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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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Abstract

We examined the effects of rapeseed variety and oil extraction method on crude protein (CP) and amino acid (AA) content in rapeseed co-products, and determined their coefficient of apparent (AID) and standardised ileal digestibility (SID) in broiler chickens. Sixteen rapeseed samples were de-oiled; four were cold-pressed producing rapeseed cake (RSC) and twelve were mild processed and hexane-extracted producing soft rapeseed meal (SRSM). One batch of the variety Compass, grown on the same farm, was processed using both methods obtaining Compass RSC and Compass SRSM. DK Cabernet rapeseed variety, grown on three different farms, was used to produce two SRSM batches and one RSC batch. All rapeseed co-products were ground through a 4 mm screen and mixed into semi-synthetic diets at a level of 500 g/kg. Day-old Ross 308 male broilers were fed a commercial diet for 14 days. A total of 96 pairs of birds were then allotted to 1 of 16 dietary treatments (n=6) and fed a test diet for 8 days. Birds were then culled allowing removal of ileal digesta from Meckel's diverticulum to the ileal-caecal junction. Digestibility of CP and AA was determined using titanium dioxide as an inert marker. The SRSM samples had an increased content of CP (419 to 560 g/kg DM) compared to RSC samples (293 to 340 g/kg DM). Both AID and SID of lysine, and SID of arginine, histidine and threonine were greater in Compass RSC compared to its SRSM counterpart ($P<0.05$). However, AID and SID of AA did not differ in both DK Cabernet SRSM, cultivated in two different farms ($P>0.05$). The SID of lysine was on average 0.03 units greater ($P<0.001$) in RSC than in SRSM. The SRSM produced from variety PR46W21 showed similar or greater AID and SID of individual AA than the RSC from four other rapeseed varieties. It is concluded that selection of rapeseed varieties and extraction method have a potential to deliver high protein dietary ingredients with a good digestibility value.

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

Abbreviations: AA, amino acid; AID, coefficient of apparent ileal digestibility; Arg, arginine; *B. napus, Brassica napus*; CP, crude protein; DM, dry matter; DMI, dry matter intake; FI, feed intake; GLS, glucosinolates; His, histidine; ; $IAAL_B$, basal ileal endogenous amino acid losses; Ile, isoleucine; Leu, leucine; Lys, lysine; Lys:CP ratio; M+C, methionine and cysteine; NDF, neutral detergent fibre; Phe, phenylalanine; RSC, rapeseed cake; RSE, rapeseed expeller; RSM, rapeseed meal; SBM, soybean meal; SEM; standard error of the difference mean; SID, coefficient of standardised ileal digestibility; SRSM, soft rapeseed meal; TAA, total amino acids; Val, valine.

1. Introduction

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

Rapeseed traditionally contains high contents of erucic acid, glucosinolates and fibre, but plant breeding improvement has delivered varieties of *B. napus* with low levels of erucic acid (<20 g/kg) and glucosinolates (<30 µmol/g) in defatted co-products in the last decades (Maison and Stein, 2014). These varieties are called “double-low” or “double zero” rapeseed in Europe, and “canola” in Australia and North America (Newkirk, 2009).

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

Rapeseed co-products are commercially produced using two main de-oiling methods: hexane extraction producing RSM and cold-pressing producing rapeseed cake (RSC). Hexane extraction involves processing at a high temperature (up to 130 ^{o}C) that provides greater extraction of the oil and results in a RSM with less than 50 g residual oil/kg (Woyengo et al. 2010; personal communication, Patrick Carre). Cold-pressing involves crushing of rapeseeds without additional heat supply, delivering a virgin oil and co-products with a high residual oil content (>170 g/kg) (Leming and Lember, 2005). The majority of the crop is crushed, heat treated and then hexane extracted in large industrial complexes, whereas a small proportion of the crop is processed by cold-pressing, mainly on farms by growers or small to medium enterprises.

Mixed varieties of rapeseed are often collected and processed by hexane extraction, which produces rapeseed co-products with potentially differing AA and crude protein (CP) digestibility. Thus, commercially available rapeseed co-products vary in digestibility of AA and CP due to the variation depending on rapeseed co-product origin including cultivar and processing, but also on the level of substitution

of RSM/RSC into a diet as well as animal species tested (Zhou et al., 2013; Qaisrani et al., 2014). Therefore, a lack of consistency in selection of rapeseed varieties leads to difficulties in estimation of nutritional value of rapeseed co-products in animal diets.

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

2. Material and methods

2.1. Rapeseed co-products and diet formulation

Thirteen varieties of oilseed rape were grown in four counties of UK and harvested in 2013. Seven rapeseed varieties were grown in Cambridgeshire (Ability, Avatar, DK Cabernet, NK Grandia, PR46W21, Quartz and Sesame), three in Lincolnshire (Excalibur, Trinity, V2750L), two in Norfolk (Compass and Incentive) and one in Suffolk (Palmedor). Eleven varieties were characterised as double low varieties, of which ten were winter types, and one was a spring type (Ability). Further diversity was derived by the inclusion of a single-low, high erucic acid oil type (Palmedor) and a relatively new type with high oleic and low linolenic oil composition with a high glucosinolate content (V2750L). Twelve rapeseed batches were de-fatted by mild hexane extraction producing a soft rapeseed meal (SRSM), and four batches were cold-pressed producing a RSC.

The hexane extraction was performed at a pilot plant (CREOL, Pessac, France). Each of the rapeseed batches was subjected to conditioning. The seeds were dried

to a moisture content of approximately 70 g/kg in a static dryer with movable containers of 1.6 x 1.2 m surface connected to a warm air generator using air at 70 °C. Unlike standard industrial processing, the seeds were softly processed by excluding the cooking step before the pressing and heat supply during the seed crushing. After conditioning, the seeds were cold-pressed at a rate of 250 kg/h using a MBU 75 press (La Mécanique Moderne, France) with a gap between pressing each batch 20 min, in order to avoid mixing the varieties. The expeller meal was then pelletized in 6 mm pellets to prevent possible differences in percolation during the extraction. Pellets were transferred immediately into the extractor. Continuous extraction was undertaken in a belt diffuser (Desmet Ballestra, Belgium). The expeller was leached by a counterflow of hexane in 6 stages. The flow of hexane at 50-55 °C was 230 L/h, resulting in the meal extraction at the rate 140 kg/h (standard deviation, SD: 12 kg/h). Subsequently, by a semi-continuous mode, the meal was forwarded to the desolventisation using a 6 tray continuous desolventiser (Desmet Ballestra, Belgium). The RT was 80 min for the following rapeseed varieties: Avatar, Compass, Incentive, Palmedor, PR46W21, Quartz, and DK Cabernet2. The variety of Ability, DK Cabernet1, V2750L, and Excalibur had a RT of 65, 86, 90, and 110 min, respectively. Direct steam was injected at 25 kg/h by the bottom tray with the temperature 102.5 °C (SD: 4.5 °C) to the mass of the de-oiled meal.

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

Compass variety grown on one farm was further processed using both methods, providing the possibility to compare the oil extraction methods without confounding effects of variety. Furthermore, DK Cabernet was grown in three different farms in Cambridgeshire; seeds from two farms were de-fatted by hexane extraction (DK

169 Cabernet SRSM1 and DK Cabernet SRSM2), whilst DK Cabernet seeds from a third
170 farm were processed through cold-pressing.
171 The resulting twelve SRSM and four RSC samples were ground using a
172 Pulverisette 15 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany) fitted with a 4
173 mm screen. Then, they were added at one inclusion rate (500 g/kg) into a semi-
174 synthetic diet consisting of wheat starch, glucose, vitamin and minerals, rapeseed oil
175 and titanium dioxide (Table 1).The diets were mixed in a commercial planetary dough
176 mixer.
177
178 *2.2. Animal study*
179 A total of 192 day-old male Ross 308 broilers were obtained from a British
180 designated breeder (PD Hook Hatcheries Ltd., Thirsk, UK) and housed in the Animal
181 Facility at the School of Biosciences, University of Nottingham. Birds were housed in
182 pairs, in cages of 37 cm wide, 42 cm tall and 30 cm deep, containing a roost. The
183 animal experiment was conducted according to protocols approved by Ethical
184 Review Committee and followed official guidelines for the care and management of
185 birds.
186 Prior to the trial period, chicks were fed a commercial diet based on wheat and
187 de-hulled SBM with content of protein 190 g/kg as-fed (Chick Starter Crumb, Dodson
188 and Horrell Ltd., Northamptonshire, UK) for 14 days. Subsequently, birds were
189 allocated to the sixteen dietary treatments in a randomized complete block design
190 with each treatment replicated six times. Each experimental diet was allocated to six
191 cages, i.e. 12 birds, for eight days. At the end of the trial, the feed intake (FI) of
192 experimental diets was measured and then all birds were culled by asphyxiation with
193 carbon dioxide followed by cervical dislocation to confirm death. The ileal region of
194 the gut was dissected out from the Meckel's diverticulum to the ileo-caecal junction
195 and the ileal contents of the two birds per cage were pooled and collected into a

196 plastic screw-top container and immediately frozen at -20 °C until subsequent
197 analysis.
198
199 *2.3. Analysis*
200 Dry matter (DM) for RSC, SRSM and diets was determined in duplicate samples
201 weighing 60 to 65 g that were dried at 100 ^{o}C in a forced air convection oven. Ileal
202 digesta was frozen and then freeze-dried when determining DM. Dried samples were
203 ground through a 0.5 mm sieve using a centrifugal mill (ZM200, Retsch GmbH,
204 Germany). The content of titanium dioxide (TiO_2) was determined using the method
205 of Short et al. (1996). The content of AA and total amino acid (TAA) in RSC, SRSM
206 and ileal digesta was determined by hydrolysis of protein, oxidisation with performic
207 acid and further neutralisation with sodium metabisulphite (Llames and Fontaine,
208 1994). The contents of AA were quantified with the internal standard method by
209 measuring the absorption of reaction products with ninhydrin. Total nitrogen (N) was
210 analysed as follows: 5 to 6 mg of RSC, SRSM and ileal digesta were weighed in
211 aluminium crucibles and burned in furnaces at 900 °C/1060 °C, using CHNS-O
212 Analyser (CE Instruments Ltd, UK) (AOAC, 2000). Sulphanilamide (cert. no.: 183407,
213 CE Instruments Ltd, UK) was used as an internal standard. The content of CP was
214 calculated by multiplying N by 6.25. Neutral detergent fibre (NDF) was assayed with
215 a heat stable amylase and expressed inclusive of residual ash (EN ISO, 2006).
216 Content of total glucosinolates was determined using high pressure liquid
217 chromatography using sinigrin as an internal standard (EN ISO, 1994).
218
219 *2.4 Calculations*
220 The lysine:crude protein ratio (Lys:CP) for each batch was calculated by
221 expressing the concentration of lysine in the sample as a percentage of the CP in the
222 samples (Gonzalez-Vega et al., 2011).

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

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

225 Where I_D = marker content in the assay diet (g/kg of DM), A_I = AA or CP content in
226 ileal digesta (g/kg of DM), A_D = AA or CP content in the assay diet (g/kg of DM), I_I=
227 marker concentration in ileal digesta (g/kg of DM).
228 Coefficient of standardised ileal digestibility (SID) in the assay diets was
229 calculated according to the following equation:

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

230 Where $IAAL_B$ = basal ileal endogenous AA losses (g/kg DMI), AA_I = AA concentration
231 in the assay diet (g/kg DM). The following $IAAL_B$ were used; arginine 0.216, histidine
232 0.209, isoleucine 0.390, leucine 0.381, lysine 0.255, methionine + cysteine 0.257,
233 phenylalanine 0.237, threonine 0.571 and valine 0.440 g/kg dry matter intake (DMI)
234 (Lemme et al. 2004, Masey O'Neill et al. 2014).
235
236 *2.5. Statistical analysis*
237 In the randomized design experiment, the digestibility values were tested using
238 one-way ANOVA with a rapeseed variety set as the treatment, and a digestibility
239 coefficient as Y-variable. An additional set of three contrasts was used to assess
240 differences between 1) Compass RSC and Compass SRSM, 2) DK Cabernet
241 SRSM1 and DK Cabernet SRSM2, and 3) RSC and SRSM across all varieties. The
242 relationships between the content of NDF, glucosinolates and FI and digestibility of
243 CP and AA were analysed by a linear regression analysis. All statistical analysis was
244 performed using GenStat (15 Edition, VSN International, Hemel Hempstead, UK).
245 Data were expressed as least squares means with differences considered
246 statistically significant at $P<0.05$.

3. Results

3.1. Rapeseed co-products

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

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

3.2. Apparent ileal digestibility

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

3.3. Standardised ileal digestibility

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

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

There was no significant correlation between the content of NDF and digestibility of CP or AA. Similarly, the content of glucosinolates in the rapeseed co-products did not show any relationship with AID of CP and AA or SID of AA (P>0.05). However,

the content of NDF showed a mild positive relationship with feed intake (coefficient of determination, r^2=0.33, P=0.02)

4. Discussion

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

4.1. Chemical composition

The content of CP and AA (with exception of methionine and cysteine) was greater in SRSM and lower in RSC compared to standard processed RSM and rapeseed expellers (RSE), reported by other researchers. A recent study of Liu et al. (2014) tested low-temperature processed canola meal (CM-LT), conventional canola meal (CM-CV) and high temperature processed canola meal (CM-HT) from the conventional prepress solvent extraction process with desolventiser/toaster temperature for production of CM-LT and CM-CV in 91-95 °C and for CM-HT in 99-105 °C. The chemical content of CM-HT, CM-LT and CM-CV resulted in a similar characteristics; such as CP was 386-409 g/kg, arginine 21.1-23.6 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 14.7-15.9 g/kg DM. Similarly, a study of Maison and Stein (2014) that characterised the AA content of seven canola meals, ten 00-RSM and five 00-RSE indicating no substantial difference in the composition of indispensable AA among all types of rapeseed co-products (such arginine 21.5-23.8 g/kg or lysine 20.7-22.1 g/kg DM).

Differences in rapeseed cultivation condition, oilseed crushing and extraction procedures influence the content of oil and protein and digestibility of components in

330 the meals (Bell, 1993; Newkirk et al., 2003a). All rapeseed varieties used in the
331 current study were grown in similar climatic condition and harvested in the South of
332 Great Britain. Thus, DK Cabernet SRSM1 and DK Cabernet SRSM2 resulted in a
333 very similar content of AA and CP. The influence of variety and environment on the
334 biochemical analysis of rapeseed co-products in UK were described elsewhere
335 (Kightley et al., 2015).
336 The effect of processing and variety caused substantial changes in the content of
337 CP and TAA. Both CP and TAA content almost doubled in the Compass SRSM
338 compared to Compass RSC, as well as averaged SRSM vs RSC. Also, the content
339 of NDF increased in Compass SRSM compared to Compass RSC. These changes
340 were due to a greater removal of oil during the hexane extraction processing
341 compared to the cold-pressing (Seneviratne et al. 2011a; 2011b).
342 Besides the increased content of CP and AA, the high temperature of de-oiling
343 process might reduce the AA content in RSM (Gonzalez-Vega et al., 2011). The
344 heating may lead to occurrence of the Maillard reaction, which causes binding of the
345 protein-bound lysine and reducing sugars, and forms deoxyketosyl-lysine derivatives
346 (Hurrell, 1990). Thus, the RT and temperature of desolventisation might be important
347 factors for the content of AA in the final co-product.
348 Newkirk et al. (2003b) showed that desolventisation/toasting of canola processed
349 at 110 °C with 150 g moisture/kg caused a significant loss of lysine, averaging at 7%
350 and in the extreme case at 11.2% in the desolventised/toasted meal compared to
351 non-toasted meal. Eklund et al. (2015) investigated the increasing residence times of
352 48, 64, 76, and 93 min in the desolventiser/toaster with combined application of
353 indirect heat (850 kPa and 140 °C) and direct unsaturated steam (15 kg/h) injection.
354 The authors observed that the content of lysine linearly decreased from 19.5 to 17.2
355 g/kg DM as the residence time increased from 48 to 93 min.
356 A more sensitive indicator for the degree of heat damage is the Lys:CP ratio in
357 feed ingredients, exposed to thermal treatments (Gonzalez-Vega et al., 2011, Kim et

358 al. 2012). In the current study, we used a relatively mild processing condition (105 °C)
359 in order to minimise the possibility of overriding the variety variation across the
360 SRSM. However, the content of lysine appeared to be slightly decreased in SRSM,
361 indicating a smaller ratio of 4.9% in Compass SRSM compared to 5.2% in Compass
362 RSC. Similarly, the average ratio of Lys:CP was greater across RSC (5.6%)
363 compared to SRSM varieties (5.1%). The ratio varied from 4.5 to 5.5% across all
364 SRSM, indicating that rapeseed variety substantially influences the content of lysine
365 in the rapeseed co-product.
366 In the present study, the content of glucosinolates varied in rapeseed co-products
367 depending on the rapeseed variety. It is important to notice that the SRSM variety
368 V2750L had a high level of glucosinolates (47.4 µmol/g DM), therefore the use of this
369 variety should be limited in the utilisation for poultry diets.
370 The content of glucosinolates was also affected by the processing method.
371 Thermal treatment is efficient in deactivating glucosinolates (Jensen et al. 1995).
372 Eklund et al. (2015) reported that the extension of RT in a toaster leads to
373 glucosinolate reduction up to 6 µmol/g DM in final RSM. However, along with
374 application of heat treatment in de-oiling, there are also negative effects on measures
375 of protein quality such as the Lys:CP or digestibility of CP and AA in the rapeseed co-
376 products.
377
378 *4.2. Digestibility*
379
380 Digestibility of CP and AA in RSC and SRSM was in a wide agreement with
381 previously published values in canola meal fed to broiler chickens (Lemme et al.,
382 2004; Woyengo et al., 2010).
383 The heat treatment during the rapeseed processing, along with the glycoproteins
384 associated with the cell wall structure, might be responsible for a small decrease in
385 AID and SID of CP and individual AA (such as lysine) in rapeseed co-product-rich

386 diets when fed to broiler chickens (Khajali and Slominski, 2012). A study of Newkirk
387 et al. (2003a) compared AID of CP and AA in rapeseed samples collected after
388 various stages of prepress-solvent extraction, and included the canola meal at 400
389 g/kg DM in broiler diets. The results showed a significant reduction in AID of CP,
390 lysine and valine by 0.07 in desolventised/toasted meal compared to expelled form.
391 In the current study, SRSM and RSC were added at 500 g/kg into diets, but such
392 large changes in AID of CP and AA between Compass RSC and SRSM were not
393 observed. This implies that both type of processing and rapeseed variety influence
394 the digestibility of individual AA in the rapeseed co-products.
395 Within the hexane extraction method, the digestibility of CP and AA in rapeseed
396 co-products might also be affected by the RT of desolventisation process. The oil
397 plants are obligated to produce the RSM with hexane losses lower than 500 ppm in
398 the final product that is below of explosivity limit of hexane (Laisney, 1984). In the
399 current study, the RT was of 80-90 min across most rapeseed varieties. The
400 variations in the RT appeared due to physical differences in the seeds characteristics
401 including content of oil or hull thickness, which overall contribute to adequate
402 requirement of RT for each variety in order to sufficiently remove the hexane from the
403 meal (Evrard and Guillaumin, 1983; Cardarelli and Crapiste, 1996). Interestingly,
404 although the RT of Excalibur was almost twice as high as the RT of Ability, the
405 digestibility of CP and AA for both SRSM was in a good agreement with SRSM of
406 other varieties.
407 There were significant variations in AID and SID of individual AA due to the effect
408 of rapeseed variety within SRSM group. As such, PR46W21 SRSM showed the
409 greatest AID of CP and AA among SRSM group, which was as high as, or greater
410 than digestibility of RSC from four rapeseed varieties. Thus, the PR46W21 rapeseed
411 variety processed by mild hexane extraction, is showing a potential of greater
412 rapeseed co-product substitution for SBM in animal diets.

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

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

5. Conclusion

The content of AA and CP was substantially changed in rapeseed co-products depending on the rapeseed variety and processing method used. Although there were some significant differences in AID and SID of AA between the cold-pressed and soft hexane extracted co-products, the current study showed that use of mild conditions in hexane extraction along with selection of the appropriate rapeseed variety (such as PR46W21) might result in as high as or greater digestibility of AA and CP in SRSM compared to cold-pressed cake. Thus, the considerably selection of rapeseed variety along with soft hexane extraction method may be beneficial to the

441 feed and livestock industry, as it might create products with greater nutritional values
442 of CP and AA. Additionally, high digestibility values of AA and CP in 500 g
443 RSC/SRSM diets suggest there is a scope to elevate the rapeseed co-products
444 addition in the poultry commercial diet.
445
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449
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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

RSC, rapeseed cake; SRSM, soft rapeseed meal.

*Target Feeds, Whitchurch, Shropshire, UK. Content per kg of complete diet: 5 g phosphorous, 0.09 g magnesium, 7.5 g calcium, 1.5 g sodium, 0.6 mg copper (as copper sulphate), 160 µg selenium (as selenium BCP), 7500 IU vitamin A, 1500 IU vitamin D3, 10 IU vitamin E (as α-tocopherol acetate), 5 mg vitamin B_1, 4 mg vitamin B_2, 4 mg vitamin B_6, 10 µg vitamin B_{12}, 9 mg pantothenic acid, 1.5 mg folic acid, 150 µg biotin, 1500 mg choline.

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

Variety	DM	NDF	GLS*	CP	TAA	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val	Lys:CP**
Rapeseed cake															
Compass	899	239	11.1	293	256	16.3	7.2	10.9	19.7	15.3	16.2	11.4	12.6	14.5	5.2
Sesame	890	249	20.5	332	293	18.4	8.6	12.4	22.1	18.3	20.6	12.7	13.9	17.1	5.5
NK Grandia	892	240	23.6	335	303	19.6	8.6	13.0	22.3	18.0	21.1	12.9	13.9	16.8	5.4
DK Cabernet	881	251	14.8	340	305	19.2	9.5	13.6	23.1	18.9	23.3	12.8	13.8	18.0	5.6
Average	890	245	17.5	325	289	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	2.81	10.7	11.4	0.73	0.47	0.57	0.74	0.81	1.50	0.35	0.31	0.75	0.83
Soft rapeseed meal															
DK Cabernet SRSM1	866	279	14.4	419	396	24.9	12.0	18.7	31.8	22.9	27.8	17.6	18.2	25.0	5.5
DK Cabernet SRSM2	864	281	12.7	457	411	25.9	12.2	17.7	32.1	24.0	28.3	17.5	19.5	23.1	5.2
Quartz	866	266	10.0	430	400	25.5	11.9	17.9	31.6	23.6	27.9	17.6	19.1	23.5	5.5
Trinity	868	271	8.3	443	399	25.8	11.7	18.3	31.2	23.7	28.7	17.4	18.5	23.9	5.3
Compass	848	283	7.4	468	386	25.0	11.9	16.8	31.3	23.0	24.5	18.6	19.4	23.2	4.9
Incentive	853	226	13.9	469	440	29.5	12.7	20.8	35.6	24.5	28.0	19.2	20.6	27.0	5.2
Excalibur	833	260	21.6	495	430	27.7	12.7	19.4	33.7	25.0	30.6	18.9	20.2	25.6	5.1
Avatar	856	255	11.3	495	410	26.1	12.9	18.7	32.9	24.3	28.2	19.3	19.7	25.4	4.9
PR46W21	822	252	25.8	507	453	30.0	13.7	19.8	35.2	27.4	33.6	19.5	21.0	25.8	5.4
Palmedor	859	269	15.3	517	451	29.9	14.5	20.9	36.4	26.6	30.8	19.9	21.1	27.8	5.1
V2750L	838	271	47.4	521	444	29.2	13.9	20.9	35.9	26.3	30.5	20.3	20.2	27.9	5.1
Ability	821	266	14.2	560	457	30.7	14.0	20.4	37.1	25.1	30.7	20.7	21.1	26.9	4.5
Average	849	265	16.9	482	423	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	3.16	12.0	7.3	0.64	0.28	0.40	0.63	0.42	0.66	0.33	0.28	0.50	0.84

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

607 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^{ab}	0.82	0.82	0.76	0.84	0.73	0.75	0.81
Sesame	0.77	0.89	0.87	0.77^{b}	0.81	0.80	0.76	0.83	0.68	0.72	0.80
NK Grandia	0.80	0.90	0.88	0.82^{a}	0.85	0.84	0.80	0.86	0.74	0.77	0.84
DK Cabernet	0.80	0.89	0.88	0.80^{ab}	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^{def}	0.87^{bcd}	0.85^{cd}	0.81^{bc}	0.84^{abcd}	0.77^{cd}	0.77^{bc}	0.84^{abc}	0.72^{bc}	0.79^{abcd}	0.80^{bcd}
DK Cabernet SRSM2	0.78^{cde}	0.88^{bc}	0.86^{bc}	0.81^{bc}	0.84^{abcd}	0.79^{bc}	0.76^{bc}	0.83^{bc}	0.74^{b}	0.78^{bcd}	0.81^{bc}
Quartz	0.74^{f}	0.85^{d}	0.83^{d}	0.77^{d}	0.81^{d}	0.75^{d}	0.73^{c}	0.81^{c}	0.69^{c}	0.74^{e}	0.77^{d}
Trinity	0.79^{bcde}	0.89^{ab}	0.87^{abc}	0.83^{ab}	0.85^{abc}	0.80^{bc}	0.80^{ab}	0.85^{ab}	0.73^{bc}	0.79^{abcd}	0.82^{ab}
Compass	0.79^{bcde}	0.88^{bc}	0.86^{bc}	0.79^{cd}	0.83^{bcd}	0.78^{bcd}	0.76^{bc}	0.84^{abc}	0.72^{bc}	0.76^{de}	0.80^{bcd}
Incentive	0.76^{ef}	0.88^{bc}	0.85^{cd}	0.81^{bc}	0.84^{abcd}	0.78^{bcd}	0.75^{bc}	0.83^{bc}	0.73^{bc}	0.78^{bcd}	0.80^{bcd}
Excalibur	0.80^{bcd}	0.89^{ab}	0.86^{bc}	0.81^{bc}	0.84^{abcd}	0.80^{bc}	0.77^{bc}	0.84^{abc}	0.75^{ab}	0.79^{abcd}	0.81^{bc}
Avatar	0.79^{bcde}	0.86^{cd}	0.85^{cd}	0.79^{cd}	0.82^{cd}	0.77^{cd}	0.75^{bc}	0.82^{bc}	0.71^{bc}	0.77^{cde}	0.78^{cd}
PR46W21	0.84^{a}	0.91^{a}	0.89^{a}	0.85^{a}	0.87^{a}	0.85^{a}	0.83^{a}	0.87^{a}	0.79^{a}	0.82^{a}	0.85^{a}
Palmedor	0.81^{abc}	0.89^{ab}	0.88^{ab}	0.83^{ab}	0.86^{ab}	0.81^{b}	0.80^{ab}	0.85^{ab}	0.75^{ab}	0.81^{ab}	0.83^{ab}
V2750L	0.81^{abc}	0.89^{ab}	0.87^{abc}	0.83^{ab}	0.85^{abc}	0.81^{b}	0.77^{bc}	0.84^{abc}	0.73^{bc}	0.80^{abc}	0.82^{ab}
Ability	0.82^{ab}	0.89^{ab}	0.87^{abc}	0.82^{abc}	0.85^{abc}	0.80^{bc}	0.79^{ab}	0.85^{ab}	0.74^{b}	0.79^{abcd}	0.82^{ab}
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

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

623 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^{bc}	0.89^{bc}	0.85^{bcd}	0.86^{ab}	0.79^{cd}	0.78^{bc}	0.86^{abc}	0.78^{bc}	0.82^{bc}
DK Cabernet SRSM2	0.89^{bc}	0.90^{abc}	0.85^{bcd}	0.87^{ab}	0.81^{bc}	0.78^{bc}	0.86^{abc}	0.80^{ab}	0.81^{bcd}
Quartz	0.86^{d}	0.86^{d}	0.82^{d}	0.84^{b}	0.77^{d}	0.75^{c}	0.83^{c}	0.75^{c}	0.78^{d}
Trinity	0.91^{ab}	0.90^{abc}	0.87^{ab}	0.88^{a}	0.82^{bc}	0.81^{ab}	0.88^{ab}	0.79^{bc}	0.83^{abc}
Compass	0.89^{bc}	0.89^{bc}	0.84^{bcd}	0.86^{ab}	0.80^{bcd}	0.78^{bc}	0.87^{ab}	0.78^{bc}	0.80^{cd}
Incentive	0.90^{ab}	0.88^{cd}	0.85^{bcd}	0.86^{ab}	0.80^{bcd}	0.77^{bc}	0.86^{abc}	0.78^{bc}	0.82^{bc}
Excalibur	0.90^{ab}	0.90^{abc}	0.85^{bcd}	0.87^{ab}	0.82^{bc}	0.79^{bc}	0.87^{ab}	0.80^{ab}	0.83^{abc}
Avatar	0.87^{cd}	0.88^{cd}	0.83^{cd}	0.84^{b}	0.79^{cd}	0.77^{bc}	0.85^{bc}	0.77^{bc}	0.80^{cd}
PR46W21	0.92^{a}	0.92^{a}	0.89^{a}	0.89^{a}	0.87^{a}	0.85^{a}	0.89^{a}	0.84^{a}	0.86^{a}
Palmedor	0.91^{ab}	0.91^{ab}	0.87^{ab}	0.88^{a}	0.83^{b}	0.82^{ab}	0.87^{ab}	0.80^{ab}	0.84^{ab}
V2750L	0.90^{ab}	0.90^{abc}	0.86^{abc}	0.87^{ab}	0.83^{b}	0.79^{bc}	0.87^{ab}	0.79^{bc}	0.84^{ab}
Ability	0.90^{ab}	0.90^{abc}	0.85^{bcd}	0.87^{ab}	0.82^{bc}	0.80^{abc}	0.87^{ab}	0.80^{ab}	0.82^{bc}
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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627 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	0.665	0.274	0.030	0.612
SEM	0.008	0.007	0.010	0.011	0.010	0.040	0.011	0.016	0.010
Contrast of DK Cabernet SRSM1 with DK Cabernet SRSM2									
p value	0.655	0.503	0.972	0.851	0.242	0.873	0.945	0.360	0.644
SEM	0.011	0.011	0.018	0.014	0.017	0.020	0.014	0.018	0.017
Contrast of average SID between RSC and SRSM									
p value	<0.001	<0.001	0.758	0.790	<0.001	0.096	0.013	0.236	0.060
SEM	0.011	0.012	0.018	0.015	0.017	0.027	0.015	0.021	0.018

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