

COMPARISON OF TWO METHODS OF PROTEIN QUALITY EVALUATION IN RICE, RYE AND BARLEY AS FOOD PROTEIN SOURCES IN HUMAN NUTRITION

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ABSTRACT

Different foods differ in their protein quality, which is characterized by the content and digestibility of individual amino acids. The Food and Agriculture Organisation has recommended replacing the method for protein quality evaluation of foods called protein digestibility corrected amino acid score (PDCAAS) with the new method - digestible indispensable amino acid score (DIAAS), in which the values of ileal amino acid digestibility obtained in pigs are used. However, the information about DIAAS values of foods are limited. Therefore, the study on growing pigs was conducted to determine true fecal protein digestibility and standardized ileal amino acid digestibility of rice, rye and barley. Using these values, the PDCAAS and DIAAS were calculated and compared. A total of 18 gilts with a T-cannula inserted in the terminal ileum were allotted to 3 diets with six replicate pigs per diet. Three semi-purified diets were formulated to contain the tested cereal grains (rice, rye, barley) as the sole nitrogen source. Chromic oxide was used as indigestible marker. Each experimental period comprised of a 7-d adaptation period followed by 24 h collection of feces and ileal digesta. The content of nitrogen, dry matter and chromic oxide was analyzed in samples of diets, feces and ileal digesta. Moreover, in the samples of diets and ileal digesta the content of amino acids was determined. Calculated ratio of crude protein to lysine was greatest in rice (4.50) followed by rye (3.65) and the lowest one in barley (3.35). True fecal protein digestibility was greater when compared with ileal amino acid digestibility for all tested samples, thus suggesting an overestimation of protein quality determined by PDCAAS. Calculated PDCAAS values for rice, rye and barley (81, 65 and 61%) were generally greater than the DIAAS values (79, 56 and 55%), especially for the poorer quality protein sources such as rye and barley in comparison with rice. The lysine was the first limiting amino acid in all tested cereal grains. Based on the DIAAS evaluation, rice is better protein source in human nutrition in comparison with rye or barley.

Keywords: PDCAAS; DIAAS; protein quality; amino acid; ileal

INTRODUCTION

Protein quality describes the nutritive value of proteins. A precise assessment of the ability of a dietary protein source to match the body's needs for individual amino acids (AA) will allow their better use (FAO, 2013).

The quality of dietary protein is a function of its individual constituent AA. The FAO/WHO Expert Consultation on Protein Quality Evaluation recommended the use of the Protein Digestibility Corrected AA Score (PDCAAS) as suitable method for protein quality evaluation (FAO, 1991). Using this method, PDCAAS is calculated by multiplying the limiting AA score (i.e. the ratio of the first-limiting AA to the same AA of the reference protein) by true fecal protein digestibility. However, the PDCAAS has limitations - the main is that fecal protein digestibility as a measure of AA availability is inaccurate due to metabolic transformations of dietary

and endogenous proteins by microbial population of the large intestine (Darragh and Hodgkinson, 2000; Gilani, 2012; Schaafsma, 2012).

Considering the number of critical reviews on this subject (Moughan, 2003; Fuller and Tomé, 2005; Hendriks et al., 2012) a new protein quality measure called digestible indispensable AA score (DIAAS) is now recommended to replace the PDCAAS for evaluating protein quality in human nutrition (FAO, 2013).

The main difference between PDCAAS and DIAAS is that dietary AA is treated as individual nutrients and their digestibility is used in calculations. The AA are absorbed only from the small intestine and their digestibility is measured as ileal digestibility (a difference between dietary AA and those appearing in terminal ileum) which is more accurate assessment of how much of the protein consumed is available to the body (Columbus and de

Lange, 2012). The apparent ileal digestibility (AID) of AA is defined as the net disappearance of ingested dietary AA from the digestive tract proximal to the distal ileum. When AID is corrected for the basal endogenous losses in pigs, the resulting value is termed standardized ileal digestibility (SID), which can be used to calculate approximate DIAAS values in humans (Stein et al., 2007). Using the DIAAS method, researchers are now able to differentiate protein sources by their ability to supply AA for use by the body (Brestenský et al., 2018).

There is no non-invasive method of ileal digesta collection applicable in humans and therefore, the number of relevant data is very limited. The pig has been recognized as a good animal model for estimating crude protein (CP) and AA digestibility in humans (Rowan et al., 1994; Deglaire et al., 2009). However, in this time there are only several studies dealing with protein quality of cereal grains or different protein sources in terms of DIAAS quality evaluation (Cervantes-Pahm et al., 2014; Mathai et al., 2017; Abellila et al., 2018).

Furthermore, that the cereal grains are the major source of energy, they can be also a good source of protein. The aim of the present study was to compare PDCAAS and DIAAS values of rice, rye and barley calculated using digestibility coefficients obtained in a series of pig experiments.

Scientific hypothesis

We tested the protein quality of different cereal grains for human nutrition by PDCAAS and DIAAS methodology. Due to the fact, that the DIAAS method is new and both methods are difficult, there is little studies which would evaluate different food sources in human nutrition from the point of view of their protein quality.

MATERIAL AND METHODOLOGY

Animals and experimental design

the Animal Care Committee of the Research Institute of Animal Production Nitra (Slovakia).

A total of 18 Large white gilts (BW, 50 ±3.5 kg) fitted with ileal T-cannulas were used throughout the study. They were allotted to 3 diets - six replicate pigs per diet. After a 14-d recovery period, an experimental period, consisting of a 7-d adaptation period followed by a 1-d (24-h) collection of ileal digesta and feces, was started.

Three semi-purified diets (Table 1) were formulated to contain the tested cereal grains (rice, rye, barley) as the sole nitrogen (N) source. Chromic oxide was added to the diets as an indigestible marker. All diets were fed twice daily at 07:00 and 16:00 h in 2 equal meals at a daily rate of 80 g.kg^{-0.75}. Water was available *ad libitum*.

Chemical analysis

The diets, feces and ileal digesta were analyzed for dry matter (DM) and total nitrogen (N) (AOAC, 1990). Chromic oxide was measured by atomic absorption spectrometry (Williams et al., 1962). The content of AA, in diets and ileal digesta, after acid hydrolysis with 6 M HCl and methionine and cysteine after oxidative hydrolysis were determined using an automatic AA analyzer (AAA 400, Ingos, Prague, Czech Republic).

Calculations

Coefficients of true fecal protein digestibility (TD) or standardized ileal AA digestibilities (SID) were calculated using the following formula:

$$TD, SID (\%) = 100 \times [(1 - N_{ex}/N_d \times Cr_d/Cr_{ex}) + N_{end}/N_d],$$

where N_{ex} is concentration of the nutrient in feces or ileal digesta, N_d is concentration of the nutrient in the diet, Cr_d is concentration of chromic oxide in the diet, Cr_{ex} is concentration of chromic oxide in feces or ileal digesta (all values in g.kg⁻¹ DM) and N_{end} is the endogenous loss of the nutrient expressed as g.kg DM⁻¹ intake.

Table 1 Composition of experimental diets.

Ingredient	Protein source		
	Rice	Rye	Barley
Rice (g.kg ⁻¹ air-dry basis)	964.0	-	-
Rye (g.kg ⁻¹ air-dry basis)	-	958.0	-
Barley (g.kg ⁻¹ air-dry basis)	-	-	972.0
Sunflower oil (g.kg ⁻¹ air-dry basis)	-	9.0	-
Limestone (g.kg ⁻¹ air-dry basis)	6.0	13.0	11.8
Monocalcium phosphate (g.kg ⁻¹ air-dry basis)	21.0	11.0	6.2
Salt (g.kg ⁻¹ air-dry basis)	3.0	3.0	4.0
Premix (g.kg ⁻¹ air-dry basis) ¹	3.0	3.0	3.0
Chromic oxide (g.kg ⁻¹ air-dry basis)	3.0	3.0	3.0

Note: ¹Provided the following per kg of diet: retinol 1.2 mg; cholecalciferol 25 mg; α-tocopherol 10 mg; menadione 0.2 mg; riboflavin 4 mg; pyridoxine 2.5 mg; d-pantothenic acid 10 mg; niacin 20 mg; folic acid 0.5 mg; biotin 0.1 mg; cyanocobalamin 30 μg; choline 500 mg; Fe 92 mg; Zn 103 mg; Mn 40 mg; Cu 19 mg; Co 0.5 mg; Se 0.16 mg.

The experimental study was performed in Laboratory of pig nutrition at National Agricultural and Food Center, Research Institute of Animal Production Nitra. All experimental procedures were reviewed and approved by

The values of endogenous N losses in feces were taken from the study by Whiting and Bezeau (1957) and the endogenous AA losses in ileal digesta from the study of Jansman et al. (2002) which were also suggested by Stein et al. (2007). For the calculation of PDCAAS and DIAAS,

indispensable AA reference pattern (reference protein) for adult humans, as defined by the FAO Expert consultation was used (FAO, 2013).

Statistic analysis

Experimental data were analyzed by General Linear Model of Statgraphics Plus package (version 3.1, Statistical Graphic Corp., Rockville, MD, USA). When the analysis of variance indicated a significant ($p < 0.05$) p -value for treatment means, the differences between means were assessed by Tukey HSD test. True fecal digestibility values were compared with weighted means of ileal digestibility of all indispensable AA by a two-sample comparison method using Student's t -test.

RESULTS AND DISCUSSION

The contents of CP in rye and barley were approximately two times greater than in rice, whereas the calculated ratio Lys : CP was greatest in rice (Table 2). Compared with other indispensable AA, all cereal grains contained relatively high amounts of leucine similarly as reported Cervantes-Pahm et al. (2014).

Mean data on true faecal protein digestibility as well as standardized ileal digestibility of AA are summarized in Table 3. Significant differences ($p < 0.05$) in both protein

and AA digestibility among the rice and other cereals (rye and barley) were found. The lowest values of both protein and AA digestibility among the tested protein sources were found in rye which was due to the ability of arabinoxylans to form highly viscous solutions, interfering with digestion or absorption along the alimentary tract (Jondreville et al., 2001). A similar effect has been attributed to mixed-linked beta-glucans of barley (Graham et al., 1989). The comparison of true fecal protein digestibility with the mean ileal AA digestibility (Table 3) showed that the fecal digestibility is not a good predictor of ileal digestibility, because the estimated values were greater than those of ileal digestibility which suggesting an overestimation of protein quality determined by means of PDCAAS. Similar results were reported also by other authors (Moughan and Donkoh, 1991; Darragh and Hodgkinson, 2000).

The calculations of PDCAAS and DIAAS values are shown in Table 4. In both cases, quite large differences in protein quality measures were found. Calculated PDCAAS values were generally greater than the DIAAS values, especially for the poorer quality proteins of rye and barley in comparison with rice. These findings were due primarily to the degree of deficiency of the first-limiting AA, which was lysine, in all tested samples and similarly its various ileal digestibility in rice, rye and barley (94.1, 73.2 and 79.4%, respectively).

Table 2 Determined crude protein and amino acid composition of rice, rye and barley.

Item	Protein source		
	Rice	Rye	Barley
Crude protein (g.kg ⁻¹ DM)	76.88	153.13	141.25
Cysteine (mg.g ⁻¹ CP)	14.9	13.6	17.0
Histidine (mg.g ⁻¹ CP)	31.2	22.5	23.7
Isoleucine (mg.g ⁻¹ CP)	44.3	28.5	30.3
Leucine (mg.g ⁻¹ CP)	93.5	65.1	64.5
Lysine (mg.g ⁻¹ CP)	40.5	36.5	33.5
Methionine (mg.g ⁻¹ CP)	23.1	11.5	16.7
Phenylalanine (mg.g ⁻¹ CP)	54.6	44.5	45.9
Tryptophan (mg.g ⁻¹ CP)	11.0	6.6	8.5
Threonine (mg.g ⁻¹ CP)	36.7	33.1	34.0
Tyrosine (mg.g ⁻¹ CP)	29.5	23.0	33.6
Valine (mg.g ⁻¹ CP)	62.8	42.1	43.9
Calculated value Lys : CP (%)	4.50	3.65	3.35

Table 3 Coefficients of true fecal digestibility of crude protein and standardized ileal digestibility of AA in pigs fed diets containing various protein sources.

Item	Protein source		
	Rice ¹	Rye ¹	Barley ¹
True fecal digestibility:			
Crude Protein (%)	95.5 ±3.2 ^b	86.0 ±3.3 ^a	86.5 ±2.6 ^a
Standardized ileal digestibility:			
Weighted AA mean (%)	92.9 ±2.1 ^b	75.7 ±3.4 ^a	80.7 ±3.6 ^a
Significance of fecal protein digestibility vs. mean ileal AA digestibility:			
<i>p</i> -value ²	0.119	<0.001	0.010

Note: ¹Values are expressed as means ±SD; ^{a,b} Means within a row not sharing a common superscript were significantly different; ²Tukey HSD test, *p* <0.05.

Table 4 Calculation of protein digestibility-corrected amino acid score and digestible indispensable amino acid score of protein sources.

Item	Amino acid										PDCAAS (%)	DIAAS (%)
	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val			
Reference pattern ¹	15	30	61	48	23	41	25	6.6	40			
Protein digestibility-corrected reference amino acid ratios²												
Rice	1.98	1.41	1.46	0.81	1.57	1.96	1.40	1.65	1.50	81		
Rye	1.29	0.82	0.92	0.65	0.94	1.42	1.14	0.87	0.91	65		
Barley	1.37	0.87	0.91	0.60	1.27	1.68	1.18	1.12	0.95	61		
Digestible amino acid reference ratios³												
Rice	1.93	1.41	1.45	0.79	1.55	1.81	1.35	1.33	1.49		79	
Rye	1.12	0.71	0.85	0.56	0.83	1.24	0.95	0.76	0.79		56	
Barley	1.25	0.86	0.88	0.55	1.25	1.52	0.98	1.02	0.92		55	

Note: ¹Reference amino acid pattern for adults, mg.g⁻¹ protein (FAO, 2013); ²Ratios of amino acids (mg.g⁻¹ crude protein) corrected for true fecal digestibility to FAO (2013) reference pattern; ³Ratios of amino acids (mg.g⁻¹ crude protein) corrected for ileal digestibility to FAO (2013) reference pattern; SAA - sulfur amino acids; AAA - aromatic amino acids.

The comparison of PDCAAS and DIAAS values showed that both methods gave the same results as for ranking proteins in terms of their quality. However, the absolute values differed. The values of DIAAS were considerably lower than the PDCAAS values. Differences between them tended to increase with decreasing ileal AA digestibility. These results suggest that protein quality evaluation based on the ileal digestibility of AA - DIAAS are more reasonable estimates of their bioavailability than PDCAAS.

CONCLUSION

The values of PDCAAS were generally greater than that of DIAAS, especially for the poorer quality proteins in rye and barley in comparison with rice. All tested parameters were greatest in rice and therefore based on the results

from the present study we can conclude that rice is better protein source in human nutrition than rye or barley.

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