Digestible Reactive Lysine in Selected Milk-Based Products

S. M. Rutherford¹ and P. J. Moughan²

¹Institute of Food, Nutrition and Human Health and

²Riddet Centre, Massey University, Palmerston North, New Zealand

ABSTRACT

Reactive lysine contents, true ileal reactive lysine digestibility, and true ileal digestible reactive lysine contents were determined in a wide range of processed milk products. A previously validated assay based on determining reactive lysine in both food and ileal digesta, after reaction of these materials with O-methylisourea, was applied. Semisynthetic diets containing milk products as the sole sources of protein and including chromic oxide as an indigestible marker were fed to growing rats. Digesta from the terminal ileum were collected postethanasia and, with samples of the diets, analyzed for reactive lysine (homoarginine) contents. True reactive lysine digestibility was determined after correcting for endogenous lysine loss at the terminal ileum of rats fed an enzyme hydrolyzed casein-based diet, followed by ultrafiltration (5000 Da) of the digesta. Digestible total lysine (determined using conventional methods) was also determined. The true ileal reactive lysine digestibility was high (>91%) in all the milk products tested, but was highest in the UHT milk (100%) and lowest in the infant formulas (91 to 93%). Total lysine digestibility (conventional measurement) significantly underestimated reactive lysine digestibility for all the products tested. The mean underestimation ranged from 1.3 to 7.1% units. The mean digestible total lysine content was significantly different from the available lysine content for most of the products examined. In some cases this difference was small (<3%), but for a number of the products (evaporated milk, whole milk protein, lactose hydrolyzed milk powder, and a sports formula) the difference was greater (6.5 to 14%). This would suggest firstly that total lysine and total lysine digestibility determined using conventional methods were inaccurate when applied to some milk-based foods, and secondly that some of the milk products have undergone lysine modification. In general, milk proteins are a highly digestible source of amino acids and lysine.

(Key words: lysine, milk, availability, digestibility)

Abbreviation key: EHC = enzymatically hydrolyzed casein.

INTRODUCTION

Lysine is a dietary essential AA that is sometimes first limiting in diets for humans, particularly diets high in cereals. Generally, milk proteins contain relatively high amounts of lysine, especially in comparison with cereal protein sources; therefore, milk protein products are a valued source of lysine. Lysine, however, is susceptible to modification during processing or prolonged storage, whereupon the side chain amino group of lysine can react with lactose or other compounds to produce nutritionally unavailable (unreactive) derivatives (Hurrell and Carpenter, 1981; Moughan, 2003). Some of these derivatives are acid labile and will revert back to lysine under the acid hydrolysis conditions used to determine lysine contents in food samples. This reversion is not quantitative and leads to inaccurate estimates of amino acid digestibility, and generally to an overestimation of the available (reactive) lysine content and the digestible lysine contents of processed foods. Numerous methods have been developed to determine chemically reactive lysine, often incorrectly referred to as available lysine, in milk products (Ferrer et al., 2003; Pereyra Gonzales et al., 2003; Ramirez-Jimenez et al., 2004), but few researchers have determined biologically available lysine in any protein sources (Batterham et al., 1990), including milk products. A method has been developed (Moughan and Rutherford, 1996; Rutherford et al., 1997) that allows accurate measurement of the available lysine content of processed foods. The assay (true ileal reactive lysine digestibility) involves feeding a test diet to an animal. Ileal digesta are collected and reactive lysine is measured in both diet and digesta. The difference between the 2 measurements reflects the reactive lysine that has been digested and absorbed. Ileal rather than fecal measurements are used, firstly, as fecal measurements are confounded by microorganisms in the hindgut that metabolize AA, and secondly, it does not appear that AA are absorbed from the hindgut in quantitatively significant amounts (Moughan, 2003). The aim of this study was to determine reactive

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Corresponding author: S. M. Rutherford; e-mail: S.M.Rutherford@massey.ac.nz.

Table 1. Crude protein, total fat, and total carbohydrate contents¹ (g/100 g of air dry weight) for the 12 protein sources.

	CP	Total fat	Total carbohydrate
Whey protein concentrate	78.5	4.9	8.6
UHT milk ²	3.5	0.5	4.8
Evaporated milk ²	8.4	8.6	11.6
Lactose-hydrolyzed milk powder ³	34	12	44.2
High-protein supplement	19.9	5.7	30.3
Sports formula ⁴	24.5	0.6	66.1
Weight gain formula ⁵	28.0	1.8	62.0
Infant formula A	11.4	29.3	57
Infant formula B	12	28	51
Infant formula C	15.4	21	56
Whole milk powder	32.2	28.8	36.2
Elderly formula ⁶	14.0	15.9	60.7

¹Data taken from the statutory label information appearing on the product.

²These products are liquids, and, as such, the protein and carbohydrate contents are g/100 mL.

³Hydrolyzed milk-based food for lactose intolerance.

⁴Milk-based formula designed for athletes and sports people.

⁵Milk-based formula designed for bodybuilders and people needing to gain BW.

⁶Milk-based formula for the elderly.

lysine content, true ileal reactive lysine digestibility, and true ileal digestible reactive lysine content in a range of high-quality milk protein sources using the new assay and to compare these values with total lysine content, true ileal total lysine digestibility, and true ileal digestible total lysine content determined using the traditional approach. Information on the available lysine content of a range of milk-based products was obtained.

MATERIALS AND METHODS

Materials

O-methylisourea was obtained from Sigma Chemicals (St. Louis, MO), and barium hydroxide octahydrate was obtained from BDH Laboratory Supplies (Poole, England). A total of 12 milk-based protein products were obtained locally. These included a whole milk powder, 3 infant formulas, whey protein concentrate, UHT milk, evaporated milk, weight gain formula, milk powder-based sports drink, milk powder-based formula for the elderly, lactose-hydrolyzed milk powder, and a milk powder-based high-protein supplement. The crude protein, total fat, and total carbohydrate contents of the 12 products (as given in the product specifications) are shown in Table 1. Enzymatically hydrolyzed casein was obtained from New Zealand Pharmaceuticals Ltd. (Palmerston North, New Zealand) and contained free AA and peptides ≤ 2000 Da. Centriprep YM-3 disposable ultrafiltration devices (with a 3000-Da MW cutoff) were

obtained from Amicon, Inc. (Beverly, MA). Laboratory rats were obtained from the Small Animal Production Unit, Massey University (Palmerston North, New Zealand).

Preparation of Protein Sources

Most products were purchased as finely ground powders. Any products purchased as liquids were freeze-dried and then ground through a 1-mm mesh.

Digestibility Study

A total of 60 male Sprague-Dawley rats, approximately 150 g BW, 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. Twelve semisynthetic test diets were formulated (Table 2) to each contain 100 g/kg of CP. An enzymatically hydrolyzed casein (EHC)-based diet was also formulated (Table 2) to allow determination of endogenous ileal lysine flows (Moughan et al., 1990; Butts et al., 1991). Chromic oxide was included (0.3%) in each diet as an indigestible marker. All diets met the nutritional requirements for the growing rat with the exception of protein (National Research Council, 1995). The animals were randomly allocated to the dietary treatments and were fed the diets for a 14-d experimental period. On each day, each rat received its respective diet as 9 meals given hourly (0830 to 1630 h); each mealtime consisted of a 10-min period during which the rats had unrestricted access to their diet. Water was available at all times. On the final day of the study, from 5.5 to 7 h after the start of feeding, the rats were asphyxiated in carbon dioxide gas and then decapitated. The 20 cm of ileum immediately anterior to the ileo-cecal junction was dissected out. The dissected ileum was washed with distilled, deionized water to remove any blood and hair, and carefully dried on absorbent paper. The digesta were gently flushed from the ileum section and freeze-dried ready for chemical analysis. The digesta of rats fed the EHC diet were adjusted to approximately pH 3 with 6 M HCl to minimize protease activity. The EHC digesta were centrifuged and ultrafiltered (3000 Da) and then freeze-dried in preparation for analysis (Butts et al., 1991).

Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University.

Chemical Analysis

Amino acid contents were determined in duplicate 5-mg protein source and digesta samples and quadruplicate 5-mg semisynthetic diet samples using a Waters ion-exchange HPLC system, utilizing postcolumn nin-

Table 2. Ingredient compositions¹ (g/kg of air dry weight) of the experimental diets.

	EHC ²	WPC ³	UHT milk	Evaporated milk	Lactose-hydrolyzed milk powder	High protein supplement	Sports formula	Weight gain formula	Infant formula A	Infant formula B	Infant formula C	WMP ⁴	Elderly formula
Wheat starch	637.0	639.6	493.9	462.7	472.9	264.5	358.8	409.9	89.8	133.7	317.6	466.0	52.7
Soybean oil	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	...	...	...	50.0	50.0
Cellulose	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	...	...	...	50.0	50.0
Sucrose	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	...	...	...	100.0	100.0
Vitamin premix ⁵	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix ⁵	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Dicalcium phosphate	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
EHC	130.0	...	...	...	...	...	...	...	...	...	...	...	...
WPC	...	127.4	...	...	...	...	...	...	...	...	...	...	...
UHT milk	...	...	273.1	...	...	...	...	...	...	...	...	...	...
Evaporated milk	...	...	...	304.3	...	...	...	...	...	...	...	...	...
Lactose-hydrolyzed milk powder	...	...	...	...	294.1	...	...	...	...	...	...	...	...
High-protein supplement	...	...	...	...	...	502.5	...	...	...	...	...	...	...
Sports formula	...	...	...	...	...	...	408.2	...	...	...	...	...	...
Weight gain formula	...	...	...	...	...	...	...	357.1	...	...	...	...	...
Infant formula A	...	...	...	...	...	...	...	...	877.2	...	...	...	...
Infant formula B	...	...	...	...	...	...	...	...	...	833.3	...	...	...
Infant formula C	...	...	...	...	...	...	...	...	...	...	649.4	...	...
WMP	...	...	...	...	...	...	...	...	...	...	...	301.0	...
Elderly formula	...	...	...	...	...	...	...	...	...	...	...	...	714.3
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

¹All diets were formulated to contain equal CP contents.²EHC = Enzymatically hydrolyzed casein diet, used for determining endogenous amino acid losses at the terminal ileum. The EHC contained free AA and small peptides (<2000 Da).³WPC = Whey protein concentrate.⁴WMP = Whole milk powder.⁵Vitamin/mineral mix was formulated to meet the requirement of the growing rat for vitamins and minerals in the final diets (National Research Council, 1995).

Table 3. Total and reactive lysine contents (mg/g of air dry weight) for 12 dairy protein sources.

	Total ¹	Reactive ¹	Overestimation (%) ²
Whole milk powder	27.5	24.4	12.8
Infant formula A	9.6	9.5	0.9
Infant formula B	10.2	10.0	2.2
Infant formula C	12.5	12.5	-0.5
Whey protein concentrate	83.0	78.6	5.6
UHT milk	32.0	31.4	1.8
Evaporated milk	26.1	21.2	23.0
Weight gain formula	25.2	24.3	3.8
Sports formula	21.4	19.5	9.7
Elderly formula	12.4	12.2	1.7
Lactose-hydrolyzed milk powder	28.3	25.5	11.1
High-protein supplement	14.6	14.4	1.9

¹Mean values based on duplicates.²Overestimation (%) = $\frac{\text{total lysine} - \text{reactive lysine}}{\text{total lysine}} \times \frac{100}{1}$.

hydrazine 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 ± 2°C in evacuated sealed tubes. Cysteine, methionine, and tryptophan were not determined, as they are destroyed during acid hydrolysis. The weight of each AA was calculated using free amino acid molecular weights.

Reactive lysine contents were determined in duplicate 5-mg protein source and digesta samples and quadruplicate 5-mg diet samples after incubation for 1, 7, and 7 d, respectively, in 0.6 M O-methylisourea, pH 10.6 (pH 11.0 for the digesta samples), at 21°C in a shaking waterbath, with the reagent to lysine ratio being >1000, according to the procedure of Moughan and Rutherford (1996). The 0.6 M O-methylisourea solution was prepared as described by Moughan and Rutherford (1996). After incubation, the samples were dried using a Speedvac concentrator (Savant Instruments, Inc., Farmingdale, NY) and analyzed for AA content as described previously.

The chromium contents of the diet and ileal digesta samples were determined in duplicate on a GBC 902 AA absorption/emission spectrophotometer (GBC Scientific NZ Ltd, Auckland, New Zealand) following the method of Costigan and Ellis (1987).

Data Analysis

Ileal total and ileal endogenous (EHC diet) AA flows at the terminal ileum were calculated using the following equation (units are µg/g of DMI):

$$\text{ileal AA flow} = \text{ileal AA} \times \frac{\text{diet chromium}}{\text{ileal chromium}}$$

True ileal AA digestibility was calculated using the following equation (units are µg/g of DMI):

true AA digestibility (%) =

$$\frac{\text{dietary AA} - (\text{ileal AA flow} - \text{endogenous AA flow}) \times 100}{\text{dietary AA}}$$

True ileal reactive lysine digestibility was calculated using the following equation (units are µg/g of DMI):

true reactive lysine digestibility (%) =

$$\frac{\text{dietary reactive lysine}^* - (\text{ileal reactive lysine flow} - \text{endogenous reactive lysine flow}) \times 100}{\text{dietary reactive lysine}}$$

The AA digestibility data were subjected to a one-way ANOVA for each amino acid singly (GLM procedure; SAS, 1999).

RESULTS

Comparison of Total and Reactive Lysine Contents for the Protein Sources

Total and reactive lysine contents were determined for the 12 milk protein products (Table 3). For the infant formulas, UHT milk, elderly formula, and high-protein supplement, the difference between total and reactive lysine contents was ≤2.2%. For the other products, total lysine overestimated reactive lysine from 3.8% for the weight gain formula to as much as 23% for evaporated milk.

*Reactive lysine was determined using the guanidination method.

Table 4. Mean (n = 5) true ileal total lysine digestibility (%) determined using conventional AA analysis and true ileal reactive-lysine digestibility (%) based on reactive lysine determined using guanidination prior to AA analysis.

	Lysine digestibility		Overall SE ³	Significance
	Total ¹	Reactive ²		
Whole milk powder	95.2	98.3	0.87	***
Infant formula A	86.9	91.0	1.71	**
Infant formula B	88.6	92.3	1.03	***
Infant formula C	89.0	93.1	1.20	**
Whey protein concentrate	96.3	98.5	0.71	***
UHT milk	99.2	100.2	0.31	*
Evaporated milk	89.6	96.7	2.02	**
Weight gain formula	96.7	99.0	0.61	***
Sports formula	95.2	98.0	0.56	**
Elderly formula	94.3	97.1	0.54	**
Lactose-hydrolyzed milk powder	96.2	98.6	0.62	***
High-protein supplement	97.4	99.9	1.11	**

¹Lysine digestibility was determined using a true ileal amino acid digestibility assay (rat), and conventional amino acid analysis was used to quantitate total lysine in the diets and digesta.

²Lysine digestibility was determined using a true ileal amino acid digestibility assay (rat) and the guanidination reaction was used to quantitate reactive lysine in the diets and digesta.

³Overall SE is the standard error for total and reactive lysine digestibility combined.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Comparison of True Ileal Lysine Digestibility Based on Either Reactive Lysine or Total Lysine

True ileal lysine digestibility based on “total” lysine was determined for the 12 milk-based protein sources using conventional amino acid analysis and compared with true ileal reactive lysine digestibility, determined after guanidination of the underivatized lysine in the diet and digesta, to form homoarginine and subsequent analysis of homoarginine by HPLC (Table 4). The reactive lysine digestibility (lysine availability) was in excess of 90% for all of the protein products tested, indicating that most of the reactive lysine was absorbed.

For all of the products tested, true ileal reactive lysine digestibility (lysine availability), determined using the new assay, was statistically significantly higher than true ileal total lysine digestibility, determined using the conventional assay. On average, total lysine digestibility underestimated lysine availability by 3.3%, and this underestimation ranged from 1.2% for UHT milk to 7.4% for evaporated milk.

Digestible total lysine (conventional analysis) and digestible reactive lysine (available lysine) contents are shown in Table 5. True ileal digestible total lysine was statistically significantly different from true ileal digestible reactive lysine (available lysine) for all of the milk protein products tested with the exception of the high-protein supplement. For the whole milk protein, whey protein concentrate, UHT milk, evaporated milk, weight gain formula, sports formula, and lactose-hy-

drolyzed milk powder, conventional analysis (digestible total lysine) overestimated digestible reactive lysine; for the infant formulas and elderly formula, conventional analysis underestimated digestible reactive lysine. For infant formula B, UHT milk, weight gain formula, and elderly formula, the difference between digestible total lysine and digestible reactive lysine was <3%. For the other milk protein products, where statistically significant differences were observed, these differences ranged from 3.2 to 13.9% but were on average approximately 4.7%.

Comparison of the Ileal Digestibility of Acid-Stable Amino Acids in Protein Sources With and Without the Guanidination Treatment

True ileal amino acid digestibility values for the acid-stable amino acids except lysine, proline, and arginine were also determined and are shown in Table 6. Overall, amino acid digestibility was high, with a mean true digestibility for all amino acids over all protein products of 91%. Generally, digestibility was lowest for glycine (69%) and highest for tyrosine (98%). The mean digestibility for all AA was lowest for the infant formula A (81%) and highest for UHT milk (97%).

A paired *t*-test was used to compare the mean true ileal digestibilities (for each AA individually) for the acid-stable AA determined using the new and conventional methods for the 12 protein products. For most (61%) of the AA, there was no significant difference

Table 5. Mean¹ digestible total and reactive lysine contents (g/kg of air dry weight) for 12 dairy protein sources

	Digestible lysine		Overall SE	Significance
	Total ¹	Reactive ²		
Whole milk protein	26.2	24.0	0.42	***
Infant formula A	8.3	8.6	0.12	**
Infant formula B	9.1	9.2	0.08	**
Infant formula C	11.1	11.7	0.14	**
Whey protein concentrate	79.9	77.5	0.58	***
UHT milk	31.7	31.4	0.06	*
Evaporated milk	23.4	20.5	0.60	***
Weight gain formula	24.4	24.1	0.12	**
Sports formula	20.4	19.1	0.22	***
Elderly formula	11.7	11.8	0.05	*
Lactose-hydrolyzed milk powder	27.2	25.1	0.38	***
High-protein supplement	14.3	14.3	0.11	NS ⁴

¹Digestible total lysine was calculated from true ileal lysine digestibility (rat), with lysine determined by conventional AA analysis and from the total lysine content in the protein source, also determined using conventional AA analysis.

²Digestible reactive lysine was calculated from true ileal reactive lysine digestibility (rat, guanidination analysis) and the reactive lysine content of the protein source, also determined using guanidination.

³Overall SE is the standard error for total and reactive lysine digestibility combined.

⁴ $P > 0.05$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

between digestibility determined either with or without guanidination prior to AA analysis. For a further 19% of the AA, there was a statistically significant difference between methods, but the actual difference between the mean digestibility for the 2 methods was <3%, which in practical terms may not be meaningful. For the remaining 20% of the AA determined, there was a statistically significant difference between the 2 methods and the difference was >3%.

DISCUSSION

It is important to have accurate information on the amount of lysine present in foods and the digestibility and availability of the lysine, as lysine is an important dietary essential AA that is often found in relatively low amounts in cereal-based diets. Lysine possesses a reactive amino group on its side chain, which is capable of reacting with other compounds present in a food to form nutritionally unavailable derivatives (e.g., Maillard products) when a food is processed or stored (Hurrell and Carpenter, 1981). Some of these products, although structurally different from lysine, can revert back to lysine when the food is subjected to the acid hydrolysis step of AA analysis, resulting in an overestimate of lysine content. Moreover, the presence of Maillard compounds in a food results in inaccurate digestibility coefficients being generated using traditional AA digestibility assays. Recently, a new assay methodology

for accurately determining ileal digestible reactive lysine (available lysine) has been developed (Moughan and Rutherfurd, 1996; Rutherfurd et al., 1997). The aim of the present study was to investigate the application of this new assay to a range of high-quality dairy protein-based products and compare results from the new assay with those found with the traditional true ileal AA digestibility assay.

Comparison of Total Lysine and Reactive Lysine Contents for 12 High-Quality Protein Sources

There was a high degree of variation among protein sources for agreement between the total lysine and reactive lysine contents. For some protein sources (the infant formulas A and C, elderly formula, and high-protein supplement), there was close agreement. In contrast, for sources such as whole milk powder, evaporated milk, and lactose-hydrolyzed milk powder, total lysine overestimated reactive lysine by $\geq 10\%$, suggesting the presence of labile lysine derivatives (Maillard products).

It is possible that during the guanidination reaction there is incomplete conversion of lysine to homoarginine, and that the determined reactive lysine values underestimate lysine content. This is unlikely, however, given that conversion of lysine to homoarginine in lysozyme (for which the lysine content is known), which was included in the present study as a quality

Table 6. Mean (n = 5) true ileal AA digestibility ($\pm$ SE) for 12 dairy protein sources.^{1,2}

	Whole milk powder		Infant formula A		Infant formula B		Infant formula C		Whey protein concentrate		UHT Milk	
	Total	Reactive	Total	Reactive	Total	Reactive	Total	Reactive	Total	Reactive	Total	Reactive
Aspartic acid	96.9 (1.05)*	93.0 (1.94)	81.3 (3.02)*	70.8 (5.89)	85.3 (1.66)*	69.5 (4.66)	81.9 (2.05)	74.6 (4.08)	95.0 (1.03)	92.7 (1.87)	98.2 (0.46)*	93.2 (1.92)
Threonine	97.9 (1.30)*	96.4 (1.71)	83.1 (2.95)*	75.5 (4.99)	86.6 (1.36)*	84.1 (1.99)	83.6 (1.65)**	79.4 (1.62)	92.4 (1.45)	92.5 (2.09)	98.7 (0.65)	97.4 (1.04)
Serine	89.9 (1.82)**	94.2 (2.22)	81.3 (3.96)	79.0 (4.87)	81.6 (2.14)	83.6 (1.49)	75.7 (3.25)*	80.1 (1.92)	92.8 (1.58)	94.5 (2.01)	92.7 (0.73)*	95.7 (1.39)
Glutamic acid	94.1 (0.86)	94.2 (1.02)	86.7 (2.27)	86.7 (2.93)	87.9 (1.03)**	91.7 (0.65)	85.6 (1.56)*	88.5 (1.13)	95.6 (0.86)	95.4 (0.92)	95.5 (0.39)	95.7 (0.52)
Glycine	76.3 (6.83)	76.0 (7.03)	42.8 (17.26)*	54.4 (13.99)	56.0 (3.95)	56.5 (4.12)	32.7 (12.25)*	43.9 (11.69)	86.2 (3.59)	86.4 (3.90)	84.3 (3.78)	81.7 (4.78)
Alanine	100.6 (0.88)***	98.0 (1.09)	72.1 (3.07)	73.4 (4.21)	79.6 (3.30)	80.0 (2.68)	76.8 (4.07)	80.3 (2.43)	97.2 (0.93)	96.8 (0.94)	101.1 (0.39)**	99.9 (0.52)
Valine	95.8 (0.83)	96.3 (0.99)	85.9 (2.36)	85.9 (2.96)	87.6 (1.23)	89.1 (1.04)	85.6 (1.53)*	88.5 (0.92)	96.0 (0.96)	96.2 (1.02)	97.1 (0.38)	97.3 (0.48)
Isoleucine	93.5 (0.97)	93.7 (1.28)	84.1 (2.73)	83.0 (3.71)	87.4 (1.15)	88.6 (0.94)	82.5 (2.01)*	85.6 (1.31)	97.1 (0.64)	97.2 (0.72)	95.6 (0.44)	95.6 (0.50)
Leucine	99.3 (0.43)	99.5 (0.48)	91.7 (1.25)	91.7 (1.61)	92.5 (0.92)	93.6 (0.80)	92.1 (0.95)*	93.6 (0.62)	98.4 (0.49)	98.5 (0.52)	99.9 (0.17)	99.9 (0.25)
Tyrosine	101.4 (0.32)	101.2 (0.44)	92.9 (0.96)	92.5 (1.44)	91.8 (1.37)	92.6 (1.44)	93.2 (1.00)	94.0 (0.69)	100.2 (0.83)	100.1 (0.78)	101.6 (0.11)	101.5 (0.22)
Phenylalanine	100.8 (0.35)*	101.1 (0.40)	91.8 (1.03)	92.1 (1.34)	91.2 (1.32)*	92.6 (1.29)	93.1 (1.10)*	94.3 (0.80)	98.5 (0.80)	98.7 (0.83)	101.2 (0.12)	101.3 (0.20)
Histidine	99.1 (0.78)***	89.7 (1.14)	88.6 (1.85)	84.2 (4.43)	88.6 (1.42)	87.2 (1.80)	90.2 (0.91)	86.7 (2.60)	95.1 (1.50)*	87.0 (2.29)	100.3 (0.19)**	92.9 (1.38)
	Evaporated milk		Weight gain formula		Sports formula		Elderly formula		Lactose-hydrolyzed milk powder		High-protein supplement	
	Total	Reactive	Total	Reactive	Total	Reactive	Total	Reactive	Total	Reactive	Total	Reactive
Aspartic acid	91.0 (2.48)	91.2 (1.98)	92.9 (1.15)*	88.8 (1.70)	90.4 (1.09)	91.4 (1.12)	88.3 (1.18)	83.0 (1.13)	96.4 (1.00)*	92.2 (1.81)	94.9 (2.75)	92.4 (2.38)
Threonine	95.9 (1.96)	95.6 (1.65)	94.8 (1.22)	93.4 (1.45)	91.6 (1.35)	91.7 (1.08)	88.3 (1.63)***	85.8 (1.46)	97.8 (1.09)	96.6 (1.89)	96.5 (3.32)*	94.6 (2.56)
Serine	88.5 (2.74)*	93.3 (1.85)	87.6 (1.79)*	89.6 (2.07)	82.1 (2.38)**	86.6 (2.86)	86.3 (1.79)	86.8 (1.37)	88.9 (1.83)***	94.9 (1.75)	91.6 (4.24)	92.7 (3.86)
Glutamic acid	92.6 (1.36)**	93.2 (1.26)	92.3 (0.79)	92.6 (0.84)	89.4 (1.09)	91.2 (0.87)	91.5 (0.94)	91.1 (0.85)	93.7 (0.76)	94.0 (1.47)	93.8 (2.17)	93.6 (2.13)
Glycine	76.4 (6.99)	77.4 (6.36)	82.2 (3.96)	82.1 (3.96)	65.3 (7.47)	65.0 (7.03)	63.8 (7.61)	64.0 (7.30)	87.4 (3.70)	86.3 (2.70)	70.0 (11.65)	72.4 (10.80)
Alanine	97.6 (1.85)**	96.4 (1.87)	96.2 (1.11)**	95.3 (1.17)	94.7 (0.91)*	93.0 (0.93)	93.7 (1.09)	92.4 (0.76)	100.2 (0.77)**	97.1 (3.28)	98.7 (2.34)**	96.6 (2.55)
Valine	94.6 (1.42)*	95.4 (1.29)	93.3 (0.88)	93.9 (0.95)	91.1 (1.01)	91.9 (1.24)	93.0 (0.93)	92.9 (0.75)	95.6 (0.78)**	96.0 (0.93)	94.9 (2.19)	95.1 (2.13)
Isoleucine	92.3 (1.68)**	93.2 (1.46)	91.5 (0.95)	92.1 (1.06)	88.9 (1.19)	89.7 (1.59)	91.9 (1.22)	91.8 (0.99)	93.1 (1.00)	93.3 (1.05)	93.8 (2.48)	93.7 (2.50)
Leucine	98.3 (0.80)*	98.7 (0.74)	97.3 (0.51)*	97.7 (0.55)	96.5 (0.51)	96.6 (0.64)	96.7 (0.51)	96.8 (0.41)	99.3 (0.36)*	99.6 (1.63)	98.2 (1.19)	98.4 (1.18)
Tyrosine	100.2 (0.70)	100.2 (0.66)	99.1 (0.46)	98.9 (0.66)	98.6 (0.38)	98.4 (0.43)	98.2 (0.31)	98.2 (0.40)	101.3 (0.26)	101.1 (0.50)	100.1 (0.84)	99.5 (1.18)
Phenylalanine	99.9 (0.70)**	100.2 (0.68)	98.0 (0.50)*	98.3 (0.58)	97.5 (0.38)	97.6 (0.49)	98.1 (0.35)*	98.3 (0.39)	100.7 (0.29)*	100.9 (0.31)	98.8 (1.06)	99.0 (1.12)
Histidine	95.2 (1.75)*	91.2 (1.73)	96.7 (0.82)*	90.4 (1.41)	96.0 (1.36)***	87.2 (1.40)	94.6 (0.69)***	86.5 (0.88)	98.9 (0.74)**	90.4 (2.73)	97.6 (1.89)***	91.2 (2.54)

¹Values were corrected for endogenous AA flow using the enzymatically hydrolyzed casein method (Moughan et al., 1990; Butts et al., 1991).²Superscripts denote statistically significant differences (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) between the mean total and reactive lysine digestibilities.

control, was in excess of 98%. At high pH, the formation of lysinoalanine may also compete with the guanidination reaction for reactive lysine. However, based on studies investigating the formation of lysinoalanine in casein at high pH (Karayiannis et al., 1979), only a maximum of about 2.5% of the reactive lysine will convert to lysinoalanine after a 6-h incubation under the guanidination conditions used here. Furthermore, given that as much as 95% of guanidination is complete in a soluble protein in the first 8 h of incubation (Rutherford and Moughan, unpublished data), the actual amounts of lysinoalanine formed during guanidination are likely to be much lower than 2.5% and only a negligible error.

It is apparent that some purified high-protein products, marketed on the basis of high-protein quality, have structural lysine damage during manufacture and storage, and for these products, the traditional total lysine determination overestimates available lysine.

Comparison of True Ileal Reactive Lysine Digestibility and Conventional Total Lysine Digestibility

For all protein sources, true ileal total lysine digestibility was significantly ($P < 0.05$) lower than true ileal reactive lysine digestibility. However, for many of the protein sources tested, including whole milk protein, whey protein concentrate, UHT milk, weight gain formula, elderly formula, sports formula, high-protein supplement, and lactose-hydrolyzed milk powder, the numerical difference between true ileal total and reactive digestibility was small ($<3\%$) and of little practical relevance. This suggests the presence of minimal amounts of acid-labile lysine derivatives, and for these particular samples, both the conventional true ileal AA digestibility assay and the new true ileal reactive lysine digestibility assay were suitable methods for determining lysine digestibility. For the infant formulas and evaporated milk, however, the difference between true ileal total and reactive lysine digestibility was $>3\%$ and as high as 7%. For these protein sources, the new assay provides more meaningful estimates of lysine availability.

It has been appreciated for some time that the conventional true ileal lysine digestibility assay overestimates lysine availability in processed feedstuffs (Batterham et al., 1990). In the present study, the digestible total lysine content was statistically significantly different from the digestible reactive lysine content for all protein sources except the high-protein supplement. Ultimately, and in relation to nutrition, it is the digestible lysine content that is of importance. For several of the protein sources (infant formula B, UHT milk, weight

gain formula, and elderly formula), the actual difference between the digestible total lysine and available lysine (digestible reactive lysine) contents was small ($<3\%$). For whole milk protein, whey protein concentrate, evaporated milk, sports formula, and lactose-hydrolyzed milk powder; the overestimation of available lysine by digestible lysine ranged from 3.2% (whey protein concentrate) to 14% (evaporated milk).

It would appear that even for purified dairy-based protein sources, such as those tested in this study, the traditional true ileal total lysine assay does not always accurately predict available lysine content. The true ileal reactive lysine digestibility assay described here measures the uptake from the small intestine of structurally unaltered lysine molecules and provides more accurate estimates of available lysine.

The True Ileal Digestibility of Acid-Stable AA in Protein Sources Determined Following Traditional AA Analysis or After Guanidination of the Protein

Although the newly developed digestible reactive lysine assay can be used to determine available lysine with accuracy (Rutherford et al., 1997), it would be useful if the other acid-stable AA could also be determined with the same procedure. To this end, a statistical comparison was conducted of the mean true ileal AA digestibility determined using either conventional AA analysis or AA analysis following guanidination. Arginine was not examined, as it coeluted with a very large ammonia peak. For most (61%) of the acid-stable AA, there were no significant differences ($P > 0.05$) between methods, and for a further 19% of the AA studied, the difference between means, although significant statistically, was $<3\%$ and arguably not meaningful in practical terms. For the remaining 20% of AA tested, the new method did not predict ileal digestibility accurately compared with the conventional method. Histidine was the AA that showed the greatest absolute differences between the 2 methods for most protein products; tyrosine showed the least difference.

It appears that the digestible reactive lysine assay can be used to determine available lysine without affecting the estimation of the majority of the acid-stable AA.

Amino Acid Quality of the Milk-Based Products

All the milk-based products tested were highly digestible. This is consistent with other milk products that have been tested in our laboratory, such as sodium and calcium caseinate, milk protein isolate, α -lactalbumin, lactic casein, and whey protein concentrates

(Rutherford and Moughan, 1998). The most poorly digested AA was glycine, with a true ileal digestibility for the 12 milk products ranging from 33 to 87% and a mean digestibility of 70%. The most highly digestible AA was tyrosine, with a digestibility ranging from 92 to 102% and a mean digestibility of 98%. The digestibility of a few of the AA was slightly >100%, reflecting complete digestion and absorption of these AA. Experimental error explains the overestimate in digestibility. The infant formulas tended to have the lowest AA digestibility, with mean true AA digestibility for the formulas ranging from 81 to 84% for the acid-stable AA (apart from lysine) tested. The cause of the relatively low digestibility is unknown but should be investigated further.

The gross reactive lysine content of the milk products ranged from 9.5 to 78.6 mg/g. This variation is largely due to the inclusion level of milk proteins into the products. The digestibility of reactive lysine was similar to the digestibility values obtained for most of the other AA and ranged from 91 to 100%. As was the case with the acid-stable AA, the digestibility of reactive lysine tended to be lowest in the infant formulas. The UHT milk was the most digestible protein source in terms of reactive lysine.

CONCLUSIONS

The traditional true ileal AA digestibility assay is inaccurate for measuring available lysine in some processed protein sources. This is particularly true for protein sources that have been heat-processed and that contain reducing sugars. The true ileal digestible reactive lysine assay used in this study to measure available lysine does accurately determine available lysine. Although for a number of the protein sources tested there was little difference between the total lysine digestibility and the reactive lysine digestibility, there were statistically significant and sizable differences in digestible reactive lysine content and digestible total lysine content for some of the milk products. Consequently, it would appear that the conventional ileal total lysine

digestibility assay may not be accurate for all processed milk products.

It would appear in general that milk provides a highly digestible protein source for inclusion into other foods.

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