

## **Report of a Sub-Committee of the 2011 FAO Consultation on “Protein Quality Evaluation in Human Nutrition” on:**

### **The assessment of amino acid digestibility in foods for humans and including a collation of published ileal amino acid digestibility data for human foods**

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NOTE: the matters expressed in this report are those of the Sub-Committee and do not necessarily reflect the opinions of members (or a consensus) of the Expert Consultation. The report was an integral part of the process, in achieving an overall consensus, as relayed in the overall report of the 2011 FAO Expert Consultation.

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# True ileal amino acid digestibility coefficients for application in the calculation of Digestible Indispensable Amino Acid Score (DIAAS) in human nutrition

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## Introduction

Traditionally in human nutrition crude protein digestibility is assumed to accurately predict individual amino acid digestibility and is used in the calculation of the PDCAAS (protein digestibility corrected amino acid score). The digestibility of crude protein in foods has largely been determined on a faecal nitrogen digestibility basis (ie over the total digestive tract) in either human subjects or by using animal models (mainly the growing rat or growing pig).

During the FAO Expert Consultation on Protein Quality Evaluation in Human Nutrition, held in Auckland, New Zealand, 31 March – 2 April 2011, arguments were rehearsed that for accuracy, protein and amino acid digestibility in humans should be determined at the terminal ileum, as a measurement of amino acid disappearance between the mouth and the end of the small intestine. The Expert Consultation recommended specifically:

1. That proteins should firstly be described on the basis of their digestible amino acid contents, with each amino acid being treated as an individual nutrient;
2. that PDCAAS be replaced by a new score, DIAAS (digestible indispensable amino acid score) where  $DIAAS \% = 100 \times [(mg \text{ of digestible indispensable amino acid in } 1 \text{ g of dietary protein}) / (mg \text{ of the same indispensable amino acid in } 1 \text{ g of reference protein})]$ ;
3. **in both cases the amounts of digestible dietary indispensable amino acids were to be determined based on amino acid composition and true ileal amino acid digestibility coefficients determined either in humans directly, the growing pig or the growing rat, in that order of preference.**

Although the physiological significance of the measurement of ileal amino acid digestibility was clearly recognised at the consultation, and indeed in earlier consultations (FAO/WHO, 1991; WHO/FAO/WHO, 2007) there were some practical concerns raised about the general availability of suitable ileal protein and amino acid digestibility data with application to humans. In this context an FAO Working Group was formed comprising Sarwar Gilani (Chair), Daniel Tomé, Paul Moughan and Barbara Burlingame (ex-officio), and the group was charged with developing a justification for the use of ileal protein and amino acid digestibility data in practice including:

1. demonstrating, based on experimental data, the nature of differences between protein digestibility and that of specific amino acids;
2. demonstrating, based on experimental data, the nature of ileo-faecal digestibility differences;

3. demonstrating that there currently exists a suitable quantum of ileal amino acid digestibility data, to allow its introduction for application in practice.

These latter three objectives form the basis of this synopsis.

## **1) Basis for determining amino acid digestibility at the terminal ileum**

### *a. Faecal versus ileal digestibility – a physiological perspective*

In simple-stomached animals possessing a well-developed hind-gut (and this includes humans), a profuse and diverse microbiota acts on undigested material entering the large bowel, with a significant degree of metabolism of protein, peptides and amino acids. Ammonia, one of the products of the bacterial breakdown of protein and amino acids, is absorbed from the hindgut, but amino acids, as such, are not considered to be absorbed from the large intestine in nutritionally meaningful amounts (Wrong *et al.*, 1981; Moughan, 2003; Moughan and Stevens, 2012). Faecal protein is largely bacterial protein, and compositionally bears no resemblance to the array of dietary amino acids remaining undigested at the end of the ileum. Given that the bacterial protein does not directly relate to the food protein and undigested food amino acids, it is illogical to determine amino acid digestibility at the faecal level. Estimates of amino acid digestibility based on analysis of faeces do not describe the amounts of amino acid absorbed. Accordingly, measurements of digestibility determined at the ileal level are critical for determining amino acid losses of both dietary and endogenous origin (Moughan, 2003; Fuller and Tomé, 2005). Faecal-ileal digestibility differences can be substantial and both amino acid and protein ileo-faecal digestibility differences have been shown across a wide range of simple-stomached species of animal (Table 1). There is no reason to believe that the human, with a well-developed colon, would be any different, and indeed albeit limited experimental evidence with humans supports this.

It should be noted that ileal values of amino acid digestibility may themselves not be completely accurate estimators of amino acid uptake as there may be unaccounted for microbial catabolism and synthesis of amino acids in the upper digestive tract. Fuller (2012) has discussed recent experimental findings on bacterial amino acid synthesis in the upper gastro-intestinal tract, where absorption of the synthesised amino acid may occur. He concludes, that although there are still uncertainties about the impact of microbial activity in the upper digestive tract, the amino acid composition of ileal digesta provides the best available basis for estimating amino acid digestibility. Also several carefully controlled studies with simple-stomached animals have demonstrated the accuracy of ileal amino acid digestibility values (refer later section).

### *b. Crude protein versus amino acid digestibility*

With the PDCAAS method a single value for the digestibility of crude protein is used to adjust dietary concentrations of dietary indispensable amino acids. The digestibility of crude protein is assumed to apply to individual dietary indispensable amino acids and this assumes that differences between protein digestibility and amino acid digestibility are minor. This is not the case. Significant differences can be observed between protein digestibility and the digestibility of specific amino acids and among amino acid digestibilities. Such differences are highlighted in the data selected from rat studies and shown in Table 2, which were reported in the FAO/WHO (1991) report on the joint FAO/WHO expert consultation on protein quality evaluation held in 1989, and are sourced from work from Sarwar Gilani's

laboratory (Sarwar Gilani, 1987). Maximum and minimum true ileal amino acid digestibility values determined in pigs along with true ileal protein digestibility for a wider range of foods are shown in Figure 1. Clearly there can be practically significant differences between crude protein digestibility and that of specific amino acids. Amino acid digestibility should be used in estimating dietary protein quality wherever possible.

*c. Endogenous amino acids present in terminal ileal digesta*

During the digestion of food very considerable quantities of proteins of body (endogenous protein) as opposed to dietary origin are voided into the digestive tract. Much of this material is recycled, with the protein being digested and the amino acids reabsorbed. Nevertheless large quantities of endogenous protein, peptides and amino acids remain unabsorbed at the end of the small intestine and these along with endogenous protein originating from the colon are largely catabolised by the colonic microflora, and (for the dietary indispensable amino acids) represent a loss of amino acids from the body. If dietary amino acid digestibility is to be determined at the terminal ileum, and given that the ileal digesta contain copious quantities of endogenous proteins, it becomes necessary to determine the endogenous amino acid component. If coefficients of amino acid digestibility are not corrected for the ileal endogenous amino acids, the resultant digestibility coefficients are referred to as ‘apparent’ coefficients, whereas if the correction is made the coefficients are termed ‘true’. True digestibility is a fundamental property of the food and is not affected by the dietary conditions under which the food is given to the subject. The apparent digestibility measure will be affected by the assay conditions and is, therefore, variable and open to error. At a set food dry-matter intake, whereby the ileal endogenous flow may be constant, the determined apparent amino acid digestibility coefficient increases markedly and curvilinearly from low to higher dietary amino acid contents. This is an artefact of the assay, and reflects a disproportionate influence of the uncorrected – for ileal endogenous amino acid flow, at the lower amino acid intakes. This effect is shown clearly by the experimental data of Donkoh and Moughan (1994) (Figure 2) in which semi-synthetic corn-starch based diets containing different amounts of meat-and-bone meal protein were fed to growing rats and ileal digesta collected from the euthanased animals. Apparent ileal N digestibility increased with increasing dietary protein content, from a low of 65% to a high of 75%, whereas true ileal N digestibility was around 77% and independent of the dietary protein content.

Clearly, determined ileal amino acid flows need to be corrected for the ileal endogenous amino acids. Traditionally, this has been done by feeding the human subject or animal (model for human) a protein-free diet, but this method has been criticised as being unphysiological (Low, 1980). Other more physiological methods (eg the enzyme hydrolysed protein/ultrafiltration method; stable isotope-labelled protein) have been developed (Moughan *et al.*, 1998; Bos *et al.*, 2002). The practical application of these methods to give ‘true’ or ‘standardised’ estimates of ileal protein and amino acid digestibility has been the subject of review (Fouillet *et al.*, 2002; Fuller and Tomé, 2005; Columbus and de Lange, 2012; Moughan and Rutherford, 2012).

## **2) Faecal and ileal nitrogen and amino acid digestibility in the pig**

An important body of comparative ileo-faecal N and amino acid digestibility data is found in work with the growing pig (Low, 1980), which appears to be a suitable animal model for nutrient digestibility studies in humans, particularly for the determination of ileal nitrogen digestibility (Pond and Houpt, 1978; Miller and Ullrey, 1987; Moughan and Rowan, 1989; Moughan *et al.*, 1992; Moughan, *et al.*, 1994; Deglaire *et al.*, 2009; Deglaire and Moughan, 2012).

*a. Comparisons of ileo-faecal nitrogen and amino acid digestibility in the pig*

There is general agreement across studies, that the ileal digestibilities of most amino acids are lower than corresponding digestibilities determined over the total digestive tract (for example see Table 3), but this finding is not universal. The amount of amino acids disappearing in the large intestine usually ranges from around 5% to 35% of the amino acid ingested. It appears that the lower the overall ileal digestibility of nitrogen or amino acids, the greater is the ileo-faecal difference in digestibility (Table 3). This is understandable as with diets containing highly digestible protein most is absorbed before the digesta enter the large intestine, whereas with protein sources of lower quality, there are larger residues to be fermented and with a proportionately greater disappearance of amino acids between the terminal ileum and rectum.

The extent of faecal digestibility over- or under-estimation varies with the amino acid, the type of dietary protein and the influence of other dietary components. Lenis (1983) surveyed the world literature from 1964 to 1982 for some 35 foods. For threonine and tryptophan the mean overestimations of apparent digestibility by the faecal method in comparison with ileal, were 10 and 11 percentage units, respectively. The ileo-faecal differences tended to be smaller for lysine. The faecal method overestimated (mean overestimation = 5.6% units) lysine digestibility for eleven foods and underestimated it (mean underestimation = 4.3% units) in ten further foods. Faecal values appear to often considerably underestimate the actual (ileal) digestibility of methionine, although the opposite has been found for cysteine. Hendriks *et al.*, (2012) have collated apparent faecal and ileal N digestibility data for the growing pig from a large number of studies. Generally faecal digestibility values were much higher than ileal digestibility values, but in a few cases the ileal N digestibility value exceeded its faecal counterpart. The extensive data set also clearly demonstrates that as apparent ileal N digestibility increased from a low of 50% to a high of 95%, the ileo-faecal difference decreased quite markedly.

Overall, the published evidence suggests that in the growing pig, ileal amino acid digestibility values are quantitatively different from faecal amino acid digestibility values, and ileal values are superior for application in practice. Similar ileo-faecal protein digestibility differences have been reported in the growing rat (Table 4).

*b. Experimental evidence for the validity of ileal amino acid digestibility coefficients*

The effect of hindgut microbial metabolism of undigested dietary amino acids, on faecal estimates of digestibility may explain the frequently reported low statistical correlations observed between pig growth performance and faecal estimates of amino acid uptake (Crampton and Bell, 1946; Lawrence, 1967; Cole *et al.*, 1970). Whereas faecal amino acid digestibility coefficients have been poor predictors of animal growth, ileal amino acid digestibility values have been shown to be sensitive in detecting small differences in protein digestibility due to the processing of foods (van Weerden *et al.*, 1985; Sauer and Ozimek, 1986) and several studies (Tanksley and Knabe, 1980; Low *et al.*, 1982; Just *et al.*, 1985; Moughan and Smith, 1985; Dierick *et al.*, 1988) have demonstrated that ileal values are accurate in describing the extent of uptake of amino acids from the gut lumen. Rutherford *et al* (1997) undertook a study to evaluate the accuracy of true ileal lysine digestibility coefficients in predicting whole body lysine deposition in the pig. The study supported the accuracy of true ileal lysine digestibility as a predictor of dietary lysine uptake from the digestive tract. Experimental evidence for the validity of the application of ileal amino acid digestibility coefficients has recently been reviewed by Columbus and de Lange (2012) who

conclude that “there is a large body of evidence to suggest that in many instances measures of ileal amino acid digestibility yield reasonable estimates of bioavailability of amino acids in foods”.

*c. The digestibility of lysine from processed foods*

For foods that have been subjected to processing and with possible damage to amino acids, the traditional ileal digestibility assay is not expected to accurately indicate the absorption of all amino acids (Moughan *et al.*, 1991). This is well exemplified for lysine. During amino acid analysis with strong hydrochloric acid, early Maillard compounds are known to partially revert to lysine. Such reversion does not occur, however, in the alimentary canal during gastric digestion. Consequently, estimates of dietary and ileal digesta lysine will be in error leading to biased ileal digestibility coefficients. Although, and at least for lysine, structurally unaltered molecules can be accurately determined chemically (eg FDNB lysine assay), there is evidence (Hurrell and Carpenter, 1981) that the unaltered or chemically available molecules may not be fully absorbed from the damaged proteins. The absorption (measured at terminal ileum) of reactive lysine has been determined in a study (Moughan *et al.*, 1996) with the growing pig (Table 5). A casein-glucose mixture was heated to produce early Maillard compounds, and the amount of epsilon-*n*-deoxy-fructosyl-lysine (blocked lysine) and lysine regenerated after acid hydrolysis in the resulting material was calculated from the determined level of furosine. The amount of unaltered or reactive lysine was found by difference between the total lysine (acid hydrolysis) and regenerated lysine. The FDNB method allowed accurate assessment of the amount of chemically reactive lysine, which was grossly overestimated by conventional amino acid analysis (acid-hydrolysed lysine), but the reactive lysine was not completely absorbed. For the amino acid lysine, and in foods that may have sustained chemical alteration of their proteins, reactive lysine as opposed to total lysine (traditional amino acid analysis) should be determined on both the food and ileal digesta, and should be used in the calculation of digestibility (Moughan and Rutherford, 1996; Rutherford and Moughan 2012). True ileal digestible reactive lysine provides an accurate assessment of lysine available to the tissues for metabolism.

### **3) Ileal protein and amino acid digestibility in the adult human**

*a. Nitrogen flow at the terminal ileum in humans*

In the adult human, total nitrogen flow at the terminal ileum ranges from 2 to 5 g/day, with endogenous and dietary nitrogen losses ranging from 0.7 to 4 g/day and 0.3 to 1 g/day, respectively. Endogenous and dietary amino acid losses are 0.6–1 g/day and 0.4–0.7 g/day, respectively (Chacko and Cummings, 1988; Mahé *et al.*, 1992; Rowan *et al.*, 1993; Fuller *et al.*, 1994; Gausserès *et al.*, 1996; Mariotti *et al.*, 1999; Gaudichon *et al.*, 2002; Moughan *et al.*, 2005a). These results show that a significant proportion of the nitrogen flow (about 40% to 50%) in the human ileum is of non-protein origin.

*b. Ileal versus faecal nitrogen digestibility in humans*

Sammons (1961) determined daily rates of faecal N output from normal human subjects and ileal N output from ileostomates given the same diet. The ileal output was 2.7 g N/d and the faecal output 1.8 g N/d, demonstrating a considerable loss of N in the large bowel of the human and suggesting quantitatively important differences in ileal and faecal N digestibility. Sandstrom *et al* (1986) gave soya- and meat-based diets to ileostomates and reported

apparent ileal digestibility coefficients for total N in the range of 80 to 85%. In comparison, in human subjects receiving soya-based diets, true faecal digestibility coefficients ranging from 90 to 98% have been reported (Istfan *et al.*, 1983; Scrimshaw *et al.*, 1983; Wayler *et al.*, 1983; Young *et al.*, 1984). Evenepoel *et al.* (1998) fed <sup>15</sup>N-labelled egg protein to human ileostomates and recorded true ileal digestibility values for crude protein in cooked and raw egg of 90.0 and 51.3%, respectively and concluded that the ileal digestibility value for cooked egg was lower than the comparable published range for faecal digestibility (92-97%). In contrast, Gibson *et al.* (1976) reported only marginally lower digestibility coefficients determined at the terminal ileum rather than across the total digestive tract for human subjects receiving highly digestible proteins. Bos *et al.* (1999) measured true ileal and faecal protein digestibility using <sup>15</sup>N-labelled milk protein and showed that the amount of N recovered at the terminal ileum peaked after 1 h and then decreased during the next 7 h with no significant amount of exogenous N recovered at the terminal ileum at the end of the 8 h, whereas the amount recovered in the faeces remained at a very low level after 24 h, peaked after 60 h and progressively decreased (figure 3). The true ileal and faecal digestibilities of the highly digestible milk protein were calculated as 95.5 % and 96.6% respectively, and were not statistically significantly different from each other.

These results from human digestibility studies, albeit small in number, are in general agreement with observations in other simple-stomached mammals.

*c. Ileal versus faecal amino acid digestibility in humans and ileal amino acid digestibility values for humans*

In the study of Rowan *et al.* (1994) five subjects with established ileostomies and six normal subjects consumed a constant diet consisting of meat, vegetables, fruit, bread and dairy products for 7 d with collection of ileostomy contents or faeces, respectively, over the final 4 d of the experimental period. Generally the apparent faecal digestibility coefficients were higher than their ileal counterparts with significant ( $P < 0.05$ ) differences being recorded for arginine, aspartic acid, glycine, phenylalanine, proline, serine, threonine and tryptophan (Table 5). The faecal digestibility of methionine was statistically significantly lower than the ileal value. Some of the differences recorded were quantitatively important (eg methionine and tryptophan), and particularly when viewed against the background of the ileal values being determined using ileostomates. Ileostomates develop a characteristic and quite extensive microflora at the end of the ileum (Vince *et al.*, 1973).

It is concluded that the use of faecal amino acid digestibility coefficients may be misleading for determining the uptake of dietary amino acids in humans, and ileal digestibility coefficients are preferred for application in humans.

Results for a set of studies determining true ileal nitrogen digestibility in healthy humans, using stable isotope-labelled protein, are reported in table 7. The values for true ileal digestibility ranged from 84 to 95%. Apparent and true ileal amino acid digestibility for mixed meals based on intact casein or on hydrolyzed casein were determined in healthy adult humans and showed differences among amino acids (Deglaire *et al.*, 2009) (Table 8). True ileal amino acid digestibility for milk and soya protein was also determined in healthy human subjects (Gaudichon *et al.*, 2002). The lowest digestibility was observed for threonine in the soya group (89.0%) and the highest was for tyrosine in the milk group (99.3%) (Table 9). A significantly lower digestibility was found for threonine, valine, histidine, tyrosine, alanine, and proline with soya protein as compared with milk protein. Nitrogen digestibility was

significantly lower in the soya group than in the milk group. In contrast, when total nitrogen digestibility was calculated from individual amino acid digestibilities, the difference between milk and soya was not statistically significant.

#### **4) General considerations**

##### *a. Findings of the 2011 FAO Expert Consultation*

The digestible indispensable amino acid score (DIAAS) approach is recommended for the evaluation of dietary protein quality in humans. In the calculation of DIAAS a value for the amount of digestible indispensable amino acid is used. With respect to the determination of dietary digestible indispensable amino acid content the 2011 FAO Consultation concluded:

“It is recommended that protein quality assessment should be based on the true ileal digestibility values of individual amino acids rather than overall (faecal) digestibility. True ileal amino acid digestibility should preferably be determined in humans. Where human data are lacking, it is recommended that ileal amino acid digestibility values from the growing pig be used, and where these data are not available, from the growing rat. When amino acid digestibility data are not available, amino acid digestibility is assumed to be equivalent to crude protein digestibility. In this case true ileal crude protein digestibility data are preferable, but where unavailable true faecal crude protein digestibility may be used”.

##### *b. Published data on true ileal amino acid digestibility in foods for humans*

A review of the literature and various data bases has been undertaken and true ileal digestibility data are presented (refer attached appendix) from work with adult humans, the growing pig and the growing laboratory rat. The dataset once again demonstrates a considerable degree of variation in digestibility among foods, and among individual amino acids and total nitrogen within a food, highlighting the need to use digestibility data for individual amino acids and specific foods wherever possible. The true ileal amino acid digestibility database presented herewith, has been gleaned from a large number of diverse studies conducted over a number of years and where different methodologies have been used. Although each study has been assessed to ensure bona-fide approaches were employed, nevertheless considerable methodological-based variation will be inherent. Also, in many cases only a rudimentary description of the food source is available. Thus the present dataset should be regarded as interim. There is a need to develop studies and to accumulate data on ileal amino acid digestibility directly determined in humans. In addition, a validated and standardised method should be developed using animal models (**either the growing pig or the growing rat**). Several currently used methodologies for obtaining pig or rat true ileal digestibility data could be considered acceptable, but the development of an agreed standardised methodology (see Moughan and Rutherfurd, 2012) would be a considerable advancement. Such an assay would be applied to form a comprehensive standardised set of tables for the true ileal amino acid digestibility of human foods.



Table 1. Comparison of ileal and faecal digestibility of dietary protein for the domestic chicken and several simple-stomached mammals

	Apparent digestibility (%)		Difference (faecal-ileal, % units)
	Ileal	Faecal	
Piglet <sup>1</sup>	90	97	+7%
Growing pig <sup>2</sup>	66	81	+22%
Pre-ruminant calf <sup>3</sup>	88	94	+7%
Chicken <sup>4</sup>	78	86	+10%
Growing rat <sup>5</sup>	69	78	+9%
Growing rat <sup>6</sup>	82	88	+6%
Growing rat <sup>7</sup>	81	77	-4%
Growing rat <sup>8</sup>	66	67	+1%

<sup>1</sup>Piglets (6 kg liveweight) fed bovine milk (Moughan *et al.*, 1990)

<sup>2</sup>Pig (45 kg liveweight) given meat and bone meal based diet (Moughan *et al.*, 1984)

<sup>3</sup>Milk fed calf (45 kg liveweight) (Moughan *et al.*, 1989)

<sup>4</sup>Overall mean amino acid digestibility for 9 amino acids and 16 diets given to 10 week old chickens (Raharjo and Farrell 1984) and based on a collection of ileal digesta or excreta.

Rat (80 g bodyweight) given a diet based either on meat and bone meal<sup>5</sup>, fish meal<sup>6</sup>, field peas meal<sup>7</sup> or barley meal<sup>8</sup> (Moughan *et al.*, 1984)

*Table 2. Faecal crude protein and amino acid digestibility (%) for selected foods, determined in the growing rat*

	<b>Protein</b>	<b>Lys</b>	<b>Met</b>	<b>Cys</b>	<b>Thr</b>	<b>Trp</b>
Pea flour	88	92	77	84	87	82
Pea (autoclaved)	83	85	62	85	78	72
Pintobean (canned)	79	78	45	56	72	70
Lentil (autoclaved)	85	86	59	75	76	63
Fababean (autoclaved)	86	85	59	75	76	63
Soyabean	90	87	82	82	84	89
Peanut	96	90	85	89	89	94
Wheat	93	83	94	97	91	96

from Sarwar Gilani (1987)

Table 3. Apparent ileal and faecal digestibilities (%) of dietary amino acids in the growing pig

a) Pigs fed a balanced cereal-based diet (Sauer and Just, 1979, n = 30).

	Ileum	Faeces	Difference (faecal-ileal, % units)
Arginine	88	92	+4
Histidine	85	92	+7
Isoleucine	81	87	+6
Leucine	83	89	+6
Lysine	85	87	+2
Methionine	85	85	0
Phenylalanine	82	89	+7
Threonine	73	85	+12
Tryptophan	79	89	+10
Valine	79	87	+8
Average	82	88	+6

b) Pigs fed wheat flour and wheat offal with digestibility determined at the terminal ileum (I) and in faeces (F) (Sauer et al., 1977).

Amino Acid	Wheat flour		Wheat offal	
	I	F	I	F
Lysine	84	86	66	76
Histidine	91	94	79	88
Methionine	94	94	78	82
Isoleucine	94	95	73	75
Leucine	95	96	75	79

c) Pigs fed raw soyabean flour (Nutrisoy) and autoclaved Nutrisoy.<sup>1</sup>

	Nutrisoy		Autoclaved Nutrisoy	
	Ileal	Faecal	Ileal	Faecal
Protein	37	77	77	90
Arginine	45	85	90	96
Histidine	44	85	83	95
Isoleucine	40	74	86	91
Leucine	37	75	86	92
Lysine	41	80	80	90
Methionine <sup>2</sup>	59	72	86	89
Cysteine	35	77	68	86
Phenylalanine	39	77	88	93
Tyrosine	34	73	85	91
Threonine	36	72	73	89
Valine	38	74	84	91

<sup>1</sup>Abstracted from Li *et al.* (1998); Two maize starch-based diets containing 200 g/kg diet of either Nutrisoy (a defatted soya flour containing active trypsin inhibitors) or autoclaved Nutrisoy (containing reduced amounts of trypsin inhibitors) were tested.

<sup>2</sup>Digestibility after correction for dietary supplementation of methionine.

*Table 4. Mean apparent digestibility of crude protein (%) as determined in ileal digesta or faeces in the growing rat (Moughan et al., 1984)*

	<b>Barley meal</b>	<b>Meat and bone meal</b>	<b>Fish meal</b>	<b>Field peas</b>
Ileal	66	69	82	81
Faecal	67	78	88	77
Difference (faecal-ileal, % units)	+1%	+9%	+6%	-4%

Table 5. Amounts of acid-hydrolysed lysine, FDNB lysine, reactive lysine and absorbed reactive lysine in a heated casein-glucose mixture.

	<b>Acid- hydrolysed<sup>1</sup></b>	<b>FDNB<sup>2</sup></b>	<b>Reactive<sup>3</sup></b>	<b>Absorbed reactive<sup>4</sup></b>
Lysine (g 100 g <sup>-1</sup> )	2.60	1.91	1.98	1.40

<sup>1</sup>After conventional amino acid analysis.

<sup>2</sup>FDNB = fluoro-dinitrobenzene.

<sup>3</sup>Lysine units remaining chemically reactive after heating, determined from furosine levels.

<sup>4</sup>Reactive lysine absorbed by the end of the small intestine.

From Moughan *et al.* (1996).

Table 6: Mean apparent ileal and faecal amino acid digestibility coefficients for adult ileostomate and healthy human subjects (65 kg body weight) receiving a meat, vegetable, cereal, and dairy-product-based diet, respectively (from Rowan et al., 1994)

Amino acid	Digestibility coefficient <sup>1</sup>		Statistical significance	Difference (Faecal-ileal, % units)
	Ileal (n 5)	Faecal (n 6)		
Arginine	90	93	*	+3
Aspartate	87	90	*	+3
Serine	87	92	***	+5
Threonine	85	89	**	+4
Proline	90	95	**	+5
Glycine	72	87	***	+15
Phenylalanine	90	91	***	+1
Methionine	93	83	***	-10
Tryptophan	77	83	*	+6

<sup>1</sup>Amino acids for which significant (P<0.05) ileo-faecal differences were found.

*Table 7: Ileal nitrogen digestibility determined in humans*

<b>Protein</b>	<b>Ileal digestibility</b>		<b>Reference</b>
	<b>Apparent</b>	<b>True</b>	
Milk protein	91	95	Mahé et al, 1994 ; Bos et al, 2003; Gaudichon et al, 2002
Fermented milk	90	-	Mahé et al, 1994
Casein	-	94.1	Deglaire et al, 2009
Soya protein	-	91.5	Bos et al, 2003; Gaudichon et al, 2002
Pea protein	-	91.5	Gausserès et al, 1997
	-	89.4	Gausserès et al, 1996
	-	90	Mariotti et al, 2001
Wheat	-	91.5	Bos et al, 2005
	-	85.0	Juillet et al, 2008
Lupin protein	-	90.0	Mariotti et al, 2002
Rapeseed protein	-	84	Bos et al, 2007

Table 8: Apparent and true ileal amino acid digestibility for mixed meals based on intact casein or on hydrolysed casein fed to adult humans (Mean values and pooled standard deviations) (Deglaire et al., 2009)

	<b>Intact casein</b>		<b>Hydrolysed casein</b>	
	<b>Apparent</b>	<b>True</b>	<b>Apparent</b>	<b>True</b>
<i>Indispensable amino acids</i>				
Histidine	0.808	0.947	0.691	0.929
Isoleucine	0.838	0.941	0.811	0.929
Leucine	0.900	0.972	0.883	0.970
Lysine	0.918	0.974	0.906	0.976
Phenylalanine	0.889	0.963	0.869	0.966
Threonine	0.757	0.933	0.708	0.925
Tyrosine	0.887	0.972	0.860	0.971
Valine	0.846	0.937	0.810	0.924
<i>Dispensable amino acids</i>				
Alanine	0.842	0.951	0.789	0.936
Aspartic acid	0.759	0.916	0.701	0.896
Glutamic acid	0.897	0.940	0.866	0.914
Proline	0.910	0.962	0.891	0.954
Serine	0.729	0.870	0.666	0.826



Table 9: True ileal digestibility (%) of dietary nitrogen and amino acids for milk or soya Protein in healthy human volunteers (from Gaudichon et al, 2002)

	<b>Milk</b>	<b>Soya</b>
Aspartate + asparagine	94.3 ± 2.1	93.2 ± 4.0
Serine	92.0 ± 2.5	93.2 ± 3.9
Glutamate + glutamine	95.3 ± 2.0	96.6 ± 2.8
Proline	96.1 ± 2.2 <sup>a</sup>	92.8 ± 3.8
Glycine	91.6 ± 4.0	90.1 ± 5.1
Alanine	95.9 ± 1.9 <sup>a</sup>	92.3 ± 2.5
Tyrosine	99.3 ± 0.4 <sup>a</sup>	96.8 ± 1.5
Threonine	93.4 ± 2.3 <sup>a</sup>	89.0 ± 4.9
Valine	95.9 ± 1.9 <sup>a</sup>	92.5 ± 3.5
Isoleucine	95.4 ± 1.8	93.5 ± 3.1
Leucine	95.1 ± 2.2	93.3 ± 3.0
Phenylalanine	95.6 ± 2.3	95.5 ± 2.3
Lysine	94.9 ± 2.7	95.0 ± 2.5
Histidine	94.9 ± 2.7 <sup>a</sup>	91.7 ± 1.7
Average amino acid digestibility <sup>b</sup>	95.3 ± 1.8	93.8 ± 3.0
Nitrogen digestibility	95.3 ± 0.9 <sup>a</sup>	91.7 ± 1.8

NOTE. Values are means ± SD, (n = 7 and n = 6 for milk and soya, respectively).

<sup>a</sup>Significantly different from soya group (ANOVA,  $P < 0.05$ ).

<sup>b</sup>Calculated from amino acid digestibilities weighted by the proportion of each amino acid in the dietary protein.

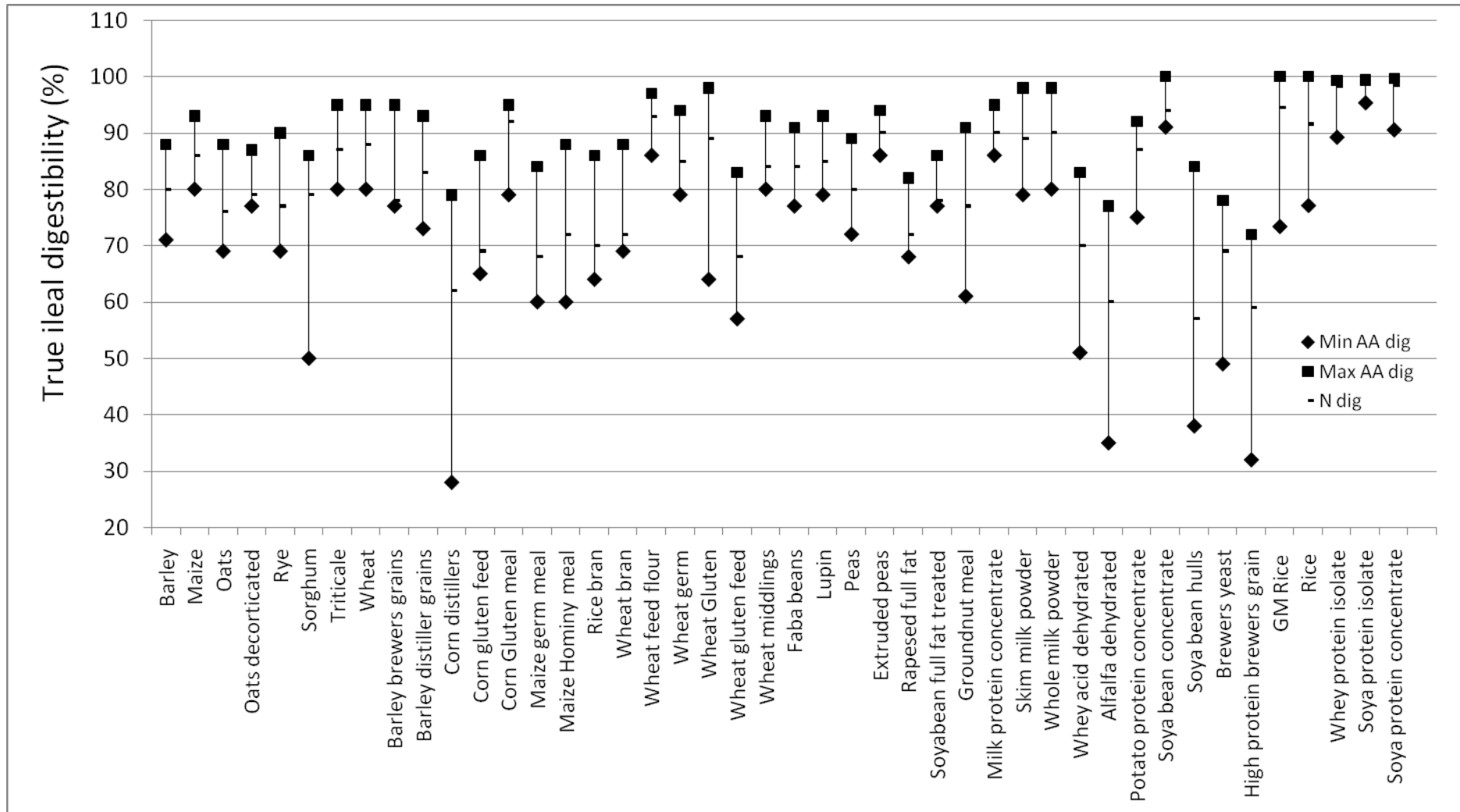


Figure 1. Maximum true ileal amino acid digestibility (■), minimum true ileal amino acid digestibility (◆), and true ileal nitrogen digestibility (◻).

Source of data: AFZ, Ajinomoto Eurolysine, Aventis Animal Nutrition, INRA, ITCF (2000); Han *et al.* (2006) and Moughan *et al.* (2005b). All values are from studies with the growing pig except for the last three datasets (whey protein isolate, soya protein isolate, soya protein concentrate) which were obtained with adult humans.

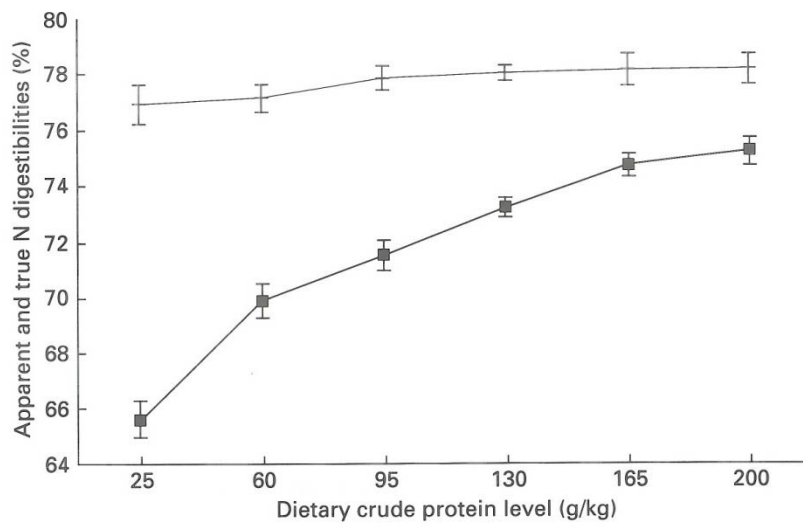


Figure 2. Effect of dietary protein content on mean apparent (■) and true (+) ileal N digestibility values for rats given a meat-and-bone-meal-based diet (Donkoh and Moughan, 1994).

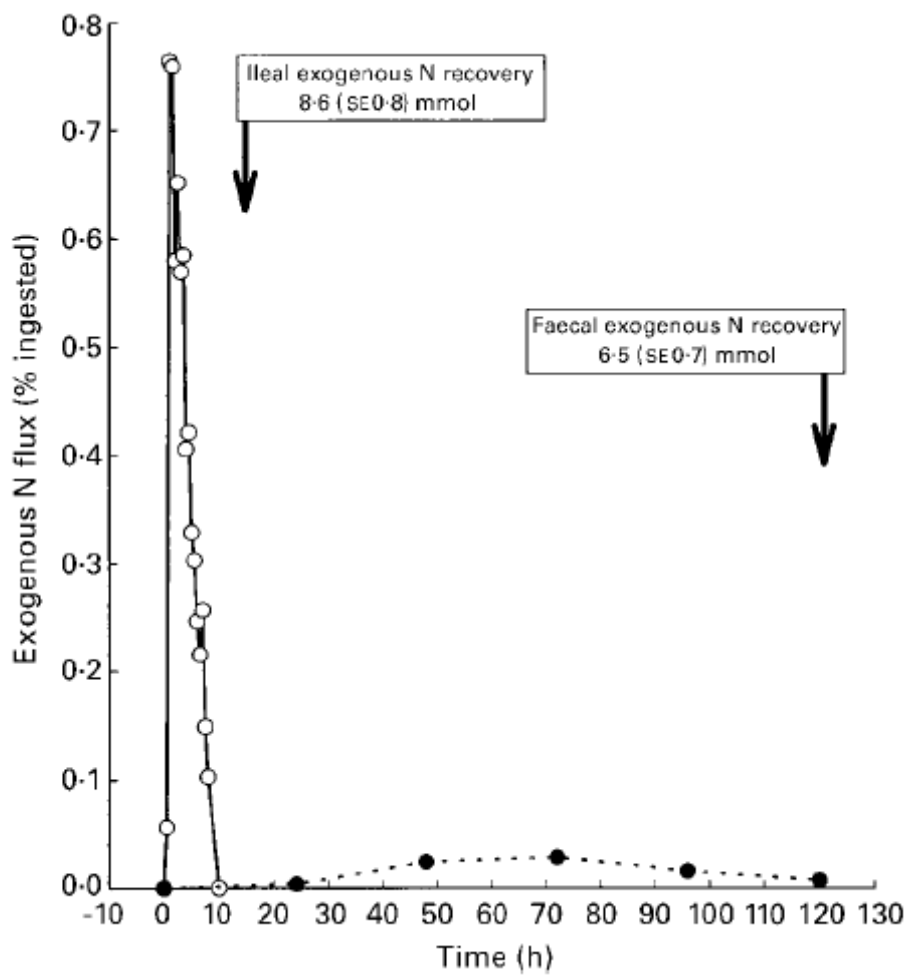


Figure 3: Exogenous nitrogen recovered in the ileum (o) and in the faeces (●) following ingestion of [15N]milk by healthy adults after an overnight fast. Each value represents the mean of five subjects (Bos et al, 1999).

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## Appendix 1: True ileal amino acid and protein digestibility (%) for selected human foods

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### Introduction

Foods are given in the table alphabetically. Within a food, digestibility data obtained from humans directly are given first, followed by predicted (regression) human data based on data obtained using the growing pig and then followed, thirdly, by digestibility data obtained using the growing laboratory rat. For the calculation of DIAAS, the 2012 FAO Report recommends using human true ileal amino acid digestibility coefficients; pig true ileal amino acid digestibility coefficients then rat true ileal amino acid digestibility coefficients, in that order of preference.

Close agreement has been shown for ileal amino acid digestibility, between the growing pig and adult human and based on first-principles the growing pig would appear to be a suitable animal model for studying protein digestion in humans. It can be argued that pig true ileal amino acid digestibility data could be used interchangeably for humans. However, small inter-species differences may exist, and the most accurate data for practical application may be human digestibility values predicted statistically (linear regression model) based on pig digestibility values. Such a regression model has been published<sup>1</sup> (albeit based on a limited number of observations) and has been applied here to derive human digestibility values based on determined pig values. Similar regression equations have not been determined for the rat, so rat true ileal amino acid digestibility data are given as determined. Several studies, but not all, have shown close agreement for true ileal amino acid digestibility between the growing rat and pig. There have been few direct rat/human ileal amino acid digestibility comparisons.

In the table, some data are mean observations from a single study, while others are means across studies. Source references for the studies are given, and a full list of the references is appended at the end of the table. It should be noted that for lysine, values denoted RL, have been determined using the true ileal reactive (available) lysine assay, involving quantification of food and digesta lysine after reaction with the reagent o-methylisourea<sup>2</sup>. These values are the preferred values for lysine digestibility.

Digestibility values are given as percentages (%)

<sup>1</sup>Deglaire, A., Bos, C., Tomé, D. and Moughan, P. J. (2009) Ileal digestibility of dietary protein in the growing pig and adult human. *British Journal of Nutrition*. 102, 1752-1759.

<sup>2</sup>Moughan, P.J. and Rutherford, S.M. (1996) A new method for determining digestible reactive lysine in foods. *Journal of Agricultural and Food Chemistry*. 44, 2202-2209.

## Key to Symbols

<sup>P</sup> Predicted from pig true ileal amino acid digestibility data based on the equations of Deglaire and Moughan *et al.* (2012)<sup>1</sup>.

<sup>D</sup> determined.

<sup>PF</sup> Endogenous amino acid losses determined using the protein-free diet method.

<sup>EHC</sup> Endogenous amino acid losses determined using the enzyme hydrolysed casein/ultrafiltration method.

<sup>HDP</sup> Endogenous amino acid losses determined using the highly digestible protein method.

<sup>WM</sup> Weighted mean based on endogenous amino acid losses determined by protein-free diet, EHC/ultrafiltration, regression, protein-free diet + parenteral amino acid infusion and highly digestible protein diet methods.

<sup>RL</sup> Lysine digestibility based on reactive lysine digestibility.

<sup>DM</sup> Data presented on a dry matter basis.

<sup>1</sup> Digestibility determined using the stable isotope techniques.

<sup>O</sup> Units are mg/g protein (N x 5.52).

<sup>Q</sup> Units are mg/g protein (N x 5.44).

<sup>1</sup>Deglaire, A. and Moughan, P.J. (2012) Animal models for determining amino acid digestibility in humans - a review. *British Journal of Nutrition*. 108, S273-S281.

	Barley <sup>1,2,4,5,19</sup>	Biscuits (CP<12%) <sup>5</sup>	Biscuits (CP>12%) <sup>5</sup>	Bovine serum albumin hydrolysate <sup>14</sup>	Bread <sup>5</sup>	Bread (wholegrain) <sup>14</sup>	Bread (Syrian, plain) <sup>14</sup>
	Human <sup>P,PF,WM</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	76	88	90	83	90	84	
Threonine	77	88	91	80	91	89	
Serine	81	94	91	80	93	93	
Glutamic acid	87	93	93	86	93	97	
Glycine	78	89	92	53	90	70	
Alanine	72	92	89	88	92	89	
Valine	80	91	90	89	91	92	
Isoleucine	81	92	92	84	92	95	
Leucine	82	93	91	91	93	95	
Tyrosine	83	90	94	92	93	95	
Phenylalanine	83	88	88	90	88	96	
Histidine	82	94	93	82	92	88	
Lysine	76	89	89	92	92	96	96
Arginine	83	93	93	92	93	91	
Cysteine	81	87	87	89	87		
Methionine	83	91	90	82	93		
Proline	91	91	90	80	91	97	
Tryptophan	80	99	86		90		
Protein	78	92	91		91		

	Bread flour <sup>14</sup>	Breakfast cereal (shredded wheat biscuit) <sub>1<sup>13</sup></sub>	Breakfast cereal (shredded wheat biscuit) <sub>2<sup>13</sup></sub>	Breakfast cereal (shredded wheat biscuit) <sub>3<sup>13</sup></sub>	Breakfast cereal (shredded wheat biscuit) <sub>4<sup>13</sup></sub>	Breakfast cereal (shredded wheat biscuit) <sub>5<sup>13</sup></sub>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid		63	54	59	64	48
Threonine		76	76	77	72	70
Serine		85	86	84	83	82
Glutamic acid		92	93	91	91	90
Glycine		61	54	55	59	44
Alanine		78	80	77	75	75
Valine		83	86	82	80	80
Isoleucine		85	89	85	84	84
Leucine		87	90	87	84	86
Tyrosine		88	88	87	85	86
Phenylalanine		91	94	91	87	90
Histidine		51	52	76	43	48
Lysine	98	84	86	81	76	68
Arginine		85	86	85	80	84
Cysteine						
Methionine		88	90	89	88	86
Proline		90	83	89	88	89
Tryptophan						
Protein						

	Breakfast cereal (shredded wheat biscuit) <sup>14</sup>	Breakfast cereal (extruded corn) <sup>13</sup>	Breakfast cereal (extruded corn) <sup>213</sup>	Breakfast cereal (flaked corn) <sup>14</sup>	Breakfast cereal (extruded wheat/oat/corn) <sup>13</sup>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	23	74	70	57	71
Threonine	42	75	80	69	84
Serine	64	84	90	80	90
Glutamic acid	85	83	85	87	96
Glycine	2	43	47	23	74
Alanine	54	87	85	86	85
Valine	63	79	81	76	90
Isoleucine	70	87	85	77	93
Leucine	78	90	90	89	94
Tyrosine	73	87	89	88	93
Phenylalanine	79	90	91	87	97
Histidine	50	71	71	74	78
Lysine	43	100	74	66	63
Arginine	73	59	50	73	89
Cysteine				66	
Methionine		96	93	85	94
Proline	80	68	77	57	95
Tryptophan				42	
Protein				67	

	Breakfast cereal (extruded wheat/oat/corn) <sup>14</sup>	Breakfast cereal (flaked wheat) 1 <sup>13</sup>	Breakfast cereal (flaked wheat) 2 <sup>13</sup>	Breakfast cereal (puffed rice) 1 <sup>13</sup>	Breakfast cereal (puffed rice) 2 <sup>13</sup>	Breakfast cereal (puffed rice) <sup>14</sup>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	76	49	62	63	49	51
Threonine	89	62	74	76	57	64
Serine	92	69	83	80	61	63
Glutamic acid	97	81	92	68	58	61
Glycine	76	20	56	50	17	15
Alanine	90	63	77	67	59	71
Valine	91	68	82	74	65	69
Isoleucine	94	74	85	75	66	69
Leucine	96	76	88	71	62	67
Tyrosine	96	75	87	70	60	68
Phenylalanine	97	80	92	75	65	70
Histidine	85	49	79	71	54	66
Lysine	96	66	66	87	90	100
Arginine	94	70	83	71	78	72
Cysteine						
Methionine		79	85	73	23	
Proline	95	69	90	60	56	40
Tryptophan						
Protein						

	Breakfast cereal (puffed wheat/rice/corn) <sup>13</sup>	Breakfast cereal (puffed wheat/rice/oat) <sup>13</sup>	Breakfast cereal (puffed wheat) <sup>13</sup>	Breakfast cereal (rolled oat) 1 <sup>13</sup>	Breakfast cereal (rolled oat) 2 <sup>13</sup>	Breakfast cereal (rolled oat) 3 <sup>13</sup>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	46	67	46	82	76	73
Threonine	60	74	71	81	77	73
Serine	78	78	83	85	80	75
Glutamic acid	83	86	91	91	88	86
Glycine	0	31	50	69	59	57
Alanine	71	76	75	82	79	72
Valine	75	80	84	86	82	78
Isoleucine	81	83	87	88	85	81
Leucine	83	84	90	88	85	81
Tyrosine	83	82	89	88	82	77
Phenylalanine	88	86	93	91	86	82
Histidine	49	68	52	74	71	67
Lysine	53	91	60	84	90	86
Arginine	80	79	87	89	85	80
Cysteine						
Methionine	82	87	88	94	89	86
Proline	78	78	89	85	83	79
Tryptophan						
Protein						



	Breakfast cereal (rolled oat) <sup>4</sup> <sup>13</sup>	Breakfast cereal (rolled oat) <sup>5</sup> <sup>14</sup>	Breakfast cereal (rolled oat/wheat/corn) <sup>13</sup>	Breakfast cereal (wheat bran) <sup>14</sup>	Calcium caseinate <sup>10</sup>	Casein <sup>23</sup>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>D,I</sup>
Aspartic acid	87	85	77	69	93	92
Threonine	84	83	75	67	92	93
Serine	88	87	81	74	83	87
Glutamic acid	92	93	89	85	89	94
Glycine	78	70	61	56	91	
Alanine	86	83	82	68	97	95
Valine	89	86	85	70	96	94
Isoleucine	91	89	87	75	92	94
Leucine	91	89	87	75	98	97
Tyrosine	90	89	85	73	100	97
Phenylalanine	93	91	90	77	100	96
Histidine	74	91	51	82	94	95
Lysine	91	91	79	85	98	97
Arginine	90	86	84	72	98	
Cysteine		93		78	83	
Methionine	92	94	91	82	83	
Proline	86		82			96
Tryptophan		84		75		
Protein		88		73		94

	Casein <sup>5,17,23</sup>	Casein <sup>16</sup>	Casein hydrolysate <sup>23</sup>	Casein hydrolysate <sup>23</sup>	Casein hydrolysate <sup>16</sup>	Cheese whey (CP<17.5%) <sup>5</sup>	Cheese whey (CP 17.5-27.5%) <sup>5</sup>
	Human <sup>P,PF</sup>	Rat <sup>D,EHC</sup>	Human <sup>D,I</sup>	Human <sup>P,I</sup>	Rat <sup>D,EHC</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>
Aspartic acid	95	93	90	91	95	88	88
Threonine	92	95	93	91	98	88	88
Serine	91	87	83	82	96	88	88
Glutamic acid	96	92	91	91	91	88	88
Glycine	95	65			80	87	88
Alanine	94	93	94	91	99	89	88
Valine	95	96	92	91	100	88	88
Isoleucine	96	91	93	91	98	87	88
Leucine	97	99	97	96	100	88	88
Tyrosine	98	99	97	98	100	90	89
Phenylalanine	98	99	97	97	98	89	87
Histidine	97	100	93	95	100	90	89
Lysine	97	97	98	97	97	90	90
Arginine	97	98			99	87	88
Cysteine	90					89	89
Methionine	97	96			97	89	92
Proline	97	98	95	94	97	89	88
Tryptophan	97					84	85
Protein	95		92	94		89	88

	Cheese whey (CP>27.5%) <sup>5</sup>	Chickpea curry <sup>8</sup>	Coconut (extracted) <sup>5</sup>	Corn <sup>1,2,3,4,5,19</sup>	Corn <sup>14</sup>	Corn flour <sup>5</sup>	Corn flour <sup>8</sup>
	Human <sup>P,PF</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF,WM</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	89	70	55	81	92	82	90
Threonine	88	80	55	78	87	76	84
Serine	88	84	55	86	93	87	93
Glutamic acid	88	89	55	86	98	91	96
Glycine	88	53	55	77	63	78	75
Alanine	88	84	55	83	96	89	95
Valine	89	83	54	84	93	85	94
Isoleucine	88	82	56	84	96	86	95
Leucine	88	88	55	88	97	92	98
Tyrosine	88	88	56	85	96	91	95
Phenylalanine	88	90	55	86	96	84	97
Histidine	87	87	53	84	87	84	94
Lysine	91	90	54	75	97	76	92
Arginine	87	91	55	86	93	84	91
Cysteine	91	72	56	82		79	85
Methionine	89	94	56	86		92	100
Proline	88	92	54	87	82	73	94
Tryptophan	85	72	57	76		78	84
Protein	88		54	81		82	

	Corn germ meal <sup>1,4</sup>	Dosa <sup>8</sup>	Egg (raw) <sup>22</sup>	Egg (cooked) <sup>22</sup>	Elderly formula <sup>9</sup>	Evaporated milk <sup>9</sup>	Evaporated milk <sup>14</sup>
	Human <sup>P,PF,WM</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>D,I</sup>	Human <sup>D,I</sup>	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>
Aspartic acid	57	90			88	91	84
Threonine	69	90			88	96	89
Serine	73	91			86	89	81
Glutamic acid	73	90			91	93	92
Glycine	60	72			64	76	69
Alanine	69	90			94	98	91
Valine	72	92			93	95	91
Isoleucine	75	91			92	92	88
Leucine	79	95			97	98	96
Tyrosine	76	90			98	100	98
Phenylalanine	81	93			98	100	99
Histidine	78	94			95	95	87
Lysine	60	95			97	97	97
Arginine	83	92			94	94	92
Cysteine	64	82					
Methionine	79	92					
Proline		97					95
Tryptophan	66						
Protein	66		51	91			

	Field beans (cooked) <sup>21</sup>	Field beans (cooked) <sup>21</sup>	Fish (chinese) <sup>21</sup>	Fish (chinese) <sup>21</sup>	Jack beans (cooked) <sup>21</sup>	Jack beans (cooked) <sup>21</sup>	Kidney beans (cooked) <sup>20</sup>
	Human <sup>P,PF</sup>	Human <sup>P,EHC</sup>	Human <sup>P,PF</sup>	Human <sup>P,EHC</sup>	Human <sup>P,PF</sup>	Human <sup>P,EHC</sup>	Human <sup>P,PF</sup>
Aspartic acid	83	85	92	92	70	72	87
Threonine	78	82	94	95	71	74	74
Serine	83	87	93	96	66	80	75
Glutamic acid	86	90	93	94	71	74	85
Glycine	76	79	87	89	64	66	100
Alanine	70	76	90	92	52	57	80
Valine	74	76	89	90	86	99	62
Isoleucine	65	71	92	93	62	63	70
Leucine	77	82	90	91	60	62	69
Tyrosine							64
Phenylalanine	65	66	83	83	70	71	72
Histidine	57	58	84	85	56	57	63
Lysine	81	82	92	93	72	74	83
Arginine	91	92	81	81	75	76	93
Cysteine							
Methionine					68	86	
Proline			85	89	100	100	100
Tryptophan							
Protein	73	77	90	91	58	61	74

	Kidney beans (cooked) <sup>20</sup>	Kiwifruit (Hayward) <sup>17</sup>	Kiwifruit (Hayward) <sup>17</sup>	Linseed <sup>5</sup>	Linseed (extracted) <sup>5</sup>	Idli <sup>8</sup>	Infant formula A <sup>9</sup>
	Human <sup>P,EHC</sup>	Human <sup>P,EHC</sup>	Human <sup>P,EHC</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	95			75	73	85	81
Threonine	81			80	78	85	83
Serine	86			74	73	86	81
Glutamic acid	100			75	73	87	87
Glycine	65			75	73	55	43
Alanine	78			75	73	85	72
Valine	65			75	73	89	86
Isoleucine	75			74	73	87	84
Leucine	70			74	73	93	92
Tyrosine	64			75	73	88	93
Phenylalanine	74			74	73	90	92
Histidine	66			74	72	89	89
Lysine	84			82	79	91	91
Arginine	86			75	73	89	82
Cysteine				85	84	42	
Methionine				86	83	54	
Proline	80			75	73	59	
Tryptophan				84	83		
Protein	78	55	62	74	73		

	Infant formula B <sup>9</sup>	Infant formula C <sup>9</sup>	Infant formula (cow milk based) <sup>15</sup>	Infant formula (goat milk based) <sup>15</sup>	Kidney beans (cooked) <sup>14</sup>	Lactalbumin <sup>10</sup>	Lactic casein <sup>10</sup>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC</sup>	Rat <sup>D,EHC</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	85	82	99	97	77	90	96
Threonine	87	84	91	88	72	95	94
Serine	82	76	98	95	80	96	90
Glutamic acid	88	86	100	99	81	95	93
Glycine	56	33	82	56	48	92	86
Alanine	80	77	96	91	74	95	97
Valine	88	86	98	98	75	96	97
Isoleucine	87	83	99	98	81	95	95
Leucine	92	92	99	99	82	96	99
Tyrosine	92	93	100	98	79	97	100
Phenylalanine	91	93	98	97	82	97	100
Histidine	89	90	91	90	81	89	96
Lysine	92	93	95	96	94	95	99
Arginine	83	84	98	95	77	97	98
Cysteine			97	92	69	96	99
Methionine			100	100	88	99	100
Proline			95	96			
Tryptophan			93	89	77		
Protein			93	92	80		

	Lentil dal <sup>8</sup>	Maize (corn) bran <sup>5</sup>	Maize roti <sup>8</sup>	Milk <sup>25</sup>	Milk protein concentrate <sup>1</sup>	Milk protein concentrate <sup>14</sup>	Milk protein isolate <sup>10</sup>
	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>D,I</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	91	70	86		91	96	96
Threonine	91	66	82		84	95	93
Serine	95	78	90		85	85	87
Glutamic acid	96	77	93		91	93	92
Glycine	67	68	59		90	68	91
Alanine	93	78	93		86	96	98
Valine	95	77	90		89	95	97
Isoleucine	95	77	91		90	92	95
Leucine	98	81	96		92	98	99
Tyrosine	96	80	94		87	99	100
Phenylalanine	97	80	95		91	100	100
Histidine	96	80	88		94	99	92
Lysine	97	68	89		93	99	98
Arginine	97	88	89		93	95	100
Cysteine	88	69	77		84	96	98
Methionine	100	83	96		87	95	100
Proline	97	75	89				
Tryptophan	82	69	77		89	97	
Protein		72		96	89	92	



	Meat protein hydrolysate <sup>14</sup>	Mung beans (cooked) <sup>21</sup>	Mung beans (cooked) <sup>21</sup>	Mung dal <sup>8</sup>	Naan <sup>8</sup>	Oats <sup>1,2,4,5</sup>	Oats (grain peeled) <sup>5</sup>
	Rat <sup>D,EHC</sup>	Human <sup>P,PF</sup>	Human <sup>P,EHC</sup>	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>P,PF,WM</sup>	Human <sup>P,PF</sup>
Aspartic acid	92	87	90	80	75	73	80
Threonine	93	84	89	83	87	70	78
Serine	92	92	96	86	91	75	84
Glutamic acid	93	90	93	89	96	83	88
Glycine	82	93	95	54	61	71	81
Alanine	96	75	81	82	90	70	79
Valine	96	80	82	88	89	79	87
Isoleucine	97	75	76	85	93	80	88
Leucine	98	80	82	94	97	82	88
Tyrosine	96			87	90	81	89
Phenylalanine	97	84	85	93	95	84	91
Histidine	91	82	84	85	93	85	92
Lysine	97	92	94	94	89	76	83
Arginine	98	89	90	87	92	88	95
Cysteine	79			70		72	80
Methionine	98	77	79	93		82	89
Proline	89	80	81	85		77	91
Tryptophan				81		77	79
Protein		82	83			74	79

	Oats (decorticated) <sup>1</sup>	Peas <sup>1,2,3,4,5</sup>	Peas <sup>11</sup>	Peas (cooked) <sup>21</sup>	Peas (cooked) <sup>21</sup>	Peas (cooked, 100°C 4 min) <sup>14</sup>	Peas (cooked, 110°C 15 min) <sup>11</sup>
	Human <sup>P,PF</sup>	Human <sup>P,PF,WM</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>P,PF</sup>	Human <sup>P,EHC</sup>	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>
Aspartic acid	81	80	74	85	86	91	79
Threonine	78	74	67	86	91	89	76
Serine	83	78	73	85	87	93	80
Glutamic acid	85	83	80	93	96	95	85
Glycine	78	77	64	90	93	74	74
Alanine	75	73	76	80	86	92	82
Valine	79	74	72	85	89	91	80
Isoleucine	81	77	74	73	74	93	81
Leucine	81	77	75	81	82	93	81
Tyrosine	83	78	69			93	74
Phenylalanine	81	76	73	70	72	94	79
Histidine	81	81	69	72	74	95	77
Lysine	77	81	88	87	90	97	90
Arginine	84	87	84	94	96	94	87
Cysteine	83	72				87	
Methionine	83	76		82	95	99	
Proline		81	61	100	100		75
Tryptophan	80	69				89	
Protein	77	78		72	74	88	

	Peas (cooked, 135°C 15 min) <sup>11</sup>	Peas (extruded) <sup>1</sup>	Pea globulins <sup>28Q</sup>	Pea globulins+ albumins <sup>28Q</sup>	Pea Flour <sup>12</sup>	Pea protein concentrate <sup>14</sup>	Peanut Roasted) <sup>14</sup>
	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF</sup>	Human <sup>D,I</sup>	Human <sup>D,I</sup>	Human <sup>D,I</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	83	90				97	92
Threonine	82	89				97	89
Serine	85	91				100	94
Glutamic acid	89	93				99	95
Glycine	75	87				86	70
Alanine	88	85				98	94
Valine	86	87				97	93
Isoleucine	88	90				99	95
Leucine	89	91				98	95
Tyrosine	84	93				98	95
Phenylalanine	86	92				99	97
Histidine	83	93				99	97
Lysine	93	92				99	94
Arginine	92	93				98	95
Cysteine		86				98	93
Methionine		84				100	100
Proline	74	91					
Tryptophan		87				99	84
Protein		89	94	90	90	97	91

	Potato crisps <sup>5</sup>	Potato fries (fat 4-12%) <sup>5</sup>	Potato fries (fat 12-18%) <sup>5</sup>	Potato fries (fat >18%) <sup>5</sup>	Potato peelings (steamed, starch <35%) <sup>5</sup>	Potato peelings (steamed, starch 35-47.5%) <sup>5</sup>	Potato peelings (steamed, starch 47.5-60%) <sup>5</sup>
	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>
Aspartic acid	47	51	51	51	58	58	58
Threonine	47	52	52	48	62	62	63
Serine	44	51	51	49	58	56	59
Glutamic acid	45	50	51	51	57	57	57
Glycine	47	49	53	51	57	57	58
Alanine	48	53	52	52	57	58	58
Valine	47	52	52	51	58	58	57
Isoleucine	44	51	51	53	58	58	59
Leucine	47	52	50	50	58	58	57
Tyrosine	47	49	53	51	57	56	57
Phenylalanine	47	52	52	51	57	58	58
Histidine	42	47	47	51	57	61	58
Lysine	48	51	52	52	63	64	62
Arginine	49	54	51	49	57	58	58
Cysteine	47	52	52	52	49	50	47
Methionine	41	47	47	52	68	70	64
Proline	44	53	51	49	57	57	58
Tryptophan	47	47	47	47	51	47	47
Protein	47	52	51	50	57	58	57

	Potato peelings (steamed, starch >60%) <sup>5</sup>	Potato protein (ash <1%) <sup>5</sup>	Potato protein (ash >1%) <sup>5</sup>	Potato protein concentrate <sup>1</sup>	Potato protein concentrate <sup>4</sup>	Potatoes (dehydrated) <sup>5</sup>	Potato (sweet, dehydrated) <sup>5</sup>
	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,WM</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>
Aspartic acid	58	81	81	89		57	51
Threonine	61	84	84	89	84	62	51
Serine	56	85	85	87		56	47
Glutamic acid	57	86	86	85		56	51
Glycine	55	80	80	82		54	50
Alanine	58	85	85	84		58	50
Valine	58	86	86	87	86	58	47
Isoleucine	56	88	88	87	87	56	55
Leucine	59	90	90	90	90	56	51
Tyrosine	58	90	89	87		58	51
Phenylalanine	56	89	89	90	89	57	53
Histidine	56	85	85	87	85	53	47
Lysine	63	88	88	87	89	62	53
Arginine	58	91	91	91	92	57	53
Cysteine	51	74	74	76		54	47
Methionine	62	89	90	90	90	66	64
Proline	56	93	93			56	47
Tryptophan	52	78	78	73	78	47	47
Protein	58	89	89	85	89	56	52

	Rajmah <sup>8</sup>	Rapeseed protein isolate <sup>23</sup>	Rapeseed protein isolate <sup>23</sup>	Refined wheat flour <sup>8</sup>	Rice <sup>6</sup>	Rice <sup>8</sup>	Rice (cooked) <sup>8,14</sup>	Rice bran <sup>1,2,4</sup>
	Rat <sup>D,EHC, RL</sup>	Human <sup>D,I</sup>	Human <sup>P,I</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>P,I,HDP</sup>	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF,WM</sup>
Aspartic acid	50			80	85	83	76	69
Threonine	61			86	82	81	71	64
Serine	64			96	93	88	75	70
Glutamic acid	74			98	91	84	66	79
Glycine	16			78	86	78	50	64
Alanine	66			91	82	90	75	71
Valine	68			94	88	92	80	66
Isoleucine	72			96	75	90	78	67
Leucine	76			99	81	92	79	67
Tyrosine	74			95	79	89	77	79
Phenylalanine	77			97	86	90	80	68
Histidine	68			97	99	90	85	77
Lysine	81			93	92	97	95	69
Arginine	79			88	90	89	85	82
Cysteine	33			92	97	85	57	65
Methionine	82			99	86	89	57	73
Proline	72			99	99	91	75	61
Tryptophan	50			83	89		86	72
Protein		87	90		90		73	65

	Rice protein concentrate <sup>14</sup>	Rye <sup>1,2,4,5</sup>	Sambar <sup>8</sup>	Sesame meal <sup>2,4</sup>	Single cell protein <sup>21</sup>	Single cell protein <sup>21</sup>	Skim milk powder <sup>1,2,4,5</sup>	Skim milk powder <sup>10,11</sup>
	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF, WM</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF, WM</sup>	Human <sup>P, PF</sup>	Human <sup>P,EHC</sup>	Human <sup>P,PF, WM</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	79	75	77		52	54	92	93
Threonine	82	69	86	83	51	53	90	93
Serine	84	76	88	87	57	59	78	84
Glutamic acid	81	87	85		59	62	86	91
Glycine	76	70	54	84	53	54	87	76
Alanine	86	66	86		49	52	88	97
Valine	84	73	90	87	55	56	88	93
Isoleucine	84	75	88	87	56	57	87	89
Leucine	83	76	95	88	58	59	95	97
Tyrosine	83	75	91	90			95	99
Phenylalanine	84	80	82	90	50	51	97	99
Histidine	86	77	91	87	63	64	94	93
Lysine	88	70	93	82	72	73	96	96
Arginine	90	78	91	89	73	74	95	99
Cysteine	71	82	79	91			85	93
Methionine	66	79	98	86	86	89	96	96
Proline		89	94		62	64	96	98
Tryptophan	85	74	70	82			89	
Protein	80	75		86	66	68	88	

	Skim milk powder (heated, 151°C, 1 min) <sup>11</sup>	skim milk powder (lactose-hydrolysed) <sup>9</sup>	Sodium caseinate <sup>7</sup>	Sodium caseinate <sup>10</sup>	Sorghum <sup>1,4,5</sup>	Soyabeans (cooked) <sup>21</sup>
	Rat <sup>D,EHC, RL</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>D,PF</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF,WM</sup>	Human <sup>P,PF</sup>
Aspartic acid	94	96	98	92	81	84
Threonine	94	98	100	93	84	81
Serine	85	89	99	86	84	86
Glutamic acid	92	94	99	89	88	87
Glycine	72	87	100	86	71	81
Alanine	98	100	100	96	81	73
Valine	94	96	100	95	86	78
Isoleucine	91	93	100	91	87	73
Leucine	98	99	100	98	88	80
Tyrosine	99	100	100	100	85	
Phenylalanine	100	100	100	100	89	80
Histidine	95	99	99	93	81	76
Lysine	88	99	100	98	79	80
Arginine	99	98	100	98	85	90
Cysteine			100	93	78	
Methionine			100	100	87	72
Proline	97		99		69	71
Tryptophan					87	
Protein			100		83	68



	Soya protein concentrate <sup>7</sup>	Soya protein concentrate <sup>10</sup>	Soya protein isolate <sup>7</sup>	Soya protein isolate <sup>10,14</sup>	Soya protein isolate <sup>27,0</sup>	Sports formula <sup>9</sup>
	Human <sup>D,PF</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>D,PF</sup>	Rat <sup>D,EHC, RL</sup>	Human <sup>D,I</sup>	Rat <sup>D,EHC, RL</sup>
Aspartic acid	97	94	97	95		90
Threonine	97	94	98	94		92
Serine	97	98	98	98		82
Glutamic acid	98	98	99	98		89
Glycine	96	91	95	85		65
Alanine	97	95	97	95		95
Valine	97	95	97	96		91
Isoleucine	97	96	97	97		89
Leucine	97	96	97	96		96
Tyrosine	99	98	99	98		99
Phenylalanine	97	97	98	97		97
Histidine	97	91	99	97		96
Lysine	98	97	99	99		98
Arginine	100	100	99	99		92
Cysteine	91	87	97	95		
Methionine	96	95	98	98		
Proline	96		98			
Tryptophan				95		
Protein	97		98	95	92	

	Sports High-protein supplement <sup>9</sup>	Sugarbeet (molasses) <sup>5</sup>	Sugarcane molasses (sugar <47.5%) <sup>5</sup>	Sugarcane molasses (sugar >47.5%) <sup>5</sup>	Sunflowerseed (solvent extracted fibre <16%) <sup>5</sup>	Sunflowerseed (solvent extracted fibre 16-20%) <sup>5</sup>
	Rat <sup>D,EHC, RL</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>
Aspartic acid	95	93	94	93	79	79
Threonine	96	99	99	99	78	78
Serine	92	93	99	99	80	80
Glutamic acid	94	93	93	92	87	86
Glycine	70	93	99	82	70	70
Alanine	99	94	92	92	76	76
Valine	95	89	99	99	79	79
Isoleucine	94	93	99	99	80	81
Leucine	98	93	78	78	79	80
Tyrosine	100	92	99	99	80	81
Phenylalanine	99	99	99	99	81	80
Histidine	98	99	99	99	79	80
Lysine	100	99	99	99	77	77
Arginine	95	99	99	99	91	91
Cysteine		99	99	64	75	76
Methionine		99	99	99	87	87
Proline		87	99	99	85	85
Tryptophan		99	99	99	81	79
Protein		94	94	94	78	78

	Sunflowerseed (solvent extracted fibre 20-24%) <sup>5</sup>	Sunflowerseed (solvent extracted fibre >24%) <sup>5</sup>	Sunflowerseed (expeller dehulled fibre<21%) <sup>5</sup>	Sunflowerseed (expeller dehulled fibre 21-32.5%) <sup>5</sup>	Triticale <sup>1,2,4,5</sup>
	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF,WM</sup>
Aspartic acid	79	79	79	79	83
Threonine	77	78	78	78	78
Serine	80	80	80	79	87
Glutamic acid	87	86	87	86	93
Glycine	71	70	71	71	82
Alanine	76	76	75	76	79
Valine	79	79	79	79	85
Isoleucine	81	81	81	81	86
Leucine	79	79	79	79	86
Tyrosine	82	81	82	80	87
Phenylalanine	80	81	81	80	87
Histidine	80	81	80	80	86
Lysine	77	77	77	77	82
Arginine	90	91	91	91	88
Cysteine	75	74	75	75	88
Methionine	85	85	87	86	88
Proline	84	85	84	84	92
Tryptophan	80	83	81	80	82
Protein	79	79	78	78	84

	UHT Milk <sup>9</sup>	Weight gain formula <sup>9</sup>	Wheat <sup>1,2,3,4,5,19</sup>	Wheat <sup>14</sup>	Wheat flour biscuit <sup>26</sup>	Wheat bran <sup>1,2,3,4,5</sup>	Wheat flour (fibre <3.5%) <sup>5</sup>
	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>P,PF,WM</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>D,I</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>
Aspartic acid	98	93	83	81		67	87
Threonine	99	95	83	86		67	86
Serine	93	88	88	90		76	93
Glutamic acid	95	92	94	96		84	94
Glycine	84	82	85	73		68	88
Alanine	100	96	80	87		61	87
Valine	97	93	85	89		72	90
Isoleucine	96	92	88	92		73	90
Leucine	100	97	88	92		76	91
Tyrosine	100	99	87	91		77	92
Phenylalanine	100	98	89	93		75	89
Histidine	100	97	88	86		80	91
Lysine	100	99	80	94		70	87
Arginine	99	95	87	88		86	92
Cysteine			88			74	86
Methionine			88			77	90
Proline			96	95		82	93
Tryptophan			88			71	89
Protein			87		90	71	89

	Wheat flour (fibre 3.5- 5.5%) <sup>5</sup>	Wheat flour <sup>8</sup>	Wheat flour <sup>8</sup>	Wheat germ <sup>1,5</sup>	Wheat roti <sup>8</sup>	Wheat gluten <sup>1,4,5</sup>	Wheat middlings (7% CF) <sup>4</sup>
	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>P,PF,WM</sup>	Human <sup>P,WM</sup>
Aspartic acid	82	87	88	81	84	84	
Threonine	80	89	92	81	88	91	71
Serine	86	94	98	84	93	94	
Glutamic acid	91	96	99	90	97	98	
Glycine	82	92	90	81	71	91	
Alanine	80	84	93	81	89	86	
Valine	84	90	96	83	91	95	79
Isoleucine	84	92	98	84	95	96	77
Leucine	86	94	99	85	98	96	78
Tyrosine	87	94	97	86	91	95	
Phenylalanine	84	95	98	86	95	96	82
Histidine	86	95	99	87	93	99	82
Lysine	82	89	94	83	91	86	76
Arginine	90	95	93	89	89	94	90
Cysteine	83	93	94	82	74	96	
Methionine	86	93	100	87	89	94	80
Proline	91	96	100	84	85	96	
Tryptophan	84	91	91	82		90	79
Protein	83	92		83		95	75

	Whey acid dehydrated <sup>1</sup>	Whey powder <sup>5</sup>	Whey powder (low lactose, ash<21%) <sup>5</sup>	Whey powder (low lactose, ash>21%) <sup>5</sup>	Whey powder part. delact. <sup>4</sup>
	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,PF</sup>	Human <sup>P,WM</sup>
Aspartic acid	72	88	91	90	
Threonine	68	89	91	91	92
Serine	58	88	91	90	
Glutamic acid	79	88	90	90	
Glycine	51	87	91	89	
Alanine	53	88	91	90	
Valine	66	88	91	91	87
Isoleucine	78	89	90	90	87
Leucine	75	88	91	90	89
Tyrosine	76	89	90	91	
Phenylalanine	80	87	90	90	96
Histidine	78	90	90	91	92
Lysine	81	91	93	92	92
Arginine	48	89	90	91	96
Cysteine	63	91	92	94	
Methionine	72	89	93	92	90
Proline	76	87	90	90	
Tryptophan	77	87	90	89	90
Protein	68	89	91	91	91

	Whey Protein concentrate <sup>7</sup>	Whey protein concentrate <sup>9,10,14</sup>	Whey protein hydrolysate <sup>10</sup>	Whey protein isolate <sup>14</sup>	Whole milk powder <sup>1,5</sup>	Whole milk powder <sup>9,14</sup>
	Human <sup>D,PF</sup>	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>	Rat <sup>D,EHC,RL</sup>	Human <sup>P,PF</sup>	Rat <sup>D,EHC,RL</sup>
Aspartic acid	98	97	86	99	92	95
Threonine	93	94	93	100	92	95
Serine	93	95	94	100	78	87
Glutamic acid	98	97	86	99	88	94
Glycine	98	89	92	97	93	72
Alanine	97	98	95	100	88	97
Valine	98	97	96	100	89	95
Isoleucine	99	98	96	100	87	92
Leucine	99	99	96	100	95	98
Tyrosine	99	100	97	100	96	99
Phenylalanine	99	100	97	100	96	100
Histidine	89	96	90	100	95	97
Lysine	97	99	94	100	91	99
Arginine	99	96	97	98	91	98
Cysteine	99	100	94	100	92	
Methionine	99	99	80	100	95	
Proline	95				98	97
Tryptophan		100		100	93	
Protein	97	95		99	89	

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