The influence of oil extraction process of different rapeseed varieties on the ileal digestibility of crude protein and amino acids in broiler chickens

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Abstract

The current study assessed the effect of rapeseed variety and oil extraction process on the apparent and standardised ileal digestibility (AID, SID) of crude protein (CP) and amino acids (AA) in rapeseed co-products in broiler chickens. PR46W21 and DK Cabernet rapeseed varieties were de-oiled by soft and standard hexane extraction, producing soft rapeseed meal (SRSM) and rapeseed meal (RSM), respectively. The soft, non-standard hexane extraction method was designed to reduce heat treatment that occurs prior to hexane extraction in order to maximise potential genetic differences in digestibility values of rapeseed co-products. The test meals were incorporated into semi-synthetic diets at a level of 500 g/kg; diets were fed to 14-day old paired chickens (n=6 pairs) for ten days, when ileal digesta was collected post-slaughter from Meckel's diverticulum to the ileal-caecal junction. The AID and SID of CP and AA were determined using titanium dioxide as inert dietary marker. The variety PR46W21 showed a greater AID and SID of CP, arginine, leucine, methionine, cysteine, phenylalanine, valine and lysine in RSM compared to the DK Cabernet RSM ($p < 0.05$). The soft processing increased AID and SID of CP, histidine and lysine in SRSM of PR46W21 and DK Cabernet compared to their RSM counterparts ($p<0.05$). An interaction between variety and processing was only observed for AID and SID of tryptophan ($p < 0.001$), as only in PR46W21 standard processing reduced the tryptophan SID compared to its soft processed counterpart. The data support the view that the selection of rapeseed variety and modification of thermal treatment during the oil extraction might improve nutritional value of rapeseed meals.

Keywords: digestibility, rapeseed meal, variety, amino acid, crude protein, broiler.

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; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; NDF, neutral detergent fibre; Phe, phenylalanine; RSM, rapeseed meal; SEM; standard error of the difference mean; SID, coefficient of standardised ileal digestibility; SRSM, soft rapeseed meal; Trp, tryptophan; Val, valine.

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1. Introduction

Global production of oilseed rape (*Brassica napus*) has substantially increased over the past decades (Carré and Pouzet, 2014). Rapeseed oil is mainly used in human nutrition and biofuel production, whereas rapeseed de-oiled meal is utilised in animal diets. Oilseed rape co-products are the second most widely fed protein ingredients to animals after soybean meal (Zhang et al., 2012). They vary from rapeseed cake, obtained after cold-pressing, to rapeseed expellers, produced by thermal seed crushing, to the most commonly used rapeseed meal (RSM), produced after thermal seed pressing and solvent extraction (Kaldmae et al., 2010; Eklund et al., 2015; Toghyani et al., 2015).

Rapeseed co-products are rich in crude protein (CP) and S-containing amino acids (AA) such as methionine and cysteine (Woyengo et al., 2010; Li et al., 2015), and are less expensive feed ingredients than soybean meal (Gonzalez-Vega and Stein, 2012). However, the chemical composition of these protein ingredients varies depending on the rapeseed variety and associated method of cultivation, environmental and climatic changes (McFadden et al., 2006) and de-oiling method including the mechanical and thermal treatments employed (Leming and Lember, 2005; Messerschmidt et al., 2014; Eklund et al., 2015). In our previous study (Kasprzak et al., 2016), we showed that the soft processing of rapeseed leads to production of rapeseed co-products with various CP and AA content depending on the rapeseed variety. Therefore, the objective of the current study was to compare the soft and standard de-oiling method in two commonly used rapeseed varieties (PR46W21 vs. DK Cabernet) on ileal digestibility of CP and AA in rapeseed co-products fed to broiler chickens.

2. Material and methods

2.1 Rapeseed co-products

Two double-low cultivars of oilseed rape (*Brassica napus*) PR46W21 and DK Cabernet were grown and harvested in Lincolnshire and Norfolk (UK Counties) in 2014, respectively. The grain samples were de-oiled at a pilot plant in Pessac (OLEAD, France) using standard or soft processing and hexane extraction, thus producing standard RSM and softly processed rapeseed meal (SRSM). The soft hexane extraction was previously described by Kasprzak et al. (2016). Briefly, prior to processing, the seeds were dried to approximately 930 g dry matter/kg using warm air at 70 ^{o}C. Standard processing involved cooking the seeds at 90 ^{o}C for a period of 44 min±1.5 min, and subsequently crushing at temperature of 79±2.3 ^{o}C using a MBU 75 press (La Mécanique Moderne, France). The cooking step was avoided for soft processing; the dried seeds were cold-pressed without heating in the same press. The resultant oilcakes from both soft and standard processes were pelletized into 5 mm pellets. Subsequently, oil was extracted by counter-flow of hexane in 6 stages in a belt diffuser (Desmet Ballestra, Belgium). The defatted cake was then forwarded to a 6 tray continuous desolventiser (Desmet Ballestra). The desolventisation in four rapeseed batches was performed for 80 min. The desolventisation temperature was 105.3 ^{o}C, 105.9 ^{o}C, 110.5 ^{o}C and 115.9 ^{o}C for PR46W21 SRSM, DK Cabernet SRSM, PR46W21 RSM and DK Cabernet RSM, respectively.

The SRSM and RSM pellets were ground by a commercial mill through a 4 mm screen. Semi-synthetic diets were then formulated containing per kg: 500 g SRSM or RSM, 200 g maize starch (Cargill, UK), 195 g dextrose (Cargill, UK), 50 g rapeseed oil (Tesco, UK), 50 g vitamin and minerals (Chicken Premix, Target Feeds, UK), and 5 g titanium dioxide (food

grade inert marker, Azelis, UK). Diet ingredients were mixed using a dough mixer, and fed as a meal.

2.2 Animal experiment

The protocol for the experiment was reviewed and approved by Ethical Review Committee, University of Nottingham, and conducted according to the UK Home Office Animal (Scientific Procedures) Act of 2010.

A total of 48 day-old male Ross 308 broilers were purchased from a British designated breeder (PD Hook Hatcheries Ltd., Thirsk, UK) and housed in the BioSupport Unit, School of Biosciences, University of Nottingham. Chickens were accommodated in pairs in cages 37 cm wide, 42 cm tall and 30 cm deep.

Birds were fed a commercial diet with crude protein at 190 g/kg as-fed (Chick Starter Crumb, Dodson and Horrell Ltd., Northamptonshire, UK) for fourteen days. Diets and water were available *ad libitum*. The amount of feed consumed and body weight of chickens were measured during the experiment. At day 15, broilers were allocated to the four experimental diets and fed for ten days. In a randomized complete block design, each dietary treatment was tested with six pairs of chickens. At the end of the trial, all chickens were culled by asphyxiation with carbon dioxide followed by cervical dislocation to confirm death. Collection of ileal digesta was undertaken as previously described (Kasprzak et al., 2016). The ileal contents of the two chickens were pooled from each cage and collected into a plastic screw-top pot and immediately frozen at -20 °C pending further analysis for dry matter (DM), total nitrogen (N) and AA.

2.3. Analysis

The DM of RSM and SRSM was measured by drying the samples at 100 °C in a forced air convection oven, whereas DM of ileal digesta was measured by freezing following freeze-drying (AOAC, 2000). The titanium dioxide (TiO_2) content was determined by the method of Short et al. (1996). The content of N was measured by the standard combustion method (AOAC, 2000); 5-6 mg of rapeseed co-products and ileal digesta were weighted in aluminium crucibles and burned in furnaces at 900 °C/1060 °C, using CHNS-O Analyser (CE Instruments Ltd., UK). The content of CP was calculated by N × 6.25. Protein solubility was measured according to a standard method ISO 14244 (2014). The AA content was measured by the hydrolysis of protein and AA derivatisation with ninhydrin as previously described by Masey O'Neill et al., (2014). The samples for tryptophan determination were similarly prepared, but the hydrolysis was performed with barium hydroxide for 16 hours in order to prevent decomposition of the amino acid. The content of neutral detergent fibre (NDF) was measured in SRSM and RSM using fibre bags according to an established method (EN ISO, 2006). The oil content was determined using continuous-wave low-resolution nuclear magnetic resonance spectrometry (ISO 1997). Phytic acid was determined using a K-PHYT kit (Megazyme, UK). The content of sinapine was determined according to a standard method used in rapeseed meal (Cai and Arntfield, 2001; Li and El Rassi, 2002). The content of tannins was measured by the method of Butler et al. (1982). Content of total glucosinolates was determined by high pressure liquid chromatography using sinigrin as an internal standard (ISO, 1994). The daily feed intake (FI) of experimental diets was recorded over three days of feeding.

2.4. Calculations and statistics

Apparent ileal digestibility (AID) and standardised ileal digestibility (SID) coefficients of CP and AA in the diets were calculated according to standard equations (Masey O'Neill et

al., 2012). The basal ileal endogenous CP and AA losses (g/kg DM intake) were corrected by the previously published values: arginine 0.216, histidine 0.209, isoleucine 0.390, leucine 0.381, lysine 0.255, methionine + cysteine 0.257, phenylalanine 0.237, threonine 0.571, valine 0.440 and tryptophan 0.082 g/kg DM intake (Lemme et al., 2004; O'Neill et al., 2014).

Coefficient of apparent total tract digestibility (ATTD) of CP and NDF in the assay diets was calculated according to the following equation:

$$\mathrm{ATTD} = 1 - \left[\frac{\mathrm{I_D} \times \mathrm{A_F}}{\mathrm{A_D} \times \mathrm{I_F}}\right]$$

Where I_D = marker content in the assay diet (g/kg of DM), A_F = CP or NDF content in excreta (g/kg of DM), A_D = CP or NDF content in the assay diet (g/kg of DM), I_F= marker concentration in excreta (g/kg of DM). The lysine (Lys) to CP ratio (Lys:CP) in the test samples as indicator of heat damage was calculated as described by Gonzalez-Vega et al. (2011).

The digestibility coefficients across four rapeseed co-products were evaluated by two-way analysis ANOVA with rapeseed variety and processing method set as factors, and digestibility coefficient as Y-variable. The statistics were done through GenStat (15 Edition, VSN International, Hemel Hempstead, UK) and reported as significant when $p < 0.05$.

3. Results

All birds were healthy throughout the experiment and daily consumed similar amounts of feed. The average FI was 99.3, 85.4, 97.4 and 90.7 g (as-is) per day of PR46W21 SRSM, PR46W21 RSM, DK Cabernet SRSM and DK Cabernet RSM diets, respectively (standard error of mean, SEM 8.16).

3.1. Chemical composition

The content of CP, AA and NDF varied in the rapeseed co-products depending on rapeseed variety and processing (Table 1). The CP content was the greatest in PR46W21 SRSM, followed by PR46W21 RSM, DK Cabernet SRSM and DK Cabernet RSM. RSM samples had smaller contents of CP than SRSM, which was also observed for most AA. Protein solubility was greater for soft processed meals than for their standard processed counterparts. The Lys:CP ratio was smaller in standard processed meals of PR46W21 and DK Cabernet variety compared to their soft processed counterparts. Although the NDF content in RP46W21 processed by both methods was very similar, the content of NDF in DK Cabernet processed through standard processing was 103 g/kg greater than for the meal processed by the soft method.

3.2. Coefficient of apparent ileal digestibility of CP and AA

Table 2 shows the AID of CP and AA for each of the four feeding treatment combinations.

Processing and variety did not show any interaction for the AID of CP and most AA. Thus, across varieties, the soft processing resulted in a significantly greater AID of CP (0.77 vs. 0.72, SEM 0.006), arginine (0.85 vs. 0.82, SEM 0.007), methionine (0.85 vs. 0.82, SEM 0.007), lysine (0.76 vs. 0.66, SEM 0.009), histidine (0.84 vs. 0.77, SEM 0.009) and threonine (0.69 vs. 0.65, SEM 0.012) than the standard processing. Likewise, across processing PR46W21 had a significantly greater AID of CP (0.76 vs. 0.73, SEM 0.006), arginine (0.85 vs. 0.82, SEM 0.007), methionine (0.85 vs. 0.81, SEM 0.007), lysine (0.73 vs. 0.69, SEM 0.009), leucine (0.81 vs. 0.77, SEM 0.010), isoleucine (0.78 vs. 0.74, SEM 0.009), histidine (0.82 vs. 0.78, SEM 0.009), threonine (0.69 vs. 0.65, SEM 0.012), valine (0.74 vs. 0.70, SEM 0.010), phenylalanine (0.80

vs. 0.76, SEM 0.009), cysteine (0.71 vs. 0.66, SEM 0.014), and methionine and cysteine (0.75 vs. 0.70, SEM 0.011) than DK Cabernet. However, processing and variety interacted for AID of tryptophan; the AID of tryptophan was reduced ($p < 0.001$) from 0.81 in SRSM to 0.53 in RSM of PR46W21, whilst it did not significantly differ between SRSM and RSM of DK Cabernet. In addition, the pair-wise comparisons would indicate that processing reduced AID of histidine and lysine to a greater extent in DK Cabernet than in PR46W21, although this was not associated with a significant interaction between processing and variety (Table 2).

3.3. Coefficient of standardised ileal digestibility of CP and AA

Table 3 shows the SID of CP and AA for each of the four feeding treatment combinations. As for AID, processing and variety did not interact for the SID of CP and most AA. Thus, across varieties, the soft processing resulted in significantly greater SID of CP (0.81 vs. 0.77, SEM 0.006), arginine (0.87 vs. 0.84, SEM 0.007), histidine (0.87 vs. 0.81, SEM 0.009), lysine (0.78 vs. 0.69, SEM 0.009), methionine (0.85 vs. 0.82, SEM 0.007) and threonine (0.75 vs. 0.72, SEM 0.012) than standard processing. Likewise, across processing, PR46W21 had a greater SID of CP (0.81 vs. 0.77, SEM 0.006), arginine (0.87 vs. 0.84, SEM 0.007), cysteine (0.72 vs. 0.68, SEM 0.014), histidine (0.86 vs. 0.82, SEM 0.009), isoleucine (0.83 vs. 0.79, SEM 0.009), leucine (0.84 vs. 0.79, SEM 0.010), lysine (0.76 vs. 0.72, SEM 0.009), methionine (0.85 vs. 0.81, SEM 0.007), methionine and cysteine (0.77 vs. 0.72, SEM 0.011), phenylalanine (0.83 vs. 0.80, SEM 0.009), valine (0.78 vs. 0.74, SEM 0.010) and threonine (0.76 vs. 0.71, SEM 0.012) than DK Cabernet ($p< 0.05$). However, similarly to AID, the only significant interaction between variety and processing was for the SID of tryptophan ($p <0.001$), arising from a reduction in tryptophan SID by 0.24 in PR46W21 RSM compared to its SRSM counterpart, but no difference within the DK Cabernet samples. In addition, the pair-wise comparisons would indicate that processing reduced SID of histidine and lysine to a greater

extent in DK Cabernet than in PR46W21, although this was not associated with a significant interaction between processing and variety (Table 3).

3.4. Coefficient of apparent total tract digestibility

Table 4 shows the coefficient of ATTD for CP and NDF. Across varieties, the soft processing resulted in greater ATTD of CP than standard processing (0.60 vs. 0.47, SEM 0.007; $p < 0.001$), whilst across processing PR46W21 had a greater CP ATTD than DK Cabernet (0.56 vs. 0.51, SEM 0.007, $p < 0.001$). However, variety and processing tended to interact for the CP ATTD ($p = 0.06$); the pair-wise comparisons indicate that standard processing had a greater effect within DK Cabernet than within PR46W21, whilst the variety effect was only observed under standard processing.

There was a significant interaction between variety and processing on ATTD of NDF. The pair-wise comparisons indicate that under soft processing conditions, ATTD of NDF for PR46W21 and DK Cabernet was similar, whilst standard processing significantly reduced NDF ATTD for PR46W21 but significantly increased NDF ATTD for DK Cabernet ($p < 0.001$).

4. Discussion

An effective way to improve the nutritional value of protein raw materials is denaturation of native protein, however extensive heating may cause AA damage (Gonzalez-Vega et al. 2011). During the production of RSM, the thermal treatment is applied from the beginning of conditioning the seed, through seed crushing until hexane extraction and desolventisation. The heating leads to occurrence of the Maillard reaction, which causes binding of the protein-bound lysine and reducing sugars, and forms deoxyketosyl-lysine derivatives as lactulosyl-lysine (Hurrell, 1990). Purcell and Walter (1982) showed that

besides the loss of lysine, thermal treatment can also reduce the content of tryptophan in heat treated sweet potatoes. Alongside, variations in thermal conditions in the oil extraction methods can also result in changes in the content of crude fat and NDF in the meal (Keith and Bell, 1991; Spragg and Mailer, 2007; Li et al., 2015). This might overall contribute to override the effect of cultivation, environment or rapeseed variety on the chemical composition of rapeseed co-products.

The standard processing caused a reduction of Lys:CP ratio in both RSM, but also substantially decreased the tryptophan content, especially for PR46W21 RSM compared to its SRSM counterpart. As degradation of lysine and tryptophan may occur in the cooking step and/or seed crushing prior to hexane extraction and desolventisation, the application of soft processing might prevent partially the loss of AA in the final meal. The variety of PR46W21 showed a greater content of CP and most AA compared to DK Cabernet. This implies that the selection of oil seed rape variety has the potential to enhance the chemical composition of the resulting defatted meal.

The PR46W21 variety showed a very similar content of NDF in both meals when processed by both soft and standard method. However, the NDF content increased in RSM compared to DK Cabernet SRSM. This was possibly due to a greater thermal treatment of DK Cabernet RSM (desolventisation temperature 115.9 ^{o}C) which may have led to a reduction in CP, protein solubility and increased the NDF content. Almeida et al. (2014) found that autoclaving canola meal at 130 ^{o}C for 45 min raised the content of NDF from 334 g/kg DM to 469 g/kg DM. Nia and Ingalls (1992) reported that the increase in content of NDF might be due to the ability of fibre to bind with dietary protein during the thermal treatment as some of the heat denaturated proteins are recovered in NDF fraction.

Although the contents of CP and AA varied slightly between the DK Cabernet and PR46W21 SRSM and RSM, they were in line with values previously published by Bell and Keith (1991) (CP 380-430 g/kg DM; lysine 24-26 g/kg DM; arginine 24-29 g/kg DM; leucine

28-33 g/kg DM), Fan et al. (1996) (CP 35-42 g/kg DM, lysine 21-24 g/kg DM; histidine 10-11 g/kg DM; phenylalanine 14-16 g/kg DM), Landero et al. (2011) (CP: 382 g/kg DM, lysine 23 g/kg DM, tryptophan 4 g/kg DM, threonine 18 g/kg DM) and Liu et al. (2014) (CP: 386-496 g/kg DM). The content of NDF in the most analysed rapeseed co-products was greater than the values reported by Parr et al. (2015) (196-279 g/kg DM) and Bell and Keith (1991) (230-250 g/kg DM), but similar to the values published by Xi et al. (2002) (320-409 g/kg DM) and Li et al. (2015) (279-410 g/kg DM).

In a previous investigation (Kasprzak et al., 2016), the soft processed PR46W21 resulted in the greatest ileal digestibility of CP (AID 0.84) and most AA such as AID of lysine 0.85, AID of arginine 0.91, AID of histidine 0.89 across SRSM of eleven different rapeseed varieties. This is consistent with the data from the current study where, across processing, the meal of PR46W21 variety had a significantly greater AID and SID of CP, arginine, isoleucine, leucine, methionine, cysteine, phenylalanine, threonine, valine, lysine and histidine (Table 2, 3) compared to DK Cabernet. It cannot be excluded that this increase in AID and SID was related to the greater content of protein in PR46W21 compared to DK Cabernet, which as such enhanced the nutritional value of the meal.

The rapeseed cooking and heat supply during crushing are crucial steps in the rapeseed processing, as they improve de-oiling process making the oil extraction more efficient and cost effective. Similarly to variations in chemical composition in the meals, the standard processing of oil extraction simultaneously reduced the digestibility value of the meal. Thus, the variations in processing condition across the oil plants and countries can lead to productions of rapeseed co-products with inconsistent nutritional values.

The overall digestibility of CP and AA in SRSM and RSM were in an agreement with previously published data for SRSM (Kasprzak et al. 2016) tested as a sole source of protein in semi-synthetic diets in broiler chickens (AID of CP 0.74-0.84; SID of lysine 0.77-0.87; SID of arginine 0.86-0.0.92). A study of Woyengo at al. (2010) testing corn and soybean meal

replaced by 30% solvent and expeller-extracted canola meal in broilers resulted also in a similar ileal digestibility (SID of CP 0.76-0.79 SID of arginine 0.80-0.84, SID of lysine 0.77-0.79, SID of leucine 0.76-0.80).

The content of fibre in diets is an important factor in animal nutrition. The processing of feedstuffs might entrap or bind the fibre with a nutrient, and consequently reducing its digestibility (Mateos et al., 2002; Garcia et al., 2008). Thus, a significantly lower ileal digestibility of CP and AA in especially DK Cabernet RSM might be a consequence of high content of analysed NDF in the meal.

Similarly to the ileal digestibility, the soft processing favoured the ATTD of CP. The ATTD of CP was much lower compared to a value reported by Gopinger et al. (2014); they tested an inclusion of 40% canola meal as a substitute for soybean meal in broiler chicken diets, which resulted in ATTD of CP at 0.74. This was a combined effect of soybean and rapeseed protein digestibility that contributed to a greater overall digestibility of protein. The use of semi-synthetic diet allows distinguishing a direct effect of the rapeseed origin of protein meals on digestibility values.

In the current study, soft processing was applied in order to maximise the potential genetic differences in the chemical composition of rapeseed meal, as standard processing might have possibly overridden the characteristics of meals. However, besides SRSM, the RSM also showed the varietal differences in the composition and digestibility values of CP and AA between two batches. Thus, the selection of rapeseed variety might be of benefits for the industry that is mostly reliant on standard hexane extraction processing.

5. Conclusion

There were considerable differences in chemical composition, AID, SID of CP and AA in rapeseed co-products depending on rapeseed variety and intensity of the de-oiling processing. Choosing variety PR46W21 over DK Cabernet, and where possible a reduction in

de-oiling process intensity, might improve the digestibility and thus nutritional value of the resulting rapeseed co-products.

Conflict of interest

We declare no conflict of interest.

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Table 1. Chemical composition of rapeseed co-products (g/kg DM)

Variety	PR46W21		DK Cabernet	
Processing	soft	standard	soft	standard
Sample name	SRSM	RSM	SRSM	RSM
DM	899	932	922	924
CP	439	411	391	378
Arg	25.4	23.4	22.7	20.1
His	12.3	10.7	11.5	9.4
Ile	16.5	17.0	16.1	15.6
Leu	29.2	28.4	27.1	25.4
Lys	24.1	21.0	23.7	18.8
Met	8.1	7.5	7.4	6.9
Cys	19.4	19.7	17.1	16.6
Met+Cys	27.5	27.2	24.4	23.4
Phe	14.8	15.3	14.9	13.9
Thr	18.0	17.1	16.9	15.8
Val	21.4	21.5	20.7	19.9
Trp	4.5	2.0	4.1	3.9
Lys:CP*	0.055	0.051	0.061	0.050
Protein solubility (%)	48.8	43.5	44.6	35.8
Oil content	58	46	78	50
NDF	325	321	330	433
Tannin catechnin equivalent	2.5	2.5	2.3	2.2
Phytic acid	26.7	25.4	14.2	18.0
Sinapin	3.9	3.9	3.6	3.1
Total glucosinolates**	7.3	4.6	6.3	2.7

Arg, arginine; CP, crude protein; Cys, cysteine; DM, dry matter; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; NDF, neutral detergent fibre; Phe, phenylalanine; RSM, rapeseed meal; SEM, standard error of the difference mean; SRSM, soft rapeseed meal; Trp, tryptophan; Val, valine. *Lys:CP ratio. ** Total glucosinolates expressed as μmol/g DM.

Table 2. Effects of rapeseed variety and processing on apparent ileal digestibility of CP and AA in rapeseed co-products in broiler chickens

Variety	PR46W21		DK Cabernet					
Processing	soft	standard	soft	standard		*p value*		
Sample name	SRSM	RSM	SRSM	RSM	SEM	*Variety*	*Processing*	*Var. x Proc.*
CP	0.78[a]	0.74[b]	0.75[ab]	0.70[c]	0.009	*<0.001*	*<0.001*	*0.830*
Arg	0.87[a]	0.84[ab]	0.83[bc]	0.80[c]	0.010	*0.002*	*0.005*	*0.863*
His	0.85[a]	0.79[bc]	0.82[ab]	0.75[c]	0.013	*0.014*	*<0.001*	*0.538*
Ile	0.79[a]	0.77[ab]	0.75[ab]	0.73[b]	0.013	*0.005*	*0.114*	*0.780*
Leu	0.82[a]	0.80[a]	0.78[ab]	0.75[b]	0.014	*0.004*	*0.080*	*0.723*
Lys	0.78[a]	0.69[b]	0.75[a]	0.63[c]	0.013	*0.004*	*<0.001*	*0.166*
Met	0.86[a]	0.84[a]	0.83[ab]	0.80[b]	0.010	*<0.001*	*0.022*	*0.548*
Cys	0.70[ab]	0.71[a]	0.66[ab]	0.65[b]	0.020	*0.018*	*0.929*	*0.603*
Met+Cys	0.75[a]	0.75[a]	0.71[ab]	0.69[b]	0.016	*0.009*	*0.510*	*0.588*
Phe	0.80[a]	0.80[a]	0.78[ab]	0.75[b]	0.013	*0.007*	*0.193*	*0.242*
Thr	0.70[a]	0.68[a]	0.67[ab]	0.62[b]	0.017	*0.014*	*0.028*	*0.418*
Val	0.75[a]	0.73[a]	0.72[ab]	0.68[b]	0.015	*0.010*	*0.088*	*0.499*
Trp	0.81[a]	0.53[d]	0.78[ab]	0.75[bc]	0.010	*<0.001*	*<0.001*	*<0.001*

Arg, arginine; CP, crude protein; Cys, cysteine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; RSM, rapeseed meal; SEM, standard error of the difference mean; SRSM, soft rapeseed meal; Trp, tryptophan; Val, valine. Values in the same row followed by different letters are significantly different ($p < 0.05$).

Table 3. Effects of rapeseed variety and processing on standardised ileal digestibility of CP and AA in rapeseed co-products in broiler chickens

Variety	PR46W21		DK Cabernet					
Processing	soft	standard	soft	standard		*p value*		
Sample name	SRSM	RSM	SRSM	RSM	SEM	*Variety*	*Processing*	*Var. x Proc.*
CP	0.83[a]	0.79[b]	0.80[ab]	0.75[c]	0.009	*0.002*	*<0.001*	*0.778*
Arg	0.89[a]	0.86[ab]	0.85[bc]	0.82[c]	0.010	*0.004*	*0.008*	*0.904*
His	0.88[a]	0.83[bc]	0.86[ab]	0.79[c]	0.013	*0.027*	*<0.001*	*0.621*
Ile	0.84[a]	0.82[ab]	0.80[ab]	0.78[b]	0.013	*0.008*	*0.113*	*0.863*
Leu	0.84[a]	0.83[a]	0.81[ab]	0.78[b]	0.014	*0.007*	*0.096*	*0.755*
Lys	0.80[a]	0.72[b]	0.77[a]	0.66[c]	0.013	*0.005*	*<0.001*	*0.195*
Met	0.86[a]	0.84[a]	0.83[ab]	0.80[b]	0.010	*<0.001*	*0.022*	*0.548*
Cys	0.72[ab]	0.73[a]	0.68[ab]	0.67[b]	0.020	*0.025*	*0.937*	*0.618*
Met+Cys	0.77[a]	0.77[a]	0.73[ab]	0.71[b]	0.016	*0.013*	*0.532*	*0.604*
Phe	0.84[a]	0.83[a]	0.81[ab]	0.78[b]	0.013	*0.009*	*0.209*	*0.293*
Thr	0.77[a]	0.74[ab]	0.74[ab]	0.69[b]	0.017	*0.026*	*0.046*	*0.437*
Val	0.79[a]	0.77[a]	0.76[ab]	0.72[b]	0.015	*0.014*	*0.097*	*0.540*
Trp	0.85[a]	0.61[c]	0.82[ab]	0.80[b]	0.010	*<0.001*	*<0.001*	*<0.001*

Arg, arginine; CP, crude protein; Cys, cysteine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; RSM, rapeseed meal; SEM, standard error of the difference mean; SRSM, soft rapeseed meal; Trp, tryptophan; Val, valine. Values in the same row followed by different letters are significantly different ($p < 0.05$).

Table 4. Effects of rapeseed variety and processing on apparent total tract digestibility of CP and NDF in rapeseed co-products in broiler chickens

Variety	PR46W21		DK Cabernet					
Processing	soft	standard	soft	standard		*p value*		
Sample name	SRSM	RSM	SRSM	RSM	SEM	*Variety*	*Processing*	*Var. x Proc.*
CP	0.61[a]	0.51[b]	0.58[a]	0.44[c]	0.009	*<0.001*	*<0.001*	*0.060*
NDF	0.26[b]	0.18[c]	0.26[b]	0.34[a]	0.013	*<0.001*	*0.840*	*<0.001*

CP, crude protein; NDF, neutral detergent fibre; RSM, rapeseed meal; SEM, standard error of the mean; SRSM, soft rapeseed meal. Values in the same row followed by different letters are significantly different ($p < 0.05$).