IDRC-TS 7e

Nutritional Standards and Methods of Evaluation for Food Legume Breeders

Prepared by the International Working Group on Nutritional Standards and Methods of Evaluation for Food Legume Breeders

J.H. Hulse, K.O. Rachie, and
L.W. Billingsley

IDRC-TS 7e • Legume Nutritional Standards

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IDRC IDRC-TS7e

Nutritional standards and methods of evaluation for food legume breeders. Ottawa, IDRC 1977. 100 p.

/IDRC pub CRDI/. Monograph on /nutrition/al /quality standard/s in /legume/ /plant breeding/ — discusses the /recommendation/s for standards of /food composition/; physical and /chemical analysis/, /food analysis/ and /biology/cal /evaluation technique/s.

UDC: 633.3 ISBN: 0-88936-137-1

Microfiche Edition $1

24657

IDRC-TS7e

26295

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J.H. Hulse,* K.O. Rachie,* and L.W. Billingsley**

This publication was prepared by the International Development Research Centre in cooperation with the International Union of Food Science and Technology, the International Union of Nutrition Sciences, the International Centre of Tropical Agriculture (CIAT), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the International Centre for Agricultural Research in Dry Areas (ICARDA).

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The Problem of Legume Protein Digestibility

R. Bressani and L.G. Elías

Legume grains in general comprise an important part of the diet of populations living in tropical and subtropical areas. Their nutritional importance is even greater, as these populations have limited availability and consumption of foods of animal origin. In Latin America, most legume grains, but mainly *Phaseolus vulgaris*, are readily accepted and consumed almost daily by low-income groups. The main dietary component is a cereal grain, although in some areas it is replaced by starchy roots. In Central America bean consumption provides from 20 to 30% of total protein intake.

Experimental evidence has indicated conclusively that legume grains are the natural protein complement to cereal grains, and when both are ingested in the appropriate ratio, the protein quality is higher than that of the individual components. For beans and corn the best ratio by weight is 3:7 (1). However, intake in such a ratio is not common, with a tendency to be significantly lower in both quantity and frequency. The reasons are not known, although availability has often been suggested as the cause. Since the importance of legume grains as sources of protein is recognized, efforts are being made throughout the world to increase their productivity in the hope that this will increase availability and finally consumption. However, other causes may be responsible for the low intakes. It has been assumed that one such cause is the relatively low digestibility of the protein of legume grains.

This paper will, therefore, attempt to analyze the low protein digestibility of legume grains and will discuss the possible reasons for it. If these are identified and could be eliminated, beans will make a better nutritional contribution than that made during the last 4000 years since their consumption apparently was initiated.

The Problem of Legume-protein Digestibility

Most nutritional and biochemical studies carried out with legume grains have dealt mainly with two factors that are important in determining their protein quality. One consists of the antiphysiological substances present in legume grains, of which the trypsin inhibitors, amylase inhibitor, and hemagglutinins are the most important. The second is the well-documented deficiency of sulfur-containing amino acids in legume grain protein. Of the two factors, the antiphysiological compounds could be responsible for low protein digestibility. However, these substances are destroyed completely or to a large extent by heat treatment; therefore, it can be assumed that in cooked foods they are not responsible for such a characteristic. On the other hand, because legume grains are heat-treated

Table 1. Apparent protein digestibility of various legume grain species fed to rats (2).

	Protein digestibility, %
Glycine max	81.1–83.0
Lens esculenta	80.2
Vigna sinensis	76.5–81.4
Cajanus cajan	76.5–76.6
Cicer arietinum	76.3
Dolichos lablab	74.5
Pisum sativum	70.7–76.0
Phaseolus vulgaris	68.7–74.2
Cajanus indicus	47.7–75.3

Table 2. Fecal nitrogen and apparent protein digestibility in human adults fed egg protein and split peas with and without added methionine (3).

Protein source	Nitrogen Intake, g	Fecal, g	Absorbed, g	Apparent protein digestibility, %
Egg	5.60	0.81	4.79	85.6
Split pea	5.47	1.16	4.31	78.8
Split pea + Met	5.95	1.19	4.76	80.0

Table 3. Fecal nitrogen of children and young dogs fed various protein foods with cooked beans (4).

Diet	Nitrogen, mg/kg per day		Fecal N of N intake,
	Intake	Fecal	%
Children			
Milk	387	70	18.1
25% Milk + 75% (maize + beans)	358	98	27.4
10% Milk + 90% (maize + beans)	353	134	38.0
100% (maize + beans)	347	107	30.8
Dogs			
Maize	520	169	36.3
Maize + black beans	635	254	40.0

Table 4. Fecal nitrogen losses from milk and *P. vulgaris* fed to children (5).

Protein source	Nitrogen balance, mg/kg per day		
	Intake	Fecal	Absorbed
Milk	236	46	190
Cooked black beans	227	81	146

before consumption and also for evaluation of their protein quality, and extended heat-treatment decreases protein digestibility, the real cause could be the thermal process used.

The protein digestibility of various legume grain species is shown in Table 1, as determined in the laboratory rat (2). These results show that there is a great variation within the same species. For example, in *Cajanus indicus* the values range from 4.7 to 75.3%. Large differences in protein digestibility are also evident between species. All values were obtained from feeding cooked samples. Therefore, it cannot be assumed the variation is due to residual levels of anti-nutritional factors, unless the heat treatment employed was less effective in some species than in others.

The results of the studies on human adults fed Alaska split pea (*Pisum sativum*) (3) are shown in Table 2. Feeding split pea at a level of nitrogen intake similar to that provided by egg, increased fecal nitrogen excretion from 0.81 to 1.16 g/day. Apparent protein digestibility for egg was 85.6% and that for split pea was 78.8%. For egg, fecal nitrogen was 14.5% of intake, but for split pea, fecal nitrogen was 21.2% of intake. The results also show that added methionine did not change fecal nitrogen excretion, which in this case represents 20% of intake. Results of various studies with children fed *P. vulgaris* in combination with other foods are shown in Table 3. Fecal nitrogen increased as milk nitrogen intake decreased and on a relative basis fecal nitrogen varied from 18.1 to 38.0% of nitrogen intake. Not all the effect can be attributed to bean protein, because it was given in combination with maize. However, nitrogen loss increased, and beans may be responsible to some degree (4).

Results in the lower section of the table were obtained with young dogs. In the case of corn, fecal nitrogen amounted to 36.3% of the intake. In the second experiment, in which 32% of the nitrogen intake was from beans, fecal nitrogen increased to 40% of the nitrogen intake (4), these figures representing significant losses of nitrogen.

Additional information for children is summarized in Table 4 (5). Fecal nitrogen for milk was equivalent to 19% of milk nitrogen intake, but for beans, it was equivalent to 36% of nitrogen intake. The losses of nitrogen in feces resulted in a nitrogen retention for milk of 80.5% of intake and only 64.3% for bean.

These results, from both animals and humans, suggest that legume grain protein is in general of low digestibility. However, it is of interest to analyze the results for apparent protein digestibility of other vegetable protein sources. Table 5 summarizes some results obtained in children fed vegetable proteins (6). Apparent protein digestibility values of these proteins ranged from 68 to 85%, which is also the range of protein digestibility values found for the legume grains. Although some of the diets contained legume grains, others did not, or were single foods. In view of this, one may ask if the low digestibility of legume grain protein is a particular characteristic of legume grains, or if it represents a characteristic common to all or almost all vegetable protein sources.

The Role of Antiphysiological Factors

In the previous section, it was suggested that antiphysiological factors, such as trypsin and amylase inhibitors, could be partially responsible for the low digestibility of the protein in legume foods. Even though these factors are destroyed by heat, it is of interest that when legume grains are fed raw, they affect protein digestibility. Table 6 summarizes the results of various investigators (7–12, 23). It is evident that raw *Phaseolus* are poorly digested with values ranging from as low as 15.6–56%. However, genera such as *Vigna* do not show low digestibility values when fed raw. *Vigna sinensis* has significantly lower levels of trypsin inhibitors than *Phaseolus* species. Although it has been indicated that no correlation seems to exist between trypsin inhibitory activity and protein digestibility (7), it is clear that the presence of such activity will interfere with the process of protein breakdown in the gastrointestinal tract. The lack of correlation may be explained by recent findings that suggest two types of trypsin inhibitory activity. One is heat-labile or true trypsin inhibitor activity, and the second is heat-resistant, probably due to tannins or polyphenols (13). However, independent of these, there are other factors and conditions that contribute to a variable degree to changes in protein digestibility.

The Role of Heat Treatment on Protein Digestibility

The variation in protein digestibility shown for legume grains is probably the result of various factors, which can be inherent in the seed; or a result of handling and storage of the seed; or result from the thermal process utilized to prepare the seed for biological evaluation.

As indicated before, the extent and length of the thermal process may damage the protein in terms of its quality, of which protein digestibility is a part. The results (Table 7) show the importance of cooking (11). The low value for the raw seed is expected because it still contains active antiphysiological compounds. In the cooked samples, the digestibility varied as the pressure used in cooking. A pressure of 15 lb/inch^2

Table 5. Apparent and true protein digestibility of various vegetable protein sources (6).

Protein source	Digestibility: Apparent, %	True, %
Milk, wheat flour, chick pea flour, and lentil flour	70	86
Milk, wheat flour chick pea flour, and soy flour	71	88
Milk, wheat flour, chick pea flour, and split pea flour	68	85
Corn and cottonseed flour	68	84
Corn and soybean flour	74	91
Corn, soybean, and cottonseed flour	74	92
Corn	69–75	—
Wheat	85	—
Rice	79	—
Milk	82	92
Egg	79	98

Table 6. Protein digestibility of raw and cooked legume foods.

Legume food	Scientific name (genus and species)	Protein digestibility Raw, %	Cooked, %	Type	Reference
Common bean (red)	*P. vulgaris*	49	71	in vitro	(10)
Common beans (red)	*P. vulgaris*	56	83	in vitro	(10)
Common beans (black)	*P. vulgaris*	55	80	in vitro	(10)
Common beans (white)	*P. vulgaris*	52	91	in vitro	(10)
Common beans	*P. vulgaris*	43.5	80.9	in vivo	(8)
Common beans (black)	*P. vulgaris*	15.6	71.2	in vivo	(11)
Common beans (white)	*P. vulgaris*	42.7	74.9	in vitro	(23)
Common beans (black)	*P. vulgaris*	41.1	68.1	in vitro	(23)
Common beans (red)	*P. vulgaris*	36.3	72.3	in vitro	(23)
Soybeans	*Glycine max*	70.1	85.4	in vivo	(7)
Soybeans	*Glycine max*	82.9	89.7	in vivo	(9)
Cowpea	*V. sinensis*	79.0	82.6	in vivo	(7)
Cowpea	*V. sinensis*	73.2	72.4	in vivo	(12)
Lima beans	*P. lunatus*	34.0	51.3	in vivo	(7)
Pigeon peas	*C. cajan*	59.1	59.9	in vivo	(7)

Table 7. Effect of various types of cooking process on the digestibility of *P. vulgaris* (black) (11).

Treatment	Digestibility
Raw	15.6
Cooked in distilled water, 85 °C, 2 h	48.7
Cooked in 0.1% CH_3COOH, 85 °C, 2 h	46.6
Cooked in 0.1% $NaHCO_3$, 85 °C, 2 h	52.9
Autoclave 15 psi, 30 min	71.2
Autoclave 15 psi, 30 min, no seed coat	73.0

Table 8. Effect of thermal treatment on the protein digestibility of *V. sinensis* (cowpea) (12).

Thermal treatment	% digestibility	Available lysine, g/16 g N
None	73.2	6.4
Autoclaved 16 psi, 121 °C, 15′	72.4	5.7
Toasted 2 30′, 210 °C	76.1	4.8
Toasted 3 20′, 230 °C	73.4	4.9
Toasted 1 30′, 240 °C	65.0	2.9
Extrusion	80.2	5.6

($1.0545/cm^2$) is much more effective than atmospheric pressure, or longer cooking time. The reason for these differences may be the degree to which the antiphysiological factors were inactivated by heat.

To eliminate this variable as responsible, similar studies were made with other thermal treatments applied to *V. sinensis* (12). This legume grain contains only small amounts of trypsin inhibitors. The results (Table 8) show that the raw seed has a protein digestibility essentially the same as the sample that was autoclaved. Excessive toasting, in terms of higher temperature and time, decreased digestibility from 76 to 65%, whereas extrusion cooking gave a product the highest digestibility of 80%. Heat obviously decreased the quality of the protein, damaging to a large extent the available lysine content as shown. However, a process such as extrusion cooking, involving a high-temperature short-time process, improved the digestibility of the protein in a way yet unknown. It is possible that this treatment is capable of destroying cell walls, thus making the contents more available to enzymatic action, or the carbohydrate fraction was made more susceptible to hydrolysis.

Cooking time under pressure is also a factor that may influence protein digestibility, because optimum conditions vary according to the legume grain species (14). For example, Table 9 shows that for *P. vulgaris* a cooking time of 45 min under pressure increased protein digestibility slightly (73–74.2%), whereas the same conditions decreased the protein digestibility of *V. sinensis*

Table 9. Effect of pressure cooking time on protein quality and digestibility of three legume grain species (14).

	Pressure cooking time, min			
	15		45	
Legume grain species	PER[a]	% PD[b]	PER	% PD
P. vulgaris (black)	0.70	73.0	0.94	74.2
V. sinensis (cowpea)	1.37	77.5	0.84	74.3
C. cajan (pigeon pea)	1.42	80.4	1.61	78.1

[a]PER, protein efficiency ratio.
[b]PD, protein digestibility.

from 77.5 to 74.3% and of *Cajanus cajan* from 80.4 to 78.1%. The changes are in general small; however, they are consistent.

The application of heat may be interpreted as having a double effect, taking place one as a consequence of the other. The first effect is to decrease and eliminate the activity of the anti-physiological factors, as is well-documented. The second is to increase amino acid availability from the protein. This is shown in Table 10 for *P. vulgaris* (8). The greatest increase in availability was observed for the sulfur-containing amino acids, methionine and cystine, amino acids that are present in high concentration in the trypsin inhibitors. It should be recalled, however, that excessive heat may decrease amino acid availability. As was shown, the effects of heat can be measured by the extent of inhibitor inactivation, by the increase in sulfur amino acid availability, and by a decrease in lysine availability.

Finally, storage time also influenced protein digestibility of legume grains. Some results are shown in Fig. 1. In these studies *P. vulgaris* was stored for 0, 3, and 6 mo. After the specified storage time, they were soaked in water and cooked under pressure for 10, 20, and 30 min. The results show that for all storage periods, longer cooking time decreased protein digestibility. Likewise, storage time also affected protein digestibility at each cooking time (15).

These results, therefore, show that there are too many factors influencing the protein digestibility of legume grains, making it absolutely necessary to study them individually and under well-controlled and known processing conditions. The lack of standard methods of processing, and of information defining or describing the conditions, may have contributed to the variation which has been reported in protein quality and digestibility. The progress of breeding programs for improved quality may be retarded unless steps are taken to standardize these conditions (16).

The Role of Water Soluble Nitrogen Fractions of Cooked Beans

To get more direct evidence on the digestibility of protein in legume grains, metabolic studies were carried out with dogs (17). They were fed six species of legume grains as part of the diet, each species being the sole source of protein providing 1.8, 2.9, or 3.7 g of protein per kilogram per day. Calorie intake was adjusted to 120 kcal/kg per day. Each level was fed for 8 days to get two nitrogen balance periods of 7 days each. The reference protein used was casein. After the 8-day trial, endogenous nitrogen excretions were determined by feeding a nitrogen-free diet. From the analysis of feces, urine, and food intake, apparent and true protein digestibility were calculated. The apparent protein digestibility for each species is shown in Fig. 2. The curves in the upper section of the figure are *P. vulgaris* of various seed coat colours. The lower graph represents three other species. The results show in both cases a significant difference in digestibility between the legume grains and casein. There are also differences between species, which persist independently of protein intake. In all species, protein digestibility increased with increased protein intake. In the upper group, red beans, and in the lower group pigeon peas, showed unexpected results in comparison with the other samples.

Table 10. Amino acid availability and protein digestibility in *P. vulgaris* (8).

Amino acid	Raw	Heated
Methionine	21.8	68.7
Cystine	36.6	80.6
Lysine	58.5	85.0
Leucine	47.6	85.7
Valine	46.0	84.8
Protein digestibility, %	43.5	80.9

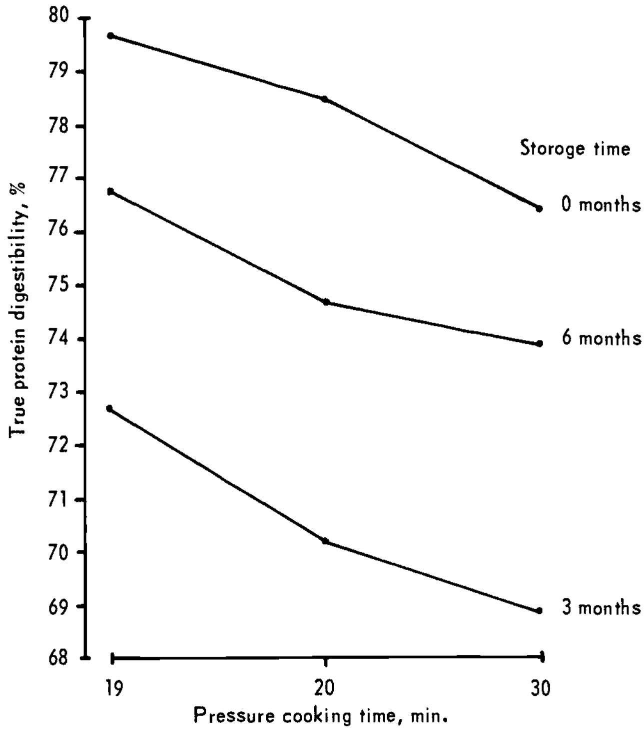

Fig. 1. Effect of storage time and cooking time on the protein digestibility of *P. vulgaris* (black).

Table 11. Fractionation of the legume grain nitrogen and of fecal nitrogen to water soluble and water insoluble nitrogen (17).

Protein source	In precooked legume grain		In feces	
	Water soluble N, %	Water insoluble N, %	Water soluble N, %	Water insoluble N, %
Black bean	15.2	84.8	35.3	64.7
Red bean	17.1	82.8	33.2	66.8
White bean	23.3	76.7	45.8	55.0
Casein	3.8	96.2	52.9	47.1
Soybean	57.4	42.6	43.7	56.2
Cowpea	40.9	59.1	49.1	50.9
Gandul	18.2	81.8	35.7	64.3
Casein	3.8	96.2	59.7	40.3

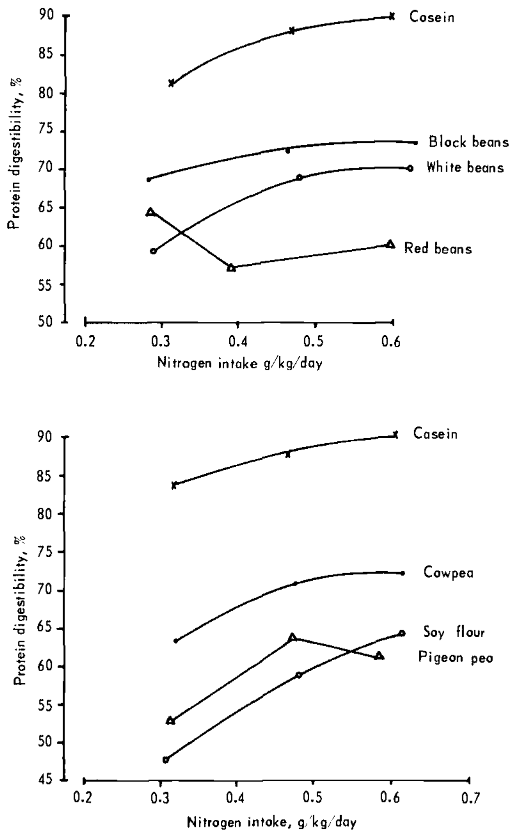

Fig. 2. Effect of protein intake on protein digestibility of some legume grain species.

During the last balance period, the feces were fractionated into water-soluble and insoluble nitrogen. The same type of nitrogen fractionation was done on the cooked legume grain flours. The results are presented in Table 11. Water-soluble nitrogen in the cooked flours ranged from 15.2% in the black bean to 57.4% in soybeans. The highest values occurred in soybean and cowpea. Insoluble nitrogen ranged from 42.6% in soybean to 84.8% in black beans. In feces, water-soluble nitrogen varied from 33.2 to 49.1% in the legume grains, and the insoluble nitrogen varied from 50.9 to 66.8%.

Using these figures, or the absolute amounts in the ingested and fecal nitrogen, the digestibility of the soluble and insoluble nitrogen fractions was calculated. These results are shown in Table 12. The values indicate that, with the exception of soybeans and cowpeas, digestibility of water-soluble nitrogen fraction was significantly lower than that of the insoluble nitrogen fraction. These in turn were higher than digestibility based on total nitrogen. These results were interpreted to mean that the protein that is more resistant to digestion is found in the water-soluble nitrogen fraction of precooked bean flour. It influences the digestibility of the fraction itself and of the

Table 12. Digestibility of the water soluble and water insoluble nitrogen of various legume grains (17).

	Apparent digestibility, %		
Protein source	Soluble N	Insoluble N	Total
Black bean	36.4	79.9	73.4
Red bean	14.2	64.3	60.1
White bean	41.2	78.7	70.3
Casein	—	94.5	89.1
Soybean	72.7	52.6	64.2
Cowpea	66.7	69.5	61.1
Pigeon pea	22.5	76.0	72.0
Casein	—	96.0	90.0

Table 13. Protein quality of various species of legume grains evaluated with and without their cooking broth (18).

Legume grain[a]	Avg wt gain[b], g Cooking broth (with)	Avg wt gain[b], g Cooking broth (without)	PER[c] Cooking broth (with)	PER[c] Cooking broth (without)
P. vulgaris (black)	6	29	0.20	0.88
P. vulgaris (red)	8	10	0.31	0.48
V. sinensis (snap pea)	32	52	1.04	1.24
C. cajan (pigeon pea)	36	51	0.98	1.46
G. max	94	113	1.69	1.80

[a]Cooking conditions: Soaking, 18 h, 15 psi, 20 min, and 121 °C; water–beans, 3:1 ratio.
[b]Twenty-eight days. Elias and Bressani (18).
[c]PER, protein efficiency ratio.

total nitrogen in the legume grain.

This interpretation, however, must be considered with some reservations. One limitation to this approach is that both the water-soluble and insoluble nitrogen fractions in the ingested bean may have undergone changes in solubility characteristics and are not identical in feces.

Therefore, a better identification is required in future work. However, the digestibility of the soluble nitrogen fraction is highly correlated with dry matter digestibility and nitrogen balance. The results of other studies seem to support the interpretation indicated above. In our laboratories for example, Molina and co-workers (15) found a negative relation between protein digestibility and a nitrogen fraction soluble in sodium chloride solution of cooked bean flours. Although the solvent used is different to that used for the dog study, the solubility values are similar. More recently Elias and co-workers (18) found (Table 13) that the protein quality of legume grains was higher when the cooking water is removed than when it is left with the grain. All samples were processed as shown at the bottom of the table. The extent of improvement in both weight gain and protein quality is in general greater with *P. vulgaris* than with cowpeas, soybeans, or pigeon peas. In view of these observations, in other studies the protein in the cooking broth was assayed for its protein digestibility, alone or together with the cooked grain. The results (Table 14) show that the lowest digestibility was that of the protein present in the broth. Although the differences between the values of beans with and without the cooking broth are small, it should be pointed out that it is difficult if not impossible to remove all cooking broth from cooked beans.

The Role of Proteins Resistant to Enzymatic Hydrolysis

Some studies have suggested that legume foods contain protein fractions that are resistant to proteolysis. For example, Jaffé and Hanning (19) found several purified bean protein fractions resistant to the hydrolytic activity of pepsin and papain. This observation could not be explained by the presence of trypsin inhibitor. In more recent studies, Seidl et al. (20) isolated a kidney bean globulin fraction that was resistant to hydrolysis by pepsin, trypsin, chymotrypsin, papain, ficin, hurain, and subtilisin. To isolate this fraction, a salt extract from beans was dialyzed against acetate buffer at pH 4.5. After denaturation of the globulin by heat or urea, only slight hydrolysis by the enzymes could be detected. Furthermore, the activity of all the enzymes on their respective substrates was inhibited by the bean globulin. This fraction amounted to about 30% of the extractable protein.

Referring to the studies on dogs in the previous section (17), the nitrogen fraction in feces named water-soluble is really a fraction soluble in salt solution because of the relatively high salt content of feces. This fraction occurred in feces in

Table 14. Protein digestibility of *P. vulgaris* evaluated with and without the cooking broth (12).

Sample	Protein digestibility, %
Cooking broth (alone)	65
Beans with cooking broth	72
Beans without cooking broth	75

Table 15. Protein quality and digestibility of black beans and its mutant white beans (*P. vulgaris*).

Legume grain[a]	Weight gain g/28 days	PER	Apparent digestibility
Black beans S-187 N (*P. vulgaris*)	33	1.16	75.0
White beans NEP-White 2 (*P. vulgaris*)	67	1.72	83.4

[a]Cooked in the autocalve at 121 °C (16 lb).

Table 16. Effect of seed coat colour on protein digestibility.

	Protein digestibility, %	
	Dogs[a]	Rats[b]
P. vulgaris (black)	72.5	68.9
P. vulgaris (red)	57.1	67.1
P. vulgaris (white)	68.9	71.2

[a]Bressani and Elias (second background paper in this book).
[b]Jaffe and Flores (11).

amounts ranging from 33.2 to 49.8%, and had digestibilities ranging from 14.2 to 72.7%, depending on the legume food studied. The lowest value was from red beans (*P. vulgaris*) and the highest from soybeans. It is possible that this nitrogen is the globulin fraction inhibiting proteolysis, and therefore, reducing protein digestibility.

Role of Seed-coat Pigments

Recent studies with bird-resistant sorghum have indicated that the pigments providing resistance also reduce the protein quality of the grains (21). Free phenolic compounds are common components in materials derived from vegetable products (22). Legume grains are no exception, and those commonly consumed are characterized by differences in the colour of the seed coat. Some evidence with respect to the colour of the seed has been obtained (23). Table 15 shows the protein digestibility of a white mutant (NEP 2), obtained by Co-60 irradiation of a black-coated bean, San Fernando variety. The digestibility was higher for the white-coated mutant than for the black-coated bean. Extensive chemical analysis including trypsin inhibitor activity, hemagglutinin concentration and amino acid analysis showed both to be essentially alike. This suggests that the pigments of the seed coat might be responsible. If the seed coat contained phenols or tannins, these could possibly react with the protein, decreasing its digestibility. Results from other studies are shown in Table 16.

The values indicate that black beans have a higher protein digestibility and red beans the lowest of the group tested, both in dogs and rats. This effect was also observed in the digestibility of the dry matter ingested, with red-coated beans giving lower values than white- and black-coated beans (11, 17). The cause-effect relation is, however, not as simple, as other factors apparently play a role, such as length and conditions of storage, altering results.

Additional evidence is shown in Table 17 by data from our laboratories (24), which show that beans contain up to 17% lignified protein. If this is not digested, obviously protein digestibility will decrease. As indicated before, storage conditions influence the amount of protein that becomes lignified both in the seed coat and in the cotyledons, which will affect protein digestibility. Tannin content in faba beans has been reported to vary from 0.34 to 0.50% expressed as gallic acid equivalent, and from 0.34 to 0.46% as tannic acid equivalent (25). Values for common beans are not yet available.

Finally, in this respect it is of interest to indicate that an alcoholic extract of black beans inhibited growth performance in rats fed a casein diet to which the extract had been added (26).

The examples given, although small in number, probably have some significance in the context of the problem of protein digestibility of legume grains, and more studies should be carried out to corroborate the information presented.

Furthermore, these data demonstrate that knowledge of the causes of the low digestibility of

Table 17. Content of "lignified protein" in *P. vulgaris* (black-coated) (24).

Condition	Lignified protein (%) of total protein: Cotyledon	Seed coat
Stored at 4 °C (9 mo)	9.2	29.5
Stored at 25 °C (9 mo)	17.2	44.2

Table 18. Rate of passage of food residues from soybean protein and casein through the GI[a] tract of pigs at 4 or 10 weeks of age (28).

Diet	Age, weeks	
	4	10
Soybean protein		
Mean rate of passage, hours	19	45
Range	14–24	40–50
Casein		
Mean rate of passage, hours	42	45
Range	36–48	40–50

[a]GI, gastrointestinal.

legume grain protein is not readily obtained, and that the problem is more complex than it appears to be. New approaches to the problem must be developed.

The Role of the Rate of Passage of Food Residues

The rate of passage of food residue through the alimentary tract has been shown to be related to the type of diet fed (27). It is well accepted that the actual acid buffering capacity of a food is dependent chiefly on its protein content. Studies in gastric secretion have shown that soybean protein is capable of maintaining gastric pH above 3.0 for about 60 min longer than milk, fish, egg, or animal meat. It has been suggested that rate of passage is related to the difference in gastric pH response to diet. Therefore, if there is a buffering action of the protein, the activation of proteolytic enzymes would be delayed, reducing the digestibility of the protein. Two popular observations seem to provide some suggestions that bean intake produces acidity in many individuals, and that there is an increase in frequency and amounts of evacuation. Both conditions will decrease protein digestibility. The results of studies by Maner et al. (28) shown in Table 18 indicate that soybean protein had a mean rate of passage in young rats of 19 h compared to 42 h when casein was fed. This difference was age-dependent, as it was not found in older rats. In children, Rosales Arzu (5) found (Table 18) an increase in the number of evacuations as well as in their weight when they came from beans as compared to milk. This increase in both number and weight also involved greater loss of fecal nitrogen, resulting in lower protein digestibility coefficients.

These observations suggest, therefore, that the proteins in legume grains may have a lower digestibility through the action that all fractions, or a specific one, may have on gastric pH and on rate of passage through the gastrointestinal tract.

Table 19. Average number of weight of evacuations of children fed milk and beans (*P. vulgaris*) (5).

Diet	No. evacuations	Weight of evacuations, g
Milk	8	70
Beans	14	148

However, among habitually bean-consuming populations, the undesirable effects of bean consumption, such as acidity, flatulence, and the number and amount of evacuations, seem to be reduced, suggesting some type of adaptation to bean intake. The lesser effects may also be due to the way in which beans are prepared for consumption.

Conclusions

From the results presented it may be concluded that in legume grains there are at least four conditions that control digestibility of the protein. These are shown in Fig. 3. The evidence indicates that some species of legume foods when raw have a low protein digestibility. However, this is not a general characteristic for all species. This low digestibility is caused mainly by the trypsin inhibitors and hemagglutinin compounds. The result of the destruction of their inhibitory activity by heat is an increase in protein digestibility. The extent of improvement depends on the method used to prepare them for cooking, on the control of heat in terms of temperature, pressure, and time. As before, conditions vary with the age and the species of legume grain. Heat destroys the action of the inhibitors, and as they contain essential amino acids, particularly cystine, the effect is also to increase amino acid availability.

An additional increase in protein digestibility probably results from destruction of the tertiary structure of certain proteins, which offer resistance to enzymatic hydrolysis. The presence of this type of protein is probably a common feature of most vegetable proteins. Likewise, increased digestibility may result from cellular wall breakdown.

Finally, further increase in protein digestibility can be obtained by minimizing, controlling, or destroying the effects of protein-complexing substances in legume grains. Although it is recognized that more evidence should be obtained on all aspects, particularly the last one, the

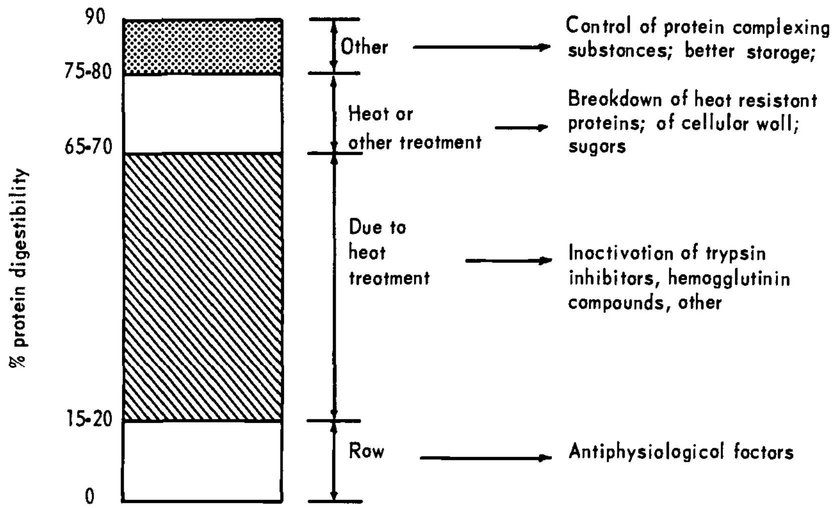

Fig. 3. Possible factors influencing the protein digestibility of legume.

evidence shows that poor storage conditions, colour of the seed coat suggesting the presence of phenolic compounds, phytic acid, sugars, saponins, and possibly other compounds, may inhibit protein digestibility and therefore, amino acid availability, reducing the efficiency of protein utilization.

Table 20 shows the practical significance of improved protein digestibility in legume grains and in vegetable proteins. The calculation shows the decrease in amount of wasted protein or wasted agricultural land that results from improved protein digestibility. Assuming an average yield of beans of about 1000 kg/ha with a protein content of 23%, the land provides 230 kg of protein. Using a protein digestibility value of 64% as obtained from human studies, and an improved protein digestibility value of 84% for the 230 kg of protein produced, 147 and 193 kg will be absorbed, respectively. This means that 83 and 37 kg of protein will be wasted in feces. The wasted protein is equivalent to 360 and 160 kg of beans, which indicates that from beans on 1 ha of land only 640 kg are of value for the low protein digestibility case, but 840 kg are of value when the digestibility increases. The picture is not complete, as it shows only the loss due to poor protein digestibility. However, if this is improved there are good possibilities to reduce protein losses in urine, through increased protein absorbed. This is shown at the bottom of the table. For this calculation, it was assumed that the amino acid pattern absorbed with digestibility 84% is the same as that with digestibility 64%. It is certain that urinary nitrogen will increase to some extent; however, for present purposes it was left the same. On this basis, nitrogen retention will increase from 37 to 82 mg/kg per day, indicating a more efficient use of the protein produced from 1 ha of land on beans. The urinary loss can also be reduced significantly by increasing the concentration of methionine in bean protein. However, improvement in the digestibility of the protein will also give a similar effect. Therefore, the understanding and solution of the problem of the low protein digestibility of legume foods, and of all vegetable proteins, is of great practical

Table 20. Efficiency of land utilization in terms of the protein from beans (*P. vulgaris*).

	Protein digestibility, 64%	Protein digestibility, 84%
Yield of beans/ha, kg	1000	1000
Yield of protein/ha, kg	230	230
Protein absorbed/ha, kg	147	193
Protein waste/ha, kg	83	83
Waste as beans/ha, kg	360	160
% land poorly utilized	36	16

NI[a]	FN[a]	UN[a]	NA[a]	NR[a]	NI	FN	UN	NA	NR
mg/kg per day					mg/kg per day				
227	81	109	146	37	227	36	109	191	82

[a]NI, nitrogen improved; FN, fecal nitrogen; UN, urinary nitrogen; NA, nitrogen absorbed; NR, nitrogen retention.

importance in terms of increasing the efficiency of land utilization for food production, particularly in view of the need to feed an increasing world population and of the position vegetable proteins are already occupying as food in the diet of the human population.

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