

NON RUMINANT NUTRITION

Excessive heating of 00-rapeseed meal reduces not only amino acid digestibility but also metabolizable energy when fed to growing pigs

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Abstract

Two experiments were conducted to test the hypothesis that both the degree of heating and the time that heat is applied will affect the concentration of DE and ME, and the apparent ileal digestibility (AID) and the standardized ileal digestibility (SID) of amino acids (AA) in 00-rapeseed meal (00-RSM) fed to growing pigs. The nine treatments were prepared using a conventional 00-RSM that was either not autoclaved or autoclaved at 110 °C for 15 or 30 min or at 150 °C for 3, 6, 9, 12, 15, or 18 min. In experiment 1, 20 growing barrows with an average initial BW of 21.2 ± 1.2 kg were randomly allotted to the 10 diets in a replicated 10 × 4 Youden square with 10 diets and four periods in each square. A corn-based basal diet and nine diets containing corn and each source of 00-RSM were formulated. Urine and fecal samples were collected for 5 d after 7 d of adaptation. In experiment 2, nine diets contained one of the nine sources of 00-RSM as the sole source of AA, and an N-free diet that was used to measure basal endogenous losses of AA and CP was formulated. Twenty growing barrows with an initial BW of 69.8 ± 5.7 kg had a T-cannula installed in the distal ileum and were allotted to a 10 × 7 Youden square design with 10 diets and 7 periods. Ileal digesta were collected on days 6 and 7 of each 7-d period. Results from the experiments indicated that there were no effects of autoclaving at 110 °C on DE and ME or on AID and SID of AA in 00-RSM, but DE and ME, and AID and SID of AA were less ($P < 0.01$) if 00-RSM was autoclaved at 150 °C compared with 110 °C. At 150 °C, there were decreases (quadratic, $P < 0.05$) in DE and ME, and in AID and SID of AA as heating time increased. In conclusion, autoclaving at 110 °C did not affect ME or SID of AA in 00-RSM, but autoclaving at 150 °C had negative effects on ME and SID of AA and the negative effects increased as heating time increased.

Key words: heat damage, metabolizable energy, Maillard reaction, rapeseed meal, standardized ileal digestibility

Introduction

00-Rapeseed meal (00-RSM) is a protein source that is low in erucic acid and glucosinolates and may be used in animal diets. After soybean meal, 00-RSM is the most commonly used oilseed meal in diets for poultry and livestock (USDA, 2019). Compared with soybean meal, 00-RSM contains less lysine,

but more sulfur-containing amino acids (AA; Kasprzak et al., 2016). However, the nutritional value may vary as a result of differences in growing conditions of the rapeseeds or the processing technology employed during crushing when oil is removed (Kasprzak et al., 2016). In most cases, rapeseeds are first mechanically pressed to remove some of the oil, and the resulting material is then solvent extracted with hexane

Abbreviations

| | |
|--------|------------------------------------|
| AA | amino acids |
| AEE | acid-hydrolyzed ether extract |
| AID | apparent ileal digestibility |
| ATTD | apparent total tract digestibility |
| CP | crude protein |
| SID | standardized ileal digestibility |
| 00-RSM | 00-rapeseed meal |

to remove additional oil. Hexane is applied at 50 to 60 °C for 90 min and the meal is then desolventized/toasted for 60 min at 100 to 110 °C before being dried and cooled for 15 min at 34 °C (Newkirk et al., 2003). Heating during desolventizing and toasting is applied to remove residual hexane and the heating also inactivates the myrosinase enzyme, which is needed for metabolism of glucosinolates. The resulting 00-RSM contains <3% ether extract and is used as animal feed, whereas the oil is used in the human food industry or in the biodiesel industry. However, variations in heat processing such as differences in temperatures and duration of heating may result in formation of sugar-AA complexes due to the Maillard reaction (Eklund et al., 2015). Maillard reaction products result in reduced standardized ileal digestibility (SID) of AA, with Lys being the most sensitive AA (González-Vega et al., 2011). There is, however, limited information about how the duration and type of heating influences digestibility of AA in 00-RSM, and there is no information about how heating affects energy

digestibility and the concentration of DE and ME in 00-RSM. Therefore, the objective of these experiments was to test the hypothesis that both the degree of heating and the time that heat is applied will affect the concentration of DE and ME and the apparent ileal digestibility (AID) and SID of AA in 00-RSM fed to growing pigs.

Materials and methods

Two experiments were conducted and the Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols for both experiments. Both experiments were conducted at the Swine Research Center at the University of Illinois at Urbana-Champaign. Pigs were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN). The 00-RSM used in both experiments originated from a single source and was divided in nine batches that were either not autoclaved or autoclaved for 15 or 30 min at 110 °C or for 3, 6, 9, 12, 15, or 18 min at 150 °C (Table 1). Autoclaving of the different batches of RSM was accomplished using a belt-autoclave (Hydrothermische Kochanlage, Amandus Kahl GmbH & Co, and Reinbek, Germany), which continuously processed the material. The autoclave has a double valve for inlet and a double valve for outlet, so that pressure and temperature can be maintained, although new product is continuously entering and processed material is continuously leaving the autoclave. The layer inside the autoclave was 5 to 7 cm thick. Before entering the autoclave, the RSM was heated

Table 1. Analyzed nutrient composition in autoclaved 00-rapeseed meal

| Item | 00-RSM ¹ | Autoclaving | | | | | | | |
|----------------------------|---------------------|-------------|-------|--------|-------|-------|-------|-------|-------|
| | | 110 °C | | 150 °C | | | | | |
| | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 |
| Duration, min | | | | | | | | | |
| GE, kcal/kg | 4,264 | 4,304 | 4,305 | 4,274 | 4,211 | 4,322 | 4,387 | 4,391 | 4,308 |
| DM, % | 92.30 | 92.39 | 92.44 | 92.04 | 92.27 | 92.25 | 92.25 | 92.60 | 92.25 |
| CP, % | 34.87 | 37.83 | 37.32 | 35.30 | 35.89 | 36.38 | 36.10 | 36.40 | 37.44 |
| AEE, ² % | 3.12 | 2.62 | 2.16 | 4.41 | 4.31 | 4.36 | 4.56 | 4.41 | 4.11 |
| Ash, % | 8.30 | 7.69 | 7.82 | 8.19 | 8.16 | 8.33 | 8.18 | 8.30 | 8.55 |
| Insoluble dietary fiber, % | 26.90 | 25.70 | 26.50 | 22.80 | 21.00 | 20.60 | 20.00 | 20.40 | 18.50 |
| Soluble dietary fiber, % | 2.60 | 2.90 | 2.80 | 7.20 | 7.90 | 8.60 | 6.00 | 7.80 | 8.50 |
| Total dietary fiber, % | 29.50 | 28.70 | 29.30 | 30.00 | 28.90 | 29.20 | 26.00 | 28.20 | 27.00 |
| Indispensable AA, % | | | | | | | | | |
| Arg | 2.06 | 2.15 | 2.13 | 1.83 | 1.62 | 1.13 | 1.14 | 1.00 | 0.89 |
| His | 0.91 | 0.98 | 0.97 | 0.90 | 0.89 | 0.88 | 0.87 | 0.88 | 0.88 |
| Ile | 1.39 | 1.45 | 1.48 | 1.41 | 1.40 | 1.42 | 1.40 | 1.46 | 1.47 |
| Leu | 2.38 | 2.54 | 2.53 | 2.41 | 2.43 | 2.46 | 2.42 | 2.48 | 2.53 |
| Lys | 2.02 | 2.09 | 2.06 | 1.73 | 1.52 | 1.20 | 1.19 | 1.16 | 1.12 |
| Met | 1.46 | 1.62 | 1.58 | 1.34 | 1.28 | 1.21 | 1.21 | 1.17 | 1.19 |
| Phe | 1.45 | 1.56 | 1.55 | 1.48 | 1.49 | 1.49 | 1.47 | 1.52 | 1.53 |
| Thr | 1.57 | 1.67 | 1.64 | 1.57 | 1.58 | 1.61 | 1.60 | 1.59 | 1.61 |
| Trp | 0.47 | 0.51 | 0.50 | 0.47 | 0.47 | 0.46 | 0.46 | 0.46 | 0.46 |
| Val | 1.75 | 1.84 | 1.88 | 1.78 | 1.75 | 1.79 | 1.75 | 1.84 | 1.84 |
| Dispensable AA, % | | | | | | | | | |
| Ala | 1.56 | 1.67 | 1.64 | 1.56 | 1.58 | 1.59 | 1.58 | 1.61 | 1.64 |
| Asp | 2.56 | 2.66 | 2.61 | 2.53 | 2.52 | 2.48 | 2.47 | 2.48 | 2.45 |
| Cys | 0.81 | 0.91 | 0.87 | 0.69 | 0.62 | 0.52 | 0.57 | 0.50 | 0.49 |
| Glu | 5.88 | 6.49 | 6.40 | 5.89 | 5.92 | 6.00 | 5.94 | 6.04 | 6.19 |
| Gly | 1.74 | 1.86 | 1.85 | 1.75 | 1.76 | 1.77 | 1.75 | 1.79 | 1.80 |
| Pro | 2.18 | 2.40 | 2.37 | 2.19 | 2.12 | 2.24 | 2.20 | 2.31 | 2.37 |
| Ser | 1.52 | 1.64 | 1.56 | 1.50 | 1.53 | 1.52 | 1.53 | 1.48 | 1.50 |

¹00-RSM = rapeseed meal without autoclaving.

²AEE = acid-hydrolyzed ether extract.

to ~100 °C by steam conditioning for 2 min. After autoclaving, the RSM entered a belt dryer (140 °C) for drying as the moisture content was increased during autoclaving to 18% to 35%, but after drying, the RSM had 9% to 10% moisture and a temperature of 40 to 60°C. Following the belt dryer, RSM was pelleted with pellets having a diameter of 3 mm (Type 33-390; Amandus Kahl GmbH & Co., Reinbek, Germany) to homogenize the material and to reduce losses during the final drying. The temperature after pelleting was 66 °C. The final drying was accomplished using a classical belt dryer (120 °C)/cooler (ambient air 20 °C) to achieve a moisture content of maximum 7%.

Experiment 1: Energy digestibility and concentrations of DE and ME

Experiment 1 was designed to determine the digestibility of energy and concentrations of DE and ME in the nine sources of 00-RSM. Twenty growing barrows with an average initial BW of 21.2 ± 1.2 kg were randomly allotted in a replicated 10 × 4 Youden Square with 10 diets and 4 periods. Thus, there were two pigs per diet in each period for a total of eight replicates per diet. Pigs were housed individually in metabolism crates that were equipped with a self-feeder, a nipple waterer, and a slatted floor. A screen and a urine pan were placed under the slatted floor to allow for the total, but separate, collection of urine and fecal materials.

A corn-based basal diet and nine diets containing corn and each source of 00-RSM were formulated (Table 2). Thus, a total of 10 diets were used. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). Feed was supplied in a daily amount of three times the maintenance energy requirement (i.e., 197 kcal ME/kg × BW^{0.60}; NRC, 2012) of the smallest pig in each replicate and divided into two equal meals that were provided at 0800 and 1600 hours. Water was available at all times. Pigs were fed experimental diets for 14 d. The initial 7 d were considered an adaptation period

Table 2. Composition of experimental diets containing corn or corn and 00-rapeseed meal, as-fed basis¹, experiment 1

| Ingredient, % | Corn | 00-Rapeseed meal |
|---------------------------------|-------|------------------|
| Corn | 97.45 | 58.40 |
| 00-Rapeseed meal | — | 40.00 |
| Ground limestone | 0.60 | 0.30 |
| Dicalcium phosphate | 1.40 | 0.75 |
| Sodium chloride | 0.40 | 0.40 |
| Vit-mineral premix ² | 0.15 | 0.15 |

¹Gross energy analyzed in the diet containing the nonautoclaved 00-rapeseed meal was 3,952 kcal GE/kg (as-fed basis) and the analyzed GE in diets containing 00-rapeseed meal autoclaved at 110 °C (15 or 30 min) or 150 °C (3, 6, 9, 12, 15, or 18 min) was 3,959, 3,983, 3,966, 4,003, 3,983, 3,982, 3,983, and 4,009 kcal/kg (as-fed basis), respectively.

²The vitamin–micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D₃ as cholecalciferol, 2,210 IU; vitamin E as DL- α tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B₁₂, 0.03 mg; pantothenic acid as calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydroiodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

to the diet. Fecal markers were fed on day 8 (chromic oxide) and day 13 (ferric oxide), and fecal collections were initiated when chromic oxide appeared in the feces and ceased when ferric oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at -20 °C immediately after collection. Urine collections were initiated on day 8 at 1600 hours and ceased on day 13 at 1600 hours. Urine buckets were placed under the metabolism crates to permit total collection and buckets were emptied every morning and a preservative of 50 mL of sulfuric acid was added to each bucket when they were emptied. The collected urine was weighed and a 20% subsample was stored at -20 °C. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a sub-sample was lyophilized before analysis (Kim et al., 2009).

All samples were analyzed in duplicate. Fecal samples were thawed and mixed within pig and diet, and then dried at 65 °C using a forced air drying oven. Samples were then ground through a 1-mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ) before analysis. Diets, ingredients, and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007), and ingredients, diets, fecal, and urine samples were analyzed for GE using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL).

The apparent total tract digestibility (ATTD) of GE and DM was calculated for each diet. Digestible energy and ME in corn were calculated by dividing the DE and ME of the corn diet by the inclusion rate of corn in that diet. The contribution of DE and ME from corn to the DE and ME of the diets containing corn and one of the sources of 00-RSM was subtracted from the DE and ME of these diets, and the DE and ME of each source of 00-RSM were calculated by difference (Adeola, 2001). The ATTD of GE and DM in the nine sources of 00-RSM was calculated using the same procedure.

Normality of data was verified and outliers were tested using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Mean values were calculated using the LSMeans statement and pig was the experimental unit. Contrast statements were used to determine effects of different heat treatment temperature (i.e., 0 vs. 110 °C; 110 vs. 150 °C) and heat treatment duration within temperature (i.e., linear effect at 110 °C; linear and quadratic effects at 150 °C). An α -value of 0.05 was used to assess significance among means and results were considered a tendency at 0.05 < P < 0.10.

Experiment 2: AA digestibility

Experiment 2 was designed to determine the AID and the SID of CP and AA in the nine sources of 00-RSM. Twenty growing barrows with an average initial BW of 69.8 ± 5.7 kg had a T-cannula installed in the distal ileum (Stein et al., 1998). Pigs were allotted to a replicated 10 × 7 Youden square design with 10 diets and seven 7-d periods in each square. There were, therefore, a total of seven observations per treatment. Pigs were placed in individual pens (1.2 × 1.5 m) that were equipped with a self-feeder, a nipple waterer, and a slatted tribar floor.

Nine diets that each contained one of the nine sources of 00-RSM as the sole source of AA were formulated (Table 3). An N-free diet that was used to determine basal endogenous losses of AA and CP was also formulated. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker. Pigs were limit fed to three times their estimated energy requirement for maintenance (i.e., 197 kcal/kg BW^{0.60}; NRC, 2012), but throughout the experiment, pigs had free access to water. The first 5 d of each period was considered the adaptation period to the diet, whereas ileal

Table 3. Ingredient composition and analyzed composition of experimental diets containing rapeseed meal, as-fed basis, experiment 2

| Item | 00-RSM ¹ | Autoclaving | | | | | | | | N-free |
|-------------------------------------|---------------------|-------------|--------|--------|--------|--------|--------|--------|--------|--------|
| | | 110 °C | | 150 °C | | | | | | |
| | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | |
| Duration, min | | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | |
| 00-Rapeseed meal | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | - |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 4.00 |
| Ground limestone | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.45 |
| Monocalcium phosphate | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 2.15 |
| Sucrose | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 20.00 |
| Cornstarch | 44.75 | 44.75 | 44.75 | 44.75 | 44.75 | 44.75 | 44.75 | 44.75 | 44.75 | 67.80 |
| Solka floc ² | — | — | — | — | — | — | — | — | — | 4.00 |
| Magnesium oxide | — | — | — | — | — | — | — | — | — | 0.10 |
| Potassium carbonate | — | — | — | — | — | — | — | — | — | 0.40 |
| Sodium chloride | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Chromic oxide | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin-mineral ³ premix | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Analyzed composition | | | | | | | | | | |
| DM, % | 91.48 | 91.88 | 91.54 | 91.38 | 91.54 | 91.9 | 92.35 | 91.70 | 91.48 | 97.69 |
| CP, % | 14.55 | 14.55 | 15.52 | 14.51 | 14.67 | 15.47 | 14.96 | 15.33 | 13.76 | 1.74 |
| Indispensable AA, % | | | | | | | | | | |
| Arg | 0.83 | 0.80 | 0.84 | 0.73 | 0.63 | 0.48 | 0.47 | 0.41 | 0.32 | 0.01 |
| His | 0.37 | 0.36 | 0.39 | 0.36 | 0.36 | 0.36 | 0.35 | 0.36 | 0.31 | 0.01 |
| Ile | 0.57 | 0.55 | 0.59 | 0.57 | 0.56 | 0.59 | 0.58 | 0.59 | 0.51 | 0.01 |
| Leu | 0.98 | 0.95 | 1.02 | 0.97 | 0.97 | 1.02 | 1.01 | 1.02 | 0.89 | 0.03 |
| Lys | 0.83 | 0.78 | 0.82 | 0.68 | 0.60 | 0.51 | 0.50 | 0.47 | 0.40 | 0.03 |
| Met | 0.25 | 0.25 | 0.27 | 0.28 | 0.23 | 0.24 | 0.23 | 0.23 | 0.19 | 0.00 |
| Phe | 0.61 | 0.58 | 0.63 | 0.57 | 0.59 | 0.63 | 0.62 | 0.62 | 0.54 | 0.02 |
| Thr | 0.63 | 0.61 | 0.65 | 0.61 | 0.61 | 0.65 | 0.63 | 0.64 | 0.56 | 0.01 |
| Trp | 0.18 | 0.18 | 0.20 | 0.19 | 0.18 | 0.19 | 0.18 | 0.19 | 0.16 | 0.02 |
| Val | 0.73 | 0.70 | 0.74 | 0.73 | 0.71 | 0.74 | 0.73 | 0.72 | 0.64 | 0.01 |
| Dispensable AA, % | | | | | | | | | | |
| Ala | 0.65 | 0.63 | 0.67 | 0.65 | 0.63 | 0.67 | 0.65 | 0.66 | 0.58 | 0.02 |
| Asp | 1.06 | 1.00 | 1.06 | 1.01 | 1.00 | 1.02 | 1.01 | 1.02 | 0.86 | 0.02 |
| Cys | 0.33 | 0.33 | 0.35 | 0.28 | 0.24 | 0.21 | 0.20 | 0.19 | 0.16 | 0.00 |
| Glu | 2.46 | 2.43 | 2.60 | 2.38 | 2.4 | 2.49 | 2.46 | 2.49 | 2.21 | 0.04 |
| Gly | 0.72 | 0.70 | 0.75 | 0.73 | 0.7 | 0.73 | 0.72 | 0.73 | 0.64 | 0.01 |
| Pro | 0.91 | 0.89 | 0.96 | 0.85 | 0.90 | 0.93 | 1.06 | 0.91 | 0.80 | 0.04 |
| Ser | 0.61 | 0.59 | 0.63 | 0.59 | 0.58 | 0.60 | 0.60 | 0.60 | 0.53 | 0.01 |

¹00-RSM = rapeseed meal without autoclaving.

²Fiber Sales and Development Corp., Urbana, OH.

³The vitamin–micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D₃ as cholecalciferol, 2,208 IU; vitamin E as DL- α tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B₁₂, 0.03 mg; D₅ pantothenic acid as D₅ calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydroiodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

digesta were collected for 9 h on days 6 and 7. A 225-mL plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected (Stein et al., 1998). Bags were removed every 30 min, or whenever full, and replaced with a new bag. Digesta were stored at -20 °C immediately after collection.

At the conclusion of the experiment, ileal digesta were thawed and mixed within animal and diet, and a subsample was collected for analysis. Samples of all diets and of each source of 00-RSM were also collected. Ileal digesta were lyophilized and finely ground before analysis. Samples of diets, ileal digesta, and ingredients were analyzed for CP (method 990.0; AOAC Int., 2007) and DM (method 930.15; AOAC Int., 2007). These samples were also analyzed for AA as previously described (Almeida et al., 2014a), and diets and

ileal digesta samples were analyzed for chromium (method 990.08; AOAC Int., 2007). Ingredient samples were also analyzed for insoluble and soluble dietary fiber according to method 991.43 (AOAC Int., 2007) using the Ankom^{TD} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY), and total dietary fiber was calculated as the sum of soluble dietary fiber and insoluble dietary fiber. Total fat was analyzed in the nine sources of 00-RSM as acid-hydrolyzed ether extract (AEE). Samples were hydrolyzed using 3N HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY). These samples were also analyzed for dry ash (method 942.05; AOAC Int., 2007).

The AID and the SID of CP and AA were calculated for the nine diets containing 00-RSM as previously described (Stein

et al., 2007). Values calculated for the nine diets also represent the values for each ingredient because the 00-RSM was the only source of AA in the diets. The basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet (Stein et al., 2007). Normality of data was verified and outliers were tested using the UNIVARIATE procedure (SAS Inst. Inc.). Data were analyzed as described for experiment 1.

Results

Experiment 1: Energy digestibility and concentrations of DE and ME

There were no differences in energy digestibility and metabolizability between the diet containing 00-RSM that was not autoclaved and the diets containing 00-RSM that were autoclaved at 110 °C (Table 4). However, the ATTD of DM and GE, and DE and ME in the diets were less ($P < 0.01$) if 00-RSM was autoclaved at 150 °C than at 110 °C. Autoclaving at 110 °C resulted in no linear effect on energy digestibility or metabolizability of diets as the duration of autoclaving increased. In contrast, the ATTD of DM and GE, and the DE and ME in diets decreased quadratically ($P < 0.05$) with increasing duration of autoclaving at 150 °C. Likewise, the ATTD of DM and GE, and the DE and ME of 00-RSM calculated on an as-fed and on a DM basis were less ($P < 0.01$) if 00-RSM was autoclaved at 150 °C than at 110 °C, but there were no differences between 00-RSM without autoclaving and 00-RSM that was autoclaved at 110 °C (Table 5). There were, however, quadratic decreases ($P < 0.05$) for ATTD of DM and GE, and DE and ME of 00-RSM calculated on an as-fed and on a DM basis, as the duration of autoclaving increased at 150 °C.

Experiment 2: AA digestibility

There were no differences in AID of CP and AA between the diet containing 00-RSM without autoclaving and the diets containing 00-RSM that was autoclaved at 110 °C, although the AID of Lys had a tendency to be less ($P = 0.08$) for 00-RSM autoclaved at 110 °C than for the nonautoclaved 00-RSM (Table 6). The AID of most AA was less ($P < 0.01$) if 00-RSM was autoclaved at 150 °C than at 110 °C. At 110 °C, there was no linear effect of autoclaving on AID of CP and AA. However, the AID of CP and most AA decreased (quadratic, $P < 0.05$) with increasing duration of autoclaving at 150 °C.

The SID of CP and all AA was less ($P < 0.01$) if 00-RSM was autoclaved at 150 °C than at 110 °C, but there were no differences between 00-RSM without autoclaving and 00-RSM that was autoclaved at 110 °C (Table 7). In contrast, the SID of CP and all AA decreased (quadratic, $P < 0.01$) as the duration of autoclaving increased at 150 °C.

Discussion

The chemical composition of the 00-RSM that was not autoclaved and the DE and ME for this RSM are in close agreement with values reported by Maison et al. (2015). Except for the SID of Met, the SID of all AA in the nonautoclaved 00-RSM is also in close agreement with values reported previously (Sauvant et al., 2004), and SID of all AA is within the range of values reported for different sources of 00-RSM (Maison and Stein; 2014). It therefore appears that the control 00-RSM was a standard conventional 00-RSM. However, the decrease in the concentrations of AA that was observed as

Table 4. Apparent total tract digestibility of GE and DM, and concentrations of DE and ME in diets containing corn or corn and nonautoclaved or autoclaved 00-rapeseed meal¹, as-fed basis, experiment 1

| Item | Autoclaving | | | | | | | | | | | | Contrast P-value | | | |
|-------------------------|---------------------|-------|-------|-------|---------------------|-------|-------------|-------|---------------------|-------|-------|------|-------------------|-----------|-----------|--------|
| | 110 °C, min | | | | | | 150 °C, min | | | | | | 110 °C vs. 150 °C | At 110 °C | At 150 °C | |
| | 00-RSM ² | | Corn | | 00-RSM ² | | Corn | | 00-RSM ² | | Corn | | | | | |
| | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | 15 | 18 | 15 | 18 | Linear | Linear | Quadratic | |
| GE intake, kcal/d | 4,451 | 4,971 | 5,202 | 5,197 | 5,201 | 4,974 | 4,307 | 4,321 | 4,393 | 4,465 | 340.6 | 0.27 | <0.001 | 0.33 | 0.07 | <0.001 |
| Fecal GE output, kcal/d | 335 | 750 | 752 | 757 | 920 | 826 | 873 | 886 | 836 | 941 | 99.1 | 0.95 | 0.02 | 0.98 | 0.33 | 0.56 |
| ATTD of DM, % | 93.2 | 85.6 | 86.7 | 85.8 | 83.4 | 84.8 | 81.4 | 81.8 | 82.1 | 81 | 0.98 | 0.59 | <0.001 | 0.42 | 0.98 | 0.002 |
| ATTD of GE, % | 91.9 | 84.9 | 85.5 | 84.9 | 82.1 | 83.7 | 79.9 | 80.5 | 80.5 | 79.5 | 1.06 | 0.81 | <0.001 | 0.65 | 0.93 | 0.001 |
| DE in diet, kcal/kg | 3,424 | 3,355 | 3,387 | 3,380 | 3,259 | 3,350 | 3,184 | 3,207 | 3,209 | 3,187 | 42.3 | 0.58 | <0.001 | 0.57 | 0.95 | 0.004 |
| Urine GE output, kcal/d | 87 | 200 | 176 | 146 | 160 | 165 | 157 | 121 | 163 | 167 | 21.75 | 0.06 | 0.68 | 0.3 | 0.13 | 0.02 |
| ME in diet, kcal/kg | 3,350 | 3,197 | 3,253 | 3,255 | 3,136 | 3,222 | 3,051 | 3,095 | 3,067 | 3,031 | 46 | 0.29 | <0.001 | 0.35 | 0.42 | 0.02 |

¹Data are least squares means of eight observations per treatment.

²00-RSM = rapeseed meal without autoclaving.

Table 5. Apparent total tract digestibility of GE and DM, and concentrations of DE and ME in corn, nonautoclaved, or autoclaved 00-rapeseed meal¹, as-fed basis, experiment 1

| Item | Autoclaving | | | | | | | | | | | | Contrast P-value | | | | | | |
|----------------|---------------------|---------------------|-------|-------|-------|-------|-------------|-------|-------|-------|-------|-------|------------------|------|-----------|------------|------------|--------|-----------|
| | 110 °C, min | | | | | | 150 °C, min | | | | | | At 110 °C | | At 150 °C | | | | |
| | 00-RSM ² | | 30 | | 75.5 | | 69 | | 72.7 | | 63.7 | | 65.1 | | 65.2 | | 62.6 | | |
| | Corn | 00-RSM ² | 15 | 30 | 77.1 | 75.8 | 73.7 | 75.5 | 69 | 72.7 | 63.7 | 65.1 | 65.2 | 62.6 | SEM | vs. 110 °C | vs. 150 °C | Linear | Quadratic |
| ATTD of GE, % | 92.2 | 75.6 | 77.1 | 75.5 | 75.5 | 73.7 | 75.5 | 69 | 72.7 | 63.7 | 65.1 | 65.2 | 62.6 | 2.51 | 0.82 | <0.001 | 0.65 | 0.93 | 0.001 |
| ATTD of DM, % | 93.5 | 73.3 | 75.8 | 73.7 | 73.7 | 71.2 | 73.3 | 67.8 | 71.2 | 63.1 | 64.1 | 64.6 | 62.1 | 2.31 | 0.6 | <0.001 | 0.42 | 0.98 | 0.001 |
| DE, kcal/kg | 3,530 | 3,161 | 3,239 | 3,220 | 3,017 | 2,919 | 3,220 | 2,919 | 3,147 | 2,731 | 2,788 | 2,793 | 2,737 | 104 | 0.58 | <0.001 | 0.57 | 0.95 | 0.004 |
| ME, kcal/kg | 3,457 | 2,873 | 3,013 | 3,017 | 2,720 | 2,936 | 3,017 | 2,720 | 2,936 | 2,508 | 2,619 | 2,547 | 2,458 | 114 | 0.28 | <0.001 | 0.34 | 0.41 | 0.02 |
| DE, kcal/kg DM | 4,000 | 3,425 | 3,506 | 3,784 | 3,171 | 3,410 | 3,784 | 3,171 | 3,410 | 2,960 | 3,023 | 3,016 | 2,967 | 113 | 0.6 | <0.001 | 0.59 | 0.9 | 0.003 |
| ME, kcal/kg DM | 3,918 | 3,113 | 3,262 | 3,264 | 2,955 | 3,182 | 2,955 | 3,182 | 2,719 | 2,839 | 2,750 | 2,750 | 2,665 | 123 | 0.3 | <0.001 | 0.35 | 0.38 | 0.01 |

¹Data are least squares means of eight observations per treatment.²00-RSM = rapeseed meal without autoclaving.

00-RSM was autoclaved at 150 °C indicates that autoclaving at 150 °C results in damage to the AA, which also is the reason for the reduced SID of AA in 00-RSM that was autoclaved at 150 °C. Similar results have been reported for autoclaved distillers dried grains with solubles, soybean meal, sunflower meal, cottonseed meal, and canola meal (Fontaine et al., 2007; González-Vega et al., 2011; Almeida et al., 2013, 2014a,b). One of the reasons for these negative effects of autoclaving is that autoclaving may result in Maillard reactions, and this reaction occurs between free amino groups of protein and carbonyl groups of reducing sugars and leads to a decrease in the availability of the AA involved. The extent of the Maillard reaction depends on the reactivity of the amine involved and the reducing sugars (Singh et al., 2007). Heat treatment may also result in crosslinking of protein with a reduced access for digestive enzymes as a result (Almeida et al., 2014a). Absorption of AA may also be reduced because of decreased transport in the intestinal lumen and proteolysis may be inhibited for AA that participate in Maillard reactions (Rerat et al., 2002). The extent of the Maillard reactions depends on water activity, temperature, pH, and time of heating (González-Vega et al., 2011). Lysine is the most reactive AA (Singh et al., 2007) because of the presence of an ε-amino group on the molecule that can react directly with reducing sugars under moist, heat conditions (Eklund et al., 2015). As a result of this reaction, the concentration of Lys and the SID of Lys and all other AA will be reduced. The extent of the reduction in SID depends on the temperature and the time that the ingredient is exposed to heat as was clearly illustrated in this experiment. The greater reduction in the SID of Lys and sulfur AA compared with the other AA that was observed in the present study confirms that these AA are most affected by heat treatment. Similar observations were reported with heat treated canola meal (Almeida et al., 2014b) and 00-RSM (Eklund et al., 2015).

The decrease in insoluble dietary fiber and the increase in soluble dietary fiber that were observed if 00-RSM was autoclaved at 110 °C or 150 °C may indicate that autoclaving solubilized some insoluble dietary fiber. Heat damage of feed ingredients is sometimes associated with an increase in analyzed values for ADF and NDF and a decrease in AA digestibility (Almeida et al., 2013, 2014b).

Results from the experiment demonstrated that heating may reduce not only the SID of AA but also DE and ME of 00-RSM. To our knowledge, this is the first time a reduction in DE and ME in 00-RSM has been demonstrated as a result of heat damage. However, the magnitude of the reduction in DE and ME indicates that the economic consequences of the reduction in DE and ME are at least as big as the consequences of reduced SID of AA. The reduction in DE and ME may be a result of sugars being bound to Lys and other AA, and therefore, these sugars are not available for absorption. Reduced absorption of AA will also reduce DE and ME and it is possible that some dietary fat gets oxidized or depolymerized during heating, which will reduce the energy value of fat (Kerr et al., 2015). Lipid molecules may be liquefied at certain temperatures and then function as a glue between particles, which will also contribute to a reduction in DE and ME of the ingredient (Liu et al., 2012). The combination of these processes appears to have a severe negative impact on the DE and ME of heat damaged 00-RSM. If heat damaged 00-RSM is included in diets for pigs it is, therefore, necessary that extra energy and extra AA are included in the diets to avoid negative impacts on pig growth performance.

Table 6. Apparent ileal digestibility (%) of CP and AA in nonautoclaved and autoclaved 00-rapeseed meal¹, experiment 2

| Item | Autoclaving | | | | | | | | | | | | | | Contrast P-value | | | | | |
|------------------|-------------|------|------|------|-------|------|-------|------------|-------|-------|--------|-------|--------|-------|------------------|--------|--------|-----------|-------|--|
| | 110°C, min | | | | | | | 150°C, min | | | | | | | None | | 110°C | | 150°C | |
| | 15 | 30 | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110°C | vs | 110°C | Linear | 110°C | Linear | 150°C | Linear | Quadratic | | |
| CP | 63.3 | 61.5 | 64.5 | 46.4 | 34.8 | 36.2 | 34.6 | 18.0 | 2.35 | 0.901 | <0.001 | 0.571 | 0.018 | 0.571 | 0.018 | <0.001 | <0.001 | | | |
| Indispensable AA | | | | | | | | | | | | | | | | | | | | |
| Arg | 82.7 | 81.7 | 83.0 | 77.1 | 56.6 | 60.3 | 54.5 | 38.0 | 2.44 | 0.901 | <0.001 | 0.754 | <0.001 | 0.754 | <0.001 | <0.001 | <0.001 | | | |
| His | 80.6 | 78.7 | 80.3 | 75.3 | 62.2 | 63.3 | 61.8 | 50.4 | 1.47 | 0.476 | <0.001 | 0.290 | 0.001 | 0.290 | 0.001 | <0.001 | <0.001 | | | |
| Ile | 69.3 | 68.4 | 69.0 | 61.0 | 47.5 | 48.4 | 47.3 | 30.1 | 1.45 | 0.746 | <0.001 | 0.678 | 0.001 | 0.678 | 0.001 | <0.001 | <0.001 | | | |
| Leu | 74.0 | 71.5 | 73.3 | 65.5 | 55.2 | 56.4 | 55.4 | 40.8 | 1.40 | 0.338 | <0.001 | 0.190 | 0.029 | 0.190 | 0.029 | <0.001 | <0.001 | | | |
| Lys | 72.8 | 66.6 | 69.3 | 55.1 | 19.2 | 19.2 | 14.6 | -8.9 | 2.67 | 0.086 | <0.001 | 0.058 | 0.001 | 0.058 | 0.001 | <0.001 | <0.001 | | | |
| Met | 39.6 | 33.9 | 40.5 | 26.1 | -0.6 | -2.4 | -2.1 | -35.7 | 3.85 | 0.597 | <0.001 | 0.281 | 0.005 | 0.281 | 0.005 | <0.001 | <0.001 | | | |
| Phe | 72.8 | 72.3 | 72.6 | 64.7 | 57.6 | 58.4 | 56.8 | 45.8 | 1.73 | 0.834 | <0.001 | 0.804 | 0.220 | 0.804 | 0.220 | <0.001 | <0.001 | | | |
| Thr | 62.1 | 58.0 | 61.4 | 51.5 | 38.9 | 40.1 | 39.3 | 21.2 | 2.09 | 0.326 | <0.001 | 0.147 | 0.079 | 0.147 | 0.079 | <0.001 | <0.001 | | | |
| Trp | 65.2 | 62.6 | 65.3 | 56.9 | 49.0 | 49.7 | 51.3 | 36.5 | 1.78 | 0.554 | <0.001 | 0.298 | 0.427 | 0.298 | 0.427 | <0.001 | <0.001 | | | |
| Val | 68.0 | 65.2 | 67.8 | 60.5 | 47.0 | 47.7 | 46.4 | 30.7 | 1.87 | 0.472 | <0.001 | 0.250 | 0.006 | 0.250 | 0.006 | <0.001 | <0.001 | | | |
| Dispensable AA | | | | | | | | | | | | | | | | | | | | |
| Ala | 65.8 | 64.3 | 65.5 | 56.5 | 39.7 | 42.7 | 39.8 | 23.5 | 2.09 | 0.689 | <0.001 | 0.580 | 0.019 | 0.580 | 0.019 | <0.001 | <0.001 | | | |
| Asp | 65.8 | 61.5 | 62.8 | 48.1 | 25.6 | 28.1 | 26.8 | 4.24 | 2.88 | 0.245 | <0.001 | 0.232 | 0.428 | 0.232 | 0.428 | <0.001 | <0.001 | | | |
| Cys | 68.5 | 64.9 | 67.2 | 51.1 | 24.3 | 24.8 | 21.5 | -3.7 | 2.68 | 0.443 | <0.001 | 0.328 | 0.006 | 0.328 | 0.006 | <0.001 | <0.001 | | | |
| Glu | 81.3 | 79.9 | 81.6 | 75.3 | 70.6 | 64.1 | 62.1 | 50.5 | 1.45 | 0.747 | <0.001 | 0.468 | 0.001 | 0.468 | 0.001 | <0.001 | <0.001 | | | |
| Gly | 57.8 | 56.9 | 55.9 | 46.7 | 23.4 | 27.2 | 25.7 | 3.9 | 4.31 | 0.994 | <0.001 | 0.748 | 0.171 | 0.748 | 0.171 | <0.001 | <0.001 | | | |
| Pro | 52.2 | 51.3 | 53.7 | 33.7 | -10.4 | 16.6 | -10.2 | -14.3 | 11.03 | 0.981 | <0.001 | 0.944 | 0.731 | 0.944 | 0.731 | <0.001 | <0.001 | | | |
| Ser | 64.5 | 60.9 | 63.4 | 53.7 | 40.6 | 42.5 | 41.1 | 22.8 | 2.12 | 0.350 | <0.001 | 0.218 | 0.077 | 0.218 | 0.077 | <0.001 | <0.001 | | | |

¹Data are least squares means of seven observations per treatment.²00-Rapeseed meal without autoclaving.

Table 7. Standardized ileal digestibility (%) of CP and AA in nonautoclaved and autoclaved 00-rapeseed meal^{1,2}, experiment 2

| Item | Autoclaving | | | | | | | | | | | | | Contrast P-value | | | |
|------------------|-------------|------|------|------------|------|------|------|------|-------|-------|-------|--------|-------|------------------|--------|-----------|--|
| | 110°C, min | | | 150°C, min | | | | | | None | | | | 110°C | | 150°C | |
| | 15 | 30 | SE | 3 | 6 | 9 | 12 | 15 | 18 | SEM | 110°C | vs | 150°C | Linear | Linear | Quadratic | |
| CP | 73.4 | 71.6 | 73.9 | 64.2 | 56.5 | 44.4 | 46.3 | 44.2 | 28.7 | 2.34 | 0.820 | <0.001 | 0.580 | 0.023 | <0.001 | | |
| Indispensable AA | | | | | | | | | | | | | | | | | |
| Arg | 87.9 | 87.2 | 88.1 | 83.0 | 77.1 | 65.6 | 69.5 | 64.9 | 51.6 | 2.45 | 0.925 | <0.001 | 0.811 | 0.001 | <0.001 | | |
| His | 84.3 | 82.5 | 83.8 | 79.0 | 74.9 | 66.1 | 67.2 | 65.7 | 54.8 | 1.47 | 0.476 | <0.001 | 0.327 | 0.001 | <0.001 | | |
| Ile | 73.8 | 73.1 | 73.4 | 65.5 | 60.1 | 51.9 | 52.9 | 51.7 | 35.1 | 1.43 | 0.750 | <0.001 | 0.741 | 0.002 | <0.001 | | |
| Leu | 78.2 | 77.4 | 77.4 | 69.7 | 66.0 | 59.3 | 60.6 | 59.4 | 45.5 | 1.29 | 0.600 | <0.001 | 0.654 | 0.025 | <0.001 | | |
| Lys | 76.8 | 70.8 | 73.3 | 59.9 | 45.7 | 25.7 | 25.8 | 21.6 | -0.49 | 2.69 | 0.095 | <0.001 | 0.070 | 0.001 | <0.001 | | |
| Met | 50.3 | 44.7 | 50.3 | 36.5 | 23.3 | 10.5 | 9.4 | 9.5 | -22.1 | 3.85 | 0.546 | <0.001 | 0.293 | 0.012 | <0.001 | | |
| Phe | 76.6 | 76.2 | 76.2 | 68.7 | 66.2 | 61.2 | 62.0 | 60.5 | 50.1 | 1.73 | 0.840 | <0.001 | 0.861 | 0.218 | <0.001 | | |
| Thr | 69.9 | 66.2 | 69.1 | 59.6 | 54.9 | 46.6 | 47.9 | 47.0 | 30.1 | 2.09 | 0.336 | <0.001 | 0.181 | 0.096 | <0.001 | | |
| Trp | 72.1 | 69.6 | 71.6 | 63.7 | 61.0 | 55.7 | 56.7 | 58.1 | 44.4 | 1.77 | 0.506 | <0.001 | 0.332 | 0.521 | <0.001 | | |
| Val | 73.3 | 70.6 | 72.9 | 65.7 | 60.4 | 52.2 | 52.9 | 51.6 | 36.6 | 1.87 | 0.489 | <0.001 | 0.293 | 0.008 | <0.001 | | |
| Dispensable AA | | | | | | | | | | | | | | | | | |
| Ala | 74.3 | 73.1 | 73.7 | 64.9 | 58.3 | 48.0 | 51.2 | 48.0 | 32.9 | 2.09 | 0.689 | <0.001 | 0.651 | 0.028 | <0.001 | | |
| Asp | 72.3 | 68.4 | 69.4 | 54.9 | 43.7 | 32.4 | 35.0 | 33.6 | 12.2 | 2.88 | 0.274 | <0.001 | 0.280 | 0.476 | <0.001 | | |
| Cys | 74.2 | 70.7 | 72.7 | 58.0 | 48.5 | 33.4 | 34.2 | 31.4 | 7.8 | 2.68 | 0.418 | <0.001 | 0.332 | 0.015 | <0.001 | | |
| Glu | 84.7 | 83.5 | 84.9 | 78.9 | 74.2 | 65.7 | 67.6 | 65.5 | 54.4 | 1.45 | 0.714 | <0.001 | 0.485 | 0.002 | <0.001 | | |
| Gly | 76.8 | 79.5 | 74.4 | 65.6 | 55.9 | 42.3 | 46.5 | 44.5 | 25.5 | 4.34 | 0.985 | <0.001 | 0.654 | 0.241 | <0.001 | | |
| Pro | 97.4 | 98.0 | 96.8 | 82.4 | 67.9 | 34.1 | 55.7 | 35.1 | 36.9 | 11.03 | 0.999 | <0.001 | 0.964 | 0.754 | <0.001 | | |
| Ser | 72.3 | 69.1 | 71.0 | 61.8 | 57.2 | 48.5 | 50.6 | 49.1 | 34.3 | 2.01 | 0.324 | <0.001 | 0.227 | 0.186 | <0.001 | | |

¹Data are least squares means of seven observations per treatment.

²Values for SID were calculated by correcting AID values for basal endogenous losses. Basal endogenous losses were determined using pigs fed the N-free diet as (g/kg DM): CP, 16.09; Arg, 0.47; His, 0.15 Ile, 0.28; Leu, 0.45; Lys, 0.36; Met, 0.29; Phe, 0.25; Thr, 0.54; Trp, 0.14; Val 0.41; Ala, 0.60; Asp, 0.76; Cys, 0.21; Glu, 0.94; Gly, 1.50; Pro, 4.50; and Ser, 0.53.

³00-Rapeseed meal without autoclaving.

Conclusion

AA digestibility and DE and ME in 00-RSM are not reduced by autoclaving at 110 °C for 15 or 30 min. However, digestibility of AA, and DE and ME are reduced if 00-RSM is autoclaved at 150 °C and the longer the duration of autoclaving is the more the SID of AA and the DE and ME are reduced. These results indicate that if crushing plants can avoid heating 00-RSM to more than 110 °C during processing, the risk of overheating is greatly reduced.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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