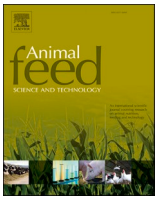
Animal Feed Science and Technology 301 (2023) 115665

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Nutritional value of Spanish *Camelina sativa* co-products for pigs

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ARTICLE INFO

Keywords:
Amino acids
Camelina sativa
Energy value
Growing pigs
Protein value

ABSTRACT

This study evaluated and compared the digestible energy (DE) and metabolizable energy (ME) and the coefficient of ileal standardized digestibility (CISD) of crude protein (CP) and amino acids (AA) in camelina expellers (CAE) and camelina meal (CAM) for growing pigs. In Exp. 1, thirty-six barrows Pietrain × (Landrace × Large White) of 61.8 ± 2.83 kg body weight were allotted to 6 diets, a basal corn-soybean meal diet and 5 diets in which a proportion of the corn and soybean meal in the basal diet was replaced by CAE (100, 200 or 300 g/kg) or CAM (100 or 200 g/kg). The experiment lasted 15 days and during the last 5 days the total amount of feces and urine were collected to calculate the energy metabolizability of diets. The CTTAD of energy and DE and ME concentration in CAE and CAM were calculated by the difference procedure as well as by the regression method. In Exp. 2, thirty-three barrows Pietrain × (Landrace × Large White) of 82.0 ± 2.57 kg body weight were allotted to three treatments, two cornstarch-based diets containing 350 g/kg CAE or 300 g/kg CAM as the sole source of CP and AA and a N-free diet. After 7 days of feeding, animals were euthanized and ileal digesta were sampled. The CISD of AA on CAE and CAM was determined using the direct method. Camelina meal had a greater concentration of CP and AA and a lower ether extract than CAE. The most abundant indispensable AA were arginine, leucine, valine, and lysine in both ingredients (26.3, 21.9, 19.1 and 16.2 g/kg dry matter (DM) in average, respectively). Camelina expellers contained 8.0 g/kg DM more soluble and 4.6 g/kg DM less insoluble fiber than CAM. The CTTAD of energy was 0.682 and 0.665 in CAE and CAM, respectively, when calculated using the difference method, and 0.665 and 0.655 in CAE and CAM, respectively, when estimated via the regression method. The DE and ME were on average greater ($P < 0.05$) for CAE compared with CAM both, using the difference or the regression method (DE, in average:14.3 MJ/kg DM and 13.1 MJ/kg DM, respectively and ME, in average: 14.1 MJ/kg DM and 12.9 MJ/kg DM, respectively). Between methods, no statistical differences were detected. The CISD of CP was greater ($P < 0.05$) in CAM compared with CAE (0.579 in CAE and 0.670 in

Abbreviations: AA, amino acids; ADF, acid detergent fiber; ANF, anti-nutritional factors; BW, body weight; CAE, camelina expellers; CAM, camelina meal; CP, crude protein; CIAD, coefficient of ileal apparent digestibility; CISD, coefficient of ileal standardized digestibility; CTTAD, coefficient of total tract apparent digestibility; DE, digestible energy; ME, metabolizable energy; DM, dry matter; EM, energy metabolizability; EE, ether extract, GE, gross energy; N, nitrogen; NDF, neutral detergent fiber; NSP, non-starch polysaccharides; TiO_2, titanium dioxide.

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https://doi.org/10.1016/j.anifeedsci.2023.115665
Received 19 January 2022; Received in revised form 17 April 2023; Accepted 28 April 2023
Available online 29 April 2023

CAM). The most digestible essential AA in both ingredients were methionine, arginine and histidine, with average digestibilities of 0.77, 0.75 and 0.83, respectively. The CISD of leucine and cysteine was greater in CAM compared with CAE ($P < 0.05$). In conclusion, CAE had greater energy value than CAM, whereas the digestibility of leucine and cysteine was less in CAE than in CAM, probably due to the greater concentration of soluble dietary fiber in CAE.

1. Introduction

Camelina (*Camelina sativa*) is an oilseed crop of the *Brassica* family that is mainly grown to obtain oil for biofuel production. Oil from camelina seeds can be mechanically expelled or solvent extracted resulting in production of co-products called camelina expellers (CAE) and camelina meal (CAM), respectively. Both ingredients have high concentrations of crude protein (CP) and dietary fiber (Pekel at al, 2015; Adhikari et al., 2016). However, the residual oil content in CAE (100–140 g/kg) is greater than in CAM (20–30 g/kg; Woyengo et al., 2016). In addition, in the solvent extraction process used to obtain CAM, camelina is subjected to more heat during oil extraction than in the mechanical process to obtain CAE. The energy value of oilseed co-products for pigs is partially dependent on the amount of residual oil in the co-products (NRC, 2012; Maison et al., 2015) and CAE is therefore expected to contain more energy than CAM. The availability of amino acids (AA) and energy may be reduced by heat whereas the concentration of heat-labile antinutritional factors (ANF) such as trypsin inhibitors can be reduced when heat is applied to the ingredient (Newkirk and Classen, 2002; Oliveira et al., 2020). Thus, the nutritive value of camelina co-products for pigs vary depending on the method of oil extraction and the amount of heat used during production.

There is limited data on the nutritional value of camelina co-products fed to pigs. The digestibility of AA and energy in CAE has been studied (Almeida et al., 2013; Kahindi et al., 2014), whereas the nutritional value of CAM has not been reported. It was, however, hypothesized that CAM has lower energy and amino acid digestibility than CAE because of the lower oil concentration and the higher processing temperatures used during production of CAM rather than CAE. Therefore, the objective of this experiment was to determine the digestible energy (DE) and the metabolizable energy (ME), as well as the coefficient of ileal standardized digestibility (CISD) of CP and AA of CAE and CAM when fed to growing pigs.

Table 1
Analyzed energy and nutrient composition of camelina expellers (CAE) or camelina meal (CAM) (g/kg, dry matter basis unless otherwise indicated)[a].

Item	Ingredient	
	CAE	CAM
Dry matter	924	914
Ash	59	61
Crude protein	370	413
Ether extract	125	14.1
Total starch	13.4	15.6
Total sugars	110	133
NDF[b]	369	376
ADF[c]	201	189
ADL[d]	50.0	48.5
Crude fiber	127	122
Gross energy, MJ/kg	21.1	19.4
Non-starch polysaccharides	257	261
Solubles	70.3	65.7
Insolubles	187	195
Antinutritional factors		
Tannins, mg/g	1.60	1.40
Erucic acid, g/kg[e]	32.8	31.0
Allyl-isothiocyanate, mg/kg	500	180
Trypsin inhibitor units/g	4.24	6.63
Minerals		
Calcium	4.75	4.61
Phosphorus	9.60	9.44
Phytate Phosphorus	5.39	5.71
Sodium, mg/kg	61.4	62.2

[a]All parameters, except antinutritional factors were analyzed in duplicate. Antinutritional factors were analyzed in simple. The considered acceptable coefficient of variation was 0.5 for dry matter and 2.0–3.0 for the rest of parameters.
[b]NDF: neutral detergent fiber.
[c]ADF: acid detergent fiber.
[d]ADL: acid detergent lignin.
[e]As a proportion of total fat.

2. Materials and methods

2.1. General

The experimental procedure was approved by the Ethics Committee of the Universitat Politècnica de València (registration number 2016/VSC/PEA/00025). Two experiments were conducted at the Centro de Investigación y Tecnología Animal of the Instituto Valenciano de Investigaciones Agrarias. The CAE and the CAM were obtained from Spanish Camelina crops and were provided by a commercial company (Camelina Company España, Madrid, Spain; Tables 1 and 2). The CAE and CAM used in both experiments originated from the same batches. Diets were fed in a mash form in both experiments and barrows that were the progeny of Pietrain males mated to Landrace × Large White females were used in both experiments.

2.2. Energy measurements (Exp. 1)

The experiment was conducted to determine the coefficient of total tract apparent digestibility (CTTAD) of energy and the concentration of DE and ME in CAE and CAM when fed to growing pigs. Thirty-six barrows of 61.8 ± 2.83 kg body weight were allotted to 6 diets (12 animals per replication). Experimental diets included a basal corn-soybean meal diet, with corn and soybean meal as the sole sources of energy and protein, and 5 diets in which a proportion of the corn and soybean meal in the basal diet was replaced by CAE (100, 200 or 300 g/kg) or CAM (100 or 200 g/kg; Tables 3 and 4). All diets contained vitamins and minerals to meet requirements for growing pigs (Fedna et al., 2013). Although in this case the digestibility of the nutrients was calculated by the total collection method, the diets contained titanium dioxide (TiO_2; 5 g/kg) for a parallel study on indigestible markers. Diets were assigned to pigs in a randomized complete block design, using replication and initial body weight as blocking factors. The experiment lasted 15 days with 10 days for adaptation and 5 days for total collection of feces and urine. During the last 6 days of adaptation and during the collection period, pigs were housed individually in stainless-steel metabolism pens (2 × 1.2 m^2) with plastic sides and plastic-covered expanded metal sheet flooring equipped with a feeder and a nipple drinker, in a temperature-controlled room. The quantity of feed provided per pig daily was calculated as 3 times the estimated requirement for maintenance ME (197 kcal ME/$kg^{0.6}$ BW; NRC, 2012) divided into two equal meals (08:30 and 15:30 h). Feed was provided mixed with water at a rate of 1.5:1 (water:feed). Feed refusals were registered, weighed, and dried to calculate feed intake. Pig weights were recorded at the beginning of the adaptation period and at the end (15 days) of the experiment. Fresh water was available at all times. Ferric oxide was used to mark the beginning and the end of the fecal collection (Woyengo et al., 2010). In the morning meals of days 11 and 16, 5 g of ferric oxide (diluted in 100 g feed) were fed, and the remaining quantity of the morning feed was offered after all the marked feed was consumed. Fecal collections commenced when the marker appeared in feces after day 11 and were terminated when the marker appeared in feces for the first time after day 16. Total collection of urine commenced on day 11 and ended on day 16. Feces were collected once daily, in the morning, and stored frozen at −20ºC. Urine was also collected once daily in the morning, using 120 mL of H_2SO_4 at 10% per bucket and day to avoid N volatilization, weighed and stored in a chamber at 4 °C until the end of the collection period, when urine was pooled per pig, mixed, subsampled and stored at −20 °C until laboratory analyses. Concentrations of DE and ME in CAE and CAM were calculated by the difference procedure as well as by the regression method (Fan and Sauer, 1995).

Table 2
Analyses of amino acid composition of camelina expellers (CAE) or camelina meal (CAM) (g/kg, dry matter basis)[a].

Item	Ingredient	
	CAE	CAM
Indispensable amino acids		
Arginine	24.9	27.7
Histidine	6.30	6.83
Isoleucine	12.0	13.7
Leucine	20.4	23.4
Lysine	15.4	17.0
Methionine	6.48	7.60
Phenylalanine	12.7	14.4
Threonine	12.0	13.5
Valine	17.7	20.5
Dispensable amino acids		
Alanine	14.5	16.7
Aspartic acid	26.5	30.2
Cysteine	5.95	6.54
Glutamic acid	61.5	72.4
Glycine	17.1	19.0
Proline	15.9	17.9
Serine	13.2	14.5
Tyrosine	7.39	8.42

[a] All parameters were analyzed in duplicate. The considered acceptable coefficient of variation was between 2.0 and 3.0 for all the amino acids.

Table 3
Ingredient composition (g/kg, as-fed basis) of experimental diets containing increasing levels of camelina expellers (CAE) or camelina meal (CAM) (Exp. 1).

Ingredient, g/kg	Diet[a]					
	Basal diet	10CAE	20CAE	30CAE	10CAM	20CAM
Corn grain	785	704	622	541	704	622
Soybean meal 45.5	180	161	143	124	161	143
CAE	-	100	200	300	-	-
CAM	-	-	-	-	100	200
Calcium carbonate	11.0	11.0	11.0	11.0	11.0	11.0
Monocalcium phosphate	11.5	11.5	11.5	11.5	11.5	11.5
Sodium chloride	4.50	4.50	4.50	4.50	4.50	4.50
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[b]	3.0	3.0	3.0	3.0	3.0	3.0

[a] Treatments: 10CAE, 20CAE, and 30CAE include 100, 200, and 300 g/kg CAE, respectively, and 10CAM and 20CAM include 100 and 200 g/kg CAM, respectively.
[b] Contains per kg of complete diet: 0.15 mg of vitamin H; 10,000 IU of vitamin A; 2000 IU of vitamin D_3; 100 mg of vitamin E; 2.00 mg of vitamin K_3; 3.75 mg of vitamin B_1; 7.00 mg of vitamin B_2; 5.25 mg of vitamin B_6; 0.03 mg of vitamin B_{12}; 51.0 mg of niacin; 30.0 mg of calcium pantothenate; 1.80 mg of folic acid; 300 mg of choline chloride; 70.0 mg of iron sulfate (from $FeSO_4 \cdot 7\ H_2O$); 45.0 mg of manganese oxide (from MnO); 125 mg of zinc oxide (from ZnO); 6.00 mg of copper sulfate (from $CuSO_4 \cdot 5\ H_2O$); 0.75 mg of calcium iodide (from $Ca(IO_3)_2$); 0.203 mg sodium selenite (from Na_2SeO_3).

Table 4
Analyses of nutrient composition (g/kg, dry matter basis unless otherwise indicated) of experimental diets containing increasing levels of camelina expellers (CAE) or camelina meal (CAM) (Exp. 1)[a].

Item, g/kg	Diet[b]					
	Basal diet	10CAE	20CAE	30CAE	10CAM	20CAM
Dry matter	87.5	87.9	88.4	89.0	87.7	88.0
Gross energy, MJ/kg	17.8	18.1	18.5	18.6	18.0	18.2
Ash	64.2	66.3	69.0	73.1	63.8	69.8
Ether extract	30.5	36.1	49.2	53.3	29.0	27.8
Crude protein	166.5	183.8	196.9	228.8	198.1	202.4
NDF[c]	98.9	134.1	143.3	189.7	165.6	153.2
ADF[c]	26.4	46.2	46.8	72.7	60.0	57.9
ADL[d]	2.0	10.1	8.1	16.1	13.6	10.6

[a] All parameters were analyzed in duplicate. The considered acceptable coefficient of variation was 0.5 for dry matter and 2.0–3.0 for the rest of parameters.
[b] Treatments: 10CAE, 20CAE, and 30CAE include 100, 200, and 300 g/kg CAE, respectively, and 10CAM and 20CAM include 100 and 200 g/kg CAM, respectively. [c]NDF: neutral detergent fiber.
[c] ADF: acid detergent fiber.
[d] ADL: acid detergent lignin.

2.3. Amino acid digestibility (Exp. 2)

Experiment 2 was conducted to determine the coefficient of ileal apparent digestibility (CIAD) and the CISD of CP and AA in CAE and CAM. Thirty-three barrows with an initial BW of 82.0 ± 2.57 kg were allotted to three treatments using initial BW as the blocking factor, with 11 pigs per diet. Pigs were housed individually in pens (2.5 × 3 m^2) with plastic sides and fully slated concrete floors in a temperature-controlled room. Experimental diets included a cornstarch-based diet containing 350 g/kg CAE or 300 g/kg CAM as the sole source of CP and AA. An N-free diet that was used to determine basal endogenous losses of CP and AA was also prepared (Tables 5 and 6). All diets contained vitamins and minerals in concentrations that exceed the requirements for growing pigs (Fedna et al., 2019) and TiO_2 (5 g/kg) was included in all diets as an indigestible marker. Feed was provided in a quantity calculated to be equal to 3 times the requirement for metabolizable energy (NRC, 2012) and daily feed provisions were divided in two equal meals (08:00 and 15:30 h). Feed was provided mixed with water at a rate of 1.5:1 (water:feed). Fresh water was available at all times. Pig weights were recorded at the beginning of the experiment. After 7 days of feeding, animals were euthanized 4.15 h after their morning meal using captive bolt penetration followed by exsanguination. The abdomen was opened, and the entire gastrointestinal tract was removed. The small and large intestines were separated and digesta samples were collected from the last 1 m (terminal ileum) by gentle flushing the intestine with deionized water as described by Ravindran et al. (2017). Ileal contents were stored at −20ºC until required for analyses. The CISD of CP and AA was determined using the direct method (Almeida et al., 2013).

2.4. Sample preparation and chemical analyses

The total amount of feces produced per pig (Exp. 1) were dried in an oven at 65ºC for 72 h. After drying, feces were pooled for each

Table 5
Ingredients composition (g/kg, as-fed basis) of experimental diets containing camelina expellers (CAE) or camelina meal (CAM), and of the N-free diet (Exp. 2).

Ingredient, g/kg	Diet[a]		
	35CAE	30CAM	N-Free
CAE	350	-	-
CAM	-	300	-
Cornstarch	434	484	667
Sucrose	150	150	200
Soybean oil	30	30	40
Cellulose[b]			40
Calcium carbonate	9.0	9.0	7.0
Monocalcium phosphate	14.0	14.0	26.0
Sodium chloride	4.5	4.5	5.0
Potassium carbonate	-	-	5.0
Magnesium oxide	-	-	1.0
Titanium dioxide	5.0	5.0	5.0
Vitamin-mineral premix[c]	4.0	4.0	4.0

[a] Treatments: 35CAE includes 300 g/kg CAE and 30CAM includes 200 g/kg CAM.
[b] Arbocel® B800 (JRS; Rosenberg, Germany)
[c] Vitamin and mineral premix supplied per kg feed: 5500 UI of vitamin A; 1100 UI of vitamin D_3; 7 mg of vitamin E; 0.5 mg of vitamin B_1; 1.4 mg of vitamin B_2; 1 mg of vitamin B_6; 8 μg of vitamin B_{12}; 0.5 mg of vitamin K_3; 5.6 mg of calcium panthotenate; 8 mg of nicotinic acid; 120 mg of choline; 80 mg of Fe (from $FeSO_4 \cdot 7\ H_2O$); 0.5 mg of calcium iodide (from $Ca(IO_3)_2$); 0.4 mg of cobalt (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$); 5 mg of copper sulfate (from $CuSO_4 \cdot 5\ H_2O$); 5 mg of Copper (from aminoacids quelate); 40 mg of manganese oxide (from MnO); 100 mg of zinc oxide (from ZnO); 0.25 mg of sodium selenite (from Na_2SeO_3).

Table 6
Analyses of nutrient composition (g/kg, dry matter basis unless otherwise indicated) of experimental diets containing camelina expellers (CAE) or camelina meal (CAM), and of the N-free diet (Exp. 2)[a].

Item, g/kg	Diet[b]		
	35CAE	30CAM	N-Free
Dry matter	92.0	91.1	91.0
Gross energy, MJ/kg	18.3	17.9	17.0
Ash	5.54	5.46	4.97
Ether extract	5.19	2.96	4.55
Crude protein	13.0	12.3	0.88
NDF[c]	12.1	10.3	3.62
ADF[d]	5.48	4.38	2.48
ADL[e]	1.78	1.06	0.77
Titanium dioxide	4.76	5.07	4.55
Indispensable amino acids			
Arginine	9.59	8.81	0.276
Isoleucine	4.85	4.35	0.269
Leucine	7.97	7.41	0.345
Lysine	7.95	6.11	0.279
Methionine	2.42	2.20	0.163
Phenylalanine	4.84	4.51	0.207
Threonine	4.96	4.47	0.187
Valine	6.54	5.90	0.252
Dispensable amino acids			
Alanine	5.73	4.91	0.257
Aspartic acid	15.5	10.8	0.424
Cysteine	3.13	2.92	0.072
Glutamic acid	27.1	22.6	0.856
Glycine	6.93	6.11	0.305
Proline	6.34	6.10	0.262
Serine	6.70	5.83	0.329
Tyrosine	2.44	2.08	0.264

[a] All parameters were analyzed in duplicate, except titanium dioxide that was analyzed in quadruplicate. The considered acceptable coefficient of variation was 0.5 for dry matter and 2.0–4.0 for the rest of parameters.
[b] Treatments: 35CAE includes 300 g/kg CAE, and 30CAM includes 200 g/kg CAM.
[c] NDF: neutral detergent fiber.
[d] ADF: acid detergent fiber.
[e] ADL: acid detergent lignin.

pig, homogenized and subsampled until analyses. Urine (Exp. 1) and ileal digesta (Exp. 2) samples were freeze-dried before analyses. All samples including camelina samples, feeds, feces, urine and ileal content were ground through a 1-mm screen using a Retch grinder (Retsch ZM 200, GmbH & Co. K. C., Haan, Germany) before analyses. Among the analyses performed, CAE, CAM, diets, feces, and freeze-dried urine and ileal digesta were dried at 103ºC for 24 h to determine laboratory dry matter (DM) content. Samples of CAE and CAM were analyzed for gross energy (GE), ether extract (EE), CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), AA, sugars, starch, minerals, ANF content (allyl-isothiocyanate, tannins, erucic acid, and trypsin inhibitors), non-starch polysaccharides profile (NSP) and minerals (calcium, phosphorus, and phytic acid). Diets were analyzed for GE, ash, CP, EE, NDF, ADF, ADL, AA (only in Exp. 2) and TiO_2 (only in Exp. 2). Feces and urine from Exp. 1 were analyzed for GE, and ileal digesta from Exp. 2 were analyzed for AA and TiO_2.

In all these samples, DM (930.15), ash (923.03), EE (920.39) and total dietary fiber (985.29) were determined according to the Association of Official Analytical Chemists (AOAC, 2000) procedures. Gross energy was determined using an adiabatic oxygen bomb calorimeter (Parr 6400, Parr Instruments Co., Moline, IL, USA). Lyophilized urine was mixed with benzoic acid before GE analysis to make sure that the whole sample was burned. Concentrations of NDF, ADF and ADL were determined sequentially using the filter bag system (Ankom Technology Corp., Macedon, NY, USA) according to Mertens (2002), AOAC procedure 973.187) (2000) and Van Soest et al. (1991), using heat-stable amylase (FAA, Ankom Technology Corp., Macedon, NY, USA), and expressed without residual ash. Total nitrogen (N) was measured by combustion (method 986.06; AOAC, 2000) using Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI, USA) and CP was estimated as N content × 6.25. Total sugars were analyzed according to the method of Yemm and Willis (1954). Amino acids were analyzed after acid hydrolysis with 6 N HCl at 110 ºC for 23 h as described by Liu et al. (1995), using a Waters (Milford, MA, USA) High Performance Liquid Chromatography (HPLC) system consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717, Waters), a fluorescence detector (Mod. 474, Waters) and a temperature control module. Aminobutyric acid was added as internal standard after hydrolyzation. Amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and separated with a C-18 reverse-phase column Waters AcQ. Tag (150 mm × 3.9 mm). Methionine and cysteine were determined separately as methionine sulfone and cysteic acid after performic acid oxidation followed by acid hydrolysis. Tryptophan was not determined. Titanium dioxide was determined according to the methodology proposed by Short et al. (1996) using spectrophotometry. Total P and Ca were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Briefly, a dried and grounded subsample of 3–5 g of each sample was weighed into a porcelain crucible and ashed at 550ºC for 3.5 h in a muffle furnace. Samples were cooled and 4 mL concentrated HCl (37%), 1 mL HNO_3 and 1 mL of Yttrium solution (100 mg/L) was added to 0.1 g-ashed sample. Samples were then filtered through a nylon 0.45 μm filter and the filtered solution was analyzed in the ICP-OES. Phytic acid was analyzed by spectrophotometry according to the method described in Haug and Lantzch (1983). Allyl isothiocyanate, tannins, erucic acid and trypsin inhibitor content in camelina co-products were analyzed using distillation and valoration (BOE 2/03/95; allyl isothiocyanate), Ultraviolet–visible Spectroscopy (UV–VIS, tannins), Gas Chromatography (UNE-EN ISO5508–1990; erucic acid) and spectrophotometry (trypsin inhibitor). The NSP was determined using Gas Chromatography, according to the methodology proposed by Englyst et al. (1994). All analyses were performed in duplicate, except for AA in the ileal digesta that were analyzed in single samples and TiO_2 in diets that were analysed in quadruplicate.

2.5. Calculations and statistical analyses

In Exp. 1, the CTTAD of energy and energy metabolizability (EM) in experimental diets were calculated according to the total collection method using the following equations:

CTTAD of energy = (energy intake – energy excretion in feces)/energy intake.

and.

EM = (energy intake – energy excretion in feces – energy excretion in urine)/energy intake,

where energy intake and excretion were measured in kcal.

The CTTAD of energy, DE and ME of CAE and CAM were determined according to the difference method and the regression method (Fan and Sauer, 1995; Zhang and Adeola, 2017). The DE and ME of CAE and CAM were calculated using the following equations:

DE (kcal/kg) = [(CTTAD of GE for CAE or CAM, %) × (GE in CAE or CAM, kcal/kg)]/100; and.

ME (kcal/kg) = [(EM for CAE or CAM, %) × (GE in CAE or CAM, kcal/kg)]/100.

In Exp. 2, the CIAD of N and AA in the different experimental diets were calculated using the following equation (Stein et al., 2007):

CIAD = 1 – [(AA concentration in ileal digesta/AA concentration in feed) × (Ti concentration in feed/Ti concentration in ileal digesta],

where AA and Ti concentration is expressed in DM.

Camelina by-products were the sole source of CP and AA in each diet, thus the CIAD of N and AA in each diet represent the CIAD of N and AA in each camelina by-product (Almeida et al., 2013). The basal endogenous losses of CP and AA were determined from pigs fed the N-free diet using the following equation (Stein et al., 2007):

Basal endogenous losses of AA (mg/kg DM intake) = AA concentration in digesta × (Ti concentration in feed/Ti concentration in digesta).

Values for the CISD of CP and AA were calculated by correcting values for the CIAD of CP and AA for the basal endogenous losses of CP and AA using the following equation (Stein et al., 2007):

CISD = CIAD + (Basal endogenous losses of AA (mg/kg DM intake) /AA concentration in digesta).

For statistical analyses, each animal was considered the experimental unit in all variables and statistical significance level was set at $P < 0.05$. Data were subjected to the REG procedure (CTTAD of energy, DE and ME determined according to the regression method;

Exp. 1) and MIXED procedure (Exp. 1 and 2) of SAS (SAS Inst. Inc. Cary, NC) with diet as the main effect, and replication and the interaction between diet and replication as random effects. The CLB statement in SAS was used to determine the 95% confidence levels for the regression coefficients used for estimating the CTTAD of energy, DE and ME of CAE and CAM. The CTTAD of energy, DE and ME of CAE and CAM obtained using the difference procedure was considered not different from those obtained using linear regression if their values were within the 95% confidence interval estimated using linear regression (Jaworski et al., 2016).

3. Results

For any of the traits studied, no effect of trial replication were observed, and no interactions were significant (data not shown). Therefore, only main effects are shown.

3.1. Camelina by-products

The analyzed composition of CAE and CAM demonstrated that CAM had a greater concentration of CP and AA than CAE (Table 1). The most abundant indispensable AA were arginine, leucine, valine and lysine in both ingredients, whereas methionine and histidine were the least abundant AA (Table 2). Both ingredients had a similar fiber content and, as expected, the concentration of EE was less in CAM than in CAE. Both ingredients contained more insoluble fiber than soluble fiber (730–750 g/kg insoluble fiber) but CAE contained more soluble and less insoluble fiber than CAM. However, CAM had a lower concentration of allyl-isothiocyanate compared with CAE and had lower levels of tannins and erucic acid expressed as percent of total fat, compared with CAE. In contrast, CAM had a greater concentration of trypsin inhibitors compared with CAE.

3.2. Energy measurements (Exp. 1)

Initial BW of animals was not different among treatment groups Table 7). Animals consumed all their daily feed allocation, with the exception of animals from treatment 30CAM. Leftovers in this treatment averaged 5.4% of the total amount of feed offered. The CTTAD of energy and EM decreased linearly ($P < 0.05$) with the inclusion of CAE or CAM in the diets (Table 7). Table 8 shows a comparison between the CTTAD of energy, DE and ME of CAE and CAM calculated by the difference (average of all the diets) and by the regression methods. When estimated via the difference procedure, the CTTAD of energy in CAE and CAM calculated using each experimental diet independently (10CAE, 20CAE and 30CAE diets and 10CAM and 20CAM diets, data not shown in the table) was not different among diets (0.689, 0.702 and 0.652 for 10CAE, 20CAE, and 30CAE diets, respectively, 0.025 of pooled SEM and 0.676 and 0.654 for 10CAM and 20CAM diets, respectively, 0.027 of pooled SEM). Similarly, the DE and ME of CAE and CAM estimated by the difference method using each of the experimental diets were also not different among diets (DE: 14.5, 14.8 and 13.8 MJ/kg DM for 10CAE, 20CAE and 30CAE diets, respectively, 0.524 of pooled SEM; ME: 14.3, 14.5 and 13.6 MJ/kg DM for 10CAE, 20CAE and 30CAE diets, respectively, $\pm$ 0.514 of pooled SEM; DE: 13.1 and 12.7 MJ/kg DM for 10CAM and 20CAM diets, respectively, 0.529 of pooled SEM; ME: 13.0 MJ/kg DM and 12.3 MJ/kg DM for 10CAM and 20CAM diets, respectively, 0.514 of pooled SEM). In average (average of the 3 and 2 diets containing CAE and CAM, respectively), the mean CTTAD, DE and ME were 0.682 and 0.665, 14.4 and 12.9 MJ/kg DM and 14.2 and 12.7 MJ/kg DM, for CAE and CAM, respectively. When estimated via the regression method, the mean CTTAD, DE and ME were 0.665 and 0.655, 14.1 and 13.2 MJ/kg DM and 14.0 and 13.1 MJ/kg DM, for CAE and CAM, respectively. Between ingredients, the CTTAD of energy was not different between CAE and CAM, but DE and ME was significantly higher in CAE compared with CAM in both methods ($P < 0.05$; except ME in the regression method for which $P = 0.063$). Between methods, the CTTAD of energy, DE and ME in both camelina by-products obtained using the difference procedure were within the 95% confidence intervals obtained for the same variables estimated using linear regression. This indicates that both procedures estimated values that were not different. Table 9 shows the regression equations obtained for estimating the energy content in CAE and CAM. The R^2 obtained was greater than 0.5 in all the cases, thus the effect size was considered medium to high for all the parameters obtained.

Table 7
The effects of including camelina expellers (CAE) or camelina meal (CAM) in diets on initial body weight (BW), coefficient of total tract apparent digestibility of energy (CTTAD), and energy metabolizability in Exp. 1.

Item	Diet[a]							
	Basal diet	10CAE	20CAE	30CAE	10CAM	20CAM	SEM[b]	P-value (diet)
Initial BW, kg	**62.2**	**61.0**	**62.7**	**61.7**	**61.2**	**62.2**	**1.35**	**0.949**
CTTAD of energy[c]	**0.876**	**0.854**	**0.836**	**0.799**	**0.854**	**0.827**	**0.004**	**< 0.001**
Energy metabolizability[c]	**0.869**	**0.846**	**0.829**	**0.792**	**0.848**	**0.820**	**0.004**	**< 0.001**

[a] Treatments: 10CAE, 20CAE, and 30CAE include 100, 200, and 300 g/kg CAE, respectively, and 10CAM and 20CAM include 100 and 200 g/kg CAM, respectively.
[b] SEM: standard error of the mean.
[c] Linear contrast for CAE and CAM diets, respectively ($P < 0.05$).

Table 8
Coefficient of total tract apparent digestibility of energy (CTTAD ± SE), digestible energy (DE, MJ/kg dry matter) and metabolizable energy (ME, MJ/kg dry matter) in camelina expellers (CAE), and camelina meal (CAM) determined with the difference and the regression methods (Exp. 1).

	Method						95% Confidence interval	
	Difference			Regression				
	CAE (n = 18)	**CAM (n = 12)**	**P-value**	**CAE (n = 24)**	**CAM (n = 18)**	**P-value**	**CAE**	**CAM**
CTTAD of energy	**0.682 ± 0.016**	**0.665 ± 0.018**	**0.415**	**0.665 ± 0.013**	**0.655 ± 0.021**	**0.357**	**0.637–0.693**	**0.611–0.699**
DE	**14.4 ± 0.318**	**12.9 ± 0.378**	**0.002**	**14.1 ± 0.293**	**13.2 ± 0.385**	**0.049**	**13.5–14.7**	**12.4–14.0**
ME	**14.2 ± 0.304**	**12.7 ± 0.365**	**0.003**	**14.0 ± 0.286**	**13.1 ± 0.378**	**0.063**	**13.4–14.5**	**12.3–13.9**

[1]The values presented are the mean CTTAD of energy, DE and ME in CAE and CAM calculated using the difference procedure for the 3 and 2 diets containing 100, 200 and 300 g/kg of CAE and 100 and 200 g/kg of CAM, respectively.

Table 9
Regression equations obtained for the estimation of the apparent digestibility of energy (CTTAD), digestible energy (DE, MJ/kg dry matter), and metabolizable energy (ME, MJ/kg dry matter) content in camelina expellers and camelina meal[a].

		SE[b]		P-value			
Dependent variable	Prediction equation	Intercept	Estimate	Intercept	Estimate	R^2	RMSE[c]
Camelina expellers							
CTTAD of energy	**0.213 x + 0.665**	**0.013**	**0.016**	**< 0.001**	**< 0.001**	**0.90**	**0.010**
DE	**1.58 x + 14.1**	**0.293**	**0.347**	**< 0.001**	**< 0.001**	**0.50**	**0.208**
ME	**1.55 x + 14.0**	**0.286**	**0.338**	**< 0.001**	**< 0.001**	**0.50**	**0.202**
Camelina meal							
CTTAD of energy	**0.222 x + 0.655**	**0.021**	**0.023**	**< 0.001**	**< 0.001**	**0.86**	**0.008**
DE	**2.38 x + 13.2**	**0.385**	**0.427**	**< 0.001**	**< 0.001**	**0.67**	**0.153**
ME	**2.40 x + 13.1**	**0.378**	**0.419**	**< 0.001**	**< 0.001**	**0.69**	**0.150**

[a] Data were subjected to linear regression analysis with the percent inclusion of camelina as the independent variable and the CTTAD, DE and ME of the diet as the dependent variable. The regression coefficients indicate the change in the CTTAD, DE and ME of the diets for each percentage point change of camelina included in the diet; therefore, the coefficient multiplied by 100 is equal to the CTTAD, DE and ME in camelina.
[b] SE: standard error.
[c] RMSE: root mean square error.

Table 10
Coefficient of ileal apparent digestibility of crude protein and amino acids in camelina expellers (CAE) and camelina meal (CAM) determined with the direct method (Exp. 2).

Item	Ingredient			
	CAE	CAM	SEM[a]	P-value
Crude protein	**0.52**	**0.61**	**0.020**	**0.008**
Amino acids				
Indispensable				
Arginine	**0.73**	**0.80**	**0.029**	**0.065**
Histidine	**0.71**	**0.78**	**0.034**	**0.180**
Isoleucine	**0.62**	**0.69**	**0.042**	**0.188**
Leucine	**0.66**	**0.76**	**0.034**	**0.033**
Lysine	**0.58**	**0.64**	**0.045**	**0.315**
Methionine	**0.81**	**0.84**	**0.006**	**0.007**
Phenylalanine	**0.67**	**0.76**	**0.039**	**0.099**
Threonine	**0.51**	**0.61**	**0.054**	**0.197**
Valine	**0.61**	**0.70**	**0.040**	**0.096**
Dispensable				
Alanine	**0.57**	**0.65**	**0.043**	**0.177**
Aspartic acid	**0.67**	**0.64**	**0.043**	**0.679**
Cysteine	**0.60**	**0.73**	**0.033**	**0.007**
Glutamic acid	**0.74**	**0.80**	**0.031**	**0.151**
Glycine	**0.36**	**0.48**	**0.086**	**0.296**
Proline	**0.53**	**0.51**	**0.059**	**0.797**
Serine	**0.54**	**0.63**	**0.048**	**0.191**
Tyrosine	**0.47**	**0.56**	**0.064**	**0.291**

[a]SEM: standard error of the mean

3.3. Ileal digestibility of amino acids (Exp. 2)

At the start of the experiment, the average BW of pigs was 82.1, 82.1 and 81.5 (± 1.143) kg in treatments 35CAE, 30CAM and N-free, respectively. All pigs consumed their daily feed allotments with the exception of one pig from treatment 35CAE, which was not able to consume the allotted feed. This pig was removed from the study.

The CIAD and CISD of CP and AA for CAE and CAM are summarized in Tables 10 and 11, respectively. The CIAD and CISD of CP were greater (P = 0.008 and P = 0.007 for CIAD and CISD, respectively) in CAM compared with CAE (0.523 and 0.579, respectively, in CAE and 0.614 and 0.670, respectively, in CAM). The CIAD of AA ranged from 0.359 to 0.809 in CAE and from 0.478 to 0.838 in CAM, and the CISD of AA ranged from 0.406 to 0.873 for CAE and from 0.528 to 0.910 for CAM. The most digestible essential AA in both ingredients were methionine, arginine and histidine. The CIAD of leucine, methionine and cysteine was greater (P < 0.05) in CAM compared with CAE, and the CISD of leucin and cysteine was greater (P < 0.05) in CAM compared with CAE. For the remaining AA, no differences between the two ingredients were observed.

4. Discussion

The CP of the CAE used in the present study is in agreement with values reported by Kahindi et al. (2014), Pekel at al. (2015) and Adhikari et al. (2016), but greater than values reported by Almeida et al. (2013) for different CAE sources. The amount of EE and NDF in CAE ranged from 120 to 200 g/kg DM and from 237 to 433 g/kg DM, respectively, in the above-mentioned studies. Values reported in our experiment for EE and NDF in CAE were within the range of these previous works. The AA profile of the CAE used in this study was also in agreement with previous values (Almeida et al., 2013; Kahindi et al., 2014; Pekel et al., 2015). Among the indispensable AA, arginine, leucine, valine and lysine were the most abundant AA in CAE, which is also in agreement with the studies by Almeida et al. (2013), Kahindi et al. (2014) and Pekel et al. (2015).

To the authors' knowledge, the chemical composition of CAM has not been previously reported. As expected, due to its lower EE content, CAM had a greater CP and AA content compared with CAE, but the AA profile was not different between the two ingredients. To our knowledge, no published data for the NSP content of camelina by-products is available in the literature. Pekel et al. (2015) reported increases in jejunal viscosity in broilers fed increasing CAM in the diet (from 0 to 200 g/kg inclusion level), associating it with the soluble NSP content in camelina by-products. The NSP content in canola meal, which is also a member of the *Brassica* family, ranged from 160 to 180 g/kg of which 15 g/kg (8–9% of the total NSP) was soluble NSP (Bell, 1993; Kocher et al., 2000). The camelina co-products used in the present experiment contained more total NSP (250–260 g/kg) than canola meal, with soluble NSP being 27.4 and. 25.2% of total NSP in CAE and CAM, respectively. The high concentration of soluble NSP may reduce voluntary feed intake and nutrient digestibility because soluble NSP increases digesta viscosity (Johansen et al., 1996), which may reduce absorption of nutrients (Agyekum and Nyachoti, 2017). The reason why the soluble NPS content for CAE was greater than in CAM is unknown, but the different processing conditions used to produce CAE and CAM may impact the solubility of fiber in the two ingredients. However, more research is needed to determine the impact of processing on solubility of fiber.

Glucosinolates are one of the major ANF in camelina by-products and they may limit feed intake, growth and dietary nutrient utilization (Woyengo et al., 2017). Total glucosinolate content in CAE usually range from 34.4 to 36.3 mol/g, although their profile is different from that of rapeseed meal. Glucosinolates from camelina are predominantly glucocamelina, whose metabolites may be protective against cancer and cardiovascular disease (Meadus et al., 2014). In addition to glucosinolates, camelina also contains the monounsaturated omega-9 fatty acid, erucic acid (C20:1 w-9), which is suspected to reduce the palatability of feed and induce myocardial lipidosis in monogastrics (Habeanu et al., 2011). Due to its potential toxic effects, the European Commission (Comission Regulation (EU) No 1275/2013 of 6 December 2013 amending Annex I to Directive 2002/32/EC) sets the allowable amount of allyl-isothiocyanate (mustard oil) in camelina seed and derivates at 4000 ppm. The allyl-isothiocyanate content in both camelina by-products used in the present study was much lower than the maximum level allowed by the European Commission. In the present study, the allyl-isothiocyanate content was greater in CAE compared with CAM, which can be attributed to the fact that solvent-extracted co-products are toasted after oil extraction to remove residual solvent, leading to a loss of some glucosinolates in CAM (Newkirk and Classen, 2002). Concentrations of erucic acid level in CAE and CAM were within reported value for camelina oil from spring genotypes (around 30 g/kg of the total fat content; Kurasiak-Popowska et al., 2020), which can be different from that of the summer genotypes. This level was slightly greater than the mean value expected for low glucosinolate and low erucic acid canola (Mejicanos et al., 2016).

Trypsin inhibitors occur naturally in plant seeds and bind to the pancreatic digestive enzymes, trypsin and chymotrypsin, resulting in increased endogenous losses and reduced digestion of AA (Schulze, 1994; Jezierny et al., 2010) and reduced feed intake (Woyengo et al., 2017) in pigs. Trypsin inhibitors in the CAE and CAM used in the present study were low (<7 TIU/g) and the final maximum concentration in diets was 1.3 TIU/g in average, which is below the concentration (3.0 TIU/mg) believed to depress feed intake of pigs (Woyengo et al., 2017).

To the best of authors' knowledge, data on the nutritional value of CAE for pigs have been reported from only a few experiments (Almeida et al., 2013; Kahindi et al., 2014; Adhikari et al., 2016; Kim et al., 2017) and only two experiments reported energy values in CAE (Kahindi et al., 2014; Kim et al., 2017). Additionally, no previous experiments reported in vivo data for the nutritional value of CAM fed to pigs. The CTTAD of energy obtained in the current experiment (from 0.655 to 0.682) for both camelina by-products is considerably lower than the value (0.82) reported for CAE by Kahindi et al. (2014). The CAE used in the present experiment had a greater concentration of NDF compared with the CAE used by Kahindi et al. (2014), whereas there were no differences between the two experiments in EE, CP, and GE. It is therefore likely that the difference in NDF could be one of the reasons for the different CTTAD of

Table 11
Coefficient of ileal standardized digestibility of crude protein and amino acids in camelina expellers (CAE) and camelina meal (CAM) determined with the direct method (Exp. 2).[a].

Item	Ingredient			
	CAE	CAM	SEM[b]	P-value
Crude protein	**0.58**	**0.67**	**0.020**	**0.007**
Amino acids				
Indispensable				
Arginine	**0.76**	**0.84**	**0.031**	**0.069**
Histidine	**0.76**	**0.83**	**0.037**	**0.193**
Isoleucine	**0.66**	**0.74**	**0.044**	**0.208**
Leucine	**0.71**	**0.82**	**0.037**	**0.039**
Lysine	**0.61**	**0.68**	**0.046**	**0.304**
Methionine	**0.87**	**0.90**	**0.009**	**0.035**
Phenylalanine	**0.71**	**0.81**	**0.043**	**0.112**
Threonine	**0.57**	**0.67**	**0.057**	**0.225**
Valine	**0.65**	**0.75**	**0.042**	**0.107**
Dispensable				
Alanine	**0.62**	**0.70**	**0.045**	**0.187**
Aspartic acid	**0.72**	**0.69**	**0.045**	**0.675**
Cysteine	**0.64**	**0.78**	**0.034**	**0.007**
Glutamic acid	**0.78**	**0.85**	**0.033**	**0.158**
Glycine	**0.41**	**0.53**	**0.090**	**0.302**
Proline	**0.64**	**0.59**	**0.066**	**0.576**
Serine	**0.53**	**0.68**	**0.050**	**0.203**
Tyrosine	**0.49**	**0.59**	**0.065**	**0.299**

[a]The basal ileal endogenous losses (g/kg dry matter intake) are: 9.62 for crude protein, 0.239 for arginine, 0.116 for histidine, 0.224 for isoleucine, 0.360 for leucine, 0.316 for lysine, 0.080 for methionine, 0.199 for phenylalanine, 0.378 for threonine, 0.311 for valine, 0.325 for alanine, 0.655 for aspartic acid, 0.133 for cysteine, 0.729 for glutamic acid, 0.572 for glycine, 0.803 for proline, 0.418 for serine, 0.079 for tyrosine.
[b]SEM: standard error of the mean

energy, but not the only one. Other factors such as the ANF content or the age of the animals, among others, could also affect the energy value of an ingredient. Greater DE and ME values for CAE (16.9 and 15.6 MJ/kg DM, respectively) compared with the present experiment were also reported by Kim et al. (2017) who used a source of CAE that had a greater concentration of NDF, but also a greater concentration of EE, than the CAE used in this experiment. Therefore, although the level of fiber can be the factor determining the CTTAD of energy of camelina by-products, a combination of the amount of other nutrients such as EE and CP and the proportion of soluble NSP affects the energy value. Although the CTTAD of energy in CAM was not different from that in CAE, DE and ME in CAM were less than in CAE, which is likely a result of the lower content of EE. The DE and ME of CAM and CAE determined in this experiment were lower than those reported for canola and rapeseed expellers and canola and rapeseed meals (FEDNA, 2019; Maison et al., 2015; Woyengo et al., 2016). When compared with soybean meal (44% CP; DE: 15.7 and ME: 14.6 MJ/kg DM), which is still the main protein source in feeds, the DE and ME of camelina by-products were also lower (FEDNA, 2019). Differences among ingredients in the chemical composition and in concentrations of ANF might explain these differences.

The CIAD and CISD of CP (0.52 and 0.58, respectively) in CAE determined in the present study were slightly lower than values previously reported (Almeida et al., 2013; Kahindi et al., 2014). For the individual AA, our results for CAE are in agreement with those by Almeida et al. (2013) with methionine and arginine being the indispensable AA with the greatest CIAD and CISD and glutamic acid being the dispensable AA with the greatest CIAD and CISD. Kahindi et al. (2014) reported lower digestibility values for all AA compared with those observed in the current experiment for CAE. Thus, AA digestibility can vary among camelina sources, as is also the case for most other feed ingredients (Maison and Stein, 2014) but also differences in the methods of calculating AA digestibility values and estimating the basal endogenous losses of AA can affect CIAD and CISD values for AA (Stein et al., 2007). The difference method was used by Kahindi et al. (2014) to estimate CP and AA digestibility, whereas in the current experiment and that of Almeida et al. (2013), the direct method was used. The direct method has been widely used in digestibility experiments for a wide variety of feed ingredients. However, with this method, the calculated CIAD of a feed ingredient is dependent on the CP level in the diet, and with CP levels lower than 14–16% the digestibility coefficients may be underestimated (Stein, 2003). In the present study, although protein levels of diets were near these values, this can be considered as a potential weakness of our dataset. Nevertheless, as suggested by Stein (2003), the effect of the dietary CP content is removed when CIAD is corrected by endogenous losses. The method for calculating or applying ileal basal endogenous losses can be also different among studies and a source of variability on the results. This was also different among the current experiment and those of Almeida et al. (2013) and Kahindi et al. (2014). In the current experiment and that of Almeida et al. (2013), the CISD of CP and AA was estimated based on basal ileal endogenous N and AA losses from pigs fed N-free diet in the same experiments, and the results were similar. In contrast, Kahindi et al. (2014) estimated an average basal ileal endogenous N and AA losses from other experiments.

The observation that the CISD of leucine, threonine, and cysteine were greater in CAM than in CAE indicates that the thermal treatment applied during production of CAM did not affect AA digestibility. However, it is possible that the greater concentration of soluble fiber and glucosinolates in CAE than in CAM negatively affected the CISD of AA in CAE.

Taking into account other protein sources commonly used in pigs, arginine, methionine, and glutamic acid were also the most digestible AA in canola co-products fed to pigs (Maison and Stein, 2014; Woyengo et al., 2016). In contrast, lysine is one of the most digestible AA in soybean meal (FEDNA, 2019). Compared with camelina by-products, the digestibility of methionine is similar or greater than that found in canola and soybean meal (around 0.80–0.90) and the CSID of isoleucine, valine and arginine was higher in camelina by-products compared with canola, but lower compared with soybean meal. According with the results of the present study, FEDNA (2019) also reported a lower AA digestibility in canola expellers compared with canola meal.

5. Conclusions

The two camelina co-products tested in this investigation had a low energy digestibility and content, but high AA digestibility compared with other camelina co-products. Camelina expellers had greater DE and ME than camelina meal, whereas the digestibility of some AA was less in camelina expellers than in camelina meal. It is possible that the greater concentration of soluble dietary fiber negatively affected AA digestibility in camelina expellers. Compared with other common protein sources such as canola or soybean meal, the energy content of camelina by-products was also lower, but the digestibility of specific AA such as methionine, isoleucine, valine and arginine can be comparable. Thus, more research is needed to elucidate the main factors affecting the nutritional value of camelina co-products fed to pigs.

CRediT authorship contribution statement

A. Cerisuelo: Conceptualization, Methodology, Data curation, Writing- Original draft preparation, Investigation, Supervision, Reviewing and Editing. **P. Ferrer:** Methodology and Investigation. **E.A. Gómez:** Data curation and Reviewing and Editing. **T. Woyengo:** Conceptualization, Methodology, Reviewing and Editing. **H.H. Stein:** Conceptualization, Methodology and Reviewing and Editing. **M. Martínez:** Methodology and Investigation. **J.L. Cano:** Conceptualization and Reviewing. **O. Piquer:** Conceptualization, Methodology, Data curation, Investigation, Reviewing and Editing.

Declaration of Competing Interest

All authors declare that there are no conflicts of interest concerning the information provided in this paper.

Acknowledgements

Financial support from the Spanish Ministry of Economy and Competitiveness, and the European Regional Development Fund (Research Project RTC-2015–265-5) is acknowledged. Also, thanks are expressed to Camelina Company España for their technical support.

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