A comparison of in vivo and in vitro estimates of protein digestibility of native and thermally processed vegetable proteins

BIBLIOTECA

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Abstract. Research was performed to determine the suitability of the rapid multienzymatic assay for in vitro protein digestibility estimation by using a group of native and thermally processed vegetable proteins which constitute the staple foods in developing countries. The in vitro digestibility was assessed by measuring the extent to which the pH of the protein suspension dropped when treated with a multienzyme system consisting of trypsin, chymotrypsin, and peptidase for 10 min, and *Streptomyces griseus* protease for 10 min more. The best correlation occurred between in vivo rat protein digestibility and the pH of the protein suspension after 15 min enzymatic treatment. The response of different types of proteins to the multienzyme assay was different, and thus distinct equations were derived for the in vitro digestibility estimation of the samples assayed. The first group included nonprocessed cereal grains and oilseeds, and cereal grain-leguminous seed mixtures. The second group was formed by leguminous seeds, and the third by thermally processed cereal and oilseed products. Although highly significant correlations between in vivo and in vitro estimates for the three groups were found, important differences occurred in the group of processed samples; therefore, more research is required with these types of samples.

Introduction

The search for more and better sources of protein to feed the growing population of the world has created the need for rapid and suitable tests to assess their protein quality. Traditional biological assays are expensive and time-consuming, and they require considerable amounts of samples which are not available. As a result, several chemical and in vitro assays for the measurement of protein quality and protein digestibility have been developed.

Protein digestibility determines the availability of the amino acids contained in food proteins. Traditionally, it has been measured by using a rat bioassay, but much research has been devoted to the development of rapid in vitro assays to overcome the inherent shortcomings of bioassays.

Hsu et al. [6] developed a multienzyme system consisting of trypsin, chymotrypsin, and peptidase for the estimation of protein digestibility. They found that the pH of the protein suspension after a 10-min digestion with the multienzyme solution was highly correlated with the in vivo apparent digestibility of rats. This method could be completed in a short period of time and

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proved to be sensitive to trypsin inhibitors, chlorogenic acid, and heat treatment. Since most of the samples were of vegetable origin, the equation derived did not accurately predict protein digestibility of meat and egg products. Satterlee et al. [13] modified the method by adding a fourth enzyme, the protease from *Streptomyces griseus*, at the end of the 10-min digestion with trypsin, chymotrypsin, and peptidase, for an additional period of 10 min, and studied a greater number of samples, including some of animal origin.

Several authors using the multienzyme method for protein digestibility have found that higher correlations between in vivo and in vitro measurements of protein digestibility are obtained when the results for similar samples are grouped together [9–12].

The purpose of this study was to compare the in vitro and in vivo apparent digestibility of a diverse group of vegetable proteins which constitute the staple foods of most of the population in developing countries, and to determine the sensitivity of the in vitro assay in assessing the effects of heat treatment on protein digestibility.

Materials and Methods

Samples and sample preparation

Protein samples were selected in order to include a group of vegetable proteins covering a wide range of protein quality. These included commercial and laboratory-prepared plant proteins such as cereal grains, leguminous seeds, oilseeds, and by-products, and mixtures of cereal grains and leguminous seeds alone and supplemented with powdered skim milk or meat meal. ANRC casein and sodium caseinate were used as reference proteins in the in vivo and in vitro assays, respectively. The samples and their nitrogen content are shown in Table 1.

Leguminous seeds were cooked according to the technique previously described [4]. Immature corn kernels were dried ($T = 40°C$) and ground. Sesame seeds (*Sesamum indicum*) were pressed in a disk mill, extracted with hexane, and ground in a hammer mill. Commercial samples included soybean meal, cottonseed meal, white wheat flour, and the corn and bean flours used in the mixtures. The rest of the samples were ground in a hammer mill to pass 60 mesh.

To determine whether leguminous seeds and thermally processed samples constituted one or two populations different from the rest of the samples, an additional lot of 17 varieties of beans (*Phaseolus vulgaris*) were studied. These varieties were obtained from the CIAT germ plasm bank, and were cultivated in INCAP's experimental farm; they had been previously characterized physically, chemically, and nutritionally [7], and were analyzed in the present study by the in vitro digestibility method.

The effect of thermal processing was investigated with three lots of samples: white wheat bread, Opaque-2 corn, and defatted cottonseed meal.

Table 1. In vivo apparent protein digestibility and pH values after multienzymatic digestion of the first set of samples

Sample	N(%)	Apparent digestibility (%)		pH		
		1[a]	2[a]	10′	15′	20′
1. Casein (ANRC)	13.88	90.7		6.75	6.21	6.16
Cereals						
2. Maize, common	1.55	83.0		7.50	6.71	6.74
3. Immature maize	1.79	78.6		7.58	7.06	6.94
4. Cornflakes	1.28	72.0		7.38	6.80	6.62
5. Cornmeal	1.43	86.5		7.11	6.51	6.43
6. Opaque-2 maize	1.39	80.3		7.52	6.87	6.74
7. White sorghum	1.33	80.6		7.62	6.95	6.91
8. Red sorghum	1.34	77.4		7.64	7.12	6.97
9. Rice	1.28	86.0	81.9	7.16	6.44	6.40
10. White wheat flour	2.05	90.7	89.4	6.95	6.34	6.22
11. Wheat, whole	2.14	81.6		7.11	6.54	6.45
Leguminous seeds						
12. Black bean 20′[b]	3.92	73.2	72.5	7.19	6.60	6.46
13. White bean 20′	4.03	74.1	71.4	7.10	6.52	6.40
14. Red bean 20′	3.77	71.2	69.0	7.28	6.65	6.49
15. Cowpea 20′	3.88	80.0	76.0	7.13	6.52	6.42
16. Pigeon pea 20′	3.49	76.4	73.7	7.16	6.59	6.47
17. Cowpea 10′	3.88		74.3	7.06	6.52	6.40
18. Black bean 30′	3.89		68.2	7.19	6.64	6.57
19. White bean 30′	3.93		75.4	7.12	6.57	6.50
Oilseeds						
20. Soybean	5.84	80.5	78.0	6.96	6.37	6.31
21. Soybean meal	7.54	83.0	79.7	6.92	6.34	6.22
22. Cottonseed meal M-J	6.50	76.9	73.4	7.06	6.41	6.31
23. Sesame seed meal	6.23	84.4		7.40	6.85	6.79
Mixtures						
24. Maize-black beans 87:13	1.62	82.6		7.22	6.88	6.61
25. Maize-black beans 70:30	2.11	79.2		7.19	6.67	6.64
26. Rice-black beans 95:5	1.35	82.3		7.12	6.58	6.51
27. Rice-black beans 80:20	1.68	80.0		7.13	6.65	6.57
28. Maize-black beans 87: 13 + 5% powdered skim milk	1.72	82.3		7.20	6.72	6.67
29. Maize-black beans 87: 13 + 10% meat meal	2.75	86.1		7.15	6.67	6.65
30. Rice-black beans 95: 5 + 5% powdered skim milk	1.55	81.5		6.95	6.48	6.39
31. Rice-black beans 95: 5 + 10% meat meal	2.54	86.3		6.93	6.40	6.34
32. Pigeon pea-immature maize 25:75	2.32	76.9		7.47	6.97	6.90
33. Maize-soybean 70:30	2.74	79.5		7.20	6.62	6.55

[a] Apparent digestibility determined in two separate assays with the same sample.
[b] Indicates cooking time of leguminous seeds, in minutes.

Each lot included three samples differing in the extent of thermal processing. A sample of bread was air-dried, while the other two were toasted to varying degrees in an oven. Opaque-2 corn samples were toasted in an internally heated rotatory drum and then ground. Two of the cottonseed meals were of commercial origin, from an expeller oil extraction process. A third meal was prepared by grinding the lintless seeds, which were then defatted with hexane and washed with acetone. All samples were ground in a Udy cyclone mill (UDY Analyzer Company, PO Box 148, Boulder, CO 80302, USA) prior to the in vitro assay.

Chemical assays

The nitrogen content of all the samples was determined by the macro-Kjeldahl method [1]. The crude protein was calculated using the appropriate factors [14]. The content of available lysine in thermally processed samples was determined by Carpenter's method [3].

In vitro digestibility experiments

The in vitro digestibility of the samples was assessed by measuring the extent to which the pH of the protein suspension dropped when treated with a multienzyme system including trypsin (porcine pancreatic, Type IX Sigma Chemical Co.), chymotrypsin (bovine pancreatic, Type II, Sigma Chemical Co.), and aminopeptidase (porcine intestinal, Grade II, Sigma Chem. Co.) as described by Hsu et al. [6] and modified by Satterlee et al. [13] by adding a fourth enzyme, *Streptomyces griseus* protease, to complete proteolysis. The enzyme solutions were freshly prepared before each series of tests. Sodium caseinate was used as a reference sample with each series of tests.

In vivo experiments

Male and female weanling rats of the Wistar strain from the INCAP colony, 21–23 days of age, were used as experimental animals. The groups were comprised of four rats of each sex. The animals were housed in individual, all-wire screen cages and were allowed free access to food and water. The basal diet consisted of: 90% cornstarch, 4% mineral mixture [5], 5% cottonseed oil, 1% cod-liver oil, and supplemented with 5 ml vitamin solution [8]. Protein samples were added by substituting the cornstarch to reach 9% protein when possible or 7% in the case of samples of low protein content.

Body weights and food consumption were recorded weekly for a total period of 28 days. Net protein ratio and protein efficiency ratio were calculated. The feces of each rat were collected during the last week of the experiment, air-dried, weighed, and analyzed for N. In vivo apparent protein digestibility was thus calculated from the formula:

$$\text{Apparent digestibility} = \frac{\text{N consumed} - \text{N feces}}{\text{N consumed}} \times 100$$

Table 2. Regression equations between pH after different incubation times and apparent in vivo rat digestibility

Variables[a]	Equation	r	Significance[b]
First assay ($n = 30$)			
1. pH after 10′	$y = 146.177 - 9.078x$	−0.418	*
2. pH after 15′	$y = 142.119 - 9.253x$	−0.421	*
3. pH after 20′	$y = 125.240 - 6.795x$	−0.308	NS
Second assay ($n = 14$)			
1. pH after 10′	$y = 354.530 - 39.249x$	−0.791	**
2. pH after 15′	$y = 372.259 - 45.606x$	−0.868	**
3. pH after 20′	$y = 367.920 - 45.604x$	−0.804	**

[a] y = apparent in vivo rat digestibility.
x = pH after different incubation times.
[b] NS, nonsignificant.
* $p < 5\%$.
** $p < 1\%$.

To test the reproducibility of the in vivo digestibility method, a group of samples were assayed at two different times. The first assay included samples of all the vegetable proteins studied, while in the second assay a more homogeneous group, including mainly leguminous seeds, was studied. CIAT varieties of *Phaseolus vulgaris* and thermally processed samples were also assayed at different times than the rest of the samples.

Results and Discussion

The in vivo digestibility and the pH values after 10, 15, and 20 min enzymatic digestion for the first set of samples are shown in Table 1. The sample distribution around the regression lines relating the in vivo digestibility to pH after 15 min enzymatic activity is shown in Figure 1. The corresponding regression after 10 min was $y = 146.17 - 9.08x$ ($r = -0.418$; significant at $p < 0.05$) and after 20 min $y = 125.24 - 6.79x$ ($r = -0.309$; NS). The numbers identifying each point correspond to the sample numbers in Table 1. It is evident that leguminous seeds and processed samples (cornflakes and cottonseed meal) deviate considerably from the rest of the samples in the first assay. The regression equations for both assays are shown in Table 2. The correlation coefficients for the second assay were highly significant for the three values of pH considered (10′, 15′, and 20′), while in the first assay the correlation coefficients were lower but yet significant after 10 and 15 min of digestion, but not significant for the 20-min pH value. This fact further confirms the suggestion that different samples behave differently when assayed by the multienzymatic method. The samples in the second assay were mainly leguminous seeds, while the samples in the first assay were a heterogeneous group of vegetable proteins. This different behavior among samples can be attributed to the fact that rapid chemical and enzymatic methods may not be sensitive to certain physical and chemical characteristics of the foods assayed

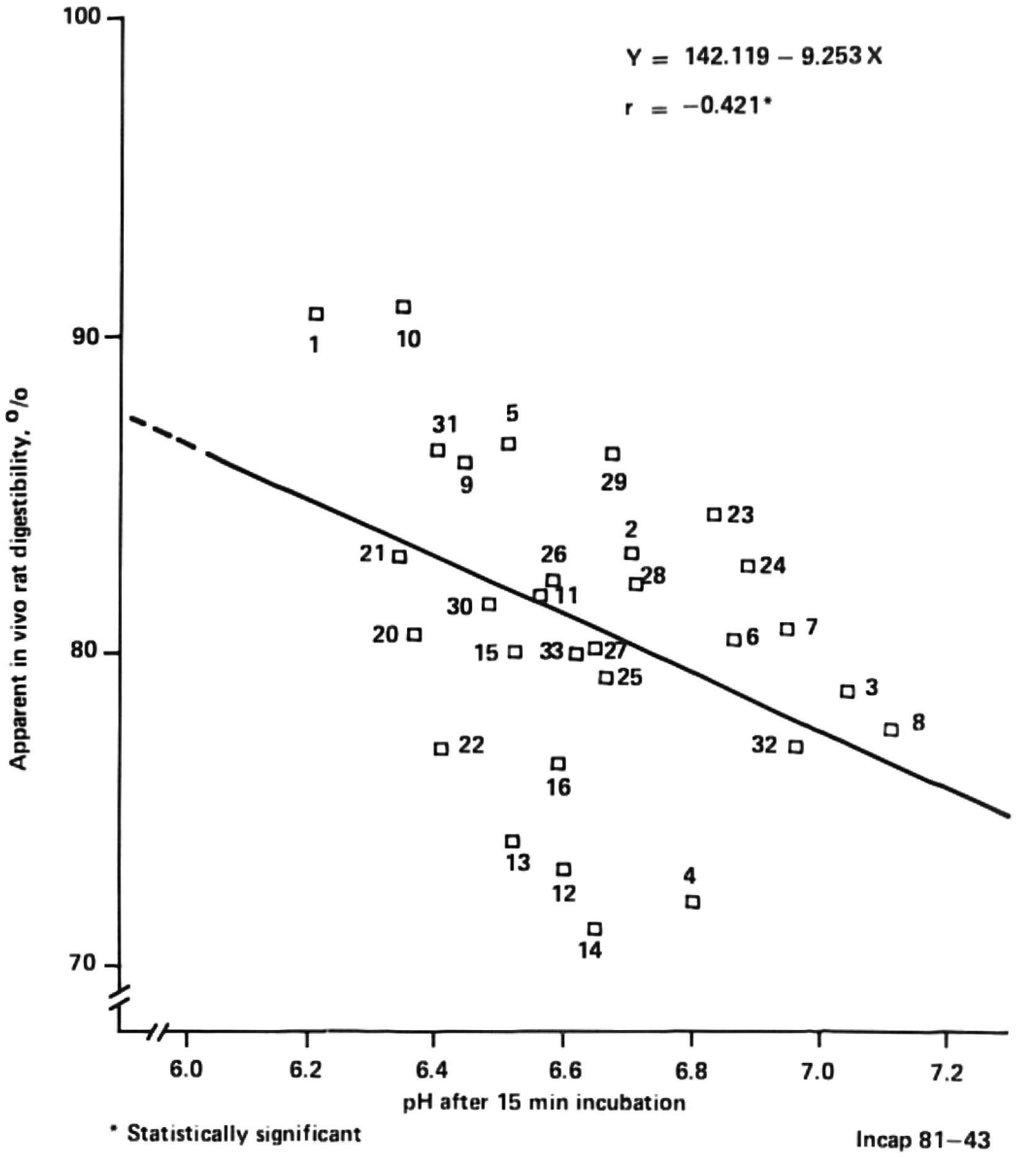

Figure 1. Relationship of pH at 15 min and apparent rat digestibility.

such as the presence of antinutritional factors and cellular walls. Similar observations have been previously reported [9–12] stressing the difficulty of finding a single equation which is suitable for accurately predicting the protein digestibility of all samples. Therefore, these results confirm the need of deriving equations for different groups of samples.

Different variables such as temperature, time of digestion, and the enzyme-substrate proportion were studied to determine optimum response. It was found that, with the exception of the pH value to be used in the in vitro digestibility calculation, the assay as described by Hsu et al. [6] and modified by Satterlee et al. [13] gave adequate results. An important finding was that, for the samples assayed, a better correlation between in vivo digestibility and pH after 15 min digestion was obtained than with either the 10-min or the 20-min pH values. In order to reduce variability due to external factors such as temperature and humidity changes and variation in electrical supply, sodium caseinate was used as a standard with every set of samples assayed enzymatically, thus permitting the correction of the pH value obtained.

The fourth enzyme, *Streptomyces griseus* protease, was added in order to

Table 3. Apparent in vivo rat digestibility and pH values after different incubation times of various *Phaseolus vulgaris* varieties from the CIAT germ plasm

Bean sample (*Phaseolus vulgaris*)		N[a](%)	Apparent in vivo digestibility (%) [7]	pH		
				10′	15′	20′
1. Sodium caseinate		14.16	90.7	6.75	6.21	6.16
2. P-757 Porrillo 1	(B)[b]	4.33	72.7	7.34	6.69	6.59
3. P-459 Jamapa	(B)	4.17	71.5	7.24	6.59	6.46
4. P-302 PI-309-804	(B)	4.27	71.4	7.25	6.68	6.55
5. P-458 Ica Tui	(B)	4.11	68.7	7.25	6.64	6.51
6. P-566 Porrillo sintético	(B)	4.49	73.3	7.26	6.60	6.49
7. P-498 Puebla 152	(B)	3.68	66.6	7.45	6.74	6.58
8. P-560 51051	(B)	4.28	71.0	7.27	6.58	6.47
9. P-675 Ica Pijao	(B)	4.54	74.5	7.16	6.52	6.42
10. P-539 Venezuela 2	(B)	4.48	68.6	7.33	6.58	6.43
11. P-512 S-166-AN	(B)	4.39	71.6	7.11	6.50	6.37
12. P-402 Brasil 2	(Br)	3.39	72.1	7.40	6.75	6.59
13. P-524 S630 BC 63	(Br)	4.40	73.7	7.16	6.54	6.39
14. P-758 Puebla 152	(Br)	3.70	66.4	7.44	6.72	6.55
15. P-755 Pompadour	(R)	4.40	72.1	7.40	6.71	6.56
16. P-392 Sanilac	(W)	4.41	76.7	7.37	6.69	6.51
17. P-756 Ex Rico	(W)	4.48	76.3	7.33	6.61	6.46
18. P-643 Nep-2	(W)	4.56	77.4	7.34	6.48	6.43

[a]Nitrogen content of cooked beans.
[b]The letter in parenthesis represents the color of the bean sample: (B), black; (Br), brown; (R), red; (W), white.

increase the extent of proteolysis and thus improve the correlation coefficients between in vivo and in vitro protein digestibility estimates of certain samples which are better digested in vivo than what is predicted in vitro, such as meat and egg proteins [14]. In our case, the 15-min pH value is superior to the 10-min value since a more complete response has been achieved. However, further enzymatic activity (20-min) results in greater uniformity among the samples and a consequent loss of discriminative ability.

The results of the multienzymatic assay and the in vivo apparent protein digestibility of the CIAT beans (*P. vulgaris*) are shown in Table 3. The regression equations between pH at different times and in vivo digestibility for the complete group of CIAT beans and for the different color groups are shown in Table 4. From these results, it seems that bean color and thus coat polyphenol content does not influence the response of bean samples to the multienzyme assay. However, examination of the regression equations indicates that the intercept and coefficient of regression for black and brown beans are similar and both higher than corresponding values for white bean cultivars. This suggests that polyphenolics influence the in vitro protein digestibility, an aspect which should be studied further with a greater number of samples in order to arrive at more definite conclusions. Finally, equations for the leguminous seeds including other species such as *Vigna sinensis* and *Cajanus cajan* are also shown. From these results, it is evident that a high

Table 4. Regression equations between pH after different incubation times and apparent in vivo digestibility of leguminous seeds

Regression equation[a]	r	Significance[b]
A. *Phaseolus vulgaris* – CIAT germ plasm ($n = 17$)		
1. $y = 252.777 - 24.721$ (pH 10′)	−0.740	**
2. $y = 289.677 - 32.811$ (pH 15′)	−0.781	**
3. $y = 323.520 - 38.689$ (pH 20′)	−0.745	**
B. *Phaseolus vulgaris* – CIAT germ plasm grouped by color		
I. Black ($n = 10$)		
1. $y = 308.964 - 32.716$ (pH 10′)	−0.923	**
2. $y = 338.550 - 40.418$ (pH 15′)	−0.895	**
3. $y = 356.233 - 43.896$ (pH 20′)	−0.826	**
II. Brown ($n = 4$)		
1. $y = 305.399 - 31.955$ (pH 10′)	−0.967	**
2. $y = 337.978 - 40.008$ (pH 15′)	−0.948	**
3. $y = 393.899 - 49.541$ (pH 20′)	−0.924	**
III. White ($n = 3$)		
1. $y = 247.352 - 23.213$ (pH 10′)	−0.996	**
2. $y = 280.195 - 30.769$ (pH 15′)	−0.929	**
3. $y = 359.080 - 43.631$ (pH 20′)	−0.982	**
C. *P. vulgaris* (CIAT) + leguminous seeds, 2nd assay ($n = 26$)		
1. $y = 224.537 - 20.960$ (pH 10′)	−0.661	**
2. $y = 287.781 - 32.586$ (pH 15′)	−0.763	**
3. $y = 313.342 - 37.158$ (pH 20′)	−0.720	**

[a] x = pH after different incubation times.
[b] y = apparent in vivo rat digestibility.
** $p < 1\%$.

correlation exists between in vivo protein digestibility and pH after 10, 15, and 20 min enzymatic digestion for the group of *Phaseolus* beans, and that including other species of leguminous seeds does not significantly affect the correlation coefficient. We can therefore conclude that leguminous seeds comprise a single population with respect to the multienzyme digestibility assay.

The regression analysis by groups of samples showed that excluding cornflakes and cottonseed meal from any calculation which included cereal grain and oilseed data improved the values of the correlation coefficients obtained noticeably. Thus, the value of r between in vivo digestibility and pH-15′ for the combined group cereal grains and oilseeds in the first assay improves from −0.574 ($p < 5\%$) to −0.747 ($p < 1\%$) when cornflakes and cottonseed meal are excluded from the calculation.

The effect of thermal processing was studied and the results are shown in Table 5. It is evident that the strongest and only highly significant ($p < 1\%$) correlation coefficient was obtained with the pH-15′ values. No significant correlation was found between available lysine and in vivo protein digestibility or pH after 10, 15, or 20 min enzymatic digestion for the complete group of processed samples. The analysis of processed samples by groups gave similar results, probably due to the small number of samples assayed in each group.

Table 5. The effect of heat treatments on the available lysine content, apparent in vivo digestibility, and pH values after different incubation times of processed samples

Sample	Nitrogen (%)	Available lysine (g/16 g N)	Apparent[a] in vivo digestibility %	pH[a]		
				10′	15′	20′
Sodium caseinate		–	90.2	6.75	6.21	6.16
Opaque-2	1.35	4.67	79.3	7.50	6.78	6.70
Opaque-2 maize 20.5′[b]	1.38	3.62	74.8	7.39	6.76	6.65
Opaque-2 maize 26′	1.43	2.76	48.9	7.56	6.98	6.83
Cornflakes	1.28	2.12	72.0	7.38	6.80	6.62
Air-dried white bread	2.34	2.23	86.5	6.96	6.31	6.19
Toasted white bread +[c]	2.38	1.90	86.6	7.02	6.33	6.22
Toasted white bread ++	2.43	1.28	82.3	7.08	6.38	6.25
Cottonseed meal M-J[d]	6.50	3.33	66.5	7.20	6.49	6.38
Cottonseed meal INCAP[e]	7.84	4.45	83.0	7.21	6.51	6.40
Cottonseed meal NAISA[d]	6.75	3.09	69.8	7.13	6.43	6.32

[a] Regression equations between pH (x) and apparent in vivo digestibility (y); $n = 11$:

$$y = 328.364 - 35.004 \text{ (pH 10')} \quad r = -0.720^{*}$$
$$y = 309.072 - 35.564 \text{ (pH 15')} \quad r = -0.744^{**}$$
$$y = 311.379 - 36.556 \text{ (pH 20')} \quad r = -0.717^{*}$$

[b] Represents toasting time.
[c] Represents the extent of toasting.
[d] Press extraction of oil.
[e] Solvent extraction of oil.

The equations used for the calculation of the in vitro digestibility of the groups of samples assayed are shown in Table 6. Tables 7–9 show the comparison between in vivo and in vitro protein digestibility for the three groups of samples. Table 7 includes nonprocessed cereal grains, oilseeds, and cereal grain-leguminous seed mixtures alone and supplemented with powdered skim milk or meat meal. Table 8 presents the results for leguminous seeds, and Table 9 for thermally processed samples. The highest differences between in vivo and in vitro digestibility values were found in the group of thermally processed samples. The in vitro method evidently overestimated the value of protein digestibility for the Opaque-2-26′ sample and for the press-extracted cottonseed meals. Similar observations have been previously reported and are attributable to the fact that in vitro methods have proved to be less sensitive than rat assays to the low availability of certain amino acids, in particular to lysine, and thus overestimate the protein digestibility of thermally processed samples [11]. We feel that more research using thermally processed samples is needed before a definite equation for calculating in vitro digestibility can be proposed. Perhaps it may even be necessary to derive equations for the different groups of thermally processed samples.

The correlation coefficients between the in vivo and in vitro estimates of protein digestibility are shown in Table 10. It is evident that highly significant correlations exist in the individual groups as well as when all of the samples are considered together ($r = 0.838, n = 60$).

Table 6. Regression equations used in the calculation of in vitro digestibility of the different groups of samples

Sample group	Equation[a]
I. Nonprocessed cereal grains and oilseeds,[b] and mixtures	$y = 150.770 - 10.250x$
II. Leguminous seeds	$y = 287.781 - 32.586x$
III. Processed cereals and oilseeds	$y = 309.072 - 35.564x$

[a] x = pH after 15-min incubation.
y = apparent in vivo digestibility.
[b] Excluding cornflakes and cottonseed meal.

Table 7. In vitro digestibility and its comparison to the apparent in vivo value – Group I[a]

Sample	pH 15′	Apparent digestibility (%)		
		In vitro	In vivo	Difference[b,c]
1. Casein	6.21	87.1	90.7	3.6
Cereals				
2. Common maize	6.71	82.0	83.0	1.0
3. Immature maize	7.06	78.4	78.6	0.2
4. Cornmeal	6.51	84.0	86.5	2.5
5. Opaque-2 maize	6.87	80.3	80.3	0.0
6. White sorghum	6.95	79.5	80.6	1.1
7. Red sorghum	7.12	77.8	77.4	−0.4
8. Rice	6.44	84.8	86.0	1.2
9. White wheat flour	6.34	85.8	90.7	4.9
10. Wheat, whole	6.54	83.7	81.6	−2.1
Oilseeds				
11. Soybean	6.37	85.5	80.5	−5.0
12. Soybean meal	6.34	85.8	83.0	−2.8
13. Sesame seed meal	6.85	80.5	84.4	3.9
Mixtures				
14. Maize-black beans 87:13	6.88	80.3	82.6	2.3
15. Maize-black beans 70:30	6.67	82.4	79.2	−3.2
16. Rice-black beans 95:5	6.58	83.3	82.3	−1.0
17. Rice-black beans 80:20	6.65	82.6	80.0	−2.6
18. Maize-black beans 87:13 + 5% powdered skim milk	6.72	81.9	82.3	0.4
19. Maize-black beans 87:13 + 10% meat meal	6.67	82.4	86.1	3.7
20. Rice-black beans 95:5 + 5% powdered skim milk	6.48	84.4	81.5	−2.9
21. Rice-black beans 95:5 + 10% meat meal	6.40	85.2	86.3	1.1
22. Pigeon pea-immature maize 25:75	6.97	79.3	76.9	−2.4
23. Maize-soybean 70:30	6.62	82.9	79.5	−3.4

[a] Cereals excluding cornflakes and oilseeds excluding cottonseed meal, mixtures; calculated using Eq. I, Table 6.
[b] In vivo dig. – in vitro digestibility.
[c] Average difference = 0.004; SD = 2.727; SE = 0.569.

Table 8. In vitro digestibility and its comparison to the apparent in vivo value – Group II[a]

Sample	pH 15′	Apparent digestibility (%)		
		In vitro	In vivo	Difference[b]
1. Casein	6.21	85.4	90.7	5.3
CIAT germ plasm samples				
2. P-757 Porrillo 1	6.69	69.8	72.7	2.9
3. P-459 Jamapa	6.59	73.0	71.5	−1.5
4. P-302 PI-309-804	6.68	70.1	71.4	1.3
5. P-458 Ica Tui	6.64	71.4	68.7	−2.7
6. P-566 Porrillo sintético	6.60	72.7	73.3	0.6
7. P-498 Puebla 152	6.74	68.2	66.6	−1.4
8. P-560 51051	6.58	73.4	71.0	−2.4
9. P-675 Ica Pijao	6.52	75.3	74.5	−0.8
10. P-539 Venezuala 2	6.58	73.4	68.6	−4.8
11. P-512 S 166 AN	6.50	76.0	71.6	−4.4
12. P-402 Brasil 2	6.75	67.8	72.1	4.3
13. P-524 S 630 BC 63	6.54	74.7	73.7	−1.0
14. P-758 Puebla 152	6.72	68.8	66.4	−2.4
15. P-755 Pompadour	6.71	69.1	72.1	3.0
16. P-392 Sanilac	6.69	69.8	76.7	6.9
17. P-756 Ex Rico	6.61	72.4	76.3	3.9
18. P-643 Nep-2	6.48	76.6	77.4	0.8
19. Black bean 20′	6.60	72.7	72.5	−0.2
20. White bean 20′	6.52	75.3	71.4	−3.9
21. Red bean 20′	6.65	71.1	69.0	−2.1
22. Cowpea 20′	6.52	75.3	76.0	0.7
23. Pigeon pea 20′	6.59	73.0	73.7	0.7
24. Cowpea 10′	6.52	75.3	74.3	−1.0
25. Black bean 30′	6.64	71.4	68.2	−3.2
26. White bean 30′	6.57	73.7	75.4	1.7

[a] Leguminous seeds, Eq. II, Table 6.
[b] In vivo dig. – in vitro digestibility.
[c] Average difference = 0.012; SD = 3.042; SE = 0.597.

Table 9. In vitro digestibility and its comparison to the apparent in vivo value – Group III[a]

Sample	pH 15′	Apparent digestibility (%)		
		In vitro	In vivo	Difference[b,c]
1. Casein	6.21	88.2	90.2	2.0
2. Opaque-2 maize	6.78	67.9	79.3	11.4
3. Opaque-2, 20.5′ maize	6.76	68.7	74.8	6.1
4. Opaque-2 26′ maize	6.98	60.8	48.9	−11.9
5. Cornflakes	6.80	67.2	72.0	4.8
6. Air-dried white bread	6.31	84.7	86.5	1.8
7. Toasted white bread +[d]	6.33	84.0	86.6	2.6
8. Toasted white bread ++[d]	6.38	82.2	82.3	0.1
9. Cottonseed meal M-J	6.49	78.3	66.5	−11.8
10. Cottonseed meal INCAP	6.51	77.6	83.0	5.4
11. Cottonseed meal NAISA	6.43	80.4	69.8	−10.6

[a] Processed cereals and oilseeds, Eq. III, Table 6.
[b] In vivo dig. – in vitro digestibility.
[c] Average difference = 0.009; SD = 7.916; SE = 2.387.
[d] + and + + refer to relative degree of toasting.

Table 10. Correlation coefficients between in vivo and in vitro digestibility values

Samples	r	Significance
Group I ($n = 23$) Nonprocessed cereal grains and oilseeds	0.683	**
Group II ($n = 26$) Leguminous seeds	0.762	**
Group III ($n = 11$) Processed cereal grains and oilseeds	0.745	**
All samples ($n = 60$)	0.838	**

**$p < 1\%$.

These results further confirm the suitability of the multienzyme assay for the rapid in vitro estimation of protein digestibility. Nevertheless, it is necessary to keep the following in mind: first, it was clear that a better estimate of protein digestibility for the samples assayed was obtained with the 15-min pH value; second, that different samples show a different response and thus should be considered separately for the in vitro digestibility calculation using the proposed equation (Table 6); and third, that more research is required in regard to the in vitro digestibility prediction of thermally processed samples.

The objective of in vitro techniques is to have a rapid, low-cost and accurate way to predict protein digestibility in humans for whom foods are intended. However, results of Bodwell et al. [2] have indicated a lack of correlation between in vitro estimates and in vivo digestibility in rats or humans on the basis of six samples. These authors, however, indicated that samples of similar origin, either from animal or vegetable origin, gave high correlations between in vitro and rat and human digestibilities. They suggested that accurate predictions by in vitro methods could be obtained only from the use of different equations. The present investigation supports this statement as discussed above.

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