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Digestibility of protein and amino acids in selected foods as determined by a rat balance method

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Abstract. Values (%) for true digestibility of crude protein and individual amino acids in 20 selected foods were determined by the rat balance (fecal) method. The products were fed as the sole source of protein in diets containing 8% crude protein ($N \times 6.25$). Lowest true protein digestibility values (79–84) were obtained for pinto beans, kidney beans and lentils; intermediate values (89–92) were obtained for chick peas, beef stew, skim milk (over heated), rolled oats, whole wheat cereal, and pea protein concentrate; and highest values (94–100) were obtained for sausage, macaroni-cheese, rice-wheat gluten cereal, skim milk, tuna, soy isolate, peanut butter, chicken frankfurters, beef salami, casein and casein + methionine. In animal foods, peanut butter and soy isolate, the differences between true digestibility of crude protein and most individual amino acids were less than 5%. However, the values for true digestibility of methionine and cystine were up to 44% lower than those of crude protein in pinto beans, kidney beans, lentils, chick peas and pea concentrate. In these legumes, digestibility of crude protein was not a good predictor of digestibility of the limiting amino acids.

Introduction

There is continuing interest in the development of accurate, precise, rapid and easily understood methods for evaluating protein quality of foods for regulatory purposes, international trade and consumer information. Methods for protein quality assessment were discussed at the third session of the Codex Committee on vegetable proteins which concerned international standards for vegetable protein products [1]. The use of an amino acid score adjusted to allow (when needed) for incomplete digestibility of protein and for unavailability of amino acids, was considered to be the preferred approach for assessing protein quality of vegetable protein products [1]. It was also noted that information on digestibility of protein and amino acids in various food products is needed to determine the nature of digestibility adjustment(s) to amino acid score(s). The desirability of using relative net protein ratio (RNPR) as a back up method was also suggested at the Codex meeting [1].

Twenty selected foods were studied to obtain more information on (a) digestibility of total nitrogen (crude protein) and individual amino acids, and (b) protein quality indices based on rat growth such as protein efficiency ratio (PER), net protein ratio (NPR), relative PER (RPER) and RNPR. Data on digestibility of crude protein and amino acids are presented in this manuscript. Data on PER, NPR, RPER, and RNPR are presented in an accompanying manuscript [2].

Materials and methods

Seventeen protein sources (ANRC casein; non-fat dried skim milk; soy protein isolate; instant whole wheat cereal; pinto beans, canned; beef salami; tuna, canned; macaroni and cheese, canned; rolled oats, instant; peanut butter, smooth; pea protein concentrate; chick peas, canned; beef stew with potatoes, canned; chicken frankfurters; rice-wheat gluten cereal; non-fat dried skim milk, overheated; and breakfast sausage) were supplied by USDA. These samples were dried and finely ground before being distributed [3]. The remaining three protein sources (ANRC casein with added L-methionine, 0.2% of the diet; kidney beans, canned-IGA Canada Ltd., Toronto, Ont.; and lentils, canned-Unico Inc., Toronto, Ont.) were prepared in our laboratory. The samples of beef salami and chicken frankfurters were partially defatted with anhydrous ether while the samples of kidney beans and lentils were freeze dried and finely ground (35 mesh) before conducting analyses.

Apparent and true digestibility of crude protein ($N \times 6.25$) and individual amino acids were determined by the rat balance (fecal) method [4]. The basal (or nitrogen-free) diet contained in g/kg diet: corn oil (Mazola, Canada Starch Co., Toronto, Ont.), 100; AIN mineral mixture 76 (Nutritional Biochemicals, Cleveland, OH), 35; AIN vitamin mixture 76, 10; choline bitartarate (Sigma Chemical Company, St. Louis, MO), 2; cellulose (Teklad Test Diets, Madison, WI), 50; chromic oxide (Fisher Scientific Company, Fair Lawn, NJ), 5; cornstarch (Canada Starch Co., Toronto, Ont.), 978. Each of the 20 protein sources was added to the basal diet at the expense of cornstarch to provide 8% dietary crude protein ($N \times 6.25$). The levels of corn oil and cellulose were varied to make the diets equal in fat content and amount of insoluble fiber [5].

Male weanling CD Sprague Dawley rats (50 ± 5 g, Charles River Canada Inc., St. Constant, Quebec) (10 per diet) were fed the 20 protein diets or a nitrogen-free diet for 28 days preceded by an adaptation period of 2 days. A randomized complete block design, using 10 blocks of 21 rats

was used. Blocking was on the basis of initial body weight, so that rats in the same block had essentially the same initial weight. The rats were housed in individual stainless steel, screen-bottom cages as reported previously [4]. Food and water were provided *ad libitum* for 28 days (14 days for the N-free diet), and records of weekly food consumption and weight gains were recorded. In the last week of the test (2nd week in the case of N-free diet and 4th week in the case of protein diets), total feces from individual rats (5 per diet) were collected, freeze dried, weighed and ground. The dried feces and diet samples were analysed for nitrogen by using a Kjeltec Auto 1030 Analyzer (Tecator AB, Hoganas, Sweden). The diet samples were analysed for moisture, and protein sources were analyzed for crude fat by the AOAC procedures [7]. Protein ($N \times 6.25$) intake and output data were determined for each rat. These data permitted the calculation of 5 individual protein digestibility values for each diet.

Protein sources and pooled feces samles were hydrolysed in duplicate with 6 *N* HCl for the determination all amino acids except methionine, cystine and/or cysteine, and tryptophan [8]. Performic acid + 6 *N* HCl hydrolysis was used to quantitatively convert methionine to methionine sulfone and cystine and/or cysteine to cysteic acid [9]. The 4.2 *N* NaOH hydrolysis was used for the determination of tryptophan [10]. AMino acid(s) in each hydrolysate were determined by ion-exchange chromatography using a Beckman 121MB analyser (Beckman Instruments, Inc., Palo Alto, CA) which was calibrated daily with amino acid standards.

Apparent and true digestibility of crude protein and amino acids were calculated according to the following formulas [8]:

Apparent protein digestibility = [PI − FP]/PI × 100,

True protein digestibility = [PI − (FP − MFP)]/PI × 100,

where PI = protein intake, FP = fecal protein, MFP = metabolic fecal protein.

Apparent amino acid digestibility = [AAI − FAA]/AAI × 100,

True amino acid digestibility = [AAI − (FAA − MFAA)]/AAI × 100,

where AAI = amino acid intake, FAA = fecal amino acid,

MFAA = metabolic fecal amino acid. The protein and amino acids in the feces of rats fed the nitrogen-free diet provided the estimates of metabolic origin.

Results

In most food products, values for true protein digestibility were 9–10 units higher than the corresponding values for apparent protein digestibility

Table 1. Values (± SE) for apparent and trude crude protein digestibilities

Food products	Crude protein digestibilities (%)	
	Apparent	True
Animal		
Casein + Met	92 ± 0.5	100 ± 0.2
Beef salami	90 ± 0.2	99 ± 0.3
Casein	90 ± 0.6	99 ± 0.6
Skim milk	86 ± 0.9	95 ± 0.9
Tuna	87 ± 0.4	97 ± 0.4
Chicken franks	92 ± 0.2	99 ± 0.2
Sausage	84 ± 0.8	94 ± 0.8
Skim milk (heated)	81 ± 0.4	90 ± 0.5
Vegetable		
Peanut butter	89 ± 0.4	98 ± 0.4
Rolled oats	82 ± 0.7	91 ± 0.6
Soy isolate	88 ± 0.6	98 ± 0.6
Chick peas	79 ± 0.3	89 ± 0.4
Pea concentrate	83 ± 1.4	92 ± 1.4
Kidney beans	72 ± 1.2	81 ± 1.2
Wheat cereal	81 ± 1.2	91 ± 1.1
Pinto beans	69 ± 1.5	79 ± 1.5
Lentils	75 ± 1.5	84 ± 1.4
Rice-wheat gluten	85 ± 0.8	95 ± 0.9
Animal–vegetable mixtures		
Macaroni-cheese	84 ± 0.9	94 ± 0.9
Beef stew	80 ± 0.6	89 ± 0.6

(Table 1). Lowest true protein digestibility values (79–84%) were obtained for pinto beans, kidney beans and lentils; intermediate values (89–92%) were obtained for chick peas, beef stew, skim milk (heated), rolled oats, whole wheat cereal, and pea protein concentrate; and highest values (94–100%) were obtained for casein + methionine, beef salami, casein, chicken frankfurters, peanut butter, soy protein isolate, tuna, skim milk, sausage, rice-wheat gluten cereal, and macaroni-cheese (Table 1).

Values for true digestibility of crude protein and individual amino acids in animal food products, legume-based foods and cereal-based foods are compared in Tables 2, 3 and 4, respectively. In animal foods, the differences between true digestibility of crude protein and most individual amino acids were small (less than 5 percentage units) (Table 2). In macaroni-cheese, true digestibility of threonine was 11 percentage units lower than the digestibility of protein, while in beef stew, true digestibility of cystine was 15 percentage units lower than true digestibility of protein (Table 2).

In all legume-based foods, except peanut butter and soy protein isolate,

Table 2. Values (%) for true digestibility of crude protein and amino acids in animal food products and animal–vegetable mixtures*.

Product	Casein	Beef salami	Skim milk	Tuna	Chicken franks	Sausage	Skim milk (heated)	Macaroni cheese	Beef stew
Protein	99	99	95	97	99	94	90	94	89
Arginine	100	99	95	98	100	97	93	93	93
Histinde	100	99	97	98	97	93	94	94	92
Isoleucine	100	100	94	99	100	90	91	94	90
Leucine	100	100	97	98	100	94	95	96	91
Lysine	100	99	96	97	99	93	88	92	90
Methionine	99	99	92	95	100	89	88	90	83
Cystine	100	100	94	96	100	87	90	88	74
Phenylalanine	100	100	99	99	100	94	98	97	90
Tyrosine	100	100	98	98	100	93	96	94	87
Threonine	100	100	95	98	100	93	93	83	86
Tryptophan	100	100	98	97	99	93	94	94	88
Valine	99	100	94	97	100	92	93	91	86
Alanine	99	99	91	96	100	93	86	88	87
Aspartic acid	98	99	94	96	100	93	91	91	87
Glutamic acid	98	99	94	97	100	95	92	96	92
Glycine	100	97	92	97	99	97	86	90	88
Proline	100	98	98	97	100	97	97	97	91
Serine	100	100	91	98	99	95	86	92	88

* Values for true digestibility were taken from Table 1. Values for true digestibility of amino acids in casein + methionine diet were 99–100%.
† Standard errors (as estimated from the analysis of variance) of means of true digestibility of all amino acids were 1.0.

Table 3. Values (%) for true digestibility of crude protein and amino acids in legume-based foods*.

Product	Peanut butter	Soy isolate	Pinto beans	Kidney beans	Lentils	Chick peas	Pea concentrate
Protein	98	98	79	81	84	89	92
Arginine	100	99	85	85	89	96	96
Histidine	99	98	82	84	83	92	94
Isoleucine	95	99	74	80	84	87	93
Leucine	99	96	78	82	84	88	93
Lysine	96	98	78	80	84	89	92
Methionine	94	94	45	44	41	74	73
Cystine	100	94	56	0	40	88	87
Phenylalanine	100	98	81	86	87	92	94
Tyrosine	99	98	67	76	79	87	96
Threonine	97	96	72	74	77	84	90
Tryptophan	99	98	70	76	73	82	91
Valine	96	96	71	76	81	83	89
Alanine	99	96	67	71	76	83	88
Aspartic acid	99	98	86	83	85	89	90
Glutamic acid	98	99	84	85	87	94	94
Glycine	99	97	73	74	76	87	91
Proline	98	98	86	84	75	93	94
Serline	99	99	82	84	84	92	93

* Values for true digestibility of protein were taken from Table 1.

† Standard errors (as estimated from the analysis of variance) of means of true digestibility of all amino acids were 1.0.

Table 4. Values (%) for true digestibility of crude protein and amino acids in cereal-based foods*.

Product	Rolled oats	Wheat cereal	Rice–Wheat gluten
Protein	91	91	95
Arginine	93	90	97
Histidine	90	92	96
Isoleucine	90	91	99
Leucine	92	91	96
Lysine	86	80	89
Methionine	85	84	88
Cystine	94	87	91
Phenylalanine	90	93	97
Tyrosine	84	87	95
Threonine	86	83	94
Tryptophan	93	89	100
Valine	89	87	95
Alanine	89	83	94
Aspartic acid	89	82	87
Glutamic acid	95	96	98
Glycine	91	87	95
Proline	95	96	98
Serine	93	91	95

* Values for true digestibility of protein were taken from Table 1.

† Standard errors (as estimated from the analysis of variance) of means of true digestibility of all amino acids were 1.0.

wide differences existed between true digestibility of protein and of individual amino acids (Table 3). In general, values for true digestibility of arginine, glutamic acid and proline were higher, while the values for limiting amino acids were lower than the values for protein. In pinto beans, kidney beans and lentils, values for true digestibility of methionine (41–45%) and cystine (0–56%), were considerably lower than the values for protein (Table 3). Similarly, in chick peas and pea protein concentrate, values for true digestibility of methionine were up to 19 percentage units lower than the values for protein. In pinto beans, kidney beans, lentils and chick peas, values for true digestibility of tryptophan were also lower (up to 11 percentage units) than those for protein (Table 3).

In cereal-based foods, values for true digestibility of lysine, threonine and methionine were up to 11, 8 and 7 percentage units lower than the values for protein, respectivley (Table 4).

Discussion

Apparent digestibility of crude protein varies with dietary protein concentration but true digestibility is independent of protein level [8, 11].

Therefore, the use of true rather than apparent digestibility of protein (and amino acids) would be more accurate in comparing different foods and in diet formulations [8].

The low true protein digestibility values for pinto beans, kidney beans and lentils (79–84%) obtained in this study were comparable to those reported for beans, peas and lentils (72–90%) [8, 12]. Differences between true digestibility of crude protein and individual amino acids in animal foods and highly digestible vegetable foods (peanut butter and soy protein isolate) were small (Tables 2–3). However, large differences between true digestibility of crude protein and limiting amino acids in poorly digestible legume-based foods (pinto beans, kidney beans and lentils) and in some cereal-based foods were noticeable (Table 4). Similar marked differences between true digestibility of crude protein and limiting amino acids in legumes and cereals have been reported [4, 8, 13–15]. In beans, peas and lentils, true digestibility values of methionine, cystine and tryptophan were up to 27 percentage units lower than the values of crude protein [8, 13]. In wheat, oat, rye and sorghum, true digestibility values of lysine were up to 14 percentage units lower than those of protein [14–15].

The lower digestibility of the limiting amino acids in cereals or legumes may be due to the occurrence of these amino acids in the less digestible parts of grain, such as the predominant occurrence of lysine in aleurone layers of cereals and high concentration of sulfur amino acids in fababean hulls [16–17]. More digestible amino acids, such as glutamic acid, occur in the highly-digestible parts of cereal endosperm or legume cotyledons [16–17]. Low digestibility of methionine in legumes may be related to steric hindrance due to bulky amino acids adjacent to methionine in peptides [18]. Methionine in such peptides (Thr–Met–Arg, Thr–Met–Lys, which are known to occur in legumes) and methionine in other peptides with bulky amino acids (Val–Met–Phe) was considerably less available for rat growth than that in the unhindered tripeptide (Ala–Met–Ala) [19].

The excretion of endogenous proteins which contain relatively high levels of methionine, cystine and lysine may influence digestibility of these amino acids in a protein source as determined by the rat balance method [20]. The increased fecal excretion of DNA and nitrogen by rats fed cooked kidney beans compared with rats fed a protein free or casein diet was considered to be due to increased turnover of mucosal cells of the intestine rather than low protein digestibility [21]. The constituent of the beans which caused the increased DNA output in feces, however, was not specified. It is possible that the presence of residual antinutritional factors (such as trypsin inhibitors, haemagglutinins, amylase inhibitors, etc.) in the cooked beans, peas and lentils tested in this study may have stimulated excretion of endogenous proteins. However, the increased excretion of endogenous pro-

teins could not solely account for the poor protein digestibility of cooked beans [29]. Other reports have suggested that high levels of dietary fiber or tannins in beans may be responsible for poor digestibility of their proteins [5, 22]. The true protein digestibility of the foods tested in this investigation was negatively correlated with their contents of food fiber ($r = -0.69$, $P < 0.01$) or food cellulose ($r = -0.82$, $P < 0.01$) [5].

Use of the balance method to determine amino acid digestibility has been criticized because of possible microbial modifications of undigested and unabsorbed nitrogenous residues in the large intestine [23]. The microbial modifications may be more pronounced in materials damaged by processing and those containing significant amount of fermentable carbohydrates which support maximum microbial growth in the large intestine [24, 31]. Measuring the disappearance of amino acids from the small intestine (ileal recovery) may provide an accurate estimate of their digestibility [25]. This was investigated by several researchers who compared the amino acid compositions of ileal and rectal digesta of pigs fed a number of protein sources and their mixtures [26–28]. In most cases, the fecal amino acid digestibility values were higher than the ileal values, especially for threonine and tryptophan (up to 16%) suggesting disappearance from the large intestine. But the fecal digestibility values for methionine were lower (5–9%) than the ileal values in pigs fed some cereal grains, suggesting synthesis in the large intestine [30]. When digestibility of all amino acids was considered, fecal values reflected the expected absorption of amino acids in the ileum [27].

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