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Apparent ileal digestibility of protein and amino acids in protein feedstuffs and trypsin activity in the small intestine in broiler chickens

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ABSTRACT: Coefficients of ileal apparent digestibility (CIAD) for crude protein (CP) and amino acids in five protein feedstuffs: corn distillers dried grains with solubles (DDGS), raw full-fat soybean (RFFSB), extruded full fat soybean (EFFSB), soybean meal (SBM), and rapeseed meal (RSM) were determined using 5-week-old male broiler chickens. Trypsin activity in digesta in the ileum and jejunum were also measured. CIAD of CP was the highest in SBM (0.73). The lowest ($P < 0.05$) CIAD of CP was determined for RFFSB (0.45). There was no significant difference in CIAD of CP between RSM (0.61) and DDGS (0.62). The CIAD values for all essential amino acids except Lys (0.52) were the lowest in RFFSB ($P < 0.05$). The CIAD of Lys was the lowest in DDGS (0.39, $P < 0.05$). The CIAD for all essential amino acids was the highest in SBM ($P < 0.05$). There was no significant difference between EFFSB and SBM ($P > 0.05$) in CIAD of Leu (0.71, 0.73), His (0.66, 0.69), Lys (0.70, 0.77), Met (0.74, 0.80), Thr (0.60, 0.62), and Val (0.65, 0.67). Both the feedstuff and the intestinal region and their interaction had a significant ($P < 0.01$) effect on trypsin activity. The CIAD of CP and amino acids were very high and comparable both in EFFSB and SBM. The CIAD values for CP and amino acids in DDGS and RSM were lower than in SBM, but they seemed to be comparable to each other.

Keywords: soybean meal; rapeseed meal; raw full fat soybean; extruded full fat soybean; DDGS

List of abbreviations: CIAD = coefficients of ileal apparent digestibility, CP = crude protein, DDGS = distillers dried grains with solubles, EFFSB = extruded full fat soybean, RFFSB = raw full-fat soybean, RSM = rapeseed meal, SBM = soybean meal, Ala = Alanine, Asp = Aspartate, Arg = Arginine, Glu = Glutamate, Gly = Glycine, His = Histidine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Pro = Proline, Ser = Serine, Thr = Threonine, Tyr = Tyrosine, Val = Valine

INTRODUCTION

Soybean meal is the primary source of very high-quality protein in diets for poultry throughout the world. Consequently, interest in the reduction of nitrogen excretion and efforts to reduce feed cost has increased through the use of by-products and alternative protein sources, the protein digestibility of which is often lower as compared with soybean meal (Perttila et al. 2002).

Among different dietary ingredients, and even among the analyzed samples, there are considerable differences in digestibility, depending on fibre content, antinutritional factors, and processing or treatment (Bedford 1996; Gilani et al. 2005). The primary effect of antinutritional factors, such as trypsin inhibitors, in feeds is related to the inactivation of trypsin and chymotrypsin activity in the small intestine (Huisman 1990). However, the breeding of soybean to reduce the content of

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trypsin inhibitors provides the opportunity to use raw full fat soybean (Hymowitz 1986; Han et al. 2005). Makkink et al. (1994) also emphasized that the dietary protein source affected jejunal trypsin and chymotrypsin activities in piglets. Valette et al. (1992) found that pancreatic enzyme secretion is affected by the source of the ingested protein.

Careful attention to the digestibility of the nutrients in the raw materials used in feed formulation can make a significant contribution towards sustainable economic and food production. From an ecological perspective, increased digestibility may well improve the utilization of dietary protein and minimize nitrogen output (Lemme et al. 2004). High utilization of crude protein and amino acids is also crucial for the feed industry.

The determination of apparent amino acid digestibility based on the analysis of ileal digest is preferred to the analysis of excreta, because microbial protein produced in the caeca can influence the obtained digestibility values (Parsons et al. 1982; Ravindran et al. 1999; Kadim et al. 2002).

The analysis of feed cost per kg broiler weight or per kg breast weight revealed considerable benefits resulting from the use of cheap by-products in the diets that were properly balanced in terms of their digestible amino acid content (Rostagno et al. 1995), because the quality of protein in these feedstuffs was affected by processing.

This study determines the coefficients of apparent ileal digestibility of crude protein and amino acids in soybean feedstuffs and the by-products, rapeseed meal (RSM), and corn distillers dried grains with solubles (DDGS). The study also evaluates the effect of soybean meal (SBM), raw full-fat soybean (RFFSB), extruded full fat soybean (EFFSB), and

Table 1. Ingredients composition of the diets (g/kg as feed base)

	DDGS	RFFSB	EFFSB	SBM	RSM
DDGS	717.9	–	–	–	–
Raw full fat soybean	–	540.4	–	–	–
Extruded full fat soybean	–	–	526.7	–	–
Soybean meal	–	–	–	464.5	–
Rapeseed meal	–	–	–	–	597.6
Corn starch	182.1	409.6	423.3	435.5	302.4
Rapeseed oil	50.0	–	–	50.0	50.0
Limestone	20.0	20.0	20.0	20.0	20.0
Dicalcium phosphate	20.0	20.0	20.0	20.0	20.0
Sodium chloride	2.0	2.0	2.0	2.0	2.0
Cr_2O_3	3.0	3.0	3.0	3.0	3.0
Premix[1]	5.0	5.0	5.0	5.0	5.0
Nutritional value					
Dry matter (g/kg)	930.89	900.17	900.04	892.75	893.74
ME (MJ/kg)	8.04	13.64	13.69	12.61	10.74
Crude protein (g/kg)	200.2	202.5	198.6	211.8	214.3
Lysine (g/kg)	7.41	12.74	12.22	12.99	11.73
Methionine (g/kg)	3.81	2.89	2.91	2.97	4.33
Ca (g/kg)	8.06	10.49	10.56	10.10	12.13
P-non-phytate (g/kg)	3.89	4.66	4.71	3.91	4.82
Na (g/kg)	1.26	0.85	0.91	0.99	1.01

DDGS = distillers dried grains with solubles, EFFSB = extruded full fat soybean, RFFSB = raw full-fat soybean, RSM = rapeseed meal, SBM = soybean meal, ME = metabolizable energy

[1]premix provided per kg diet: retinol 13 500 IU, cholecalciferol 499.80 IU, alpha tocopherol 35.10 mg, menadione 3.00 mg, thiamine 2.25 mg, riboflavine 6.00 mg, pyridoxine 5.10 mg, cobalamine 0.02 mg, calcium pantothenate 11.01 mg, niacinamide 32.49 mg, folic acid 1.50 mg, biotin 0.26 mg, betaine 45.0 mg, choline chloride 250.2 mg, Fe 75.00 mg, Cu 15.00 mg, Mn 115.20 mg, Zn 108.00 mg, Se 0.30 mg, I 1.05 mg, Co 0.25 mg

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RSM on trypsin activity. The aim was to compare the crude protein and amino acid digestibility of different protein feedstuffs available for the use in the commercial poultry feed industry.

MATERIAL AND METHODS

Diets. Apparent ileal digestibility of amino acids was determined for corn DDGS, RSM, RFFSB, EFFSB, and SBM. The diets contained the assay feedstuffs as the sole source of protein. The diets were based on corn starch and a proportion of assay feedstuff and premix. All diets were formulated to contain approximately 200 g of crude protein (CP)/kg. Chromium oxide was used as an indigestible marker in the diets. The composition and nutritional values of the experimental diets are shown in Table 1. The characteristic of the tested feeds is presented in Table 2.

Birds and their management. The experiment was conducted on 150 male Ross 308 chickens obtained from a conventional farm at 30 days of age with an average weight of 1650 g. The chickens were distributed among 30 cages in two-floor test batteries, resulting in 5 chicks per cage. Each feedstuff was tested on 5 birds, with six replicates (30 birds in total). For the first three days after placement in balance cages, the chickens were fed the same commercial grower diets as on the farm due to acclimation and stress reduction. Access to drinking water and feed was provided *ad libitum*. As shown in Table 1, the birds were on the experimental diets for four days (days 33–37 of age). On day 37, the birds were starving for 12 h and after this period they were fed again and 4 h after the reestablishment of the feed, they were slaughtered. This treatment was necessary to ensure the fulfillment of the digestive tract, includ-

Table 2. Nutrient content in test feedstuff (g/kg as fed basis)

	DDGS	RFFSB	EFFSB	SBM	RSM
Crude protein	298.8	389.9	402.1	470.0	359.9
Dry matter	957.9	926.7	924.3	918.4	923.3
Crude fat	160.0	177.5	178.3	16.6	29.1
Neutral detergent fibre	296.7	136.6	72.6	125.7	242.5
TIA	0.095	0.494	0.073	0.104	0.484
Essential amino acids					
Arg	16.0	34.8	36.6	36.2	27.8
His	8.6	10.7	10.1	11.6	11.2
Leu	33.0	28.9	34.0	29.3	25.3
Ile	10.9	17.4	16.5	17.7	12.9
Lys	10.3	27.2	27.2	28.6	24.4
Met	5.3	4.6	6.1	4.7	5.5
Phe	13.4	18.3	18.4	22.2	16.1
Thr	11.0	14.6	15.0	13.8	13.4
Val	14.5	17.4	18.4	15.7	15.4
Nonessential amino acids					
Ala	19.9	15.9	16.9	14.6	13.2
Asp	20.6	44.2	46.9	40.9	24.4
Glu	43.3	66.1	67.6	56.0	47.0
Gly	11.3	14.8	16.3	15.9	17.1
Pro	19.9	15.2	17.5	24.5	21.6
Ser	13.7	19.1	19.7	17.4	13.2
Tyr	10.9	12.2	11.2	14.6	11.7

DDGS = distillers dried grains with solubles, EFFSB = extruded full fat soybean, RFFSB = raw full-fat soybean, RSM = rapeseed meal, SBM = soybean meal, TIA = trypsin inhibitor activity (mg of trypsin inhibited 1 g of sample)

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ing the ileum, by digesta. All the birds were then slaughtered in accordance with legislation. The abdominal cavity was opened immediately, and the gut content from the last third of the ileum to 3 cm proximal to the ileocecal junction was placed into Petri dishes and frozen at –30°C for subsequent processing. For each cage, the digesta of the birds was collected in a single Petri dish. The digesta samples were subsequently freeze-dried, finely ground, and analyzed for N, amino acids, Cr_2O_3, and dry matter (DM) content.

Chemical analyses. To measure the content of amino acids in the diets and digesta, oxidative acid hydrolysis was used (HCl, c = 6 mol/l). The chromatographic analysis of the hydrolysate samples was performed in an AAA 400 analyzer (Ingos, Prague, Czech Republic) using Na-citrate buffers and ninhydrin detection to determine the amounts of specific amino acids. The N content was analyzed using the Kjeldahl method.

Calculation. The coefficient of ileal apparent digestibility of the feed nutrient was calculated with the following formula:

$$CIAD = (100 - (100 \times Id \times AAdc/Idc \times AAd))/100$$

where:

Id = content of the indicator in DM of diet
AAdc = amino acid content in DM of digesta
Idc = indicator content in DM of digesta
AAd = amino acid content of DM of diet

Trypsin activity. In ten chickens per treatment, two chickens from each cage were randomly selected and digesta from 5-cm sections in the middle part of the jejunum and the central part of the ileum were used to determine trypsin activity. Samples of the digesta were gently pushed from the intestine and immediately diluted 10-fold, based on the sample weight, with ice-cold phosphate-buffered saline (pH 7.0) and homogenized for 10 min in the refrigerator. The samples were then centrifuged at 1500 *g* at 4°C for 10 min. The supernatant was transferred to Eppendorf tubes and stored at –30°C for the use in enzyme activity assays.

The trypsin activity was analyzed using the modified method of constant time (Almirall et al. 1995). Nα-benzoyl-DL-arginine-p-nitroaniline (BAPNA) diluted with 50mM Tris-HCl was used as a substrate. 1 ml of substrate was incubated at 37°C for 5 min. Then, 0.1 ml of supernatant (digesta samples) was added, and the solution was incubated for 10 min. 1 ml of acetic acid was added to stop the reaction. Trypsin activity was determined by measuring the absorbance of the sample at 405 nm. One unit of trypsin activity was defined as 1 mmol of BAPNA hydrolyzed per min at 37°C and pH 8.2 in the presence of 10 mmoles $CaCl_2$/l.

Statistical analysis. CIAD data obtained from these experiments were analyzed using One-Way Analysis of Variance, followed by the Duncans' Multiple Range Test. The effect of the diets and part of the small intestine and interaction between the diets and the part of the intestine were evaluated using Two-Way Analysis of Variance. The data were analyzed using the software package Unistat (Version 6.5, 2013).

RESULTS

CIAD of crude protein and amino acids. The CIAD values for CP and amino acids are shown in Table 3. The CIAD of CP was the highest in SBM (0.73), and the difference vs the RFFSB, DDGS, and RSM was statistically significant ($P < 0.05$). The CIAD of CP was the lowest in RFFSB (0.45), and again, the difference vs the other feedstuffs was also statistically significant ($P < 0.05$). There were no significant differences in the CIAD of CP and between EFFSB, RSM, and DDGS.

The highest CIAD of essential amino acids was found in SBM. Except for Lys, the CIAD of all essential amino acids were the lowest in RFFSB ($P < 0.05$). The CIAD of Lys was the lowest in DDGS ($P < 0.05$). There was no significant difference between EFFSB and SBM ($P > 0.05$) in CIAD of Leu, His, Lys, Met, Thr, and Val. The CIAD values for RSM were consistently lower than those found for SBM, except in the cases of Met and His. In a comparison between RSM and DDGS, there were no significant differences found in the CIAD for His, Ile, Met, Phe, and Thr. The CIAD values for Arg and Lys were significantly ($P < 0.05$) higher in RSM. The CIAD values for Leu and Val were significantly ($P < 0.05$) higher in DDGS. In soybean feedstuffs, the highest CIAD was found for Arg (from 0.61 RFFSB to 0.83 SMB). In RSM, the highest CIAD was for Met (0.80), followed by Arg (0.74). In DDGS, the highest CIAD was calculated for Leu (0.79), followed by Met (0.77).

The amino acid with the lowest CIAD in soybean feedstuffs and RSM was Thr. In DDGS, the lowest CIAD was found for Lys (0.39). The CIAD for Lys

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Table 3. Coefficients of apparent ileal digestibility

	DDGS	RFFSB	EFFSB	SBM	RSM	SEM
Crude protein	0.62[b]	0.45[a]	0.66[b]	0.73[c]	0.61[b]	0.017
Essential amino acids						
Arg	0.67[b]	0.61[a]	0.77[c]	0.83[d]	0.74[c]	0.015
His	0.65[b]	0.55[a]	0.66[b]	0.69[b]	0.65[b]	0.013
Leu	0.79[d]	0.45[a]	0.71[b,c]	0.73[c]	0.68[b]	0.022
Ile	0.70[b]	0.48[a]	0.71[b]	0.79[c]	0.67[b]	0.019
Lys	0.39[a]	0.52[b]	0.70[d]	0.77[d]	0.64[c]	0.030
Met	0.77[b]	0.45[a]	0.74[b]	0.80[b]	0.80[b]	0.024
Phe	0.71[b]	0.50[a]	0.69[b]	0.79[c]	0.71[b]	0.017
Thr	0.52[b]	0.37[a]	0.60[c]	0.62[c]	0.47[b]	0.018
Val	0.64[c]	0.37[a]	0.65[c]	0.67[c]	0.53[b]	0.021
Nonessential amino acids						
Ala	0.74[d]	0.44[a]	0.65[c]	0.67[c]	0.58[b]	0.020
Asp	0.52[a]	0.51[a]	0.71[b]	0.71[b]	0.55[a]	0.019
Glu	0.76[c]	0.63[a]	0.76[c]	0.77[c]	0.70[b]	0.010
Gly	0.52[b]	0.40[a]	0.63[c]	0.66[c]	0.61[c]	0.018
Pro	0.66[c]	0.35[a,b]	0.58[b,c]	0.78[c]	0.13[a]	0.059
Ser	0.64[c]	0.42[a]	0.67[c,d]	0.70[d]	0.53[b]	0.019
Tyr	0.72[c]	0.39[a]	0.65[b]	0.78[d]	0.67[b]	0.024

DDGS = distillers dried grains with solubles, EFFSB = extruded full fat soybean, RFFSB = raw full-fat soybean, RSM = rapeseed meal, SBM = soybean meal, SEM = standard error of the mean

[a–d]different superscripts indicate a statistically significant difference between groups ($P < 0.05$)

was the highest in SBM (0.77), which was almost twice the value found for this amino acid in corn DDGS (0.39).

The CIAD values for nonessential amino acids are shown in Table 3. In general, the highest CIAD values were found in SBM. Except for Tyr, there were no significant differences in the CIAD values between SBM and EFFSB. The lowest CIAD values were found in RFFSB, the CIAD of Ala, Gly, Ser, and Tyr were the lowest in this feedstuff. The values obtained were significantly different vs those in the other feedstuffs ($P < 0.05$). In comparison with DDGS and RSM, there were no significant differences in the CIAD of Asp, but for Ala, Glu, Pro, Tyr and Ser, the CIAD were significantly ($P < 0.05$) lower in RSM than in DDGS.

Trypsin activity. Table 4 presents trypsin activity in digesta from the jejunum and the ileum for soybean feedstuffs and RSM. Both the feedstuff and the intestinal region had significant ($P < 0.01$) effects on trypsin activity, as well as interaction between the feedstuff and intestinal region ($P < 0.01$). In the jejunum, significantly ($P < 0.01$) higher trypsin activity was found in SBM and RSM com-

Table 4. Trypsin activity in jejunum and ileum (U/ml)

		RFFSB	EFFSB	SBM	RSM
Trypsin activity	jejunum	369.3[a]	428.7[a]	572.8[b]	518.9[b]
	ileum	423.0[a,b]	341.8[a]	396.6[b]	439.3[b]
P-value	diet			$P < 0.01$	
	intestine			$P < 0.01$	
	interaction diet × intestine			$P < 0.01$	

EFFSB = extruded full fat soybean, RFFSB = raw full-fat soybean, RSM = rapeseed meal, SBM = soybean meal

[a,b]different superscripts indicate a statistically significant difference between groups ($P < 0.05$)

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pared with RFFSB and EFFSB. In the ileum, the lowest trypsin activity was found in EFFSB, and this activity was significantly ($P < 0.05$) lower in comparison with that found in SBM and RSM. In all the assayed foodstuffs except RFFSB, the trypsin activity was lower in the ileum than in the jejunum.

DISCUSSION

Clarke and Wiseman (2005) demonstrated that the coefficients of ileal apparent digestibility for individual amino acids varied widely among soybean samples. However, it did not correlate with trypsin inhibitor activity (TIA) levels, indicating that other factors also affect amino acid digestibility of RFFSB and SBM. Clark and Wiseman (2007) reported that the concentration of ileal apparent digestible Lys and CIAD of Lys of EFFSB is influenced by the processing temperature. They also showed similar improvements in other amino acids both in ileum apparent digestible concentration and CIAD. Foltyn et al. (2013) reported that the replacement of SBM by EFFSB significantly ($P < 0.05$) decreased amino acids CIAD when the level of EFFSB was higher than 40 g/kg in feed. However, in this study, the amino acids CIAD of EFFSB and SBM was very similar. This may mean that the individual components of the feed mixture can interact digestibility of amino acids.

The CIAD values obtained for RSM were in most cases lower than those for SBM. It confirms the result of the study of Bell (1984) who reported that RSM protein is well digested, but the protein digestion coefficient and the availability of amino acids are lower than for SBM. Factors that contribute to lower amino acid digestibility in RSM may include higher levels of hulls and tannins in this ingredient (Bell 1993).

Lys is the most limiting amino acid for growth (de Araujo et al. 2014). A very low CIAD value for Lys was found in DDGS. Batal and Dale (2006) reported an average Lys digestibility coefficient of 0.51 with a range from 0.18 to 0.66 in DDGS. Low digestibility of Lys in DDGS is associated with the destruction of Lys during processing. Arguably, the destruction may well occur in the soluble components of DDGS, such as the reducing sugars (Batal and Dale 2006; Soares et al. 2012). The low amino acid digestibility is evident for amino acids that are present in smaller amounts in DDGS (Lys, Met) (Foltyn et al. 2014). In formulating feedstuff including DDGS, attention must be paid to Lys level and/or digestible Lys level and only high quality DDGS should be used.

Makkink et al. (1994) also reported that trypsin activity in the jejunum is affected by protein sources. Arg and Lys are amino acids that specifically cause the release of trypsinogen in a pancreatic tissue homogenate, because they contain sites of tryptic cleavage (Niederau et al. 1986). This may explain the lower CP and amino acids digestibility in DDGS because the content of Lys and Arg was the lowest in this feedstuff. Trypsin inhibitors contained in soybean inhibit the proteolytic action by combining with trypsin to form a stable compound (Kunitz 1947). Raw soybean or trypsin inhibitor (TI) ingestion by chicks caused a reduction in the trypsin activity in the small intestine (Gertler and Nitsan 1970). After feeding EFFSB, the trypsin activity was lower in comparison with SBM.

The CIAD of nutritients are influenced by individual feed ingredients and the type of bird or methods by which CIAD values are determined (Adedokun et al. 2009). The results of CIAD from this study indicate lower digestibility of assay feedstuff than the findings presented by other authors (Perttila et al. 2002; Ravindran et al. 2005; de Coca-Sinova et al. 2008; Adedokun et al. 2009; Kong and Adeola 2010).

The CIAD of CP and amino acids were very high and comparable in both EFFSB and SBM. In general, the CIAD of CP and amino acids in DDGS and RSM were lower than in SBM, but they seem to be comparable to each other. The CIAD of nutrients of RFFSB was the lowest in comparison with other feeds ($P < 0.05$). Diet formulation on a digestible amino acid basis should improve the precision of diet formulation when protein feedstuffs with different digestibility are fed.

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