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# Effects of heat treatment on the apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs

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ABSTRACT

An experiment was conducted to determine effects of heat damage on the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in canola meal fed to growing pigs. The second objective was to develop regression equations to predict the concentration of SID AA from the nutrient composition of canola meal. Ten growing pigs (initial body weight: 26.5 ± 0.7 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. One batch of canola meal was divided into 4 batches that were either not autoclaved or autoclaved at 130 °C for 20, 30, or 45 min. Four diets were formulated with canola meal being the only source of AA and CP in each diet. A N-free diet also was formulated and used to determine the basal endogenous losses of CP and AA in the pigs. The AID of CP and all AA was reduced (quadratic, $P < 0.01$) as a result of increasing time of autoclaving. Autoclaving of canola meal also reduced (quadratic, $P < 0.01$) the SID of CP and all AA. The concentration (g/kg) of SID lysine in canola meal may be predicted by regression equations using the concentration (g/kg) of reducing sugars in the meal ($r^2 = 0.96$). Likewise, the concentrations of SID AA for most AA may be predicted from the nutrient composition of canola meal. In conclusion, heat damage reduces both the concentration and the digestibility of AA in canola meal. Regression equations developed in this experiment may be used to predict the concentration of SID AA in canola meal.



## 1. Introduction

Canola meal, the product remaining after oil has been solvent extracted from canola, is the second most used protein source for feeding of poultry and livestock (Canola Council of Canada, 2009). The final step in producing canola meal involves desolventizing and toasting of the meal, which may last between 35 and 50 min and requires steam (*i.e.*, moisture) and temperatures that vary from 95 to 115 °C (Canola Council of Canada, 2009; Unger, 2011). Consequently, differences in processing of canola meal may result in variations in the nutritional composition of canola meal among different processing plants because Maillard reactions may occur as a result of the combination of heat and moisture applied to the meals

*Abbreviations:* AA, amino acids; ADIN, acid detergent insoluble N; AID, coefficient of ileal apparent digestibility; CP, crude protein; DDGS, distillers dried grains with solubles; DM, dry matter; ME, metabolizable energy; NDF, neutral detergent fiber; SID, coefficient of ileal standardized digestibility.

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containing amino acids (AA) and reducing sugars (Nursten, 2005). These reactions result in a decrease in the concentration and digestibility of AA, and lysine is the AA most affected by heat damage (Carvalho et al., 2009; González-Vega et al., 2011; Newkirk, 2011). Conventional AA analysis may overestimate the concentration of lysine available for the pig in heat-damaged feed ingredients because some of the lysine that participates in the Maillard reactions is recovered during the acid hydrolysis step, although this lysine is not released *in vivo* (Williams et al., 2006). The use of reactive lysine, determined by the furosine procedure, has been suggested as an approach to evaluate the nutritional quality of corn distillers dried grains with solubles (DDGS), but this procedure has not been used in canola meal (Pahm et al., 2008; Kim et al., 2012). Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of CP and AA in canola meal have been determined (Stein et al., 2005; Woyengo et al., 2010; Trindade Neto et al., 2012), but effects of heat damage on the AID and SID of CP and AA in canola meal fed to growing pigs have not been determined. We hypothesized that heat damage decreases the concentration and digestibility of AA in canola meal and that the concentration of SID AA in heat-damaged canola meal may be predicted from regression equations developed using the nutrient composition of heat-damaged canola meal. Therefore, the main objective of this experiment was to determine the effects of heat damage on the AID and SID of CP and AA in canola meal fed to growing pigs. The second objective was to develop regression equations to predict the concentration of SID AA in canola meal.

## 2. Materials and methods

The protocol for this experiment was reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiment were the offspring of G-performer boars and F-25 females (Genetiporc, Alexandria, MN).

### 2.1. Animals, housing, and experimental design

Ten growing pigs (initial body weight: 26.5 ± 0.7 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. Pigs were individually housed in a controlled environment (27 °C, 76% humidity) in pens (1.2 m × 1.5 m) that were equipped with a feeder and a nipple waterer.

### 2.2. Diets and feeding

Canola meal was obtained from the University of Illinois Feed Mill (Champaign, IL) and divided into 4 batches that were either not autoclaved or autoclaved at 130 °C for 20, 30, or 45 min (Table 1). Four diets were formulated with canola meal being the only source of AA and CP in each diet (Tables 2 and 3). Each diet contained 1 of the 4 batches of canola meal. A N-free diet also was formulated and used to determine the basal endogenous losses of CP and AA in the pigs. Diets were supplied with vitamins and minerals to meet or exceed the requirement estimates for growing pigs (NRC, 1998). Chromic oxide was included (4 g/kg) in the diets and used as an indigestible marker.

The amount of feed provided daily was calculated as 2.5 times the maintenance requirement of energy (*i.e.*, 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Pigs were fed once daily at 0800 h. At the beginning of each period, feed allowance was adjusted based on the body weight of each pig. Water was available at all times.

### 2.3. Sample collection

Each period lasted 7 d. The initial 5 d were considered an adaptation period to the diet. On d 6 and d 7, ileal digesta were collected for 8 h. A plastic bag (232 mL) was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were replaced whenever they were filled with digesta, or at least once every 30 min, and immediately frozen at −20 °C to prevent bacterial degradation of AA in the digesta.

### 2.4. Chemical analyses

At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized, ground to pass a 1 mm screen, and analyzed. A sample of each diet and of each batch of canola meal was collected at the time of diet mixing. Diets, ingredients, and ileal samples were analyzed for AA by ion-exchange chromatography with postcolumn derivatization with ninhydrin. Cysteine and Methionine were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 N HCL for 24 h at 110 °C and quantified with the internal standard (Norleucine) by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110 °C (Commission Directive, 2000). Diets, ingredients, and ileal samples were also analyzed for DM (Method 935.29; AOAC International, 2007), and for CP following the Dumas procedure (Method 968.06; AOAC International, 2007). Diets and ileal samples were also analyzed for chromium (Method 990.08; AOAC International, 2007). Ingredients were

**Table 1**
Chemical composition of canola meal (g/kg, unless otherwise specified; as-fed basis).

| Item | Canola meal | | | |
|---|---|---|---|---|
| | Non-autoclaved | Autoclaved at 130 °C | | |
| | | 20 min | 30 min | 45 min |
| Dry matter | 909 | 894 | 898 | 884 |
| Ash | 75 | 75 | 77 | 76 |
| Crude protein | 368 | 365 | 369 | 369 |
| Acid detergent fiber | 200 | 236 | 227 | 313 |
| Neutral detergent fiber | 334 | 422 | 420 | 469 |
| Acid detergent lignin | 80 | 107 | 110 | 165 |
| Acid detergent insoluble N | 4 | 8 | 9 | 18 |
| Reducing sugars | 51 | 42 | 43 | 33 |
| Fat[a] | 37 | 33 | 38 | 20 |
| Total glucosinolates, μmol/g | 16 | – | – | – |
| Ca | 6 | 6 | 7 | 6 |
| P | 11 | 10 | 10 | 10 |
| Lysine:CP ratio[b], g/100 g | 52 | 43 | 41 | 37 |
| Furosine | 0 | 0 | 0 | 0 |
| Reactive lysine[c] | 19 | 15 | 15 | 13 |
| $L^*$[d] | 529 | 472 | 476 | 451 |
| $a^*$[d] | 58 | 63 | 60 | 64 |
| $b^*$[d] | 126 | 89 | 88 | 74 |
| Indispensable amino acid | | | | |
| Arginine | 22 | 20 | 19 | 17 |
| Histidine | 10 | 9 | 9 | 9 |
| Isoleucine | 14 | 14 | 13 | 14 |
| Leucine | 26 | 26 | 25 | 26 |
| Lysine | 19 | 16 | 15 | 14 |
| Methionine | 7 | 7 | 7 | 7 |
| Phenylalanine | 15 | 15 | 14 | 14 |
| Threonine | 16 | 16 | 16 | 16 |
| Tryptophan | 5 | 5 | 5 | 5 |
| Valine | 18 | 18 | 17 | 17 |
| All indispensable | 153 | 144 | 140 | 139 |
| Dispensable amino acid | | | | |
| Alanine | 17 | 16 | 16 | 16 |
| Aspartic acid | 27 | 26 | 26 | 26 |
| Cysteine | 9 | 8 | 8 | 7 |
| Glutamic acid | 63 | 62 | 61 | 62 |
| Glycine | 19 | 18 | 18 | 18 |
| Proline | 22 | 21 | 21 | 21 |
| Serine | 16 | 16 | 16 | 16 |
| All dispensable | 171 | 167 | 165 | 167 |
| Total amino acids | 324 | 312 | 305 | 306 |

[a] Fat, acid hydrolyzed ether extract.
[b] Calculated by expressing the concentration of lysine in the sample relative to the concentration of the CP in the sample (g/100 g; Stein et al., 2009).
[c] Reactive lysine (g/kg) = [lysine (g/kg) – (Furosine (g/kg) ÷ 0.32 × 0.40)]; Pahm et al. (2008).
[d] $L^*$, lightness; $a^*$, redness; and $b^*$, yellowness.

analyzed for ash (Method 942.05; AOAC International, 2007), acid detergent fiber (ADF; Method 973.18; AOAC International, 2007), neutral detergent fiber (NDF; Holst, 1973), acid detergent lignin (Method 973.18 (A–D); AOAC International, 2007), furosine as described by Kim et al. (2012), total reducing sugars (Dubois et al., 1956), acid detergent insoluble N (ADIN; Method 990.03; AOAC International, 2007), Ca and P by inductively coupled plasma (ICP) spectroscopy (Method 985.01; AOAC International, 2007), total fat by acid hydrolysis using 3 N HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06; AOAC International, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN), and for total glucosinolates (Method ISO 9167-1:1992, Eurofins, Des Moines, IA). Minolta $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness) values for each batch of canola meal were determined (8 mm aperture, D65 light source, and 0° observer, Minolta Camera Co., Osaka, Japan).

### 2.5. Calculations and statistical analysis

The AID and SID values were calculated as previously described (Stein et al., 2007). The lysine:CP ratio in each canola meal sample was calculated by expressing the concentration of lysine in the sample relative to the concentration of the CP in the sample (g/100 g; Stein et al., 2009), and the concentration of reactive lysine was calculated as previously described

**Table 2**
Ingredient composition of experimental diets (g/kg, as-fed basis).

| Ingredient (%) | Canola meal | | | | N-free diet |
|---|---|---|---|---|---|
| | Non-autoclaved | Autoclaved at 130 °C | | | |
| | | 20 min | 30 min | 45 min | |
| Canola meal | 420.0 | 420.0 | 420.0 | 420.0 | – |
| Maize starch | 421.5 | 421.5 | 421.5 | 421.5 | 664.0 |
| Sucrose | 100.0 | 100.0 | 100.0 | 100.0 | 200.0 |
| Solka floc[a] | – | – | – | – | 50.0 |
| Soybean oil | 34.0 | 34.0 | 34.0 | 34.0 | 40.0 |
| Ground limestone | 7.0 | 7.0 | 7.0 | 7.0 | – |
| Monocalcium phosphate | 6.5 | 6.5 | 6.5 | 6.5 | 30.0 |
| NaCl | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Magnesium oxide | – | – | – | – | 1.0 |
| Potassium carbonate | – | – | – | – | 4.0 |
| Chromic oxide | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Vitamin-mineral premix[b] | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |

[a] Fiber Sales and Development Corp., Urbana, OH, USA.
[b] Provided the following per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin $D_3$ as cholecalciferol, 2204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin $B_{12}$, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

using furosine to indicate the concentration of regenerated lysine (Kim et al., 2012). Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC). The presence of outliers was evaluated using the UNIVARIATE procedure of SAS. The model included diet as a fixed effect and pig and period as random effects. Linear and quadratic effects of increasing time of heat treatment on the AID and SID of AA were analyzed by orthogonal polynomial contrasts. Regression equations to estimate the concentration of SID AA were developed using the REG procedure in SAS. The pig was the experimental unit and significance among means was assessed with an $\alpha$ level of 0.05.

**Table 3**
Analyzed nutrient composition of experimental diets (g/kg, as-fed basis).

| Item | Canola meal | | | | N-free diet |
|---|---|---|---|---|---|
| | Non-autoclaved | Autoclaved at 130 °C | | | |
| | | 20 min | 30 min | 45 min | |
| Dry matter | 918.6 | 912.4 | 910.1 | 913.6 | 926.6 |
| Crude protein | 171.7 | 152.4 | 150.9 | 150.4 | 1.9 |
| Indispensable amino acid | | | | | |
| Arginine | 9.6 | 8.2 | 7.9 | 7.1 | – |
| Histidine | 4.2 | 3.9 | 3.8 | 3.7 | – |
| Isoleucine | 5.9 | 5.7 | 5.6 | 5.5 | – |
| Leucine | 11.3 | 10.7 | 10.5 | 10.3 | – |
| Lysine | 8.3 | 6.6 | 6.4 | 5.6 | – |
| Methionine | 3.1 | 2.8 | 2.7 | 2.7 | – |
| Phenylalanine | 6.4 | 6.1 | 6 | 5.8 | – |
| Threonine | 7.1 | 6.7 | 6.5 | 6.5 | – |
| Tryptophan | 2.1 | 2 | 1.9 | 2 | – |
| Valine | 7.7 | 7.3 | 7.2 | 7 | – |
| All indispensable | 65.7 | 60 | 58.5 | 56.2 | – |
| Dispensable amino acid | | | | | |
| Alanine | 7.3 | 6.8 | 6.7 | 6.6 | – |
| Aspartic acid | 11.8 | 11 | 10.7 | 10.5 | – |
| Cysteine | 3.8 | 3.3 | 3.2 | 2.9 | – |
| Glutamic acid | 27.7 | 26.3 | 25.7 | 25.3 | – |
| Glycine | 8.2 | 7.7 | 7.5 | 7.4 | – |
| Proline | 9.5 | 9 | 8.6 | 8.5 | – |
| Serine | 7.2 | 6.7 | 6.5 | 6.5 | – |
| All dispensable | 75.5 | 70.8 | 68.9 | 67.7 | – |

**Table 4**
Coefficient of apparent ileal digestibility of crude protein and amino acids in canola meal subjected to increasing levels of heat treatment by growing pigs.[a]

| Item | Canola meal | | | | SEM | P-value[b] | |
|---|---|---|---|---|---|---|---|
| | Non-autoclaved | Autoclaved at 130 °C | | | | | |
| | | 20 min | 30 min | 45 min | | Linear | Quadratic |
| Crude protein | 0.597 | 0.486 | 0.513 | 0.227 | 0.03 | <0.01 | <0.01 |
| Indispensable amino acid | | | | | | | |
| Arginine | 0.787 | 0.741 | 0.767 | 0.586 | 0.03 | <0.01 | <0.01 |
| Histidine | 0.770 | 0.739 | 0.748 | 0.562 | 0.01 | <0.01 | <0.01 |
| Isoleucine | 0.692 | 0.652 | 0.659 | 0.440 | 0.01 | <0.01 | <0.01 |
| Leucine | 0.749 | 0.713 | 0.714 | 0.528 | 0.01 | <0.01 | <0.01 |
| Lysine | 0.619 | 0.483 | 0.494 | 0.129 | 0.02 | <0.01 | <0.01 |
| Methionine | 0.811 | 0.778 | 0.781 | 0.640 | 0.01 | <0.01 | <0.01 |
| Phenylalanine | 0.743 | 0.716 | 0.721 | 0.530 | 0.01 | <0.01 | <0.01 |
| Threonine | 0.632 | 0.592 | 0.592 | 0.362 | 0.01 | <0.01 | <0.01 |
| Tryptophan | 0.663 | 0.640 | 0.631 | 0.441 | 0.01 | <0.01 | <0.01 |
| Valine | 0.674 | 0.625 | 0.634 | 0.390 | 0.01 | <0.01 | <0.01 |
| Mean | 0.720 | 0.670 | 0.679 | 0.462 | 0.01 | <0.01 | <0.01 |
| Dispensable amino acid | | | | | | | |
| Alanine | 0.630 | 0.542 | 0.563 | 0.277 | 0.02 | <0.01 | <0.01 |
| Aspartic acid | 0.639 | 0.554 | 0.566 | 0.277 | 0.01 | <0.01 | <0.01 |
| Cysteine | 0.694 | 0.652 | 0.646 | 0.402 | 0.01 | <0.01 | <0.01 |
| Glutamic acid | 0.791 | 0.765 | 0.766 | 0.606 | 0.01 | <0.01 | <0.01 |
| Glycine | 0.467 | 0.342 | 0.360 | −0.029 | 0.06 | <0.01 | <0.01 |
| Serine | 0.660 | 0.616 | 0.621 | 0.406 | 0.01 | <0.01 | <0.01 |
| Mean | 0.647 | 0.579 | 0.587 | 0.323 | 0.02 | <0.01 | <0.01 |

[a] Data are means of 10 observations.
[b] Linear and quadratic effects of time of autoclaving.

## 3. Results

Pigs recovered well after the surgery. Feed intake was normalized within a week post-surgery. Pigs remained healthy during the experiment, and no feed refusals were observed.

The concentrations of ADF were 200.0, 236.3, 227.3, and 313.0 g/kg in non-autoclaved canola meal, and canola meal autoclaved for 20, 30, or 45 min, respectively (Table 1). The non-autoclaved canola meal contained 333.6 g/kg NDF, whereas the autoclaved canola meal contained 421.8, 419.5, and 468.8 g/kg NDF (20, 30, and 45 min, respectively). Non-autoclaved canola meal contained 80.2 g/kg lignin, but the concentrations of lignin in canola meals that were autoclaved for 20, 30, or 45 min were 107.4, 109.6, and 164.5 g/kg, respectively. The concentration of ADIN in the non-autoclaved canola meal was 3.7 g/kg, but autoclaving canola meal for 20, 30, or 45 min resulted in ADIN concentrations of 8.0, 8.8, and 17.5 g/kg, respectively. The concentration of reducing sugars in autoclaved canola meal was 42.0, 43.4, and 33.1 g/kg (20, 30, and 45 min, respectively), whereas the concentration of reducing sugars in non-autoclaved canola meal was 50.5 g/kg. The concentration of lysine was 13.6 g/kg in the canola meal autoclaved for 45 min vs. 19.2 g/kg in non-autoclaved canola meal. Non-autoclaved canola meal contained 0.16 g/kg furosine, but autoclaved canola meal contained 0.33, 0.33, and 0.25 g/kg furosine (20, 30, and 45 min, respectively). The concentration of reactive lysine was 19.0 g/kg in non-autoclaved canola meal, whereas canola meal that was autoclaved for 20, 30 and 45 min contained 15.3, 14.7, and 13.3 g/kg reactive lysine, respectively. Lightness (color $L^*$) was 52.88 in non-autoclaved canola meal, 47.19 in canola meal autoclaved for 20 min, 47.63 in canola meal autoclaved for 30 min, and 45.08 in canola meal autoclaved for 45 min.

The AID of CP and all AA was reduced (quadratic, $P < 0.01$) as a result of increasing time of autoclaving (Table 4). Autoclaving of canola meal also reduced (quadratic, $P < 0.01$) the SID of CP and all AA (Table 5).

A regression equation that uses the concentration (g/kg) of reactive lysine as an independent variable may be used to predict the concentration (g/kg) of SID lysine in heat-damaged canola meal (SID lysine = −16.6 + 1.60 × reactive lysine; $r^2 = 0.83$; Table 6). The concentration (g/kg) of SID lysine in canola meal may also be calculated using the concentration (g/kg) of reducing sugars (SID lysine = −16.5 + 0.59 × reducing sugars; $r^2 = 0.97$). The concentration of lignin (g/kg) may be used to predict the concentration (g/kg) of SID Methionine (SID Methionine = 7.6 − 0.02 × lignin; $r^2 = 0.93$), whereas the concentration of ADF in combination with the concentration of reducing sugars may be used to predict the concentration of SID Threonine [SID Threonine = 31.6 − (0.06 × ADF) − (0.15 × reducing sugars); $r^2 = 0.89$] and SID Tryptophan [SID Tryptophan = 9.9 − (0.018 × ADF) − (0.05 × reducing sugars); $r^2 = 0.88$].

**Table 5**
Coefficient of standardized ileal digestibility of crude protein and amino acids in canola meal subjected to increasing levels of heat treatment by growing pigs.[a]

| Item | Canola meal | | | | SEM | P-value[b] | |
|---|---|---|---|---|---|---|---|
| | Non-autoclaved | Autoclaved at 130 °C | | | | | |
| | | 20 min | 30 min | 45 min | | Linear | Quadratic |
| Crude protein | 0.717 | 0.620 | 0.648 | 0.345 | 0.03 | <0.01 | <0.01 |
| Indispensable amino acid | | | | | | | |
| Arginine | 0.846 | 0.810 | 0.839 | 0.666 | 0.03 | <0.01 | <0.01 |
| Histidine | 0.813 | 0.785 | 0.796 | 0.610 | 0.01 | <0.01 | <0.01 |
| Isoleucine | 0.754 | 0.716 | 0.724 | 0.506 | 0.01 | <0.01 | <0.01 |
| Leucine | 0.803 | 0.768 | 0.771 | 0.586 | 0.01 | <0.01 | <0.01 |
| Lysine | 0.682 | 0.562 | 0.574 | 0.208 | 0.02 | <0.01 | <0.01 |
| Methionine | 0.851 | 0.822 | 0.826 | 0.685 | 0.01 | <0.01 | <0.01 |
| Phenylalanine | 0.802 | 0.778 | 0.784 | 0.596 | 0.01 | <0.01 | <0.01 |
| Threonine | 0.715 | 0.679 | 0.682 | 0.452 | 0.01 | <0.01 | <0.01 |
| Tryptophan | 0.739 | 0.719 | 0.714 | 0.520 | 0.01 | <0.01 | <0.01 |
| Valine | 0.740 | 0.694 | 0.704 | 0.462 | 0.01 | <0.01 | <0.01 |
| Mean | 0.779 | 0.734 | 0.744 | 0.530 | 0.01 | <0.01 | <0.01 |
| Dispensable amino acid | | | | | | | |
| Alanine | 0.742 | 0.661 | 0.684 | 0.401 | 0.02 | <0.01 | <0.01 |
| Aspartic acid | 0.714 | 0.633 | 0.647 | 0.360 | 0.01 | <0.01 | <0.01 |
| Cysteine | 0.756 | 0.723 | 0.718 | 0.483 | 0.01 | <0.01 | <0.01 |
| Glutamic acid | 0.833 | 0.809 | 0.811 | 0.652 | 0.01 | <0.01 | <0.01 |
| Glycine | 0.692 | 0.581 | 0.604 | 0.220 | 0.06 | <0.01 | <0.01 |
| Serine | 0.743 | 0.705 | 0.712 | 0.498 | 0.01 | <0.01 | <0.01 |
| Mean | 0.747 | 0.686 | 0.696 | 0.436 | 0.02 | <0.01 | <0.01 |

[a] Data are means of 10 observations; values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI), which were determined by feeding pigs a N-free diet: CP, 22.32; Arg, 0.62; His, 0.20; Ile, 0.40; Leu, 0.65; lysine, 0.57; Met, 0.13; Phe, 0.42; Thr, 0.64; Trp, 0.17; Val, 0.55; Ala, 0.89; Asp, 0.96, Cys, 0.26; Glu, 1.27; Gly, 2.01; and Ser, 0.65.
[b] Linear and quadratic effects of time of autoclaving.

## 4. Discussion

Canola meal contains glucosinolates, which are antinutritional factors that may reduce feed intake because of their bitter taste, and which also may decrease growth performance (Seneviratne et al., 2010). Canola meal should not be included in diets for pigs at inclusion levels that result in dietary total concentration of glucosinolates that exceed 2 μmol/g (Arntfield and Hickling, 2011). Total glucosinolates in the non-autoclaved canola meal used in this experiment was 1.58 μmol/g and even at relatively high inclusion levels of canola meal (420 g/kg) in the experimental diets, the calculated concentration of glucosinolates in the mixed diets (0.66 μmol/g) was well below the maximum recommended levels. Therefore, we did not expect any negative effects of glucosinolates on feed intake of pigs during the experiment.

During the desolventization and toasting processes of canola meal, injection of steam and temperatures ranging from 95 to 115 °C during an average of 30 min are commonly used (Canola Council of Canada, 2009). Processing, however, may not be

**Table 6**
Linear regressions to predict the concentration (g/kg) of standardized ileal digestible (SID) AA in canola meal fed to pigs.[a]

| Dependent variable | Intercept | | | Independent variables[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | SE | P-value | $X_1$ | Estimate | SE | P-value | $X_2$ | Estimate | SE | P-value | $r^2$ |
| SID Arginine | −2.01 | 1.63 | 0.23 | RS | 0.41 | 0.03 | <0.01 | – | – | – | – | 0.76 |
| SID Histidine | 10.2 | 0.19 | <0.01 | Lignin | −0.03 | 0.001 | <0.01 | – | – | – | – | 0.89 |
| SID Isoleucine | 6.67 | 3.02 | 0.03 | NDF | 0.02 | 0.009 | 0.07 | ADIN | −0.50 | 0.09 | <0.01 | 0.72 |
| SID Leucine | 23.8 | 1.44 | <0.01 | NDF | 0.01 | 0.006 | 0.02 | Lignin | −0.09 | 0.009 | <0.01 | 0.92 |
| SID | −16.6 | 1.89 | <0.01 | RL | 1.60 | 0.12 | <0.01 | – | – | – | – | 0.83 |
| Lysine | −16.5 | 0.85 | <0.01 | RS | 0.59 | 0.02 | <0.01 | – | – | – | – | 0.96 |
| SID Methionine | 7.63 | 0.01 | <0.01 | Lignin | −0.02 | 0.0007 | <0.01 | – | – | – | – | 0.93 |
| SID Phenylalanine | 18.0 | 0.53 | <0.01 | ADF | −0.03 | 0.002 | <0.01 | – | – | – | – | 0.84 |
| SID Threonine | 31.6 | 5.47 | <0.01 | ADF | −0.06 | 0.01 | <0.01 | RS | −0.15 | 0.07 | 0.03 | 0.89 |
| SID Tryptophan | 9.93 | 1.50 | <0.01 | ADF | −0.018 | 0.0028 | <0.01 | RS | −0.05 | 0.02 | <0.01 | 0.88 |
| SID Valine | 10.2 | 1.60 | <0.01 | NDF | 0.02 | 0.004 | <0.01 | ADIN | −0.55 | 0.05 | <0.01 | 0.93 |

[a] $n = 39$ observations; for all models $P < 0.01$.
[b] ADIN, acid detergent insoluble nitrogen; RS, reducing sugars; and RL, reactive lysine (g/kg) = [lysine (g/kg) − (Furosine (g/kg) ÷ 0.32 × 0.40)].

consistent among processors and this may create some variability in the nutritional composition of canola meal (Spragg and Mailer, 2007). The concentration of lysine in different sources of canola meal ranges from 16.4 to 24.0 g/kg (Spragg and Mailer, 2007; Newkirk, 2011). Because some of this variation may be a result of heat damage and because the concentration of lysine in canola meal used in this experiment ranged from 13.6 to 19.2 g/kg, we believe that heat damage caused by autoclaving in this experiment is equivalent to heat damage that may be caused by processing conditions at different processing plants.

The concentrations of DM, CP, and AA in the non-autoclaved canola meal used in the experiment were in agreement with previously published values (Mariscal-Landín et al., 2008; NRC, 2012). The increase in analyzed ADF and lignin observed as a result of increased time of autoclaving also has been observed for Italian ryegrass (Miao et al., 1994). The analyzed concentrations of ADF and lignin may increase because some melanoidins, which are polymers originating from Maillard reactions, may be analyzed as ADF or lignin (Marlett and Johnson, 1985; Miao et al., 1994). Consequently, heat treatment of feed ingredients is expected to increase analyzed values of ADF, NDF, and lignin. Our results for the concentration of ADIN in heat-damaged canola meal are in agreement with previous observations in which the concentration of ADIN increased as a result of heat damage (Cromwell et al., 1993; Schroeder et al., 1996; Seifdavati and Taghizadeh, 2012). This observation indicates that ADIN may be used as an indicator of heat damage in canola meal. The concentrations of reducing sugars and lysine were concomitantly reduced as time of autoclaving increased, and this was expected and clearly reflects the occurrence of Maillard reactions as a result of heat damage, in which the carbonyl group of reducing sugars reacts with the epsilon amino group of lysine to form Amadori compounds and other Maillard reaction products (Nursten, 2005). We observed that the concentration of furosine increased with autoclaving of canola meal up to 30 min, but the concentration of furosine was slightly reduced in canola meal that was autoclaved for 45 min. The reason for these observations is that furosine is a product of Amadori compounds subjected to acid hydrolysis (Boucher et al., 2009). Amadori compounds are formed during the early Maillard reaction stage, but if the reaction progresses to more advanced stages, the Amadori compounds are converted to advanced Maillard reaction products (Nursten, 2005). Therefore, autoclaving of canola meal for 45 min likely resulted in conversion of Amadori compounds to advanced Maillard reaction products, thus causing a reduction in the concentration of furosine. Although we observed a numerical decrease in the lysine concentration of canola meal as time of autoclaving increased, the concentration of CP remained unaffected regardless of the degree of heat damage. Thus, the calculated lysine:CP ratio also decreased as time of autoclaving increased. The lysine:CP ratio may be used as an indicator of heat damage in feed ingredients and the current results support this assumption (Cozannet et al., 2010; González-Vega et al., 2011; Kim et al., 2012). A change in color of canola meal (*i.e.*, less yellow and more brown) also has been observed after the desolventization and toasting processes (Newkirk et al., 2003), which is likely a result of the formation of advanced Maillard reaction products such as pre-melanoidins and melanoidins that give a brown pigmentation to heat-damaged feeds (Nursten, 2005).

Values for the AID of CP and AA that were determined in this experiment for non-autoclaved canola meal are in agreement with previously published values (Fan and Sauer, 1995; Stein et al., 2005; NRC, 2012). The observation that the AID of most AA is reduced due to autoclaving is in agreement with data for autoclaved SBM (Fontaine et al., 2007; González-Vega et al., 2011). Values for the SID of AA in the non-autoclaved canola meal determined in this experiment are also in agreement with previously reported values (Sauvant et al., 2004; Stein et al., 2005; Woyengo et al., 2010; Rostagno et al., 2011). The reason the SID of lysine is reduced more than the SID of other AA is that in the presence of heat, moisture, and pressure, reducing sugars condense with the epsilon amino group of lysine (Nursten, 2005). This reaction initiates a series of other reactions. After lysine has reacted with reducing sugars, Amadori compounds are generated, which reduces the calculated digestibility as well as the concentration of lysine, whereas formation of the advanced Maillard reaction products (*i.e.*, pre-melanoidins and melanoidins), results in reduced concentration of lysine in the ingredient. The observed reduction in SID of other AA may be associated with their direct participation in Maillard reactions (*e.g.*, Cysteine and Arginine) or with the formation of cross-links that impair digestibility (Finot et al., 1990; Ledl and Schleicher, 1990).

Regression equations developed in the experiment to predict the concentration of SID lysine in heat-damaged canola meal have a relatively high $r^2$, which indicates that variations are well explained by the models. The concentration of reactive lysine, calculated by the furosine procedure, is a good predictor for the concentration of SID lysine in heat-damaged canola meal. To the best of our knowledge, this is the first time that the usefulness of reactive lysine, determined by the furosine procedure, to predict the concentration of SID lysine in canola meal fed to pigs is demonstrated. The concentration of SID lysine in DDGS is also accurately ($r^2 = 0.90$) predicted from the concentration of reactive lysine (Kim et al., 2012). The concentration of analyzed reducing sugars is also a good predictor of the concentration of SID lysine in canola meal that has been heat-damaged. Regression equations developed in this experiment are valid if the same source of canola meal is used, as was the case in this experiment. Further research, however, needs to be conducted to determine if the use of reducing sugars as a predictor for the concentration of SID lysine in different sources of canola meal is applicable.

## 5. Conclusions

Results of this experiment confirm that the concentration and digestibility of AA in canola meal are reduced as a consequence of heat damage. This indicates that some of the variations in AA concentration and digestibility among different sources of canola meal fed to pigs are likely caused by differences in processing, specifically, the desolventization step, which

likely causes Maillard reactions. Therefore, standardization of desolventization steps among processing plants may be beneficial to feed manufacturers and to the livestock industry, as it may create a product that is less variable in AA composition and digestibility. Regression equations developed in this experiment may be used to predict the concentration of SID AA in canola meal.

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