Canadian Journal of Animal Science
Revue canadienne de science animale

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Journal:	<i>Canadian Journal of Animal Science</i>
Manuscript ID	CJAS-2015-0207.R7
Manuscript Type:	Article
Date Submitted by the Author:	29-Mar-2017
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Keywords:	Broiler chickens, expeller pressed black-seeded napus canola, heat treatment, AMEn, ileal digestible amino acids

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Meal residual oil level and heat treatment after oil extraction affects the nutritive value of expeller pressed canola meal for broiler chickens

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Abstract

This experiment determined the effects of expeller pressed canola meal (EPCM) residual oil (10 vs 14%) and heat treatment at 115 °C for 25 minutes on the nitrogen-corrected apparent metabolizable energy (AMEn) value and amino acid (AA) digestibility for broilers. Day old male chicks (6 per cage) were fed 6 test diets (5 cages per diet) from 14 to 21 days using the substitution method in a 2x2 factorial design. Increasing EPCM oil content from 10 to 14% increased ($P < 0.05$) EPCM AMEn value by 287 kcal kg⁻¹. There was heat treatment by oil level interaction ($P < 0.05$) on standardized ileal digestibility (SID) of arginine, isoleucine, leucine, lysine, phenylalanine, threonine, and valine for EPCM where heat treatment of the low oil EPCM reduced ($P \leq 0.0012$) the digestibility of all these AA, but heat treatment of the high oil EPCM only reduced lysine digestibility. In conclusion, heat treatment of EPCM reduced its AMEn value and digestibility of some AA. The AA digestibility of EPCM with low oil were reduced more by heat treatment than EPCM with high oil content, implying that heat treatment negative effects on AA digestibility increases with a decrease in residual oil content in EPCM.

BRYAN ET AL.-DIGESTIBILITY OF HEATED EXPELLER PRESSED CANOLA MEAL

Keywords: Broiler chickens, expeller pressed black-seeded napus canola, heat treatment, AMEn, ileal digestible amino acids

There is interest globally to develop more renewable energy from oil seed (Natural Resources Canada. 2010). In 2008, the Canadian government launched a program called the National Renewable Diesel Demonstration Initiative which used off graded canola seed as feedstock for biodiesel production (Natural Resource Canada 2010). Canola oil is a good feedstock for biodiesel production. Traditionally the oil is extracted from the seeds with hexane after pre-pressing. However small scale and on-farm biodiesel producers who use canola seed often use an expeller to extract the oil from the seeds. During oil extraction using the expelling process, a screw press is used to apply high pressure to disrupt the seed cell membrane and squeeze out the oil. The pressing process only removes some of the oil, unlike solvent extraction which uses hexane to dissolve almost all the oil after seed membrane disruption. The meal produced by expelling process has different nutritional quality from traditional solvent extracted canola meal (Thacker and Petri 2009*a,b*; Woyengo et al. 2010). Also, the EPCM has higher oil than regular canola meal because of the method of oil extraction (Thacker and Petri 2009*a*).

Results from previous work on the nutritive value of canola meal from biodiesel production (Leming and Lember 2005; Thacker and Petri 2009*a*; Woyengo et al. 2010) showed that the energy value of EPCM for poultry was greater than that for solvent extracted canola meal. The higher energy values were mainly due to the oil content of the meal (Leming and Lember 2005; Woyengo et al. 2010). Nutrient digestibility of canola meals is also influenced by the method of oil extraction and heat exposure during processing (Newkirk et al. 2003*a,b*).

During oil extraction by expeller process, the seeds are not preheated and cooked, and the extracted meal is not toasted (Leming and Lember 2005). The preheating, cooking and toasting stages are important for oil extraction, the inactivation of meal myrosinase enzyme and the removal of the excess solvent. However, the heat that is applied during conventional canola meal processing can reduce the AA digestibility of the meals, especially during the toasting stage (Newkirk et al. 2003a,b).

It is unclear if applying heat to the meals after oil extraction by expeller process can have the same effects as those seen during solvent extraction. To date, the relationship between AA digestibility and the variations commonly seen in EPCM with different oil content is not well researched. It is still not clear from the literature if the oil content of canola meal can interact with heat treatment during meal processing to influence the apparent metabolizable energy (AME) and nutrient digestibility when fed to broiler chickens. The objective of this study was to determine the effects of heat treatment and oil content of expeller pressed black-seeded *Brassica napus* canola meals (IFN #5-03-870) for broiler chickens on SID AA and the AME values.

MATERIALS AND METHODS

Animal Care

The experimental protocols were approved by the Dalhousie Faculty of Agriculture Animal Care and Use Committee. All broilers were managed according to guidelines provided by the Canadian Council on Animal Care (2009).

Meal Preparation

Black-seeded *Brassica napus* canola was grown at the Kentville Research and Development Centre in Nova Scotia. After cleaning the seeds, they were expeller pressed by Atlantic Oil

Seed Processing Ltd. (Summerside, PEI). The test meals were prepared as outlined in Figure 1, and as described below:

- A. The clean seeds were expelled to produce a meal (IFN #5-03-870) with 12% residual oil.
- B. The 12% oil meal was mixed with petroleum ether at a ratio of 3:1 (weight/weight). The mixture was stirred for a 1-minute duration at 0, 14, 29, 44, and 59 minutes during the 1-hour mixing period.
- C. After mixing the ether was poured off and the meal saturated with ether was placed on an absorbent pad (Universal pad model no S-17293 Uline, Brampton, Canada) and gently squeezed by hand to remove excess ether. The meal was then transferred to a new absorbent pad and placed in a fume hood overnight to air dry. The oil content in the dried meal was 3%.
- D. To produce a 100 kg batch of the meal with 10% residual oil (LOM), 77.8 kg of the meal with 12% oil and 22.2 kg of the meal with 3% oil were mixed.
- E. The 14% residual oil meal (HOM) was produced by adding 2% by weight of the unfiltered expelled oil to the 12% oil expelled meal.
- F. The heated 10% oil meal (LOM+H) and the heated 14% oil meal (HOM+H) were created by heating a portion of the meal from step E and F above to 115 ± 3 °C for 25 minutes using an industrial drying oven (Model ST33ATUL208V9KW, JPW Design Manufacturing, Trout Run, USA).
- G. The other portions of the meals from step E and F which did not undergo heat treatment were designated as unheated 10% oil meal (LOM-H) and unheated 14% oil meal (HOM-H).

Diet preparation

The diets presented in Table 1 were in mash form and contained 0.5% chromic oxide as an indigestible marker. The diets were a nitrogen-free diet, a corn-soybean meal basal diet and four substitution diets composed of 70% basal and 30% EPCM (LOM+H, LOM-H, HOM+H or HOM-H). The basal grower diet and the substitution diets were formulated to contain 3,150 kcal kg⁻¹ of AMEn per kilogram of dry matter (DM), and 20% crude protein (CP) on DM basis. The basal and test diets were formulated to meet National Research Council (1994) nutrient requirement for broilers at 3 to 6 weeks of age, except for energy which was 50 kcal less than the requirement.

Animal husbandry

One hundred and eighty male 1 day-old Ross 308 broiler chicks were obtained from a local hatchery (Burtts Corner, NB). Upon arrival, the birds were weighed and housed in 30 cages (6 birds per cage) in an environmentally controlled room. The cages were mobile Specht pullet two-tier battery cages (Specht Ten Elsen GmbH Sonsbeck, Germany) with four cages per tier. Each cage measured 60 x 48 x 48 cm and had two nipple drinkers. At the start of the experiment, the temperature and lighting of the room were 32 °C and 20 lux. The temperature was reduced by 1 °C every two days until a temperature of 25 °C was reached. The lighting period was a 24 h cycle, and the intensity was reduced by 5 lux every four days until 5 lux was reached. The temperature and light intensity remained at 25 °C and 5 lux respectively until the end of the trial. All birds were fed a common broiler starter diet [which on DM basis, contained 3,050 kcal kg⁻¹ AMEn, 23% CP, 1.4% SID lysine, 0.6% SID methionine, 1% calcium and 0.5% available phosphorus] in mash form from hatch to 14 days of age. At 15 days of age, all birds in each cage were weighed. The birds had free access to feed and water during the experiment.

Sample and performance data collection

Feed consumed was recorded throughout the trial. Clean excreta samples were collected on days 20 and 21 from beneath all cages and placed in individual containers. Body weights were taken on day 21 after which six birds per cage were killed by cervical dislocation. The intestinal contents were collected from the Meckel's diverticulum to about 1 cm anterior to the ileal-cecal junction, by gently flushing with distilled water. The ileal digesta samples collected from the six individual birds within a cage were pooled into one container. Feed samples were taken from the starter, grower, and nitrogen free diets. All samples were stored at -20°C until analyzed.

Sample analysis

Before DM of the ileal digesta, excreta, feeds, and canola meal samples were determined; samples were prepared and dried as follows. Forty grams per duplicate of feed and ingredient samples were weighed out in aluminum sample containers then placed in a hot air oven at 60°C for 24 h to dry. Ileal digesta and excreta samples were weighed out in 35 g duplicates and then frozen (-20°C). The frozen digesta and excreta samples were freeze-dried without supplemental plate heating to avoid AA damage. All dried samples were ground using a coffee grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT). The ground sample dry matter was determined by drying at 135°C for 2 h using method 935.29 [Association of Official Analytical Chemists (AOAC), 2000].

Crude protein of all samples was calculated as % nitrogen x 6.25, and nitrogen was determined according to AOAC (2000; method 990.03) with a Leco Nitrogen analyzer (Leco Corporation, St. Joseph, MI), using EDTA as the calibration standard. Gross energy (GE) values of the samples were determined using a Parr Adiabatic bomb calorimeter (Parr

Instrument Company, Moline, Illinois, USA.). The concentration of chromic oxide in feed, digesta, and excreta was determined by the method of (Fenton and Fenton (1979) using a Bausch and Lomb Spectronic 501 spectrometer (model 33.51.03 9 Milton Ray Company USA). The AA profiles of the test ingredients, diets, and ileal digesta samples were analyzed by HPLC using a Sykam S430 AA analyzer (Sykam, Eresing, Germany) according to the method described by Woyengo et al. (2010). During AA sample preparation, 100 mg of sample was digested in 4 mL of 6 N HCl at 110°C for 24 h then neutralized with four mL of 25% NaOH (wt/vol). After cooling to room temperature, the mixture was equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and then analyzed. Samples for analysis of sulfur-containing AA underwent performic acid oxidation before acid hydrolysis and HPLC analysis.

Calculations and statistical analysis

The ileal CP and basal ileal endogenous AA losses, apparent ileal digestibility, SID, AME of test diets, AMEn of the test ingredients, digestibility of diets and ingredients were calculated using the following formulas described by Woyengo et al. (2010).

The diets AME, apparent retention of DM and N, and apparent ileal digestibility of AA were calculated using the following equations:

Diets AME= GE value of diet-(GE value of excreta x (% Cr₂O₃ in diets/% Cr₂O₃ in excreta))

Apparent retention of DM or N for diets = 100-[100 x {(% Cr₂O₃ in diets/% Cr₂O₃ in excreta) x (% DM or N in diets/% DM or N in excreta)}].

Apparent ileal digestibility of AA or CP for diets= [1-{(% Cr₂O₃ in diets/% Cr₂O₃ in ileal digesta) x (% AA or CP in ileal digesta/% AA or CP in diet)}].

The digestibilities of nutrients in the meals were calculated based on the by difference method as described by MacLeod et al. (2008) using the following formula:

$$D_A = D_B + (D_D - D_B)/P_A,$$

Where D_A = AME, digestibility of CP or AA, or retention of GE or Nitrogen in assay feedstuff (EPCM); D_B = AME, digestibility of CP or AA or retention of GE or Nitrogen in basal diet; D_D = AME, digestibility of CP or AA, or retention of GE or Nitrogen in assay diet (test meal + basal diet mix); and P_A = proportion (30 %) assay feedstuff.

The basal ileal endogenous CP and AA losses, the SID of AA for the meals and AMEn were calculated using the following equations:

Basal ileal endogenous losses of N or AA (mg kg^{-1} DM) = [CP or AA in ileal digesta (mg kg^{-1})] x {[Cr₂O₃ in diets (mg kg^{-1})]/[Cr₂O₃ in ileal digesta (mg kg^{-1})]} (Moughan et al. 1992).

SID of AA for meal = apparent ileal digestibility of AA for the meal (%) + {[AA basal ileal endogenous losses in mg kg^{-1} DM]/(AA content in test meals in mg kg^{-1})] x 100} (Lemme et al. 2004).

Test meal AMEn (kcal kg^{-1}) = Test meal AME – (8.22 x ANR), where ANR is apparent nitrogen retained (g kg^{-1}) (King et al. 1997).

All data were subjected to analysis of variance using the Proc Mixed procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). Digestibility data were analyzed as a 2 x 2 factorial arrangement in a completely randomized design to identify the main effects of meal oil level and heat treatment and their interactions. If significant ($P \leq 0.05$) main effects or interactions were found, the Tukey-Kramer test was used to compare differences among the least square means at $\alpha \leq 0.05$ (Gbur et al. 2012).

RESULTS AND DISCUSSION

Chemical composition

The analyzed nutrient composition of the test ingredients and the diets used in this study is shown in Table 2. The DM values of the EPCM used in this study were within a range of values

(89.6 to 98.2%) that were reported by Leming and Lember (2005) for EPCM. The DM level is an indication that the meal's moisture contents were typical to what would be found in the industry. Heat treatment of the meals did not seem to have any effect on the moisture level.

The GE value of the meals varied and the oil level did not seem to have a huge impact on the GE value. When expressed on a 100% DM basis, all meals GE values in this study except for LOM+H, were within a range of values (5,135 to 5,631 kcal kg⁻¹ DM) that were reported by Leming and Lember (2005) and Woyengo et al. (2010) for expeller extracted canola meal having 12.3 to 13% oil. The heated canola meals had lower GE value than unheated meals at both oil levels. The GE value of a feed ingredient is derived from the carbohydrate, protein and fat content of the ingredient. A reduction in any of these components often results in a reduction in energy of the ingredient (Newkirk et al. 2003a). Loss of energy yielding components such as CP due to heat treatment of the meal after oil extraction may have resulted in the difference seen between the heated and non-heated meals GE values. The difference in oil level could also explain the slightly lower GE value of LOM+H meal compared to what Leming and Lember (2005) and Woyengo et al. (2010) reported for EPCM. The crude fat content of the meals was as expected and the difference between the heat treated meals within each group of oil level was 0.1%, implying that the meal and added oil were mixed thoroughly during the preparation of meals with different oil levels. The CP values of the meals were within the range of CP values previously reported for EPCM (Woyengo et al. 2010; Toghyani et al. 2015). On a DM basis, the CP values were similar to values reported for biodiesel press cakes (Thacker and Petri 2009a).

The lysine and methionine levels in the meals ranged from 1.58 to 1.70% and 0.58 to 0.53%, respectively. Both lysine and methionine concentrations were less than the values (2.31

and 0.68%) that were reported for lysine and methionine, respectively, in EPCM (Woyengo et al. 2010). It is possible that differences in the geographical location where the seeds were grown could be one reason for low lysine and methionine values of the EPCM fed in the current study compared with values reported by Woyengo et al. (2010) for EPCM. There was a reduction in the content of CP and all AA in the meals following heat treatment. The CP and AA composition of EPCM is known to be influenced by heat during processing (Newkirk et al. 2003a; Classen et al. 2004; Toghyani et al. 2015). Anderson-Hafemann et al. (1993) and Leming and Lember (2005) evaluated the effects of processing on canola meal AA content and observed that heat processing reduced the AA content of the meals. The analyzed CP, lysine and methionine values were greater than the calculated values, which could have been due to underestimation of the AA and CP levels in the feedstuffs that were used to formulate the diets.

Apparent retention of DM and CP

The canola meals' apparent retention of DM values ranged from 73 to 81% (Table 3). There were no interactions between heat and oil level on the apparent retention of DM for the meals. The HOM had higher ($P = 0.0066$) apparent retention of DM than LOM. Higher DM digestibility indicates that the birds were digesting and absorbing more nutrients from HOM. Chickens can digest and use canola oil as an energy source quite well. Thus, the meals with higher oil content were more digestible. There was no effect of oil level on the apparent retention of CP for EPCM. Heat treatment of the meals reduced the apparent retention of DM and CP, which could have been due to a decrease in nutrient digestibility by heat treatment as evidenced by the reduced AA digestibility.

Apparent metabolizable energy

The analyzed AMEn value of the basal diet in Table 2 was higher than the calculated value in Table 1. However, the test diet AMEn values were similar to the EPCM substitution diets reported by Woyengo et al. (2010). The AMEn of EPCM was influenced ($P = 0.0036$) by the oil content and the heat treatment ($P < 0.001$), but the interaction of oil content and heat treatment was not significant ($P=0.1613$) for the AMEn of EPCM. The AMEn value for LOM was lower ($P < 0.05$) than that for HOM, implying that the 4% oil difference between the LOM and the HOM resulted in a 287 kcal kg⁻¹ difference in AMEn.

Energy yielding components in canola meal include protein, fat and carbohydrates. Fiber, which constitute high proportion of carbohydrates in canola meal is poorly digested by poultry. Protein digestibility was reduced by the addition of oil in the canola meal. Thus, the greater AMEn value for HOM than for LOM could have been due to the greater content and digestibility of oil in the former than in the latter. Woyengo et al. (2010) also reported greater AMEn value for canola meal with high oil content than for canola meal with low oil content. Heat treatment reduced AMEn value of canola meal, and this could have been due to reduced nutrient digestibility by heat treatment of the meal. Newkirk et al. (2003a) also reported lower AMEn value for canola meal that had been subjected to heat treatment through toasting than for canola meal that had not been toasted.

Basal ileal endogenous AA and CP losses

The basal ileal endogenous CP loss in mg kg⁻¹ of DM intake (DMI) in Table 4 was similar to the values (8,184-6,514 mg kg⁻¹ DM) reported by Toghyani et al. (2015). The predominant basal ileal endogenous AA were threonine, glutamic acid, serine, aspartic acid and histidine. Of these AA, threonine and serine are constituents of mucin (Lien et al. 2001), which

could explain their abundance in basal endogenous secretions. Overall tryptophan was the least abundant AA. Some individual AA values in the current study were higher than previously reported values for nitrogen-free diet, but their proportions within the endogenous flow were similar to what other studies have reported (Toghyani et al. 2015).

Standardized ileal digestible AA and CP

The SID of CP and AA for EPCM from this study are presented in Table 5. Expeller pressed canola meal with high residual oil level significantly ($P < 0.03$) improve SID of serine, threonine, tryptophan, alanine, glycine and proline when compared to meals with low oil level. Heat treatment reduced ($P < 0.05$) the SID of histidine, methionine, tryptophan, cysteine, tyrosine and CP, which could be due to the formation of indigestible complexes in the meals from the heat treatment.

It should be noted that a brownish color change was observed during heat treatment of the meals. The same color change was detected by Newkirk et al. (2003a) after toasting canola meal and Almeida et al. (2014) reported a change from less yellow to more brown in heat-treated canola meal using the Minolta L* a* b* color scoring system. The color change is an indication that some level of Maillard reaction had taken place during heating (Almeida et al. 2014). This reaction can occur during oil extraction if the temperature of the expeller press surpass 150 °C, but it was more likely to have occurred in this study when EPCM was heat treated. At elevated temperatures, the amino group of proteins plus free amino acids reacts with the carbonyl group of reducing sugar to form glycosylamino and Amadori compounds. The Amadori compound dissociates and copolymerizes with more amino acids which yields the brown pigments called melanoidin. As a consequence of melanoidin formation in EPCM, the digestibility of amino acids and sugars are reduced (Newkirk et al. 2003a; Almeida et al. 2014).

Of the five AA whose SID was reduced by the heat treatment, only tryptophan was affected ($P = 0.0014$) by the oil content of the meals such that tryptophan SID was reduced in EPCM with the higher oil level. Other researchers have also reported a reduction in AA digestibility of canola meal after heat treatment (Anderson-Hafemann et al. 1993; Newkirk et al. 2003a). Newkirk et al. (2003a) and Spragg and Mailer (2007) suggested that AA, especially lysine, tend to participate more in Maillard reaction at 105° C due to the very reactive nature of their amino group.

There was an interaction ($P < 0.05$) between heat treatment and oil level on the SID of all AA except histidine, methionine, tryptophan, cysteine, cysteine and CP in EPCM. The isoleucine, lysine, phenylalanine, valine and glutamic acid digestibilities in EPCM were not affected by the meal's oil content when the meal was not heated. The LOM-H had greater ($P < 0.05$) SID of leucine, serine, threonine, alanine, aspartic acid, and glycine than LOM+H, HOM-H, or HOM+H. An increase in dietary level of oil from 10 to 14% resulted in decreased ($P < 0.05$) digestibility of leucine, serine, threonine, alanine, aspartic acid, glycine and proline. Heat treatment of the meals with high residual oil level further reduced leucine, serine, threonine, alanine, aspartic acid, glycine and proline digestibilities. The SID of arginine, on the other hand, was only significantly reduced when the heat was applied to the LOM.

Dietary fat is known to reduce gut transit time, which results in an increase in the digestion of nutrients. The digestion of AA in solvent extracted canola meal, which has lower oil content than EPCM, tends to be lower than that in EPCM for poultry and pigs (Woyengo et al. 2010; Maison and Stein 2014). The opposite effect was seen in the present study for EPCM with respect to the level of residual oil in the meals. The 10% oil meal had higher digestibility of most of the AA. Seneviratne et al. (2011) and Maison and Stein (2014) fed young pigs

EPCM which varied in oil content from 14.3 to 24.2% and 8.27 to 13.84% respectively. In both studies, the EPCM with the higher oil content (24.2% and 13.84%) had lower AA digestibility, but these results were opposite to what Grageola et al. (2013) reported for AA digestibility of EPCM with 10.5% and 20% in older pigs. The reasons for the reduction in AA digestibility in response to increased oil content in EPCM fed in this study, and in the studies of Seneviratne et al. (2011) and Maison and Stein (2014) is not clear and it requires further investigation. From the author's knowledge, this is the first study that shows how the heat treatment of expeller pressed black-seeded *Brassica napus* canola meals after expelling, and the level of oil retained in the meal can influence the SID of AA and AME value of meal for broiler chickens.

In conclusion, heat treatment of EPCM reduced the AMEn and AA digestible, possibly due to the creation of indigestible complexes between AA and other components of meals during the heating process. An increase in the oil content in the EPCM for broiler chickens resulted in increased AMEn values and reduced SID of some AA. Expeller pressed canola meal can be a good source of AA and energy for broiler chickens. The digestibility values obtained in this study can be used to formulate poultry diets.

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Table 1. Ingredients and nutrient composition of experimental diets (as-fed basis)

Ingredients (%)	Grower diets		
	Basal	Test	N-Free
Corn	65.80	46.06	0.00
Cornstarch	0.00	0.00	20.00
Soybean meal	30.20	21.14	0.00
Canola meals ^a	0.00	30.00	0.00
Dextrose	0.00	0.00	64.00
Cellulose	0.00	0.00	5.00
Soybean oil	0.00	0.00	5.00
Limestone ground	1.60	1.12	1.30
Mono-dicalcium phosphate	0.80	0.56	1.90
Chromic oxide	0.50	0.35	0.50
Vitamin/mineral premix ^b	0.50	0.35	0.50
Iodized salt	0.40	0.28	0.00
Methionine premix ^c	0.20	0.14	0.00
Sodium bicarbonate	0.00	0.00	0.80
Potassium chloride	0.00	0.00	0.30
Potassium carbonate	0.00	0.00	0.30
Magnesium oxide	0.00	0.00	0.20
Choline chloride	0.00	0.00	0.30
Calculated composition (%)			
Crude protein	20	20	0.00
Apparent metabolizable energy AMEn (kcal kg ⁻¹)	3,150	3,150	3,150
Standardized ileal digestible lysine	1.10	1.10	0.00
Standardized ileal digestible methionine	0.40	0.40	0.00
Calcium	0.90	0.85	0.90
Non-phytate phosphorus	0.40	0.40	0.50

Note: Starter diet contained: 44.5% corn, 38.7 5 soybean meal, 10% wheat, 3.2% tallow-poultry grease blend, 1.7% limestone, 0.6% mono-dicalcium phosphate, 0.5% vitamin/mineral premix, 0.4% iodized salt, 0.4% methionine premix, 23% crude protein, 1.4% standardized ileal digestible lysine, 0.6% standardized ileal digestible methionine, 1% calcium, 0.5% non-phytate phosphorus and 3,050 kcal kg⁻¹ apparent metabolizable energy

^aExpeller pressed canola meals with 10 or 14% residual and expeller pressed canola meals with 10 or 14% residual that were heated at 115°C for 25 minutes.

^bSupplied per kilogram of diet: 9,750 IU vitamin A, 2,000 IU vitamin D₃, 25 IU vitamin E, 3 mg vitamin K, 8 mg riboflavin, 14 mg DL Ca- pantothenate, 0.012 mg vitamin B₁₂, 30 mg niacin, 1mg folic acid, 801 mg choline, 0.3 mg biotin, 4.5 mg pyridoxine, 3 mg thiamine, 70 mg manganese, 80 mg zinc, 80 mg iron, 1.25 mg iodine, 25 mg copper; 0.15 mg selenium, 50 mg ethoxyquin, 500 mg calcium carbonate and 1,543 mg wheat middlings.

^cContains 500g kg⁻¹ DL- methionine and 500g kg⁻¹ wheat middlings.

Table 2. Analyzed nutrient composition of diets and expeller pressed canola meals (as fed basis)

Items	Diets					Meals			
	Basal	LOM-H	LOM+H	HOM-H	HOM+H	LOM+H ^a	LOM-H ^b	HOM+H ^c	HOM-H ^d
Dry matter	89	91	90	90	91	93	91	92	92
GE (kcal kg ⁻¹)	4,177	4,301	4,317	4,351	4,348	4,540	4,796	4,740	5,016
AME _n (kcal kg ⁻¹)	3,244	2,972	2,872	3,095	2,921	2,004	2,336	2,168	2,747
CP	21.30	25.00	23.90	23.20	23.50	31.30	33.20	29.40	30.20
Fat	-	-	-	-	-	10.20	10.30	14.10	14.00
Calcium	-	-	-	-	-	0.56	0.56	0.52	0.52
Phosphorus	-	-	-	-	-	1.26	1.26	1.23	1.23
Indispensable amino acids (%)									
Arginine	1.58	1.68	1.64	1.66	1.60	2.07	2.10	1.95	1.99
Histidine	0.74	0.81	0.80	0.78	0.76	0.99	1.05	0.94	0.97
Isoleucine	0.83	0.83	0.85	0.85	0.86	1.09	1.05	0.97	1.06
Leucine	1.88	1.89	1.89	1.86	1.87	2.14	2.18	1.96	2.08
Lysine	1.28	1.44	1.41	1.39	1.35	1.78	1.85	1.70	1.71
Methionine	0.41	0.46	0.50	0.44	0.45	0.54	0.58	0.53	0.54
Phenylalanine	1.07	1.07	1.06	1.07	1.05	1.23	1.24	1.13	1.19
Serine	1.24	1.32	1.29	1.27	1.23	1.50	1.56	1.39	1.45
Threonine	0.90	1.06	1.05	1.02	1.00	1.45	1.47	1.34	1.41
Tryptophan	0.18	0.26	0.26	0.23	0.22	0.29	0.35	0.29	0.29
Valine	0.97	1.04	1.07	1.06	1.08	1.50	1.46	1.36	1.44
Dispensable amino acids (%)									
Alanine	1.05	1.14	1.15	1.08	1.10	1.39	1.43	1.26	1.35
Aspartic acid	2.38	2.38	2.31	2.33	2.23	2.44	2.52	2.27	2.37
Cysteine	0.32	0.43	0.43	0.43	0.41	0.62	0.66	0.56	0.64
Glycine	0.94	1.16	1.15	1.09	1.08	1.65	1.71	1.53	1.61
Glutamic acid	4.23	4.57	4.47	4.40	4.33	5.42	5.59	5.04	5.23
Proline	1.51	1.69	1.66	1.62	1.60	2.02	2.13	1.92	2.01
Tyrosine	0.70	0.74	0.71	0.72	0.71	0.90	0.90	0.82	0.86

^aExpeller pressed black canola meal with 10% residual oil
^bExpeller pressed black canola meal with 10% residual oil heated at 115°C for 25 minutes
^cExpeller pressed black canola meal with 14% residual oil
^dExpeller pressed black canola meal with 14% residual oil heated at 115°C for 25 minutes

Table 3. Effects of treatments of expeller pressed black canola meal on AMEn^a value and apparent retention of CP and DM in 21-day old broilers

Treatments	Nutrient apparent retention		
	DM (%)	CP (%)	Meal AMEn (kcal kg ⁻¹ as fed)
Meal oil			
LOM ^b	75 ^b	67	2,170 ^b
HOM ^c	79 ^a	66	2,457 ^a
SEM ^d	0.9	1.7	59.5
Heat treatment			
Yes (+H ^e)	75 ^b	61 ^b	2,086 ^b
No (-H ^f)	79 ^a	72 ^a	2,541 ^a
SEM	0.9	1.7	59.5
Two-way interaction meal oil*heat treatment			
LOM-H	77	72	2,336
LOM+H	73	60	2,004
HOM-H	81	72	2,747
HOM+H	77	63	2,168
SEM	1.2	2.4	84.2
ANOVA P value			
Meal oil	0.006	0.740	0.003
Heat	0.006	<.004	<.001
Meal oil * Heat	0.960	0.517	0.161

Note: Means within a column with different lowercased italic letters per main effects or interactions are significantly different based on (Tukey-Kramer test $\alpha \leq 0.05$)

^aApparent metabolizable energy nitrogen corrected

^bExpeller pressed canola meal with 10% oil content

^cExpeller pressed canola meal with 14% oil content

^dStandard error of mean

^eMeals heated at 115°C for 25 minutes

^fNo heat treatment of meals

Table 4. Basal ileal endogenous losses of crude protein and amino acids of 21 days old Ross 308 broilers birds fed a nitrogen-free diet from day 15 to 21

Amino Acids	Flow, mg kg ⁻¹ of DMI ^a
Methionine	95
Lysine	307
Cysteine	174
Threonine	454
Tryptophan	59
Isoleucine	239
Arginine	353
Valine	361
Leucine	409
Phenylalanine	236
Serine	453
Glycine	318
Histidine	812
Aspartic acid	540
Glutamic acid	722
Proline	406
Alanine	263
Tyrosine	189
NH ₃	222
Crude protein	7177

Note: Data are means of 5 pens of broilers with 6 broilers per pen.

^aDry mater intake

Table 5. Effects of treatments on the standardized ileal crude protein and amino acid digestibility coefficients of expeller pressed canola meal in 21-day old broilers

Treatments	Indispensable amino acids digestibility coefficients (%)											Dispensable amino acids digestibility coefficients (%)								
	ARG	HIS	ILE	LEU	LYS	MET	PHE	SER	THR	TRP	VAL	ALA	ASP	CYS	GLY	GLU	PRO	TYR	CP	
Meal oil																				
LOM ^a	83	56	67	74	77	86	75	69	69	85a	66	79	74	72	76	83	70	75	69	
HOM ^b	81	54	66	71	74	83	72	62	61	75b	64	73	69	68	69	80	64	74	69	
SEM ^c	2.1	3.5	2.2	1.7	1.5	1.4	1.9	1.9	1.8	1.9	2.0	1.4	1.9	3.0	1.7	1.4	1.5	2.5	1.7	
Heat treatment																				
Yes (+H ^d)	77	45 <i>b</i>	57	65	68	80 <i>b</i>	65	55	54	70 <i>b</i>	57	69	61	61 <i>b</i>	63	75	58	67 <i>b</i>	64 <i>b</i>	
No (-H ^e)	87	64 <i>a</i>	75	81	83	89 <i>a</i>	82	76	76	90 <i>a</i>	74	83	82	79 <i>a</i>	82	88	76	82 <i>a</i>	74 <i>a</i>	
SEM	2.1	3.5	2.2	1.7	1.5	1.4	1.9	1.9	1.8	1.9	2.0	1.4	1.9	3.0	1.7	1.4	1.5	2.5	1.7	
Two-way interaction meal oil*heat treatment																				
LOM-H	91 <i>a</i>	66	80 <i>a</i>	86 <i>a</i>	87 <i>a</i>	91	87 <i>a</i>	83 <i>a</i>	84 <i>a</i>	97	79 <i>a</i>	89 <i>a</i>	87 <i>a</i>	75	89 <i>a</i>	92 <i>a</i>	83 <i>a</i>	86	75	
LOM+H	74 <i>b</i>	46	54 <i>c</i>	63 <i>c</i>	67 <i>b</i>	81	63 <i>c</i>	54 <i>c</i>	68 <i>b</i>	72	54 <i>c</i>	68 <i>c</i>	60 <i>c</i>	60	63 <i>c</i>	74 <i>b</i>	57 <i>c</i>	64	62	
HOM-H	83 <i>ab</i>	63	71 <i>ab</i>	76 <i>b</i>	79 <i>a</i>	86	78 <i>ab</i>	69 <i>b</i>	54 <i>c</i>	82	69 <i>ab</i>	77 <i>b</i>	76 <i>b</i>	82	75 <i>b</i>	85 <i>a</i>	70 <i>b</i>	79	74	
HOM+H	80 <i>ab</i>	44	61 <i>bc</i>	67 <i>b</i>	68 <i>b</i>	79	67 <i>b</i>	56 <i>c</i>	54 <i>c</i>	67	60 <i>bc</i>	69 <i>bc</i>	62 <i>c</i>	62	63 <i>c</i>	76 <i>b</i>	59 <i>c</i>	69	65	
SEM	2.9	5.0	3.1	2.4	2.1	2.0	2.5	2.6	2.5	2.7	2.8	2.0	2.6	4.3	2.4	2.0	2.2	3.5	2.3	
ANOVA P value																				
Meal oil	0.688	0.658	0.760	0.212	0.108	0.101	0.357	0.027	0.004	0.001	0.488	0.013	0.108	0.308	0.011	0.142	0.027	0.774	0.704	
Heat	0.002	0.001	<.001	<.001	<.001	<.002	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.008	<.001	<.001	<.001	<.004	<.003	
Interaction	0.041	0.882	0.015	0.013	0.037	0.391	0.022	0.008	0.003	0.076	0.011	0.005	0.026	0.569	0.008	0.024	0.003	0.077	0.539	

Note: Means whin a column with different lowercased italic letters per main effects or interactions are significantly different based on (Tukey-Kramer test $\alpha \leq 0.05$) where ARG =Arginine, Histidine = HIS, ILE = Isoleucine, LEU = Leucine, LYS =Lysine, MET =Methionine, PHE = Phenylalanine, SER =Serine, THR =Threonine, TRP = Tryptophan, VAL = Valine, ALA= Alanine, ASP =Aspartic acid, CYS =Cysteine , GLY = Glycine, GLU =Glutamic acid, PRO= Proline, TYR =Tyrosine and CP=crude protein

^aExpeller pressed canola meal with 10% oil content

^bExpeller pressed canola meal with 14% oil content

^cStandard error of mean

^dMeals heated at 115°C for 25 minutes

^eNo heat treatment of meals

Figure 1. Flow chart for meal preparation