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Available lysine and digestible amino acid contents of proteinaceous foods of India

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Abstract

Cereals and legumes are staple foods in India and are limiting in lysine and sulphur amino acids, respectively. Available lysine loss, due to Maillard-type reactions that may occur during food preparation, exacerbates the problem of lysine deficiency particularly in cereals. Consequently, determining the contents of digestible essential amino acids, particularly lysine, is important. True ileal digestibilities of most amino acids (including total and reactive lysine) were determined for ten food ingredients and eleven foods commonly consumed in India. Semi-synthetic diets each containing either an ingredient or the prepared food as the sole protein source were formulated to contain 100 $g\,kg^{-1}$ protein (75 $g\,kg^{-1}$ for rice-based diets) and fed to growing rats. Titanium dioxide was included as an indigestible marker. Digesta were collected and the amino acid content (including reactive lysine) of diets and ileal digesta determined. Available (digestible reactive) lysine content ranged from 1·9–15·4 $g\,kg^{-1}$ and 1·8–12·7 $g\,kg^{-1}$ across the ingredients and prepared foods respectively. True ileal amino acid digestibility varied widely both across ingredients and prepared foods for each amino acid (on average 60–92 %) and across amino acids within each ingredient and prepared food (overall digestibility 31–96 %). Amino acid digestibility was low for many of the ingredients and prepared foods and consequently digestibility must be considered when assessing the protein quality of poorer quality foods. Given commonly encountered daily energy intakes for members of the Indian population, it is estimated that lysine is limiting for adults in many Indian diets.

Key words: **Available lysine: Amino acids: India: Protein: Protein quality**

India, like many other developing countries has not as yet eliminated the problem of under-nourishment in its poorer communities particularly with respect to protein. In these communities protein intakes tend to be low, and to exacerbate the problem Indian diets are generally cereal and legume based, the proteins of which tend to be more poorly digested than North American protein-based foods (57–75 % versus 88–94 %), largely due to the presence of high levels of insoluble fibre and anti-nutritional factors(1). Cereals tend to be limiting in lysine while legumes are limiting in sulphur containing amino acids.

Ensuring food and nutritional security is a challenge for India, given its large population and high levels of poverty and malnutrition. National Nutrition Monitoring Bureau of India (NNMB) data have revealed a gradual decline in daily per capita intake of protein from 62·9 g in 1975–1979 to 48·8 g in 2004–2005. In countries like India, where dietary amino acid supply may potentially limit protein metabolism, it is imperative to accurately monitor the dietary supply of 'available amino acids' in relation to the dietary requirement(2). Consequently, it is essential to have a fundamental understanding of the digestible amino acid content of Indian foods and the extent to which the digestible amino acids meet the requirements of people in India. Protein digestibility-corrected amino acid score (PDCAAS), scores proteins based on their ability to meet the deemed amino acid requirement of humans for the first limiting amino acid and in the case of cereal-based diets that is likely to be lysine.

While the amino acid composition of many foods consumed in developing countries, including India, is known there is scant data about the digestibility of those amino acids and it is important that this gap in knowledge be addressed. Currently, PDCAAS uses faecal nitrogen digestibility values to correct amino acid scores to PDCAAS values. However, a single nitrogen digestibility value may not reflect the digestibility of all dietary amino acids(2). Moreover, faecal amino acid digestibility values are often higher than ileal amino acid digestibility values, particularly for poorly digested protein sources(2,3). Consequently, PDCAAS values may be more accurate when derived using true ileal amino acid digestibility values as opposed to faecal nitrogen digestibility values(4,5).

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Lysine is of particular interest since it is often first limiting in cereal-based diets and is susceptible to chemical modification during processing or cooking to form nutritionally unavailable derivatives[4,6]. Furthermore, lysine intakes have been shown to be marginal in low socio-economic group Indians[7,8], which is of even greater concern given that the daily requirement for lysine has been shown to be approximately 30 $mg\,kg^{-1}\,day^{-1}$ for healthy Indian men[7] or higher (44 $mg\,kg^{-1}\,day^{-1}$) where there is chronic undernourishment[8]. This value is more than double the requirement estimate laid out in 1985 by the WHO/FAO/UNU expert consultation group. Values for digestible lysine based on true ileal digestible total lysine are likely to overestimate the nutritional value of foods and food ingredients that have been processed or cooked. Instead true ileal digestible reactive lysine provides a more accurate measure of available lysine in such foods[4]. A method has been developed[9,10] that allows the accurate determination of the available lysine (true ileal digestible reactive lysine) content of processed foods (Biolysine™). The objective of this study was to determine true ileal amino acid digestibility and available (digestible reactive) lysine contents for a range of typical cereal- and legume-based foods from India.

Materials and methods

Samples

Ten raw ingredients (wheat flour, rice, maize flour, black gram beans, refined flour, mung beans, lentils, chickpeas, kidney beans and pigeon peas) and eleven prepared foods (wheat roti, cooked rice, maize roti, dosa, idli, naan, mung dal, lentil dal, sambar, chickpea curry and rajmah) were each collected from six households selected in the Punjab region of India. The raw ingredients and prepared foods were pooled across households, freeze dried and ground using a standard kitchen food processor. The dried samples were then air freighted to Massey University, New Zealand where they were further ground through a 1 mm mesh and stored at −20°C prior to analysis. The ingredients used to prepare the food dish are shown in Table 1.

Preparation of 0·6M O-methylisourea solution

A 0·6 M O-methylisourea solution was prepared as described by Moughan and Rutherfurd[9].

Digestibility study

Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University, Palmerston North, New Zealand. Male Sprague-Dawley rats of approximately 200 g bodyweight were housed individually in stainless steel wire-bottomed cages in a room maintained at 22 ± 2°C with a 12 h light/dark cycle. Twenty one semi-synthetic test diets were formulated. The crude protein content of the raw ingredients and prepared foods ranged from 77 to 279 $g\,kg^{-1}$ DM. For samples that had a crude protein content less than 100 $g\,kg^{-1}$, the diets consisted of the raw ingredient or prepared food, a proprietary vitamin mix (50 $g\,kg^{-1}$), a proprietary mineral mix (50 $g\,kg^{-1}$) and an indigestible marker titanium dioxide (3 $g\,kg^{-1}$). For the remainder of the foods, diets were prepared by adding a proprietary vitamin mix, a proprietary mineral mix and an indigestible marker (titanium dioxide) to each of the foods and then diluting as appropriate with a mixture of soybean oil, purified cellulose, sugar and cornstarch in ratios of 10:5:15:70 to reduce the crude protein content to 100 $g\,kg^{-1}$. The concentrations of vitamin mix, mineral mix and titanium dioxide in the final diets were 50 $g\,kg^{-1}$, 50 $g\,kg^{-1}$, 3 $g\,kg^{-1}$ respectively. A basal diet containing 100 $g\,kg^{-1}$ protein was also formulated, using casein as the sole protein source, to meet the nutritional requirements for the growing rat for all nutrients except protein[11]. The latter diet contained 118 $g\,kg^{-1}$ lactic casein, 50 $g\,kg^{-1}$ proprietary vitamin premix, 50 $g\,kg^{-1}$ proprietary mineral premix, 100 $g\,kg^{-1}$ soybean oil, 100 $g\,kg^{-1}$ sucrose, 50 $g\,kg^{-1}$ purified cellulose and 529 $g\,kg^{-1}$ cornstarch. Over the first eleven days of the 14-day experimental period, all the rats were fed the basal casein-based diet. The rats were then randomly allocated to the test diets such that there were five animals per diet and the animals were fed their respective test diets for a further three days. The test diets were not fed for the entire experimental period as they may not have met the rat's requirement for all nutrients. On each day, each rat had unrestricted access to its respective diet from 09.00 hours to 12.00 hours. Water was available at all times. On the final day of the study, between three and four hours after the start of feeding, the rats were asphyxiated using carbon dioxide gas and then decapitated. The stomach contents were checked for faecal matter and no sign of coprophagy was observed. The twenty centimetres of ileum immediately anterior to the ileo-caecal junction was dissected out.

Table 1. Ingredients in the prepared foods.

Food	Ingredients
Cereal-based	
Wheat roti	Wheat flour, water
Maize roti	Maize flour, water
Boiled rice	Rice, water
Naan	Refined wheat flour, oil
Legume-based	
Chickpea curry	Chickpeas, water, onion, garlic, tomatoes, salt, chillies and spices
Lentil dal	Lentils (dehusked and split), water, salt, red chillies and spices. Onion and garlic (optional)
Mung dal	Mung beans (dehusked and split), water, oil, salt, red chillies and spices. Onion and garlic (optional)
Rajmah	Kidney beans, water, onion, garlic, tomatoes, salt, chillies and spices
Sambar	Pigeon peas (dehusked and split), water, oil, salt, red chillies and spices, seasonal vegetables, tamarind extract
Cereal-legume based	
Idli	Rice, black gram beans (split and dehusked), water
Dosa	Rice, black gram beans (split and dehusked), oil

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The dissected ileum was washed with distilled deionised water to remove any blood and hair and carefully dried on an absorbent paper towel. The digesta were gently flushed from the ileum section with distilled deionised water from a syringe. The digesta were then freeze-dried ready for chemical analysis.

Chemical analysis

Dry matter, ash, crude protein, crude fibre and total fat were determined according to the methods described by AOAC[12]. Protein content was estimated from the nitrogen content using a nitrogen to protein conversion factor of 6·25.

Amino acid contents were determined in duplicate five mg ingredient, prepared food and ileal digesta samples and quadruplicate five mg diet samples following hydrolysis in 6 M glass-distilled HCl containing 0·1 % phenol for 24 h at 110 ± 2°C in evacuated sealed tubes. The liberated amino acids were derivatised with *o*-phthalaldehyde (OPA) (proline was not detected in the assay as it does not react with OPA). The derivatives were then separated on an Agilent 1200SL HPLC system equipped with a C_{18} reverse-phase HPLC column and quantified using fluorescence detection (excitation λ 338 nm, emission λ 450 nm). Proline was detected using Accutag derivatisation (Waters Millipore, Milford, Ma) and fluorescence detection (excitation λ 245 nm, emission λ 395 nm). Cysteine and methionine were determined using performic acid oxidation followed by HCl acid hydrolysis as described above and quantified using Accutag derivatisation (Waters Millipore, Milford, Ma) and fluorescence detection. Tryptophan was determined using alkali hydrolysis in 4·5 M NaOH containing 5 % (w/v) maltodextrin at 110 ± 2°C for 20 h. Tryptophan was then quantified using absorbance at 280 nm. 5-methyl tryptophan was used as an internal standard. The weight of each amino acid was calculated using free amino acid molecular weights. It should be noted that for some rats there was insufficient digesta material for the determination of methionine, cysteine and tryptophan.

Reactive lysine contents were determined in duplicate five mg ingredient, prepared food, digesta and diet samples after guanidination by incubation for seven days in 0·6 M O-methylisourea, pH 10·6 (pH 11·0 for the digesta samples), at 21 ± 2°C in a shaking waterbath, with a reagent to lysine ratio greater than 1000[9]. After incubation, the samples were dried using a Speedvac concentrator (Savant Instruments, Inc, Farmingdale, NY, USA) and analysed for homoarginine content using a Waters ion-exchange HPLC system, utilising post-column OPA derivatisation and detection using fluorescence (excitation λ 338 nm, emission λ 450 nm), following hydrolysis as described above for the other amino acids.

The titanium contents of the diet and ileal digesta samples were determined in duplicate based on the method of Short *et al.*[13]. Samples were ashed before being digested in 60 % (v/v) sulphuric acid and then incubated with 30 % hydrogen peroxide and the absorbance read at 405 nm.

Data Analysis

Ileal amino acid (AA) flows were calculated using the following equation (units are $\mu g\,g^{-1}$ dry matter (DM)):

$$\begin{aligned}&\text{Ileal AA flow } (\mu g\,g^{-1} \text{ dry matter intake (DMI)})\\&= \text{AA concentration in ileal digesta}\\&\times \text{Dietary titanium/Ileal titanium}\end{aligned}$$

True ileal amino acid digestibility[1] was calculated as follows (units are $\mu g\,g^{-1}$ DMI):

$$\begin{aligned}&\text{True digestibility } (\%)\\&= [\text{Dietary AA intake} - (\text{Ileal AA flow}\\&- \text{Endogenous AA flow}^2)] / \text{Dietary AA intake} \times 100\end{aligned}$$

[1]True ileal glycine digestibility values were not calculated since the enzyme hydrolysed casein/ultrafiltration method underestimates endogenous ileal glycine losses.

[2]Based on endogenous amino acid flows for the growing rat as reported by Rutherfurd and Moughan[14] and based on the enzyme hydrolysed casein/ultrafiltration method[15].

True ileal reactive lysine (RL) digestibility was calculated as follows (units are $\mu g\,g^{-1}$ DMI):

$$\begin{aligned}&\text{True ileal RL digestibility } (\%)\\&= [\text{Dietary RL}^1 \text{ intake} - (\text{Ileal RL}^1 \text{ flow}\\&- \text{Endogenous lysine flow}^2)] / \text{Dietary RL}^1 \text{ intake} \times 100\end{aligned}$$

[1]Reactive lysine determined using the guanidination method.

[2]Based on the endogenous lysine flow reported by Rutherfurd and Moughan[14] where for the enzyme hydrolysed casein/ultrafiltration method[15] endogenous reactive lysine is equivalent to endogenous total lysine.

True ileal digestible reactive lysine content of the foods was calculated as follows:

$$\begin{aligned}&\text{True ileal digestible reactive lysine content } (g\,kg^{-1})\\&= \text{Reactive lysine content of the food } (g\,kg^{-1})\\&\times \text{True ileal reactive lysine digestibility } (\%)\end{aligned}$$

True ileal digestible amino acid content of the food was calculated as follows:

$$\begin{aligned}&\text{True ileal digestible amino acid content of the food } (g\,kg^{-1})\\&= \text{Amino acid content of the food } (g\,kg^{-1})\\&\times \text{True ileal amino acid digestibility } (\%)\end{aligned}$$

The amino acid digestibility data were subjected to a one-way analysis of variance for each amino acid singly (GLM Procedure)[16].

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Results

The determined proximate composition of the ten Indian food ingredients and eleven common Indian food dishes prepared from similar ingredients is presented in Table 2. Crude protein ranged from 77 to 279, crude fibre from 7 to 71, total fat from 11 to 154 and ash from 3 to 64 g kg^{-1} DM across ingredients and prepared foods. The nitrogen free extractive (NFE) ranged from 576 to 884 g kg^{-1} DM and demonstrated as expected, that carbohydrates were the main chemical component of the foods tested.

The true ileal digestible reactive (available) lysine content varied markedly across ingredients and prepared foods from 1·9 g kg^{-1} DM for rice to 15·4 g kg^{-1} DM for lentils for the ingredients and 1·8 g kg^{-1} DM for maize roti to 12·7 g kg^{-1} DM for lentil dal for the prepared foods (Table 3). For the cereal ingredients and cereal-based prepared foods the available lysine content ranged from 1·9 g kg^{-1} DM for rice to 3·1 g kg^{-1} DM for wheat flour for the ingredients and from 1·8 g kg^{-1} DM for maize roti to 2·7 g kg^{-1} DM for wheat roti for the prepared foods. For the legume ingredients and legume-based prepared foods the available lysine content ranged from 10·3 g kg^{-1} DM for pigeon peas to 15·4 g kg^{-1} DM for lentils for the ingredients and 9·7 g kg^{-1} DM for chickpea curry to 12·7 g kg^{-1} DM for lentil dal for the prepared foods. For the prepared foods containing both cereals and legumes the available lysine ranged from 4·2 g kg^{-1} DM for dosa to 4·9 g kg^{-1} DM for idli.

The true ileal digestible total lysine content was also determined and compared with the true ileal digestible reactive (available) lysine content (Table 3). For seven of the eleven prepared foods, true ileal digestible total lysine significantly ($P<0·05$) overestimated true ileal digestible reactive (available) lysine content. This overestimation ranged from 1 % for lentil dal to 27 % for idli with the mean overestimation being 12 %. For the other prepared foods, cooked rice, maize roti, rajmah and chickpea curry there was no significant difference ($P>0·05$) between digestible total lysine and digestible reactive lysine contents. For the ingredients, there was no significant ($P>0·05$) difference between digestible total and reactive lysine contents for most of the ingredients. The exceptions were wheat flour, rice, kidney beans and pigeon peas where for wheat flour, kidney beans and pigeon peas digestible total lysine significantly ($P<0·05$) overestimated digestible reactive (available) lysine by between 6 and 12 %.

The true ileal digestibilities of amino acids (including reactive lysine) for the eleven commonly prepared Indian foods and the ten Indian food ingredients are given in Table 4. The overall true ileal amino acid digestibility determined across all amino acids for each food ingredient ranged from 31 % for black gram beans to 96 % for wheat flour with an overall mean digestibility across all ingredients of 77 %. For the prepared foods, overall amino acid digestibility ranged from 67 % for rajmah to 95 % for lentil dal with a mean overall amino acid digestibility calculated across all foods of 86 %. The least digestible amino acid across all the prepared foods was cysteine (mean value of 69 %) and the most digestible amino acids were lysine and leucine (mean value of 92 %). For the ingredients, the least digestible amino acid was aspartic acid

Table 2. Determined nutrient composition[1] (g kg^{-1} DM) of the eleven prepared Indian foods and ten Indian food ingredients.

	Crude protein[2]	Crude fibre	Total fat	Ash	NFE[3]
Prepared Indian food					
Wheat roti	124	23	32	17	821
Cooked rice	77	9	11	5	884
Maize roti	104	25	58	15	811
Dosa	121	12	94	13	762
Idli	146	11	19	16	805
Naan	123	7	59	6	793
Mung dal	213	66	102	64	612
Lentil dal	228	31	108	45	601
Sambar	212	28	101	52	602
Chickpea curry	187	64	154	51	600
Rajmah	233	65	74	53	629
Indian food ingredient					
Wheat flour	138	24	30	16	755
Rice	77	9	11	3	794
Maize flour	108	21	57	14	752
Black gram beans	269	17	23	37	602
Refined flour	135	11	19	6	730
Mung beans	249	71	22	38	598
Lentils	274	21	21	20	618
Chickpeas	265	55	58	32	590
Kidney beans	279	61	28	41	576
Pigeon peas	256	24	29	41	604

[1] Analysis was conducted in duplicate.
[2] Protein content was estimated from the nitrogen content using a nitrogen to protein conversion factor of 6·25.
[3] Nitrogen free extractive was calculated as follows (units are g kg^{-1}):

$$\text{Nitrogen free extractive (g kg}^{-1}\text{ DM)} = \text{Total sample weight} - (\text{Moisture} + \text{Ash} + \text{Crude protein} + \text{Crude fibre} + \text{Ether extract})$$

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Table 3. Mean (n = 5) true ileal digestible total and reactive (available) lysine contents[1] (g kg^{-1} DM) for the eleven prepared Indian foods and ten Indian food ingredients.

	Digestible lysine		Overall	Statistical
	Total[2]	Reactive[3]	SE	Significance[4]
Prepared Indian food				
Wheat roti	2·95	2·66	0·02	***
Cooked rice	2·00	1·98	0·03	NS
Maize roti	1·74	1·82	0·02	NS
Dosa	4·85	4·21	0·07	**
Idli	6·22	4·90	0·08	***
Naan	2·59	2·20	0·06	**
Mung dal	12·37	11·38	0·07	***
Lentil dal	12·81	12·65	0·07	***
Sambar	11·47	11·15	0·11	*
Chickpea curry	9·81	9·66	0·11	NS
Rajmah	10·13	10·25	0·23	NS
Indian food ingredient				
Wheat flour	3·35	3·11	0·04	*
Rice	1·87	1·92	0·03	*
Maize flour	2·27	2·34	0·03	NS
Black gram beans	6·03	7·00	0·80	NS
Refined flour	2·19	2·18	0·02	NS
Mung beans	12·57	11·96	0·37	NS
Lentils	15·20	15·42	0·22	NS
Chickpeas	15·27	15·12	0·15	NS
Kidney beans	11·33	10·66	0·35	**
Pigeon peas	11·68	10·32	0·28	***

[1] The true ileal digestibility values used to calculate the true ileal digestible amino acid content were determined after correction for endogenous lysine flow determined using the enzyme hydrolysed casein/ultrafiltration method[15] and using values as reported by Rutherfurd and Moughan[14].
[2] Determined based on the analysis of total lysine in diet and digesta of rats fed the experimental diets.
[3] Determined based on the analysis of reactive lysine (guanidination reaction) in diet and digesta of rats fed the experimental diets.
[4] NS Not significant $P > 0·05$, *$0·05 > P > 0·01$, **$0·01 > P > 0·001$, ***$P < 0·001$.

(mean value of 60 %) and the most digestible was methionine (mean value of 89 %).

The true ileal digestible amino acid contents of the materials is presented in Table 5. There was considerable variation in the digestible amino acid content across both the prepared foods and food ingredients, with on average a 4·2- and 5·0-fold range in digestible amino acid contents across foods and amino acids for the prepared foods and ingredients respectively.

Discussion

The lysine intake of people in India, particularly children, is likely to be marginal, because firstly, food intake is generally low [17]. Secondly, cereals and legumes tend to be staple foods for many Indians[17] and cereals are low in lysine while legume intake relative to cereals tends to be low[18]. Thirdly, lysine is prone to undergo chemical modification when foods undergo heat processing (such as cooking) to form Maillard-type products which are generally nutritionally unavailable and will therefore further reduce the available lysine content. In this study, the available content of lysine and the ileal digestible amounts of other amino acids were determined in eleven commonly prepared Indian foods and ten Indian food ingredients.

Overall, true ileal digestible total lysine overestimated available lysine (true ileal digestible reactive lysine) for more than half the prepared foods and just under half of the food ingredients and in many cases this overestimation was large (>8 % for seven of the prepared foods and ingredients). This is consistent with previous results[19–21] and given that the available lysine assay (Biolysine™) only requires a different step in the chemical analysis rather than a separate bioassay, this method should be the preferred one for determining lysine availability in foods. The traditional (total lysine based) ileal digestibility assay will lead to considerable error for some foods.

There was considerable variation in the available lysine content of the Indian food ingredients and prepared foods with 8-fold differences in the available lysine content across the ingredients and prepared foods. As expected, the available lysine content was lowest for the cereal-based prepared foods (1·8–2·7 g kg^{-1} DM), intermediate in the prepared foods containing cereals and legumes (4·2–4·9 g kg^{-1} DM) and highest in the legume-based prepared foods (9·7–12·7 g kg^{-1} DM). For the cereals and legume ingredients respectively, the range in available lysine content was small with a 1·6-fold and 1·5-fold difference in the available lysine content across cereal ingredients and cereal-based prepared foods respectively and a 1·4-fold and 1·3-fold difference in available lysine content across legume ingredients and prepared foods respectively. The inclusion of legumes into cereal-based diets increased the available lysine content considerably (approximately double) and clearly fortification of cereal-based diets with legumes is a useful approach for increasing the lysine intakes of the Indian population.

Overall true ileal amino acid digestibility varied markedly across both ingredients and prepared foods and was often relatively low. It is clear that for even the most digestible ingredients and prepared foods amino acid digestion and absorption was far from complete and therefore must be taken into account when determining available amino acid content. Amino acid digestibility also varied considerably across amino acids within each ingredient and prepared food, with the difference between the lowest and highest digestibilities across amino acids within ingredients or prepared foods ranging from 12 % units (88–100 %) for wheat flour to 85 % units (0–85 %) for black gram beans for the ingredients and 15 % units (82–97 %) for lentil dal to 49 % units (33–82 %) for rajmah. This highlights the potential error in using a single digestibility value (eg. crude protein digestibility) to predict amino acid digestibility in general. Protein digestibility-corrected amino acid scores (PDCAAS) are commonly calculated using a single digestibility factor (true faecal nitrogen digestibility). Depending on the limiting amino acid in each ingredient or prepared food, the use of true faecal nitrogen digestibility values could result in an inaccurate assessment of protein quality.

For the foods containing legumes, true ileal amino acid digestibility determined across all amino acids was lower for the food ingredients than for the prepared foods, which is most likely due to the presence of anti-nutritional factors[1]. For example, the overall mean amino acid digestibilities for

Table 4. Mean (n = 5) true ileal amino acid digestibility[1] (%) for the eleven prepared Indian foods and ten raw Indian food ingredients[1].

	Prepared Indian foods											
Amino acid	Wheat roti	Cooked rice	Maize roti	Dosa	Idli	Naan[2]	Mung dal	Lentil dal	Sambar	Chickpea curry	Rajmah	Overall SE
Aspartic acid	84	74	86	90	85	75	80	91	77	70	50	2·4
Threonine	88	70	82	90	85	87	83	91	86	80	61	2·6
Serine	93	72	90	91	86	91	86	95	88	84	64	1·8
Glutamic acid	97	60	93	90	87	96	89	96	85	89	74	1·5
Alanine	89	75	93	90	85	90	82	93	86	84	66	1·7
Valine	91	78	90	92	89	89	88	95	90	83	68	1·9
Isoleucine	95	75	91	91	87	93	85	95	88	82	72	2·0
Leucine	98	82	96	95	93	97	94	98	95	88	76	1·3
Tyrosine	91	81	94	90	88	90	87	96	91	88	74	1·8
Phenylalanine	95	79	95	93	90	95	93	97	82	90	77	1·2
Histidine	93	84	88	94	89	93	85	96	91	87	68	1·9
Lysine[3]	91	96	90	95	91	89	95	97	94	90	81	1·4
Arginine	89	84	89	92	89	92	87	97	91	91	79	1·4
Cysteine[4]	74	48	77	82	63	–	70	88	79	72	33	4·4
Methionine[4]	89	54	96	92	81	–	93	100	98	94	82	2·3
Proline[4]	85	75	89	97	88	–	85	97	94	92	72	2·6
Tryptophan[5]	–	–	77	–	–	–	81	82	70	72	50	4·2
Overall digestibility	91	74	89	91	86	91	86	95	87	84	67	2·1

	Indian food ingredients										
Amino acid	Wheat flour	Rice	Maize flour	Black gram beans	Refined flour	Mung beans	Lentils	Chickpeas	Kidney beans	Pigeon peas	Overall SE
Aspartic acid	88	83	90	0	80	34	82	82	28	36	4·6
Threonine	92	81	84	22	86	43	89	83	38	53	4·7
Serine	98	88	93	20	96	63	92	86	47	54	3·0
Glutamic acid	99	84	96	20	98	54	89	89	43	49	3·0
Alanine	93	90	95	27	91	60	90	86	52	57	2·9
Valine	96	92	94	19	94	60	90	85	42	50	3·0
Isoleucine	98	90	95	14	96	52	87	83	34	46	3·4
Leucine	99	92	98	29	99	62	90	88	50	60	2·7
Tyrosine	97	89	95	44	95	69	86	90	47	62	3·2
Phenylalanine	98	90	97	20	97	60	89	89	43	49	3·1
Histidine	99	90	94	36	97	62	93	90	53	71	2·9
Lysine[3]	94	97	92	42	93	78	96	92	62	66	2·5
Arginine	93	89	91	46	88	70	94	93	66	70	2·1
Cysteine[4]	94	85	85	17	92	67	93	82	30	55	3·4
Methionine[4]	100	89	100	85	99	86	105	96	54	77	1·3
Proline[4]	100	91	94	55	99	64	99	94	28	74	2·7
Tryptophan[5]	91	–	84	–	83	76	90	71	–	–	4·5
Overall digestibility	96	89	93	31	93	62	91	87	45	58	3·1

[1] Values were corrected for endogenous amino acid flows determined using the enzyme hydrolysed casein/ultrafiltration method[15] and as reported by Rutherfurd and Moughan[14].
[2] Insufficient digesta material for the determination of cysteine, methionine, proline and tryptophan.
[3] Based on reactive lysine determined using the guanidination method.
[4] n = 5 for all ingredients except wheat flour (n = 4) and kidney beans (n = 2) and all prepared foods except wheat roti (n = 4), dosa (n = 1) and idli (n = 2) where there was insufficient material to analyse digesta from all the rats.
[5] Insufficient digesta material for the determination of tryptophan.

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Table 5. Mean (n = 5) true ileal digestible amino acid contents[1] (g kg^{-1} DM) for the eleven prepared Indian foods and ten Indian food ingredients.

Amino acid	Wheat roti	Cooked rice	Maize roti	Dosa	Idli	Naan[2]	Mung dal	Lentil dal	Sambar	Chickpea curry	Rajmah	Overall SE
	Prepared Indian foods											
Aspartic acid	6·0	4·5	5·5	11·8	13·0	5·8	22·6	23·0	16·7	15·2	14·4	0·33
Threonine	3·6	1·7	3·0	3·9	4·3	4·4	7·2	7·5	6·5	5·5	6·4	0·13
Serine	5·2	2·3	3·9	4·9	5·5	6·6	8·9	9·2	8·3	7·1	8·6	0·11
Glutamic acid	39·5	7·2	17·9	18·0	19·8	52·4	35·0	33·4	37·9	25·5	27·8	0·34
Alanine	4·2	2·9	6·4	4·7	5·1	4·5	7·4	7·2	6·9	5·8	6·2	0·09
Valine	5·4	3·2	4·1	6·1	6·8	6·5	10·4	9·2	8·1	6·7	8·5	0·13
Isoleucine	4·2	2·0	2·9	4·3	4·8	5·3	7·8	7·5	6·3	5·9	6·6	0·11
Leucine	5·6	3·1	8·3	6·0	7·4	7·8	12·6	11·2	10·8	9·4	11·1	0·10
Tyrosine	2·9	2·1	3·2	3·5	3·3	4·6	5·9	5·5	5·1	4·5	5·8	0·07
Phenylalanine	5·3	2·6	4·1	5·5	6·4	6·9	11·7	9·1	16·3	8·7	9·9	0·09
Histidine	2·5	1·2	2·1	2·6	2·9	2·9	5·0	4·3	6·1	3·6	4·2	0·06
Arginine	4·9	4·1	3·5	6·3	6·9	5·5	12·1	17·6	11·1	13·1	12·6	0·11
Cysteine[3]	3·2	1·0	1·8	2·1	1·0	–	1·4	3·0	2·1	2·9	0·7	0·13
Methionine[3]	2·9	1·4	2·3	3·8	2·1	–	3·3	2·4	3·0	3·5	2·6	0·13
Proline[4]	11·3	2·9	7·7	5·7	3·9	–	6·8	8·6	8·4	7·0	6·6	0·32
Tryptophan[4]	2·4	–	0·7	–	–	–	2·6	1·6	1·3	1·8	1·6	0·07

Amino acid	Wheat flour	Rice	Maize flour	Black gram beans	Refined flour	Mung beans	Lentils	Chickpeas	Kidney beans	Pigeon peas	Overall SE
	Indian food ingredients										
Aspartic acid	5·3	4·1	4·9	0	3·7	8·0	19·8	22·7	8·0	7·7	0·97
Threonine	3·1	1·7	2·7	1·8	2·5	3·3	7·5	7·4	4·0	4·4	0·28
Serine	6·3	3·0	4·7	2·9	5·5	7·8	10·9	11·0	6·8	6·3	0·30
Glutamic acid	36·9	8·8	17·3	8·9	40·7	20·2	31·2	35·0	16·6	24·1	1·05
Alanine	4·0	3·3	7·2	0	3·3	6·2	8·4	8·9	5·4	5·8	0·22
Valine	5·5	3·6	4·4	2·9	4·8	6·2	8·6	8·4	5·2	4·8	0·30
Isoleucine	4·0	2·0	3·1	2·6	3·6	4·4	7·1	7·9	3·4	3·6	0·27
Leucine	5·5	5·4	9·5	1·4	4·4	9·4	12·0	13·3	7·6	7·7	0·35
Tyrosine	3·9	2·2	3·7	5·2	3·8	3·6	4·8	5·6	2·8	3·5	0·14
Phenylalanine	5·3	2·5	4·5	2·4	5·0	7·9	9·1	12·0	5·7	11·6	0·36
Histidine	2·3	1·0	2·2	2·9	1·9	3·9	4·5	5·3	3·4	5·5	0·14
Arginine	5·7	4·3	4·3	2·4	4·0	10·8	20·5	21·7	9·8	10·5	0·23
Cysteine[3]	3·8	2·1	2·2	0·4	3·5	1·6	3·0	4·2	0·9	1·5	0·08
Methionine[3]	2·7	2·6	2·5	4·7	2·6	3·6	2·3	4·7	2·2	2·5	0·04
Proline[3]	14·4	3·3	8·1	6·2	14·3	6·8	9·2	9·9	2·6	8·2	0·21
Tryptophan[4]	2·1	–	0·8	–	1·6	2·9	2·2	2·3	–	–	0·09

[1] True ileal digestible lysine content is presented in Table 3 as the true ileal digestible reactive lysine content.
[2] Insufficient digesta material for the determination of cysteine, methionine, proline and tryptophan.
[3] n = 5 for all ingredients except wheat flour (n = 4) and kidney beans (n = 2) and all prepared foods except wheat roti (n = 4), dosa (n = 1) and idli (n = 2) where there was insufficient material to analyse digesta from all the rats.
[4] Insufficient digesta material for the determination of tryptophan.

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mung dal, sambar, and rajmah were markedly higher than for their main ingredients (mung beans, pigeon peas and kidney beans). Similarly, Wu *et al.*[22] reported true faecal protein digestibility values of 16 and 79 % respectively for raw kidney beans and kidney beans cooked at 100°C for 2 h. The increase in the mean amino acid digestibility is most likely due to the destruction of anti-nutritional factors present in the legume ingredients during cooking[23]. For the cereal-based ingredients and prepared foods, wheat roti, cooked rice, maize roti, naan were more poorly digested than their respective major ingredients wheat flour, rice, maize flour, wheat flour. Kubota *et al.*[24] have also reported that cooking reduces the digestion of prolamin, a major storage protein in rice. The decrease in mean amino acid digestibility observed for the cereal-based prepared foods is possibly due to Maillard-type reactions occurring during cooking[6] and the subsequent formation of indigestible limit peptides[25]. For lentil dal and chickpea curry each made from lentils and chickpeas respectively, the overall mean amino acid digestibility of the prepared food was similar to that of the respective major ingredients.

There was considerable variation in the true ileal digestible amino acid contents for each amino acid, including lysine, across both the prepared foods (on average a 4·6-fold difference) and food ingredients (on average a 5·4-fold difference). As expected the amino acid profile of the prepared foods generally reflected that observed for the main ingredients included in each of the prepared foods.

Diet surveys conducted by the National Nutrition Monitoring Bureau (NNMB) of India during 2001–2002[17] revealed that among 1–6 year-old children, the consumption of various foods like cereals and pulses was less than the recommended levels. The intake among the adults was higher, where the recommended intake of cereals was met, but the intake of legumes was still inadequate. Furthermore, while adults received 80–90 % of the recommended dietary protein intake only 30 % of children consumed protein adequate diets. The net availability (per capita per day) of cereals in India has increased from 394·9 g in 1951 to 439·3 g in 2007 but at the same time the net availability of legumes which are a significant source of lysine for Indians has declined from 60·7 g in 1951 to 29·4 g in 2007[18]. In an attempt to put the results of the present study into context, the available lysine intake of the Indian population for a range of commonly consumed prepared food combinations based on the prepared foods tested in the present study was estimated and is presented in Table 6. The daily lysine intakes were estimated in two ways, firstly, based on the reported average daily energy intake of the Indian population (2034 kcal day^{-1})[26] and secondly based on cereal and legume supply data[18]

Table 6. Estimated daily lysine intake (g day^{-1}) and lysine adequacy for the Indian population receiving a diet derived from selected foods tested in this study.

	Based on cereal and legume supply in India				Based on reported daily energy intakes			
Percentage of legume-based food to cereal-based food[4]	Lys intake[1] 10:90	Adequacy[3] 10:90	Lys intake[1] 20:80	Adequacy[3] 20:80	Lys intake[2] 10:90	Adequacy[3] 10:90	Lys intake[2] 20:80	Adequacy[3] 20:80
Sambar/idli	2·24	1·05	2·42	1·14	2·51	1·18	2·70	1·27
Sambar/dosa	1·90	0·90	2·04	0·96	1·96	0·93	2·11	0·99
Mung dal/wheat roti	1·35	0·64	1·59	0·75	1·50	0·71	1·76	0·83
Lentil dal/wheat roti	1·38	0·65	1·66	0·78	1·53	0·72	1·83	0·87
Chickpea curry/wheat roti	1·32	0·62	1·53	0·72	1·46	0·69	1·68	0·79
Rajmah/wheat roti	1·29	0·61	1·47	0·69	1·44	0·68	1·64	0·77
Mung dal/cooked rice	1·27	0·60	1·69	0·80	1·44	0·68	1·91	0·90
Lentil dal/cooked rice	1·33	0·63	1·81	0·85	1·51	0·71	2·03	0·96
Sambar/cooked rice	1·17	0·55	1·52	0·72	1·34	0·63	1·72	0·81
Chickpea curry/cooked rice	1·22	0·58	1·59	0·75	1·38	0·65	1·77	0·83
Mung dal/maize roti	0·99	0·47	1·24	0·58	1·08	0·51	1·34	0·63
Lentil dal/maize roti	1·02	0·48	1·30	0·62	1·11	0·52	1·41	0·67
Chickpea curry/maize roti	0·97	0·46	1·19	0·56	1·05	0·50	1·28	0·60
Rajmah/maize roti	0·94	0·44	1·12	0·53	1·02	0·48	1·22	0·58
Mung dal/naan	1·13	0·53	1·35	0·64	1·20	0·57	1·44	0·68
Lentil dal/naan	1·16	0·55	1·41	0·67	1·23	0·58	1·50	0·71
Chickpea curry/naan	1·11	0·52	1·30	0·62	1·18	0·56	1·38	0·65
Rajmah/naan	1·08	0·51	1·24	0·59	1·15	0·54	1·33	0·63

[1] Based on a daily food intake of 468·7 g day^{-1} (439·3 g day^{-1} of cereals and 29·4 g day^{-1} of legumes)[18] and assuming a DM content of 90 % calculated as follows:

$$\text{Daily lysine intake (g day}^{1}) = \text{Avlys}_{(\text{legume-based food})} \times \text{Daily DM intake} \times \text{Percentage}_{(\text{legume-based food})} + \text{Avlys}_{((\text{cereal-based food})} \times \text{Daily DM intake} \times \text{Percentage}_{(\text{cereal-based food})}$$

Where Avlys is the determined available lysine content (g g^{-1}) of the prepared food, percentage is the percentage of either the legume-based prepared food or cereal-based prepared food in the combined food and daily DM intake = 421·8 g DM day^{-1} (468·7 g day^{1}x 90 % DM).

[2] Based on the reported daily energy intake of 2034 kcal day^{-1}[26] and calculated as follows:

$$\text{Daily lysine intake} = \text{Avlys}_{(\text{legume-based food})} \times \text{Daily DM intake} \times \text{Percentage}_{(\text{legume-based food})} + \text{Avlys}_{(\text{cereal-based food})} \times \text{Daily DM intake} \times \text{Percentage}_{(\text{cereal-based food})}$$

Where Avlys is the determined available lysine content (g g^{-1}) of the prepared food, percentage is the percentage of either the legume-based prepared food or cereal-based prepared food in the combined food and daily DM intake is calculated as follows:

$$\text{Daily DM intake} = \text{Daily energy intake (kcal day}^{-1})^{(26)} / \text{Energy content of the combined food (kcal g}^{-1}\text{DM)}$$

[3] Adequacy was calculated for a 70 kg adult based on a lysine requirement of 30 mg kg^{-1} day^{-1}[27] as follows:

$$\text{Adequacy} = \text{Lysine intake (g day}^{-1}) / \text{Lysine requirement (g day}^{-1})$$

[4] Percentage of each prepared food in the combined food and based on the wet weight.

and assuming 10 % food wastage. In both cases it was assumed that for each day each meal consisted of a legume-based prepared food and a cereal-based prepared food (for example, cooked rice with sambar) the proportions of which were assumed to be between 10 % to 20 % legume-based prepared food and correspondingly between 90 % to 80 % cereal-based prepared food (these figures were based on the relative legume and cereal availability in India[18]). A similar analysis (data not shown) was conducted for the sulphur amino acids (methionine plus cysteine) which tend to be first limiting in legumes. For a seventy kilogram adult human, the lysine requirement is 2·12 g day^{-1}[27]; and the methionine plus cysteine requirement 0·98 g day^{-1}[27]; however, the estimated daily lysine intake for all of the prepared food combinations, with the exception of sambar/idli was lower than 2·12 g day^{-1} ranging from 0·94 g day^{-1} for rajmah/maize roti (10:90) to 2·04 g day^{-1} for sambar/dosa (20:80) when based on cereal and legume availability per capita and ranging from 1·02 g day^{-1} for rajmah/maize roti (10:90) to 2·1 g day^{-1} for sambar/dosa (20:80) when based on daily energy intake. In contrast, the daily intake of sulphur amino acids estimated based on the daily energy intake of the Indian population ranged from 1·30 g day^{-1} for sambar/cooked rice (10:90) to 2·88 g day^{-1} for chickpea curry/wheat roti (10:90) and would be sufficient to meet the daily methionine plus cysteine requirement for a 70 kg man for all of the prepared food combinations examined. Although it is recognised that it is unlikely that Indians will consume the same prepared food combination for each meal in a day, consumption of any of the prepared food combinations, excluding sambar/idli, would lead to an insufficient daily lysine intake and in many cases supplying less than three quarters of the daily lysine requirement. The similar outcome, as to whether analysis was based on food supply data or reported energy intakes, gives some confidence in the data. For the scenario above both protein and energy would be limiting. However, even if food intakes were sufficient to meet energy requirements it was calculated that lysine would still be limiting for between 40–80 % of the food combinations evaluated as part of this study.

Conclusion

The available lysine content was highly variable across ingredients and prepared foods. In addition, digestible total lysine overestimated available lysine (digestible reactive lysine) for many of the ingredients and prepared foods. This has important implications for dietary protein quality assessment. Available (true ileal digestible reactive) lysine values are generally likely to be lower than true faecal lysine digestibility values because faecal lysine digestibility usually overestimates ileal lysine digestibility and digestible total lysine often over estimates digestible reactive (available) lysine. True ileal amino acid digestibility varied widely both across ingredients and prepared foods for each amino acid and also across amino acids within each ingredient and prepared food. True ileal nitrogen digestibility was a poor predictor of amino acid digestibility for many amino acids in the ingredients and prepared foods tested in this study. Amino acid digestibility was often far less than complete and consequently amino acid digestibility must be taken into account when assessing the protein quality of poorer quality foods and ingredients such as those often consumed in developing countries such as India.

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SR co-designed the study, ran the animal trials and laboratory analysis and co-wrote the manuscript. KB co-designed the study and arranged for the collection and preparation of the samples and co-wrote the manuscript. PM co-designed the study and co-wrote the manuscript. The authors state that there are no conflicts of interest. Funding for the work was provided by the Riddet Institute.

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