Determination of ileal digestible and apparent metabolizable energy contents of expeller-extracted and solvent-extracted canola meals for broiler chickens by the regression method

Changsu Kong¹ and Olayiwola Adeola²

Abstract
The present study was conducted to determine ileal digestible energy (IDE), metabolizable energy (ME), and nitrogen-corrected ME (MEₙ) contents of expeller- (EECM) and solvent-extracted canola meal (SECM) for broiler chickens using the regression method. Dietary treatments consisted of a corn–soybean meal reference diet and four assay diets prepared by supplementing the reference diet with each of canola meals (EECM or SECM) at 100 or 200 g/kg, respectively, to partly replace the energy yielding sources in the reference diet. Birds received a standard starter diet from day 0 to 14 and the assay diets from day 14 to 21. On day 14, a total of 240 birds were grouped into eight blocks by body weight and randomly allocated to five dietary treatments in each block with six birds per cage in a randomized complete block design. Excreta samples were collected from day 18 to 20 and ileal digesta were collected on day 21. The IDE, ME, and MEₙ (kcal/kg DM) of EECM or SECM were derived from the regression of EECM- or SECM-associated IDE, ME and MEₙ intake (Y, kcal) against the intake of EECM or SECM (X, kg DM), respectively. Regression equations of IDE, ME and MEₙ for the EECM-substituted diet were Y = −21.2 + 3035X (r² = 0.946), Y = −1.0 + 2807X (r² = 0.884) and Y = −2.0 + 2679X (r² = 0.902), respectively. The respective equations for the SECM diet were Y = 20.7 + 2881X (r² = 0.962), Y = 27.2 + 2077X (r² = 0.875) and Y = 24.7 + 2013X (r² = 0.901). The slope for IDE did not differ between the EECM and SECM whereas the slopes for ME and MEₙ were greater (P < 0.05) for the EECM than for the SECM. These results indicate that the EECM might be a superior energy source for broiler chickens compared with the SECM when both canola meals are used to reduce the cost of feeding.

Keywords: Broiler chickens, Expeller-extracted canola meal, Ileal digestible energy, Metabolizable energy

Background
Canola meal is a by-product of canola seed oil extraction and is a good source of essential amino acids for broiler chickens leading to its frequent use in poultry diet formulation as a protein source (Newkirk 2009). There are two extraction methods for obtaining canola oil from canola seed. Solvent extraction is the most common method and uses solvent to improve oil-extraction efficiency, resulting in a meal with <5 % residual oil. On the other hand, the expeller extraction is suggested to be less efficient for oil extraction because the oil is extracted only mechanically, thus leaving more oil (8–15 %) in the meal compared with solvent extraction method (Spragg and Mailer 2007). Moreover, it does not leave any solvent residues which would remain in the solvent-extracted meal. In addition, processing temperature and moisture contents also differ depending on extraction method. The processing conditions for expeller-extracted canola meal

*Correspondence: changsukong@gmail.com
¹ Department of Animal Science and Technology, Konkuk University, Seoul 05029, Republic of Korea
² Full list of author information is available at the end of the article

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(EECM) are less in moisture (<12 % vs. 15–18 %) and higher in temperature (up to 160 °C vs. 95–115 °C) than for solvent-extracted canola meal (SECM), respectively (Newkirk 2009).

Processing conditions for canola oil extraction as well as residual oil in the meal can affect the nutritive values of canola meal (Woyengo et al. 2010b; Khajali and Slominski 2012). It has been reported that EECM had greater amino acid (AA) digestibility and metabolizable energy (ME) than SECM fed to growing pigs (Woyengo et al. 2010a; Maison and Stein 2014). However, there is a dearth of studies which evaluated the nutritive values of EECM for broilers. Woyengo et al. (2010b) reported that doubly extracted EECM compared with SECM fed to broilers had more standardized ideal digestible AA and N-corrected apparent ME (AMEn) by using the difference method. Toghyani et al. (2014) reported that ileal digestible energy (IDE), apparent ME (AME), and AMEn values vary depending on processing conditions and chemical composition of EECM fed to broilers. In view of the dearth of data, the objective of the present study was to determine IDE, ME and MEn of single-extracted EECM and SECM fed to broiler chickens using the regression method.

Methods
All protocols for the experiment were reviewed and approved by the Purdue University Animal Care and Use Committee.

Animals and experimental diets
Day-old male broiler chicks of the Ross 308 (Aviagen, Huntsville, AL, USA) strain were obtained from a local hatchery, tagged with identification numbers, and housed in electrically heated battery cages (model SB 4 T, Alternative Design Manufacturing, Siloam Springs, AR, USA) in an environmentally controlled room. Battery brooder temperatures from day 0 to 7 and day 7–14 were kept at 35 and 32 °C, respectively. All birds received a mash standard broiler starter diet from day 0 to 14 (Table 1). On day 14, the birds were weighed individually and 240 birds were grouped into eight blocks by body weight and randomly allocated to five dietary treatments in each block with six birds per cage in a randomized complete block design using the Experimental Animal Allotment Program of Kim and Lindemann (2007). Birds were provided ad libitum access to water and experimental diets from day 14 to 21 and the battery brooder temperatures were maintained at 27 °C.

The analyzed chemical composition of EECM and SECM used in the present study are presented in Table 2. Dietary treatments consisted of a corn-SBM reference diet and four assay diets. In the reference diet (Table 3), corn, SBM, corn starch, and soybean oil were used as energy yielding sources. The four assay diets were prepared by supplementing the reference diet with each of canola meals (EECM or SECM) at 100 or 200 g/kg, respectively, to partly replace the energy yielding sources in the reference diet. The ratio of the energy yielding sources remained constant in all treatments to enable determination of the energy value of expeller extracted and solvent extracted canola meals by the regression method (Kong and Adeola 2014).

### Table 1 Ingredient composition of starter diet fed from d 0 to 14

| Item                  | Ingredients (g/kg) |
|-----------------------|--------------------|
| Corn                  | 542.2              |
| Soybean meal          | 360.0              |
| Soybean oil           | 50.0               |
| Monocalcium phosphate | 16.5               |
| Limestone             | 16.5               |
| Salt                  | 4.0                |
| Vitamin-mineral premix| 3.0                |
| a-Methionine          | 3.8                |
| l-Threonine           | 1.1                |
| l-Lysine HCl          | 2.9                |
| Total                 | 1000               |
| Calculated nutrients and energy |       |
| Crude protein (g/kg)  | 226.4              |
| Metabolizable energy  | 3143               |
| Calcium (g/kg)        | 9.5                |
| Phosphorus (g/kg)     | 7.2                |
| Non-phytate phosphorus (g/kg) | 4.7                |
| Total indispensable amino acids (g/kg) |       |
| Arginine              | 14.6               |
| Histidine             | 5.9                |
| l-Isoleucine          | 9.2                |
| Leucine               | 18.9               |
| Lysine                | 14.3               |
| Methionine            | 8.3                |
| Methionine + Cysteine | 10.8               |
| Phenylalanine         | 10.5               |
| Phenylalanine + Tyrosine | 19.1          |
| Threonine             | 8.3                |
| Tryptophan            | 3.0                |
| Valine                | 10.2               |

* Supplied the following per kilogram of diet: vitamin A, 5484 IU; vitamin D3, 2643 IU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; l, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg
Sample collection and chemical analyses

Excreta samples were collected twice daily from day 18 to 20. During collection, waxed paper was placed in trays under the cages, and excreta on the paper were collected. The excreta samples were pooled per cage over the 2 days and stored in a freezer at −20 °C. On day 21, all birds were euthanized by asphyxiation with carbon dioxide and ileal digesta were collected from the distal two-thirds of ileum by gently rinsing with distilled water. The collected ileal digesta from six birds within a cage were pooled and stored in the freezer at −20 °C.

At the completion of the experiment, ileal digesta and excreta samples were thawed and placed in a forced-air oven at 55 °C for 96 h and ground using a mill grinder (Retsch ZM 100, Retsch GmbH & Co., Haan, Germany). Gross energy (GE) of diets, excreta and ileal digesta samples was determined in adiabatic bomb calorimeter (Parr 1261, Parr Instruments Co., Moline, IL, USA) using benzoic acid as a calibration standard. Dry matter analysis of samples was conducted by drying the samples in a drying oven at 105 °C for 24 h (method 934.01; AOAC 2005). Nitrogen contents of the diets, excreta, and ileal digesta were determined using the combustion method (Model FP2000, Leco Corp., St. Joseph, MI) with EDTA as a calibration standard. Chromium concentrations in the diets, ileal, and excreta samples were determined using the method of Fenton and Fenton (1979).

Calculations

The coefficients of ileal digestibility and metabolizability of DM, N and energy in the experimental diets were calculated using the index method with chromic oxide as the index.
an indigestible index (Kong and Adeola 2014). The IDE and AME contents of experimental diets were then calculated as the product of respective coefficients and the gross energy (kcal/kg). The AME was corrected to zero N retention using the factor of 8.22 kcal/g of N (Hill and Anderson 1958). The IDE, ME or MEₙ of the test ingredients (SECM and EECM) were calculated using the regression method described in the study by Adeola and Ileleji (2009). Gross energy of the assay diets was corrected for non-energy yielding fractions and then the substitution rates of the canola meals were corrected for energy contributions of energy-yielding ingredients and canola meals in the assay diet. The energy contribution-corrected substitution rate was multiplied with dietary IDE, ME or MEₙ intake to determine EECM- or SECM-associated corresponding energy intake (kcal). This was then regressed against the intake of EECM or SECM (kg DM) to determine the IDE, ME or MEₙ (kcal/kg DM) of the canola meal samples using slopes of the regression lines.

Statistical analysis
Data for the ileal digestibility and total tract retention of energy were analyzed using the GLM procedures of SAS (SAS Institute Inc., Cary, NC, USA). The model included diet and block as the independent variables and individual cage served as the experimental unit. The orthogonal polynomial contrast was used to examine the relationship between energy utilization response criteria and graded concentrations of either EECM or SECM. The EECM- or SECM-associated IDE, ME or MEₙ intake (kcal) was regressed against the intake of EECM or SECM for cage of six birds, respectively. Slopes and intercepts derived from 3 cages of 0, 100, or 200 g of canola meal substitution in each block were generated using the SLOPE and INTERCEPT functions in Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA), respectively. The intercept and slope data were analyzed as a one-way ANOVA in a completely randomized design using canola meal type as the independent variable and the intercept or slope as the dependent variable with 1 df for canola meal type and 14 df for the error term (Adeola and Ileleji 2009). In this analysis, experimental unit was a block of 3 cages of 0, 100, or 200 g of canola meal type substitution. Statistical significance level was set at 5%.

Results
The CP contents in EECM and SECM were analyzed to be similar (398.7 vs. 402.0 g/kg, on DM basis), which was also reflected in the analyzed values of AA for the respective ingredients. The EECM had greater GE (5789 vs. 4895 kcal/kg, on DM basis) and ether extract (EE) (138.7 vs. 24.3 g/kg, on DM basis) contents than SECM whereas the value of neutral detergent fiber (NDF) was greater in the SECM than in EECM (265.3 vs. 233.8 g/kg). Regardless of the canola type, the body weight gain, feed intake, and feed efficiency of the birds were not influence (P > 0.05) by the supplementation of canola meal to the reference diet (Table 4).

The data presented in Table 5 show the ileal digestibility and total tract retention of DM, N and energy of experimental diets used in the current study. There were linear (P < 0.05) and quadratic (P < 0.05) decreases in ileal digestibility of DM and energy as well as IDE as the EECM level in the diets increased from 0 to 200 g/kg. As the SECM substitution into the reference diet increased from 0 to 200 g/kg, all the response criteria measured linearly increased (P < 0.05) with the exception of the N retention which was not affected by the substitution of SECM.

The regression of IDE, ME and MEₙ intake associated with either EECM or SECM against the intake of substituted canola meals depicted in Figs. 1 or 2, respectively. Regression equations of IDE, ME and MEₙ for the EECM...
substituted diet were $Y = -21.2 + 3035X$ ($r^2 = 0.946$), $Y = -1.0 + 2807X$ ($r^2 = 0.884$) and $Y = -2.0 + 2679X$ ($r^2 = 0.902$), respectively. The respective equations for the SECM diet were $Y = 20.7 + 2881X$ ($r^2 = 0.962$), $Y = 27.2 + 2077X$ ($r^2 = 0.875$) and $Y = 24.7 + 2013X$ ($r^2 = 0.901$).

Comparisons of slopes and intercepts for the IDE, ME, MEn regressions between the SECM and EECM are presented in Table 6. The slopes for ME and ME$_n$ were greater ($P < 0.05$) for the EECM than for the SECM, whereas slope for IDE did not differ between the EECM and SECM.

Table 5 Dry matter (DM), energy, and nitrogen (N) digestibility and total tract retention of broilers fed the experimental diets containing expeller-extracted canola meal (EECM) or solvent extracted canola meal (SECM) levels at 0, 100, 200 g/kg

| Item | 0 | 100 | 200 | 100 | 200 | SEM | P value | L$^a$ | Q$^a$ | L$^b$ | Q$^b$ |
|------|---|-----|-----|-----|-----|-----|---------|-------|-------|-------|-------|
| Ileal DM digestibility (%) | 72.1 | 66.9 | 67.1 | 71.3 | 67.9 | 0.598 | <0.001 | 0.002 | <0.001 | 0.086 |
| Total tract DM retention (% of DM intake) | 66.9 | 64.9 | 62.2 | 66.3 | 60.4 | 1.623 | 0.057 | 0.852 | 0.012 | 0.200 |
| Ileal N digestibility (%) | 82.9 | 80.8 | 82.3 | 82.3 | 80.5 | 0.701 | 0.530 | 0.046 | 0.028 | 0.499 |
| Total tract N retention (% of N intake) | 55.8 | 57.0 | 54.0 | 56.6 | 50.3 | 2.828 | 0.655 | 0.550 | 0.191 | 0.325 |
| Total tract N retention (mg/g of DM intake) | 22.2 | 22.7 | 21.5 | 22.5 | 20.0 | 1.126 | 0.656 | 0.550 | 0.191 | 0.325 |
| Ileal energy digestibility (%) | 76.0 | 72.1 | 72.1 | 75.7 | 73.1 | 0.520 | <0.001 | 0.007 | 0.001 | 0.087 |
| Total tract energy retention (% energy intake) | 73.7 | 72.1 | 70.1 | 73.2 | 68.3 | 1.227 | 0.052 | 0.933 | 0.007 | 0.016 |
| IDE (kcal/kg of DM) | 3485 | 3358 | 3399 | 3498 | 3399 | 24 | 0.024 | 0.013 | 0.024 | 0.078 |
| AME (kcal/kg of DM) | 3379 | 3355 | 3304 | 3382 | 3178 | 57 | 0.362 | 0.846 | 0.024 | 0.156 |
| AME$_n$ (kcal/kg of DM) | 3196 | 3168 | 3127 | 3198 | 3013 | 49 | 0.332 | 0.914 | 0.018 | 0.141 |

SEM standard error of the mean, IDE ileal digestive energy, AME apparent metabolizable energy, AME$_n$ nitrogen-corrected AME

* Linear (L) and quadratic (Q) contrasts for the EECM

* Linear (L) and quadratic (Q) contrasts for the SECM

Discussion

The general steps of solvent-extraction method include flaking, cooking, pressing, and solvent-extraction of seeds followed by desolventizing, toasting, and drying of the extracted meal whereas the steps for expeller-extraction method only include flaking, cooking, and pressing of seeds (Spragg and Mailer 2007). The difference in extraction method could influence the composition of two types of canola meal. In the current study, the EECM had 18.4 and 471 % greater GE and EE, respectively, than SECM, which could be due to the difference in extraction method because an additional oil-extraction process by solvent is performed for the SECM. Woyengo et al. (2010b) reported greater GE (5199 vs. 4895) is fully accounted for by the 114 g/kg DM greater EE in EECM than in SECM (138.7 vs. 24.3) and the 31.5 g/kg DM lower NDF in EECM than in SECM (238.8 vs. 265.3) using gross energies of 9.2 and 4.5 kcal/g for EE and NDF.

Regardless of the type of canola meals, the influences by the addition of graded canola meal were not observed. These results might be attributed to the short experimental period in which the effect of treatments on the growth performance may not be obvious (Woyengo et al. 2010b). Substitution of two canola meals at 100 or 200 g/kg of diet for the energy supplying ingredients (corn, soybean meal, soybean oil, and cornstarch) in the reference diet linearly decreased ileal DM and energy digestibility as well as the IDE of diet. This may be attributed to performed (single extraction for the present study vs. double extraction for the previous study) to maximize oil recovery.

The greater NDF content in the SECM compared with the EECM observed in the current study is in agreement with the study by Woyengo et al. (2010b). One of common Maillard reaction products is neutral detergent insoluble nitrogen (Khajali and Slominski 2012) which falls in NDF fraction (van Soest 1994). Maillard reaction can occur during both oil extraction processes due to high temperature of the expeller pressing process for the EECM and desolventizing and toasting processes for the SECM, but the extent of the Maillard reaction may be less for the EECM than for the SECM because the expeller pressing process is faster compared with desolventizing and toasting processes (Landero et al. 2012).
the level of fiber in both canola meals used in the current study because both EECM and SECM had relatively greater contents of fiber compared with corn and SBM (NRC 2012). Due to the physical presence of fiber in the gastrointestinal tract, fiber may have a detrimental influence on the utilization of nutrients in broiler chickens (Pettersson and Aman 1989; Choct and Annison 1990). The physical barrier of the cell walls of fiber can encapsulate potentially available nutrients. Furthermore, the viscous properties of fiber may interfere with the digestion process and thereby reduce the digestibility of other nutrients (Choct and Annison 1992; Steenfeldt 2001). Consequently, the fiber components have negative impact on the digestibility of nutrients in the broiler chickens and lead to lower energy utilization. High dietary fiber may increase the passage rate of digesta (Khajali and Slominski 2012), which adversely affect the digestibility of nutrient.

In the current study, the EECM compared with SECM had 35 or 33 % more ME (2807 vs. 2077 kcal/kg) or MEn (2679 vs. 2013 kcal/kg) estimated by the regression method, respectively. Using the difference method, Woyengo et al. (2010b) also reported greater AME (3039 vs. 2005 kcal/kg, on DM basis) and AMEn (2694 vs. 1801 kcal/kg, on DM basis) contents in EECM than in SECM fed to broilers. This in part could result from the difference in the fat content between the EECM and SECM. The concentration of ether extract in EECM used in the present study or the study by Woyengo et al. (2010b) was 471 (13.87 vs. 2.43 %) or 217 % (5.54 vs. 12.03 %) greater than in SECM, respectively.

The significant differences in ME or MEn between the SECM and EECM were observed in the present study whereas the difference in the IDE was not significant. The reason for these results was not clear. Speculatively, difference in the fermentation of undigested dietary fibrous contents might have contributed to such difference in ME values. Because microbes in the ceca of broiler chickens are able to ferment undigested dietary fiber contents
entering from the ileum into short chain fatty acids which could be absorbed by broiler chickens and used as energy (Sugahara et al. 2004).

**Conclusion**

In conclusion, the results showed that the EECM used in the current study had more fat as well as less NDF and consequently more GE content compared with the SECM. In addition, the ME and ME<sub>i</sub> contents of the EECM were greater than those of the SECM, which indicates that the EECM might be a superior energy source for broiler chickens compared with the SECM when both canola meals are used to reduce the cost of feeding.

**Authors’ contributions**

CK: Conducted analysis, calculations, analysis of the data, and wrote the manuscript. OA: Supervised the experimental work and manuscript preparation. Both authors read and approved the final manuscript.

**Author details**

1 Department of Animal Science and Technology, Konkuk University, Seoul 05029, Republic of Korea. 2 Department of Animal Sciences, Purdue University, West Lafayette, IN 47907-2054, USA.

**Acknowledgements**

The authors thank Pat Jaynes and Anna Goad (Purdue University, West Lafayette, IN) for their varied roles in the conduct of and contribution to this study. The authors gratefully acknowledge Dr. Martin Nyachoti (University of Manitoba, Winnipeg, Manitoba, Canada) for providing expeller-extracted canola meal. This paper was supported by the KU Research Professor Program of Konkuk University.

**Competing interests**

The authors declare that they have no competing interests.

Received: 19 November 2015 Accepted: 11 May 2016 Published online: 23 May 2016

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