

## Available (Ileal Digestible Reactive) Lysine in Selected Cereal-Based Food Products

SHANE M. RUTHERFURD,<sup>\*,†</sup> NOUR MOHAMMAD TORBATINEJAD,<sup>§</sup> AND  
 PAUL J. MOUGHAN<sup>‡</sup>

Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand,  
 Department of Animal Science, Gorgan University of Agricultural Sciences & Natural Resources,  
 Gorgan, Iran, and Riddet Centre, Massey University, Palmerston North, New Zealand.

True ileal total lysine digestibility was determined and compared with true ileal reactive lysine digestibility when applied to 20 cereal-based breakfast foods. Semisynthetic diets each containing a breakfast cereal as the sole protein source were formulated and fed to growing rats. Titanium dioxide was included as an indigestible marker. Digesta were collected from the rats and total (conventional amino acid analysis) and reactive (guanidination) lysine were determined in both diets and digesta. The true ileal reactive lysine digestibility ranged from 53 to 108% and was significantly higher than the true ileal total lysine digestibility for most of the breakfast cereals. Available lysine content (digestible reactive lysine content) ranged from 0.21 to 3.5 g/kg across the breakfast cereals. The conventional measure of digestible total lysine content significantly overestimated (on average 37%) available lysine for the majority of the cereals. Breakfast cereals undergo a significant degree of lysine modification probably as a result of processing during manufacture.

**KEYWORDS:** Lysine; digestibility; availability; ileal; cereals

### INTRODUCTION

Although cereals are an important source of dietary protein for humans, they tend to contain lower amounts of the amino acid lysine compared to other protein sources such as milk or meat. Consequently, lysine is often the first limiting amino acid in diets that are high in cereals.

A unique property of the amino acid lysine is that it possesses a reactive side chain amino group which can react with a variety of chemical entities, particularly reducing sugars, to produce biologically unavailable lysine derivatives. These reactions occur when protein sources are manufactured or stored for prolonged periods of time, with the rate of reaction being greatly accelerated during processing, particularly heat processing (1, 2). Simple sugars are often added to cereal-based breakfast foods during processing as sweetening agents, which is expected to exacerbate the extent of lysine side chain reactions. Erbersdobler and Hupe (3) reported that some 20% of lysine was inactivated and 10% was destroyed in a single processed breakfast cereal. Moreover, a recent study from our laboratory (4) found large differences between the total lysine and the chemically reactive lysine contents of 20 commercial breakfast cereal products indicating that considerable lysine damage had occurred. Given

that cereals are an important staple food, such deterioration in nutrient quality is a matter of concern.

When lysine reacts with other compounds, it is expected, in addition to the formation of biologically unavailable lysine derivatives, that overall protein digestibility is also adversely affected (2) meaning that otherwise reactive lysine (available) units may not be released and absorbed from the parent protein. Thus, the digestible reactive lysine content of a processed food may be considerably lower than the reactive lysine content, which is lower in turn than total lysine. It has been reported that "ready-to-eat" cereals based on maize, wheat, rice, or oats tend to have low overall protein digestibilities (5), and it is possible that Maillard product formation is at least partly responsible for such a reduction in amino acid digestibility. It is important, therefore, to determine digestible reactive lysine in addition to the chemically reactive lysine content. The latter can be accomplished by applying an in-vivo ileal digestibility assay which determines the disappearance of reactive lysine units from the upper digestive tract of the laboratory rat (6). The new assay has been validated (7) and applied to a range of processed food products including milk-based foods (8) and animal feeds (9).

The presently reported study follows that of Torbatinejad et al. (4) and aims to determine the in-vivo digestible reactive (available) lysine content of 20 processed cereal-based breakfast foods using the true ileal reactive lysine digestibility assay.

\* Author to whom correspondence should be addressed. Phone: 64 6 350 5894; fax: 64 6 350 5657; e-mail: S.M.Rutherford@massey.ac.nz.

<sup>†</sup> Institute of Food, Nutrition and Human Health, Massey University.

<sup>§</sup> Gorgan University of Agricultural Sciences & Natural Resources.

<sup>‡</sup> Riddet Centre, Massey University.

## MATERIALS AND METHODS

**Cereal Samples.** Twenty commercially available packaged breakfast cereal products were selected as described fully by Torbatinejad et al. (4). In brief, six different batches each of 20 cereal products (120 samples in total) were purchased from supermarkets in Palmerston North, New Zealand. Equal weights of each batch were pooled for each cereal product and the resulting 20 composite samples were ground through a 1-mm mesh and were stored at  $-20^{\circ}\text{C}$  prior to being tested for true ileal digestible reactive lysine content using an in-vivo digestibility assay.

The major ingredients of the cereal products as reported on the statutory labels given on the packages consisted of either wheat, corn, oatmeal, or rice (4). The proximate composition of each of the breakfast cereals was reported by Torbatinejad et al. (4). In brief, the crude protein content of the breakfast cereals ranged from 52 to 253, crude fiber from 4 to 38, total fat from 14 to 144, and ash from 7 to 32 g/kg DM. The nitrogen free extractive (NFE) ranged from 678 to 908 g/kg DM.

**Preparation of 0.6 M O-Methylisourea Solution.** A 0.6 M O-methylisourea solution was prepared as described by Moughan and Rutherford (6), based on the procedures of Chervenka and Wilcox (10), Shields et al. (11), Mauron and Bujard (12), and Kassell and Chow (13).

**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 150 g body weight were housed individually in stainless steel wire-bottomed cages in a room maintained at  $22 \pm 2^{\circ}\text{C}$ , with a 12-h light/dark cycle. Twenty semisynthetic test diets were formulated. The protein content of the cereal products ranged from 5.2% to 25.3%, and in the main, the experimental diets consisted of the cereal product alone with the addition of titanium dioxide (0.3%). For cereal products that had a crude protein content greater than 10%, dilution with soybean oil and cornstarch was used to reduce the crude protein content to 100 g/kg. A basal diet containing 100 g/kg protein was also formulated using skim milk powder as the protein source and met the nutritional requirements for the growing rat for all nutrients except protein (14). The latter diet contained 42.5% skim milk powder, 5% proprietary vitamin premix, 5% proprietary mineral premix, 5% soybean meal, 10% sucrose, 5% purified cellulose, and 27.2% cornstarch. Titanium dioxide (0.3%) was added to all diets as an indigestible marker. For the first 10 days of the experimental period, all the rats were fed the basal skim milk powder based diet. The test diets were then randomly allocated to the rats, and the animals were fed the diets for the final 4 days. The test diets were not fed for the entire experimental period as they may not have met the rat's requirement for all vitamins and minerals. On each day, each rat had unrestricted access to its respective diet from 0830 to 1130 h. Water was available at all times. On the final day of the study, between 3 and 4 h after the start of feeding, the rats were asphyxiated using carbon dioxide gas and then were decapitated. The 20 cm of ileum immediately anterior to the ileo-caecal junction was dissected out. The dissected ileum was washed with distilled deionized water to remove any blood and hair and was carefully dried on an absorbent paper towel. The digesta were gently flushed from the ileum section with distilled deionized water from a syringe. The digesta were then freeze-dried ready for chemical analysis.

**Chemical Analysis.** Amino acid contents were determined in duplicate 5 mg diet and digesta samples using a Waters ion-exchange HPLC system, utilizing postcolumn ninhydrin derivatization and detection using absorbance at 570 and 440 nm, following hydrolysis in 6 M glass-distilled HCl containing 0.1% phenol for 24 h at  $110 \pm 2^{\circ}\text{C}$  in evacuated sealed tubes. Cysteine, methionine, and tryptophan were not determined as they are destroyed, at least in part, during acid hydrolysis. The weight of each amino acid was calculated using free amino acid molecular weights.

Reactive lysine contents were determined in duplicate 5 mg digesta and diet samples by incubation for 7 days in 0.6 M O-methylisourea, pH 10.6 (pH 11.0 for the digesta samples), at  $21^{\circ}\text{C}$  in a shaking waterbath, with the reagent to lysine ratio being greater than 1000 according to the procedure of Moughan and Rutherford (6). After incubation, the samples were dried using a Speedvac concentrator

(Savant Instruments, Inc, Farmingdale, NY) and were analyzed for homoarginine content in the same manner as for the amino acid content described above. The reactive lysine content of the breakfast cereals themselves was those determined by Torbatinejad et al. (4).

The titanium contents of the diet and ileal digesta samples were determined in duplicate. Titanium was determined on the basis of the method of Short et al. (15). Samples were ashed before being digested in 60% (v/v) sulfuric acid and then were incubated with 30%  $\text{H}_2\text{O}_2$ , and the absorbance was read at 405 nm.

**Data Analysis.** Ileal and endogenous ileal amino acid flows were calculated using the following equation (units are  $\mu\text{g g}^{-1}$  dry matter intake (DMI)):

Ileal amino acid flow =

$$\text{Amino acid concentration in ileal digesta} \times \frac{\text{Dietary titanium}}{\text{Ileal titanium}}$$

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

$$\text{True digestibility (\%)} = \frac{[\text{Dietary amino acid intake} - (\text{Ileal amino acid flow} - \text{Endogenous amino acid flow})]}{\text{Dietary amino acid intake}} \times \frac{100}{1}$$

Endogenous amino acid flow is based on the endogenous amino acid flows for the growing rat reported by Rutherford and Moughan (16).

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

$$\text{True ileal reactive lysine digestibility (\%)} = \frac{[\text{Dietary reactive lysine intake} - (\text{Ileal reactive lysine flow} - \text{Endogenous lysine flow})]}{\text{Dietary reactive lysine intake}} \times \frac{100}{1}$$

Reactive lysine was determined using the guanidination method, and endogenous lysine flow is based on the endogenous lysine flow reported by Rutherford and Moughan (16).

True ileal digestible reactive lysine content of the cereals was calculated as follows (units are  $\text{g kg}^{-1}$ ):

$$\text{True ileal digestible reactive lysine content} = \frac{\text{Reactive lysine content in the cereal} \times \text{True ileal reactive lysine digestibility (\%)}}{1}$$

True ileal digestible amino acid content of the cereals was calculated as follows (units are  $\text{g kg}^{-1}$ ):

$$\text{True ileal digestible amino acid content of the cereals} = \frac{\text{Amino acid content in the cereal} \times \text{True ileal amino acid digestibility (\%)}}{1}$$

The amino acid digestibility data were subjected to a one-way analysis of variance for each amino acid singly (GLM Procedure) (17).

## RESULTS

The amino acid composition of the 20 cereal-based products examined in this study has been presented in a previous paper (4).

**True Ileal Total and Reactive Lysine Digestibility for 20 Cereal-Based Foods.** True ileal total lysine digestibility, which is based on conventional amino acid analysis of both diets and digesta, was determined in 20 breakfast cereals and was compared to true ileal reactive lysine digestibility, which is based on using the guanidination reaction to determine the reactive lysine contents in both diets and digesta (Table 1). The mean reactive lysine digestibility (lysine availability) across all cereals was 80% but ranged from 53% for cereal 13 (a puffed wheat product) to 108% for cereal 6 (a flaked corn product). For half

**Table 1.** Mean ( $n = 5$ ) True Ileal Total and Reactive Lysine Digestibility (%) for 20 Selected Cereal-Based Foods

| cereal | lysine digestibility |                       | overall SE | statistical significance <sup>c</sup> |
|--------|----------------------|-----------------------|------------|---------------------------------------|
|        | total <sup>a</sup>   | reactive <sup>b</sup> |            |                                       |
| 1      | 68                   | 84                    | 4.0        | **                                    |
| 2      | 72                   | 86                    | 4.8        | *                                     |
| 3      | 67                   | 81                    | 3.6        | *                                     |
| 4      | 74                   | 76                    | 7.0        | NS                                    |
| 5      | 64                   | 68                    | 5.7        | NS                                    |
| 6      | 71                   | 108                   | 3.3        | ***                                   |
| 7      | 77                   | 74                    | 14.9       | NS                                    |
| 8      | 54                   | 63                    | 5.5        | NS                                    |
| 9      | 40                   | 66                    | 7.2        | ***                                   |
| 10     | 58                   | 66                    | 7.0        | NS                                    |
| 11     | 84                   | 87                    | 5.6        | NS                                    |
| 12     | 63                   | 90                    | 4.2        | ***                                   |
| 13     | 55                   | 53                    | 18.4       | NS                                    |
| 14     | 67                   | 91                    | 1.2        | ***                                   |
| 15     | 52                   | 60                    | 8.7        | NS                                    |
| 16     | 82                   | 84                    | 5.7        | NS                                    |
| 17     | 77                   | 90                    | 3.8        | **                                    |
| 18     | 72                   | 86                    | 3.4        | **                                    |
| 19     | 81                   | 91                    | 1.5        | **                                    |
| 20     | 79                   | 79                    | 3.6        | NS                                    |

<sup>a</sup> Total lysine digestibility was determined using the true ileal amino acid digestibility assay in the rat using traditional amino acid analysis to determine the total lysine content of the diets and digesta. <sup>b</sup> Reactive lysine digestibility was determined using the true ileal amino acid digestibility assay in the rat using guanidination and homoarginine analysis to determine the reactive lysine content of the diets and digesta. <sup>c</sup> NS, not significant,  $P > 0.05$ ; \*,  $0.05 > P > 0.01$ ; \*\*,  $0.01 > P > 0.001$ ; \*\*\*,  $P < 0.001$ .

of the cereals tested, true ileal reactive lysine digestibility was significantly ( $P < 0.05$ ) higher than true ileal total lysine digestibility determined using conventional amino acid analysis. For these cereals, total lysine digestibility underestimated lysine digestibility by between 12% and 65% with the average underestimation being 31%. For three breakfast cereals (8, 10, and 15), the difference between total lysine digestibility and reactive lysine digestibility was large (17%, 14%, and 14%, respectively) although these differences were not statistically ( $P < 0.05$ ) different.

**True Ileal Digestible Total and Reactive Lysine Content for 20 Cereal-Based Foods.** The true ileal digestible total lysine content was determined and compared with the true ileal digestible reactive lysine (available lysine) content for the 20 selected breakfast cereals (Table 2). For 15 of the 20 cereal products, digestible total lysine content significantly ( $P < 0.05$ ) overestimated digestible reactive lysine content (available lysine). This overestimation ranged from 16% for cereal product 1 (a shredded wheat product) to 77% for product 11 (a puffed rice product). The mean overestimation was 40%. For the other five cereals, there was no significant difference ( $P > 0.05$ ) between digestible total lysine content and digestible reactive lysine content. However, for these five cereal products, there were some sizable numerical differences (4–79%, mean 47%) between digestible total and reactive lysine contents.

**True Ileal Amino Acid Digestibility for the 20 Cereal-Based Foods.** True ileal amino acid digestibility values for the amino acids other than lysine for the 20 breakfast cereals are given in Table 3. Glycine digestibility was not determined since, in protein sources that contain low levels of protein, endogenous glycine may be underestimated using the enzymatically hydrolyzed casein method as bile acids may be included in the endogenous amino acid fraction. The overall true ileal amino acid digestibility across amino acids for each cereal ranged from 61% for cereal 12 (a puffed rice product) to 89% for cereal 8

**Table 2.** Mean ( $n = 5$ ) Digestible Total and Reactive Lysine Contents (Available Lysine) (g/kg) for 20 Cereal-Based Foods

| cereal | digestible lysine  |                       | overall SE | statistical significance <sup>c</sup> |
|--------|--------------------|-----------------------|------------|---------------------------------------|
|        | total <sup>a</sup> | reactive <sup>b</sup> |            |                                       |
| 1      | 1.8                | 1.6                   | 0.08       | *                                     |
| 2      | 1.7                | 1.3                   | 0.08       | **                                    |
| 3      | 1.3                | 0.8                   | 0.05       | **                                    |
| 4      | 2.0                | 1.4                   | 0.13       | *                                     |
| 5      | 1.4                | 1.1                   | 0.09       | *                                     |
| 6      | 0.7                | 0.6                   | 0.02       | *                                     |
| 7      | 0.4                | 0.2                   | 0.05       | NS                                    |
| 8      | 1.1                | 0.7                   | 0.07       | *                                     |
| 9      | 0.9                | 0.7                   | 0.11       | NS                                    |
| 10     | 1.1                | 0.7                   | 0.10       | **                                    |
| 11     | 1.1                | 0.6                   | 0.05       | **                                    |
| 12     | 0.8                | 0.7                   | 0.04       | *                                     |
| 13     | 0.4                | 0.3                   | 0.10       | NS                                    |
| 14     | 1.4                | 1.0                   | 0.02       | ***                                   |
| 15     | 0.8                | 0.5                   | 0.09       | NS                                    |
| 16     | 3.6                | 2.5                   | 0.19       | *                                     |
| 17     | 3.8                | 3.2                   | 0.15       | *                                     |
| 18     | 3.7                | 3.5                   | 0.15       | NS                                    |
| 19     | 3.7                | 2.8                   | 0.06       | ***                                   |
| 20     | 3.2                | 1.9                   | 0.08       | ***                                   |

<sup>a</sup> Digestible total lysine was calculated from the true ileal total lysine digestibility determined using the true ileal amino acid digestibility assay (rat) using traditional amino acid analysis to determine the total lysine content of the diets and digesta and from the total lysine content of the cereal also determined using traditional amino acid analysis. <sup>b</sup> Digestible reactive lysine was calculated from the true ileal reactive lysine digestibility determined using the true ileal amino acid digestibility assay (rat) using guanidination and amino acid analysis to determine the reactive lysine content of the diets and digesta and from the reactive lysine content of the cereal also determined using guanidination and amino acid analysis. <sup>c</sup> NS, not significant,  $P > 0.05$ ; \*,  $0.05 > P > 0.01$ ; \*\*,  $0.01 > P > 0.001$ ; \*\*\*,  $P < 0.001$ .

(an extruded wheat product) with a mean overall digestibility of 79%. The least digestible amino acid across all cereals was histidine (63%) and the most digestible amino acid was phenylalanine (88%).

**True Ileal Digestible Amino Acid Content for the 20 Cereal-Based Foods.** True ileal digestible amino acid content of the 20 breakfast cereals is presented in Table 4. There was considerable variation in digestible amino acid content between the 20 cereal products, with a greater than 10-fold range in digestible amino acid content across cereal products for glutamic acid, proline, histidine, and arginine. For threonine, serine, valine, isoleucine, leucine, tyrosine, and phenylalanine, there was a relatively lower (5-fold) difference in digestible amino acid content across products.

## DISCUSSION

Lysine is prone to undergo chemical modification when foodstuffs are processed (Maillard reaction). This modified lysine is generally nutritionally unavailable, and its presence leads to an overestimate of determined available lysine in foodstuffs when traditional techniques such as the true ileal amino acid digestibility assay are used. In this study, a new and accurate method (6) for determining available lysine (true ileal digestible reactive lysine assay) was applied to 20 commercially available cereal-based breakfast foods.

**True Ileal Total and Reactive Lysine Digestibility for the 20 Cereal-Based Foods.** In processed foods, such as breakfast cereals, a proportion of the lysine will have inevitably been chemically modified to form Maillard products (1, 2). Torbatinejad et al. (4) reported sizable differences between the amounts of total lysine and reactive lysine in cereal foods

**Table 3.** Mean ( $n = 5$ ) True Ileal Amino Acid Digestibility (%) for the 20 Cereal-Based Breakfast Products<sup>a</sup>

| amino acid    | cereal |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | overall SE |
|---------------|--------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|------------|
|               | 1      | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |            |
| aspartic acid | 63     | 54 | 59 | 64 | 48 | 74 | 70 | 71 | 49 | 62 | 63 | 49 | 46 | 67 | 46 | 82 | 76 | 73 | 87 | 77 | 3.9        |
| threonine     | 76     | 76 | 77 | 72 | 70 | 75 | 80 | 84 | 62 | 74 | 76 | 57 | 60 | 74 | 71 | 81 | 77 | 73 | 84 | 75 | 3.8        |
| serine        | 85     | 86 | 84 | 83 | 82 | 84 | 90 | 90 | 69 | 83 | 80 | 61 | 78 | 78 | 83 | 85 | 80 | 75 | 88 | 81 | 3.0        |
| glutamic acid | 92     | 93 | 91 | 91 | 90 | 83 | 85 | 96 | 81 | 92 | 68 | 58 | 83 | 86 | 91 | 91 | 88 | 86 | 92 | 89 | 1.6        |
| proline       | 90     | 83 | 89 | 88 | 89 | 68 | 77 | 95 | 69 | 90 | 60 | 56 | 78 | 78 | 89 | 85 | 83 | 79 | 86 | 82 | 2.7        |
| alanine       | 78     | 80 | 77 | 75 | 75 | 87 | 85 | 85 | 63 | 77 | 67 | 59 | 71 | 76 | 75 | 82 | 79 | 72 | 86 | 82 | 2.8        |
| valine        | 83     | 86 | 82 | 80 | 80 | 79 | 81 | 90 | 68 | 82 | 74 | 65 | 75 | 80 | 84 | 86 | 82 | 78 | 89 | 85 | 2.5        |
| isoleucine    | 85     | 89 | 85 | 84 | 84 | 87 | 85 | 93 | 74 | 85 | 75 | 66 | 81 | 83 | 87 | 88 | 85 | 81 | 91 | 87 | 2.2        |
| leucine       | 87     | 90 | 87 | 84 | 86 | 90 | 90 | 94 | 76 | 88 | 71 | 62 | 83 | 84 | 90 | 88 | 85 | 81 | 91 | 87 | 1.8        |
| tyrosine      | 88     | 88 | 87 | 85 | 86 | 87 | 89 | 93 | 75 | 87 | 70 | 60 | 83 | 82 | 89 | 88 | 82 | 77 | 90 | 85 | 2.0        |
| phenylalanine | 91     | 94 | 91 | 87 | 90 | 90 | 91 | 97 | 80 | 92 | 75 | 65 | 88 | 86 | 93 | 91 | 86 | 82 | 93 | 90 | 1.7        |
| histidine     | 51     | 52 | 76 | 43 | 48 | 71 | 71 | 78 | 49 | 79 | 71 | 54 | 49 | 68 | 52 | 74 | 71 | 67 | 74 | 51 | 4.7        |
| arginine      | 85     | 86 | 85 | 80 | 84 | 59 | 50 | 89 | 70 | 83 | 71 | 78 | 80 | 79 | 87 | 89 | 85 | 80 | 90 | 84 | 3.0        |

<sup>a</sup> Values were corrected for endogenous amino acid flow using the enzymatically hydrolyzed casein method (26, 27) reported by Rutherford and Moughan (16).

**Table 4.** Mean ( $n = 5$ ) True Ileal Digestible Amino Acid Content (g/kg) for the 20 Cereal-Based Breakfast Products

| amino acid    | cereal |      |      |      |      |      |     |      |      |      |     |     |      |      |      |      |      |      |      |      | overall SE |
|---------------|--------|------|------|------|------|------|-----|------|------|------|-----|-----|------|------|------|------|------|------|------|------|------------|
|               | 1      | 2    | 3    | 4    | 5    | 6    | 7   | 8    | 9    | 10   | 11  | 12  | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   |            |
| aspartic acid | 4.2    | 3.5  | 3.1  | 3.6  | 3.2  | 2.9  | 2.4 | 7.0  | 2.9  | 3.8  | 3.4 | 2.7 | 1.5  | 4.1  | 3.1  | 7.3  | 8.0  | 7.9  | 9.4  | 6.5  | 0.23       |
| threonine     | 2.5    | 2.5  | 1.9  | 1.9  | 2.3  | 1.4  | 1.3 | 4.5  | 1.8  | 2.3  | 1.5 | 1.1 | 0.9  | 1.9  | 2.4  | 2.7  | 3.1  | 3.1  | 3.4  | 2.4  | 0.10       |
| serine        | 4.0    | 4.3  | 3.1  | 3.4  | 4.0  | 2.2  | 2.1 | 8.1  | 2.6  | 4.0  | 2.3 | 1.8 | 1.8  | 3.0  | 4.1  | 3.9  | 4.4  | 4.5  | 4.8  | 3.7  | 0.11       |
| glutamic acid | 28.2   | 34.4 | 24.9 | 24.4 | 32.4 | 10.0 | 8.8 | 77.7 | 17.9 | 32.9 | 8.0 | 7.2 | 10.8 | 19.5 | 29.8 | 21.3 | 25.6 | 24.3 | 23.8 | 20.6 | 0.31       |
| proline       | 9.3    | 9.9  | 8.3  | 8.2  | 10.4 | 3.5  | 3.2 | 25.8 | 5.5  | 10.4 | 1.9 | 1.8 | 3.4  | 5.5  | 9.3  | 5.3  | 6.3  | 5.7  | 5.6  | 4.8  | 0.18       |
| alanine       | 3.8    | 3.7  | 2.9  | 2.8  | 3.4  | 3.5  | 3.1 | 6.1  | 2.8  | 3.3  | 2.2 | 2.1 | 2.1  | 2.8  | 3.6  | 4.2  | 4.7  | 4.8  | 5.6  | 4.1  | 0.12       |
| valine        | 4.1    | 4.3  | 3.3  | 3.4  | 4.0  | 2.2  | 2.0 | 7.9  | 2.8  | 4.0  | 2.4 | 2.3 | 1.9  | 3.2  | 4.4  | 4.6  | 5.3  | 5.4  | 5.8  | 4.6  | 0.10       |
| isoleucine    | 3.1    | 3.5  | 2.6  | 2.7  | 3.2  | 1.7  | 1.6 | 7.1  | 2.2  | 3.3  | 1.7 | 1.6 | 1.5  | 2.5  | 3.6  | 3.4  | 4.0  | 3.9  | 4.2  | 3.3  | 0.07       |
| leucine       | 6.4    | 7.2  | 5.8  | 5.3  | 6.8  | 5.9  | 5.5 | 14.9 | 4.8  | 6.8  | 3.4 | 3.3 | 4.0  | 5.2  | 7.3  | 6.9  | 8.2  | 8.3  | 8.7  | 6.7  | 0.12       |
| tyrosine      | 3.0    | 3.2  | 2.6  | 2.4  | 3.0  | 2.0  | 2.0 | 6.8  | 2.1  | 3.1  | 1.9 | 1.9 | 1.7  | 2.5  | 3.5  | 3.3  | 3.9  | 3.9  | 4.1  | 3.2  | 0.06       |
| phenylalanine | 4.3    | 5.0  | 3.7  | 3.5  | 4.6  | 2.5  | 2.3 | 10.4 | 3.0  | 4.6  | 2.2 | 2.1 | 2.2  | 3.4  | 4.8  | 4.6  | 5.5  | 5.5  | 5.7  | 4.6  | 0.07       |
| histidine     | 1.0    | 1.2  | 1.7  | 1.0  | 1.3  | 1.4  | 1.2 | 4.3  | 1.5  | 2.5  | 1.0 | 0.8 | 0.3  | 1.2  | 1.2  | 1.9  | 2.2  | 1.8  | 2.3  | 1.3  | 0.10       |
| arginine      | 4.4    | 4.0  | 2.9  | 3.8  | 3.6  | 1.4  | 0.8 | 6.1  | 3.5  | 3.7  | 3.0 | 3.3 | 1.8  | 3.5  | 4.6  | 6.9  | 6.9  | 7.5  | 8.5  | 6.0  | 0.12       |

suggesting the presence of acid-labile lysine derivatives. Erbersdobler and Hupe (3) also reported lysine damage in breakfast cereals. The presence of Maillard products (modified lysine residues) in dietary proteins is believed to reduce the effectiveness of digestive enzymes, resulting in the presence of undigested peptides (limit peptides) at the terminal ileum (18) and a proportionally higher concentration of modified lysine in the digesta compared to the diet. Consequently, total lysine digestibility is expected to underestimate the actual lysine digestibility. Reactive lysine digestibility focuses only on the lysine that has remained intact during processing and as such is an accurate measure of lysine digestibility.

For the majority of the cereal products, total lysine digestibility underestimated lysine digestibility in comparison to reactive lysine digestibility. This underestimation was quantitatively large, being on average 33%. Reactive lysine digestibility also ranged widely across breakfast cereals, likely reflecting variation in processing methods used and ingredient composition.

For cereal 6 (a flaked corn product), the mean true ileal digestibility of reactive lysine was 108% which from a theoretical viewpoint is not possible. In this study, endogenous amino acid losses were determined using an enzymatically hydrolyzed casein diet (EHC) containing 10% peptides while the cereal 6 diet contained only 6% protein. Since the protein/peptide level in a diet influences the amount of endogenous amino acids at the terminal ileum (19), it is likely that the endogenous lysine in the rats fed the cereal 6 diet was lower than that determined using the EHC diet, which would lead to an overestimate of the true reactive lysine digestibility.

**True Ileal Digestible Total and Reactive Lysine Contents for the 20 Cereal-Based Foods.** There have been few reports in the literature describing available lysine contents of breakfast cereals. Clarke and Kennedy (20) reported available lysine data for two breakfast cereals, but their estimates were based on the faecal digestibility of total lysine and are likely to be misleading (21). While several workers have examined total lysine levels and in-vitro protein digestibility in processed wheat-based cereals (22, 23), biologically available lysine in breakfast cereals has not been investigated. In the present work, digestible total lysine content overestimated digestible reactive lysine content (available lysine) in 15 of the cereal foods tested. The degree of overestimation was considerable, being greater than 20% for at least 60% of the cereal products tested. For many of the breakfast cereals tested, there was a considerable amount of lysine damage, and for such foods, digestible total lysine is an inaccurate measure of available lysine. Given the public perception that breakfast cereals are high-quality healthy foods, it is somewhat surprising that the protein quality of many of the breakfast cereals was so poor. Lysine is naturally low in cereals and as such any further losses from processing should be viewed with some concern.

**True Ileal Amino Acid Digestibility for the 20 Cereal-Based Foods.** The true ileal digestibility of the acid-stable amino acids (apart from lysine) was determined. Overall, amino acid digestibility was low (79%), which concurs with the low lysine availability observed. Khan and Eggum (24) also reported a reduction in overall protein digestibility in breakfast cereals after processing. There appeared to be some reasonably highly digestible cereal products with products 1, 2, 3, 7, 8, 10, 16,



17, 19, and 20 having mean true ileal amino acid digestibilities above 80%. These cereals were predominately wheat and rolled oat based cereal foods. In contrast, there were several cereal-based products that were poorly digested, and these included cereals 9, 11, 12, and 13 all of which had mean amino acid digestibilities ranging from 61% to 74%. These cereal products generally contained significant amounts of puffed rice. It is likely that the type of processing method used and differences in the cereal bases used resulted in the differences in lysine damage and the varying amino acid digestibility among the cereal products. Without more detailed information, however, it is difficult to draw definitive conclusions.

**True Ileal Digestible Amino Acid Content for the 20 Cereal-Based Foods.** Overall, there was considerable variation (more than a 3-fold difference) in the true ileal digestible amino acid content for each amino acid across the breakfast cereal products. This variation was predominantly a result of the different cereal bases used to formulate each of the breakfast cereal products. For example, wheat and the wheat-based cereals (1–5, 8–10, 13–15) both have high amounts of glutamic acid and proline (4). Furthermore, amino acid digestibility will impact the digestible amino acid contents across the cereal products.

## CONCLUSION

True ileal total lysine digestibility, on the basis of conventional amino acid analysis of diets and digesta, is an inaccurate measure of lysine availability in processed protein sources (25). The reactive lysine digestibility assay used in this study and based on the guanidination of lysine in both diets and digesta is an alternative accurate measure of lysine availability (7). For most of the breakfast cereals tested in this study, the traditional measure of true ileal digestible total lysine considerably overestimated available lysine.

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