

**PROTEIN DIGESTIBILITY OF METHIONINE
SUPPLEMENTED COMMON BEANS (*Phaseolus vulgaris*)
IN ADULT HUMAN SUBJECTS¹**

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SUMMARY

The study reports on the protein digestibility of five cultivars of common beans (*Phaseolus vulgaris*) of different color, tested in young adult human subjects, as the sole dietary protein source. Cheese protein was used as reference protein. The cooked beans provided 0.65 g protein/kg/day, and energy intake was adjusted to 45 kcal/kg/day. Results indicated apparent protein digestibility to vary from 49.6 to 62.1%^o. White colored beans showed the highest value, while black beans gave the lowest. Cheese protein showed an apparent protein digestibility of 76.2%^o. The true protein digestibility was calculated using the endogenous nitrogen excretion values obtained in the study, as well as literature values. Digestibility increased as expected, but it is still low as compared to other protein sources. A high correlation was found between dry matter digestibility and protein digestibility.

Fecal nitrogen was fractionated between soluble and insoluble nitrogen in a 0.02 N NaOH solution. This assay was also carried out in the cooked beans, and findings revealed that the soluble nitrogen fraction was highly correlated with protein digestibility, with a correlation coefficient of -0.94. This fraction, still to be identified,

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could very well be responsible for the low digestibility values found for common beans in human subjects.

INTRODUCTION

From the chemical composition point of view, it is well documented that food grain legumes have a relatively high nutritive value as compared to cereal grains, foods of great significance in the diet of large population groups in Latin America and other parts of the world.

Various investigators have reported on the protein digestibility of food legumes in general. Most of the work has been conducted in experimental animals and shows that apparent protein digestibility of food legumes is relatively low (1-4). The reasons for such low values have not been established, although various hypotheses have been proposed, among them, the presence of residual trypsin inhibitors and lectins (5), as well as of proteins resistant to enzymatic actions (6, 7). Other reasons are the rapid transit time in the intestinal tract due to fiber and fermentable carbohydrate (8) and to the action of polyphenolic compounds in common beans (9).

The protein digestibility values of common beans, as obtained from human subjects are not readily available, and because of their nutritional importance in human diets, it is of practical interest to address this problem.

MATERIALS AND METHODS

Human Subjects

For the digestibility studies, 12 adult male human subjects were selected on the basis of overall good health as determined by physical, medical, and parasitological examinations. Their height and weight are described in Table 1. The subjects performed their normal activities throughout the study, which included laboratory analysis, maintenance and messenger duties. The 12 subjects were assigned to three groups of four each according to body weight, which on the average did not differ more than 0.3 kg.

Experimental Design

The digestibility study was performed in two assays with an interval of four months between assays. The experimental design was a modified latin square as shown in Table 2. Each assay was developed in four continuous experimental periods. During the first three, each group of individuals was fed the protein source randomly assigned. In both studies the experimental periods lasted five days in the first assay and six days in the second, of which two and three were for adaptation, respectively, and three days for fecal and urinary collections. During the fourth period, all subjects were fed a low nitrogen-containing diet for three days, collecting feces and urine during the last two days. Therefore, the assays

TABLE 1

PHYSICAL CHARACTERISTICS OF THE EXPERIMENTAL SUBJECTS

Subjects	Age	Height cm	Phase I Weight, kg ¹		Phase II Weight, kg ²	
			Initial	Final	Initial	Final
CE	28	164	64.7	63.6	66.4	65.2
FM	25	157	55.4	53.4	55.7	53.4
AO	29	158	52.7	51.3	50.9	49.5
VR	19	164	48.6	48.1	50.1	49.3
AG	30	169	58.1	58.4	58.0	58.4
MR	29	167	58.1	57.1	61.3	60.1
OB	23	157	55.0	53.5	58.2	57.2
RS	22	157	53.4	53.2	56.1	56.3
MM	32	160	59.0	58.8	59.5	59.5
SE	19	167	55.7	54.3	57.7	55.4
JP	23	167	56.4	57.1	59.6	59.0
RC	32	157	49.9	48.8	49.7	48.0
$\bar{x} \pm SE$	30	162	55.5 ± 1.2	54.7 ± 1.2	56.9 ± 1.4	55.9 ± 1.48
SD			4.29	4.42	4.89	5.15
Coefficient variation			7.73	8.08	8.59	9.21

1 $t = 0.20$ NS.

2 $t = 0.21$ NS.

lasted a total of 18 and 21 days, respectively. Protein intake was adjusted to 0.65 g/kg/day and calories to 48-50 kcal/kg/day. During the first assay, the red and black beans from Jalpatagua and Jutiapa, as well as cheese were used, the latter as reference protein. In the second one, a white and a black cultivar from Ipala, and a 50:50 mixture by weight were fed. All bean samples were first soaked in water for 18 hours, using a ratio of water:beans of 3:1. The soaked beans with the soaking water were then cooked for 30 min at 15 lb pressure (121°C), and the cooked beans were ground with the cooking water in a Waring Blender. From this lot, weighed portions to provide 0.65 g protein per kg/day were placed in polyethylene containers refrigerated in a cold room, at -4°C, and removed daily as needed. A similar and representative sample was used for chemical analysis. As stated, the control protein was white cheese prepared commercially and it was handled for feeding purposes as the bean purée. Linear regression and other statistical analyses of the data were performed according to Snedecor (10) and Cochran and Core (11).

Diets

The basal low protein diet is shown in Table 3 and, as may be observed, it was made up of foods which provided variety, supplemental

TABLE 2

EXPERIMENTAL DESIGN UTILIZED TO DETERMINE PROTEIN DIGESTIBILITY OF RED AND BLACK BEAN CULTIVARS (*Phaseolus vulgaris*)¹ WITH COOKING BROTH, AND OF A CONTROL PROTEIN (ASSAY I), AND OF WHITE AND BLACK BEAN CULTIVARS (*P. vulgaris*)¹ WITH COOKING BROTH (ASSAY II), AS WELL AS BEAN COLOR MIXTURES

Assay I					
Experimental subjects		Experimental period			
No.	Identification	1	2	3	4
4	(CE, FM, AO, VR)	C	R	B	LND
4	(MM, SE, JP, RC)	R	B	C	LND
4	(AG, MR, OB, RS)	B	C	R	LND
Days in experimental period		5	5	5	3
Days in balance		3	3	3	2

Assay II					
4	(CE, FM, AO, VR)	W	B:W	B	LND
4	(MM, SE, JP, RC)	W:B	B	W	LND
4	(AG, MR, OB, RS)	B	W	B:W	LND
Days in experimental period		6	6	6	3
Days in balance		3	3	3	2

Bean protein supplemented with 0.5 g o/o DL-methionine.

B = Black bean.

W = White bean.

R = Red bean.

C = Control protein (fresh cheese).

W:B = White:black mixture 50:50.

LND = Low nitrogen diet.

nutrition and contained low nitrogen levels. This diet provided most of the calories intake, which was adjusted to the individuals' need with starch cookies, candy and soft drinks, after taking into consideration the energy contributed by the beans fed at 0.65 g protein/kg body weight/day. Beans were provided in three equal quantities for breakfast (7:00 a.m.), lunch (12:00) and dinner (5:00 p.m.). Each bean portion was fried with 25 g of oil and 10 g of chopped fresh onion before feeding. With each bean portion fed, the subjects also ingested a capsule containing the equivalent amount of DL-methionine added at the level of 0.50/o of the ingested protein. This level was calculated on the basis of the level found to give maximum protein quality improvement in rat studies (4, 12). Besides consuming the foods in the diets indicated above, all subjects were given a complete vitamin and mineral capsule.

TABLE 3
INGREDIENTS OF THE LOW-PROTEIN BASAL DIETS

Ingredients	Grams
Soluble coffee	3
Sugar	25
Apple jelly	50
Bread ¹	300
Margarine	60
Soup ²	240
Guisquil (Chayote)	100
Cooked pineapple	100
Apple (with skin)	100
Artificial fruit beverage	4 glasses
Mineral and vitamin supplement ³	1 tablet
Calorie sources to fill the requirements:	
Carbonated drinks (Pepsi-Cola, T.M. ³)	1
Refreshment	Variable
Cookies ¹	Variable
Candies	Variable

- 1 Prepared with wheat starch (Jolly Joan. Ener-G Foods Inc., P. O. Box 24723, Seattle, WA 98124, USA).
- 2 Corn starch based (Maizena Duryea. Productos de Maíz y Alimentos, S. A., Km. 3-1/2 Carretera al Atlántico, Guatemala, C. A.) and margarine, seasoned with aromatic herbs. Consumed without the herbs.
- 3 UNICAP-T, T.M. previously described (15).

Fecal Analysis

The fecal samples collected were homogenized in a Waring Blender with a fixed level of water, and a representative sample was withdrawn for dry matter analysis by dehydration (corrected for the water added) and for nitrogen, by the Kjeldahl method (13). The nitrogen content of feces was fractionated into soluble and insoluble N by extracting a weighed sample with 0.02 N NaOH followed by centrifugation and analysis of nitrogen in the residue and in the extract. The same procedure was applied to a cooked bean sample.

RESULTS

The nitrogen balance data of the subjects fed the different bean cultivars and the control protein, fresh cheese, are presented in Table 4. At the level of nitrogen intake—which varied from 106.7 to 110.1 mg per kg body weight per day (0.67 - 0.69 g protein/kg/day)—all nitrogen sources

TABLE 4

**NITROGEN BALANCE OF HUMAN SUBJECTS FED VARIOUS BEAN
CULTIVARS AND FRESH CHEESE**

Nitrogen source	Nitrogen, mg/kg/day				
	Intake	Fecal	Urine	Absorbed	Retained
Fresh cheese	110.1±0.59	25.8±1.5	80.8±3.7	83.8±1.8	3.4±3.7
Red bean (J)	106.7±0.34	47.2±5.0	81.8±6.1	59.6±4.9	-22.2±6.8
Black bean (J)	106.8±0.53	53.8±3.1	75.7±3.5	52.9±3.1	-22.6±3.8
White beans (I)	109.6±0.52	41.4±3.1	87.2±5.5	68.1±3.4	-19.0±7.2
White:black beans (50:50)	108.4±0.30	46.0±2.8	81.2±6.2	62.3±3.0	-18.9±8.1
Black bean (I)	108.1±0.52	50.3±2.2	80.2±5.3	57.7±5.3	-22.8±6.4
Low nitrogen diet	19.8±1.2	25.8±1.4	47.7±2.7	—	—

with the exception of fresh cheese, gave negative nitrogen balances. This result was due to the very large loss of nitrogen in the feces of subjects fed beans as compared to the fecal nitrogen loss when fed cheese. It should be pointed out that fecal nitrogen loss from the basal low nitrogen diet was the same as that from fresh cheese. On the other hand, urinary nitrogen loss was less variable among nitrogen food sources, with bean values varying between 75.7 to 87.2 mg/kg/day, and 80.8 from fresh cheese. On the basal low nitrogen diet, the nitrogen urinary loss was slightly less than half when compared to that from the food nitrogen sources under study.

Apparent protein digestibilities values are shown in Table 5. Apparent protein digestibility was 76.20% for fresh cheese, while for the bean cultivars studied, the values ranged from 49.60% for the black coated Jalpatagua cultivar, to 62.10% for the white bean. The 50:50 blend of white and black gave a protein digestibility of 57.40%, similar to that expected from the average of the white and black (I) bean cultivars. Using the fecal nitrogen excretion from the basal low nitrogen diet, true protein digestibility was also calculated (Table 6). The value of fresh cheese was 100% and ranged from 73.8 to 85.80% for the bean samples under study. The Table also shows the true protein digestibility values using an endogenous nitrogen loss of 12 mg/kg/day. As the data show, values are lower than those obtained when using 25.8 mg, the value from this study, since this nitrogen is added to the fecal nitrogen loss. This can be taken as added proof of the low protein digestibility of beans.

TABLE 5

AVERAGE VALUES¹ FOR APPARENT NITROGEN DIGESTIBILITY OF DIETS BASED ON FRESH CHEESE AND BEANS IN ADULT MEN

Experimental subjects	Red beans (R)	Black beans (Jalpatagua) (BJ)	White beans (W)	White:black (50:50) beans (W:B)	Black beans (Ipala) (BI)	Cheese (C)
CE	83.4	55.2	66.6	60.1	47.8	82.2
FM	53.2	47.3	52.9	50.8	55.9	77.8
AO	45.9	50.4	47.6	55.3	44.5	72.2
VR	74.9	46.6	82.6	82.9	64.5	77.6
AG	51.5	39.9	59.0	46.3	54.6	72.2
MR	42.7	40.6	50.1	49.9	42.7	67.6
OB	39.2	71.3	57.8	57.9	48.0	73.2
RS	58.6	63.5	58.7	54.6	61.7	75.2
MM	46.6	45.4	61.7	53.0	48.3	69.1
SE	82.0	44.5	76.0	58.9	55.6	78.9
JP	35.3	35.7	64.9	61.4	54.1	77.4
RC	55.4	55.9	67.5	58.4	62.7	84.1
$\bar{x} \pm SE$	55.7 \pm 4.6	49.6 \pm 2.9	62.1 \pm 2.9	57.4 \pm 2.6	53.4 \pm 2.1	76.2 \pm 1.4
SD	16.2	10.2	10.1	9.1	7.2	4.9
o/o Coef. var.	29.1	20.6	16.3	16.0	13.5	6.5

1 F = 9.45**.

Duncan: C W W:B R BI BJ.

TABLE 6

AVERAGE TRUE PROTEIN DIGESTIBILITY

Protein source	Apparent protein digestibility o/o	True protein digestibility, o/o	
		1	2
Cheese	76.2	100.0	87.5
Red beans	55.7	79.9	67.0
Black beans (J)	49.6	73.8	60.9
White	62.1	85.8	73.2
W:B (50:50)	57.4	81.4	68.6
Black beans (I)	53.4	77.4	64.6

1 Using 25.8 mg N/kg/day as endogenous nitrogen excretion.

2 Using 12.0 mg N/kg/day as endogenous nitrogen excretion.

Table 7 summarizes the dry matter digestibility of the diets fed, and ranged from 85.9 to 92.0% for all food nitrogen sources. Only the fresh cheese value was statistically different from those of the bean samples, which were very similar among them. A positive highly significant correlation was found between protein and dry matter digestibility ($Y = -182.52 + 2.74X$) ($r = 0.74$).

The fecal nitrogen from the subjects fed red beans, one black bean (J), cheese and the nitrogen low basal diet was fractionated into soluble and insoluble and average values are given in Table 8. While the soluble and insoluble fecal nitrogen from the low-nitrogen diet and the cheese diet were essentially the same, they were from 1.5 to twice as large in the feces of the subjects fed beans. From the nitrogen balance data the relationship between nitrogen intake and nitrogen retention was calculated by linear regression analysis (14), using two points. One, when the subjects were fed the low-nitrogen diet and the second, when they were fed the bean-containing diet at an intake of 0.65 g protein/kg/day assuming that the response was linear (Table 9). The regression coefficients were low and were correlated with the NPU calculated from the nitrogen balance at 0.65 g protein/kg/day and when the low protein diet was fed divided by nitrogen intake.

DISCUSSION

The results of the present research with young adult human subjects, confirm previous observations carried out with experimental animals (1-4) on the low digestibility of bean protein, and of other studies reported from human subjects (15). It is possible that because of this low digestibility none of the subjects of many of the bean diets consumed

TABLE 7

PERCENTAGES¹ OF DRY MATTER DIGESTIBILITY² OF BEAN AND CHEESE-BASED DIETS IN ADULT MEN

Experimental subjects	Red beans (R)	Black beans (Jalpatagua) (BJ)	White beans (W)	White:black (50:50) beans (W:B)	Black beans (Ipala) (BI)	Cheese (C)
CE	82.3	87.3	90.6	87.8	87.9	97.5
FM	95.9	84.3	86.5	84.5	87.7	92.5
AO	83.6	83.7	85.6	85.7	87.8	93.2
VR	87.9	85.9	94.2	94.4	87.9	92.5
AG	82.9	81.9	90.3	86.2	87.2	91.5
MR	82.7	86.7	87.7	89.6	86.3	90.1
OB	82.0	93.7	88.7	88.9	87.2	92.0
RS	85.9	90.4	88.0	86.5	88.3	91.5
MM	82.3	80.6	86.9	85.3	84.1	90.5
SE	95.9	85.9	87.5	88.8	85.9	93.0
JP	83.6	83.3	90.3	89.4	86.6	93.0
RC	87.9	88.2	90.4	87.7	89.1	92.0
$\bar{x} \pm SE$	86.0 \pm 1.4	85.9 \pm 1.0	89.0 \pm 0.7	88.0 \pm 0.8	87.2 \pm 0.4	92.0 \pm 0.3
SD	5.03	3.65	2.3	2.66	1.35	0.97
o/o Coef. var.	5.8	4.2	2.5	3.0	1.5	1.0

1 F = 6.79**.

2 o/o digestibility = $\frac{\text{Dry matter intake} - \text{dry matter excreted}}{\text{dry matter intake}} \times 100$

DMS = 2.45 (p < 0.05).

C W W:B BI R BJ

TABLE 8

**FRACTIONATION OF FECAL NITROGEN FROM HUMAN SUBJECTS FED
COMMON BEANS, CHEESE AND A NITROGEN-LOW DIET**

Diet	Fecal nitrogen (mg/kg/day)		
	Total	Soluble	Insoluble
Nitrogen-low diet	24.4	17.8	6.6
Cheese	25.8	20.6	5.2
Red beans	47.2	35.7	11.1
Black beans (J)	53.8	37.8	16.0

reached nitrogen equilibrium, much less positive nitrogen balance even when intake was as high as 0.65 g P/kg/day. The results are even more surprising because of the accepted deficiency in sulfur amino acids in bean protein (12). Thus, the lack of response to methionine addition may have been due again to the low digestibility of the protein or to the addition of an inadequate amount of the amino acid. Various investigators have studied the effect of methionine supplementation to food legumes as a means to improve their efficiency of utilization. Zzulka and Calloway (16) showed that methionine supplementation of soy protein improved nitrogen balance when nitrogen intake from soy was 1.5 and 3 g/day with a total intake of 9 g N/day. However, when soy nitrogen intake was 6.0 g/day, no response was observed. This finding corroborates that the protein level of intake is important in determining the effect of methionine supplementation, as has been demonstrated quite often with rats. On the other hand, an excess of methionine supplementation can decrease nitrogen balance as showed by Scrimshaw and Young (17) with soy protein supplemented with 1.10% methionine. This is also a well known effect, since an excess of one amino acid may induce a deficiency of the second most limiting amino acid. The difference with the studies mentioned and the one reported in this paper is that soy protein is much more digestible than bean protein, and nitrogen balance was attained with a lower nitrogen intake than with that found with common beans, since even when intake was 0.65 g protein/kg/day, the subjects were not in equilibrium, much less in a positive nitrogen balance. This occurred in contrast to balance studies on subjects fed mixtures of corn and beans, wherein nitrogen equilibrium was reached when intake was 0.60 g protein/kg/day (15). Since the subjects were fed a low nitrogen diet and the bean diet at an intake of 0.65 g protein/kg/day, and since, theoretically, there is a straight line relationship between these two extremes, the relationship between nitrogen intake and nitrogen balance was estimated by linear regression analysis. The results are given in Table 9, and show that in order to obtain nitrogen equilibrium, an intake which varied from 155 to 174 mg/kg/day was needed as compared to an intake of 105 mg from cheese.

TABLE 9

REGRESSION BETWEEN NITROGEN INTAKE AND NITROGEN RETAINED IN ADULT HUMAN FED
BEAN-BASED DIETS (*Phaseolus vulgaris*)

Assay	Leguminous seed	NR = a + b N intake	r ¹	Necessary for NB mg/kg/día	D.F. ²	Level ³	NPU
I	Red bean	-59.03 + 0.34	0.66	174	10	*	29.3
I	Black bean (Jalpatagua)	-59.30 + 0.36	0.77	163	10	**	26.2
II	White bean	-61.42 + 0.38	0.66	158	10	*	29.4
II	White bean:black bean (50:50)	-61.47 + 0.39	0.62	155	10	*	30.0
II	Black bean (Ipala)	-60.6 + 0.35	0.65	172	10	*	26.3
I	Cheese	-62.78 + 0.59	0.92	105	10	***	51.7

- 1 Correlation coefficient.
 2 Degrees of freedom.
 3 Level of statistical significance:
 * Significant at 5^o/o.
 ** Significant at 1^o/o.
 *** Significant at 0.1^o/o.

The average true protein digestibility was calculated using the nitrogen excretion on a nitrogen-low diet (Table 6). As the data show, the average value obtained in the first assay was 24.7 ± 1.5 , and 26.9 ± 1.2 mg/kg/day in the second. Even with the value of the present study, which is high, the digestibility still remained low. If the value reported in the literature is used (14), around 12 mg/kg/day, true digestibility is lower than when using the value obtained in the present study.

In order to learn the reasons for the low digestibility of bean protein, the fecal matter nitrogen was fractionated into soluble and insoluble in 0.02 N NaOH solution. As indicated previously, both fractions in feces from beans were higher than those measured in feces from the low nitrogen diet or from the cheese diet. Previous studies with dogs (1) suggested that the soluble fraction was highly undigestible and, in the present study, the effect of this fraction on protein digestibility was analyzed by linear regression analysis. The calculation showed a regression of the soluble nitrogen fraction of protein digestibility of the beans to be $Y = 95.81 - 1.17X$, with a correlation coefficient of -0.94 , which is highly significant. This then suggests that such fraction is responsible for the low digestibility values observed. Assuming that part of the soluble nitrogen is from bacterial origin, one can therefore subtract this amount from the total fecal nitrogen from beans. Such a correction is equivalent to the calculation of true protein digestibility and it increases the digestibility value from around 60 to 75%, which is still low when compared to other nitrogen food sources. Two additional factors may be of interest. One is that white beans showed a higher digestibility than black and red colored beans. The second is that in these studies the beans were fed with their respective cooking broth, which has been shown to decrease protein digestibility and quality (9).

The chemical nature of the soluble nitrogen fraction in cooked beans and of the equivalent nitrogen fraction in the feces, as well as the nature of the seed coat color and cooking broth should, therefore, be studied in order to understand the reasons for the low digestibility values reported for food legumes, and in particular for common beans.

RESUMEN

DIGESTIBILIDAD PROTEINICA DEL FRIJOL COMUN (*Phaseolus vulgaris*) EN HUMANOS ADULTOS

El estudio informa acerca de la digestibilidad de la proteína de cinco cultivares de frijol común (*Phaseolus vulgaris*) de diferente color, los que se sometieron a prueba en adultos jóvenes como fuente única de proteína. Se utilizó proteína de queso como proteína de referencia. Los frijoles cocidos proporcionaron 0.65 g de proteína/kg/día, y la ingesta de energía se ajustó a 45 Kcal/kg/día. Los resultados indicaron que la digestibilidad de la proteína aparente varió de 49.6 a 62.1%; los frijoles blancos mostraron los valores más altos, y los frijoles negros, los más bajos. La proteína del queso acusó una digestibilidad de proteína aparente de 76.2%. La digestibilidad de la proteína verdadera se calculó utilizando los valores de excreción de nitrógeno endógeno obtenidos en el estudio, así como los valores citados en la literatura. Como se esperaba, la digestibilidad aumentó, pero aun así fue baja, comparada con otras

fuentes de proteína. Se encontró una alta correlación entre la digestibilidad de la materia seca y la digestibilidad de la proteína.

El nitrógeno fecal se fraccionó en nitrógeno soluble e insoluble en una solución de NaOH 0.02 N, lo que también se hizo con los frijoles cocidos. El fraccionamiento de nitrógeno soluble en estos últimos estaba altamente correlacionado con la digestibilidad de la proteína, con un coeficiente de correlación de -0.94 . Esta fracción, aún por ser identificada, podría muy bien ser la responsable de los bajos valores de digestibilidad encontrados en humanos para el frijol común.

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