Standardized ileal digestibility of amino acids from several cereal grains and protein-rich feedstuffs in broiler chickens at the age of 30 days[*]

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ABSTRACT

The standardized ileal amino acid (AA) digestibility (SID) of 3 cereals: wheat, barley and maize; 3 feeds with a medium protein content: faba beans, field pea and full-fat rape seeds; and 4 by-products: solvent-extracted rapeseed meal (RSM), rapeseed expeller cake (RSC), maize distillers dried grains with solubles (DDGS) and soyabean meal (SBM) was measured in 30-day-old broiler chickens. Digestibility values were determined with the direct method using semi-synthetic diets containing Cr_2O_3 as an indigestible marker. Each diet was offered *ad libitum* for 5 days to 4 replicate cages of 6 broilers and the ileal contents were collected by the slaughter method. A protein-free diet was fed to assess ileal endogenous AA losses (IEAA). These IEAA values were used to obtain SID coefficients for each of the tested ingredients. Considering the SID values of 14 of the most important AA, the test ingredients were ranked as follows. Within cereal grains: maize > wheat > barley. The exception was the SID of cysteine in maize which was lower compared with that in wheat. Within native seeds: faba beans = peas > rape seeds. However, the SID of methionine was greater in rape as compared with legume seeds. Within by-product feeds: SBM > RSC > RSM > DDGS. However, SID values for valine, phenylalanine and serine in DDGS were comparable to those obtained for SBM and higher than in the two rapeseed products.

KEY WORDS: chickens, ileal digestibility, amino acids, plant feeds

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INTRODUCTION

   The digestibility of AA in feeds for broiler chickens is commonly accepted as a sensitive indicator of the biological availability of these nutrients (Ravindran and Bryden, 1999; Lemme et al., 2004). Therefore, assessment of AA digestibility of raw materials is essential if broilers are to be fed with balanced diets assuring adequate provision of AA available for maintenance and production goals. A growing consensus also exists around the need to correct the apparent digestibility for AA of non-dietary origin. Since the additivity of apparent digestibility values in diet formulations remains questionable (Ravindran and Bryden, 1999), the standardized (true) digestibility of AA is preferred. Hence the determination of unabsorbed endogenous amino acids originating from digestive enzymes, mucoproteins and desquamated cells becomes very important (Lemme et al., 2004). Methods that have gained greater acceptance in the quantification of endogenous AA losses in poultry include feeding protein-free diets or feeding highly digestible protein sources, e.g., enzyme-hydrolysed (EHC) or guanidinated (GuC) casein (Ravindran and Bryden, 1999).

   Present knowledge about the physiological aspects of measuring AA digestibilities in poultry feed ingredients clearly suggests that ileal digesta sampling after sacrificing the birds should be applied to determine these values (Kadim et al., 2002; Lemme et al., 2004). In this method birds have free access to the test diets, which represents a common feeding pattern. Additionally, a major advantage of this approach is that birds of different age classes can be used. It is increasingly recognized that in birds, the digestibility of AA increases with age (Ten Doeschate et al., 1993; Batal and Parsons, 2002; Jamroz et al., 2002; Rezvani et al., 2007; Adedokun et al., 2008). A number of studies have shown that for some AA, coefficients determined by the ileal digesta-based method using market-age broilers (42-43 days) or by excreta analysis with adult cockerels are not applicable for feed formulations for younger chicks (Ten Doeschate et al., 1993; Huang et al., 2005; Garcia et al., 2007). This has been demonstrated for such plant ingredients as maize, wheat, soyabean and canola meals, and some cereal by-products. To date, however, reliable ileal digestibility values for AA in common feed ingredients, expressed either as apparent (Ravindran et al., 2005) or standardized (EHC method) coefficients (Lemme et al., 2004), are still limited to the data generated using 6-week-old broilers.

   Current research projects undertaken in our laboratory are aimed at testing locally-grown crops and by-product ingredients for ileal digestibility of their AA in broiler chickens younger than 42 days. These studies also involve validation of the assessed digestibility values in the growth experiments with birds of the given age class. The present paper reports on part of an ongoing study that has been carried

out with 30-day-old chickens with the aim of determining the ileal digestibility coefficients of AA in a variety of feeds, including common cereals, legume seeds, oilseed rape, and several plant-based by-products. In order to confirm that the presence of various anti-nutritional substances as well as processing technology may cause distinct impairment in the digestibility of individual AA, comparative analysis of the data is presented for each group of ingredients assayed.

MATERIAL AND METHODS

Raw materials and diets

A total of 10 feed ingredients was tested (Table 1), comprising 3 cereals: maize, wheat and barley; 3 samples of feeds with a medium (20-28%) crude protein (CP): native faba bean, field pea and winter rape (*Brassica napus*) rape seeds,

Table 1. Chemical composition of raw feeds used in the experiment, g/kg DM (unless otherwise stated)

Item	Feeds[1]									
	Ma	Wh	Ba	Fab	Pea	Rap	SBM	RSM	RSC	DDGS
Dry matter, g/kg	868.6	887.4	870.3	859.6	882.4	920.0	896.8	884.6	927.8	921.1
Crude protein	90.1	135.9	120.4	280.1	224.8	201.4	488.6	371.9	353.8	304.0
Crude fat	44.3	15.2	19.1	10.1	13.7	446.1	19.2	34.7	99.3	144.5
Crude ash	13.6	16.0	21.0	36.5	31.5	40.8	67.7	71.4	71.9	59.2
Crude fibre	33.7	29.6	49.3	86.1	75.2	167.3	53.0	168.1	172.2	113.3
Amino acids, g/16 g N										
arginine	4.2	4.7	4.9	10.2	8.7	8.0	7.2	6.3	6.1	3.8
histidine	2.6	2.5	2.3	2.5	2.3	2.6	2.7	2.9	2.5	2.4
isoleucine	3.3	3.1	3.3	4.2	4.2	3.9	4.3	4.0	3.9	3.4
leucine	11.2	6.1	6.6	7.5	7.1	6.9	7.6	7.2	6.8	11.9
lysine	2.9	2.7	3.5	6.8	7.6	6.1	6.2	5.6	5.6	2.2
methionine	2.3	1.5	1.7	0.6	1.0	2.0	1.3	1.9	2.1	1.8
phenylalanine	4.8	4.4	4.7	4.4	4.8	4.1	4.8	4.2	4.0	5.2
threonine	3.6	2.9	3.4	3.5	3.9	4.9	4.1	4.8	4.6	3.6
tryptophan	0.7	1.0	1.2	0.9	1.0	1.5	1.2	1.6	1.4	0.9
valine	4.6	4.1	4.7	4.6	4.7	5.3	4.8	5.3	5.1	4.8
alanine	7.5	3.4	3.8	4.1	4.5	4.5	4.4	4.5	4.4	7.2
aspartic acid	7.6	5.2	6.3	11.2	11.7	7.9	11.1	7.8	7.7	5.7
cystine	1.9	1.9	2.1	1.1	1.4	2.2	1.3	2.1	2.4	1.8
glutamic acid	17.9	28.0	21.7	16.5	16.5	15.2	17.6	16.6	15.8	13.0
glycine	3.7	3.9	3.7	4.3	4.5	5.5	4.3	5.4	5.2	3.5
proline	9.6	10.3	10.2	4.2	4.1	6.0	5.4	6.1	6.5	8.1
serine	4.7	4.6	4.1	5.1	4.9	4.8	5.5	4.9	4.6	4.9
tyrosine	1.8	1.2	1.2	3.0	2.6	3.3	3.3	3.0	2.8	3.9

[1] Ma - maize, Wh - wheat, Ba - barley, Fab - faba beans, Pea - pea seeds, Rap - full-fat rape seeds, SBM - soyabean meal, RSM - solvent-extracted rapeseed meal, RSC - rapeseed expeller cake, DDGS - maize distillers dried grains with solubles

and 4 samples with a high CP content. The materials classified as high protein ingredients (>30% CP in dry matter) included three locally obtained by-products: rapeseed meal (RSM), produced by prepress-solvent extraction technology with desolventizing-toasting as the final stage of meal processing; rapeseed expeller cake (RSC), obtained as a residue after oil pressing from mildly cooked and flaked seeds; maize distillers dried grains with solubles (DDGS), a co-product of ethanol production, with a moderately dark colour on visual evaluation; and a sample of Brazilian soyabean meal (SBM). Ingredients were purchased from commercial feed millers. Cereals, grain legumes and rape were cultivated in the south-central region of the country. Before dietary incorporation the test components were ground using a cutting mill (Pulverisette-15, Fritsch GmbH, Germany) fitted with a 3 mm sieve.

Eleven experimental diets were formulated. Test feeds served as the sole source of CP in the assay diets. The ingredient composition of these diets and calculated values for some nutrients are presented in Table 2. Cr_2O_3 oxide (POCH S.A. 250570421) was included in all experimental diets (0.5% on an air-dry matter basis) as an indigestible marker. In the case of cereals, diets were based on 940 g of test cereal/kg, had the same Ca and P supplementation, and rape seed oil was

Table 2. Composition of diets containing tested feeds and the protein-free diet (PFD) used in the experiment, g/kg

Item	Diet[1]										
	Ma	Wh	Ba	Rap	Fab	Pea	SBM	RSM	RSC	DDGS	PFD
Components											
test feeds	940	940	940	730	745	907	415	547	550	650	-
maize starch	-	-	-	227	152	10	416	280	324	218	687
rapeseed oil	17	17	17	-	60	40	20	72	20	20	55
sucrose	-	-	-	-	-	-	60	70	70	70	170
cellulose	-	-	-	-	-	-	46	-	-	-	45
dicalcium phosphate	20	20	20	20	20	20	20	13	18	20	20
limestone	10	10	10	10	10	10	10	5	5	9	10
NaCl	3	3	3	3	3	3	3	3	3	3	3
premix[2]	5	5	5	5	5	5	5	5	5	5	5
chromic oxide	5	5	5	5	5	5	5	5	5	5	5
Calculated[3]											
ME, MJ/kg	13.43	12.8	11.35	16.90	11.71	11.71	11.71	11.70	11.72	11.72	14.81
crude protein[4]	74 (73)	113 (114)	99 (100)	135 (137)	179 (181)	180 (178)	182 (184)	180 (182)	180 (181)	182 (183)	0 (1.5)

[1] refer to footnote 1 for Table 1 for information on test feeds; [2] premix provided per kg of diet; mg: retinyl palmitate 6.6, cholecalciferol 0.08, dl-α-tocopheryl acetate 40, menadion 2.25, thiamin 2.0, riboflavin 7.25, pyridoxine 4.25, cyanocobalamine 0.03, biotin 0.1, folic acid 1.0, nicotinic acid 40, calcium pantothenate 12, choline chloride 450, Fe 65, Zn 65, Mn 100, Cu 15, I 0.8, Se 0.25, Co 0.4, diclazuril 1; [3] calculated as a sum of crude protein content and energy value of feed ingredients; [4] values in parenthesis represent the analysed concentrations

added to reduce dustiness. Diets containing feedstuffs of medium and high CP levels were formulated, where possible, to be isoenergetic and isonitrogenous by varying the amounts of maize starch (Sigma-Aldrich S4126), rape seed oil, sucrose and purified cellulose (Sigma-Aldrich C8002). For full-fat rape seeds this was not possible. A protein-free diet (PFD) was used to determine the basal ileal endogenous losses of AA. The composition of this diet was that given by Kadim et al. (2002). All of the diets were prepared in the form of a dry mash.

Birds and housing

Male and female Ross 308 broiler chickens (initial number, 280 birds, sex ratio 1:1) were raised in battery brooders and fed on a commercial starter diet (22% CP) during the first 18 days posthatching. Feed and water were offered *ad libitum*. On day 19 of age, the chickens were weighed and birds with extreme body weights were rejected. Then experimental birds were randomly assigned to 44 wire cages, with 3 males and 3 females per cage (0.13 m^2 of cage space per bird), and housed under constant light at a temperature of 24°C. The broilers were submitted to a 6-days period of acclimatization period to the cages, during which they were offered the same starter diet. At the start of the digestibility assay the birds were 24 days-old (average body weight 850±7 g).

All animal care and experimental procedures were reviewed and approved by the Local Ethics Committee for Animal Experimentation.

Experimental procedure

Chickens were distributed onto the experimental diets with 4 replicate cages for each diet. The ileal contents were collected on day 30. Feeding of the birds and the digesta sampling and processing were carried out using procedures adapted from Kadim and Moughan (1997). In brief, the birds were allowed to consume *ad libitum* the respective diets for 5 days and were deprived of feed for the next 24 h. The chickens were then given free access to their diet for 1 h and feed intake in birds placed on the PFD diet was recorded. All chickens were killed by a lethal injection of sodium thiopentone (Thiopental, Sandoz GmbH, Austria) at 4 h following the start of feeding. After euthanasia, the abdominal cavity was opened and the portion of the small intestine from the vitelline diverticulum to approximately 2 cm proximal to the ileo-caecal junction was removed. The contents of the distal ileum (20 cm adjacent to the ileo-caecal junction) were flushed with deionised water, pooled within a cage (6 birds), deep-frozen (-20°C) and freeze-dried to await analysis.

Chemical analyses

The samples of the respective feeds were ground to pass a 1 mm sieve screen before analysis. The freeze-dried ileal digesta samples were ground with a mortar and pestle. All analyses were performed in duplicate. The concentrations of proximate nutrients in test feedstuffs, and dry matter (DM) and CP (N × 6.25) in diets and lyophilized digesta were analysed according to AOAC (2000) procedures. The chromium contents of diet and ileal digesta samples were measured by the method of Saha and Gilbreath (1991) using a Carl Zeiss 1N atomic absorption spectrophotometer after wet mineralization of the samples in $HNO_3/HClO_4$ (1:1.5) mixture. Prior to AA analyses, all samples were hydrolysed in 6 *N* HCl at 110°C for 22 h. For determination of methionine and cystine, preliminary oxidation with performic acid was carried out. Tryptophan was assayed following alkaline hydrolysis of samples in a barium hydroxide solution (110°C, 18 h). All AA were determined by HPLC after postcolumn ninhydrin derivatization (AOAC, 2000) using a Beckman 126AA System Gold analyser. Amino acid concentrations were corrected for incomplete recovery resulting from hydrolysis.

Calculations and statistical analysis

The ileal endogenous loss of each AA (IEAA) was determined based on the ileal flow in chickens fed the protein-free diet using the formula proposed by Moughan et al. (1992):

$$\text{IEAA (mg/kg DM intake)} = [\text{AA in digesta, mg/kg DM}] \times [(\text{diet Cr, mg/kg DM}) / (\text{digesta Cr, mg/kg DM})]$$

The coefficients of apparent ileal digestibility of amino acids (AID) were calculated using Cr_2O_3 as the inert marker according to the formula of Kadim and Moughan (1997). By correcting the AID for IEAA, coefficients of standardized ileal digestibility (SID) for AA were calculated according to the following equation:

$$\text{SID (\%)} = \text{AID (\%)} + [(\text{IEAA} / \text{AA in diet, mg/kg DM}) \times 100]$$

Digestibility data for each of the three groups of feed ingredients were subjected to analysis of variance using the Statistica ver. 6 software system (www.statsoft.com). If the F-ratio indicated significance, the differences between means were separated using Tukey's HSD test. The level of significance was set at $P < 0.05$.

RESULTS

The digestibility coefficients (SID) and the comparison of the assessed values are shown for 14 representative AA selected because of their nutritional essentiality and/or importance for broiler chickens (Smulikowska and Rutkowski, 2005).

Endogenous amino acid outputs. The endogenous amino acid flows at the terminal ileum for the broilers are presented in Table 3. Methionine, tryptophan and cystine were the AA with the lowest values, respectively, 1.5, 1.7 and 1.8% of total AA flow. The most abundant AA in the ileal endogenous protein were glutamic acid, aspartic acid, leucine and proline, ranging from 12.8 to 7.9% of the total AA flow.

Table 3. Ileal endogenous amino acid flows determined in chickens fed protein-free diet, mg/kg DM intake

Item	Mean	SE	Item	Mean	SE
Arginine	282	10.4	Valine	378	28.6
Histidine	211	17.6	Alanine	229	5.3
Isoleucine	288	10.7	Aspartic acid	578	19.0
Leucine	498	41.5	Cystine	104	10.9
Lysine	270	10.2	Glutamic acid	752	33.4
Methionine	89	0.8	Glycine	284	38.2
Phenylalanine	283	12.3	Proline	465	57.0
Threonine	299	17.7	Serine	382	10.6
Tryptophan	101	12.3	Tyrosine	361	35.0
			Total amino acids	5854	194.5

Digestibility values of amino acids in cereals. For maize the SID values of most considered AA were statistically (P<0.05) or numerically higher compared with barley (Table 4). The digestibility of lysine, methionine, tryptophan, arginine, phenylalanine and tyrosine in wheat was lower (P<0.05) than in maize, but not significantly different from that in barley. In contrast, for cystine in wheat a higher (P<0.05) SID was obtained compared with the other two cereals. Differences between maize and wheat in the digestibility of isoleucine, valine, leucine, glycine and serine, as well as between wheat and barley for methionine, tryptophan, arginine, valine and glycine, though in some cases sizeable, were not confirmed by statistical analysis. The SID coefficients for threonine, isoleucine and histidine also did not differ significantly among the cereals tested. It was found, however, that the average coefficient for indispensable AA was higher (P<0.05) in maize compared to wheat and barley.

Digestibility values of amino acids in faba beans, pea and rape seeds. There were no statistically significant differences in SID between legume seeds studied among any essential AA and serine (Table 5). The digestibility of cystine in faba beans was higher, whereas values for glycine and tyrosine were significantly

Table 4. Coefficients of standardized ileal digestibility of amino acids for maize (Ma), wheat (Wh) and barley (Ba), %

Amino acid	Feeds			SEM
	Ma	Wh	Ba	
Lysine	85.9[a]	78.3[b]	79.2[b]	1.27
Methionine	89.2[a]	81.5[b]	77.7[b]	1.53
Cystine	75.3[a]	80.0[b]	73.9[a]	0.91
Threonine	75.2	76.7	78.2	0.77
Tryptophan	94.6[a]	86.5[b]	82.6[b]	1.60
Arginine	93.5[a]	82.6[b]	79.3[b]	1.88
Isoleucine	88.9	86.1	85.0	0.75
Valine	90.6[a]	86.1[ab]	83.5[b]	1.13
Histidine	91.3	87.9	90.2	0.68
Leucine	90.8[a]	87.5[a]	80.9[b]	1.32
Phenylalanine	89.7[a]	85.6[b]	85.0[b]	0.73
Glycine	80.5[a]	79.8[ab]	76.3[b]	1.02
Serine	85.9[a]	86.1[a]	80.3[b]	0.93
Tyrosine	84.8[a]	78.4[b]	77.8[b]	1.21
Mean for essential AA[1]	89.0[a]	83.9[b]	82.2[b]	0.93

[1] mean of 10 amino acids (excluding values for cystine, glycine, serine and tyrosine); [a,b] values in a row with different superscripts are significantly different at P<0.05

Table 5. Coefficients of standardized ileal digestibility of amino acids for faba beans (Fab), pea (Pea) and full-fat rape seeds (Rap), %

Amino acid	Feeds			SEM
	Fab	Pea	Rap	
Lysine	87.6[a]	87.3[a]	81.2[b]	1.00
Methionine	86.2[a]	83.8[a]	93.1[b]	1.32
Cystine	82.3[a]	77.1[b]	76.1[b]	0.98
Threonine	80.0[a]	81.3[a]	77.7[b]	0.72
Tryptophan	81.2	77.6	83.1	1.34
Arginine	88.1[a]	90.6[a]	86.4[b]	0.69
Isoleucine	84.6	84.9	84.0	0.47
Valine	83.2	83.5	82.0	0.52
Histidine	87.4	87.2	86.6	0.48
Leucine	86.0	84.8	83.8	0.48
Phenylalanine	81.3	82.7	83.3	0.59
Glycine	79.3[a]	83.7[b]	81.3[ab]	0.77
Serine	83.4	83.2	79.2	0.81
Tyrosine	82.9[a]	87.7[b]	87.4[b]	0.73
Mean for essential AA[1]	84.6	84.4	84.1	0.43

[1] for explanations see Table 4

[a,b] values in a row with different superscripts are significantly different at P<0.05

lower than those in peas. Differences in SID between rape and legume seeds were not consistent for individual AA. With respect to rape seeds, the digestibility of lysine, threonine and arginine, and cystine, valine, leucine and serine was significantly or numerically lower compared with that in both legumes. Conversely, the value for methionine in rape was significantly the highest. However, SID

values calculated as an overall mean for the ten essential AA did not differ among any of the examined seeds.

Digestibility values of amino acids in by-product feeds. In respect to the SID of lysine, significant differences were observed between all evaluated by-products, with the highest value for SBM (Table 6). In this group of feed ingredients DDGS had the lowest (P<0.05) digestibility of lysine, methionine, threonine and arginine, respectively 14.4, 8.2, 17.2 and 12.6% units lower than in SBM. SID values for valine, phenylalanine and serine in DDGS were comparable, however, to those obtained for SBM and higher (P<0.05) than in the two rapeseed by-products.

Table 6. Coefficients of standardized ileal digestibility of amino acids for rapeseed meal (RSM), rapeseed expeller cake (RSC), maize distillers dried grains with solubles (DDGS) and soyabean meal (SBM), %

Amino acid	Feeds				
	RSM	RSC	DDGS	SBM	SEM
Lysine	78.1[a]	81.2[b]	72.8[c]	87.2[d]	1.39
Methionine	90.0[a]	91.4[a]	83.1[b]	91.3[a]	0.97
Cystine	74.0[a]	75.0[a]	74.0[a]	84.3[b]	1.30
Threonine	76.4[a]	75.5[a]	67.5[b]	84.7[c]	1.62
Tryptophan	83.1[a]	81.4[a]	81.2[a]	88.3[b]	0.95
Arginine	82.8[a]	83.7[a]	76.0[b]	88.6[c]	1.22
Isoleucine	79.8[a]	82.6[a]	82.2[a]	86.5[b]	0.75
Valine	78.2[a]	80.3[a]	83.8[b]	85.7[b]	0.83
Histidine	83.1[a]	86.9[b]	80.0[a]	87.1[b]	0.84
Leucine	81.2[a]	81.7[a]	83.5[a]	86.3[b]	0.69
Phenylalanine	83.3[a]	82.3[a]	86.9[b]	87.2[b]	0.73
Glycine	77.2[a]	81.7[b]	81.3[b]	86.4[c]	0.92
Serine	78.6[a]	79.1[a]	84.0[b]	85.2[b]	1.06
Tyrosine	83.2[a]	84.3[a]	84.2[a]	87.0[b]	0.52
Mean for essential AA[1]	81.5[a]	82.7[a]	79.7[a]	87.3[b]	0.79

[1] for explanations see Table 4; [a,b,c,d] values in a row with different superscripts are significantly different at P<0.05

There were significant differences between rapeseed ingredients in terms of the digestibility of lysine, histidine and glycine, which was lower (P<0.05) in RSM compared with that in RSC. The SID of methionine in both RSM and RSC, and histidine in RSC was similar to that in SBM. However, the SID coefficients for the remaining AA in the rapeseed products were lower (P<0.05) than values obtained for SBM. Consequently, among the tested by-products the overall mean coefficient for the essential AA was significantly higher for SBM.

DISCUSSION

The primary aim of the present study was to determine the ileal digestibility coefficients for the purpose of evaluating the capacity of 30-day-old broilers to utilize the AA from tested feeds. In addition to this, the IEAA losses in birds at a particular age were estimated.

Quantification of IEAA was based on feeding a protein-free diet (PFD); hence, the obtained data may be referred to as basal (inevitable) losses related to dry matter intake and independent of the raw material or diet composition (Lemme et al., 2004; Adedokun et al., 2007). There is evidence showing that the basal IEAA outputs in broiler chickens are age dependent. Higher IEAA flows (PFD method) in chickens at a very early age (5 days), relative to those at day 15 or 21, were given in a recent paper of Adedokun et al. (2007). In a study involving 14 and 42-day-old broilers, however, Ravindran and Hendriks (2004) found a significant increase in the ileal endogenous flow of most AA with advancing age. That may be an indication that using IEAA flows determined with birds of a particular age to correct the apparent AA digestibility estimates for broilers of a different age could result in less accurate standardized digestibility values. It is noteworthy that in this study IEAA flows were substantially greater than those determined by Adedokun et al. (2007) with 15 or 21-day-old broilers fed a protein-free diet, but lower when compared with values obtained in the study of Kadim et al. (2002) for birds at 39 days of life. In the current experiment, glutamic acid and aspartic acid dominated the AA profile of endogenous protein, and the flow rates of sulphur-containing AA were the lowest. This is consistent with previous data determined for growing chickens using the PFD or peptide alimentation methods (Kadim et al., 2002; Ravindran and Hendriks, 2004; Adedokun et al., 2007). The relatively high levels of glutamic acid and aspartic acid, and proline could be ascribed to the high content of mucin glycoproteins in the small intestine which are rich in these AA (Lien et al., 1997).

In the present work the raw materials were conventionally divided into three groups based on the relative similarity of protein concentrations, though some distinctions in CP content were visible (Table 1). A statistical examination of differences in AA digestibility was carried out with the assumption that the use of ileal digestibility corrected for basal endogenous AA secretions allows feed ingredients to be compared even if they differ in protein content (Lemme et al., 2004). On the other hand, due to the methodological differences in terms of bird age and corrections of digestibility estimates for IEAA losses, it is difficult to directly compare results from this study with literature data. Also variations in the antinutritional factor concentrations of the ingredients tested by various authors and differences in processing conditions, in the case of processed materials, can

cause discrepancies in the assessed values.

Overall, it is interesting to note that in the present study relatively low SID values of threonine (<80%) were calculated for most of the tested ingredients. This resulted, at least in part, from the relatively low content of this AA in the ileal endogenous protein (Table 3). Several studies with broilers between weeks 5 and 6 of life have shown that threonine is a major essential AA in endogenous secretions (PDF method), with flows reaching above 700 mg/kg DM intake (Kadim et al., 2002; Ravindran et al., 2004).

The current results with barley and wheat confirm the impaired digestibility of nutrients in young birds fed diets containing considerable amounts of cereals rich in soluble non-starch polysaccharides (Annison and Choct, 1991). Of the three cereals evaluated, maize had the highest SID for the majority of the indispensable AA, with a relatively low value for cystine. The SID of AA in wheat was in most cases numerically higher than that in barley. These trends are in general agreement with recently published data on cereals in broilers between 4 and 6 weeks of life (Lemme et al., 2004; Huang et al., 2005, 2007).

It is known that anti-nutritional properties of some substances that occur naturally in entire seeds of legumes and rape may adversely affect protein digestion and AA absorption in young poultry. Such compounds as tannins and trypsin inhibitors in legumes, and phytates, non-starch polysaccharides (NSP) and tannins in rape are present in the raw material in various amounts depending on species, cultivar, geographical location, growing season, and weather conditions (Gilani et al., 2005). In the study reported herein no significant differences between faba beans and field peas in the digestibility of most AA were found. Although the anti-nutritional substances were not analysed, lack of considerable differences in their concentrations (particularly condensed tannins) is the most likely reason for the relative similarity in the essential AA digestibilities of the tested legumes. The high fibre level, and presumably the relatively high proportion of NSP and phytates, were the major factors that could have contributed to the lower digestibility of lysine, threonine, arginine, cystine and some other AA in oilseed rape. In contrast with the present results, Ravindran et al. (2005) found the apparent ileal digestibility of AA in Australian samples of faba beans to be numerically lower compared with that in pea seeds. Also the coefficients of digestibility reported by those authors for full-fat seeds of canola (*Brassica campestris*) were higher than those for peas and beans, however, observed differences were not tested for statistical significance. On the other hand, in the combined data set released by Lemme et al. (2004) identical values have been proposed as coefficients of standardized ileal digestibility of AA in both faba beans and pea seeds.

As anticipated, the superiority of the SBM over the other three by-product ingredients was observed for the SID of most AA considered in this report.

Similar patterns of the AA ileal digestibility between common oilseed meals and other plant by-products, including maize DDGS, have been reported in previous experiments with broilers at different ages (Huang et al., 2005; Ravindran et al., 2005; Adedokun et al., 2008). Heat treatment is necessary to deactivate myrosinase and to reduce the glucosinolate content of the rapeseed protein products. On the other hand it is known that high temperatures during processing lower the RSM protein value for poultry by decreasing the content and digestibility of AA. The latter has been recently clearly documented by Newkirk et al. (2003) for solvent-extracted canola meals produced in Canada. Using 28-day-old chickens the authors showed substantial reduction in the apparent ileal digestibilities of AA in toasted canola meal samples compared with those in non-toasted meals. Considering the foregoing findings it is very probable that in this study the desolventizing/toasting process was the main factor responsible for the lower SID coefficients of lysine and some other AA in RSM compared with the respective values in RSC. Of the AA under consideration, methionine in both RSM and RSC was digested very well (≥90%), and cystine and threonine were the least digestible AA in this study. This is similar to what Newkirk et al. (2003) reported for some samples of toasted and non-toasted canola meals tested with growing broilers. Also noteworthy is that in the current experiment, the SID coefficients for some AA in RSM were higher than those obtained by Adedokun et al. (2008) for conventional canola meal tested with 21-day-old chickens and using the same method of standardization. It is not unlikely that some of these differences could partially result from the advancing age of birds.

The major problem facing the utilization of maize distillers dried grains with solubles in broiler diets is the high variability of AA concentrations and digestibility among different DDGS sources. From the review of the composition and the use of DDGS as a feed component for poultry, Świątkiewicz and Koreleski (2008) reported that variations in the digestibility of AA, especially lysine, methionine, threonine and cystine, in maize DDGS samples are mainly related to the excessive heating during drying procedures, which may have resulted in the darker colour of the DDGS. This is supported by the recent work of Adedokun et al. (2008), who using the PFD method determined the standardized ileal digestibility of AA in two different maize DDGS samples. In 3-week-old broilers these authors found the digestibility of all essential AA in dark DDGS to be considerably lower compared with light-coloured DDGS, with the difference in the SID of lysine amounting to 13 percentage points (52 vs 65%, respectively). In the present study the SID values of most AA in DDGS were notably higher than those for both dark and light samples tested by Adedokun et al. (2008) with 21-day-old chickens. Considering the close similarity in the assay methodology and SID calculation used in both studies, the better digestibility of AA in DDGS evaluated in the current experiment can likely

be attributed to the age-associated effects.

Consumer concerns about the use of soyabean meal obtained from genetically protein sources in poultry diets, including such feeds as grain legumes and by-products deriving from non-GM plant materials. It seems that for more effective utilization of these ingredients in broiler feeding, a dietary formulation based on AA ileal digestibility estimates determined with chickens of different age classes should be considered. Validation of the digestibility data reported herein was performed in a growth experiment with chickens between 3 and 6 weeks of age fed compound diets comprised of wheat, mix of faba beans, peas and DDGS, and one of two rapeseed components (RSM or RSC) as the main protein source. Comparative analysis indicated that formulating these diets on a SID amino acid basis yielded improved weight gains, feed efficiency and breast meat percentage compared with their total AA counterparts (Szczurek, 2008).

CONCLUSIONS

In conclusion, data obtained in the present experiment showed various ability of broilers at 30 days of life to digest the amino acids (AA) from the tested feeds, and both the presence of anti-nutritional substances and processing factors could contribute to the observed differences. Based on the ileal digestibility coefficients of the 14 most important AA, standardized for age-specific ileal endogenous amino acids losses, the evaluated ingredients can be ranked as follows. Within cereal grains: maize > wheat > barley; within native seeds: faba beans = peas > rape; within by-product feeds: soyabean meal > rapeseed expeller cake > solvent-extracted rapeseed meal > maize distillers dried grains with solubles (DDGS). The AA digestibilities for rapeseed meal and DDGS were higher than existing literature values generated with younger birds using a similar assay technique. This implies that age-associated effects should be considered for obtaining reliable information on AA digestibility in these ingredients for broiler chickens.

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