

Scotland's Rural College

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Effects of rapeseed variety and oil extraction method on the content and ileal digestibility of crude protein and amino acids in rapeseed cake and softly processed rapeseed meal fed to broiler chickens

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## 27 Highlights

- 28 • Thirteen varieties of rapeseed were de-oiled by hexane extraction and cold-pressing.
- 29 • Twelve soft rapeseed meal (SRSM) and four rapeseed cakes (RSC) were fed to
- 30 chickens.
- 31 • Content of crude protein (CP) and amino acids (AA) varied depending on RSC and
- 32 SRSM.
- 33 • Digestibility of CP and AA depended on a rapeseed variety and processing method.

34 **Abstract**

35

36 We examined the effects of rapeseed variety and oil extraction method on crude protein  
37 (CP) and amino acid (AA) content in rapeseed co-products, and determined their coefficient  
38 of apparent (AID) and standardised ileal digestibility (SID) in broiler chickens. Sixteen  
39 rapeseed samples were de-oiled; four were cold-pressed producing rapeseed cake (RSC)  
40 and twelve were mild processed and hexane-extracted producing soft rapeseed meal  
41 (SRSM). One batch of the variety Compass, grown on the same farm, was processed using  
42 both methods obtaining Compass RSC and Compass SRSM. DK Cabernet rapeseed  
43 variety, grown on three different farms, was used to produce two SRSM batches and one  
44 RSC batch. All rapeseed co-products were ground through a 4 mm screen and mixed into  
45 semi-synthetic diets at a level of 500 g/kg. Day-old Ross 308 male broilers were fed a  
46 commercial diet for 14 days. A total of 96 pairs of birds were then allotted to 1 of 16 dietary  
47 treatments (n=6) and fed a test diet for 8 days. Birds were then culled allowing removal of  
48 ileal digesta from Meckel's diverticulum to the ileal-caecal junction. Digestibility of CP and  
49 AA was determined using titanium dioxide as an inert marker. The SRSM samples had an  
50 increased content of CP (419 to 560 g/kg DM) compared to RSC samples (293 to 340 g/kg  
51 DM). Both AID and SID of lysine, and SID of arginine, histidine and threonine were greater  
52 in Compass RSC compared to its SRSM counterpart ( $P<0.05$ ). However, AID and SID of AA  
53 did not differ in both DK Cabernet SRSM, cultivated in two different farms ( $P>0.05$ ). The SID  
54 of lysine was on average 0.03 units greater ( $P<0.001$ ) in RSC than in SRSM. The SRSM

55 produced from variety PR46W21 showed similar or greater AID and SID of individual AA  
56 than the RSC from four other rapeseed varieties. It is concluded that selection of rapeseed  
57 varieties and extraction method have a potential to deliver high protein dietary ingredients  
58 with a good digestibility value.

59 *Keywords:* digestibility, broiler, rapeseed cake, rapeseed meal, amino acid.

60 *Abbreviations:* AA, amino acid; AID, coefficient of apparent ileal digestibility; Arg, arginine; *B.*

61 *napus*, *Brassica napus*; CP, crude protein; DM, dry matter; DMI, dry matter intake; FI, feed

62 intake; GLS, glucosinolates; His, histidine; ; IAAL<sub>B</sub>, basal ileal endogenous amino acid

63 losses; Ile, isoleucine; Leu, leucine; Lys, lysine; Lys:CP ratio; M+C, methionine and cysteine;

64 NDF, neutral detergent fibre; Phe, phenylalanine; RSC, rapeseed cake; RSE, rapeseed

65 expeller; RSM, rapeseed meal; SBM, soybean meal; SEM; standard error of the difference

66 mean; SID, coefficient of standardised ileal digestibility; SRSM, soft rapeseed meal; TAA, total

67 amino acids; Val, valine.

68

## 69 **1. Introduction**

70

71 The strong dependence of the British livestock sector on imported protein-rich feeds  
72 such as soybean meal (SBM), is prompting investigations into the nutritional value of home-  
73 grown protein alternatives for animal production. As the European Union is the greatest  
74 producer of *Brassica napus* (*B. napus*) rapeseed worldwide (USDA, 2015), rapeseed co-  
75 products are of considerable interest as a protein source in animal diets. Compared to SBM,  
76 rapeseed meal (RSM) contains considerably less lysine but more sulphur-containing amino  
77 acids (AA) (Khajali and Slominski, 2012). The indices for the quality of rapeseed protein may  
78 be as high as those of animal protein (e.g. eggs) and far higher than those of other legume  
79 or cereal sources (e.g. peas and wheat, respectively) with a high content of indispensable  
80 AA (Thompson et al., 1982; Friedman, 1996).

81 Rapeseed traditionally contains high contents of erucic acid, glucosinolates and fibre, but  
82 plant breeding improvement has delivered varieties of *B. napus* with low levels of erucic acid  
83 (<20 g/kg) and glucosinolates (<30  $\mu\text{mol/g}$ ) in defatted co-products in recent decades  
84 (Maison and Stein, 2014). These varieties are called “double-low” or “double zero” rapeseed  
85 in Europe, and “canola” in Australia and North America (Newkirk, 2009).

86 Rapeseed co-products are currently used as a protein ingredient in animal diets;  
87 however the nutritional value, measured by protein digestibility, varies and is often reported  
88 as being lower than that of SBM (Adedokun et al., 2008). The low digestibility of protein in  
89 rapeseed has been associated with components such as enzyme inhibitors, phenolic  
90 compounds, glucosinolates and dietary fibre (Rayner and Fox, 1976; Bell, 1993). Moreover,  
91 the nutritional value of rapeseed protein is influenced by many different factors that are  
92 closely related to the concentration of components and the processing technology employed.  
93 The concentration of components in rapeseed co-products (e.g. protein, fibre and oil) might  
94 differ considerably depending on the seed cultivars, growing conditions, harvesting time,  
95 seed storage conditions, seed drying temperature and further processing such as de-hulling,  
96 heat treatment, oil removal method and pelleting (Bell, 1993; Newkirk et al., 2003a, Liu et al.  
97 2014).

98 Rapeseed co-products are commercially produced using two main de-oiling methods:  
99 hexane extraction producing RSM and cold-pressing producing rapeseed cake (RSC).  
100 Hexane extraction involves processing at a high temperature (up to 130 °C) that supports  
101 greater extraction of the oil and results in a RSM with less than 50 g residual oil/kg  
102 (Woyengo et al. 2010; personal communication, Patrick Carre). Cold-pressing involves  
103 crushing of rapeseeds without additional heat supply, delivering a virgin oil and co-products  
104 with a high residual oil content (>170 g/kg) (Leming and Lember, 2005). The majority of the  
105 crop is crushed, heat treated and then hexane extracted in large industrial complexes,

106 whereas a small proportion of the crop is processed by cold-pressing, mainly on farms by  
107 growers or small to medium enterprises.

108 Mixed varieties of rapeseed are often collected and processed by hexane extraction,  
109 which produces rapeseed co-products with potentially differing AA and crude protein (CP)  
110 digestibility. Thus, commercially available rapeseed co-products vary in digestibility of AA  
111 and CP due to the variation depending on rapeseed co-product origin including cultivar and  
112 processing, but also on the level of substitution of RSM/RSC into a diet as well as animal  
113 species tested (Zhou et al., 2013; Qaisrani et al., 2014). Therefore, a lack of consistency in  
114 selection of rapeseed varieties leads to difficulties in estimation of nutritional value of rapeseed co-  
115 products in animal diets.

116 A recent investigation at a rapeseed pilot plant (CREOL, Pessac, France) showed that  
117 decreasing the residence time (RT) in the desolventiser/toaster during the hexane extraction  
118 led to production of RSM with a greater content and digestibility of lysine, measured in pigs  
119 (Eklund et al. 2015). The reduction of heat treatment in rapeseed processing has the  
120 potential to improve digestibility of AA in the final co-products. The aim of the present study  
121 was to compare the effects of soft processing by hexane extraction or cold pressing of  
122 Western rapeseed varieties on content and digestibility of CP and AA in rapeseed co-  
123 products fed to broiler chickens.

124

## 125 **2. Material and methods**

126

### 127 *2.1. Rapeseed co-products and diet formulation*

128 Thirteen varieties of oilseed rape were grown in four South Eastern counties of the  
129 United Kingdom (UK) and harvested in 2013. Seven rapeseed varieties were grown in  
130 Cambridgeshire (Ability, Avatar, DK Cabernet, NK Grandia, PR46W21, Quartz and  
131 Sesame), three in Lincolnshire (Excalibur, Trinity, V2750L), two in Norfolk (Compass and

132 Incentive) and one in Suffolk (Palmedor). Eleven varieties were characterised as double low  
133 varieties, of which ten were winter, and one was spring (Ability). Further diversity was  
134 derived by the inclusion of a single-low, high erucic acid oil variety (Palmedor) and a  
135 relatively new variety with high oleic and low linolenic oil composition with a high  
136 glucosinolate content (V2750L). Twelve rapeseed batches were de-fatted by mild hexane  
137 extraction producing a soft rapeseed meal (SRSM), and four batches were cold-pressed  
138 producing a RSC.

139 The hexane extraction was performed at a pilot plant (CREOL, Pessac, France). Each of  
140 the rapeseed batches was subjected to conditioning. The seeds were dried to a moisture  
141 content of approximately 70 g/kg in a static dryer with movable containers of 1.6 x 1.2 m  
142 surface connected to a warm air generator using air at 70 °C. Unlike standard industrial  
143 processing, the seeds were softly processed by excluding the cooking step before the  
144 pressing and heat supply during the seed crushing. After conditioning, the seeds were cold-  
145 pressed at a rate of 250 kg/h using a MBU 75 press (La Mécanique Moderne, France) with a  
146 gap between pressing each batch 20 min, in order to avoid mixing the varieties. The expeller  
147 meal was then pelletized in 6 mm pellets to prevent possible differences in percolation  
148 during the extraction. Pellets were transferred immediately into the extractor. Continuous  
149 extraction was undertaken in a belt diffuser (Desmet Ballestra, Belgium). The expeller was  
150 leached by a counterflow of hexane in 6 stages. The flow of hexane at 50-55 °C was 230  
151 L/h, resulting in the meal extraction at the rate 140 kg/h (standard deviation, SD: 12 kg/h).  
152 Subsequently, by a semi-continuous mode, the meal was forwarded to the desolventisation  
153 unit using a 6 tray continuous desolventiser (Desmet Ballestra, Belgium). The RT was 80  
154 min for the following rapeseed varieties: Avatar, Compass, Incentive, Palmedor, PR46W21,  
155 Quartz, and DK Cabernet<sup>2</sup>. The variety of Ability, DK Cabernet<sup>1</sup>, V2750L, and Excalibur had  
156 a RT of 65, 86, 90, and 110 min, respectively. Direct steam was injected at 25 kg/h by the  
157 bottom tray with the temperature 102.5 °C (SD: 4.5 °C) to the mass of the de-oiled meal.



158 The cold-pressing was performed at a local plant in Norfolk (UK). The seeds were  
159 crushed at rate of 50 kg/h by a Kern Kraft KK40 press (Egon Keller GmbH, Remscheid,  
160 Germany). The rate of pressing led to an increased temperature of exiting RSC to 55 °C.  
161 The cake was expelled through a 10 mm sieve plate, as pellets.

162 Compass variety grown on one farm was further processed using both methods,  
163 providing the possibility to compare the oil extraction methods without confounding effects of  
164 variety. Furthermore, DK Cabernet had been grown in three different farms in  
165 Cambridgeshire; seeds from two farms were de-fatted by hexane extraction (DK Cabernet  
166 SRSM1 and DK Cabernet SRSM2), whilst DK Cabernet seeds from a third farm were  
167 processed through cold-pressing.

168 The resulting twelve SRSM and four RSC samples were ground using a Pulverisette 15  
169 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany) fitted with a 4 mm screen. Then, they  
170 were added at one inclusion rate (500 g/kg) into a semi-synthetic diet consisting of wheat  
171 starch, glucose, vitamin and minerals, rapeseed oil and titanium dioxide (Table 1). The diets  
172 were mixed in a commercial planetary dough mixer.

173

## 174 *2.2. Animal study*

175 A total of 192 day-old male Ross 308 broilers were obtained from a British designated  
176 breeder (PD Hook Hatcheries Ltd., Thirsk, UK) and housed in the Animal Facility at the  
177 School of Biosciences, University of Nottingham. Birds were housed in pairs, in cages of 37  
178 cm wide, 42 cm tall and 30 cm deep, containing a roost. The animal experiment was  
179 conducted according to protocols approved by Ethical Review Committee and followed  
180 official guidelines for the care and management of birds.

181 Prior to the trial period, chicks were fed a commercial diet based on wheat and de-hulled  
182 SBM (190 g CP/kg as-fed; Chick Starter Crumb, Dodson and Horrell Ltd., Northamptonshire,  
183 UK) for 14 days. Subsequently, birds were allocated to the sixteen dietary treatments in a

184 randomized complete block design with each treatment replicated six times. Each  
185 experimental diet was allocated to six cages, i.e. 12 birds in total, for eight days. At the end  
186 of the trial, the feed intake (FI) of experimental diets was measured and then all birds were  
187 culled by asphyxiation with carbon dioxide followed by cervical dislocation to confirm death.  
188 The ileal region of the gut was dissected out from the Meckel's diverticulum to the ileo-  
189 caecal junction and the ileal contents of the two birds per cage were pooled and collected  
190 into a plastic screw-top container and immediately frozen at -20 °C until subsequent  
191 analysis.

192

### 193 *2.3. Analysis*

194 Dry matter (DM) for RSC, SRSM and diets was determined in duplicate with samples  
195 weighing 60 to 65 g that were dried at 100 °C in a forced air convection oven. Ileal digesta  
196 was frozen and then freeze-dried when determining DM. Dried samples were ground  
197 through a 0.5 mm sieve using a centrifugal mill (ZM200, Retsch GmbH, Germany). The  
198 content of titanium dioxide (TiO<sub>2</sub>) was determined using the method of Short et al. (1996).  
199 The total amino acid (TAA) content in RSC, SRSM and ileal digesta was determined by  
200 hydrolysis of protein, oxidation with performic acid and further neutralisation with sodium  
201 metabisulphite (Llames and Fontaine, 1994). The contents of AA were quantified with the  
202 internal standard method by measuring the absorption of reaction products with ninhydrin.  
203 Total nitrogen (N) was analysed as follows: 5 to 6 mg of RSC, SRSM and ileal digesta were  
204 weighed in aluminium crucibles and burned in furnaces at 900 °C/1060 °C, using CHNS-O  
205 Analyser (CE Instruments Ltd, UK) (AOAC, 2000). Sulphanilamide (cert. no.: 183407, CE  
206 Instruments Ltd, UK) was used as an internal standard. The content of CP was calculated by  
207 multiplying N by 6.25. Neutral detergent fibre (NDF) was assayed with a heat stable amylase  
208 and expressed inclusive of residual ash (EN ISO, 2006). Content of total glucosinolates was

209 determined using high pressure liquid chromatography using sinigrin as an internal standard  
210 (EN ISO, 1994).

211

#### 212 *2.4 Calculations*

213 The lysine:crude protein ratio (Lys:CP) for each batch was calculated by expressing the  
214 concentration of lysine in the sample as a percentage of the CP in the samples (Gonzalez-  
215 Vega et al., 2011).

216 Coefficient of apparent ileal digestibility (AID) of CP and AA in the assay diets was  
217 calculated according to the following equation:

$$218 \quad AID = 1 - \left[ \frac{I_D \times A_I}{A_D \times I_I} \right]$$

219 Where  $I_D$  = marker content in the assay diet (g/kg of DM),  $A_I$  = AA or CP content in ileal  
220 digesta (g/kg of DM),  $A_D$  = AA or CP content in the assay diet (g/kg of DM),  $I_I$  = marker  
221 concentration in ileal digesta (g/kg of DM).

222 Coefficient of standardised ileal digestibility (SID) in the assay diets was calculated  
223 according to the following equation:

$$224 \quad SID = AID + \left[ \frac{IAAL_B}{AA_I} \times 100\% \right]$$

225 Where  $IAAL_B$  = basal ileal endogenous AA losses (g/kg DMI),  $AA_I$  = AA concentration in the  
226 assay diet (g/kg DM). The following  $IAAL_B$  were used; arginine 0.216, histidine 0.209,  
227 isoleucine 0.390, leucine 0.381, lysine 0.255, methionine + cysteine 0.257, phenylalanine  
228 0.237, threonine 0.571 and valine 0.440 g/kg dry matter intake (DMI) (Lemme et al. 2004,  
229 Masey O'Neill et al. 2014).

230

#### 231 *2.5. Statistical analysis*

232 In the randomized design experiment, the digestibility values were tested using one-way  
233 ANOVA with a rapeseed variety set as the treatment, and a digestibility coefficient as Y-

234 variable. An additional set of three contrasts was used to assess differences between 1)  
235 Compass RSC and Compass SRSM, 2) DK Cabernet SRSM1 and DK Cabernet SRSM2,  
236 and 3) RSC and SRSM across all varieties. The relationships between the content of NDF,  
237 glucosinolates and FI and digestibility of CP and AA were analysed by a linear regression  
238 analysis. All statistical analysis was performed using GenStat (15 Edition, VSN International,  
239 Hemel Hempstead, UK). Data were expressed as least squares means with differences  
240 considered statistically significant at  $P < 0.05$ .

241

### 242 **3. Results**

243

#### 244 *3.1. Rapeseed co-products*

245 The chemical composition of RSC and SRSM is shown in Table 2. DK Cabernet SRSM1  
246 and DK Cabernet SRSM2 had similar amounts of CP and a sum of TAA without tryptophan.  
247 Compass SRSM had greater CP and TAA values (468 and 386 g/kg DM) than its RSC  
248 counterpart (293 and 256 g/kg DM). The content of TAA in rapeseed co-products varied  
249 substantially depending on rapeseed varieties; ranging from 256 to 305 g/kg in RSC, and  
250 from 396 to 457 g/kg DM in SRSM, while the content of CP varied from 293 to 340 g/kg in  
251 RSC and from 419 to 560 g/kg DM in SRSM. The average ratio of Lys:CP was lower across  
252 SRSM (5.1%) compared to RSC (5.6%). Similarly, the content of lysine appeared to be  
253 slightly decreased in SRSM, with 4.9% in Compass SRSM compared to 5.2% in Compass  
254 RSC. The soft hexane extraction lowered the content of glucosinolates (7.4  $\mu\text{mol/g DM}$ ) in  
255 Compass SRSM compared to cold-pressed Compass RSC (11.1  $\mu\text{mol/g DM}$ ). All rapeseed  
256 co-products had the content of glucosinolates below 30  $\mu\text{mol/g DM}$ , with the exception of  
257 V2750L SRSM with 47.4  $\mu\text{mol/g DM}$ . The contents of NDF ranged from 226 to 283 and 239  
258 to 251 g/kg DM for SRSM and RSC, respectively.

259 The FI of rapeseed diets varied depending on a rapeseed variety origin. Across the RSC  
260 varieties, the FI was 108, 109, 127 and 131 g as-fed/day for Sesame, NK Grandia, DK  
261 Cabernet and Compass RSC, respectively. Among the SRSM, the FI was 136, 139, 141,  
262 145, 149, 150, 152, 154, 155, 155, 161, 161 g as-fed/day for Excalibur, Incentive, Quartz,  
263 V2750L, Trinity, DK Cabernet SRSM2, DK Cabernet SRSM1, Palmedor, PR46W21,  
264 Compass, Ability and Avatar, respectively.

265

### 266 *3.2. Apparent ileal digestibility*

267 Apparent ileal digestibility coefficients for CP and AA are shown in Table 3. The AID of  
268 all CP and AA was almost identical between DK Cabernet SRSM1 and DK Cabernet  
269 SRSM2. The AID of lysine was greater by 0.04 units in Compass RSC compared to its  
270 SRSM counterpart ( $P=0.002$ ). Within RSC, the AID of CP and AA did not differ markedly  
271 between the varieties used (with the exception of AID of isoleucine). However, AID of CP  
272 and AA in SRSM significantly varied among the varieties, being the greatest for PR46W21  
273 and lowest for Quartz within the SRSM group. Average AID of lysine was greater ( $P<0.001$ )  
274 and AID of valine was smaller ( $P<0.001$ ) for the four sources of RSC compared to twelve  
275 sources of SRSM.

276

### 277 *3.3. Standardised ileal digestibility*

278 Similarly to AID, SID of AA did not differ substantially between DK Cabernet SRSM1 and  
279 DK Cabernet SRSM2 within SRSM group (Table 4). The SID of arginine, histidine, lysine  
280 and threonine was greater by 0.03, 0.04, 0.05 and 0.04 units for Compass RSC compared to  
281 Compass SRSM ( $P<0.05$ ). Standardised ileal digestibility coefficients of all AA were  
282 significantly different among the twelve SRSM varieties, whereas none of SID of AA  
283 changed markedly among the four RSC varieties. Standardised ileal digestibility coefficient  
284 of AA was the greatest in PR46W21 and lowest in Quartz among SRSM varieties ( $P<0.05$ ).

285 The average SID of arginine, histidine, lysine and phenylalanine was greater in RSC  
286 compared to SRSM ( $P < 0.05$ ).

287

288 *3.4. Relationships between the chemical composition, feed intake and digestibility of*  
289 *rapeseed co-products*

290 There was no significant correlation between the content of NDF and digestibility of CP  
291 or AA. Similarly, the content of glucosinolates in the rapeseed co-products did not show any  
292 relationship with AID of CP and AA or SID of AA ( $P > 0.05$ ). However, the content of NDF  
293 showed a positive relationship with feed intake (coefficient of determination,  $r^2 = 0.32$ ,  
294  $P = 0.022$ )

295

#### 296 **4. Discussion**

297

298 Rapeseed co-products contain glucosinolates and NDF, which are anti-nutritional factors  
299 that may reduce FI (Seneviratne et al. 2010, Eklund et al., 2015). Although a high inclusion  
300 of rapeseed co-products was used in diets, we did not observe any negative effect of  
301 glucosinolates or NDF on FI.

302

##### 303 *4.1. Chemical composition*

304 The content of CP and AA (with the exception of methionine and cysteine) was greater in  
305 SRSM and lower in RSC compared to standard processed RSM and rapeseed expellers  
306 (RSE), reported by other researchers. A recent study of Liu et al. (2014) tested low-  
307 temperature processed canola meal (CM-LT), conventional canola meal (CM-CV) and high  
308 temperature processed canola meal (CM-HT) from the conventional prepress solvent  
309 extraction process with desolventiser/toaster temperature for production of CM-LT and CM-  
310 CV of 91-95 °C and for CM-HT of 99-105 °C. The chemical content of CM-HT, CM-LT and

311 CM-CV resulted in a similar characteristics; thus CP was 386-409 g/kg, arginine 21.1-23.6  
312 g/kg, histidine 9.7-10.9 g/kg, leucine 25.8-28.1 g/kg, lysine 20.3-23.3 g/kg or phenylalanine  
313 14.7-15.9 g/kg DM. Similarly, a study of Maison and Stein (2014) that characterised the AA  
314 content of seven canola meals, ten 00-RSM and five 00-RSE indicating no substantial  
315 difference in the composition of indispensable AA among all types of rapeseed co-products  
316 (such as arginine 21.5-23.8 g/kg or lysine 20.7-22.1 g/kg DM).

317 Differences in rapeseed cultivation conditions, oilseed crushing and extraction  
318 procedures influence the content of oil and protein and digestibility of components in the  
319 meals (Bell, 1993; Newkirk et al., 2003a). All rapeseed varieties used in the current study  
320 were grown in similar climatic condition and harvested in the South East of Great Britain.  
321 Thus, DK Cabernet SRSM1 and DK Cabernet SRSM2 resulted in a very similar content of  
322 AA and CP. The influence of variety and environment on the biochemical analysis of  
323 rapeseed co-products in UK were described elsewhere (Kightley et al., 2015).

324 The effect of processing and variety caused substantial changes in the content of CP  
325 and TAA. Both CP and TAA content almost doubled in the Compass SRSM compared to  
326 Compass RSC, as well as mean SRSM vs RSC. Also, the content of NDF increased in  
327 Compass SRSM compared to Compass RSC. These changes were due to a greater  
328 removal of oil during the hexane extraction processing compared to the cold-pressing  
329 (Seneviratne et al. 2011a; 2011b).

330 Besides the increased content of CP and AA, the high temperature of de-oiling process  
331 might reduce the AA content in RSM (Gonzalez-Vega et al., 2011). The heating may lead to  
332 occurrence of the Maillard reaction, which causes binding of the protein-bound lysine and  
333 reducing sugars, and forms deoxyketosyl-lysine derivatives (Hurrell, 1990). Thus, the RT  
334 and temperature of desolventisation might be important factors for the content of AA in the  
335 final co-product.

336 Newkirk et al. (2003b) showed that desolventisation/toasting of canola processed at 110  
337 °C with 150 g moisture/kg caused a significant loss of lysine, averaging 7% and, in the  
338 extreme case, 11.2% in the desolventised/toasted meal compared to non-toasted meal.  
339 Eklund et al. (2015) investigated the increasing residence times of 48, 64, 76, and 93 min in  
340 the desolventiser/toaster with combined application of indirect heat (850 kPa and 140 °C)  
341 and direct unsaturated steam (15 kg/h) injection; it was observed that the content of lysine  
342 linearly decreased from 19.5 to 17.2 g/kg DM as the residence time increased from 48 to 93  
343 min.

344 A more sensitive indicator for the degree of heat damage is the Lys:CP ratio in feed  
345 ingredients (Gonzalez-Vega et al., 2011, Kim et al. 2012). In the current study, we used a  
346 relatively mild processing condition (105 °C) in order to minimise the possibility of overriding  
347 the variety variation across the SRSM. However, the content of lysine appeared to be slightly  
348 decreased in SRSM, indicating a smaller ratio of 4.9% in Compass SRSM compared to 5.2%  
349 in Compass RSC. Similarly, the average ratio of Lys:CP was greater across RSC (5.6%)  
350 compared to SRSM varieties (5.1%). The ratio varied from 4.5 to 5.5% across all SRSM,  
351 indicating that rapeseed variety substantially influences the content of lysine in the rapeseed  
352 co-product.

353 In the present study, the content of glucosinolates varied in rapeseed co-products  
354 depending on the rapeseed variety. It is important to notice that the SRSM variety V2750L  
355 had a high level of glucosinolates (47.4 µmol/g DM), therefore the use of this variety should  
356 be limited in poultry diets.

357 The content of glucosinolates was also affected by the processing method. Thermal  
358 treatment is efficient in deactivating glucosinolates (Jensen et al. 1995). Eklund et al. (2015)  
359 reported that the extension of RT in a toaster leads to glucosinolate reduction up to 6 µmol/g  
360 DM in final RSM. However, along with application of heat treatment in de-oiling, there are



361 also negative effects on measures of protein quality such as the Lys:CP or digestibility of CP  
362 and AA in the rapeseed co-products.

363

#### 364 *4.2. Digestibility*

365

366 Digestibility of CP and AA in RSC and SRSM was in broad agreement with previously  
367 published values in canola meal fed to broiler chickens (Lemme et al., 2004; Woyengo et al.,  
368 2010).

369 Heat treatment during rapeseed processing, along with the glycoproteins associated with  
370 the cell wall structure, might be responsible for a small decrease in AID and SID of CP and  
371 individual AA (such as lysine) in rapeseed co-product-rich diets when fed to broiler chickens  
372 (Khajali and Slominski, 2012). A study of Newkirk et al. (2003a) compared AID of CP and AA  
373 in rapeseed samples collected after various stages of prepress-solvent extraction, and  
374 included canola meal at 400 g/kg DM in broiler diets. The results showed a significant  
375 reduction in AID of CP, lysine and valine by 0.07 units in desolventised/toasted meal  
376 compared to the expeller form. In the current study, SRSM and RSC were added at 500 g/kg  
377 into diets, but such large changes in AID of CP and AA between Compass RSC and SRSM  
378 were not observed. This implies that both type of processing and rapeseed variety influence  
379 the digestibility of individual AA in the rapeseed co-products.

380 Within the hexane extraction method, the digestibility of CP and AA in rapeseed co-  
381 products might also be affected by the RT during the desolventisation process. The oil plants  
382 are obliged to produce the RSM with hexane losses lower than 500 ppm in the final product  
383 that is below of explosivity limit of hexane (Laisney, 1984). In the current study, the RT was  
384 of 80-90 min across most rapeseed varieties. The variations in the RT appeared to be due to  
385 physical differences in seed characteristics including content of oil or hull thickness, which  
386 overall contribute to adequate requirement of RT for each variety in order to remove

387 sufficiently the hexane from the meal (Evrard and Guillaumin, 1983; Cardarelli and Crapiste,  
388 1996). Interestingly, although the RT of Excalibur was almost twice as high as the RT of  
389 Ability, the digestibility of CP and AA for both SRSM was in a good agreement with SRSM of  
390 other varieties.

391 There were significant variations in AID and SID of individual AA due to the effect of  
392 rapeseed variety within SRSM group. As such, PR46W21 SRSM showed the greatest AID of  
393 CP and AA among SRSM group which was as high as, or greater, than digestibility of RSC  
394 from four rapeseed varieties. Thus, the PR46W21 rapeseed variety processed by mild  
395 hexane extraction shows potential for greater rapeseed co-product substitution for SBM in  
396 animal diets.

397 The content of dietary fibre and anti-nutritional factors in rapeseed co-products might be  
398 responsible for the differences in digestibility of AA and CP (Khajali and Slominski, 2012).  
399 The cell wall constituents of rapeseed hull such as pectin, cellulose and hemicellulose may  
400 bind AA released during protein hydrolysis and thereby decreases the AA absorption in the  
401 small intestine (Howard et al 1986, Bjerregaard et al 1991). Grala et al. (1999) reported a  
402 decrease in AID of CP and AA due to the association of protein to the fibre matrix in the  
403 rapeseed hulls diet fed to pigs. Similarly, Eklund et al. (2015) showed a close linear  
404 relationship between SID of CP and AA and the contents of NDF and glucosinolates in RSM  
405 fed to pigs. In contrast to previous studies, we did not observe any negative effect of NDF or  
406 glucosinolates on digestibility of CP and AA in rapeseed co-products fed to broiler chickens.

407 A recent increase in small and medium oil plants focusing on production of high quality  
408 virgin oil (Ghazani et al. 2014), is giving new perspectives to deliver rapeseed co-products  
409 with high quality rapeseed protein – derived from a single rapeseed variety. The present  
410 study showed that the choice of rapeseed variety and processing is important to increase the  
411 content of protein in the co-products as well as deliver a product with a consistent nutritional  
412 value.

413

414 **5. Conclusion**

415

416 The content of AA and CP was substantially changed in rapeseed co-products  
417 depending on the rapeseed variety and processing method used. Although there were some  
418 significant differences in AID and SID of AA between the cold-pressed and soft hexane  
419 extracted co-products, the current study showed that use of mild conditions in hexane  
420 extraction along with selection of the appropriate rapeseed variety (such as PR46W21)  
421 might result in as high as or greater digestibility of AA and CP in SRSM compared to cold-  
422 pressed cake. Thus, selection of rapeseed variety along with soft hexane extraction method  
423 may be beneficial to the feed and livestock industry, as it might create products with greater  
424 nutritional values in terms of CP and AA. Additionally, high digestibility values of AA and CP  
425 in RSC and SRSM suggests there is scope to increase the inclusion of rapeseed co-  
426 products in poultry commercial diets.

427 Conflict of interest statement for manuscript entitled "Effects of rapeseed variety and oil extraction method  
428 on the content and ileal digestibility of crude protein and amino acids in rapeseed cake and softly processed  
429 rapeseed meal fed to broiler chickens"

430

431 On behalf of all authors of this article, I would like to declare that none of the authors has a personal,  
432 financial or other relationship with other people or organisations that could inappropriately affect or  
433 bias the content of the paper.

434

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442

443

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448 **References**

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 559 and growth performance of weaned pigs. *Anim. Feed Sci. Tech.* 179, 112-120.

560

561 Table 1. Dietary formulation

Ingredient	g/kg diet
RSC/SRSM	500
Wheat Starch	200
Glucose (Dextrose)	195
Vitamins and Minerals Premix*	50
Rapeseed Oil	50
Titanium dioxide	5

562 RSC, rapeseed cake; SRSM, soft rapeseed meal.

563 \*Target Feeds, Whitchurch, Shropshire, UK. Content per kg of complete diet: 5 g phosphorous, 0.09 g  
 564 magnesium, 7.5 g calcium, 1.5 g sodium, 0.6 mg copper (as copper sulphate), 160 µg selenium (as  
 565 selenium BCP), 7500 IU vitamin A, 1500 IU vitamin D3, 10 IU vitamin E (as α-tocopherol acetate), 5  
 566 mg vitamin B<sub>1</sub>, 4 mg vitamin B<sub>2</sub>, 4 mg vitamin B<sub>6</sub>, 10 µg vitamin B<sub>12</sub>, 9 mg pantothenic acid, 1.5 mg  
 567 folic acid, 150 µg biotin, 1500 mg choline.

568

569 Table 2. Contents of crude protein, amino acids, neutral detergent fibre and glucosinolates in  
 570 rapeseed cake and soft rapeseed meal (g/kg DM as not stated otherwise)

Variety	D M	N DF	GL S*	C P	TA A	Ar g	Hi s	Ile	Le u	Ly s	M+ C	Ph e	Th r	Va l	Lys:C P**
Rapeseed cake															
Compass	89 9	23 9	11. 1	29 3	25 6	16 .3	7. 2	10 .9	19 .7	15 .3	16. 2	11 .4	12 .6	14 .5	5.2



Sesame	89 0	24 9	20. 5	33 2	29 3	18 .4	8. 6	12 .4	22 .1	18 .3	20. 6	12 .7	13 .9	17 .1	5.5
NK Grandia	89 2	24 0	23. 6	33 5	30 3	19 .6	8. 6	13 .0	22 .3	18 .0	21. 1	12 .9	13 .9	16 .8	5.4
DK Cabernet	88 1	25 1	14. 8	34 0	30 5	19 .2	9. 5	13 .6	23 .1	18 .9	23. 3	12 .8	13 .8	18 .0	5.6
Average	89 0	24 5	17. 5	32 5	28 9	18 .4	8. 5	12 .5	21 .8	17 .6	20. 3	12 .5	13 .5	16 .6	5.6
SEM	3. 6	3. 2	2.8 1	10 .7	11 .4	0. 73	0. 47	0. 57	0. 74	0. 81	1.5 0	0. 35	0. 31	0. 75	0.83
Soft rapeseed meal															
DK Cabernet SRSM1	86 6	27 9	14. 4	41 9	39 6	24 .9	12 .0	18 .7	31 .8	22 .9	27. 8	17 .6	18 .2	25 .0	5.5
DK Cabernet SRSM2	86 4	28 1	12. 7	45 7	41 1	25 .9	12 .2	17 .7	32 .1	24 .0	28. 3	17 .5	19 .5	23 .1	5.2
Quartz	86 6	26 6	10. 0	43 0	40 0	25 .5	11 .9	17 .9	31 .6	23 .6	27. 9	17 .6	19 .1	23 .5	5.5
Trinity	86 8	27 1	8.3 3	44 9	39 8	25 .8	11 .7	18 .3	31 .2	23 .7	28. 7	17 .4	18 .5	23 .9	5.3
Compass	84 8	28 3	7.4 8	46 6	38 6	25 .0	11 .9	16 .8	31 .3	23 .0	24. 5	18 .6	19 .4	23 .2	4.9
Incentive	85 3	22 6	13. 9	46 9	44 0	29 .5	12 .7	20 .8	35 .6	24 .5	28. 0	19 .2	20 .6	27 .0	5.2
Excalibur	83 3	26 0	21. 6	49 5	43 0	27 .7	12 .7	19 .4	33 .7	25 .0	30. 6	18 .9	20 .2	25 .6	5.1
Avatar	85 6	25 5	11. 3	49 5	41 0	26 .1	12 .9	18 .7	32 .9	24 .3	28. 2	19 .3	19 .7	25 .4	4.9
PR46W21	82 2	25 2	25. 8	50 7	45 3	30 .0	13 .7	19 .8	35 .2	27 .4	33. 6	19 .5	21 .0	25 .8	5.4
Palmedor	85 9	26 9	15. 3	51 7	45 1	29 .9	14 .5	20 .9	36 .4	26 .6	30. 8	19 .9	21 .1	27 .8	5.1
V2750L	83 8	27 1	47. 4	52 1	44 4	29 .2	13 .9	20 .9	35 .9	26 .3	30. 5	20 .3	20 .2	27 .9	5.1
Ability	82 1	26 6	14. 2	56 0	45 7	30 .7	14 .0	20 .4	37 .1	25 .1	30. 7	20 .7	21 .1	26 .9	4.5
Average	84 9	26 5	16. 9	48 2	42 3	27 .5	12 .8	19 .2	33 .7	24 .7	29. 1	18 .9	19 .9	25 .4	5.1
SEM	5. 0	4. 5	3.1 6	12 .0	7. 3	0. 64	0. 28	0. 40	0. 63	0. 42	0.6 6	0. 33	0. 28	0. 50	0.84

571 Arg, arginine; CP, crude protein; DM, dry matter; His, histidine; Ile, isoleucine; Leu, leucine; Lys,  
572 lysine; M+C, methionine and cysteine; NDF, neutral detergent fibre; Phe, phenylalanine; SEM,  
573 standard error of the difference mean; TAA, total amino acids; Val, valine; \*GLS, glucosinolates  
574 expressed as  $\mu\text{mol/g DM}$ ; \*\*Lys:CP ratio expressed as %.

575  
576

Table 3. AID of crude protein and amino acids in rapeseed co-products for broiler chickens

Rapeseed variety	CP	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val	TAA
Rapeseed cake											
Compass	0.79	0.89	0.87	0.78 <sub>ab</sub>	0.82	0.82	0.76	0.84	0.73	0.75	0.81
Sesame	0.77	0.89	0.87	0.77 <sub>b</sub>	0.81	0.80	0.76	0.83	0.68	0.72	0.80
NK Grandia	0.80	0.90	0.88	0.82 <sub>a</sub>	0.85	0.84	0.80	0.86	0.74	0.77	0.84

DK Cabernet	0.80	0.89	0.88	0.80 <sub>ab</sub>	0.83	0.82	0.81	0.84	0.71	0.77	0.82
Average	0.79	0.89	0.87	0.79	0.83	0.82	0.78	0.84	0.72	0.75	0.82
SEM	0.018	0.011	0.011	0.020	0.016	0.016	0.030	0.016	0.024	0.023	0.016
p value	0.426	0.387	0.137	0.045	0.150	0.262	0.245	0.307	0.107	0.101	0.154
Soft rapeseed meal											
DK Cabernet SRSM1	0.77 <sub>def</sub>	0.87 <sub>bcd</sub>	0.85 <sub>cd</sub>	0.81 <sub>bc</sub>	0.84 <sub>a</sub>	0.77 <sub>cd</sub>	0.77 <sub>bc</sub>	0.84 <sub>abc</sub>	0.72 <sub>bc</sub>	0.79 <sub>a</sub>	0.80 <sub>bcd</sub>
DK Cabernet SRSM2	0.78 <sub>cd</sub>	0.88 <sub>bc</sub>	0.86 <sub>bc</sub>	0.81 <sub>bc</sub>	0.84 <sub>a</sub>	0.79 <sub>bc</sub>	0.76 <sub>bc</sub>	0.83 <sub>bc</sub>	0.74 <sub>b</sub>	0.78 <sub>b</sub>	0.81 <sub>bc</sub>
Quartz	0.74 <sub>f</sub>	0.85 <sub>d</sub>	0.83 <sub>d</sub>	0.77 <sub>d</sub>	0.81 <sub>d</sub>	0.75 <sub>d</sub>	0.73 <sub>c</sub>	0.81 <sub>c</sub>	0.69 <sub>c</sub>	0.74 <sub>e</sub>	0.77 <sub>d</sub>
Trinity	0.79 <sub>bcde</sub>	0.89 <sub>ab</sub>	0.87 <sub>abc</sub>	0.83 <sub>ab</sub>	0.85 <sub>a</sub>	0.80 <sub>bc</sub>	0.80 <sub>ab</sub>	0.85 <sub>ab</sub>	0.73 <sub>bc</sub>	0.79 <sub>a</sub>	0.82 <sub>ab</sub>
Compass	0.79 <sub>bcde</sub>	0.88 <sub>bc</sub>	0.86 <sub>bc</sub>	0.79 <sub>cd</sub>	0.83 <sub>b</sub>	0.78 <sub>bcd</sub>	0.76 <sub>bc</sub>	0.84 <sub>abc</sub>	0.72 <sub>bc</sub>	0.76 <sub>d</sub>	0.80 <sub>bcd</sub>
Incentive	0.76 <sub>ef</sub>	0.88 <sub>bc</sub>	0.85 <sub>cd</sub>	0.81 <sub>bc</sub>	0.84 <sub>a</sub>	0.78 <sub>bcd</sub>	0.75 <sub>bc</sub>	0.83 <sub>bc</sub>	0.73 <sub>bc</sub>	0.78 <sub>b</sub>	0.80 <sub>bcd</sub>
Excalibur	0.80 <sub>bc</sub>	0.89 <sub>ab</sub>	0.86 <sub>bc</sub>	0.81 <sub>bc</sub>	0.84 <sub>a</sub>	0.80 <sub>bc</sub>	0.77 <sub>bc</sub>	0.84 <sub>abc</sub>	0.75 <sub>ab</sub>	0.79 <sub>a</sub>	0.81 <sub>bc</sub>
Avatar	0.79 <sub>bcde</sub>	0.86 <sub>cd</sub>	0.85 <sub>cd</sub>	0.79 <sub>cd</sub>	0.82 <sub>c</sub>	0.77 <sub>cd</sub>	0.75 <sub>bc</sub>	0.82 <sub>bc</sub>	0.71 <sub>bc</sub>	0.77 <sub>c</sub>	0.78 <sub>cd</sub>
PR46W21	0.84 <sub>a</sub>	0.91 <sub>a</sub>	0.89 <sub>a</sub>	0.85 <sub>a</sub>	0.87 <sub>a</sub>	0.85 <sub>a</sub>	0.83 <sub>a</sub>	0.87 <sub>a</sub>	0.79 <sub>a</sub>	0.82 <sub>a</sub>	0.85 <sub>a</sub>
Palmedor	0.81 <sub>abc</sub>	0.89 <sub>ab</sub>	0.88 <sub>ab</sub>	0.83 <sub>ab</sub>	0.86 <sub>a</sub>	0.81 <sub>b</sub>	0.80 <sub>ab</sub>	0.85 <sub>ab</sub>	0.75 <sub>ab</sub>	0.81 <sub>a</sub>	0.83 <sub>ab</sub>
V2750L	0.81 <sub>abc</sub>	0.89 <sub>ab</sub>	0.87 <sub>abc</sub>	0.83 <sub>ab</sub>	0.85 <sub>a</sub>	0.81 <sub>b</sub>	0.77 <sub>bc</sub>	0.84 <sub>abc</sub>	0.73 <sub>bc</sub>	0.80 <sub>a</sub>	0.82 <sub>ab</sub>
Ability	0.82 <sub>ab</sub>	0.89 <sub>ab</sub>	0.87 <sub>abc</sub>	0.82 <sub>abc</sub>	0.85 <sub>a</sub>	0.80 <sub>bc</sub>	0.79 <sub>ab</sub>	0.85 <sub>ab</sub>	0.74 <sub>b</sub>	0.79 <sub>a</sub>	0.82 <sub>ab</sub>
Average	0.79	0.88	0.86	0.81	0.84	0.79	0.77	0.84	0.73	0.79	0.81
SEM	0.017	0.012	0.012	0.016	0.014	0.017	0.026	0.014	0.020	0.016	0.015
p value	<0.001	<0.001	<0.001	0.001	0.008	<0.001	0.023	0.014	0.003	<0.001	<0.001

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Table 3. AID of crude protein and amino acids in rapeseed co-products for broiler chickens (continued)

Rapeseed variety	CP	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val	TAA
Contrast of Compass RSC with Compass SRSM											
p value	0.738	0.064	0.342	0.230	0.315	0.002	0.862	0.765	0.664	0.114	0.392
SEM	0.014	0.008	0.007	0.010	0.011	0.010	0.040	0.011	0.016	0.010	0.014
Contrast of DK Cabernet SRSM1 with DK Cabernet SRSM2											

p value	0.578	0.620	0.482	0.877	0.846	0.225	0.883	0.933	0.274	0.532	0.454
SEM	0.015	0.011	0.011	0.018	0.014	0.017	0.020	0.014	0.018	0.017	0.014
Contrast of average AID between RSC and SRSM											
p value	0.767	0.003	0.012	0.007	0.022	<0.001	0.339	0.696	0.051	<0.001	0.339
SEM	0.017	0.011	0.012	0.018	0.015	0.017	0.027	0.015	0.021	0.018	0.016

581 AID, coefficient of apparent ileal digestibility; Arg, arginine; CP, crude protein; DM, dry matter; His,  
582 histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; M+C, methionine and cysteine; NDF, neutral  
583 detergent fibre; Phe, phenylalanine; RSC, rapeseed cake; SEM, standard error of the difference  
584 mean; SRSM, soft rapeseed meal; TAA, total amino acids; Val, valine. Values in the same column  
585 followed by different letters are significantly different ( $p < 0.05$ ).  
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Table 4. SID of amino acids in rapeseed co-products for broiler chickens

Rapeseed variety	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val
Rapeseed cake									
Compass	0.92	0.93	0.85	0.86	0.85	0.79	0.88	0.82	0.81
Sesame	0.91	0.91	0.83	0.84	0.83	0.79	0.87	0.76	0.77
NK Grandia	0.93	0.93	0.88	0.88	0.87	0.83	0.90	0.82	0.83
DK Cabernet	0.92	0.92	0.86	0.87	0.85	0.83	0.87	0.80	0.82
Average	0.92	0.92	0.86	0.86	0.85	0.81	0.88	0.80	0.81
SEM	0.011	0.010	0.020	0.016	0.016	0.030	0.016	0.024	0.023
p value	0.451	0.166	0.084	0.174	0.256	0.339	0.319	0.079	0.112
Soft rapeseed meal									
DK Cabernet SRSM1	0.89 <sup>bc</sup>	0.89 <sup>bc</sup>	0.85 <sup>bc</sup> <sub>d</sub>	0.86 <sup>a</sup> <sub>b</sub>	0.79 <sup>cd</sup>	0.78 <sup>bc</sup>	0.86 <sup>ab</sup> <sub>c</sub>	0.78 <sup>b</sup> <sub>c</sub>	0.82 <sup>bc</sup>
DK Cabernet SRSM2	0.89 <sup>bc</sup>	0.90 <sup>ab</sup> <sub>c</sub>	0.85 <sup>bc</sup> <sub>d</sub>	0.87 <sup>a</sup> <sub>b</sub>	0.81 <sup>bc</sup>	0.78 <sup>bc</sup>	0.86 <sup>ab</sup> <sub>c</sub>	0.80 <sup>a</sup> <sub>b</sub>	0.81 <sup>bc</sup> <sub>d</sub>
Quartz	0.86 <sup>d</sup>	0.86 <sup>d</sup>	0.82 <sup>d</sup>	0.84 <sup>b</sup>	0.77 <sup>d</sup>	0.75 <sup>c</sup>	0.83 <sup>c</sup>	0.75 <sup>c</sup>	0.78 <sup>d</sup>
Trinity	0.91 <sup>ab</sup>	0.90 <sup>ab</sup> <sub>c</sub>	0.87 <sup>ab</sup>	0.88 <sup>a</sup>	0.82 <sup>bc</sup>	0.81 <sup>ab</sup>	0.88 <sup>ab</sup>	0.79 <sup>b</sup> <sub>c</sub>	0.83 <sup>ab</sup> <sub>c</sub>
Compass	0.89 <sup>bc</sup>	0.89 <sup>bc</sup>	0.84 <sup>bc</sup> <sub>d</sub>	0.86 <sup>a</sup> <sub>b</sub>	0.80 <sup>bc</sup> <sub>d</sub>	0.78 <sup>bc</sup>	0.87 <sup>ab</sup>	0.78 <sup>b</sup> <sub>c</sub>	0.80 <sup>cd</sup>
Incentive	0.90 <sup>ab</sup>	0.88 <sup>cd</sup>	0.85 <sup>bc</sup> <sub>d</sub>	0.86 <sup>a</sup> <sub>b</sub>	0.80 <sup>bc</sup> <sub>d</sub>	0.77 <sup>bc</sup>	0.86 <sup>ab</sup> <sub>c</sub>	0.78 <sup>b</sup> <sub>c</sub>	0.82 <sup>bc</sup>
Excalibur	0.90 <sup>ab</sup>	0.90 <sup>ab</sup> <sub>c</sub>	0.85 <sup>bc</sup> <sub>d</sub>	0.87 <sup>a</sup> <sub>b</sub>	0.82 <sup>bc</sup>	0.79 <sup>bc</sup>	0.87 <sup>ab</sup>	0.80 <sup>a</sup> <sub>b</sub>	0.83 <sup>ab</sup> <sub>c</sub>
Avatar	0.87 <sup>cd</sup>	0.88 <sup>cd</sup>	0.83 <sup>cd</sup>	0.84 <sup>b</sup>	0.79 <sup>cd</sup>	0.77 <sup>bc</sup>	0.85 <sup>bc</sup>	0.77 <sup>b</sup> <sub>c</sub>	0.80 <sup>cd</sup>
PR46W21	0.92 <sup>a</sup>	0.92 <sup>a</sup>	0.89 <sup>a</sup>	0.89 <sup>a</sup>	0.87 <sup>a</sup>	0.85 <sup>a</sup>	0.89 <sup>a</sup>	0.84 <sup>a</sup>	0.86 <sup>a</sup>
Palmedor	0.91 <sup>ab</sup>	0.91 <sup>ab</sup>	0.87 <sup>ab</sup>	0.88 <sup>a</sup>	0.83 <sup>b</sup>	0.82 <sup>ab</sup>	0.87 <sup>ab</sup>	0.80 <sup>a</sup> <sub>b</sub>	0.84 <sup>ab</sup>

V2750L	0.90 <sup>ab</sup>	0.90 <sup>ab</sup> <sub>c</sub>	0.86 <sup>ab</sup> <sub>c</sub>	0.87 <sup>a</sup> <sub>b</sub>	0.83 <sup>b</sup>	0.79 <sup>bc</sup>	0.87 <sup>ab</sup>	0.79 <sup>b</sup> <sub>c</sub>	0.84 <sup>ab</sup>
Ability	0.90 <sup>ab</sup>	0.90 <sup>ab</sup> <sub>c</sub>	0.85 <sup>bc</sup> <sub>d</sub>	0.87 <sup>a</sup> <sub>b</sub>	0.82 <sup>bc</sup>	0.80 <sup>ab</sup> <sub>c</sub>	0.87 <sup>ab</sup>	0.80 <sup>a</sup> <sub>b</sub>	0.82 <sup>bc</sup>
Average	0.90	0.90	0.85	0.87	0.82	0.80	0.87	0.80	0.82
SEM	0.012	0.012	0.017	0.014	0.017	0.026	0.014	0.020	0.016
p value	<0.001	0.001	0.005	0.014	<0.001	0.034	0.021	0.008	0.003

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Table 4. SID of amino acids in rapeseed co-products for broiler chickens (continued)

Rapeseed variety	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val
Contrast of Compass RSC with Compass SRSM									
p value	0.010	0.002	0.289	0.778	<0.001	0.66	0.27	0.03	0.61
SEM	0.008	0.007	0.010	0.011	0.010	0.04	0.01	0.01	0.01
Contrast of DK Cabernet SRSM1 with DK Cabernet SRSM2									
p value	0.655	0.503	0.972	0.851	0.242	0.87	0.94	0.36	0.64
SEM	0.011	0.011	0.018	0.014	0.017	0.02	0.01	0.01	0.01
Contrast of average SID between RSC and SRSM									
p value	<0.001	<0.001	0.758	0.790	<0.001	0.09	0.01	0.23	0.06
SEM	0.011	0.012	0.018	0.015	0.017	0.02	0.01	0.02	0.01

610 Arg, arginine; CP, crude protein; DM, dry matter; His, histidine; Ile, isoleucine; Leu, leucine; Lys,  
611 lysine; M+C, methionine and cysteine; NDF, neutral detergent fibre; Phe, phenylalanine; RSC,  
612 rapeseed cake; SEM, standard error of the difference mean; SID, coefficient of standardised ileal  
613 digestibility; SRSM, soft rapeseed meal; TAA, total amino acids; Val, valine. Values in the same  
614 column followed by different letters are significantly different ( $p < 0.05$ ).

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