AME_n were 1469 and 1283 kcal·kg^{-1}, respectively. According to Sauvant et al. (2004), the AME_n of solvent-extracted soybean meal (CP = 48%) was 2223 kcal·kg^{-1} in broiler chickens while the AME_n of pelleted full-fat toasted soybean was 3274 kcal·kg^{-1} on an as-fed basis. According to NRC (1994), the AME_n of solvent-extracted soybean meal was 2440 kcal·kg^{-1} (CP = 48.5%) while the AME_n of heat processed soybeans was 3300 kcal/kg, on an as-fed basis. Therefore, theoretically, the AME_n of mechanically-pressed soybean meal might be within the range of 2223 - 3300 kcal·kg^{-1}. According to Powell et al. (2011), the ME_n of EE-SBM (ether extract value = 8.1% on an as-fed basis) was 2673 kcal·kg^{-1} (on an as-fed basis) which was calculated using "$ME_n = 37.5 \times CP + 46.39 \times$ ether-extract value + $14.9 \times$ nitrogen-free extract value" equation. However, in the current study the AME_n of 7 (1469 kcal·kg^{-1}) and 11% (1283 kcal·kg^{-1}) MPSBM were not in 2223 - 3300 kcal·kg^{-1} range. The reason for the low AME_n is not clear. However, the

high gross energy contents of excreta materials showed that the birds could not utilize the energy in residual oil soybean meals well, resulting in higher levels in excreta.

4.6.3 Ileal digestible nutrients

4.6.3.1 Standardized ileal crude protein digestibility (%)

The standardized ileal CP digestibility of MPSBM was affected ($P<0.05$) by enzyme and heat main effects (Table 4.8). Heat treatment improved ($P<0.05$) the ileal CP digestibility of MPSBM. When compared to no-enzyme and lipase treatments, carbohydrase or protease enhanced ($P<0.05$) the ileal CP digestibility. The protease was superior ($P<0.05$) to carbohydrase, in improving the CP digestibility MPSBM in broilers. The reported standardized ileal CP digestibilties were greater than apparent CP digestibilities (Table 4.6).

4.6.3.2 Standardized ileal essential amino acid digestibility (%)

The standardized phenylalanine digestibility was influenced ($P<0.05$) by heat and enzyme main effects (Table 4.9). Heat treatment increased ($P<0.05$) the ileal phenylalanine digestibility. When compared to no-enzyme treatment, carbohydrase, protease and lipase improved ($P<0.05$) the ileal phenylalanine digestibility of MPSBM.

The standardized ileal isoleucine digestibility was affected ($P<0.05$) by the heat and enzyme main effects (Table 4.10). Heat enhanced ($P<0.05$) the ileal isoleucine digestibility in broiler chickens. When compared to no-enzyme and carbohydrase enzyme treatments, protease or lipase upgraded ($P<0.05$) the isoleucine digestibility in MPSBM.

The standardized ileal arginine digestibility was influenced by enzyme main effect ($P<0.05$) and two-way interaction ($P\leq0.05$) between oil and heat (Table 4.11).

Table 4.8 Effects of oil levels, heat and enzymes on standardized ileal crude protein digestibility (%) of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	82±0.8	66±0.8	82±0.8	64±0.8	82±0.5	63±0.7	74±0.4 c
Carbohydrase	84±0.8	68±0.8	83±0.8	66±0.8	84±0.5	67±0.5	75±0.4 b
Protease	88±0.8	69±0.8	86±0.8	68±0.8	87±0.5	68±0.5	76±0.4 a
Lipase	81±0.8	65±0.8	84±0.8	64±0.8	82±0.5	63±0.7	73±0.4 c
Oil x Enzyme							
No Enzyme	73±0.6		73±0.6				
Carbohydrase	76±0.6		75±0.6				
Protease	78±0.6		77±0.6				
Lipase	73±0.6		74±0.6				
Oil	75±0.3		74±0.3				
Heat	84±0.3 a	66±0.3 b					
Oil x Heat	84±0.4	67±0.4	84±0.4	65±0.4			
Source of variation	Pr>F						
Oil	0.0550						
Heat	<.0001						
Oil x Heat	0.1257						
Enzyme	<.0001						
Oil x Enzyme	0.1376						
Heat x Enzyme	0.3538						
Oil x Heat x Enzyme	0.2913						

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

Table 4.9 Effects of oil levels, heat and enzymes on standardized ileal phenylalanine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±1	62±1	81±1	63±1	81±0.7	63±0.7	72±0.5 c
Carbohydrase	86±1	66±1	85±1	67±1	85±0.7	67±0.7	76±0.5 ab
Protease	87±1	69±1	85±1	67±1	86±0.7	68±0.7	77±0.5 a
Lipase	84±1	66±1	83±1	67±1	83±0.7	63±0.7	75±0.5 b
Oil x Enzyme							
No Enzyme	71±0.7		72±0.7				
Carbohydrase	76±0.7		76±0.7				
Protease	78±0.7		76±0.7				
Lipase	75±0.7		74±0.7				
Oil	75±0.3		75±0.3				
Heat	84±0.3 a	66±0.3 b					
Oil x Heat	84±0.5	66±0.5	83±0.5	66±0.5			
Source of variation	Pr>F						
Oil	0.4212						
Heat	<.0001						
Oil x Heat	0.3268						
Enzyme	<.0001						
Oil x Enzyme	0.2851						
Heat x Enzyme	0.6071						
Oil x Heat x Enzyme	0.6488						

[a-c]Means ± SE in the same group: heat, enzyme main effects with no common letters are significantly different (α = 0.05).

Table 4.10 Effects of oil levels, heat and enzymes on standardized ileal isoleucine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	84±1	61±1	83±1	62±1	84±1	62±1	73±0.7 b
Carbohydrase	83±1	63±1	82±1	63±1	82±1	63±1	73±0.7 b
Protease	87±1	69±1	87±1	67±1	87±1	68±1	77±0.7 a
Lipase	89±1	65±1	87±1	65±1	88±1	65±1	77±0.7 a
Oil x Enzyme							
No Enzyme	73±1		72±1				
Carbohydrase	73±1		73±1				
Protease	78±1		77±1				
Lipase	77±1		76±1				
Oil	75±0.5		75±0.5				
Heat	85±0.5 a	64±0.5 b					
Oil x Heat	86±0.7	65±0.7	85±0.7	64±0.7			
Source of variation	Pr>F						
Oil	0.4681						
Heat	<.0001						
Oil x Heat	0.5449						
Enzyme	<.0001						
Oil x Enzyme	0.9986						
Heat x Enzyme	0.1269						
Oil x Heat x Enzyme	0.7649						

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

Table 4.11 Effects of oil levels, heat and enzymes on standardized ileal arginine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±0.7	62±0.7	82±0.7	61±0.7	82±0.5	62±0.5	72±0.4 b
Carbohydrase	83±0.7	66±0.7	85±0.7	64±0.7	84±0.5	65±0.5	74±0.4 a
Protease	86±0.7	67±0.7	85±0.7	64±0.7	85±0.5	66±0.5	75±0.4 a
Lipase	80±0.7	62±0.7	81±0.7	64±0.7	81±0.5	63±0.5	72±0.4 b
Oil x Enzyme							
No Enzyme	72±0.5		72±0.5				
Carbohydrase	75±0.5		74±0.5				
Protease	76±0.5		75±0.5				
Lipase	71±0.5		73±0.5				
Oil	74±0.3		73±0.3				
Heat	83±0.3	64±0.3					
Oil x Heat	83±0.4 a	64±0.4 b	83±0.4 a	63±0.4 b			
Source of variation	Pr>F						
Oil	0.7498						
Heat	<.0001						
Oil x Heat	0.0500						
Enzyme	<.0001						
Oil x Enzyme	0.0751						
Heat x Enzyme	0.1526						
Oil x Heat x Enzyme	0.1378						

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

The heated 7% MPSBM gave a greater ($P<0.05$) ileal arginine digestibility than non-heated 7% MPSBM. A similar relationship was seen in 11% MPSBM where heated 11% MPSBM exhibited a higher ($P<0.05$) arginine digestibility than non-heated meal. When compared to no-enzyme and lipase treatments, addition of carbohydrase or protease enhanced ($P<0.05$) the ileal arginine digestibility of MPSBM. Carbohydrase and protease were superior ($P<0.05$) to lipase, in improving the arginine digestibility.

The standardized ileal leucine digestibility was influenced ($P<0.05$) by heat x enzyme, oil x enzyme interaction effects (Table 4.12). In oil x enzyme interaction, when compared to no-enzyme, carbohydrase and protease enzyme treatments, lipase supplementation improved ($P<0.05$) the leucine digestibility of 7% MPSBM. In 11% MPSBM, compared to no-enzyme treatment, lipase enhanced ($P<0.05$) the ileal digestible leucine coefficients, however, no difference ($P>0.05$) was seen among carbohydrase, protease and lipase enzyme treatments. In the heat x enzyme interaction effect ($P<0.05$), when compared to carbohydrase, protease or no-enzyme treatments, lipase improved ($P<0.05$) the leucine digestibility in heated MPSBM. None of the enzyme treatments upgraded ($P>0.05$) the leucine digestibility in non-heated MPSBM.

The standardized ileal lysine digestibility was influenced ($P<0.05$) by the oil x heat and heat x enzyme interaction effects (Table 4.13). For the oil x heat interaction, the heated 7% and 11% MPSBM showed a greater ($P<0.05$) lysine digestibility than the respective non-heated MPSBM. The lysine digestibility in heated 7% MPSBM was higher ($P<0.05$) than that of heated 11% MPSBM. Similarly, non-heated 7% MPSBM showed a greater ($P<0.05$) lysine digestibility than non-heated 11% MPSBM.

Table 4.12 Effects of oil levels, heat and enzymes on standardized ileal leucine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±1	64±1	79±1	63±1	80±0.6 c	63±0.6 d	72±0.5
Carbohydrase	83±1	62±1	83±1	63±1	83±0.6 b	63±0.6 d	73±0.5
Protease	84±1	65±1	82±1	62±1	83±0.6 bc	63±0.6 d	73±0.5
Lipase	90±1	67±1	86±1	64±1	88±0.6 a	65±0.6 d	76±0.5
Oil x Enzyme							
No Enzyme	72±0.6 bc		71±0.6 c				
Carbohydrase	73±0.6 bc		73±0.6 bc				
Protease	74±0.6 b		72±0.6 bc				
Lipase	78±0.6 a		75±0.6 b				
Oil	74±0.3		73±0.3				
Heat	84±0.3	64±0.3					
Oil x Heat	84±0.5	64±0.5	83±0.5	63±0.5			
Source of variation	Pr>F						
Oil	0.0011						
Heat	<.0001						
Oil x Heat	0.7092						
Enzyme	<.0001						
Oil x Enzyme	0.0155						
Heat x Enzyme	0.0008						
Oil x Heat x Enzyme	0.7202						

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

Table 4.13 Effects of oil levels, heat and enzymes on standardized ileal lysine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	87±0.6	68±0.6	85±0.6	65±0.6	86±0.4 ab	66±0.4 d	76±0.3
Carbohydrase	87±0.6	71±0.6	86±0.6	68±0.6	86±0.4 ab	70±0.4 c	80±0.3
Protease	89±0.6	71±0.6	87±0.6	69±0.6	88±0.4 a	70±0.4 c	79±0.3
Lipase	86±0.6	68±0.6	85±0.6	66±0.6	85±0.4 b	67±0.4 d	76±0.3
Oil x Enzyme							
No Enzyme	77±0.4		75±0.4				
Carbohydrase	79±0.4		77±0.4				
Protease	80±0.4		78±0.4				
Lipase	77±0.4		75±0.4				
Oil	78±0.2		76±0.2				
Heat	86±0.2	68±0.2					
Oil x Heat	87±0.3 a	70±0.3 c	86±0.3 b	67±0.3 d			
Source of variation	Pr>F						
Oil	<.0001						
Heat	<.0001						
Oil x Heat	0.0210						
Enzyme	<.0001						
Oil x Enzyme	0.9318						
Heat x Enzyme	0.0127						
Oil x Heat x Enzyme	0.5572						

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

In the heat x enzyme interaction, when compared to no-enzyme treatment, there was no advantage ($P>0.05$) in adding enzymes to improve the lysine digestibility in heated MPSBM. The heated meals supplemented with lipase gave lower ($P<0.05$) lysine digestibility, when compared to meals supplemented with protease. However, in non-heated meals, when compared to no-enzyme or lipase enzyme treatment, either carbohydrase or protease improved ($P<0.05$) lysine digestibility.

The standardized ileal valine digestibility was affected ($P<0.05$) by the two-way interaction between heat and enzymes (Table 4.14). When compared to no-enzyme, carbohydrase and lipase enzyme treatments, protease supplementation enhanced ($P<0.05$) the valine digestibility in heated MPSBM. In non-heated meals, protease enzyme upgraded ($P<0.05$) the valine digestibility, when compared to no-enzyme.

The standardized ileal histidine digestibility was influenced ($P<0.05$) by the oil, heat and enzyme main effects (Table 4.15). Heat increased ($P<0.05$) the ileal histidine digestibility of MPSBM. When compared to no-enzyme, lipase and carbohydrase enzyme treatments, protease enhanced ($P<0.05$) the ileal histidine digestibility of the two meals. The 7% MPSBM gave a greater ($P<0.05$) histidine digestibility than 11% MPSBM.

The standardized ileal methionine digestibility was affected ($P<0.05$) by the three-way interaction among oil x heat x enzyme (Table 4.16). When compared to no-enzyme and lipase enzyme treatments, protease improved ($P<0.05$) the methionine digestibility in heated 7% MPSBM. There was no difference ($P>0.05$) between carbohydrase and protease enzyme treatments. In non-heated 7% MPSBM, when compared to no-enzyme, carbohydrase or lipase enzyme treatments, protease increased ($P<0.05$) the ileal methionine digestibility in broiler chickens. When compared to no-enzyme, carbohydrase and lipase,

Table 4.14 Effects of oil levels, heat and enzymes on standardized ileal valine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	84±1	64±1	83±1	65±1	83±0.8 b	65±0.8 d	74±0.5
Carbohydrase	83±1	68±1	84±1	67±1	83±0.8 b	68±0.8 cd	76±0.5
Protease	88±1	69±1	87±1	68±1	87±0.8 a	69±0.8 c	78±0.5
Lipase	83±1	67±1	81±1	67±1	82±0.8 b	67±0.8 cd	75±0.5
Oil x Enzyme							
No Enzyme	74±0.8		73±0.8				
Carbohydrase	76±0.8		76±0.8				
Protease	79±0.8		77±0.8				
Lipase	75±0.8		74±0.8				
Oil	76±0.4		75±0.4				
Heat	84±0.4	67±0.4					
Oil x Heat	84±0.5	67±0.5	84±0.5	67±0.5			
Source of variation	Pr>F						
Oil	0.2882						
Heat	<.0001						
Oil x Heat	1.0000						
Enzyme	<.0001						
Oil x Enzyme	0.7843						
Heat x Enzyme	0.0248						
Oil x Heat x Enzyme	0.6278						

[a-d]Means ± SE in the heat x enzyme interaction effect with no common letters are significantly different (α = 0.05).

Table 4.15 Effects of oil levels, heat and enzymes on standardized ileal histidine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	85±1	65±1	83±1	63±1	84±0.7	64±0.7	74±0.5 b
Carbohydrase	85±1	67±1	83±1	66±1	84±0.7	66±0.7	75±0.5 b
Protease	87±1	68±1	86±1	70±1	87±0.7	69±0.7	78±0.5 a
Lipase	87±1	66±1	84±1	66±1	86±0.7	66±0.7	76±0.5 b
Oil x Enzyme							
No Enzyme	75±0.7		73±0.7				
Carbohydrase	76±0.7		75±0.7				
Protease	78±0.7		78±0.7				
Lipase	76±0.7		75±0.7				
Oil	76±0.3 a		75±0.b				
Heat	85±0.3 a	66±0.3 b					
Oil x Heat	86±0.5	66±0.5	84±0.5	66±0.5			
Source of variation	Pr>F						
Oil	0.0211						
Heat	<.0001						
Oil x Heat	0.0977						
Enzyme	<.0001						
Oil x Enzyme	0.5739						
Heat x Enzyme	0.2469						
Oil x Heat x Enzyme	0.5999						

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

Table 4.16 Effects of oil levels, heat and enzymes on standardized ileal methionine digestibility coefficient of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	86±0.6 b	70±0.6 def	84±0.6 b	67±0.6 f	85±0.4	68±0.4	77±0.3
Carbohydrase	87±0.6 ab	70±0.6 de	85±0.6 b	72±0.6 cd	86±0.4	71±0.4	79±0.3
Protease	89±0.6 a	74±0.6 c	90±0.6 a	71±0.6 de	90±0.4	73±0.4	81±0.3
Lipase	85±0.6 b	71±0.6 de	84±0.6 b	69±0.6 ef	85±0.4	70±0.4	78±0.3
Oil x Enzyme							
No Enzyme	78±0.4		76±0.4				
Carbohydrase	79±0.4		79±0.4				
Protease	81±0.4		80±0.4				
Lipase	78±0.4		77±0.4				
Oil	79±0.2		78±0.2				
Heat	86±0.2	70±0.2					
Oil x Heat	87±0.3	71±0.3	86±0.3	70±0.3			
Source of variation	Pr>F						
Oil	0.0002						
Heat	<.0001						
Oil x Heat	0.3523						
Enzyme	<.0001						
Oil x Enzyme	0.2045						
Heat x Enzyme	0.1072						
Oil x Heat x Enzyme	0.0020						

[a-f]Means ± SE in the oil x heat x enzyme interaction effect with no common letters are significantly different (α = 0.05).

protease enhanced ($P<0.05$) the methionine digestibility in heated 11% MPSBM. Both carbohydrase and protease were superior ($P<0.05$) to no-enzyme treatment, in improving the ileal methionine digestibility.

The standardized ileal threonine digestibility had significant ($P<0.05$) heat and enzyme main effect differences (Table 4.17). Heat treatment improved ($P<0.05$) the ileal threonine digestibility. When compared to no-enzyme, carbohydrase or lipase enzyme treatment, protease increased ($P<0.05$) the threonine digestibility.

The standardized ileal tryptophan digestibility was affected ($P<0.05$) by the oil level, heat application and the use of enzymes (Table 4.18). Heat treatment enhanced ($P<0.05$) the tryptophan digestibility. The 7% MPSBM gave a greater ($P<0.05$) tryptophan digestibility than 11% MPSBM. When compared to no-enzyme, lipase and carbohydrase treatments, protease enhanced ($P<0.05$) the tryptophan digestibility of MPSBM. Carbohydrase and lipase enzyme treatments were similar ($P>0.05$) in tryptophan digestibility in MPSBM.

Table 4.17 Effects of oil levels, heat and enzymes on standardized ileal threonine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	85±1	69±1	84±1	69±1	85±0.7	69±0.7	77±0.5 b
Carbohydrase	85±1	71±1	83±1	70±1	84±0.7	71±0.7	77±0.5 b
Protease	88±1	73±1	87±1	73±1	88±0.7	73±0.7	80±0.5 a
Lipase	83±1	68±1	86±1	69±1	85±0.7	69±0.7	77±0.5 b
Oil x Enzyme							
No Enzyme	77±0.7		76±0.7				
Carbohydrase	78±0.7		76±0.7				
Protease	81±0.7		80±0.7				
Lipase	76±0.7		78±0.7				
Oil	78±0.3		77±0.3				
Heat	85±0.3 a	70±0.3 b					
Oil x Heat	85±0.5	70±0.5	85±0.5	70±0.5			
Source of variation	Pr>F						
Oil	0.5274						
Heat	<.0001						
Oil x Heat	0.7860						
Enzyme	<.0001						
Oil x Enzyme	0.0737						
Heat x Enzyme	0.1293						
Oil x Heat x Enzyme	0.7325						

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

Table 4.18 Effects of oil levels, heat and enzymes on standardized ileal tryptophan digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	80±1	62±1	80±1	61±1	80±0.7	61±0.7	71±0.5 c
Carbohydrase	85±1	64±1	84±1	61±1	85±0.7	63±0.7	74±0.5 b
Protease	87±1	67±1	86±1	65±1	86±0.7	66±0.7	76±0.5 a
Lipase	82±1	63±1	82±1	61±1	82±0.7	62±0.7	72±0.5 bc
Oil x Enzyme							
No Enzyme	71±0.7		71±0.7				
Carbohydrase	75±0.7		73±0.7				
Protease	77±0.7		75±0.7				
Lipase	73±0.6		72±0.7				
Oil	74±0.3 a		73±0.3 b				
Heat	83±0.3 a	63±0.3 b					
Oil x Heat	84±0.5	64±0.5	83±0.5	62±0.5			
Source of variation	Pr>F						
Oil	0.0223						
Heat	<.0001						
Oil x Heat	0.0960						
Enzyme	<.0001						
Oil x Enzyme	0.8573						
Heat x Enzyme	0.1491						
Oil x Heat x Enzyme	0.9962						

[a-c]Means ± SE in the same group: oil, heat, enzyme main effects with no common letters are significantly different (α = 0.05).

4.6.3.2 Standardized ileal non-essential amino acid digestibility (%)

The standardized ileal serine digestibility was influenced ($P<0.05$) by heat and enzyme main effects (Table 4.19). Heat treatment enhanced the serine digestibility. Although the enzyme effect was significant ($P<0.05$) according to the ANOVA table, Tukey Kramer option did not differentiate the differences among means.

The standardized ileal asparagine digestibility was affected ($P<0.05$) by heat main effects (Table 4.20). Heat treatment increased ($P<0.05$) the asparagine digestibility of MPSBM.

The standardized ileal cysteine digestibility was influenced ($P<0.05$) by heat and enzyme main effects (Table 4.21). Heat treatment enhanced ($P<0.05$) the cysteine digestibility in MPSBM. When compared to no-enzyme treatment, carbohydrase and protease enzymes improved ($P<0.05$) the ileal cysteine digestibility. There was no difference ($P>0.05$) in cysteine digestibility between carbohydrase and protease enzyme treatments.

The standardized ileal glutamine digestibility was influenced ($P<0.05$) by heat and enzyme main effects (Table 4.22). There was a strong trend ($P = 0.052$) in oil x heat, two-way interaction. Heat treatment enhanced ($P<0.05$) the ileal glutamine digestibility in MPSBM. When compared to no-enzyme treatment, protease upgraded ($P<0.05$) the ileal glutamine digestibility. Carbohydrase, protease and lipase enzymes showed differences ($P<0.05$) in glutamine digestibility in broiler chickens.

The standardized ileal glycine digestibility was affected ($P<0.05$) by heat and enzyme main effects (Table 4.23). Heat improved ($P<0.05$) the ileal glycine digestibility in MPSBM. When compared to no-enzyme, carbohydrase or lipase enzyme treatment, protease upgraded ($P<0.05$) the ileal glycine digestibility in MPSBM. There was no difference ($P>0.05$) in glycine digestibility between carbohydrase and lipase enzyme treatments.

Table 4.19 Effects of oil levels, heat and enzymes on standardized ileal serine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±1	66±1	81±1	66±1	81±0.6	66±0.6	74±0.4
Carbohydrase	81±1	67±1	80±1	66±1	81±0.6	67±0.6	74±0.4
Protease	84±1	68±1	82±1	67±1	83±0.6	67±0.6	75±0.4
Lipase	80±1	69±1	81±1	67±1	81±0.6	68±0.6	74±0.4
Oil x Enzyme							
No Enzyme	73±0.6		74±0.6				
Carbohydrase	74±0.6		73±0.6				
Protease	76±0.6		74±0.6				
Lipase	75±0.6		74±0.6				
Oil	75±0.3		74±0.3				
Heat	81±0.3 a	67±0.3 b					
Oil x Heat	82±0.4	67±0.4	81±0.4	67±0.4			
Source of variation	Pr>F						
Oil	0.0667						
Heat	<.0001						
Oil x Heat	0.8506						
Enzyme	0.0410*						
Oil x Enzyme	0.2298						
Heat x Enzyme	0.1667						
Oil x Heat x Enzyme	0.5401						

[a-b]Means ± SE in the heat main effect with no common letters are significantly different (α = 0.05).
*Tukey Kramer option did not differentiate the enzyme effect that was different in the ANOVA.

Table 4.20 Effects of oil levels, heat and enzymes on standardized ileal asparagine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	82±1	63±1	80±1	63±1	81±0.6	63±0.6	72±0.4
Carbohydrase	82±1	65±1	81±1	64±1	81±0.6	64±0.6	73±0.4
Protease	81±1	64±1	83±1	62±1	82±0.6	63±0.6	73±0.4
Lipase	81±1	63±1	81±1	63±1	81±0.6	63±0.6	72±0.4
Oil x Enzyme							
No Enzyme	72±0.6		72±0.6				
Carbohydrase	74±0.6		73±0.6				
Protease	73±0.6		73±0.6				
Lipase	72±0.6		72±0.6				
Oil	73±0.3		72±0.3				
Heat	81±0.3 a	63±0.3 b					
Oil x Heat	81±0.4	64±0.4	81±0.4	63±0.4			
Source of variation	Pr>F						
Oil	0.2200						
Heat	<.0001						
Oil x Heat	0.5054						
Enzyme	0.1695						
Oil x Enzyme	0.9078						
Heat x Enzyme	0.2170						
Oil x Heat x Enzyme	0.3652						

[a-b]Means ± SE in the heat main effect with no common letters are significantly different (α = 0.05).

Table 4.21 Effects of oil levels, heat and enzymes on standardized ileal cysteine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	82±2	61±1	81±2	60±1	81±0.7	60±0.7	71±0.5 c
Carbohydrase	83±1	65±1	83±1	64±1	83±0.7	65±0.7	74±0.5 ab
Protease	86±1	65±1	85±1	64±1	85±0.7	65±0.7	75±0.5 a
Lipase	82±2	62±1	82±2	62±1	82±0.7	62±0.7	72±0.5 bc
Oil x Enzyme							
No Enzyme	71±0.7		70±0.7				
Carbohydrase	74±0.7		74±0.7				
Protease	76±0.7		74±0.7				
Lipase	72±0.7		72±0.7				
Oil	73±0.4		73±0.4				
Heat	83±0.4 a	63±0.4 b					
Oil x Heat	83±0.5	63±0.5	83±0.5	63±0.5			
Source of variation	Pr>F						
Oil	0.2400						
Heat	<.0001						
Oil x Heat	0.9369						
Enzyme	<.0001						
Oil x Enzyme	0.8407						
Heat x Enzyme	0.2003						
Oil x Heat x Enzyme	0.8886						

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

Table 4.22 Effects of oil levels, heat and enzymes on standardized ileal glutamine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	82±1	62±1	81±1	62±1	82±0.7	62±0.7	72±0.5 bc
Carbohydrase	83±1	63±1	83±1	64±1	83±0.7	64±0.7	73±0.5 b
Protease	87±1	65±1	84±1	66±1	86±0.7	65±0.7	76±0.5 a
Lipase	81±1	62±1	79±1	63±1	80±0.7	63±0.7	71±0.5 c
Oil x Enzyme							
No Enzyme	72±0.7		72±0.7				
Carbohydrase	73±0.7		74±0.7				
Protease	76±0.7		75±0.7				
Lipase	72±0.7		71±0.7				
Oil	73±0.3		73±0.3				
Heat	83±0.3 a	63±0.3 b					
Oil x Heat	83±0.5	63±0.5	82±0.5	64±0.5			
Source of variation	Pr>F						
Oil	0.6168						
Heat	<.0001						
Oil x Heat	0.0517						
Enzyme	<.0001						
Oil x Enzyme	0.8030						
Heat x Enzyme	0.1863						
Oil x Heat x Enzyme	0.3962						

[a-c]Means ± SE in the same group: heat, enzyme main effects with no common letters are significantly different (α = 0.05).

Table 4.23 Effects of oil levels, heat and enzymes on standardized ileal glycine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±0.8	61±0.8	81±0.8	62±0.8	81±0.6	62±0.6	71±0.4 b
Carbohydrase	83±0.8	61±0.8	82±0.8	61±0.8	83±0.6	61±0.6	72±0.4 b
Protease	87±0.8	65±0.8	86±0.8	65±0.8	86±0.6	65±0.6	76±0.4 a
Lipase	82±0.8	61±0.8	82±0.8	61±0.8	82±0.6	61±0.6	71±0.4 b
Oil x Enzyme							
No Enzyme	71±0.6		72±0.6				
Carbohydrase	72±0.6		72±0.6				
Protease	76±0.6		76±0.6				
Lipase	71±0.6		71±0.6				
Oil	73±0.3		73±0.3				
Heat	83±0.3 a	62±0.3 b					
Oil x Heat	84±0.4	62±0.4	83±0.4	62±0.4			
Source of variation	Pr>F						
Oil	0.9193						
Heat	<.0001						
Oil x Heat	0.1356						
Enzyme	<.0001						
Oil x Enzyme	0.6062						
Heat x Enzyme	0.4009						
Oil x Heat x Enzyme	0.8336						

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

The standardized ileal proline digestibility was influenced ($P<0.05$) by heat and enzyme main effects (Table 4.24). There was no advantage ($P>0.05$) in adding enzymes to improve the ileal proline digestibility of MPSBM. Heat improved ($P<0.05$) the proline digestibility of MPSBM.

The standardized ileal tyrosine digestibility was influenced ($P<0.05$) by oil x heat and heat x enzyme interaction effects (Table 4.25). The ileal tyrosine digestibility of heated 7% MPSBM was greater ($P<0.05$) than non-heated 7% MPSBM. A similar trend was seen in 11% MPSBM. The tyrosine digestibility of heated 7% MPSBM was greater ($P<0.05$) than heated 11% MPSBM. However, no difference ($P>0.05$) in ileal tyrosine digestibility, was seen between non-heated 7 and 11% MPSBM. When compared to carbohydrase and no-enzyme treatment, protease or lipase enhanced the tyrosine digestibility in heated MPSBM. The reported digestibilities of heated meals supplemented with either carbohydrase, protease or lipase were different ($P<0.05$). When compared to no-enzyme treatment, either carbohydrase, protease or lipase enhanced ($P<0.05$) the tyrosine digestibility in non-heated MPSBM.

The standardized ileal NH_3 digestibility was influenced ($P<0.05$) by oil, heat and enzyme main effects (Table 4.26). The 7% MPSBM showed a greater ($P<0.05$) NH_3 digestibility than 11% MPSBM. Heat treatment increased ($P<0.05$) the ileal NH_3 digestibility. When compared to no-enzyme treatment, protease enhanced ($P<0.05$) the NH_3 digestibility in 7 and 11% MPSBM.

The standardized ileal alanine digestibility was affected ($P<0.05$) by oil main effect and heat x enzyme interaction effect (Table 4.27). The 7% MPSBM gave a greater ($P<0.05$) alanine digestibility than 11% MPSBM, regardless of the heat and enzyme treatments.

Table 4.24 Effects of oil levels, heat and enzymes on standardized ileal proline digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	83±2	61±2	82±1	62±1	83±0.6	61±0.6	72±0.4 ab
Carbohydrase	84±1	61±2	84±1	62±1	84±0.6	62±0.6	73±0.4 a
Protease	82±2	62±1	80±1	59±1	81±0.6	61±0.6	70±0.4 b
Lipase	81±1	61±1	82±2	62±1	81±0.6	62±0.6	71±0.4 ab
Oil x Enzyme							
No Enzyme	72±0.6		72±0.6				
Carbohydrase	73±0.6		73±0.6				
Protease	72±0.6		70±0.6				
Lipase	71±0.6		72±0.6				
Oil	72±0.3		72±0.3				
Heat	82±0.3 a	61±0.3 b					
Oil x Heat	83±0.4	61±0.4	82±0.4	61±0.4			
Source of variation	Pr>F						
Oil	0.6289						
Heat	<.0001						
Oil x Heat	0.2137						
Enzyme	0.0159						
Oil x Enzyme	0.1175						
Heat x Enzyme	0.2248						
Oil x Heat x Enzyme	0.5048						

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

Table 4.25 Effects of oil levels, heat and enzymes on standardized ileal tyrosine digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±0.5	61±0.5	81±0.6	61±0.5	81±0.4 c	61±0.4 f	71±0.3
Carbohydrase	83±0.5	68±0.5	82±0.6	69±0.5	82±0.4 c	68±0.4 d	75±0.3
Protease	87±0.5	69±0.5	86±0.6	66±0.5	86±0.4 a	68±0.4 d	77±0.3
Lipase	85±0.5	65±0.5	83±0.6	65±0.5	84±0.4 b	65±0.4 e	75±0.3
Oil x Enzyme							
No Enzyme	71±0.4		71±0.4				
Carbohydrase	75±0.4		75±0.4				
Protease	78±0.4		76±0.4				
Lipase	75±0.4		74±0.4				
Oil	75±0.1		74±0.1				
Heat	83±0.2	66±0.2					
Oil x Heat	84±0.3 a	66±0.2 c	83±0.3 b	65±0.3 c			
Source of variation	Pr>F						
Oil	0.0098						
Heat	<.0001						
Oil x Heat	0.0405						
Enzyme	<.0001						
Oil x Enzyme	0.0762						
Heat x Enzyme	<.0001						
Oil x Heat x Enzyme	0.0873						

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

Table 4.26 Effects of oil levels, heat and enzymes on standardized ileal NH_3 digestibility coefficients of mechanically-pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	84±1	68±1	84±1	66±1	84±0.6	67±0.6	76±0.4 b
Carbohydrase	85±1	69±1	85±1	68±1	85±0.6	68±0.6	77±0.4 ab
Protease	87±1	71±1	86±1	68±1	87±0.6	70±0.6	78±0.4 a
Lipase	85±1	67±1	84±1	66±1	85±0.6	67±0.6	76±0.4 b
Oil x Enzyme							
No Enzyme	76±0.6		75±0.6				
Carbohydrase	77±0.6		76±0.6				
Protease	79±0.6		77±0.6				
Lipase	76±0.6		75±0.6				
Oil	77±0.3 a		76±0.3 b				
Heat	85±0.3 a	68±0.3 b					
Oil x Heat	85±0.4	69±0.4	85±0.4	67±0.4			
Source of variation	Pr>F						
Oil	0.0408						
Heat	<.0001						
Oil x Heat	0.1376						
Enzyme	0.0004						
Oil x Enzyme	0.8553						
Heat x Enzyme	0.6815						
Oil x Heat x Enzyme	0.8357						

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

Table 4.27 Effects of oil levels, heat and enzymes on standardized ileal alanine digestibility coefficients of mechanically pressed *Glycine max* meal in 21day old broilers.

	Oil x Heat x Enzyme						
	7% oil meal		11% oil meal		Heat x Enzyme		
	Heat	No Heat	Heat	No Heat	Heat	No Heat	Enzyme
Enzyme treatments							
No Enzyme	81±0.6	63±0.6	80±0.6	62±0.6	81±0.4 b	63±0.4 c	72±0.3
Carbohydrase	85±0.6	62±0.6	82±0.6	61±0.6	83±0.4 a	62±0.4 c	72±0.3
Protease	83±0.6	63±0.6	81±0.6	62±0.6	82±0.4 ab	63±0.4 c	72±0.3
Lipase	83±0.6	64±0.6	82±0.6	62±0.6	83±0.4 ab	63±0.4 c	73±0.3
Oil x Enzyme							
No Enzyme	72±0.4		71±0.4				
Carbohydrase	74±0.4		71±0.4				
Protease	73±0.4		72±0.4				
Lipase	73±0.4		72±0.4				
Oil	73±0.2 a		72±0.2 b				
Heat	82±0.2	62±0.2					
Oil x Heat	83±0.3	63±0.3	81±0.3	62±0.3			
Source of variation	Pr>F						
Oil	<.0001						
Heat	<.0001						
Oil x Heat	0.2469						
Enzyme	0.1295						
Oil x Enzyme	0.5284						
Heat x Enzyme	0.0045						
Oil x Heat x Enzyme	0.2169						

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

When compared to no-enzyme treatment, carbohydrase enhanced ($P<0.05$) the ileal alanine digestibilities in heated 7 and 11% MPSBM. The ileal alanine digestibilities reported in heated 7 and 11% MPSBM supplemented with either carbohydrase, protease or lipase were not different ($P>0.05$). No improvement in alanine digestibilities ($P>0.05$) was observed in non-heated meals supplemented with either carbohydrase, protease or lipase enzyme.

Heat treatment improved ($P<0.05$) ileal phenylalanine, isoleucine, histidine, threonine, tryptophan, serine, asparagine, cysteine, glutamine, glycine, proline and NH_3 digestibilities. When the heat x enzyme interaction was significant ($P<0.05$) for a particular amino acid, heated meals supplemented with any enzyme gave greater ($P<0.05$) amino acid digestibilities than non-heated meals. When the oil x heat x enzyme interaction was significant ($P<0.05$) for a particular amino acid in 7 or 11% MPSBM, heated meals supplemented with any enzyme gave better ($P<0.05$) amino acid digestibilities than those in non-heated meals. Hence, heat treatment has enhanced the SIAAD of 7 and 11% MPSBM. Soybean meal has been known to contain trypsin inhibitors as anti-nutritional factors (Birk and Gertler 1961, Liener and Tomlinson 1981). These trypsin inhibitors inhibit the pancreatic protease enzyme activity in the intestine and reduce the protein breakdown in the intestine of chickens (Alumot and Nitsan 1961).

The analyzed urease activity in heated 7 (0.11 increase in pH) and 11% (0.12 increase in pH) MPSBM indicated that most of the trypsin inhibitors present in MPSBM were destroyed during heat treatment as the urease acitivities were in the acceptable range of 0.05 - 0.20 (American Feed Manufacturers Association 1979). Therefore, in the current study, inactivation of trypsin inhibitors with heat treatment, must have improved the SIAAD in MPSBM. The SIAAD of heated meals ranged from 81 to 86% while the SIAAD

of heated meals ranged from 81 to 86% while SIAAD of non-heated meals ranged from 61 to 70%. In a review article, Martins et al. (2001) mentioned that at an initial stage of heating, Maillard reaction takes place between reducing sugars and free amino groups. These free amino groups can be Ɛ-amino group of lysine or other α-amino groups of amino acids (Martins et al. 2001). However, in the current study, the SIAAD were not reduced due to the Maillard reaction. This was confirmed as brown colour pigments were not seen in MPSBM after the heat treatment.

According to Gonzalez-Vega et al. (2011), feeding conventional solvent-extracted soybean meal (ether-extract value = 1.35) autoclaved at 125 °C for 30 min, reduced ($P<0.05$) the SIAAD in growing pigs. They found that standardized ileal essential amino acid digestibilities of non-heated soybean meal and autoclaved soybean meal ranged from 89 to 98% and 83 - 93%, respectively. Moreover, the standardized ileal non-essential amino acid digestibilities of non-heated and autoclaved soybean meals ranged from 88 to 98% and 80 - 87%, respectively. When the soybean meal was autoclaved, meal was heated under pressure in the presence of moisture and subjected to high temperature. It has been found that Maillard reaction rate is high when the humidity is high (Schwartz and Lea 1952). As autoclaving involves high humidity, Maillard reaction can be expected during autoclaving and Gonzalez-Vega et al. (2011) observed brown color pigments in autoclaved soybean meal. Therefore, the reduced SIAAD in autoclaved soybean meal (Gonzalez-Vega et al. 201a) might be due to high pressure and Maillard reaction.

The SIAAD of conventional soybean meal (ether extract value = 1.31) oven-dried at 125 °C for 30 min, were not reduced ($P<0.05$) with the heat treatment, when compared to those found in non-heated conventional soybean meal (Gonzalez-Vega et al. 2011). Gonzalez-

Vega et al. (2011) confirmed no Maillard reaction took place as they did not observe brown color pigments in oven-dried soybean meal. In the current study, MPSBM were oven-dried at 130 °C for 30 min. This time-temperature combination might not have given the required conditions to cause the Maillard reaction in MPSBM as confirmed by the findings of Gonzalez-Vega et al. (2011).

The ileal arginine, lysine and tyrosine digestibilities of heated 7 and 11% MPSBM were greater ($P<0.05$) than those found in either non-heated oil meals. The heated meals supplemented with carbohydrase or lipase enhanced ($P<0.05$) the leucine digestibility. The lysine digestibility was upgraded with either carbohydrase or protease supplementation in non-heated meals. The valine digestibilities of heated and non-heated meals were improved ($P<0.05$) with protease. The methionine digestibility was enriched ($P<0.05$) with the protease supplementation in heated and non-heated 7% MPSBM and heated and non-heated 11% MPSBM. Moreover, carbohydrase enhanced ($P<0.05$) the methionine digestibility in non-heated 11% MPSBM. Carbohydrase supplementation enhanced ($P<0.05$) the alanine digestibility in heated 7 and 11% MPSBM. The tyrosine digestibilities of heated meals were improved ($P<0.05$) with protease or lipase while in non-heated meals either carbohydrase, protease or lipase enhanced ($P<0.05$) the tyrosine digestibility. The cysteine, glutamine, glycine, proline, NH_3 phenylalanine, isoleucine, arginine threonine and tryptophan digestibilities were enriched ($P<0.05$) with protease enzyme. Carbohydrase improved ($P<0.05$) the phenylalanine, arginine, tryptophan and cysteine digestibilities of 7 and 11% MPSBM. Supplementation of lipase upgraded ($P<0.05$) the phenylalanine and isoleucine digestibilities of meals. Therefore, enzyme treatment has improved the SIAAD of 7 and 11% MPSBM.

4.7 Conclusions

The highest AME_n for 7% MPSBM was reported when the meal was supplemented with lipase, without heat treatment. The heat and carbohydrase enzyme treatment gave the highest AME_n for 11% MPSBM. The AME_n of 1469 and 1283 $kcal \cdot kg^{-1}$ (on an as-fed basis) for 7 and 11% MPSBM respectively, could be used in practical broiler ration formulations. Heating MPSBM at 130 °C for 30 min, improved the SIAAD of 7 and 11% MPSBM. Addition of either carbohydrase, protease or lipase enhanced the SIAAD, except for serine and asparagine. The majority of amino acid digestibilities were improved with protease supplementation. There was an effect of oil level on SIAAD of histidine, tryptophan, NH_3 and alanine. The AME_n and IDAA of MPSBM were summarized in Table 4.28 and 4.29.

Table 4.28 AME_n and IDAA of mechanically-pressed *Glycine max* meal (on a DM basis).

Oil level (%)	Heat treatment	Enzyme	AME_n	DM (%)	IDAA (%)				
					Methionine	Threonine	Leucine	Isoleucine	Tryptophan
7	Yes	NE	1120	98	0.44	1.33	2.36	1.21	0.34
		C	1775	98	0.45	1.32	2.43	1.20	0.36
		P	1364	98	0.46	1.36	2.44	1.24	0.37
		L	1198	98	0.45	1.30	2.61	1.28	0.35
	No	NE	1357	91	0.36	1.03	1.82	0.88	0.25
		C	1687	91	0.37	1.07	1.79	0.91	0.26
		P	1577	91	0.39	1.09	1.85	0.99	0.27
		L	1318	91	0.37	1.02	1.91	0.94	0.26
11	Yes	NE	1515	94	0.40	1.26	2.29	1.25	0.34
		C	1671	94	0.41	1.25	2.40	1.23	0.36
		P	1766	94	0.43	1.32	2.36	1.31	0.36
		L	1430	94	0.40	1.29	2.48	1.31	0.35
	No	NE	1587	90	0.32	0.96	1.72	0.89	0.23
		C	1657	90	0.35	0.98	1.74	0.91	0.24
		P	1378	90	0.34	1.02	1.69	0.97	0.25
		L	1904	90	0.33	0.97	1.75	0.94	0.23

NE = No-enzyme, C = carbohydrase, P = protease, L = Lipase
AME_n = Nitrogen-corrected apparent metabolizable energy
SIAAD = Standardized ileal amino acid digestibility
IDAA = Ileal digestible amino acid
DM = Dry matter

Table 4.29 IDAA of mechanically-pressed *Glycine max* meal (on a DM basis).

Oil level (%)	Heat treatment	Enzyme	DM (%)	IDAA (%)					
				Arginine	Valine	Histidine	Lysine	Phenylalanine	Cysteine
7	Yes	NE	98	2.37	1.33	1.03	2.10	1.50	0.48
		C	98	2.42	1.32	1.03	2.07	1.60	0.49
		P	98	2.50	1.40	1.06	2.13	1.61	0.50
		L	98	2.35	1.31	1.05	2.07	1.56	0.49
	No	NE	91	1.83	1.02	0.76	1.68	1.18	0.35
		C	91	1.93	1.08	0.78	1.78	1.26	0.38
		P	91	1.95	1.10	0.79	1.78	1.31	0.38
		L	91	1.83	1.07	0.76	1.71	1.28	0.36
11	Yes	NE	94	2.37	1.40	0.99	2.11	1.50	0.45
		C	94	2.46	1.42	0.99	2.15	1.57	0.46
		P	94	2.46	1.47	1.02	2.17	1.58	0.47
		L	94	2.35	1.38	1.00	2.11	1.53	0.46
	No	NE	90	1.68	1.03	0.70	1.53	1.10	0.33
		C	90	1.75	1.08	0.72	1.60	1.16	0.35
		P	90	1.77	1.08	0.76	1.63	1.16	0.35
		L	90	1.77	1.07	0.72	1.56	1.15	0.34

NE = No-enzyme, C = carbohydrase, P = protease, L = Lipase DM = Dry matter
AME_n = Nitrogen-corrected apparent metabolizable energy
SIAAD = Standardized ileal amino acid digestibility
IDAA = Ileal digestible amino acid

CHAPTER 5: THE EFFECTS OF OIL LEVELS, HEATING AND ENZYMES ON THE NUTRITIVE VALUE OF MECHANICALLY-PRESSED CARINATA (*BRASSICA CARINATA*) MEAL IN 21 DAY OLD BROILER CHICKENS

5.1 Abstract

An experiment was conducted to determine the effects of oil levels, heat treatment and enzymes on nitrogen-corrected apparent metabolizable energy (AME_n) of mechanically-pressed carinata meal (MPCARIM). The trial was a completely randomized design with a factorial arrangement of treatments: 2 oil levels (12.5 or 16.5%) x 3 heat treatments (dry-heat, wet-heat or no-heat) x 4 enzyme treatments (carbohydrase, protease, lipase or no-enzyme), using 750 Ross-308 broilers (6 birds/cage and 5 replicates/treatment). AME_n was determined using excreta and diets. AME_n data were analyzed using Proc Mixed procedure of SAS. AME_n of MPCARIM was affected ($P<0.05$) by the three-way interaction among oil levels, heat and enzyme treatments. Wet-heat and carbohydrase gave the highest AME_n for 12.5% MPCARIM while for 16.5% MPCARIM, the highest AME_n occurred, when the meal was wet-heated and supplemented with lipase. Under different enzyme treatments, in most cases, higher AME_n were reported in wet-heated 12.5 and 16.5% meals. The AME_n of 2399 and 1868 kcal/kg for 12.5 and 16.5% MPCARIM respectively (on an as-fed basis), could be used in practical broiler ration formulations.

Keywords: amino acid digestibility, broilers, carinata meal, energy, enzyme, heat, mechanical pressing

5.2 Introduction

The oil content of brown-seeded carinata seed was in the range of 30.5-34.8% (Getinet et al. 1995). This is even greater than the oil content of soybean seed, which was one of the major sources of biofuels, according to the International Energy Agency (2011). There is potential use for carinata seeds as a source of biodiesal (Cardone et al. 2003) to meet some of the energy demand in the world. Small-scale biofuel producers press carinata seeds mechanically to produce oil for biofuels. In the oil extraction process, the resultant by-product is the mechanically-pressed carinata meal (MPCARIM); however, nutritional composition varies under different processing conditions. The CP content of brown-seeded

Brassica carinata meal ranged from 44.3 to 50% (Getinet et al. 1995). This high CP content suggests that the meal can be used as a protein supplement for broiler chickens. When the carinata seeds are pressed by mechanical methods, a considerable amount of oil is left in the meal. Getinet et al. (1995) found the oil contained 16.1 - 20.4% and 11.7 - 17.3% linoleic and linolenic fatty acids respectively, as a percent of total fatty acids. Therefore, the carinata meal could be a good source of energy and essential fatty acids for broiler chickens. The high residual oil and crude protein in the meal do not necessarily reflect the digestibility of these nutrients by poultry. The nutritive value of carinata meal should be expressed in terms of AME_n and SIAAD, which is a clear indication of how competently the birds can utilize the meal. Unlike camelina meal, the carinata meal was found to contain a greater glucosinolate content of 134-188 μmol/g (Getinet et al. 1995). These glucosinolates could be reduced by heat treatment (Jensen et al. 1995) or water treatment (Tyagi 2002). However, heat or water treatment may affect the nutritive value of MPCARIM. A search of the literature did not find information on residual oil content of MPCARIM. However, when different processing conditions are used to extract oil from carinata seeds, the resulting meals may have different residual oil contents. These different residual oil contents may affect the nutritive value of MPCARIM in broiler chickens. Meng and Slominski (2005) and Zanella et al. (1999) observed that enzyme supplementation improved the nutrient digestibility of diets. Therefore, this evidence suggests that the incorporation of different enzymes, like carbohydrase, protease and lipase, into MPCARIM, may improve the nutritive value. Hence, it is important to determine the effects of oil levels, heat and enzyme treatments on the nutritive value of MPCARIM.

5.3 Objectives

1. To determine the effect of residual oil levels (12.5 or 16.5%) in MPCARIM on AME_n, for broiler chickens
2. To determine the effect of heat on AME_n of MPCARIM with 12.5 or 16.5% residual oil, for broiler chickens
3. To determine the effect of dietary enzyme supplementation (carbohydrase, protease, lipase or no-enzyme) on AME_n of MPCARIM with 12.5 or 16.5% residual oil, for broiler chickens

5.4 Hypotheses

1. AME_n of MPCARIM with 16.5% residual oil will be higher than MPCARIM with 12.5% residual oil.
2. Heat will increase AME_n in either 12.5 or 16.5% residual oil meals.
3. Enzyme supplementation will increase AME_n of MPCARIM with 12.5 or 16.5% residual oil.

5.5 Materials and methods

5.5.1 Preparation of 12.5% and 16.5% residual oil carinata meals

Carinata seeds were pressed, using an expeller-press from Atlantic Oilseeds, Kinkora, Prince Edward Island and this process produced carinata oilseed cake. Finally, carinata oilseed cake was hammer-milled. The product was carinata meal with 25.5% residual oil. The carinata crude oil, which was expelled during the oil extraction process, was collected and stored in a cool environment. However, the objective was to produce two oil meals with 12.5% and 16.5% residual oil levels using, 25.5% residual oil meal. One half of 25.5% residual oil meal was further pressed, using a micro-scale oil press (Anton-fries vegetable

oil press P500R, Maschinenbau GmbH, Meitingen-Herbertshofen, Germany) in order to produce 12.5% residual oil carinata meal. The other half of the same meal was further pressed, using the same micro-scale oil press to produce 16.5% residual oil carinata meal.

5.5.2 Preparation of heat-treated and wet-heated 12.5% and 16.5% residual oil carinata meals

Both water (Tyagi 2002) and heat (Jensen et al. 1995) treatments reduced glucosinolates present in oil seed meals produced from *Brassica* oil seeds. As *Brassica carinata* belongs to the Brassicaceae family, carinata meal was processed using heat or water treatments to eliminate glucosinolates. Each 12.5% and 16.5% residual oil carinata meal was divided into three parts. One part was heat-treated at 100 °C for 30 min. The second portion was water soaked in a ratio of 1:5 w/v (meal:water) for 24 h and then dried at 50 °C. The third was not heat processed or water soaked.

5.5.2.1 Preparation of heat-treated 12.5% and 16.5% residual oil carinata meals

Each 12.5% and 16.5% residual oil carinata meal was heat processed at 100 °C for 30 min, using a drying oven (model ST33ATUL208V9KW, JPW Design Manufacturing, Trout Run, Pennsylvania, USA). The carinata meal was uniformly spread on stainless steel trays (88.9 cm x 88.9 cm x 2.54 cm) and placed in the drying oven. Then, the oven was allowed to reach 100 °C and maintained at that temperature for 30 min. The trays were then removed and allowed to cool to room temperature. Once cooled, the meal was transferred into Rubbermaid containers. Finally, the two heated oil meals were separately mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, USA).

5.5.2.2 Preparation of wet-heated 12.5% and 16.5% residual oil carinata meals

Each 12.5% and 16.5% residual oil carinata meal was water soaked in a ratio of 1:5 w/v (meal:water) (EFSA 2008) for 24 hours. Five hundred grams of carinata meal was placed in 4 L beakers. Then, 2.5 L of water was added into the beaker and the mixture was kept for 24 h, then spread on stainless steel trays (88.9 cm x 88.9 cm x 2.54 cm) lined with parchment paper. The trays were placed in the drying oven (model ST33ATUL208V9KW, JPW Design Manufacturing, Trout Run, Pennsylvania, USA) which was allowed to reach 60 °C and maintained at that temperature until the meal dried. When the meal was dry, the trays were removed from the oven and allowed to cool to room temperature. Finally, the dried meal was ground using a hammer mill (model 11881, Christy and Norris Limited, Process Engineers, Chelmsford, England).

5.5.3 Preparation of grower test diets

Six test meal ingredients (dry-heated carinata meal with 12.5% residual oil, non-heated carinata meal with 12.5% residual oil, wet-heated carinata meal with 12.5% residual oil, dry-heated carinata meal with 16.5% residual oil, non-heated carinata meal with 16.5% residual oil and wet-heated carinata meal with 16.5% residual oil) were supplemented with four enzyme treatments; carbohydrase, protease, lipase and no enzymes to prepare 24 test diets. A basal grower diet (Table 5.1) was also prepared. Twenty five treatment diets were tested. Each test diet consisted of 69.5% basal diet and one of six test meal ingredients at 30% inclusion level. All grower diets contained 0.5% chromic oxide as an inert marker. Test diets were mixed in a Hobart, bowl type, mixer (model L.800, The Hobart manufacturing Co. Ltd, Don Mills, Ontario, Canada). The basal diet was formulated to contain 20% CP and 2964 kcal·kg^{-1} AME_n (Table 5.1). The starter diet was formulated to

have 23% CP and 3050 kcal·kg^{-1} AME_n. The starter and basal grower diets were formulated using the MIXIT-WIN professional feed formulation program (version 6.22, Agricultural Software Consultants, Inc.). The carbohydrase, protease and lipase enzymes were obtained from Genencor, Danisco Division, Denmark. Carbohydrase is a mixture of amylase and xylanase, while protease and lipase were pure enzymes.

Table 5.1 Composition of diets formulated to determine the nitrogen-corrected apparent metabolizable energy of mechanically-pressed carinata meal (as fed basis).

		Grower Diets		
	Starter Diet	Basal	Test Diet (No enzyme)	Test diet (With enzyme)
Ingredients as fed basis (%)				
Corn	44.5	65.8	41.8	41.7
Soybean meal	38.7	30.2	24.3	24.3
Mechanically-pressed carinata meal[Z]	-	-	30.0	30.0
Wheat	10.0	-	-	-
Tallow-grease blend	3.2	-	-	-
Ground limestone	1.7	1.6	1.6	1.6
Mono-dicalcium phosphate	0.6	0.8	0.8	0.8
Chromic oxide	-	0.5	0.5	0.5
Enzyme[Y]	-	-	-	0.05
Vitamin/mineral premix[X]	0.5	0.5	0.5	0.5
Iodized salt	0.4	0.4	0.4	0.4
Methionine premix[W]	0.4	0.2	0.1	0.1
Total	100	100	100	100
Calculated Analysis				
AME_n (kcal·kg^{-1})	3050	2964	-	-
Protein %	23	20	-	-
Lysine %	1.4	1.1	-	-
Methionine %	0.6	0.4	-	-
Calcium %	1	0.9	-	-
Phosphorus %	0.5	0.4	-	-

[Z]Mechanically-pressed carinata meal: 12.5 or 16.5%

[Y] Enzyme (1000 g tonne^{-1} feed): Carbohydrase: Xylanase 2400 μ·kg^{-1} feed and Amylase 240 μ·kg^{-1} feed, protease 5000 μ·kg^{-1} feed or Lipase, 3300 μ·kg^{-1} feed (Genencor, A Danisco Division, Denmark)

[X]Vitamin-mineral premix (Amount per kilogram): Vitamin A (1.00x10^9 IU kg^{-1}), 1.56 g; Vitamin D3 premix (3.00x10^7 IU kg^{-1}), 16 g; Vitamin E (5x10^5 IU kg^{-1}), 10 g; Vitamin K (33%), 1.8 g; Riboflavin (80%), 1.9 g; DL Ca-pantothenate (45%), 6 g; Vitamin B12 (1000 mg kg^{-1}), 4.6 g; Niacin (98%), 6 g; Folic acid (3%), 26.6 g; Choline chloride (60%), 267 g; Biotin (400 ppm), 60 g; Pyridoxine (990000 mg kg^{-1}), 1 g; Thiamine (970000 mg kg^{-1}), 0.6 g; Manganous oxide (56%), 23.4 g; Zinc oxide (80%), 20.78 g; Copper sulfate (25%), 20 g; Selenium premix (1000 mg kg^{-1}), 14.85 g; Ethoxyquin (60%), 16.6 g; Ground corn, 401.31 g; Ground limestone, 100 g.

[W]Methionine premix: DL-Methionine (50%), Ground corn (50%).

5.5.4 Animal husbandry

Seven hundred and fifty, Ross-308, male, day-old broiler chickens were obtained from Clark's Chick Hatchery Ltd, Burtts Corner, New Brunswick, Canada. The experiment was conducted in a controlled environment room at the Atlantic Poultry Research Center at Bible Hill, Truro, Nova Scotia, Canada.

The temperature, light intensity and lighting schedule (lights hr on/off) inside the controlled environment room were maintained, as described in Chapter 3, Section 3.5.4.

The birds were weighed and randomly assigned to 125 cages with six birds per cage. A standard starter diet was fed to all birds from Day 1 to Day 14. From Day 15 to 21 days of age, the cages were randomly assigned to one of the 25 test diets (including the basal diet) in five replicate cages per treatment (125 cages in total). Feed was provided *ad libitum* from feed troughs and was measured into the feeders, as needed. The remaining feed in the feeders was weighed on Days 15 and 21. At 0, 14, and 21 days of age, the birds from each cage were weighed as a group. Water was provided *ad libitum* from a nipple system. Birds were monitored for physical and behavioral changes daily, following the principles established by the Canadian Council on Animal Care (2009), under the guidance of the Dalhousie Animal Care and Use Committee.

5.5.5 Sample collection

At 19, 20 and 21 days of age, representative samples of clean excreta materials were collected from the cleaned trays underneath each cage. The excreta samples were frozen in a -20 °C freezer, then freeze-dried (Chapter 3, Section 3.5.6).

5.5.6 Chemical analysis

Dry matter content of test diets and carinata meal ingredient samples were determined (method, 934.01; AOAC 2005) as described in Chapter 3, Section 3.5.6. Excreta samples were freeze-dried as described in Chapter 3, Section 3.5.6. All freeze-dried samples were ground with a coffee grinder (model 43-1964-8, Lancaster™, China). CP content of test diets, test meal ingredients and excreta samples was determined (method 990.03; AOAC 2005), as described in Chapter 3, Section 3.5.6. Gross energy content of the dried excreta, test diet samples and carinata meals was determined, using a Parr 6300 adiabatic bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA). Chromic oxide contents of test diets, dried ileal content and excreta samples were determined (Fenton and Fenton 1979), using a Bausch and Lomb Spectronic (model 501, Milton Roy Company, Ivyland, USA).

5.5.7 Calculations

AME_n of test diets, basal diets and carinata meals were calculated (Leeson and Summers 2001), as described in Chapter 3, Section 3.5.7.

5.5.8 Statistical Analysis

The experimental design was a completely randomized design with 2 x 3 x 4 factorial arrangement with two residual oil levels (12.5% or 16.5%), three heat treatments (dry-heat, wet-heat and no-heat) and four types of enzyme treatments (No-enzyme, carbohydrase, protease or lipase). The statistical model statement of the experiment was as follows.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} was the response variable (AME_n, apparent CP digestibility and apparent DM digestibility).

μ was the overall mean of the response variable (AME_n, apparent CP digestibility and apparent DM digestibility).

α_i was the effect of i^{th} level of residual oil in meal (12.5% or 16.5%).

β_j was the effect of j^{th} heat treatment (dry-heated. wet-heated or non-heated).

γ_k was the effect of k^{th} enzyme (no-enzyme, carbohydrase, protease or lipase).

$(\alpha\beta)_{ij}$ was the two-way interaction effect of i^{th} level of meal residual oil and j^{th} heat treatment.

$(\alpha\gamma)_{ik}$ was the two-way interaction effect of i^{th} level of meal residual oil and k^{th} enzyme.

$(\beta\gamma)_{jk}$ was the two-way interaction effect of j^{th} heat treatment and k^{th} enzyme.

$(\alpha\beta\gamma)_{ijk}$ was the three-way interaction effect of i^{th} level of meal residual oil, j^{th} heat treatment and k^{th} enzyme.

ε_{ijkl} was the residual error.

The AME_n, apparent CP digestibility and apparent DM digestibility of test meal ingredients data were subjected to analysis of variance, using the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). If main effects or interaction effects were significant ($P<0.05$), the least square means were compared ($\alpha = 0.05$), using the Tukey-Kramer option (Gbur et al. 2012).

5.6 Results and Discussion

5.6.1 Analyzed nutrient composition of carinata test meals and diets

The analyzed nutrient compositions of MPCARIM were given in Table 5.2. The CP content of MPCARIM ingredients ranged from 37 to 41%. The CP contents of MPCARIM reported in the current study (37 - 41%) were less than those reported by Getinet et al. (1995) (44.3 - 50%). The gluocosinolate contents of MPCARIM (Table 5.2) in both dry-heated 12.5% and 16.5% MPCARIM, were greater than those in non-heated 12.5 and 16.5% MPCARIM. The reason is unknown. However, wet-heat treatment reduced the glucosinolates dramatically, in both 12.5 and 16.5% MPCARIM. This might be due to the volatilization of glucosinolate breakdown products, mainly isothiocyanate. A "radish like" strong smell was experienced when the carinata meal was being water soaked, supporting this assumption. The test diets containing 12.5% residual oil carinata meal (Table 5.3) and 16.5% residual oil carinta meal (Table 5.4) were not balanced as formulated feed. Therefore, the high CP of the carinata meals raised the test diet CP content. The analyzed CP content (Table 5.3) of the basal diet (20%) was similar to the calculated analysis (Table 5.1) while the analyzed AME_n of the basal diet (Table 5.3) was less than the calculated analysis (Table 5.1).

Table 5.2 Analyzed nutrient composition (as-fed basis) of dry-heated, wet-heated and non-heated 12.5% low oil carinata meal (LOM) and 16.5% high oil carinata meal (HOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of carinata meal using 21 day old broilers.

	LOM			HOM		
	No-heat	Dry-heat	Wet-heat	No-heat	Dry-heat	Wet-heat
DM (%)	92	94	95	92	94	94
Crude protein (%)	40	41	41	37	40	39
Gross energy (kcal·kg^{-1})	4747	4898	4905	5301	5276	5381
Crude fat (%)	12.5	12.6	12.4	16.5	16.6	16.6
Glucosinolates (μmol·g^{-1})	155	177	3	152	156	7

Table 5.3 Analyzed nutrient composition of diets (as-fed basis) with 12.5% low oil carinata meal (LOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of carinata meal using 21 day old broilers.

	Diets											
	Basal diet	Diets with non-heated LOM				Diets with dry-heated LOM				Diets with wet-heated LOM			
		C	P	L	NE	C	P	L	NE	C	P	L	NE
DM (%)	87	89	90	90	90	91	91	91	91	91	91	92	91
CP (%)	20	26	27	27	27	28	28	27	28	28	28	28	28
AME_n (kcal·kg^{-1})	2471	2400	2177	2316	2164	2254	2304	2287	2244	2493	2457	2558	2467
Gross energy (kcal·kg^{-1})	3784	4047	4080	4098	4095	4151	4161	4177	4157	4161	4164	4222	4146

C = carbohydrase, P = protease, L = lipase, NE = no-enzyme

Carbohydrase: Xylanase 2400 μ·kg^{-1} feed and Amylase 240 μ·kg^{-1} feed, protease 5000 μ·kg^{-1} feed or Lipase, 3300 μ·kg^{-1} feed

Table 5.4 Analyzed nutrient composition of diets (as-fed basis) with 16.5% high oil carinata meal (HOM) used to determine the effects of oil levels, heat and enzyme treatment on nutritive value of carinata meal using 21 day old broilers.

	Diets											
	Diets with non-heated HOM				Diets with dry-heated HOM				Diets with wet-heated HOM			
	C	P	L	NE	C	P	L	NE	C	P	L	NE
DM (%)	90	90	90	90	90	90	91	90	90	90	91	90
CP (%)	26	25	25	26	27	27	26	27	27	27	27	20
AME_n ($kcal \cdot kg^{-1}$)	2493	2586	2425	2396	2377	2499	2515	2377	2404	2551	2665	2634
Gross energy ($kcal \cdot kg^{-1}$)	4099	4132	4113	4121	4138	4148	4172	4136	4139	4155	4187	4156

C = carbohydrase, P = protease, L = lipase, NE = no-enzyme

Carbohydrase: Xylanase 2400 $\mu \cdot kg^{-1}$ feed and Amylase 240 $\mu \cdot kg^{-1}$ feed, protease 5000 $\mu \cdot kg^{-1}$ feed or Lipase, 3300 $\mu \cdot kg^{-1}$ feed

5.6.2 Apparent dry matter digestibility (%)

The apparent DM digestibility of MPCARIM was influenced ($P<0.05$) by a three-way interaction among the oil levels, heat and enzymes (Table 5.5). None of the enzymes improved ($P>0.05$) the DM digestibility in 12.5% wet-heated, dry-heated and non-heated meals, when compared to wet-heated, dry-heated and non-heated meals with no-enzyme, respectively. A similar trend was seen in 16.5% wet-heated, dry-heated and non-heated MPCARIM, where enzyme supplementation was not effective ($P>0.05$) in improving the DM digestibility, when compared to respective wet-heated, dry-heated and non-heated meal with no-enzyme. For a particular heat treatment, either in 12.5 or 16.5% MPCARIM, no difference ($P>0.05$) occurred among carbohydrase, protease and lipase treatments, except in 12.5% non-heated meal. The 12.5% non-heated meal supplemented with carbohydrase resulted in a greater ($P<0.05$) DM digestibility compared to the 12.5% non-heated meal with protease enzyme. However, no difference ($P>0.05$) was seen between carbohydrase and lipase enzymes or protease and lipase enzyme treatments. In general, enzyme supplementation did not improve the DM digestibility of either the wet-heated, dry-heated or non-heated MPCARIM, with 12.5 or 16.5% residual oil level. There were no values for DM digestibility of MPCARIM found in the literature.

5.6.2 Apparent crude protein digestibility (%)

The apparent CP digestibility of MPCARIM was influenced ($P<0.05$) by enzymes (Table 5.6). When compared to no-enzyme treatment, either carbohydrase or protease enzyme supplementation improved ($P<0.05$) the apparent CP digestibility of MPCARIM. However, among carbohydrase, protease and lipase enzymes, carbohydrase was superior ($P<0.05$) to protease and lipase.

Table 5.5 Effects of oil level, heat and enzymes on the apparent dry matter digestibility (%) of mechanically- pressed *Brassica carinata* meal in 21day old broilers.

	Oil x Heat x Enzyme					
	12.5% oil meal			16.5% oil meal		
	Wet heat	Dry heat	No heat	Wet heat	Dry heat	No heat
Enzyme treatment						
No-enzyme	83±2 abcd	77±2 d	79±2 bcd	86±2 abcd	80±2 bcd	85±2 abcd
Carbohydrase	86±2 abcd	77±2 d	88±2 ab	79±2 bcd	80±2 bcd	87±2 abc
Protease	84±2 abcd	78±2 cd	77±2 d	82±2 bcd	82±2 bcd	92±2 a
Lipase	85±2 abcd	79±2 b-d	83±2 abcd	87±2 abc	85±2 abcd	88±2 ab
Oil		81±0.5			84±0.5	
Heat	84±0.6	80±0.6	85±0.6			
Oil x Heat	84±0.9	78±0.9	82±0.9	83±0.9	82±0.9	88±0.9
Oil x Enzyme						
No-enzyme		80±1			84±1	
Carbohydrase		83±1			82±1	
Protease		80±1			85±1	
Lipase		82±1			87±1	
Heat x Enzyme					Enzyme	
No-enzyme	84±1	79±1	82±1		82±0.7	
Carbohydrase	83±1	79±1	87±1		83±0.7	
Protease	83±1	80±1	84±1		82±0.7	
Lipase	86±1	82±1	86±1		84±0.7	
Source of variation	Pr>F					
Oil	<.0001					
Heat	<.0001					
Oil x Heat	0.0202					
Enzyme	0.0006					
Oil x Enzyme	0.0049					
Heat x Enzyme	0.0003					
Oil x Heat x Enzyme	0.0135					

[a-d]Mean±SE in the oil x heat x enzyme interaction with no common letters are significantly different (α = 0.05).

Table 5.6 Effects of oil level, heat and enzymes on the apparent crude protein digestibility (%) of mechanically- pressed *Brassica carinata* meal in 21day old broilers.

	Oil x Heat x Enzyme					
	12.5% oil meal			16.5% oil meal		
	Wet heat	Dry heat	No heat	Wet heat	Dry heat	No heat
Enzyme treatment						
No-enzyme	44±1	45±1	45±1	45±1	44±1	45±1
Carbohydrase	51±1	47±1	50±1	48±1	47±1	51±1
Protease	45±1	49±1	45±1	46±1	47±1	49±1
Lipase	43±1	44±1	48±1	46±1	47±1	47±1
Oil		46±0.4			47±0.4	
Heat	46±0.5	46±0.5	47±0.5			
Oil x Heat	46±0.6	46±0.6	47±0.6	46±0.6	46±0.6	48±0.6
Oil x Enzyme						
No-enzyme		45±0.7			44±0.7	
Carbohydrase		49±0.7			48±0.7	
Protease		46±0.7			47±0.7	
Lipase		45±0.7			47±0.7	
Heat x Enzyme					Enzyme	
No-enzyme	45±1	44±1	45±1		44±0.5 c	
Carbohydrase	50±1	47±1	50±1		49±0.5 a	
Protease	46±1	48±1	47±1		47±0.5 b	
Lipase	44±1	45±1	47±1		46±0.5 bc	
Source of variation	Pr>F					
Oil	0.3893					
Heat	0.1085					
Oil x Heat	0.9490					
Enzyme	<.0001					
Oil x Enzyme	0.3431					
Heat x Enzyme	0.1416					
Oil x Heat x Enzyme	0.1401					

[a-c]Mean±SE in the enzyme main effect with no common letters are significantly different (α = 0.05).

The meal supplemented with lipase or no-enzyme gave similar (P>0.05) apparent CP digestibility. However, in the current study, the reported apparent CP digestibilities of MPCARIM were fairly low. Bryan (2013) observed low apparent CP digestibility for 14% residual oil yellow canola meal (52%) and 10% residual oil yellow canola meal (43%). He found higher standardized ileal CP digestibility values for both 10 and 14% residual oil yellow canola meals. Moreover, the standardized ileal CP digestibilities for MPCM (Chapter 3, Section 3.6.2.1) were greater than the reported apparent CP digestibilies. Hence, the elucidation of standardized crude protein digestibility for MPCARIM is needed as this is the best determinant of crude protein digestibility of a meal.

5.6.3 Nitrogen-corrected apparent metabolizable energy

The AME_n of MPCARIM was affected (P<0.05) by a three-way interaction among the oil levels, heat and enzymes (Table 5.7). Enzyme treatment did not improve (P>0.05) the AME_n of 12.5% wet-heated MPCARIM, when compared to the no-enzyme treatment. A similar trend was observed in 12.5% dry-heated carinata meal, where carbohydrase, protease or lipase did not improve AME_n (P>0.05), when compared to the no-enzyme treatment. However, when compared to protease and no-enzyme treatment, carbohydrase enzyme supplementation improved (P<0.05) the AME_n of 12.5% non-heated carinata meal and the AME_n reported for 12.5% meals with protease or lipase were similar (P>0.05). The AME_n of 16.5% wet-heated meals were not affected (P>0.05) by the enzyme treatments. When compared to dry-heated meal with no-enzyme treatment, there was no difference (P>0.05) in AME_n in meals supplemented with carbohydrase, protease or lipase. Protease improved (P<0.05) the AME_n of 16.5% non-heated meal, when compared to the no-enzyme treatment. There were no differences (P>0.05) in AME_n among carbohydrase, protease or

Table 5.7 Effects of oil level, heat and enzymes on the nitrogen-corrected apparent metabolizable energy (kcal·kg^{-1}) content of mechanically- pressed *Brassica carinata* meal in 21day old broilers (on a DM basis).

	Oil x Heat x Enzyme					
	12.5% oil meal			16.5% oil meal		
	Wet heat	Dry heat	No heat	Wet heat	Dry heat	No heat
Enzyme treatment						
No-enzyme	2409±130 b-e	1591±130 fg	1386±130 g	3128±130 a	2175±130 c-f	2245±130 c-f
Carbohydrase	2699±145 a-d	1629±130 fg	2362±130 b-e	2616±130 a-d	2175±130 c-f	2605±130 a-d
Protease	2373±130 b-e	1812±130 e-g	1434±130 g	2818±130 a-c	2628±130 a-d	2949±130 ab
Lipase	2640±130 a-d	2021±145 d-g	2072±149 d-g	3132±130 a	2585±130 a-d	2351±130 b-e
Oil		2036±39			2617±38	
Heat	2727±48	2077±47	2175±47			
Oil x Heat	2530±67	1763±67	1813±67	2923±70	2391±65	2537±65
Oil x Enzyme						
No-enzyme		1795±75			2516±75	
Carbohydrase		2230±78			2465±83	
Protease		1873±75			2798±75	
Lipase		2244±81			2689±75	
Heat x Enzyme					Enzyme	
No-enzyme	2769±92	1883±92	1815±92		2158±53	
Carbohydrase	2658±111	1902±92	2483±92		2348±57	
Protease	2596±92	2220±92	2192±92		2336±55	
Lipase	2886±92	2303±98	2211±97		2467±53	
Source of variation	Pr>F					
Oil	<.0001					
Heat	<.0001					
Oil x Heat	0.0457					
Enzyme	0.0013					
Oil x Enzyme	0.0001					
Heat x Enzyme	0.0001					
Oil x Heat x Enzyme	0.0086					

[a-g]Mean±SE in the oil x heat x enzyme interaction with no common letters are significantly different ($\alpha = 0.05$).

lipase enzyme treatments in 16.5% non-heated carinata meals.

In most cases, the 12.5 and 16.5% MPCARIM achieved comparatively higher AME_n values for wet-heated meals than the dry-heated and non-heated meals under different enzyme treatments. One of the reasons might be due to the starch gelatinization. In the current study, after soaking the meal for 24 h, it was poured into trays with water and heated at 60 °C as described in Section 5.5.2.2, until the meal dried. During heating, starch in the meal, might have been gelatinized because according to Ratnayake and Jackson (2006), when corn starch was heated with water, at or below 80 °C, all starch granules were gelatinized. When the starch is heated with water, starch granules absorb water and swell, finally separating into amylose and amylopectin. Therefore, when the carinata meal was heated with water, breakdown of starch into amylose and amylopectin should have been completed to some extent. Hence, a greater AME_n in wet-heated 12.5 and 16.5% MPCARIM could be expected.

The AME_n for 12.5 and 16.5% MPCARIM in the current study were 1868 and 2399 $kcal \cdot kg^{-1}$ respectively, (as-fed basis). Therefore, the increase in AME_n with an addition of 1% carinata oil was 133 $kcal \cdot kg^{-1}$ which is fairly high. Bryan (2013) showed a fairly high increment in AME_n in black canola meal, with the addition of an extra 1% canola oil. According to his findings, the AME_n of 10 and 14% residual oil black canola meal were 2245 and 2752 $kcal \cdot kg^{-1}$, respectively on an as-fed basis. A 1% addition of canola oil gave an extra 127 $kcal \cdot kg^{-1}$ of AME_n in mechanically-pressed black canola meal.

5.7 Conclusions

The highest AME_n for 12.5% MPCARIM occurred when the meal was wet-heated and supplemented with carbohydrase enzyme. Wet heat and lipase enzyme treatment gave the greatest AME_n for 16.5% MPCARIM. Under different enzyme treatments, in most cases, better AME_n were reported in wet-heated 12.5 and 16.5% meals than dry-heated and non-heated meals. The AME_n of 2399 and 1868 $kcal \cdot kg^{-1}$ for 16.5 and 12.5% MPCARIM respectively (on an as-fed basis), could be used in practical broiler ration formulations. The AME_n of MPCARIM were summarized in Table 5.8.

Table 5.8 AME_n of mechanically-pressed *Brassica carinata* meal (on a DM basis).

Oil level (%)	Heat treatment	Enzyme	DM (%)	AME_n ($kcal \cdot kg^{1}$)
12.5	Wet-heat	NE	95	2409
		C	95	2699
		P	95	2373
		L	95	2640
	Dry-heat	NE	94	1591
		C	94	1629
		P	94	1812
		L	94	2021
	No-heat	NE	92	1386
		C	92	2362
		P	92	1434
		L	92	2072
16.5	Wet-heat	NE	94	3128
		C	94	2616
		P	94	2818
		L	94	3132
	Dry-heat	NE	94	2175
		C	94	2175
		P	94	2628
		L	94	2585
	No-heat	NE	92	2245
		C	92	2605
		P	92	2949
		L	92	2351

NE = No-enzyme, C = Carbohydrase, P = Protease, L = Lipase

CHAPTER 6: PRODUCTION PERFORMANCE OF BROILER CHICKENS FED GRADED LEVELS OF MECHANICALLY-PRESSED CAMELINA (*CAMELINA SATIVA*) MEAL FROM 0-36 DAYS

6.1 Abstract

The oil from camelina seeds is commonly extracted by mechanical-pressing. The resultant-byproduct is mechanically-pressed camelina meal (MPCM) which can be a potential energy and protein supplement in broiler diets. This experiment was conducted to determine the effect of feeding MPCM on production performance of broiler chickens. Camelina seeds were pressed using an expeller-press to produce meal with 2 residual oil levels. Each meal was incorporated at 0, 5, 10 and 15% in starter, grower and finisher diets, producing 8 treatment diets for each phase. The trial was a randomized complete block design with 2 residual oil levels (13.5 or 16.5%) x 4 inclusion levels (0, 5, 10, or 15%) factorial arrangement. A total of 2560 day old Ross 308 male broiler chicks were randomly placed within 64 (8 pens/treatment) floor pens (40 birds/pen). Feed and water were provided *ad libitum*. The feed consumption (FC) per pen was measured. The birds were group weighed per pen at Day 0, 15, 25 and 35. The body weight gain (BWG) ($bird^{-1} \cdot day^{-1}$), FC ($bird^{-1} \cdot day^{-1}$) and feed conversion ratio (FCR) were calculated. The data were analyzed using Proc Mixed procedure of SAS, Inc, with the day as the repeated factor. For both oil levels, during the starter, grower and finisher phases, 15% reduced ($P<0.05$) the BW and BWG of the birds when compared to 0% inclusion. During the starter and grower phases, 15% and in the finisher phase, 10% inclusion reduced ($P<0.05$) the FC, compared to 0%, for both oil meals. Within each phase, there was no difference ($P>0.05$) in FCR among the birds fed camelina meal containing diets and 0% meal inclusion diet, regardless of the oil level in the meal. It was recommended to incorporate MPCM at 10% in starter, grower and finisher broiler diets.

Keywords: Body weight, broilers, feed consumption, weight gain, FCR, camelina meal

6.2 Introduction

The oil from camelina seeds can be extracted either by solvent extraction or mechanical pressing where the most popular method is mechanical pressing. Mechanical pressing produces camelina oil which is considered to be healthy for human consumption as the mechanical meals do not involve organic solvents in the extraction process. In extracting oil from camelina seeds by mechanical pressing, the resultant by-product is camelina meal with a variable nutritional composition. Mechanical extraction of camelina oil leaves a considerable amount of oil in the meal. The residual oil content of MPCM meal was 13.6%

on an as-fed basis (Pekel et al. 2009). According to Ryhanen et al. (2007) and Almeida et al. (2013), the residual oil content was 17% and 11% respectively, on an as fed basis. Therefore, MPCM is considered as a good source of energy and lipid for broiler chickens. This meal contains a high CP content which comprises both indispensable and dispensable amino acids. According to Ryhanen et al. (2007) camelina expeller cake contained 35.6% CP on a DM basis. Pekel et al. (2009) and Almeida et al. (2013) found that the CP content of MPCM was 38%, on a DM basis. Camelina meal was found to be a good source of essential fatty acids. Cherian et al. (2009) determined that, out of the total fatty acid content in the camelina meal, alpha-linolenic acid and linoleic acid comprised 29.6 and 23.4%, respectively, which constituted more than 50% of total fatty acids in the meal. Because of the attractive nutrient composition present in the MPCM, it is considered as a good source of protein and energy supplement for broiler chickens. In the past, little research has been conducted to investigate the effect of feeding graded levels of MPCM on the production performance of broiler chickens. Ryhanen et al. (2007) observed that the inclusion of *Camelina sativa* expeller cake in diets at 5 and 10% exerted significant negative effects on the production performance of broiler chickens. From evaluation of the growth performance and carcass characteristics of birds, Aziza et al. (2010) recommended that the broiler diets can be supplemented with camelina meal, up to 10%, without significant negative effects. However, no comparision of camelina oil levels has been found in the literature. Therefore, a production trial was conducted to determine the effects of four inclusion levels (0, 5, 10 and 15%) of MPCM with 13.5 and 16.5% residual oil, on the production performance (body weight, body weight gain, feed consumption and feed conversion ratio) of Ross 308 male broiler chickens.

6.3 Materials and methods

6.3.1 Preparation of 13.5% and 16.5% residual oil camelina meal

Camelina seeds were pressed using an expeller-press in Prince Edward Island to produce camelina oilseed cake, which was then hammer-milled. The product was camelina meal with 13.1% residual oil. Crude oil was added to one half of 13.1% residual oil meal and mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, United States) in order to produce 13.5% residual oil meal. Camelina crude oil was added to the other half of the 13.1% residual oil camelina meal and mixed in a Marion mixer to produce a uniform 16.5% residual oil meal.

6.3.2 Preparation of test diets

The AME_n contents of camelina meal, reported in Chapter 3, were used to formulate starter, grower and finisher broiler diets. However, when the growth trial was initiated, the ileal digestible amino acid (IDAA) contents of camelina meal had not been determined. Therefore, IDAA contents of camelina meal were not used to formulate broiler diets. No IDAA contents of camelina meal were found in the literature. However, both the camelina: *Camelina sativa* and canola: *Brassica napus* belong to the family, Brassicaceae and it was hypothesized that the broiler chickens may digest the amino acids present in camelina meal efficiently, similar to canola meal. Therefore, faecal amino acid digestibility coefficients for methionine, cysteine, threonine, tryptophan and lysine of pre-pressed solvent-extracted canola meal (Leeson and Summers 2001) were used to calculate faecal digestible amino acid contents of camelina meal. These faecal digestible amino acid contents of camelina meal were used to formulate the broiler diets. All the diets were isocaloric and isonitrogenous within the starter (0 to 14 days), grower (15 to 25 days) and finisher (26 to

36 days) phases and were formulated according to Ross 308 broiler nutrient specifications, that met or exceeded the NRC (1994) nutrient requirements for each stage. The 13.5 or 16.5% residual oil camelina meal was included at 0, 5, 10 or 15% levels in the starter, grower and finisher diets, resulting in 24 diets. As the AME_n content of camelina meal was significantly improved ($P<0.05$) with the carbohydrase enzyme in the digestibility study (Chapter 3), all the 24 test diets were supplemented with superzyme^{TM}-OM enzyme complex (Canadian Bio-systems Inc., Calgary, Alberta, Canada). All diets were mixed using a vertical mixer controlled by the LV feeds computer batching system (L.V. Control Manufacturing Limited, Winnipeg, Manitoba, Canada). The starter diets were made in a mash form. The grower and finisher diets were pelleted into 3 mm thickness using a California pellet mill (model 94103, California Pellet Mill Co., San Fransisco, California, United States of America). All starter diets (Table 6.1) were formulated to contain 3025 $kcal \cdot kg^{-1}$ AME_n and 23% CP. The eight grower diets (Table 6.2) had 3150 $kcal \cdot kg^{-1}$ AME_n and 20 % CP. The finisher diets were formulated to contain 3200 $kcal \cdot kg^{-1}$ AME_n and 18% CP (Table 6.3). All diets were prepared using MIXIT-WIN professional feed formulation program (version 6.22, Agricultural Software Consultants, Inc.).

Table 6.1 Ingredient and calculated analyses of starter broiler diets containing mechanically-pressed camelina meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Ingredient							
Corn	51.11	45.02	41.47	37.91	45.19	41.79	38.40
Soybean meal	40.65	39.73	36.69	33.65	39.83	36.88	33.94
Camelina meal	…….	5.00	10.00	15.00	5.00	10.00	15.00
Ani/veg fat[Z]	3.46	5.41	6.99	8.56	5.15	6.46	7.78
Dicalcium phosphate	1.64	1.71	1.66	1.61	1.71	1.66	1.60
Limestone	1.42	1.44	1.45	1.47	1.44	1.45	1.46
Met-px[Y]	0.64	0.60	0.60	0.60	0.60	0.61	0.61
Vit-min-px[X]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.43	0.45	0.45	0.45	0.45	0.45	0.45
Coban[W]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[V]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Superzyme™-OM[U]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine-HCl	0.02	0.01	0.07	0.13	0.01	0.07	0.13
Calculated analysis							
AME_n ($kcal \cdot kg^{-1}$)	3025	3025	3025	3025	3025	3025	3025
Tryptophan %	0.24	0.25	0.24	0.24	0.25	0.24	0.24
Threonine %	0.88	0.89	0.87	0.85	0.89	0.87	0.85
Lysine %	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Methionine+ Cysteine %	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Protein %	23	23	23	23	23	23	23
Ca %	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Available P%	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Na %	0.19	0.19	0.19	0.19	0.19	0.19	0.19

[Z]Ani/veg fat: animal fat (80%), vegetable fat (20%)

[Y]Met-px (methionine premix): DL-Methionine (50%), Ground corn (50%)

[X]Vit-min-px (vitamin-mineral premix) (Amount per kilogram of diet): Vitamin A (1.00×10^9 IU kg^{-1}), 7.8 g; Vitamin D3 premix (3.00×10^7 IU kg^{-1}), 80 g; Vitamin E (5×10^5 IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (80%), 9.59 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 23 g; Niacin (98%), 30 g; Folic acid (3%), 133 g; Choline chloride (60%), 1335 g; Biotin (400 ppm), 300 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (970000 mg kg^{-1}), 3 g; Manganous oxide (56%), 117 g; Zinc oxide (80%), 103.9 g; Copper sulfate (25%), 100 g; Selenium premix (1000 mg kg^{-1}), 74.25 g; Ethoxyquin (60%), 83 g; Ground corn, 2006.55 g; Ground limestone, 500 g.

[W]Coban: Coccidiostat; Bio Agri Mix LP, Mitchell, Ontario, Canada

[V]Stafac: Antibiotic; Bio Agri Mix LP, Mitchell, Ontario, Canada

[U]Superzyme™-OM: Cellulase, 2800 CMC units/g; Mannanase, 400 MAN units/g,; Galactanase, 50 GAL units/g; Xylanase, 1000 XYL units/g; Glucanase, 600 GLU units/g; Amylase, FAA units/g; Protease, 200 HUT units/g (Canadian Bio-systems Inc., Calgary, Alberta, Canada)

Table 6.2 Ingredient and calculated analyses of grower broiler diets containing mechanically-pressed camelina meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Ingredient							
Corn	54.36	48.07	44.60	41.04	48.24	44.92	41.53
Soybean meal	35.79	35.00	31.95	28.91	35.10	32.14	29.20
Camelina meal	…….	5.00	10.00	15.00	5.00	10.00	15.00
Ani/veg fat[Z]	5.19	7.17	8.72	10.29	6.91	8.19	9.51
Dicalcium phosphate	1.43	1.51	1.45	1.40	1.51	1.45	1.40
Limestone	1.17	1.20	1.21	1.22	1.20	1.21	1.22
Pellet binding agent[Y]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Met-px[X]	0.54	0.50	0.50	0.50	0.50	0.51	0.51
Vit-min-px[W]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.42	0.43	0.43	0.42	0.43	0.43
Coban[V]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[U]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Superzyme^{TM}-OM[T]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine-HCl	0.00	0.00	0.02	0.08	0.00	0.02	0.09
Calculated analysis							
AME_n (kcal·kg^{-1})	3150	3150	3150	3150	3150	3150	3150
Tryptophan %	0.22	0.23	0.22	0.21	0.23	0.22	0.22
Threonine %	0.81	0.82	0.80	0.78	0.82	0.80	0.77
Lysine %	1.12	1.13	1.10	1.10	1.13	1.10	1.10
Methionine+ Cysteine %	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Protein %	21	21	21	21	21	21	21
Ca %	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Available P %	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Na %	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[Z]Ani/veg fat: animal fat (80%), vegetable fat (20%)

[Y]Pellet binding agent: Lignisol

[X]Met-px (methionine premix): DL-Methionine (50%), Ground corn (50%)

[W]Vit-min-px (vitamin-mineral premix) (Amount per kilogram of diet): Vitamin A ($1.00x10^9$ IU kg^{-1}), 7.8 g; Vitamin D3 premix ($3.00x10^7$ IU kg^{-1}), 80 g; Vitamin E ($5x10^5$ IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (80%), 9.5 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 23 g; Niacin (98%), 30 g; Folic acid (3%), 133 g; Choline chloride (60%), 1335 g; Biotin (400 ppm), 300 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (970000 mg kg^{-1}), 3 g; Manganous oxide (56%), 117 g; Zinc oxide (80%), 103.9 g; Copper sulfate (25%), 100 g; Selenium premix (1000 mg kg^{-1}), 74.25 g; Ethoxyquin (60%), 83 g; Ground corn, 2006.55 g; Ground limestone, 500 g

[V]Coban: Coccidiostat; Bio Agri Mix LP, Mitchell, Ontario, Canada

[U]Stafac: Antibiotic; Bio Agri Mix LP, Mitchell, Ontario, Canada

[T]Superzyme^{TM}-OM: Cellulase, 2800 CMC units/g; Mannanase, 400 MAN units/g,; Galactanase, 50 GAL units/g; Xylanase, 1000 XYL units/g; Glucanase, 600 GLU units/g; Amylase, FAA units/g; Protease, 200 HUT units/g (Canadian Bio-systems Inc., Calgary, Alberta, Canada)

Table 6.3 Ingredient and calculated analyses of finisher broiler diets containing mechanically-pressed camelina meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Ingredient							
Corn	54.03	47.59	44.17	40.76	47.73	44.45	41.18
Soybean meal	26.66	26.19	23.13	20.07	26.29	23.33	20.37
Wheat	10.00	10.00	10.00	15.00	10.00	10.00	10.00
Camelina meal	…….	5.00	10.00	10.00	5.00	10.00	15.00
Ani/veg fat[Z]	4.7	6.56	8.01	9.47	6.32	7.53	8.74
Dicalcium phosphate	1.31	1.40	1.34	1.29	1.40	1.34	1.29
Limestone	1.21	1.23	1.24	1.25	1.23	1.24	1.25
Pellet binding agent[Y]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Met-px[X]	0.54	0.49	0.49	0.49	0.50	0.50	0.50
Vit-min-px[W]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.43	0.43	0.43	0.43	0.43	0.43
Superzyme^{TM}-OM[V]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine-HCl	0.10	0.07	0.13	0.19	0.07	0.13	0.19
Calculated analysis							
AME_n (kcal·kg^{-1})	3200	3200	3200	3200	3200	3200	3200
Tryptophan %	0.19	0.20	0.19	0.19	0.20	0.19	0.19
Threonine %	0.68	0.70	0.68	0.66	0.69	0.67	0.65
Lysine %	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Methionine+ Cysteine %	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Protein %	18	18	18	18	18	18	18
Ca %	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Available P %	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Na %	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[Z]Ani/veg fat: animal fat (80%), vegetable fat (20%)

[Y]Pellet binding agent: Lignisol

[X]Met-px (methionine premix): DL-Methionine (50%), Ground corn (50%)

[W]Vit-min-px (vitamin-mineral premix) (Amount per kilogram of diet): Vitamin A ($1.00x10^9$ IU kg^{-1}), 7.8 g; Vitamin D3 premix ($3.00x10^7$ IU kg^{-1}), 80 g; Vitamin E ($5x10^5$ IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (80%), 9.5 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 23 g; Niacin (98%), 30 g; Folic acid (3%), 133 g; Choline chloride (60%), 1335 g; Biotin (400 ppm), 300 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (970000 mg kg^{-1}), 3 g; Manganous oxide (56%), 117 g; Zinc oxide (80%), 103.9 g; Copper sulfate (25%), 100 g; Selenium premix (1000 mg kg^{-1}), 74.25 g; Ethoxyquin (60%), 83 g; Ground corn, 2006.55 g; Ground limestone, 500 g

[V]Superzyme^{TM}-OM: Cellulase, 2800 CMC units/g; Mannanase, 400 MAN units/g,; Galactanase, 50 GAL units/g; Xylanase, 1000 XYL units/g; Glucanase, 600 GLU units/g; Amylase, FAA units/g; Protease, 200 HUT units/g (Canadian Bio-systems Inc., Calgary, Alberta, Canada)

6.3.3 Animal husbandry

Two thousand five hundred and sixty, Ross-308, male, newly hatched, broiler chickens obtained from Clark's Chick Hatchery Ltd, Burtts Corner, New Brunswick, were grown in floor pens (2.996 m^2). The experiment was conducted in four rooms at the Atlantic Poultry Research Center, Dalhousie University, Truro Campus, Nova Scotia, Canada. Upon arrival of the chicks, light intensity inside the room was 20 lux. When the birds were 5 days old, the light intensity was reduced from 20 lux to 15 lux and maintained at 15 lux until the birds were 7 days old. At 7 days of age, the light intensity was reduced from 15 lux to 10 lux and maintained at 10 lux until the birds reach 9 days old. At 9 days of age, the light intensity was reduced from 10 lux to 5 lux and maintained at 5 lux until the end of experiment (36 days).

On the first day, 24 h of light was provided. When the chicks were 2 days old, 23 h of light and 1 h of darkness were provided. Sixteen hours of light and 8 h of darkness were supplied when the birds were 4 days old and this was maintained until 28 days of age. Then, 17 h of light and 7 h of darkness were given. Eighteen hours of light and 6 h of darkness were provided at 32 days of age and it was maintained until the end of the experiment.

When the birds were one day old, the temperature was reduced from 30.5 °C to 30 °C. At 3 days of age, the temperature was reduced to 29 °C and was maintained at 29 °C until the birds reached 5 days old. At 5 days of age, the temperature was reduced to 28.5 °C and maintained at 28.5 °C until the birds reached 7 days of age. At 7 days of age, the temperature was lowered to 28 °C and maintained at that temperature until Day 10, when the temperature was reduced to 27 °C. The temperature was reduced from 27 °C to 25 °C by 1 °C every 2 days until the chicks were 14 days old. The temperature of 25 °C was

maintained until day 17, when it was reduced to 24 °C and maintained until Day 19. At 19 days of age, the temperature was lowered to 23 °C and maintained until 22 days of age. At 22 days, the temperature was reduced to 22.5 °C which was maintained until the end of the experiment.

Broiler chicks were randomly placed within 64 floor pens (40 birds·pen^{-1}), with 765 cm^{2}·bird^{-1} of floor area. The starter diets were fed from 0 to 14 days of age. The grower diets were fed from 15 to 24 days of age and the finisher diets were fed from 25 to 35 days of age. Initially, feed was provided on a 52 cm x 44 cm x 5 cm cardboard tray, placed on the litter and from a tube feeder placed in each pen. The cardboard tray was removed after one week. Feed, provided *ad libitum* from tube feeders was measured into the feeders as needed. The remaining feed in the feeders was weighed on each weigh day and as mortality occurred. Water was provided *ad libitum* through a nipple drinking system. Birds were group weighed per pen at Day 0, 15, 25 and 36. Mortalities were recorded as they occurred.

6.3.4 Production performance data collection

The body weight and feed consumption per pen were measured at each weigh day and the body weight gain·bird^{-1}·day^{-1} and feed consumption·bird^{-1}·day^{-1} were calculated. Body weight gain and feed consumption were used to calculate the feed conversion ratio. For each production phase, the mortalities were expressed as a percentage.

6.3.5 Statistical analysis

The production data were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). For repeated measures analysis, the factor time was added. In repeated measures analysis, five covariance structures, compound symmetry, heterogeneous compound symmetry, toeplitz, heterogeneous toeplitz and ante-dependence

were compared. The covariance structure which gave the smallest corrected Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC) numbers, was selected for the ANOVA test (SAS Institute Inc., Cary, NC). Three orthogonal polynomials: linear, quadratic and cubic, were tested to determine the relationship between the meal inclusion levels and the response variable for each oil level within each growth phase, using Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). The experimental design was a randomized complete block design with 2 x 4 factorial arrangement, with two residual oil levels (13.5% and 16.5%) and four camelina meal inclusion levels (0%, 5%, 10% and 15%). A row consisted of 8 pens was considered as a block. The eight treatments were randomly allocated into 8 pens in one block. In one room there were 2 blocks. Since 4 rooms were used for the growth study, there were 8 blocks in total. The block effect was considered as a random effect. The statistical model of the experiment was as follows.

$$y_{ijkl} = \mu + \mathrm{p}_i + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} was the response variable (body weight, body weight gain·bird^{-1}·day^{-1}, feed consumption·bird^{-1}·day^{-1} and feed conversion ratio).

μ was the overall mean of response variable data.

p_i was the effect of i^{th} block.

α_j was the effect of j^{th} level of residual oil (13.5% and 16.5%) in camelina meal.

β_k was the effect of k^{th} meal inclusion level (0, 5, 10, and 15%).

γ_l was the effect of l^{th} day (15, 25 and 36 days).

$(\alpha\beta)_{jk}$ was the two-way interaction effect of j^{th} level of residual oil in meal and k^{th} level of meal inclusion.

$(\alpha\gamma)_{jl}$ was the two-way interaction effect of j^{th} level of residual oil in meal and effect of l^{th} day.

$(\beta\gamma)_{kl}$ was the two-way interaction effect of k^{th} level of meal inclusion and l^{th} day.

$(\alpha\beta\gamma)_{jkl}$ was the three-way interaction effect of j^{th} level of residual oil in meal, k^{th} level of meal inclusion and l^{th} day.

ε_{ijkl} was the residual error.

If main effects or interaction effects were significant ($P<0.05$), the least square means were compared ($\alpha = 0.05$) using the Tukey-Kramer option (Gbur et al. 2012).

6.4 Results and Discussion

6.4.1 Analyzed nutrient composition of diets

The calculated analyses of all starter (Table 6.1), grower (Table 6.2) and finisher (Table 6.3) diets were described in Section 6.3.2 and the determined values of diets are listed in Table 6.4. The determined values for CP in all the starter diets were greater than the calculated analysis. The determined total phosphorous contents in all the starter diets were greater than the calculated analysis which was based on available phosphorous. The analyzed calcium content in all the starter diets, except for the diet containing 15% of 13.5% residual oil camelina meal, was lower than the calculated calcium content. The starter diet which contained 16.5% residual oil camelina meal at 15%, had lower determined value for sodium compared to the calculated value. The determined values for crude protein, phosphorous and sodium in all the grower and finisher diets, were greater than the calculated analysis. However, the analyzed calcium content in all the grower diets,

was lower than the calculated calcium content. Among all the finisher diets, the determined calcium content was greater than the calculated analysis only in the diet which contained 5% of 16.5% residual oil camelina meal.

Table 6.4 Determined values[a] of starter, grower and finisher broiler diets containing mechanically-pressed camelina meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Starter diet							
Dry matter %	88.0	88.8	89.1	89.7	88.4	89.0	89.7
Protein %	24.1	23.6	25.2	24.6	23.3	26	24.7
Calcium %	0.92	0.98	0.99	1.09	0.86	0.96	0.88
Phosphorous* %	0.73	0.83	0.81	0.85	0.73	0.81	0.78
Magnesium%	0.16	0.19	0.20	0.22	0.18	0.19	0.21
Potassium %	1.06	1.06	1.03	1.02	1.03	1.06	1.05
Sodium %	0.20	0.20	0.25	0.23	0.19	0.20	0.18
Fat %	5.8	7.7	10.0	11.9	8.2	10.4	12.4
Grower diet							
Dry matter %	87.0	86.9	87.2	87.3	86.9	88.2	86.9
Protein %	21.2	21.7	22.6	21.5	22.3	23.4	22.4
Calcium %	0.75	0.84	0.73	0.85	0.76	0.75	0.80
Phosphorous* %	0.67	0.70	0.68	0.69	0.69	0.75	0.70
Magnesium%	0.17	0.18	0.18	0.19	0.17	0.19	0.19
Potassium %	1.00	1.03	0.90	0.95	1.02	1.03	0.95
Sodium %	0.19	0.19	0.20	0.18	0.22	0.19	0.19
Fat %	7.1	8.9	11.0	12.9	9.3	11.2	13.9
Finisher diet							
Dry matter %	86.9	87.1	87.2	87.8	87.3	87.6	87.8
Protein %	18.5	19.0	20.4	19.6	19.1	20.0	19.6
Calcium %	0.72	0.74	0.75	0.83	0.95	0.82	0.72
Phosphorous* %	0.61	0.66	0.68	0.70	0.65	0.66	0.68
Magnesium%	0.15	0.17	0.18	0.18	0.16	0.17	0.18
Potassium %	0.80	0.89	0.91	0.81	0.85	0.85	0.83
Sodium %	0.18	0.19	0.19	0.19	0.18	0.19	0.20
Fat %	6.8	8.8	10.5	13.0	9.7	11.1	12.8

[a]Means of duplicate determinations *Total phosphorous

6.4.2 Feed consumption

The effects of oil, day and inclusion levels of MPCM on feed consumption of broiler chickens, from Day 0 to Day 36 (Table 6.5) indicated the two-way interaction (inclusion x day) was different (P<0.05). When compared to 0% inclusion, 15% inclusion of MPCM in the starter (Day 1 to Day 15) and grower (Day 15 - Day 25) diets, reduced (P<0.05) the

Table 6.5 Effects of oil, day and inclusion levels of mechanically-pressed camelina meal on feed consumption (g·bird^{-1}·day^{-1}) from Day 0 - 36 in broiler chickens.

		Days		
		0 - 15	15 - 25	25 - 36
Inclusion (%)				
0		39±0.3f	125±0.8d	208±1a
5		39±0.3f	125±0.8d	203±1ab
10		38±0.3f	123±0.8d	199±1bc
15		36±0.3g	116±0.8e	194±1c
Source of variation	Pr>F			
Oil	0.5662			
Inclusion	<.0001			
Oil x Inclusion	0.5108			
Day	<.0001			
Oil x Day	0.2390			
Inclusion x Day	<.0001			
Oil x Inclusion x Day	0.4622			

[a-g] Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

feed consumption·bird^{-1}·day^{-1}. In both periods, there were no differences (P>0.05) in the feed consumption of birds fed 5 and 10% of camelina meal diets. However, there was a reduction (P<0.05) in feed consumption for the birds fed the diet containing 15% of MPCM compared to birds fed the 5 and 10% MPCM. Pekel et al. (2009) found that 10% inclusion of camelina meal containing 13.5% residual oil, reduced (P<0.05) feed intake during 21 days in broiler chickens (Cobb x Avian-48), when compared to the control diet with no camelina meal. Ryhanen et al. (2007), found the feed consumption per bird per day was significantly reduced during the starter period (Day 1 - Day 14), when the birds were fed

the diets containing 5% (P=0.01) and 10% (P<0.001) of camelina expeller cake, compared to a diet with no camelina meal. In the current study, the feed consumptions were greater than those reported by Ryhanen et al. (2007) for feed consumption at 5% (33 $g \cdot day^{-1}$) and 10% (32 $g \cdot day^{-1}$) levels of meal inclusion. In the present study, from Day 25 to Day 36 (finisher phase), 10% and 15% inclusion of MPCM lowered (P<0.05) the feed consumption of birds when compared to 0%. The feed consumption observed at 5% meal inclusion was not different (P>0.05) when compared to 10% inclusion, but was higher (P<0.05) than that of 15% inclusion. However, the feed consumption of birds fed diets containing 10 and 15% inclusion of MPCM was not different (P>0.05). The estimated glucosinolate levels in 13.5 and 16.5% MPCM were 40.5 and 37.5 $\mu mol \cdot g^{-1}$. In the current study, the observed reduction in feed consumption with the inclusion of camelina meal may be due to the reduced palatability caused by the glucosinolates present in the camelina meal. Previous studies using rapeseed meal (McNeill et al. 2004) and camelina meal (Ryhanen et al. 2007) suggested that the reduction (P<0.05) in feed intake of broiler chickens was due to the glucosinolates present in those meals.

In the present study, during the starter, grower and finisher phases, there was a linear decreasing trend (P<0.05) between the feed consumption and the inclusion levels of either 13.5 or 16.5% residual oil meals. The current study indicated that starter and grower diets can include MPCM at 10% with no significant negative effects on feed consumption. However, during the finisher phase, MPCM can only be incorporated at 5%.

6.4.3 Body weight

The effects of oil, day and inclusion levels of MPCM on body weight of broiler chickens from Day 0 to Day 36 (Table 6.6) showed that the two-way interaction (inclusion x day)

was different (P<0.05). When compared to 0% inclusion, 15% inclusion of MPCM, reduced (P<0.05) the body weight of birds, during all the phases of growth. In the starter and grower phases, there was no difference (P>0.05) in the body weights between the birds fed 5 and 10% camelina meal incorporated diets, whereas a reduction (P<0.05) in body weight was observed at 15% inclusion of MPCM, compared to the two lower levels of inclusion. Pekel et al. (2009) observed that 10% inclusion of camelina meal reduced (P<0.05) the body weights at Day 21, when compared to the diet without camelina meal. When the results of the body weights were considered over the three phases, MPCM could be incorporated in the starter, grower and finisher diets at 10%, without causing significant adverse effects on the body weight.

Table 6.6 Effects of oil, day and inclusion levels of mechanically-pressed camelina meal on body weight (g) from Day 0 - 36 in broiler chickens.

		Day		
		15	25	36
Inclusion (%)				
0		464±3f	1377±9d	2652±12ab
5		472±3f	1396±8d	2679±13a
10		463±3f	1362±8d	2606±13b
15		430±4g	1283±9e	2480±13c
Source of variation	Pr>F			
Oil	0.6919			
Inclusion	<.0001			
Oil x Inclusion	0.5139			
Day	<.0001			
Oil x Day	0.7797			
Inclusion x Day	<.0001			
Oil x Inclusion x Day	0.7255			

[a-g] Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

6.4.4 Body weight gain

The effects of oil, day and inclusion levels of MPCM on body weight gain of broilers from Day 0 to Day 36 (Table 6.7) indicated the two-way interaction (inclusion x day) was different ($P<0.05$). During the starter, grower and finisher phases, when compared to 0% inclusion, 15% inclusion of MPCM reduced ($P<0.05$) the body weight gain of birds. Among the birds fed the diets at 5, 10 and 15% inclusion of both meals, there was no difference ($P>0.05$) in the body weight gain of the birds fed 5 and 10% of camelina meal, whereas 15% inclusion showed a lower ($P<0.05$) body weight gain over the 5 and 10% inclusion. During the starter, grower and finisher phases, there was a decreasing linear trend ($P<0.05$) between the body weight gain and the increasing inclusion levels of MPCM. When birds fed the diets containing camelina meal were compared with control birds, MPCM can be incorporated in the starter, grower and finisher diets at 10% without affecting the body weight gain. The body weight gain results followed the same trend as body weight.

Table 6.7 Effects of oil, day and inclusion levels of mechanically-pressed camelina meal on body weight gain (g·bird^{-1}·day^{-1}) from Day 0 - 36 in broiler chickens.

		Days		
		0 - 15	15 - 25	25 - 36
Inclusion (%)				
0		30±0.2e	91±0.6c	128±1a
5		30±0.2e	92±0.6c	128±1a
10		30±0.2e	90±0.6c	124±1ab
15		27±0.2f	85±0.6d	120±1b
Source of variation	Pr>F			
Oil	0.6764			
Inclusion	<.0001			
Oil x Inclusion	0.4835			
Day	<.0001			
Oil x Day	0.5800			
Inclusion x Day	<.0001			
Oil x Inclusion x Day	0.6443			

[a-f] Mean±SE in the oil x inclusion x day interaction with no common letters are significantly different (α=0.05).

6.4.5 Feed conversion ratio

The effects of oil, day and inclusion levels of MPCM on feed conversion ratio of broiler chickens from Day 0 to Day 36 (Table 6.8) indicated the two-way interaction (inclusion x day) was different (P<0.05). During the starter phase, when compared to birds fed no MPCM, there was no difference (P>0.05) in FCR of birds fed diets containing MPCM. The FCR of the birds ranged from 1.28 to 1.31 in the starter period. The FCR observed at 5% inclusion was not different (P>0.05) from the 10% inclusion, but was lower (P<0.05) than that of 15% inclusion. However, the FCR of birds fed diets containing 10 and 15% inclusion of two oil meals was not different (P>0.05). There was a quadratic relationship (P<0.05) between FCR and meal inclusion levels of MPCM in the starter phase.

Table 6.8 Effects of oil, day and inclusion levels of mechanically-pressed camelina meal on FCR of broiler chickens from Day 0 - 36.

		Days		
		0 - 15	15 - 25	25 - 36
Inclusion (%)				
0		1.30±0.01cd	1.37±0.01b	1.63±0.01a
5		1.28±0.01d	1.35±0.01b	1.60±0.01a
10		1.28±0.01cd	1.37±0.01b	1.60±0.01a
15		1.31±0.01c	1.36±0.01b	1.61±0.01a
Source of variation	Pr>F			
Oil	0.4586			
Inclusion	0.0002			
Oil x Inclusion	0.0653			
Day	<.0001			
Oil x Day	0.1878			
Inclusion x Day	0.0469			
Oil x Inclusion x Day	0.9750			

[a-d] Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

During the grower and finisher phases, there was no difference (P>0.05) in FCR among all the treatment groups. In the grower phase, the FCR ranged from 1.35 to 1.37, whereas in the finisher phase, the FCR ranged from 1.60 to 1.63. During the grower phase, there was a quadratic relationship (P<0.05) between FCR and inclusion levels of 13.5% MPCM while it was a cubic relationship (P<0.05) for 16.5% MPCM. During the finisher phase, a quadratic relationship was seen between FCR and meal inclusion levels of 13.5% MPCM. However, neither linear, quadratic nor cubic relationship was seen between FCR and 16.5% meal inclusion levels in the finisher phase. When the FCR results were considered, MPCM can be incorporated into starter, grower and finisher diets at 15% without deleterious effects on FCR. The similar FCR suggest that, birds have utilized the diets containing camelina meal similar to those fed the control diet. Ryhanen et al. (2007) observed a negative effect when incorporating camelina expeller cake in the FCR of birds. They

reported an increase in FCR of birds fed the diets at 5% (P=0.002) and 10% (P<0.001) of camelina expeller cake during the first 14 days. However, when compared to the control with no camelina expeller cake, only the 10% (P=0.01) inclusion of camelina expeller cake impaired the FCR of the birds from Day 15 to Day 37. As observed in the present study, Pekel et al. (2009) found no difference (P>0.05) in FCR between control birds and the birds fed camelina meal.

The present study demonstrated that the feed consumption, body weight and FCR of birds have been improved when compared to those reported in previous research. This might be due to the improved nutrient utilization of the diets by the birds with the use of superzyme^{TM}-OM, which consisted of a blend of enzymes, cellulase, mannanase, galactanase, xylanase, glucanase, amylase and protease.

6.4.6 Mortality of the birds

The total mortality in the growth study was 2.8%. During the starter phase, 39 mortalities were recorded which was 1.5%. The mortalities recorded during the grower (8 birds) and finisher phases (24 birds) were 0.3 and 0.9%, respectively. The post-mortem investigations during the grower and finisher periods revealed that most mortalities occurred due to congested lungs, flips and ascites. During the starter phase, dead birds were sent for post-mortem investigations. However, investigations were not conducted in a timely manner at the pathology laboratory and accurate diagnosis of the pathology was not provided. The mortalities for the starter, grower and finisher phases were not statistically analyzed as the mortality data did not satisfy the normality assumption. When the number of mortalities occurred in birds fed no MPCM was compared with other mortalities, no treatment effects could be identified during all the growth phases (Table 6.9).

Table 6.9 Number of mortalities from Day 0-36.

	Starter*		Grower**		Finisher***		Total (%)	
	13.5% meal	16.5% meal	13.5% meal	16.5% meal	13.5% meal	16.5% meal	13.5% meal	16.5% meal
Inclusion (%)								
0	4	5	0	3	6	1	0.4	0.4
5	6	2	1	1	4	5	0.4	0.3
10	5	6	0	1	2	2	0.3	0.4
15	9	2	1	1	4	0	0.6	0.1

*Starter: 0-14 days
**Grower: 15-24 days
***Finisher: 25-36 days

6.5 Conclusions

Equal feed conversion ratio suggests that the birds utilized the diets incorporated with graded levels of MPCM, similar to the birds not fed MPCM. Body weight gain reflected feed consumption because all FCR were equal. The residual oil content in the meal did not affect the production performance of the birds. However, since the body weight and body weight gain were reduced significantly at 15% meal inclusion, it was recommended to incorporate mechanically-pressed camelina meal up to 10% in starter, grower and finisher broiler diets.

CHAPTER 7: PRODUCTION PERFORMANCE OF BROILER CHICKENS FED GRADED LEVELS OF MECHANICALLY-PRESSED SOYBEAN (*GLYCINE MAX*) MEAL FROM 0-35 DAYS

7.1 Abstract

When the soybean seeds are mechanically pressed, the resultant by-product is the mechanically-pressed soybean meal (MPSBM) which is a potential energy and protein supplement for broiler chickens. Research on the use of MPSBM in broiler diets is very limited and no recommended meal inclusion levels have been established for starter, grower and finisher diets. Therefore, an experiment was conducted to determine the effect of feeding MPSBM on production performance of broiler chickens. The trial was a randomized complete block design with 2 residual oil levels (9 or 13%) x 4 inclusion levels (0, 5, 10, or 15%) in a factorial arrangement. A total of 2560 day old Ross 308 male broiler chicks were randomly placed within 64 (8 pens/treatment) floor pens with 40 birds in each pen. Feed and water were provided *ad libitum*. The feed consumption (FC) per pen was measured. The birds were group weighed per pen at Day 0, 15, 25 and 35. The body weight gain (BWG) (bird·day^{-1}), FC (bird·day^{-1}) and feed conversion ratio (FCR) were calculated. The data were analyzed using Proc Mixed procedure of SAS, Inc, with the day as the repeated factor. For both oil levels, during the starter, grower and finisher phases, 15% reduced ($P<0.05$) the body weight and BWG of the birds when compared to 0%. Though there was no difference ($P>0.05$) in FC during the starter phase, 15% reduced ($P<0.05$) the FC, compared to 0%, during the grower and finisher phases, for both oil meals. Within each phase, there was no difference ($P>0.05$) in FCR, regardless of the oil level in the meal. It was recommended to incorporate MPSBM up to 10% into starter, grower and finisher broiler diets.

Keywords: Body weight, broilers, feed consumption, weight gain, FCR, soybean meal

7.2 Introduction

Currently, there is a consumer demand for mechanically-extraced soybean oil. Moreover, mechanical extraction of soybean oil is employed in small-scale biofuel industries. In extracting oil from soybean seeds by mechanical means for any of these purposes, the resultant by-product is a soybean meal or press cake with varying nutritional compositions. Soybean seeds can be mechanically-processed either by screw-pressing or extruded-expelling (Nelson et al. 1987). When compared to the screw-pressed and extruded-expelled soybean meal, the solvent-extracted soybean meal contains a lower residual oil content (1.2% as-fed basis) (Wang and Johnson 2001). The greater oil content remaining after

extruded-expelling (7.2% as-fed basis) and screw-pressing (6.3% as-fed basis) (Wang and Johnson 2001), makes this soybean meal an attractive energy supplement in broiler diets. Extruded-expelled and screw-pressed soybean meals contain a CP content of 42.5 and 43.2%, respectively, on an as-fed basis (Wang and Johnson 2001). Therefore, meals produced after mechanical extraction, can be used as protein supplements in broiler diets. Research evaluating the feeding of MPSBM to broiler chickens is very limited. This is due to MPSBM being less popular than solvent-extracted soybean meal. The amino acids present in solvent-extracted soybean meal are highly digestible by broiler chickens. All the amino acids had a higher than 80% SIAAD in 21 days old broiler chickens (Adedokun et al. 2008). This is one of the reasons solvent-extracted soybean meal is considered as the main protein supplement in commercial poultry diets. Powell et al. (2011) evaluated the expeller-extruded soybean meal in broiler chickens, found no significant differences in body weight, average daily gain, average daily feed intake and gain:feed intake ratio at 49 days between groups of birds fed solvent-extracted or expeller-extruded soybean meal. They did not evaluate birds fed with different inclusion levels of expeller-extruded soybean meal. There are no recommended inclusion levels of MPSBM established for the starter, grower and finisher phases in broiler diets. Therefore, a production performance study was conducted to determine the effects of inclusion levels (0, 5, 10 or 15%) of MPSBM with 9 or 13% residual oil, on the production performance (body weight, body weight gain, feed consumption and feed conversion ratio) of Ross 308, male, broiler chickens.

7.3 Materials and methods

7.3.1 Preparation of 9 and 13% residual oil soybean meals

Soybean seeds were pressed using an expeller-press from Agri Bio Fuel Ltd, Bible Hill, Truro, Nova Scotia and soybean oilseed cakes were produced. The soybean oilseed cakes were hammer-milled to produce soybean meal with 9% residual oil. Soybean crude oil was added to 9% residual oil soybean meal and mixed in a Marion mixer (Rapids Machinery Company, Marion, Iowa, United States) to produce 13% residual oil soybean meal.

7.3.2 Preparation of test diets

The AME_n content for soybean meal (reported in Chapter 4) was used to formulate starter, grower and finisher broiler diets. However, when the growth trial was intiated, the IDAA contents of soybean meal had not been determined. Therefore, IDAA values of soybean meal could not be used to formulate the broiler diets. Hence, faecal amino acid digestibility coefficients for methionine, cysteine, threonine, tryptophan and lysine of pre-pressed solvent-extracted soybean meal were used to calculate faecal digestible amino acid contents of high oil residue soybean meal. These faecal digestible amino acid contents of soybean meal were used to formulate the broiler diets. All diets were isocaloric and isonitrogenous within the starter (0 to 14 days), grower (15 to 24 days) and finisher (25 to 35 days) phases and formulated according to Ross 308, broiler nutrition specifications which met or exceeded the NRC (1994) nutrient requirements for each stage. Both 9 and 13% MPSBM were heated at 130 °C for 30 min to destroy the trypsin inhibitors and lectins. The heated 9% MPSBM and 13% MPSBM were included at 0, 5, 10 and 15% levels in the starter, grower and finisher diets, resulting in 24 diets. As the supplementation of carbohydrase enzyme increased ($P<0.05$) the AME_n of the soybean meal in the digestibility

study (Chapter 4), all the diets for this growth study were supplemented with Superzyme^{TM}-OM supplied by Canadian Bio-Systems Inc. All the diets were mixed using a vertical mixer controlled by LV feeds computer batching system (L.V. Control Manufacturing Limited, Winnipeg, Manitoba, Canada). The starter diets were made as a mash form. The grower and finisher diets were pelleted using a 3 mm die in a California pellet mill (model 94103, California Pellet Mill Co., San Fransisco, California, United States).

All the starter diets (Table 7.1) were formulated to contain 3025 kcal·kg^{-1} AME_n and 23% CP. The eight grower diets (Table 7.2) had 3150 kcal/kg AME_n and 20 % CP. An AME_n content of 3200 kcal·kg^{-1} and 18% CP were formualted for the eight finisher diets (Table 7.3). All diets were formulated using the MIXIT-WIN professional feed formulation program (version 6.22, Agricultural Software Consultants, Inc.).

Table 7.1 Ingredient and calculated analyses of starter broiler diets containing mechanically-pressed soybean meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Ingredient							
Corn	51.11	49.05	46.98	44.86	48.44	45.77	43.07
Soybean meal	40.65	36.47	32.28	28.11	36.98	33.30	29.64
Mechanically-pressed soybean meal	…….	5.00	10.00	15.00	5.00	10.00	15.00
Ani/veg fat[Z]	3.46	4.59	5.73	6.88	4.67	5.87	7.08
Dicalcium phosphate	1.64	1.65	1.65	1.66	1.64	1.65	1.66
Limestone	1.42	1.45	1.48	1.52	1.45	1.48	1.51
Met-px[Y]	0.64	0.68	0.72	0.76	0.71	0.78	0.85
Vit-min-px[X]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.43	0.43	0.43	0.43	0.43	0.43	0.43
Coban[W]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[V]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Superzyme™ -OM[U]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine-HCl	0.02	0.06	0.10	0.14	0.05	0.08	0.11
Calculated analysis							
AME_n ($kcal \cdot kg^{-1}$)	3025	3025	3025	3025	3025	3025	3025
Protein (%)	23	23	23	23	23	23	23
Tryptophan %	0.24	0.23	0.21	0.20	0.23	0.22	0.20
Threonine %	0.88	0.86	0.83	0.83	0.86	0.84	0.83
Lysine %	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Methionine+ Cysteine %	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Ca %	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Available P %	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Na %	0.19	0.19	0.19	0.19	0.19	0.19	0.19

[Z]Ani/veg fat: animal fat (80%), vegetable fat (20%)

[Y]Met-px (methionine premix): DL-Methionine (50%), Ground corn (50%)

[X]Vit-min-px (vitamin-mineral premix) (Amount per kilogram of diet): Vitamin A (1.00×10^9 IU kg^{-1}), 7.8 g; Vitamin D3 premix (3.00×10^7 IU kg^{-1}), 80 g; Vitamin E (5×10^5 IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (80%), 9.5 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 23 g; Niacin (98%), 30 g; Folic acid (3%), 133 g; Choline chloride (60%), 1335 g; Biotin (400 ppm), 300 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (970000 mg kg^{-1}), 3 g; Manganous oxide (56%), 117 g; Zinc oxide (80%), 103.9 g; Copper sulfate (25%), 100 g; Selenium premix (1000 mg kg^{-1}), 74.25 g; Ethoxyquin (60%), 83 g; Ground corn, 2006.55 g; Ground limestone, 500 g

[W]Coban: Coccidiostat; Bio Agri Mix LP, Mitchell, Ontario, Canada

[V]Stafac: Antibiotic; Bio Agri Mix LP, Mitchell, Ontario, Canada

[U]Superzyme™ -OM: Cellulase, 2800 CMC units/g; Mannanase, 400 MAN units/g,; Galactanase, 50 GAL units/g; Xylanase, 1000 XYL units/g; Glucanase, 600 GLU units/g; Amylase, FAA units/g; Protease, 200 HUT units/g (Canadian Bio-systems Inc., Calgary, Alberta, Canada)

Table 7.2 Ingredient and calculated analyses of grower broiler diets containing mechanically-pressed soybean meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Ingredient							
Corn	54.36	52.34	50.27	48.20	51.73	49.07	46.40
Soybean meal	35.79	31.59	27.41	23.23	32.10	28.43	24.76
Mechanically-pressed soybean meal	…….	5.00	10.00	15.00	5.00	10.00	15.00
Ani/veg fat[Z]	5.19	6.30	7.43	8.57	6.37	7.58	8.78
Dicalcium phosphate	1.43	1.43	1.44	1.45	1.43	1.44	1.44
Limestone	1.17	1.20	1.24	1.27	1.20	1.23	1.26
Pellet binding agent[Y]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Met-px[X]	0.54	0.58	0.62	0.66	0.61	0.68	0.75
Vit-min-px[W]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.41	0.41	0.40	0.41	0.41
Coban[V]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[U]	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Superzyme^{TM} -OM[T]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine-HCl	0.00	0.02	0.05	0.09	0.01	0.04	0.07
Calculated analysis							
AME_n (kcal·kg^{-1})	3150	3150	3150	3150	3150	3150	3150
Protein (%)	21	21	21	21	21	21	21
Tryptophan %	0.22	0.21	0.19	0.18	0.21	0.19	0.18
Threonine %	0.81	0.78	0.76	0.73	0.79	0.76	0.74
Lysine %	1.12	1.10	1.10	1.10	1.10	1.10	1.10
Methionine+ Cysteine %	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Ca %	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Available P %	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Na %	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[Z]Ani/veg fat: animal fat (80%), vegetable fat (20%)

[Y]Pellet binding agent: Lignisol,[X]Met-px (methionine premix): DL-Methionine (50%), Ground corn (50%)

[W]Vit-min-px (vitamin-mineral premix) (Amount per kilogram of diet): Vitamin A ($1.00x10^9$ IU kg^{-1}), 7.8 g; Vitamin D3 premix ($3.00x10^7$ IU kg^{-1}), 80 g; Vitamin E ($5x10^5$ IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (80%), 9.5 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 23 g; Niacin (98%), 30 g; Folic acid (3%), 133 g; Choline chloride (60%), 1335 g; Biotin (400 ppm), 300 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (970000 mg kg^{-1}), 3 g; Manganous oxide (56%), 117 g; Zinc oxide (80%), 103.9 g; Copper sulfate (25%), 100 g; Selenium premix (1000 mg kg^{-1}), 74.25 g; Ethoxyquin (60%), 83 g; Ground corn, 2006.55 g; Ground limestone, 500 g

[V]Coban: Coccidiostat; Bio Agri Mix LP, Mitchell, Ontario, Canada

[U]Stafac: Antibiotic; Bio Agri Mix LP, Mitchell, Ontario, Canada

[T]Superzyme^{TM} -OM: Cellulase, 2800 CMC units/g; Mannanase, 400 MAN units/g,; Galactanase, 50 GAL units/g; Xylanase, 1000 XYL units/g; Glucanase, 600 GLU units/g; Amylase, FAA units/g; Protease, 200 HUT units/g (Canadian Bio-systems Inc., Calgary, Alberta, Canada)

Table 7.3 Ingredient and calculated analyses of finisher broiler diets containing mechanically-pressed soybean meal (% as fed).

	Control	13.5% residual oil meal			16.5% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Ingredient							
Corn	54.03	49.14	47.28	45.38	48.57	46.14	43.69
Soybean meal	26.66	24.97	20.69	16.41	25.46	21.66	17.86
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Mechanically-pressed soybean meal	…….	5.00	10.00	15.00	5.00	10.00	15.00
Ani/veg fat[Z]	4.70	6.13	7.16	8.20	6.19	7.28	8.38
Dicalcium phosphate	1.31	1.45	1.45	1.46	1.45	1.45	1.45
Limestone	1.21	1.25	1.28	1.31	1.25	1.28	1.31
Pellet binding agent[Y]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Met-px[X]	0.54	0.53	0.57	0.61	0.56	0.64	0.71
Vit-min-px[W]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.43	0.43	0.43	0.43	0.43	0.43
Superzyme^{TM}-OM[V]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine-HCl	0.10	0.05	0.09	0.13	0.04	0.08	0.11
L-Threonine	0.00	0.00	0.00	0.02	0.00	0.00	0.01
L-Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calculated analysis							
AME_n (kcal·kg^{-1})	3200	3200	3200	3200	3200	3200	3200
Protein (%)	18	18	18	18	18	18	18
Tryptophan %	0.19	0.19	0.17	0.16	0.19	0.17	0.16
Threonine %	0.68	0.69	0.66	0.65	0.69	0.67	0.65
Lysine %	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Methionine+ Cysteine %	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Ca%	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Available P %	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Na %	0.18	0.18	0.18	0.18	0.18	0.18	0.18

[Z]Ani/veg fat: animal fat (80%), vegetable fat (20%)

[Y]Pellet binding agent; Lignisol

[X]Met-px (methionine premix): DL-Methionine (50%), Ground corn (50%)

[W]Vit-px (vitamin-mineral premix) (Amount per kilogram of diet): Vitamin A ($1.00x10^9$ IU kg^{-1}), 7.8 g; Vitamin D3 premix ($3.00x10^7$ IU kg^{-1}), 80 g; Vitamin E ($5x10^5$ IU kg^{-1}), 50 g; Vitamin K (33%), 9 g; Riboflavin (80%), 9.5 g; DL Ca-pantothenate (45%), 30 g; Vitamin B12 (1000 mg kg^{-1}), 23 g; Niacin (98%), 30 g; Folic acid (3%), 133 g; Choline chloride (60%), 1335 g; Biotin (400 ppm), 300 g; Pyridoxine (990000 mg kg^{-1}), 5 g; Thiamine (970000 mg kg^{-1}), 3 g; Manganous oxide (56%), 117 g; Zinc oxide (80%), 103.9 g; Copper sulfate (25%), 1000 g; Selenium premix (1000 mg kg^{-1}), 74.25 g; Ethoxyquin (60%), 83 g; Ground corn, 2006.55 g; Ground limestone, 500 g

[V]Superzyme^{TM}-OM: Cellulase, 2800 CMC units/g; Mannanase, 400 MAN units/g,; Galactanase, 50 GAL units/g; Xylanase, 1000 XYL units/g; Glucanase, 600 GLU units/g; Amylase, FAA units/g; Protease, 200 HUT units/g (Canadian Bio-systems Inc., Calgary, Alberta, Canada)

7.3.3 Animal husbandry

Two thousand five hundred and sixty, Ross 308, male, newly hatched, broiler chickens were obtained from Clark's Chick Hatchery Ltd, Burtts Corner, New Brunswick. The experiment was conducted in four rooms in the Atlantic Poultry Research Center, Dalhousie University, Truro, Nova Scotia. The temperature, light intensity and lighting schedule described in Chapter 6, Section 6.3.3 were used.

Chicks were randomly placed within 64 floor pens, with forty birds in each pen. The total area per pen was 2.996 m^2 (1.4 m x 2.14 m). The floor area per bird was 765 cm^2. The starter diets were fed from 0 to 14 days of age, the grower diets from 15 to 24 days of age and the finisher diets from 25 to 34 days of age. Before the arrival of the birds, in each pen, feed was provided on a 52 cm x 44 cm x 5 cm cardboard tray which was placed on the litter and in a tube feeder. The cardboard tray with feed was kept for one week until the birds become familiar with the tube feeder in each pen. Feed was provided *ad libitum* through tube feeders and was measured into the feeders as needed. The remaining feed in the feeders was weighed on each weigh day and as mortality occurred. Water was provided *ad libitum* through a nipple drinking system. Birds were group weighed per pen on 0, 14, 24 and 34 days of age. Mortalities were recorded as they occurred and dead birds were necropsied by a veterinary pathologist.

7.3.4 Production performance data collection

The body weight and feed consumption per pen were measured at each weigh day. The body weight gain·$bird^{-1}$·day^{-1} and feed consumption·$bird^{-1}$·day^{-1} calculated. The data for feed consumption and body weight gain were used to calculate the feed conversion ratio. The mortalities were expressed as a percentage for each growth period.

7.3.5 Statistical analysis

Feed consumption·bird^{-1}·day^{-1}, body weight, body weight gain·bird^{-1}·day^{-1} and FCR were subjected to analysis of variance (ANOVA) by the Proc Mixed procedure using SAS 9.3 (SAS Institute Inc., Cary, NC). The factor of day was added for repeated measures analysis. In repeated measures analysis, five covariance structures, compound symmetry, heterogeneous compound symmetry, toeplitz, heterogeneous toeplitz and ante-dependence were compared. The covariance structure which provided the smallest corrected Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC) numbers, was selected to run the ANOVA test (SAS Institute Inc., Cary, NC). Three orthogonal polynomials: linear, quadratic and cubic, were tested to determine the relationship between the meal inclusion levels and the response variable for each oil level within each growth phase, using Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). The experimental design was a randomized complete block design with 2 x 4 factorial arrangement with two residual oil levels (9% and 13%) and four high oil residue soybean meal inclusion levels (0%, 5%, 10% and 15%). A row consisted of 8 pens was considered as a block. The eight treatments were randomly allocated into 8 pens in one block. In one room there were 2 blocks. Since 4 rooms were used for the growth study, there were 8 blocks in total. The block effect was considered as a random effect. The statistical model statement of the experiment was as follows.

$$y_{ijkl} = \mu + \mathrm{p}_i + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \varepsilon_{ijkl}$$

Where:

y_{ijkl} was the response variable (body weight, body weight gain·bird^{-1}·day^{-1}, feed consumption·bird^{-1}·day^{-1} and feed conversion ratio).

μ was the overall mean of response variable data.

p_i was the effect of i^{th} block.

α_j was the effect of j^{th} level of residual oil (9% and 13%) in soybean meal.

β_k was the effect of k^{th} meal inclusion level (0, 5, 10, and 15%).

γ_l was the effect of l^{th} day (15, 25 and 35 days).

$(\alpha\beta)_{jk}$ was the two-way interaction effect of j^{th} level of residual oil in meal and k^{th} level of meal inclusion.

$(\alpha\gamma)_{jl}$ was the two-way interaction effect of j^{th} level of residual oil in meal and effect of l^{th} day.

$(\beta\gamma)_{kl}$ was the two-way interaction effect of k^{th} level of meal inclusion and l^{th} day.

$(\alpha\beta\gamma)_{jkl}$ was the three-way interaction effect of j^{th} level of residual oil in meal, k^{th} level of meal inclusion and l^{th} day.

ε_{ijkl} was the residual error.

If main effects or interaction effects were significant ($P<0.05$), the least square means were compared ($\alpha = 0.05$) using the Tukey-Kramer option (Gbur et al. 2012).

7.4 Results and Discussion

7.4.1 Analyzed nutrient compositon of diets

The calculated analyses of all starter (Table 7.1), grower (Table 7.2) and finisher (Table 7.3) diets were compared to the determined values of diets (Table 7.4). The determined values for CP and total phosphorous in all the starter diets were greater than the calculated CP and available phosphorous, while the analyzed calcium content in all the soybean incorporated starter diets was lower than the calculated calcium content. The determined sodium content was lower in the starter diets, except for the control diet and diets

containing 10% inclusion of 9% MPSBM and 5% inclusion of 13% MPSBM. The analyzed crude protein and total phosphorous contents in all the grower diets were higher than the calculated crude protein and available phosphorous, where the determined calcium content in all the grower diets was below the calculated analysis.

Table 7.4 Determined compositions[a] of starter, grower and finisher broiler diets containing mechanically-pressed soybean meal (% as fed).

	Control	9% residual oil meal			13% residual oil meal		
	0%	5%	10%	15%	5%	10%	15%
Starter diet							
Dry matter %	89.4	89.8	90.3	90.7	89.0	89.8	90.4
Protein %	24.7	25.3	24.0	25.2	24.8	24.0	25.2
Calcium %	1.12	0.90	0.97	0.92	1.02	0.97	0.93
Phosphorous* %	0.79	0.69	0.72	0.70	0.75	0.72	0.68
Magnesium%	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Potassium %	1.00	1.05	1.05	1.06	1.07	1.07	1.05
Sodium %	0.27	0.17	0.20	0.18	0.21	0.18	0.17
Fat %	6.0	7.3	7.8	10.4	7.4	9.2	10.5
Grower diet							
Dry matter %	87.2	87.2	87.7	87.8	86.3	87.6	90
Protein %	21.0	21.8	22.0	21.0	21.3	21.4	22.8
Calcium %	0.79	0.78	0.80	0.82	0.62	0.81	0.53
Phosphorous* %	0.63	0.64	0.64	0.64	0.62	0.63	0.66
Magnesium%	0.16	0.16	0.16	0.16	0.16	0.16	0.17
Potassium %	0.91	0.93	0.95	0.93	0.90	0.93	1.01
Sodium %	0.19	0.17	0.17	0.17	0.20	0.19	0.17
Fat %	7.3	9.1	10.3	11.8	9.4	11.4	13.8
Finisher diet							
Dry matter %	86.6	87.7	87.3	87.1	87.0	87.8	88.1
Protein %	18.7	19.9	18.9	18.7	19.0	18.9	19.8
Calcium %	0.70	0.93	0.90	0.70	0.83	0.83	0.86
Phosphorous* %	0.57	0.66	0.65	0.62	0.60	0.62	0.63
Magnesium%	0.14	0.16	0.16	0.16	0.16	0.16	0.16
Potassium %	0.79	0.83	0.86	0.85	0.82	0.83	0.87
Sodium %	0.19	0.21	0.20	0.21	0.18	0.21	0.21
Fat %	7.1	8.8	10.5	11.6	9.1	11.1	13.3

[a]Means of duplicate determinations
*Total phosphorous

The analyzed sodium content was poorer than that of the calculated analysis in the grower diets, except for the control diet and the diets containing 5 and 10% inclusion of 13% MPSBM. The analyzed CP, total phosphorous and sodium in finisher diets were greater than the calculated values for the same nutrients. However, only the diets containing 5 and 10% inclusion of 9% MPSBM and 15% inclusion of 13% MPSBM showed a greater value for calcium than the calculated calcium content.

7.4.2 Feed consumption

The effects of oil, day and inclusion levels of MPSBM on feed consumption of broiler chickens from Day 0 to Day 35 (Table 7.5) showed the two-way interaction (inclusion x day), was significant ($P<0.05$). From Day 0 to 14 days of age (starter), there was no treatment effect ($P>0.05$) on feed consumption of birds. During the starter period, the feed consumption·bird^{-1}·day^{-1} ranged from 36 - 37 g. A linear decreasing relationship ($P<0.05$) was seen between the feed consumption and the 9% MPSBM inclusion levels, during the starter phase. However, there was no linear, quadratic or cubic relationship ($P>0.05$) between feed consumption and 13% MPSBM inclusion levels. Powell et al. (2011) observed a 30 g average daily feed intake in birds during the starter period (Day 0 to Day 14) when the birds were fed a diet containing 38% expeller-extruded soybean meal. The feed consumption reported by Powell et al. (2011) was lower than the values for all inclusion levels of two residual oil meals reported in the present study. From Day 15 to Day 24 (grower phase), 15% inclusion of MPSBM reduced ($P<0.05$) the feed consumption of birds compared to birds fed the control diet. There was no difference ($P>0.05$) in feed consumption of birds fed diets containing 5 and 10% of soybean meal. During the grower phase, there was a decreasing linear trend ($P<0.05$) between the feed consumption and

inclusion levels of MPSBM. According to Powell et al. (2011), birds fed a grower diet containing 34% inclusion of expeller-extruded soybean meal from Day 15 to Day 35, had an average daily feed intake per bird of 101 g. However, in the present study the daily feed consumption per bird, during the grower phase (Day 15 to Day 24), ranged from 125 - 136 g, which was greater than the value reported by Powell et al. (2011). In the current study, from Day 25 to Day 35 (finisher phase), the same trend occurred as in the grower phase for feed consumption.

Table 7.5 Effects of oil, day and inclusion levels of mechanically-pressed soybean meal on feed consumption ($g \cdot bird^{-1} \cdot day^{-1}$) from Day 0-35 in broiler chickens.

		Days		
		0 - 15	15 - 25	25 - 35
Inclusion (%)				
0		37±0.3e	136±1c	212±2a
5		37±0.3e	133±1c	210±2a
10		37±0.3e	129±1cd	203±2ab
15		36±0.3e	125±1d	198±2b
Source of variation	Pr>F			
Oil	0.4848			
Inclusion	<.0001			
Oil x Inclusion	0.6373			
Day	<.0001			
Oil x Day	0.1715			
Inclusion x Day	<.0001			
Oil x Inclusion x Day	0.7455			

[a-e]Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

In the grower and finisher phases, the birds fed the diet with 15% inclusion of MPSBM had the lowest feed consumption, which was lower (P<0.05) than those fed the 5% meal diet. The feed consumption at 15% meal inclusion was not different (P>0.05) when compared to 10% meal inclusion. The average daily feed intake$\cdot bird^{-1}$ during the finisher phase (from day 36 to Day 49) was 166 g, when the birds were fed the finisher diet containing 32% inclusion of expeller-extruded soybean meal Powell et al. (2011).

However, in the present study, the feed consumption·bird^{-1}.day^{-1} for the finisher phase (Day 25 to Day 35), ranged from 198 g to 212 g, which were higher than reported by Powell et al. (2011) during a later finisher phase. In general, during the starter, grower and finisher phases, the feed consumption of birds reported by Powell et al. (2011) was comparatively lower than those reported in the present study. The reduced feed intake observed by Powell et al (2011), may have been due to the reduced palatability of the feed with higher inclusion (more than 30%) of expeller-extruded soybean meal within the starter, grower and finisher diets, compared to the present study. The lower feed intake observed by Powell et al. (2011) at more than 30% inclusion of soybean meal, can be expected, as a significant reduction ($P<0.05$) was observed in the present study, when 15% inclusion of MPSBM was fed. However, Powell et al. (2011) did not evaluate the production performance of broiler chickens fed graded levels of expeller-extruded soybean meal. Therefore, the present study addresses the knowledge gap concerning the production performance at lower inclusion levels of MPSBM. When the feed consumption results of the present study were considered, it was concluded that during the starter phase, MPSBM can be incorporated at 15% in the starter diet, without having significant negative effects on feed consumption. However, during the grower and finisher phases, MPSBM can be incorporated into diets at 10%.

7.4.3 Body weight

The effects of oil, day and inclusion levels of MPSBM on body weight of broiler chickens from Day 0 to Day 35 (Table 7.6) revealed the two-way interaction (inclusion x day) was significant ($P<0.05$). During the starter, grower and finisher phases, the birds fed a diet with 15% inclusion of MPSBM had ($P<0.05$) lowered body weight when compared to the

control group. In all three phases, the body weight decreased linearly (P<0.05) with increasing inclusion levels of MPSBM. During the starter, grower and finisher phases, the body weights recorded at 5 and 10% meal inclusion, were not different (P>0.05). However, the body weights of birds fed diets containing 10 and 15% soybean meal were not different (P>0.05). When the body weights of the birds were taken into consideration, it could be concluded that MPSBM can be incorporated in the starter, grower and finisher diets at 10%, without exerting significant deleterious effects on body weight.

Table 7.6 Effects of oil, day and inclusion levels of mechanically-pressed soybean meal on body weight (g) from Day 0-35 in broiler chickens.

		Day		
		15	25	35
Inclusion (%)				
0		465±5e	1444±12c	2622±21a
5		455±5e	1421±12c	2603±21a
10		442±5ef	1394±12cd	2532±21ab
15		430±5f	1345±12d	2455±21b
Source of variation	Pr>F			
Oil	0.0934			
Inclusion	<.0001			
Oil x Inclusion	0.8851			
Day	<.0001			
Oil x Day	0.1896			
Inclusion x Day	<.0001			
Oil x Inclusion x Day	0.5685			

[a-f] Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

7.4.4 Body weight gain

The effects of oil, day and inclusion levels of MPSBM on body weight gain of broiler chickens from Day 0 to Day 35 (Table 7.7) showed two-way interaction, (inclusion x day) was significant (P<0.05). From Day 0 to Day 15, 15% inclusion of MPSBM reduced (P<0.05) the body weight gain per bird, per day, when compared to birds fed the no

MPSBM diet. However, there was no difference (P>0.05) in the body weight gain (27-29 $g \cdot bird^{-1} \cdot day^{-1}$) of birds fed MPSBM incorporated diets. During the starter phase, the body weight gain diminished linearly (P<0.05), with the increasing meal inclusion in the diet.

Table 7.7 Effects of oil, day and inclusion levels of mechanically-pressed soybean meal on body weight gain ($g \cdot bird^{-1} \cdot day^{-1}$) from Day 0-35 in broiler chickens.

		Days		
		0 - 15	15 - 25	25 - 35
Inclusion (%)				
0		30±0.3e	98±0.8c	131±1a
5		29±0.3ef	97±0.8c	131±1a
10		28±0.3ef	95±0.8c	126±1ab
15		27±0.3f	91±0.8d	123±1b
Source of variation	Pr>F			
Oil	0.0640			
Inclusion	<.0001			
Oil x Inclusion	0.9391			
Day	<.0001			
Oil x Day	0.1165			
Inclusion x Day	<.0001			
Oil x Inclusion x Day	0.1439			

[a-f] Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

According to Powell et al. (2011), the average daily gain of broiler chickens fed 38% expeller-extruded soybean meal during the starter phase (Day 0 - Day 14) was 23 g per bird, which was lower than the values reported in the present study for all the inclusion levels for both residual oil meals. During the grower phase, when compared to the control diet, the diet with 15% inclusion of MPSBM reduced (P<0.05) the body weight gain in birds fed MPSBM. The weight gain at 5 and 10% meal inclusion was higher (P<0.05) than that of birds fed 15%. There was a decreasing linear relationship (P<0.05) between the weight gain and the meal inclusion levels of MPSBM. The average daily gain of the birds from Day 15 to Day 35, fed the solvent-extracted or expeller-extruded soybean meal diet, reported by Powell et al. (2011) was 62 g per bird. This was lower than the body weight

gain (91 - 98 g) reported in the present study during Day 15 to Day 25. From Day 25 to Day 35, incorporation of MPSBM at 15% in the finisher diet, reduced ($P<0.05$) the body weight gain, when compared to the control. Among the three MPSBM treatments, 15% inclusion had the lowest body weight gain, which was lower ($P<0.05$) than the 5% inclusion but similar ($P>0.05$) body weight gain when compared to 10% meal inclusion. The body weight gain had a decreasing linear relationship ($P<0.05$) with meal inclusion levels of MPSBM. According to Powell et al. (2011), during a later finisher phase (from Day 36 to Day 49), the average daily gain of a bird was 78 g, which was lower than the values (123 - 131 g) reported in the present study during the finisher phase (Day 25 - Day 35). The body weight gain results followed the same trend as body weight. When the body weight gain results were considered, MPSBM can be incorporated in the starter, grower and finisher diets at 10% without significant negative effects on body weight gain.

7.4.5 Feed conversion ratio

The effects of oil, day and MPSBM inclusion on feed conversion ratio (FCR) of broiler chickens from Day 0 to Day 35 (Table 7.8) showed the two-way interaction (inclusion x day) was significant ($P<0.05$). There was no difference ($P>0.05$) in FCR of birds during starter, grower and finisher phases, among all inclusion levels. During the starter phase, the FCR of the birds ranged from 1.26 to 1.31. There was a linear decreasing trend ($P<0.05$) between FCR and 9% MPSBM inclusion levels. However, there was neither a linear, quadratic nor cubic relationship between FCR and meal inclusion levels of 13% MPSBM. From Day 15 to 25, the FCR was in the range of 1.36 - 1.39. There was a cubic relationship between the FCR and 13% MPSBM inclusion levels while a quadratic relationship ($P<0.05$) was seen between the FCR and 9% MPSBM inclusion levels. During the finisher

phase, the FCR of birds ranged from 1.60 to 1.62. There was neither linear, quadratic nor cubic relationship (P>0.05) between FCR and 13% MPSBM inclusion levels while a quadratic relationship (P<0.05) was observed for the FCR and 9% MPSBM inclusion levels. Among the birds fed three MPSBM incorporated diets, there was no difference (P>0.05) in FCR during starter, grower and finisher phases. When the FCR was considered, MPSBM can be incorporated in starter, grower and finisher diets at 15% inclusion, without having significant adverse effects on FCR.

Table 7.8 Effects of oil, day and inclusion levels of mechanically-pressed soybean meal on FCR of broiler chickens from Day 0-35.

		Days		
		0 - 15	15 - 25	25 - 35
Inclusion (%)				
0		1.26±0.01d	1.39±0.01b	1.62±0.01a
5		1.29±0.01d	1.38±0.01b	1.60±0.01a
10		1.30±0.01d	1.36±0.01bc	1.61±0.01a
15		1.31±0.01cd	1.37±0.01b	1.61±0.01a
Source of variation	Pr>F			
Oil	0.1211			
Inclusion	0.6905			
Oil x Inclusion	0.0724			
Day	<.0001			
Oil x Day	0.3292			
Inclusion x Day	0.0075			
Oil x Inclusion x Day	0.8175			

[a-d] Mean±SE in the inclusion x day interaction with no common letters are significantly different (α=0.05).

In the present study, the feed consumption and body weight gain of the birds, were greater than those reported by Powell et al. (2011). In the current study, toasting of soybean meal might have increased the palatability of the soybean meal which may have caused an increase in feed consumption of birds. The greater weight gain of the birds reported in the

current study, might be due to the improved nutrient digestion and utilization of the diets with the use of superzyme^{TM} -OM enzyme.

7.4.6 Mortality of birds

Eighty six mortalities were recorded from Day 0 to Day 35. As a percentage, this represented 3.4% out of 2560 birds. Most of the mortalities occurred during the starter period (2.0%). According to post-mortem examination, the mortalities which occurred during the starter phase were mainly due to yolk sac infections, dehydrated vents, septicemia, omphalitis, fibrinous pericarditis, swollen pale liver and early ascites. During the grower period, 0.7% mortality occurred with the main causes being swollen liver, probable flips, fibrinous pericarditis, yellow liver and chronic pericarditis. The post-mortem investigations indicated that most of the mortalities observed during the finisher phase (0.7%) were because of suppurative arthritis, tibial dyschondroplasia, fibrous pericarditis, probable flips, ascites, chronic fibrinous pericarditis and osteomyelitis. However, the mortality data within the starter, grower and finisher phases were not statistically analyzed as the data did not satisfy the normality assumption. Therefore, the incidence of moratalities due to any treatment effect, could not be statistically proven. As no noticeable differences in mortalities were observed among birds fed diets with or without MPSBM, it was assumed that mortalities did not occur in a pattern related to treatments (Table 7.9).

Table 7.9 Number of mortalities from Day 0-35.

	Starter*		Grower**		Finisher***		Total (%)	
	9% meal	13% meal	9% meal	13% meal	9% meal	13% meal	9% meal	13% meal
Inclusion (%)								
0	6	6	3	1	2	1	0.4	0.3
5	5	9	3	1	6	2	0.6	0.5
10	4	6	1	1	1	0	0.2	0.3
15	7	7	5	2	2	5	0.6	0.6

*Starter: 0-14 days
**Grower: 15-24 days
***Finisher: 25-35 days

7.5 Conclusions

Within the starter, grower and finisher phases, the FCR were similar in birds fed diets containing 0, 5, 10 and 15% MPSBM. The body weight gain reflected the feed consumption because all FCR were equal. The residual oil content in the mechanically-pressed soybean meal did not affect the production performance of birds. However, the body weight, body weight gain and feed consumption except in the starter phase, were reduced significantly at 15% meal inclusion. It was recommended to incorporate mechanically-pressed soybean meal up to 10% into the starter, grower and finisher broiler diets.

CHAPTER 8: CONCLUSION

The dry-heat treatment was not effective in destroying glucosinolates in MPCM. The wet-heat treatment reduced the glucosinolates in MPCARIM, dramatically. Therefore, wet-heat was more effective than dry-heat, in destroying glucosinolates. The trypsin inhibitors in MPSBM were inactivated by heat. Heating MPCM to destroy glucosinolates was not seemed to be essential as birds fed graded levels of non-heated MPCM showed satisfactory production performance in growth study. However, heating the MPSBM is necessary to eliminate trypsin inhibitors as these anti-nutritional factors reduce the protein digestion and growth in broiler chicekns.

The actual AME_n of MPCM and MPSBM was not what was predicted from the oil content. The MPCM and MPSBM with low residual oil level gave higher AME_n than meals with high residual oil level. Therefore, there was no positive relationship between increased meal residual oil level and increased AME_n for MPCM and MPSBM. However, the MPCARIM with high residual oil level gave higher AME_n than meal with low residual oil level. Therefore, there was a positive relationship between increased meal residual oil level and increased AME_n for MPCARIM. Although no AME_n are reported for growing chickens for MPCM and MPCARIM to compare, AME_n of MPCM reported in the current study were assumed to be low, while AME_n of MPCARIM were fairly reasonable. The AME_n of MPSBM was not what was predicted from the oil content, according to the literature. The highest AME_n were reported in MPCARIM with MPSBM being second and MPCM was the lowest. The residual oil levels affected some SIAAD of MPCM and MPSBM where high residual oil meal had lower SIAAD than low residual oil meal.

The heat main effect reduced the AME_n of MPCM and MPSBM for which the reason was unclear. The better AME_n of MPCARIM were reported in wet-heated meals than dry-heated and non-heated meals. The heat treatment did not affect SIAAD of MPCM. The SIAAD of MPSBM were improved with heat treatment as heat inactivated trypsin inhibitors. The SIAAD of MPSBM were greater than those of MPCM.

Generally, enzymes improved AME_n of MPCM, MPSBM and MPCARIM with or without heat. The carbohydrase and lipase were the key enzymes contributed to improve AME_n of MPCM and MPSBM. This is acceptable, as with carbohydrase, non-starch polysaccharides in MPCM and MPSBM will get degraded and release energy containing components. The lipase will breakdown fat and covert into energy.

In most cases, enzyme supplementation enhanced SIAAD of MPCM and MPSBM. When compared to protease, carbohydrase and lipase were more effective in improving SIAAD of MPCM. The carbohydrase and lipase may degrade non-starch polysaccharides and fat respectively, thereby releasing proteins to react with intestinal protease enzymes. In MPSBM, in most cases, protease was superior to carbohydrase and lipase. The protease will react on protein peptides bonds and improve amino acid digestibility.

The birds fed graded levels of MPCM and MPSBM showed satisfactory production performance with superzyme^{TM}-OM enzyme. It was recommended to incorporate MPSBM and MPCM up to 10% in starter, grower and finisher diets, supplemented with superzyme^{TM}-OM enzyme complex.

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