Comparative ileal digestibility of amino acids in 00-rapeseed meal and rapeseed meal fed to growing male broilers

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ABSTRACT Rapeseed meal (RSM) is a commonly used protein source in poultry diet but its usage is limited due to antinutritional factors, the glucosinolates (GLS) and erucic acid. The 00-rapeseed meal (00-RSM) is the developed variety of rapeseed with reduced GLS and erucic acid content through genetic selection. The present study was conducted with the objective of comparing the standardized ileal digestibility (SID) of amino acids (AA) in 00-RSM and RSM when fed to growing broilers. Three samples of each ingredient were collected from different sources. Two hundred and fifty two day-old male broilers (Hubbard × Hubbard) were fed a corn-soybean meal based starter diet in crumble form from day 1 to 13. On day 14, all chicks were individually weighed and randomly distributed to 42 replicate pens (6 birds in each pen). Six test diets (2 ingredient × 3 samples) with approximately 20% crude protein were made in mash form in such a way that the 6 test ingredients served as the sole source of AA in one diet. The endogenous AAs (EAA) were determined by feeding a nitrogen-free diet to six replicate pens. Each test diet was fed to six replicates of broiler chicks from 14 to 21 days of age. Results indicated that the SID of all AA differed ($P < 0.001$) among 00-RSM samples. Among RSM samples, the SID of AA varied for arginine, methionine ($P < 0.01$), histidine, leucine, lysine, aspartic acid, and phenylalanine ($P < 0.05$). A greater ($P < 0.05$) SID of all AA except arginine, histidine, phenylalanine, cysteine, and glutamic acid was observed in 00-RSM compared with RSM. In conclusion, 00-RSM had greater SID of AA compared with RSM and it is nutritionally superior to RSM to be used in broiler diets.

Key words: 00-rapeseed meal, rapeseed meal, ileal digestibility, broiler

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INTRODUCTION

Interest in determining the amino acid (**AA**) digestibility has been increased since Likuski and Dorrell (1978) and Sibbald (1979) developed the rapid bioassay to access AA digestibility. Presence of essential AA, protein digestibility, and bioavailable AA are the key features in assessing the protein's quality (Gilani et al., 2005). The term bioavailable AA may be defined as the portion of AA that can be digested, absorbed, and utilized by the animal. However, Ravindran et al. (2005) documented that under certain situations, AAs are absorbed in a form not suitable for protein synthesis and such AAs make no contribution to the protein status of the animal. The concentration of digestible AA in a diet or feed ingredient is a better measure of protein quality than total AA (McNab, 1989). Standardized ileal digestibility (**SID**) is the most authentic way to express

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Table 1. Ingredient composition of nitrogen free diet and experimental feeds.

Ingredients (g/kg)	N-free diet	00-Rapeseed meal			Rapeseed meal		
		Source 1	Source 2	Source 3	Source 1	Source 2	Source 3
Test ingredient	–	520	517	528	508	505	561
Dextrose	640	359	362	351	370	374	317
Soybean oil	50	60	60	60	60	60	60
Di-calcium pohosphate	19	19	19	19	19	19	19
Limestone	13	10	10	10	10	10	10
Choline chloride	3	3	3	3	3	3	3
Salt	2	2	2	2	2	2	2
Celite[†]	20	20	20	20	20	20	20
Vit - min premix[‡]	7	7	7	7	7	7	7
Corn starch	169	–	–	–	–	–	–
Arbocel (cellulose)[#]	50	–	–	–	–	–	–
Sodium bicarbonate	15	–	–	–	–	–	–
Potassium chloride	12	–	–	–	–	–	–

[†]Cellulose (James River Co., NJ) used as acid insoluble ash as an external marker.
[#]Arbocel, insoluble raw fiber concentrate, Holzmuhle, Rosenberg,Germany.
[‡]Provided per kg of diet: retinyl acetate, 4,400 IU; cholecalciferol, 118 μg; DL-α-tocopheryl acetate, 12 IU; menadione sodium bisulphite, 2.40 mg; thiamine, 2.5 mg; riboflavin, 4.8 mg; niacin, 30 mg; D-pentothenic acid, 10 mg; pyridoxine, 5 mg; biotin, 130 μg; folic acid, 2.5 mg; cyanocobalamin, 19 μg; manganese, 85 mg (from $MnSo_4.H_2O$); Iron, 80 mg (from $FeSo_4.H_2O$); Zinc, 75 mg (from ZnO); Copper, 6 mg (from $CuSo_4.5H_2O$); Iodine, 1 mg (from ethylene diamine dihydroiodide); Selenium, 130 μg (from Na_2SeO_3).

AA digestibility (Ravindran et al., 1999) because values for SID of AA are additive in mixed diets. Values for SID of AA are therefore widely acknowledged as the correct way to describe the protein quality of feed ingredients and diets (Garcia et al., 2007; Adedokun et al., 2008).

00-Rapeseed meal (**00-RSM**) and rapeseed meal (**RSM**) are commonly used protein sources in poultry diets (Newkirk, 2009; Kim et al., 2012; Toghyani et al., 2014) and are the cost effective substitute of soybean meal for poultry (Mushtaq et al., 2007; Toghyani et al., 2015). The 00-rapeseed (also known as canola in Canada and the United States) is a variety of rapeseed that has lower concentrations of glucosinolates (**GLS**) and erucic acid in oil than traditional rapeseed. Quality of 00-RSM and RSM may be affected because of processing methods (Newkirk and Classen, 2002) and seasonal as well as cultivar conditions (Bell, 1993; Ravindran et al., 2014). In addition, these factors may also alter proportion of antinutritional factors and nutrient composition (Tripathi and Mishra, 2007). Therefore, determining digestibility of nutrients in 00-RSM and RSM can aid in formulating diets containing these ingredients for broiler chickens (Gopinger et al., 2014). Keeping in view the insufficient literature comparing the SID of AA between 00-RSM and RSM, the present study was conducted to make an AAs SID comparison in 00-RSM and RSM fed to growing broilers.

MATERIALS AND METHODS

All procedures were performed according to the Local Experimental Animal Care Committee, and approved by the Faculty of Animal Husbandry, University of Agriculture, Faisalabad, Pakistan.

Bird Management

A total of 252 day-old male broiler chickens (Hubbard x Hubbard) were received from commercial hatchery (SB Hatchery, Rawalpindi, Pakistan) and placed in cages. All chicks were reared under identical managemental conditions according to the Hubbard management guide (2014). Brooding room temperature was maintained at 33°C and it was 24°C at day 21. Continuous fluorescent light was provided to chicks. Humidity of the experimental room varied from 60 to 65%. Clean water was available at all times. A corn-soybean meal based starter diet was provided in crumble form to meet the nutritional requirements (NRC, 1994) from day 1 to 13. On day 14, all chicks were individually weighed and randomly distributed to 42 pens (6 birds in each pen). The mean body weight among replicates was within a range of ±10 g.

Diet Allocation

Six diets (2 ingredients × 3 samples) were formulated with one of the test ingredients providing all AA in one diet (Table 1). The proportion of each test ingredient in the diets was adjusted to maintain approximately 20% CP in all diets (Ravindran et al., 2005). Three samples of each of 00-RSM and RSM were obtained from local suppliers and analyzed for proximate composition (Table 2) and AA profile (Table 3). To calculate SID of AA, a nitrogen-free diet (**NFD**) was formulated and fed to six replicate pens to determined endogenous AA (**EAA**) losses (Adedokun et al., 2007). Each test diet was randomly assigned to six replicate pens (36 birds/diet). Celite (acid insoluble ash) was added to diets as an indigestible marker at the rate of 2% (Ravindran et al., 2005).

Table 2. Proximate composition of feed ingredients (880 g/kg DM).

Nutrient (g/kg)	00-Rapeseed meal Source 1	00-Rapeseed meal Source 2	00-Rapeseed meal Source 3	Rapeseed meal Source 1	Rapeseed meal Source 2	Rapeseed meal Source 3
Crude protein	370	359.7	361.7	364.3	377.6	329.5
Ash	61.9	64.9	62.3	80.3	71.8	87
Crude fiber	94	105	99.8	76.4	90.1	84.7
Ether extract	6.6	8	11.4	7.4	13.3	38.6
Acid insoluble ash	12.9	13.9	6.7	20.1	11.7	32.4
Nitrogen free extract	346.9	342.7	344.8	351.5	327.1	340.2
Glucosinolates, μmol/g	31	39	25	83	89	110

Table 3. Total amino acid profile of feed ingredients (as received basis).

Amino acid (g/kg)	00-Rapeseed meal Source 1	00-Rapeseed meal Source 2	00-Rapeseed meal Source 3	Rapeseed meal Source 1	Rapeseed meal Source 2	Rapeseed meal Source 3
Indispensable AA						
Arginine	22.1	22.5	25.7	23.3	22.5	19.5
Histidine	11.0	10.9	11.8	10.9	10.5	9.1
Isoleucine	17.0	14.9	16.6	15.3	14.2	12.0
Leucine	26.0	26.0	28.2	25.6	24.7	22.0
Lysine	20.7	21.6	21.8	18.8	17.3	17.2
Methionine	8.6	8.6	9.0	8.0	8.0	6.6
Phenylalanine	16.5	16.6	18.2	16.4	16.5	13.2
Threonine	16.7	16.7	17.4	15.3	15.2	14.4
Valine	20.2	20.2	21.7	19.6	18.9	13.8
Dispensable AA						
Alanine	16.2	16.4	17.5	15.7	15.5	14.9
Aspartic acid	24.6	25.2	27.5	23.2	22.5	23.1
Cysteine	10.1	10.0	10.4	10.7	10.5	8.4
Glycine	19.1	19.4	20.4	18.8	18.7	17.2
Glutamic acid	70.0	70.9	77.5	72.5	71.5	61.3
Serine	14.7	14.9	15.9	14.1	14.0	14.7

Ileal Digesta Collection

On day 21, all birds were euthanized by intravenous injection of Ketamax (Ketamine hydrochloride, Rotex Med, Germany). The contents of the ileum from the vitelline diverticulum (formerly named as Meckel's diverticulum) to a point approximately 40 mm anterior to the ileo-caecal junction (Bandegan et al., 2009) were collected in plastic bags by gently flushing with distilled water and air pressure. Ileal digesta of all birds in a replicate were pooled, immediately stored at −20°C, and subsequently freeze dried. Dried ileal digesta were then ground using a coffee grinder (MC3001 coffee grinder; Moulinex Ltd. Weston, Ontario, Canada) to pass through a 0.5 mm sieve and stored in plastic tubes at −4°C for chemical analyses (Bandegan et al., 2009).

Chemical Analysis

Raw ingredients, test diet, and ileal digesta samples were analyzed for dry matter (AOAC, 2000; procedure 934.01) and crude protein (N x 6.25) by Leco nitrogen analyzer (model FP-528, Leco Corporation, St. Joseph, MI). Acid insoluble ash of diet and ileal digesta samples were determined by the procedure used by Siriwan et al. (1993). The AA profile of the test ingredients, diets, and ileal digesta were determined by the procedure used by Palliyeguru et al. (2010) using an AA analyzer (Biochrom 30 plus, Biochrom Ltd. Cambridge, UK). Briefly, samples were oxidized with hydrogen peroxide-formic acid-phenol solution. Sodium disulphite was used to decompose the excess oxidation reagent. After oxidation, samples were hydrolyzed using 6 *M* HCl for 24 hours. The pH of hydrolysate was adjusted to 2.20, centrifuged, and filtered. The AAs in solution were separated using AA analyzer at 570 nm (AOAC, 2000). Raw ingredients were also analyzed for crude fat (AOAC, 2006; procedure 920.39), crude fiber (AOAC, 2006; procedure 978.10), and ash content (AOAC, 2006; procedure 942.05) (Table 2). Glucosinolates content of 00-RSM and RSM samples was determined by the procedure reported by Krishna and Ranjhan (1981).

Calculations

The EAA concentration was calculated as milligrams of AA flow per kg DM intake as described by Moughan et al. (1992).

$$\text{leal AA flow, mg/kg DMI} = \left[\text{AA in ileal digesta mg/kg}\left(\frac{\text{Diet marker, mg/kg}}{\text{Ileal marker, mg/kg}}\right)\right]$$

Table 4. Concentration of endogenous amino acid losses used to standardize the amino acid digestibility.

Amino acid	Endogenous AA concentration
	(mg/kg DMI)
Indispensable AA	
Arginine	179
Histidine	189
Isoleucine	349
Leucine	341
Lysine	225
Methionine	49
Phenylalanine	202
Threonine	412
Valine	396
Dispensable AA	
Alanine	108
Aspartic acid	168
Cysteine	141
Glycine	120
Glutamic acid	237
Serine	136

$$\text{Apparent ileal AA digestibility (AIAAD), \%} = \left[1 - \left(\frac{\text{Marker in diet}}{\text{Marker in ileal digesta}}\right) \times \left(\frac{\text{AA in ileal digesta}}{\text{AA in diet}}\right)\right] \times 100$$

The endogenous ileal AA losses were used (Table 4) to calculate SID of AA by using following equation.

$$\text{Standardized ileal AA digestibility (SIAAD), \%} = \text{AIAAD, \%} + \left[\left(\frac{\text{Ileal AA flow, g/kg of DMI}}{\text{AA in raw material, g/kg of DMI}}\right)\right] \times 100$$

Statistical Analysis

Values for apparent ileal digestibility (**AID**) and SID of AA were analyzed using the Generalized Liner Model (GLM) procedure of SAS (2008) under a completely randomized design. Treatment means were separated by Tukey's test. The level of significance was set as $P \leq 0.05$.

RESULTS

Proximate composition and GLS content of 00-RSM and RSM indicated that there are variations not only between ingredients but also among samples of the same ingredient (Table 2). The SID of CP and AA within the ingredients were also different ($P < 0.05$). In case of 00-RSM the SID of AA highly varied ($P < 0.001$) for all tested AA (Table 5).

Variability of SID of AA were observed among the three sources of RSM but differences among 00-RSM sources were greater ($P < 0.001$). The digestibility varied for CP ($P < 0.001$), methionine (**Met**), arginine (**Arg**) ($P < 0.01$), histidine (**His**), leucine (**Leu**), lysine (Lys), phenylalanine (**Phe**), and aspartic acid (**Asp**) ($P < 0.05$) among RSM sources. The SID of AA was similar ($P > 0.05$) for isoleucine (**Ile**), threonine (**Thr**), valine (**Val**), alanine (**Ala**), cysteine (**Cys**), glycine (**Gly**), glutamic acid (**Glu**), and serine (**Ser**) among the RSM samples (Table 6).

The comparison between 00-RSM and RSM for SID of AA (Table 7) demonstrated that the SID of AA in 00-RSM was greater for Val ($P < 0.01$) and Ile, Leu, Lys, Meh, Thr, Ala, Asp, Gly, and Ser ($P < 0.05$). However, Arg, His, Phe, Cys, and Glu resulted in similar digestibility ($P > 0.05$).

Table 5. Standardized ileal amino acid digestibility coefficient of different 00-rapeseed meal sources (as received basis).

Amino acid	00-Rapeseed meal Source 1	Source 2	Source 3	Mean	SEM	Significance
Indispensable AA						
Crude protein	0.762[b]	0.757[b]	0.827[a]	0.782	0.023	***
Arginine	0.872[b]	0.854[c]	0.928[a]	0.884	0.022	***
Histidine	0.854[b]	0.842[b]	0.901[a]	0.866	0.018	***
Isoleucine	0.832[b]	0.819[b]	0.875[a]	0.842	0.017	***
Leucine	0.853[b]	0.840[b]	0.896[a]	0.863	0.017	***
Lysine	0.828[b]	0.810[c]	0.873[a]	0.837	0.019	***
Methionine	0.908[b]	0.905[b]	0.936[a]	0.916	0.010	***
Phenylalanine	0.858[a]	0.812[b]	0.882[a]	0.851	0.021	***
Threonine	0.765[b]	0.745[c]	0.829[a]	0.780	0.025	***
Valine	0.816[b]	0.826[b]	0.863[a]	0.835	0.014	***
Dispensable AA						
Alanine	0.840[b]	0.831[b]	0.877[a]	0.849	0.014	***
Aspartic acid	0.789[b]	0.767[c]	0.857[a]	0.804	0.027	***
Cysteine	0.788[b]	0.769[b]	0.862[a]	0.806	0.028	***
Glycine	0.782[b]	0.766[b]	0.850[a]	0.799	0.026	***
Glutamic acid	0.875[b]	0.866[b]	0.923[a]	0.888	0.018	***
Serine	0.777[b]	0.748[c]	0.829[a]	0.785	0.023	***

[a–c]Mean values sharing different superscripts within rows differ significantly.
***$P < 0.001$.
SEM = Standard error of mean.

Table 6. Standardized ileal amino acid digestibility coefficient of different rapeseed meal sources (as received basis).

Amino acid	Rapeseed meal Source 1	Source 2	Source 3	Mean	SEM	Significance
Indispensable AA						
Crude protein	0.717[a]	0.698[a]	0.630[b]	0.681	0.027	***
Arginine	0.863[a]	0.866[a]	0.81[b]	0.848	0.017	**
Histidine	0.823[a,b]	0.799[b]	0.833[a]	0.818	0.010	*
Isoleucine	0.786	0.764	0.745	0.765	0.012	NS
Leucine	0.814[a]	0.777[a,b]	0.772[b]	0.788	0.013	*
Lysine	0.777[a]	0.756[a,b]	0.739[b]	0.757	0.011	*
Methionine	0.884[a]	0.858[a,b]	0.843[b]	0.862	0.012	**
Phenylalanine	0.811[a]	0.795[a,b]	0.760[b]	0.789	0.015	*
Threonine	0.712	0.682	0.690	0.695	0.009	NS
Valine	0.764	0.747	0.737	0.749	0.008	NS
Dispensable AA						
Alanine	0.803	0.769	0.778	0.783	0.010	NS
Aspartic acid	0.741[a]	0.710[a,b]	0.668[b]	0.707	0.021	*
Cysteine	0.752	0.761	0.770	0.761	0.005	NS
Glycine	0.737	0.726	0.709	0.724	0.008	NS
Glutamic acid	0.857	0.827	0.830	0.838	0.010	NS
Serine	0.704	0.677	0.669	0.683	0.010	NS

[a,b]Mean values sharing different superscripts within rows differ significantly.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
NS = Non-significant.
SEM = Standard error of mean.

Table 7. Comparison of standardized ileal amino acid digestibility coefficient between 00-rapeseed meal and rapeseed meal (as received basis).

Amino acid	00-Rapeseed meal	Rapeseed meal	SEM	Significance
Indispensable AA				
Crude protein	0.782[a]	0.681[b]	0.027	*
Arginine	0.884	0.848	0.015	NS
Histidine	0.866	0.818	0.014	NS
Isoleucine	0.842[a]	0.765[b]	0.020	*
Leucine	0.863[a]	0.788[b]	0.019	*
Lysine	0.837	0.757	0.020	*
Methionine	0.916[a]	0.862[b]	0.014	*
Phenylalanine	0.851	0.789	0.018	NS
Threonine	0.780[a]	0.695[b]	0.022	*
Valine	0.835[a]	0.749[b]	0.020	**
Dispensable AA				
Alanine	0.849[a]	0.783[b]	0.017	*
Aspartic acid	0.804[a]	0.707[b]	0.027	*
Cysteine	0.806	0.761	0.016	NS
Glycine	0.799[a]	0.724[b]	0.021	*
Glutamic acid	0.888	0.838	0.014	NS
Serine	0.785[a]	0.683[b]	0.025	*

[a,b]Mean values sharing different superscripts within rows differ significantly.
*$P < 0.05$.
**$P < 0.01$.
NS = Non-significant.
SEM = Standard error of mean.

DISCUSSION

00-rapeseed and rapeseed are oil seed crops; after oil extraction, 00-RSM and RSM are used as protein sources in poultry and livestock feed (Meng and Slominski, 2005). Rapeseed meal contains anti-nutritional factors, GLS, erucic acid, phytic acid, and some soluble non-starch polysaccharides. These factors affect the performance of poultry birds. By genetic selection, scientists developed new rapeseed variety having <2% erucic acid in oil and not more than 30 μmol/g aliphatic GLS in meal (Mushtaq et al., 2007; Khajali and Slominski, 2012). This new variety was named as 00-rapeseed to differentiate it from older rapeseed variety (Newkirk, 2009). Due to these antinutritional factors, the digestibility of RSM is leading to poor bird performance. The AA digestibility comparison between RSM and

00-RSM demonstrated the difference between the two ingredients.

The SID of Cys in RSM was close to Evonik (2010) but the SID of all other AA was less than the values published by Evonik (2010) and NRC (1994). The reason for this observation may be differences in processing conditions. Although processing may improve the SID of AA, use of elevated temperature during processing may results in damage to AA (Fenwick and Curtis, 1980) and some AA may become unavailable due to Millard reactions (Mushtaq et al., 2007).

Newkirk (2009) and Kim et al. (2012) documented higher AID of AA in 00-RSM than observed in the present study. The reason for this difference may be differences in sample source or nutrient composition of 00-RSM as nutrient profile was not mentioned. Variation in nutrient composition may also be due to different seed varieties, agronomic practices, and environmental and soil conditions in which seeds were grown (Newkirk, 2011; Ravindran et al., 2014). The relatively better Arg, His, and Met digestibilities were in agreement with findings of Fan et al. (1996) who also documented higher digestibility's of these AAs in pigs. Oil extractions also affect SID of AA in 00-RSM. Woyengo et al. (2010) documented higher Arg, Ile, Leu, Phe, and Glu digestibility in canola expellers compared with solvent extracted canola meal.

The reduced SID of AA in RSM compared with 00-RSM is likely due to higher GLS content in RSM. Erucic acid is included in the oil fraction after processing and is, therefore, not of concern in feeding of the meal. As a consequence, the main antinutritional factor in RSM is the GLS. The GLS are commonly named goitrogens, which are present in all varieties of rapeseed (Fenwick and Curtis, 1980). All types of GLS have a common structural skeleton, but different GLS have different side chains (-R chain). On the basis of side chain, GLS are classified as alkenyl-GLS and indolyl-GLS. These GLS are considered non-toxic; however, their hydrolytic products are toxic (Benn, 1977). The enzymes responsible for GLS hydrolysis are the myrosinases (Thioglycoside glycohydrolase), which are present in all plant tissues that contain GLS (Buchwaldt et al., 1986). During processing, cellular disruption causes release of myrosinases that hydrolyze the GLS into glucose and aglucone. At neutral pH, aglucone releases sulphate ions and rearranges itself into isothiocyanate (Underhill, 1980). Myrosinase activity has also been observed in gastrointestinal bacteria of poultry and other animal species (Bougon et al., 1988).

In conclusion, 00-RSM is a better choice for poultry feed compared with RSM.

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