

# Amino acid utilization and body composition of growing pigs fed processed soybean meal or rapeseed meal with or without amino acid supplementation

T. G. Hulshof<sup>1,2</sup>, A. F. B. van der Poel<sup>2</sup>, W. H. Hendriks<sup>2</sup> and P. Bikker<sup>1†</sup>

<sup>1</sup>Wageningen Livestock Research, Wageningen University & Research, PO Box 338, 6700 AH Wageningen, The Netherlands; <sup>2</sup>Animal Nutrition Group, Wageningen University & Research, PO Box 338, 6700 AH Wageningen, The Netherlands

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*Feed ingredients used in swine diets are often processed to improve nutritional value. However, (over-)processing may result in chemical reactions with amino acids (AAs) that decrease their ileal digestibility. This study aimed to determine effects of (over-)processing of soybean meal (SBM) and rapeseed meal (RSM) on post-absorptive utilization of ileal digestible AAs for retention and on body AA composition of growing pigs. Soybean meal and RSM were processed by secondary toasting in the presence of lignosulfonate to obtain processed soybean meal (pSBM) and processed rapeseed meal (pRSM). Four diets contained SBM, pSBM, RSM or pRSM as sole protein source. Two additional diets contained pSBM or pRSM and were supplemented with crystalline AA to similar standardized ileal digestible (SID) AA level as the SBM or RSM diet. These diets were used to verify that processing affected AA retention by affecting ileal AA digestibility rather than post-absorptive AA utilization. The SID AA levels of the protein sources were determined in a previous study. In total, 59 pigs were used (initial BW of 15.6 ± 0.7 kg) of which five were used to determine initial body composition at the start of the experiment. In total, 54 pigs were fed one of six experimental diets and were slaughtered at a BW of 40 kg. The organ fraction (i.e. empty organs plus blood) and carcass were analyzed separately for N and AA content. Post-absorptive AA utilization was calculated from AA retention and SID AA intake. An interaction between diet type, comprising effects of processing and supplementing crystalline AA, and protein source was observed for CP content in the organ fraction, carcass and empty body and for nutrient retention. Processing reduced CP content and nutrient retention more for SBM than for RSM. Moreover, processing reduced (P < 0.001) the lysine content in the organ fraction for both protein sources. Supplementing crystalline AA ameliorated the effect of processing on these variables. Thus, the data indicated that processing affected retention by reducing digestibility. Correcting AA retention for SID AA intake was, therefore, expected to result in similar post-absorptive AA utilization which was observed for the RSM diets. However, post-absorptive AA utilization was lower for the pSBM diet than for the SBM diet which might be related to an imbalanced post-absorptive AA supply. In conclusion, processing negatively affected nutrient retention for both protein sources and post-absorptive utilization of SID AA for retention for SBM. Effects of processing were compensated by supplementing crystalline AA.*

**Keywords:** amino acid, body composition, nutrient retention, post-absorptive utilization, toasting with lignosulfonate

## Implications

The main protein sources used in animal feed have undergone some form of (thermal) processing which may reduce their digestible amino acid (AA) content. In this study, we demonstrated that additional processing, in the current study this was over-processing, of commercial soybean meal (SBM) or rapeseed meal (RSM) slightly altered AA composition of body protein in growing pigs and reduced the efficiency of using digested AAs for retention. Effects of over-processing

can be ameliorated when crystalline AAs are supplemented based on the ileal digestible AA content in over-processed feed ingredients.

## Introduction

Processing is commonly applied to feed ingredients used in swine diets to improve nutritional value, whereas over-processing may result in chemical reactions that reduce nutritional value. Research into effects of (over-)processing of protein sources has focused on (ileal) AA digestibility (Van Barneveld *et al.*, 1994a; González-Vega *et al.*, 2011;

† E-mail: paul.bikker@wur.nl

Almeida *et al.*, 2014; Hulshof *et al.*, 2016a). For protein sources with a high AA availability such as SBM, there is close agreement between ileal digestibility and availability measured using the slope–ratio assay and based on feed conversion efficiency on a carcass basis. For protein sources with a low AA availability such as cottonseed meal, however, ileal digestibility overestimated AA availability (Batterham *et al.*, 1990b) because the absorbed AA could not be fully utilized by the pig. The latter was also reported for heat-treated fish meal (Wiseman *et al.*, 1991) and heat-treated field peas (Van Barneveld *et al.*, 1994b). The negative effects of processing on digestibility and availability are, at least partly, caused by the formation of Maillard reaction products which are formed by the reaction between amino groups and reducing sugars (Hurrell and Carpenter, 1981). Lysine is often the first limiting AA in diets for growing pigs and the most susceptible AA to react with sugars because of its free  $\epsilon$ -amino group. Reactive lysine is thought to provide a better estimation of lysine availability than total lysine because the latter includes the lysine reverted back from early Maillard reaction products during acid hydrolysis (Moughan and Rutherford, 2008). Chemical reactions during processing may result in an imbalance among available AA and between AA and net energy, which was demonstrated to affect organ weight, carcass composition and the gain to feed ratio (Hulshof *et al.*, 2016b). Conde-Aguilera *et al.* (2010) reported that pigs were able to alter the AA composition of retained protein when fed a diet deficient in sulfur AA. However, the effects of processing of SBM and RSM on the utilization of ileal digestible AA for retention and the metabolic flexibility of an animal to cope with changes in post-absorptive AA availability have received little attention.

The current study aimed at determining the effects of (over-)processing of SBM and RSM on (post-absorptive) utilization of ileal digestible AA for retention and on body AA composition of growing pigs. It was hypothesized that processing may reduce AA retention because of reduced ileal digestibility of AA but not via an effect on utilization of ileal digested AA for retention. It was further hypothesized that processing and subsequent AA availability would not affect body AA composition.

## Material and methods

### *Animals and housing*

This study was approved by the Animal Care and Use Committee of Wageningen University and Research Centre (Wageningen, the Netherlands). A total of 59 growing gilts (Tempo  $\times$  Topigs 40 from Van Haaren, Horssen, the Netherlands) with an average initial BW of  $15.6 \pm 0.7$  kg at the day of arrival (day 1) were used in the experiment. Pigs were fed a commercial diet and were gradually adapted during the 1<sup>st</sup> week to a mixture of SBM and RSM diets (50:50) and to a feeding level of 3.0 times net energy requirements for maintenance ( $321 \text{ kJ net energy/kg BW}^{0.75}$ ; Agricultural Research Council, 1981). After this week, pigs were allotted either to the initial slaughter group (ISG-pig) or

to an experimental diet and subsequently to a pen. The housing conditions of the pigs are described elsewhere (Hulshof *et al.*, 2016b).

### *Experimental design and diets*

The study consisted of a randomized complete block design with BW at day 8 as blocking factor and nine blocks consisting alternately of seven (five blocks) and six (four blocks) pigs. From each BW block consisting of seven pigs, one pig was randomly chosen and allotted to the ISG-pigs. The five ISG-pigs were divided over two rooms and housed in groups of two and three pigs, respectively, until slaughter at day 9. The remaining 54 pigs (average BW of  $17.1 \pm 1.0$  kg on day 8) were randomly allocated to one of six experimental diets within a block and assigned to a pen in one of four rooms as described previously (Hulshof *et al.*, 2016b).

All six isoenergetic (9.96 MJ of net energy/kg) experimental diets (Research Diet Services, Wijk bij Duurstede, the Netherlands) consisted of a basal CP-free component based on gelatinized potato starch. Three experimental diets contained commercially toasted/desolventized SBM (*Glycine max*), whereas the other three experimental diets contained RSM (*Brassica napus* 00-variety referring to low levels of erucic acid and glucosinolates) as sole protein source ( $2 \times 3$  factorial treatment arrangement). Soybean meal and RSM (supplied by Feed Valid B.V., Poederrijen, the Netherlands) were used either as received, referred to as SBM and RSM diet, respectively, or processed by secondary toasting in the presence of lignosulfonate as described by Hulshof *et al.* (2016a) to obtain processed soybean meal (pSBM) and processed rapeseed meal (pRSM). This processing treatment, normally applied in ruminant nutrition to produce rumen by-pass protein, was used to induce protein damage through the Maillard reaction. The SBM and RSM diets were supplemented with crystalline L-Ile, L-Leu, L-Lys, DL-Met, L-Thr, L-Trp and L-Val to meet 90% of the requirements for standardized ileal digestible (SID) lysine (SID lysine to net energy (NE) ratio of 0.98 g/MJ) for growing pigs (Centraal Veevoeder Bureau (CVB), 2011) and the other indispensable AA as percentage of lysine according to Institut National de la recherche agronomique recommendations (Gloaguen *et al.*, 2013). The remaining two experimental diets contained pSBM and pRSM and were supplemented with crystalline AA to meet the SID AA concentration in the SBM and RSM diets, respectively, based on the SID AA concentration in the pSBM and pRSM protein sources (Hulshof *et al.*, 2016a). These diets were formulated to compensate for the losses in SID AA by processing and are referred to as pSBM + AA and pRSM + AA diet. L-Glutamine was added to these diets to account for the loss in SID of dispensable AA. The manufacturing of the experimental diets have been described previously (Hulshof *et al.*, 2016b). The chemical composition of the feed ingredients, the composition of the experimental diets, as well as the calculated SID AA concentration and SID AA to SID lysine ratio of the experimental diets are provided in Tables 1 to 3, respectively. Pigs were fed the experimental diets twice a day in equal portions at 0800 and 1600 h from day 9 until a BW of  $40 \pm 2$  kg was reached.

**Table 1** Analyzed nutrient composition of the four protein sources: soybean meal (SBM), processed SBM (pSBM),<sup>1</sup> rapeseed meal (RSM) or processed RSM (pRSM)<sup>1,2</sup>

Analyzed nutrient composition (g/kg DM) <sup>3</sup>	Protein source			
	SBM	pSBM <sup>4</sup>	RSM	pRSM <sup>4</sup>
DM (g/kg as-is)	900	895	895	897
Ash	73	72	75	77
CP	531	511	383	371
Acid-hydrolyzed ether extract	28	28	39	42
Starch	10	10	7	7
Total sugars	134	122	102	96
Indispensable amino acids (g/100 g CP)				
Arginine	7.3	6.4	5.8	5.0
Histidine	2.8	2.7	2.7	2.7
Isoleucine	4.6	4.6	3.9	3.9
Leucine	7.8	7.8	6.9	7.0
Lysine	6.3	4.6	5.6	4.3
OMIU-reactive lysine	6.0	3.7	5.0	3.1
Methionine	1.3	1.3	1.9	1.9
Phenylalanine	5.2	5.1	4.0	4.0
Threonine	4.0	3.9	4.4	4.4
Tryptophan	1.3	1.3	1.3	1.3
Valine	4.9	4.9	5.2	5.2
Dispensable amino acid (g/100 g CP)				
Alanine	4.5	4.4	4.4	4.5
Aspartic acid	11.5	11.4	7.3	7.2
Cysteine	1.4	1.3	2.2	2.1
Glutamic acid	18.2	18.0	16.2	16.1
Glycine	4.3	4.3	5.1	5.2
Proline	4.8	4.8	5.8	5.7
Serine	4.9	4.8	4.1	4.1
Tyrosine	3.6	3.6	3.1	3.1

DM = dry matter; OMIU = *O*-methylisourea.<sup>1</sup>Adapted from Hulshof *et al.* (2016a).<sup>2</sup>Processing consisted of addition of lignosulfonate to SBM and RSM and secondary toasting at 95 ± 2°C for 30 min.<sup>3</sup>Unless stated otherwise.<sup>4</sup>pSBM and pRSM include lignosulfonate at 7% and 5% (w/w), respectively.

### Slaughter procedure, sample preparation and chemical analysis

The five ISG-pigs were killed at day 9 (average BW before slaughter 18.2 ± 0.6 kg) and the 54 pigs receiving the experimental diets were killed at a BW of 40 ± 2 kg by electrical stunning followed by hanging the pig by the ankles and exsanguination by cutting the carotid artery. Blood and the empty visceral organs, referred to as organ fraction, were collected together in a bag as described previously (Hulshof *et al.*, 2016b) and frozen at -20°C. The carcass, including head, teeth, feet, hair and skin, was collected in plastic bags, and frozen at -20°C. The frozen organ fraction and carcass were cut separately in small blocks using a band saw (Butcher Boy B 14-9, Montebello, CA, USA) and homogenized using a commercial butcher mincer (Alexanderwerk, Remscheid, Germany). The organ fraction and carcass were subsampled after mincing and stored at -20°C pending analysis. The organ fraction and carcass of all pigs ( $n = 59$ )

were analyzed separately for moisture by freeze-drying, ash (method International Organization for Standardization (ISO) 5984; ISO, 2002) after 3 h heating at 550°C, ether extract (method ISO 6492; ISO, 1999) by extraction with petroleum ether and N (method ISO 5983-1; ISO, 2005a) by the Kjeldahl method with CP calculated as  $N \times 6.25$ . Samples of the organ fraction and carcass of the five ISG-pigs and six pigs per experimental diet representative for the BW blocks ( $n = 41$ ) were defatted by ether extraction and subsequently separately analyzed for total AA profile (method ISO 13903; ISO, 2005b) by acid hydrolysis at 110°C for 23 h and ion-exchange chromatography with post-column derivatization with ninhydrin, including sulfur containing AA measured as cysteic acid and methionine sulfone after oxidation with performic acid; tryptophan (method ISO 13904; ISO, 2005c) was measured by alkaline hydrolysis at 110°C for 20 h and ion-exchange chromatography with fluorescence detection.

### Calculations and statistical analysis

The nutrient retention in the organ fraction and carcass of pigs fed the experimental diets was calculated from the composition at 40 ± 2 kg and the initial composition at day 9 based on the composition of the ISG-pigs (Supplementary Table S1). The AA composition of protein in the empty body of the pigs was calculated from the AA composition in the organ fraction and carcass. The nutrient and AA retentions in the empty body were calculated from the retention in the organ fraction (Supplementary Table S2) and carcass (Supplementary Table S3). The post-absorptive N and AA utilizations were calculated as the ratio between the amount of N and individual AA retained in the empty body and the daily intake of SID N and individual AA. The daily intake of SID N and individual AA was based on the AA concentration in the diet excluding crystalline AA, the SID of AA from the protein sources as reported in Hulshof *et al.* (2016a) and considering 100% digestibility of crystalline AA. Reactive lysine was analyzed using the guanidination reaction with *O*-methylisourea (OMIU) and its concentration in the experimental diets is reported in Table 2. Lysine in the empty body was considered to be reactive. The post-absorptive OMIU-reactive lysine utilization was calculated from the lysine retained in the empty body and the daily intake of SID OMIU-reactive lysine.

The AA composition, nutrient retention and post-absorptive N and AA utilization were statistically analyzed using a two-tailed GLM (PROC GLM) in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) with pig as experimental unit. The model included the fixed effects of protein source, diet type and their interaction to test the effect of SBM/RSM and the effect of processing and supplementing crystalline AA on response variables. Body weight block was included in the random statement of the GLM. Least squares means were calculated per experimental diet and statistical differences between least squares means were determined using a *post hoc* Tukey test. The response variables were tested for normality using studentized residuals and non-normal data were log

**Table 2** Ingredient and analyzed nutrient composition<sup>1</sup> of six experimental diets containing soybean meal (SBM), processed SBM<sup>2</sup> (pSBM), pSBM supplemented with crystalline amino acid (AA) (pSBM + AA<sup>3</sup>), rapeseed meal (RSM), processed RSM<sup>2</sup> (pRSM) or pRSM supplemented with crystalline AA (pRSM + AA<sup>3</sup>) fed to growing pigs

Ingredient composition (g/kg as-fed)	Diet					
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA
Gelatinized potato starch	420.1	420.1	385.0	447.4	447.4	430.8
SBM	350.0	350.0	350.0	–	–	–
RSM	–	–	–	350.0	350.0	350.0
Lignosulfonate <sup>4</sup>	26.75	26.75	26.75	18.81	18.81	18.81
Dextrose	100.0	100.0	100.0	100.0	100.0	100.0
Arbocel	49.9	49.9	49.9	–	–	–
Soybean oil	20.0	20.0	23.5	35.0	35.0	36.0
Limestone	11.37	11.37	11.37	7.40	7.40	7.38
Monocalcium phosphate	8.68	8.68	8.79	7.97	7.97	7.99
Vitamin–mineral premix <sup>5</sup>	5.0	5.0	5.0	5.0	5.0	5.0
Salt	3.68	3.68	3.68	3.55	3.55	3.55
Potassium carbonate <sup>6</sup>	–	–	3.41	7.79	7.79	9.30
Titanium dioxide	2.5	2.5	2.5	2.5	2.5	2.5
L-Arginine HCl (83% arginine)	–	–	3.20	–	–	1.47
L-Histidine (99% histidine)	–	–	0.84	–	–	0.35
L-Isoleucine (99% isoleucine)	–	–	0.91	1.66	1.66	2.04
L-Leucine (99% leucine)	–	–	1.40	3.12	3.12	3.82
L-Lysine HCl (78% lysine)	0.10	0.10	5.45	4.52	4.52	7.01
DL-Methionine (99% methionine)	1.58	1.58	1.86	1.40	1.40	1.54
L-Cysteine (99% cysteine)	–	–	0.49	–	–	0.45
L-Phenylalanine (98% phenylalanine)	–	–	0.91	–	–	0.35
L-Threonine (98% threonine)	0.34	0.34	1.21	1.68	1.68	2.20
L-Tryptophan (98% tryptophan)	–	–	0.35	0.52	0.52	0.62
L-Tyrosine (98% tyrosine)	–	–	0.56	–	–	0.31
L-Valine (98% valine)	–	–	1.01	1.68	1.68	2.31
L-Glutamine (99% glutamine)	–	–	11.91	–	–	6.20
Analyzed nutrient composition						
DM (g/kg)	894	901	898	887	894	896
CP (g/kg DM)	184	186	215	147	148	161
Total sugars (g/kg DM)	156	150	144	139	133	129
Net energy <sup>7</sup> (MJ/kg feed)	9.9	9.5	9.7	10.0	9.9	10.0
Indispensable AAs (g/100 g CP)						
Arginine	6.9	6.2	6.7	5.1	4.5	4.9
Histidine	2.5	2.3	2.5	2.3	2.2	2.3
Isoleucine	4.4	4.5	4.4	4.7	4.6	4.6
Leucine	7.5	7.6	7.3	8.4	8.5	8.2
Total lysine	6.0	4.6	6.1	7.3	6.1	6.8
OMIU-reactive lysine	4.8	3.2	3.3	4.0	2.9	2.9
Methionine	2.1	2.0	1.7	2.5	2.5	2.3
Phenylalanine	5.0	5.0	4.8	3.6	3.6	3.5
Threonine	4.1	4.1	4.0	5.1	5.1	5.0
Tryptophan	1.3	1.3	1.3	1.6	1.6	1.5
Valine	4.7	4.8	4.7	5.8	5.7	5.8
Dispensable AAs (g/100 g CP)						
Alanine	4.3	4.3	3.9	4.0	4.0	3.7
Aspartic acid	11.0	11.1	9.7	6.6	6.5	6.0
Cysteine	1.4	1.3	1.3	1.9	1.9	1.9
Glutamic acid	17.6	17.8	21.3	14.7	14.7	17.4
Glycine	4.1	4.2	3.7	4.6	4.6	4.3
Proline	5.1	5.1	4.4	5.5	5.5	5.1
Serine	4.9	5.0	4.4	3.9	3.9	3.5
Tyrosine	3.3	3.3	3.3	2.5	2.5	2.6

DM = dry matter; OMIU = O-methylisourea.

<sup>1</sup>Adapted from Hulshof *et al.* (2016b).<sup>2</sup>Processing consisted of the addition of lignosulfonate to SBM and RSM and secondary toasting at 95 ± 2°C for 30 min.<sup>3</sup>The pSBM + AA and pRSM + AA diets were formulated on standardized ileal digestible AA levels in the pSBM and pRSM, respectively, and supplemented with crystalline AA to meet standardized ileal digestible AA levels in the SBM and RSM diets, respectively.<sup>4</sup>Lignosulfonate typically contains 35% to 42% Mg-lignosulfonate, 15% to 25% xylose, 3% to 7% glucose, 3% to 7% mannose, 3% to 7% rhamnose, 12% to 41% water (add up to 100%). The CP level was analyzed as 3.2 g/kg as-is.<sup>5</sup>The vitamin–mineral premix provided per kilogram complete diet: vitamin A, 10 000 IU; vitamin D<sub>3</sub>, 2000 IU; vitamin E, 40 mg; vitamin K<sub>3</sub>, 1.5 mg; thiamin, 1.0 mg; riboflavin, 4.0 mg; pyridoxine, 1.5 mg; vitamin B<sub>12</sub>, 20 µg; niacin, 30 mg; D-pantothenic acid, 15 mg; choline chloride, 150 mg; folic acid, 0.4 mg; biotin, 0.05 mg; Fe, 100 mg as iron sulfate; Cu, 160 mg as copper sulfate; Mn, 30 mg as manganese oxide; Zn, 70 mg as zinc sulfate; Co, 0.21 mg as cobalt sulfate; I, 0.70 mg as potassium iodate; Se, 0.25 mg as sodium selenite; anti-oxidant, 125 mg.<sup>6</sup>Added to maintain a constant diet cation/anion balance.<sup>7</sup>Net energy = 10.8 × apparent total tract digestible CP + 36.1 × apparent total tract digestible acid-hydrolyzed ether extract + 13.7 × starch + 12.4 × sugars + 9.6 × apparent total tract digestible non-starch polysaccharides (CVB, 2011). Apparent total tract digestibility of the protein sources is given in Hulshof *et al.* (2016b).

**Table 3** Calculated standardized ileal digestible (SID) amino acid (AA) concentration (g/kg as-is)<sup>1</sup> of six experimental diets containing soybean meal (SBM), processed SBM<sup>2</sup> (pSBM), pSBM supplemented with crystalline AAs (pSBM + AA<sup>3</sup>), rapeseed meal (RSM), processed RSM<sup>2</sup> (pRSM) or pRSM supplemented with crystalline AAs (pRSM + AA<sup>3</sup>) fed to growing pigs

AAs <sup>4</sup>	Diet					
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA
Histidine	3.65 (43)	3.00 (58)	3.89 (42)	2.52 (31)	2.20 (36)	2.59 (32)
Isoleucine	6.39 (75)	5.96 (115)	6.86 (74)	5.14 (64)	4.83 (78)	5.30 (66)
Leucine	10.50 (124)	9.98 (193)	11.30 (122)	9.33 (116)	8.98 (146)	9.60 (120)
Lysine	8.50 (100)	5.17 (100)	9.23 (100)	8.07 (100)	6.17 (100)	7.97 (100)
OMIU-reactive lysine	8.97 (106)	5.02 (97)	9.09 (98)	7.95 (98)	5.69 (92)	7.53 (94)
Methionine	3.26 (38)	3.04 (59)	3.07 (33)	3.07 (38)	2.96 (48)	3.01 (38)
Methionine + cysteine	5.14 (60)	4.47 (86)	4.95 (54)	5.07 (63)	4.66 (76)	5.07 (64)
Phenylalanine	7.14 (84)	6.75 (131)	7.57 (82)	3.80 (47)	3.53 (57)	3.92 (49)
Phenylalanine + tyrosine	11.77 (139)	11.21 (217)	12.69 (137)	6.24 (77)	5.77 (94)	6.52 (82)
Threonine	5.64 (66)	5.17 (100)	5.98 (65)	5.43 (67)	5.05 (82)	5.48 (69)
Tryptophan	1.84 (22)	1.60 (31)	1.97 (21)	1.75 (22)	1.65 (27)	1.74 (22)
Valine	6.61 (78)	6.14 (119)	7.22 (78)	6.20 (77)	5.77 (94)	6.45 (81)

<sup>1</sup>Based on data published in Hulshof *et al.* (2016a).

<sup>2</sup>Processing consisted of the addition of lignosulfonate to SBM and RSM and secondary toasting at 95 ± 2°C for 30 min.

<sup>3</sup>The pSBM + AA and pRSM + AA diets were formulated on SID AA levels in pSBM and pRSM, respectively, and supplemented with crystalline AAs to meet SID AA levels in the SBM and RSM diets, respectively.

<sup>4</sup>The ratio between SID individual AAs and SID lysine is provided in parentheses. The requirements for SID AAs to SID lysine were as follows: 31 for histidine, 52 for isoleucine, 101 for leucine, 30 for methionine, 60 for methionine + cysteine, 54 for phenylalanine, 94 for phenylalanine + tyrosine, 65 for threonine, 22 for tryptophan and 70 for valine (Gloaguen *et al.*, 2013).

transformed and these *P*-values are included in the tables. The presented least squares means are of non-log transformed data. The *P*-values <0.05 were considered significant and *P*-values between 0.05 and 0.10 were considered indicative of a trend.

## Results

All pigs remained healthy during the whole experimental period. With regard to the nutrient composition of the protein sources and experimental diets, processing lowered the content of total sugars, arginine, total lysine and OMIU-reactive lysine in the protein sources (Table 1) and consequently in the diets (Table 2).

### Body composition

An interaction effect between diet type, comprising effects of processing and supplementing crystalline AA, and protein source was observed for the CP concentration in the organ fraction (*P* = 0.044; Table 4), carcass (*P* < 0.001; Table 5) and empty body (*P* < 0.001; Table 6). Processing decreased the CP concentration in the organ fraction, carcass and empty body with greater effects for SBM than for RSM and supplementing pSBM and pRSM with crystalline AA restored the CP concentration. The arginine, methionine, threonine and cysteine concentration in the organ fraction was lower (*P* < 0.05), and the histidine concentration in the carcass was greater (*P* < 0.05) for the SBM diets than for the RSM diets. Diet type affected the lysine (*P* < 0.001), cysteine (*P* = 0.046), glutamic acid (*P* = 0.003) and serine (*P* = 0.003) concentration in the organ fraction, and the tryptophan concentration (*P* = 0.027) in the carcass. Diet

type tended to affect (*P* = 0.067) the lysine content in the carcass. Processing lowered the concentrations of these AA and supplementing crystalline AA restored the concentrations. An interaction effect between diet type and protein source was observed for the histidine content in the empty body (*P* = 0.023; Table 6). Processing lowered the histidine content in the empty body for SBM, whereas this was the opposite for RSM. Supplementing crystalline AA resulted in an intermediate histidine content for both protein sources. A diet type effect was observed for the lysine (*P* = 0.015) and tryptophan (*P* = 0.043) content in the empty body with processing decreasing the content and supplementing crystalline AA increasing the content to levels equal to those for the SBM and RSM diets.

### Nutrient retention

An interaction effect between diet type and protein source was observed for nutrient retention (*P* < 0.05; *P* = 0.079 for ether extract; Table 7). Processing decreased water, ash, N and AA retention but the differences were smaller for RSM compared with SBM. Supplementing crystalline AA restored nutrient retention for both protein sources. Processing increased fat retention in the empty body and supplementing crystalline AA resulted in a fat retention similar to the SBM and RSM diets (*P* < 0.001 for diet type).

### Post-absorptive utilization of Nitrogen and amino acids

An interaction effect between diet type and protein source was observed for the post-absorptive utilization of N and all AA (*P* < 0.05; Table 8). Processing of SBM decreased the post-absorptive utilization of N and all AA, whereas processing of RSM only decreased the post-absorptive utilization of

**Table 4** Effect of protein source, diet type and their interaction on the amino acid (AA) composition of protein in the organ fraction of growing pigs fed one of six experimental diets: SBM, pSBM, pSBM + AA, RSM, pRSM or pRSM + AA<sup>1,2</sup>

Items	Diet						SEM	P-value		
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA		Protein source	Diet type	Protein source × diet type
CP (g/kg as-is)	146.3 <sup>a</sup>	138.1 <sup>c</sup>	144.3 <sup>ab</sup>	141.5 <sup>abc</sup>	139.5 <sup>bc</sup>	142.3 <sup>abc</sup>	1.2	0.080	<0.001	0.044
AAAs (g/100 g CP)										
Arginine	4.66	4.78	4.65	4.89	4.77	4.82	0.06	0.013	0.743	0.118
Histidine	3.15	3.09	3.11	3.16	3.10	3.11	0.03	0.813	0.218	0.975
Isoleucine	2.95	2.98	2.99	2.97	2.96	2.99	0.02	0.815	0.383	0.621
Leucine	9.07	8.95	9.08	9.07	9.02	9.04	0.06	0.850	0.292	0.651
Lysine	6.48 <sup>a</sup>	5.88 <sup>b</sup>	6.46 <sup>a</sup>	6.41 <sup>a</sup>	6.03 <sup>b</sup>	6.52 <sup>a</sup>	0.09	0.495	<0.001	0.442
Methionine	1.39	1.40	1.39	1.42	1.43	1.43	0.02	0.024	0.884	0.924
Phenylalanine	4.76	4.73	4.77	4.79	4.73	4.75	0.03	0.802	0.170	0.665
Threonine	3.74	3.75	3.76	3.81	3.77	3.79	0.02	0.038	0.762	0.506
Tryptophan	1.19	1.19	1.16	1.19	1.17	1.16	0.01	0.385	0.083	0.451
Valine	6.05	6.13	6.11	6.08	6.07	6.09	0.04	0.632	0.630	0.441
Alanine	6.40	6.48	6.49	6.44	6.47	6.46	0.05	0.979	0.476	0.792
Aspartic acid	9.10	9.00	9.07	9.08	8.89	9.00	0.07	0.245	0.137	0.838
Cysteine	1.12 <sup>ab</sup>	1.09 <sup>b</sup>	1.14 <sup>ab</sup>	1.13 <sup>ab</sup>	1.14 <sup>ab</sup>	1.15 <sup>a</sup>	0.01	0.014	0.046	0.275
Glutamic acid	11.17 <sup>ab</sup>	11.04 <sup>ab</sup>	11.19 <sup>ab</sup>	11.30 <sup>a</sup>	10.98 <sup>b</sup>	11.32 <sup>a</sup>	0.07	0.266	0.003	0.297
Glycine	6.13	6.32	6.15	6.21	6.24	6.22	0.07	0.696	0.237	0.411
Proline	4.57	4.73	4.73	4.67	4.70	4.66	0.05	1.000	0.167	0.225
Serine	4.15 <sup>ab</sup>	4.06 <sup>ab</sup>	4.14 <sup>ab</sup>	4.19 <sup>a</sup>	4.05 <sup>b</sup>	4.17 <sup>ab</sup>	0.03	0.458	0.003	0.662
Tyrosine	2.72	2.78	2.81	2.81	2.77	2.76	0.06	0.833	0.951	0.484

SBM = soybean meal; RSM = rapeseed meal.

<sup>a,b,c</sup>Least squares means within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>Diet type included the factor processing (pSBM and pRSM) and processing plus supplementing with crystalline AAs to standardized ileal digestible AA levels in the SBM (pSBM + AA) and RSM (pRSM + AA) diets. Processing consisted of addition of lignosulfonate to SBM and RSM and secondary toasting at  $95 \pm 2^\circ\text{C}$  for 30 min.

<sup>2</sup>Least squares means are reported for nine pigs for CP and six pigs for individual AAs.

N, leucine, phenylalanine, glutamic acid and serine. Supplementing pSBM with crystalline AA increased the post-absorptive utilization but the pSBM + AA diet had a lower post-absorptive utilization for retention of N, leucine, valine and glutamic acid. Supplementing pRSM with crystalline AA increased the post-absorptive utilization of N and AA with the post-absorptive utilization for retention of aspartic acid and glutamic acid being higher for the pRSM + AA diet than for the RSM diet.

## Discussion

In the current study, it was hypothesized that processing may reduce AA retention by a reduced ileal digestibility of AA but not by reduced post-absorptive AA utilization for retention. This was verified by supplementing diets containing pSBM and pRSM with crystalline AA based on SID AA levels in the SBM and RSM diet. According to our hypothesis, the AA supplementation was expected to restore N and AA retentions.

In general, the effects of processing on AA composition of body protein and nutrient retention were greater for SBM than for RSM. This might be related to a different degree of heat damage and a different level of crystalline AA supplementation between both protein sources and their respective diets.

## Body amino acid composition

The body AA composition of pigs fed the SBM and RSM diets was comparable with values reported in other studies (Kyriazakis *et al.*, 1993; Bikker *et al.*, 1994). Processing reduced the SID AA concentration in the diets (Hulshof *et al.*, 2016a). The subsequent decrease in body protein content and lysine concentration in body protein, mainly in the organ fraction, may have been caused by an inadequate supply of lysine and other AA and/or an imbalance between dietary AA. Batterham *et al.* (1990a) reported an increase in lysine content in whole body protein by an increase in dietary lysine level at constant AA to lysine ratio. Moreover other AA, for example, leucine, methionine, phenylalanine and tyrosine, in body protein were increased with increasing dietary lysine level, whereas glycine and arginine were reduced. This can be related to the deposition of different types of proteins. For example, muscle protein in the carcass has a different AA pattern compared with protein in bone and adipose tissue with the latter being relatively low in lysine (Wünsche *et al.*, 1983). Changing the AA profile by adding crystalline methionine at the expense of corn starch was also reported to affect the AA composition of body protein (Chung and Baker, 1992; Conde-Aguilera *et al.*, 2010). Moreover, an increase in dietary methionine reduced the amount of collagen, that is, glycine and proline, in the empty body

**Table 5** Effect of protein source, diet type and their interaction on the amino acid (AA) composition of protein in the carcass of growing pigs fed one of six experimental diets: SBM, pSBM, pSBM + AA, RSM, pRSM or pRSM + AA<sup>1,2</sup>

Items	Diet						SEM	P-value		
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA		Protein source	Diet type	Protein source × diet type
CP (g/kg as-is)	174.5 <sup>a</sup>	145.0 <sup>c</sup>	173.6 <sup>a</sup>	160.9 <sup>b</sup>	151.2 <sup>c</sup>	160.1 <sup>b</sup>	1.7	<0.001	<0.001	<0.001
AA (g/100 g CP)										
Arginine	6.42	6.25	6.37	6.39	6.34	6.32	0.09	0.982	0.487	0.698
Histidine	2.87 <sup>a</sup>	2.71 <sup>ab</sup>	2.81 <sup>a</sup>	2.51 <sup>b</sup>	2.77 <sup>ab</sup>	2.66 <sup>ab</sup>	0.07	0.011	0.703	0.016
Isoleucine	3.80	3.58	3.71	3.68	3.70	3.82	0.10	0.664	0.409	0.390
Leucine	6.87	6.47	6.73	6.64	6.67	6.87	0.15	0.780	0.290	0.327
Lysine	7.03	6.50	7.01	6.80	6.84	7.12	0.16	0.609	0.067	0.242
Methionine	2.08	1.97	2.05	2.00	2.00	2.11	0.05	0.919	0.157	0.355
Phenylalanine	3.85	3.64	3.81	3.68	3.78	3.79	0.08	0.795	0.473	0.138
Threonine	3.80	3.61	3.80	3.71	3.75	3.86	0.08	0.614	0.230	0.383
Tryptophan	0.92 <sup>ab</sup>	0.85 <sup>b</sup>	0.97 <sup>a</sup>	0.89 <sup>ab</sup>	0.90 <sup>ab</sup>	0.91 <sup>ab</sup>	0.02	0.375	0.027	0.073
Valine	4.51	4.25	4.49	4.41	4.41	4.51	0.10	0.751	0.204	0.442
Alanine	6.19	5.99	6.19	6.14	6.15	6.03	0.09	0.829	0.578	0.247
Aspartic acid	8.15	7.72	8.16	7.97	7.99	8.28	0.16	0.609	0.093	0.362
Cysteine	0.98	0.94	0.95	0.99	0.94	1.00	0.04	0.474	0.412	0.698
Glutamic acid	13.48	12.90	13.46	13.35	13.33	13.55	0.25	0.535	0.290	0.554
Glycine	8.26	8.33	8.30	8.55	8.22	7.64	0.27	0.458	0.268	0.219
Proline	5.78	5.87	5.98	5.92	5.86	5.54	0.15	0.413	0.776	0.154
Serine	3.78	3.63	3.82	3.73	3.72	3.79	0.07	0.962	0.195	0.559
Tyrosine	2.88	2.72	2.83	2.78	2.81	2.91	0.07	0.642	0.341	0.339

SBM = soybean meal; RSM = rapeseed meal.

<sup>a,b,c</sup>Least squares means within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>Diet type included the factor processing (pSBM and pRSM) and processing plus supplementing with crystalline AAs to standardized ileal digestible AA levels in the SBM (pSBM + AA) and RSM (pRSM + AA) diets.

<sup>2</sup>Least squares means are reported for nine pigs for CP and six pigs for individual AAs.

(Chung and Baker, 1992). Thus, processing in the current study might have resulted in the deposition of different types of proteins, with the organ fraction being most susceptible to these effects. The effects of processing on body AA composition were ameliorated by supplementing crystalline AA.

#### Limiting dietary amino acids

The experimental diets were formulated to be equally limiting in SID isoleucine, leucine, lysine, methionine, methionine + cysteine, threonine and valine. The SBM diet complied well with these requirements, whereas lysine and methionine + cysteine were first limiting in the pSBM and pSBM + AA diet, respectively (Table 3). The three RSM diets may have been first limiting in aromatic AA (phenylalanine and tyrosine), which were not supplemented to the RSM diet. The SID AA concentration was slightly different between the SBM/RSM and pSBM + AA/pRSM + AA diets because of small differences between AA concentration used for diet formulation and for calculating the actual SID AA concentration.

#### Nutrient retention and post-absorptive utilization of standardized ileal digestible amino acid for retention

Each AA had a different post-absorptive utilization for retention which was also dependent on protein source.

This is in accordance with Batterham (1992) who reported different post-absorptive utilizations for the retention of lysine, threonine, methionine and tryptophan for cottonseed meal, meat-and-bone meal and SBM.

In the current study, it was expected that supplementation with crystalline AA to processed protein sources would not restore nutrient retention when, for example, absorbed AA could not fully be utilized by the pig. This was previously reported for cottonseed meal (Batterham *et al.*, 1990b), heat-treated fish meal (Wiseman *et al.*, 1991) and heat-treated field peas (Van Barneveld *et al.*, 1994b). For example, early Maillard reaction products, resulting from the reaction between sugars and the free  $\epsilon$ -amino group of lysine, were reported to be absorbed in the small intestine (Moughan *et al.*, 1996) but were unavailable for protein synthesis in growing pigs (Rerat *et al.*, 2002). The results of the current study, however, indicated that supplementing the pSBM and pRSM diets with crystalline AA increased nutrient retention to similar levels as for the SBM and RSM diets. Moreover, the SID lysine and SID OMIU-reactive lysine concentrations of the protein sources were similar (Hulshof *et al.*, 2016a) and the post-absorptive utilization of lysine and OMIU-reactive lysine were comparable within each experimental diet (Table 8) indicating that the absorption of early Maillard reaction products may have played a minor role. Thus, the

**Table 6** Effect of protein source, diet type and their interaction on the amino acid (AA) composition of protein in the empty body<sup>1</sup> of growing pigs fed one of six experimental diets: SBM, pSBM, pSBM + AA, RSM, pRSM or pRSM + AA<sup>2,3</sup>

Items	Diet						SEM	P-value		
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA		Protein source	Diet type	Protein source × diet type
CP (g/kg as-is)	169.6 <sup>a</sup>	143.8 <sup>c</sup>	168.1 <sup>a</sup>	157.4 <sup>b</sup>	149.2 <sup>c</sup>	157.0 <sup>b</sup>	1.5	<0.001	<0.001	<0.001
AAs (g/100 g CP)										
Arginine	6.16	6.00	6.11	6.14	6.09	6.08	0.08	0.774	0.390	0.709
Histidine	2.92 <sup>a</sup>	2.78 <sup>ab</sup>	2.87 <sup>ab</sup>	2.61 <sup>b</sup>	2.82 <sup>ab</sup>	2.74 <sup>ab</sup>	0.06	0.013	0.789	0.023
Isoleucine	3.67	3.48	3.60	3.56	3.58	3.68	0.08	0.728	0.375	0.357
Leucine	7.20	6.89	7.10	7.04	7.04	7.21	0.13	0.751	0.312	0.430
Lysine	6.95 <sup>ab</sup>	6.40 <sup>b</sup>	6.93 <sup>ab</sup>	6.74 <sup>ab</sup>	6.71 <sup>ab</sup>	7.02 <sup>a</sup>	0.14	0.564	0.015	0.180
Methionine	1.97	1.88	1.94	1.91	1.91	2.00	0.04	0.835	0.158	0.297
Phenylalanine	3.98	3.82	3.96	3.86	3.93	3.94	0.06	0.818	0.480	0.207
Threonine	3.80	3.63	3.80	3.73	3.76	3.85	0.07	0.555	0.223	0.390
Tryptophan	0.96 <sup>ab</sup>	0.91 <sup>b</sup>	1.00 <sup>a</sup>	0.94 <sup>ab</sup>	0.94 <sup>ab</sup>	0.95 <sup>ab</sup>	0.02	0.346	0.043	0.105
Valine	4.74	4.57	4.74	4.69	4.67	4.76	0.08	0.759	0.297	0.625
Alanine	6.22	6.08	6.23	6.19	6.20	6.10	0.08	0.825	0.678	0.252
Aspartic acid	8.29	7.94	8.31	8.15	8.14	8.39	0.14	0.692	0.092	0.446
Cysteine	1.00	0.97	0.98	1.01	0.98	1.02	0.03	0.389	0.399	0.805
Glutamic acid	13.14	12.58	13.10	13.01	12.95	13.20	0.21	0.519	0.190	0.521
Glycine	7.94	7.99	7.96	8.16	7.91	7.41	0.22	0.451	0.253	0.231
Proline	5.60	5.68	5.79	5.72	5.67	5.41	0.13	0.397	0.800	0.137
Serine	3.83	3.70	3.87	3.80	3.77	3.85	0.06	0.900	0.129	0.647
Tyrosine	2.85	2.73	2.83	2.79	2.81	2.89	0.06	0.640	0.320	0.428

SBM = soybean meal; RSM = rapeseed meal.

<sup>a,b,c</sup>Least squares means within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>Empty body is the sum of the organ fraction and carcass.

<sup>2</sup>Diet type included the factor processing (pSBM and pRSM) and processing plus supplementing with crystalline AAs to standardized ileal digestible AA levels in the SBM (pSBM + AA) and RSM (pRSM + AA) diets.

<sup>3</sup>Least squares means are reported for nine pigs for CP and six pigs for individual AAs.

decrease in nutrient retention was caused by a reduction in AA digestibility. Correcting N and AA retention for SID N and AA intake (i.e. referred to as post-absorptive utilization) would, therefore, not be affected by processing. Processing of SBM, however, reduced the utilization of SID AA for retention. This was not because energy or AA other than lysine were limiting in the pSBM diet. The NE level was similar between diets (Table 2) and the AA to lysine ratio were above maintenance requirements in the pSBM diet (Table 3). Utilization of AA has been shown to depend on the indispensable to dispensable AA ratio with higher utilizations at a lower indispensable to dispensable AA ratio (Lenis *et al.*, 1999). The SID indispensable AA to SID dispensable AA ratio of the SBM and pSBM diets were, however, similar for the SBM and pSBM diets (47 : 53 and 46 : 54, respectively). The reduced post-absorptive AA utilization might result from a relatively higher amount of SID AA being used for maintenance for the pSBM diet compared with the SBM diet. The post-absorptive AA utilization above maintenance were calculated using maintenance requirements given by Fuller (1991) and Van Milgen *et al.* (2008). This calculation, however, gave similar results for the SBM and pSBM diets as in Table 8 (data not shown) indicating that AA of the pSBM diet were less efficiently used for growth than of the SBM diet. As lysine was first limiting in the pSBM diet, other AA could not

be fully utilized resulting in an increased oxidation of these AA. The involvement of lysine in metabolic pathways used to remove excess AA, such as deamination via amino-transferase and in the citric acid cycle via acetyl CoA (Berg *et al.*, 2002), might result in an increased catabolism of lysine which subsequently reduces the post-absorptive utilization for retention. An increased catabolism of AA may result in an increased liver weight which was reported for pigs fed the pSBM diet (Hulshof *et al.*, 2016b). Another possibility for the reduced post-absorptive utilization of AA for the pSBM diet is an overestimation of SID lysine of the pSBM protein source. The SID lysine was calculated based on values of a previous study (Hulshof *et al.*, 2016a). However, the same batches of protein sources were used for both studies and the quality of the protein sources, as measured by analyzing the OMIU-reactive and total lysine contents, had not decreased over time. It is, therefore, unlikely that the digestibility of lysine was decreased by the additional formation of lysine complexes, such as Maillard reaction products during the time between the two studies. The diets in both studies contained the same feed ingredients in similar proportions and, therefore, digestibility of the diet was expected to be similar as well. As correcting AA retention for SID AA intake for the RSM and pRSM diets resulted in similar post-absorptive utilizations, it is likely that SID AA levels were not

**Table 7** Effect of protein source, diet type and their interaction on nutrient retention (g/day) in the empty body of growing pigs fed one of six experimental diets: SBM, pSBM, pSBM + AA, RSM, pRSM or pRSM + AA<sup>1,2</sup>

Items	Diet						SEM	P-value		
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA		Protein source	Diet type	Protein source × diet type
Water	390.5 <sup>a</sup>	223.2 <sup>c</sup>	374.5 <sup>a</sup>	299.1 <sup>b</sup>	229.3 <sup>c</sup>	310.3 <sup>b</sup>	6.3	<0.001	<0.001	<0.001
Ash	17.8 <sup>a</sup>	12.6 <sup>b</sup>	17.1 <sup>a</sup>	13.6 <sup>b</sup>	12.8 <sup>b</sup>	13.9 <sup>b</sup>	0.6	<0.001	<0.001	0.001
Ether extract	96.4 <sup>c</sup>	118.0 <sup>ab</sup>	89.2 <sup>c</sup>	110.2 <sup>b</sup>	124.1 <sup>a</sup>	110.2 <sup>b</sup>	3.2	<0.001	<0.001	0.079
N	16.6 <sup>a</sup>	8.3 <sup>c</sup>	15.6 <sup>a</sup>	12.0 <sup>b</sup>	9.3 <sup>c</sup>	12.3 <sup>b</sup>	0.2	<0.001	<0.001	<0.001
AAs										
Arginine	6.53 <sup>a</sup>	3.20 <sup>c</sup>	5.88 <sup>a</sup>	4.51 <sup>b</sup>	3.54 <sup>c</sup>	4.61 <sup>b</sup>	0.15	<0.001	<0.001	<0.001
Histidine	3.32 <sup>a</sup>	1.61 <sup>c</sup>	2.98 <sup>a</sup>	1.97 <sup>bc</sup>	1.80 <sup>bc</sup>	2.20 <sup>b</sup>	0.09	<0.001	<0.001	<0.001
Isoleucine	3.94 <sup>a</sup>	1.84 <sup>d</sup>	3.49 <sup>a</sup>	2.59 <sup>bc</sup>	2.10 <sup>cd</sup>	2.87 <sup>b</sup>	0.14	<0.001	<0.001	<0.001
Leucine	7.64 <sup>a</sup>	3.61 <sup>c</sup>	6.83 <sup>a</sup>	5.09 <sup>b</sup>	4.07 <sup>c</sup>	5.54 <sup>b</sup>	0.22	<0.001	<0.001	<0.001
Lysine	7.48 <sup>a</sup>	3.31 <sup>c</sup>	6.81 <sup>a</sup>	4.93 <sup>b</sup>	3.91 <sup>c</sup>	5.52 <sup>b</sup>	0.23	<0.001	<0.001	<0.001
Methionine	2.15 <sup>a</sup>	1.02 <sup>d</sup>	1.92 <sup>a</sup>	1.42 <sup>bc</sup>	1.13 <sup>cd</sup>	1.60 <sup>b</sup>	0.07	<0.001	<0.001	<0.001
Phenylalanine	4.24 <sup>a</sup>	2.02 <sup>d</sup>	3.84 <sup>a</sup>	2.78 <sup>bc</sup>	2.30 <sup>cd</sup>	3.00 <sup>b</sup>	0.12	<0.001	<0.001	<0.001
Threonine	4.02 <sup>a</sup>	1.90 <sup>d</sup>	3.68 <sup>a</sup>	2.70 <sup>bc</sup>	2.19 <sup>cd</sup>	2.97 <sup>b</sup>	0.12	<0.001	<0.001	<0.001
Tryptophan	1.01 <sup>a</sup>	0.46 <sup>d</sup>	0.98 <sup>a</sup>	0.67 <sup>bc</sup>	0.53 <sup>cd</sup>	0.71 <sup>b</sup>	0.03	<0.001	<0.001	<0.001
Valine	4.99 <sup>a</sup>	2.38 <sup>c</sup>	4.56 <sup>a</sup>	3.38 <sup>b</sup>	2.69 <sup>c</sup>	3.62 <sup>b</sup>	0.15	<0.001	<0.001	<0.001
Alanine	6.55 <sup>a</sup>	3.21 <sup>c</sup>	6.02 <sup>a</sup>	4.50 <sup>b</sup>	3.60 <sup>c</sup>	4.56 <sup>b</sup>	0.15	<0.001	<0.001	<0.001
Aspartic acid	8.81 <sup>a</sup>	4.18 <sup>c</sup>	8.08 <sup>a</sup>	5.92 <sup>b</sup>	4.72 <sup>c</sup>	6.49 <sup>b</sup>	0.23	<0.001	<0.001	<0.001
Cysteine	1.08 <sup>a</sup>	0.52 <sup>d</sup>	0.95 <sup>ab</sup>	0.76 <sup>bc</sup>	0.57 <sup>cd</sup>	0.80 <sup>b</sup>	0.04	0.001	<0.001	0.002
Glutamic acid	13.95 <sup>a</sup>	6.62 <sup>c</sup>	12.70 <sup>a</sup>	9.52 <sup>b</sup>	7.54 <sup>c</sup>	10.17 <sup>b</sup>	0.39	<0.001	<0.001	<0.001
Glycine	8.29 <sup>a</sup>	4.30 <sup>c</sup>	7.65 <sup>a</sup>	6.02 <sup>b</sup>	4.53 <sup>c</sup>	5.27 <sup>bc</sup>	0.30	<0.001	<0.001	<0.001
Proline	5.92 <sup>a</sup>	3.12 <sup>d</sup>	5.73 <sup>a</sup>	4.26 <sup>b</sup>	3.35 <sup>cd</sup>	4.01 <sup>bc</sup>	0.17	<0.001	<0.001	<0.001
Serine	4.04 <sup>a</sup>	1.94 <sup>c</sup>	3.76 <sup>a</sup>	2.76 <sup>b</sup>	2.17 <sup>c</sup>	2.94 <sup>b</sup>	0.10	<0.001	<0.001	<0.001
Tyrosine	3.01 <sup>a</sup>	1.41 <sup>d</sup>	2.71 <sup>a</sup>	1.99 <sup>bc</sup>	1.61 <sup>cd</sup>	2.21 <sup>b</sup>	0.10	<0.001	<0.001	<0.001

SBM = soybean meal; RSM = rapeseed meal; AA and AAs = amino acid(s).

<sup>a,b,c,d</sup>Least squares means within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>Diet type included the factor processing (pSBM and pRSM) and processing plus supplementing with crystalline amino acids (AAs) to standardized ileal digestible AA levels in the SBM (pSBM + AA) and RSM (pRSM + AA) diets.

<sup>2</sup>Least squares means are reported for nine pigs for water, ash, ether extract and N, and six pigs for individual AAs.

overestimated. The similar post-absorptive AA utilizations for the RSM and pRSM diets indicate that there was no additional effect of processing of RSM beyond effects on digestibility.

The reduced post-absorptive utilization of AA for the pSBM + AA diet compared with the SBM diet might be explained by a shift in first limiting AA. Methionine + cysteine became limiting for nutrient retention in the pSBM + AA diet which may explain why other AA could not be used with the same efficiency as for the SBM diet. In general, the utilization of methionine + cysteine for retention is lower than of lysine because methionine is used for several metabolic processes and, thus, has higher requirements for maintenance than lysine which is mainly used for protein synthesis and, thus, for retention (Chung and Baker, 1992; Heger *et al.*, 2002). The higher post-absorptive utilization of aspartic acid and glutamic acid for the pRSM + AA diet compared with the RSM diet might also be explained by a shift in first limiting AA. Phenylalanine + tyrosine were less limiting in the pRSM + AA diet compared with the RSM diet (Table 3), which presumably resulted in a higher utilization of these AA. Moreover, catabolism of AA might have been lower as well as the liver weight was lower for the pRSM + AA

diet than for the RSM diet (Hulshof *et al.*, 2016b). Thus, supplementing crystalline AA restored the post-absorptive utilization of SID AA for retention to a large extent.

In conclusion, (over-)processing of SBM and RSM reduced nutrient retention by reducing AA digestibility. Processing of SBM negatively affected the utilization of SID AA for retention which might be related to an imbalanced post-absorptive AA supply. Supplementing crystalline AA based on SID AA content in pSBM largely restored utilization but effects may have been influenced by a limiting methionine + cysteine supply. Processing of RSM did not affect post-absorptive AA utilization, whereas supplementing crystalline AA slightly increased utilization presumably because phenylalanine was less limiting. Although processing reduced the protein content in the organ fraction, carcass and empty body, the AA composition of protein in the carcass and empty body was rather constant. Processing reduced the lysine content of protein in the organ fraction. Supplementing crystalline AA restored AA composition and nutrient retention for both protein sources. In practice, supplementing crystalline AA to diets containing over-processed feed ingredients can be a useful tool to compensate for the negative effects of processing on nutrient retention.

**Table 8** Effect of protein source, diet type and their interaction on the utilization (%) of standardized ileal digestible N and individual amino acids (AAs) for retention<sup>1</sup> in the empty body of growing pigs fed one of six experimental diets: SBM, pSBM, pSBM + AA, RSM, pRSM or pRSM + AA<sup>2,3</sup>

Items	Diet							P-value		
	SBM	pSBM	pSBM + AA	RSM	pRSM	pRSM + AA	SEM	Protein source	Diet type	Protein source × diet type
N	65.9 <sup>a</sup>	38.2 <sup>c</sup>	60.7 <sup>b</sup>	67.0 <sup>a</sup>	57.9 <sup>b</sup>	67.6 <sup>a</sup>	1.2	<0.001	<0.001	<0.001
AAs										
Arginine	53.7 <sup>b</sup>	31.2 <sup>c</sup>	45.2 <sup>b</sup>	68.4 <sup>a</sup>	63.1 <sup>a</sup>	66.5 <sup>a</sup>	2.1	<0.001	<0.001	0.002
Histidine	80.3 <sup>a</sup>	47.6 <sup>b</sup>	68.8 <sup>a</sup>	69.9 <sup>a</sup>	72.2 <sup>a</sup>	73.7 <sup>a</sup>	2.9	0.013	<0.001	<0.001
Isoleucine	54.1 <sup>a</sup>	27.3 <sup>d</sup>	45.9 <sup>abc</sup>	44.9 <sup>bc</sup>	38.1 <sup>c</sup>	47.4 <sup>ab</sup>	2.1	0.555	<0.001	<0.001
Leucine	64.0 <sup>a</sup>	32.1 <sup>c</sup>	54.5 <sup>b</sup>	49.0 <sup>b</sup>	40.1 <sup>c</sup>	50.7 <sup>b</sup>	2.0	0.034	<0.001	<0.001
Lysine	77.6 <sup>a</sup>	56.8 <sup>bc</sup>	66.7 <sup>ab</sup>	54.6 <sup>c</sup>	55.8 <sup>bc</sup>	60.8 <sup>bc</sup>	2.7	<0.001	0.003	<0.001
OMIU-reactive lysine	73.3 <sup>a</sup>	58.4 <sup>b</sup>	67.6 <sup>ab</sup>	55.6 <sup>b</sup>	60.7 <sup>b</sup>	64.4 <sup>ab</sup>	2.8	0.011	0.072	0.004
Methionine	58.0 <sup>a</sup>	29.6 <sup>d</sup>	56.5 <sup>a</sup>	41.4 <sup>bc</sup>	33.8 <sup>cd</sup>	46.6 <sup>b</sup>	2.0	<0.001	<0.001	<0.001
Cysteine	50.6 <sup>a</sup>	32.3 <sup>b</sup>	45.4 <sup>a</sup>	34.1 <sup>b</sup>	29.5 <sup>b</sup>	34.3 <sup>b</sup>	2.1	<0.001	<0.001	0.013
Methionine + cysteine	55.3 <sup>a</sup>	30.4 <sup>c</sup>	52.2 <sup>a</sup>	38.5 <sup>bc</sup>	32.2 <sup>c</sup>	41.6 <sup>b</sup>	2.0	<0.001	<0.001	<0.001
Phenylalanine	52.3 <sup>bc</sup>	26.5 <sup>d</sup>	45.7 <sup>c</sup>	65.6 <sup>a</sup>	57.5 <sup>b</sup>	66.8 <sup>a</sup>	2.2	<0.001	<0.001	0.002
Threonine	62.3 <sup>a</sup>	32.4 <sup>e</sup>	55.5 <sup>ab</sup>	44.8 <sup>cd</sup>	38.5 <sup>de</sup>	47.8 <sup>bc</sup>	1.9	<0.001	<0.001	<0.001
Tryptophan	48.0 <sup>a</sup>	25.6 <sup>d</sup>	44.8 <sup>a</sup>	34.4 <sup>bc</sup>	28.4 <sup>cd</sup>	35.6 <sup>b</sup>	1.6	<0.001	<0.001	<0.001
Valine	66.4 <sup>a</sup>	34.3 <sup>d</sup>	57.0 <sup>b</sup>	49.2 <sup>bc</sup>	41.3 <sup>cd</sup>	49.4 <sup>bc</sup>	2.0	0.001	<0.001	<0.001
Alanine	101.6 <sup>a</sup>	55.1 <sup>c</sup>	100.9 <sup>ab</sup>	100.0 <sup>ab</sup>	87.9 <sup>b</sup>	110.7 <sup>a</sup>	3.1	<0.001	<0.001	<0.001
Aspartic acid	50.3 <sup>c</sup>	27.1 <sup>d</sup>	53.2 <sup>c</sup>	81.3 <sup>b</sup>	73.0 <sup>b</sup>	99.7 <sup>a</sup>	2.8	<0.001	<0.001	<0.001
Glutamic acid	47.7 <sup>a</sup>	25.0 <sup>d</sup>	32.9 <sup>c</sup>	50.9 <sup>a</sup>	42.3 <sup>b</sup>	41.5 <sup>b</sup>	1.6	<0.001	<0.001	<0.001
Glycine	128.7 <sup>ab</sup>	76.9 <sup>d</sup>	137.0 <sup>a</sup>	113.5 <sup>abc</sup>	95.6 <sup>cd</sup>	108.6 <sup>bc</sup>	5.7	0.085	<0.001	0.001
Proline	73.9 <sup>ab</sup>	43.7 <sup>c</sup>	82.7 <sup>a</sup>	73.7 <sup>ab</sup>	69.3 <sup>b</sup>	82.4 <sup>ab</sup>	3.0	0.002	<0.001	<0.001
Serine	49.4 <sup>b</sup>	25.6 <sup>c</sup>	50.5 <sup>b</sup>	60.7 <sup>a</sup>	51.5 <sup>b</sup>	70.7 <sup>a</sup>	2.0	<0.001	<0.001	<0.001
Tyrosine	57.2 <sup>bc</sup>	28.1 <sup>d</sup>	48.0 <sup>c</sup>	73.3 <sup>a</sup>	63.9 <sup>ab</sup>	74.5 <sup>a</sup>	2.9	<0.001	<0.001	<0.001

SBM = soybean meal; RSM = rapeseed meal; OMIU = *O*-methylisourea.

<sup>a,b,c,d,e</sup>Least squares means within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>Utilization was calculated as the ratio between the amount of nutrient retained (g/day) divided by the daily intake of standardized ileal digestible nutrient (g/day).

<sup>2</sup>Diet type included the factor processing (pSBM and pRSM) and processing plus supplementing with crystalline AAs to standardized ileal digestible AA levels in the SBM (pSBM + AA) and RSM (pRSM + AA) diets.

<sup>3</sup>Least squares means are reported for nine pigs for N and six pigs for individual AAs.

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## Supplementary material

For supplementary materials referred to in this article, please visit <https://doi.org/10.1017/S1751731116002548>

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