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STUDIES ON THE PROTEIN DIGESTIBILITY OF COMMON BEANS (*Phaseolus vulgaris*) IN ADULT HUMAN SUBJECTS

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I. INTRODUCTION

Food legumes constitute the main protein source for many population groups in developing countries. In Latin America common beans (Phaseolus vulgaris) is the food legume of preference by the population, consumed by all socioeconomic groups, but mainly by the rural and low income populations. For these populations beans may provide up to 32% of the daily protein intake (1). Furthermore, the importance of beans to the diet resides in the fact that they increase the quality of the protein consumed which is mainly derived from cereal grains, such as corn and rice (2, 3). As is well known, these cereal grains are low in protein content, and are poorly utilized because of essential amino acid deficiencies, such as lysine, tryptophan and sometimes threonine. The complementary protein effect of food legumes to cereal protein is well eatablished. Food legume protein provides the deficient amino acids in cereal protein while the latter supplies the sulfur amino acid deficiency in food legumes (2, 3, 4). This effect is evident in Figure 1 for various examples between four different food legumes with either corn or rice. As shown there is a point of maximum protein quality which is close to a 7:3 mixture by weight. The protein from food legumes is even more important for diets based on tubers, as shown in Table 1 for an example with cassava and plantain flour. The rats on cassava, plantain flour or corn starch alone lost weight and around 20% of beans by weight were needed to promote growth (5).

Because of the importance of beans in diets as a source of important nutrients, many efforts are being made to improve their availability to the consumer by increasing and stabilizing production, and in imporoving its nutritive value for increased biologfcal utilization. In this respect, much attention has been given to the antiphysiological factors and to the sulfur amino acid deficiency in food legumes as important constraints in their

utilization, but very few efforts have been made to understand and solve the low digestibility of its protein, which if increased might result in increased biological available protein and nutritive value (5). This paper summarizes some of the results we have obtained in the study of the low digestibility of the protein of food legumes, in particular for common beans.

II. PROTEIN DIGESTIBILITY OF CEREAL/BEAN AND STARCHY FOOD/BEAN DIETS

As part of the program to establish protein requirements for human adults fed diets based on common beans and cereal grains and common beans and starchy foods, a series of nitrogen balance studies have been carried out with young adult human subjects. These studies were carried out with groups of 8-12 individuals fed variable levels of protein at a fixed and adequate intake of energy from the bean/cereal or bean/starchy food mixture. From the relationship between nitrogen intake and nitrogen retention, amounts of protein for nitrogen equilibrium were determined by simple regression analysis. Protein digestibilities were calculated when the individuals were consuming 0.6 g protein kg/day. A summary of the results is shown in Table 2. As can be seen, protein digestibility in diets based on beans and starch, beans and plantain and beans and cassava were 60.0, 52.5 and 55.7%, respectively (7, 8, 9, 10). In the starch study all the protein was derived from common beans, while in the study with cassava and plantain, some of the protein ingested came from these carbohydrate sources but the greatest amount came from beans. All digestibility values were low, and mainly representing that from bean protein. In the experiments with corn and rice shown in the lower section of the Table, apparent protein digestibility was also low, although somewhat higher than in the starchy food/bean studies. In the former, beans provided from 40 to 50% of the dietary protein and cereal grains the difference.

In the studies with rice the nutritional significance of two interventions were tested as shown in Table 3. One was to increase energy intake from 45 to 50 kcal/kg/day; this, however, did not change the results. Digestibility of the protein was still low. The second intervention was to add 10% skim-milk to the 60:40, rice:bean mixture. As shown in the Table, apparent protein digestibility increased, effect which was attributed to the high digestibility of milk protein (10).

Further indirect evidence that the low digestibility values are due to beans may be reached by observing the results of protein digestibility of cereal grains fed alone (11). These are shown in Table 4.

The Table shows the apparent protein digestibility of lime-treated corn and rice when fed to human subjects providing the only protein source of the diet at two levels of intake. For corn, the protein digestibility varied from 76 to 78% while that of rice varied from 79 to 82%, values significantly higher than those obtained when bean protein was part of the diet. Thus, the conclusion is that the low values are due to the poor utilization of bean protein, this becoming an important problem to solve.

III. SOME ANIMAL STUDIES

The low digestibility of food legumes, particularly in common beans, was pointed out by Jaffé many years ago (12), therefore, the results shown in Table 5 are really not new. The results in the Table were obtained with a total of 57 common bean cultivars (13, 14, 15). The average values on the samples classified by color of the seed coat, show white beans to have a digestibility of 76.6%, while red show a digestibility of 72.4%, black 71.5% and brown 70.7%. The overall figure is 72.7%. Although these values are higher than those reported previously for man, still they are low. The Table also shows the protein quality of the samples as NPR. It is interesting to

point out that the protein quality of the white beans is higher than that of the colored beans. Table 6 shows the correlation of protein digestibility to protein quality for the 57 samples grouped again by color and for all samples together. All correlations were not statistically significant. This was not expected since higher digestibility implies higher protein quality due to an increase in total available amino acids, that is an increased amount of amino acids are absorbed,unless bean proteins containing sulfur amino acids are not as digestible as other proteins in beans or the pattern absorbed is low in sulfur amino acids. Correlations by color, between protein quality and protein digestibility were not evident either. These data then confirm that protein digestibility in common beans is a problem and that color of the seed coat probably plays an important role.

IV. STUDIES WITH HUMANS

In order to confirm in humans the results obtained in rats, samples of beans of different color were selected for digestibility studies in humans (16). These materials were cooked in an autoclave at 15 lb pressure for 30 minutes after an overnight soak. The cooked samples were dried and analyzed for residual antiphysiological factors. These were low as expected. The subjects, 24 in total, were fed these bean preparation under standard digestibility test, at an intake of 0.65 g P/kg/day and 45 kcal/kg/day. Besides the 4 color samples the individuals were fed a 50/50 mixture by weight of a white and a black coated cultivar. The control protein in these studies was white cheese, also fed at a level of 0.65 g P/kg/day (16). The results are shown in Table 7. White beans had a digestibility of 62.1%, followed by the mixture white/black, the red, and the two black bean cultivars. All values were significantly lower than that found for the white cheese.

These results then agree very well with those from the rat studies, although the values are lower. Thus bean proteins are not altogether available to the human, and factors must be present responsible for the effect observed.

In an attempt to learn more about the reasons for the low values, the nitrogen in the feces was fractionated by a simple procedure of extraction with 0.02 N NaOH solution into two fractions, the soluble and insoluble nitrogen. The results are shown in Table 8 which indicate that the total nitrogen excreted, as well as the soluble and insoluble fractions of the total nitrogen are significantly higher when the protein source fed was common beans than when the protein source was cheese or a nitrogen low diet. The values for cheese and for the nitrogen free diet were essentially alike. It should be indicated that nitrogen intake was constant, therefore, this is not the cause of the higher fecal nitrogen excretion. No identification has been made as yet of the soluble and insoluble fecal nitrogen in 0.02 N NaOH.

The question is if the nitrogen excreted comes from specific protein fractions in the bean, and studies are now underway to test this possibility. Therefore, one possibility which could explain the low digestibility of bean protein is that certain fractions may be resistant to enzymatic action in the intestinal tract (17, 18).

V. EFFECT OF PROCESSING AND STORAGE

Other factors could also be responsible for the low protein digestibility of beans and one which is receiving attention is that of processing. Beans must be heat processed before consumption to destroy the well known antiphysiological factors. The results of various studies suggest that the process of destruction of these factors may affect protein digestibility.

An example of the effect of soaking time and of cooking time on protein digestibility is shown in Table 9. Both soaking time and cooking time were responsible for a small but consistent decrease in protein digestibility. The extent of the decrease is practically the same. Cooking time at all soaking times decreased digestibility from 72.4 to 68.9%, while soaking time at all cooking times decreased digestibility from 71.9 to 68.7%, a decrease in both cases of about 5% (19).

Storage time also plays a role in decreasing digestibility as shown in Figure 2, probably through an interaction with processing (19). As is known there is a tendency for beans to become hard-to-cook upon prolonged and improper storage conditions of high temperature and high relative humidity. Although the biochemical mechanism leading to this condition is not known, the cooking process may induce the formation of protein units resistant to digestion. Some evidence in this respect is shown in Figure 3. A salt-soluble protein fraction from cooked beans increases with respect to cooking time, at all times of storage. On the other hand, cooking time decreases protein digestibility, however, the higher decrease and lower digestibility took place in those cases with a higher amount of salt-soluble proteins to protein digestibility is negative with a correlation coefficient of -0.59, significant at the 5% level. These findings are in agreement with the studies carried out in humans and it may mean this fraction to be relatively indigestible contributing to the low digestibility values found. Studies along these lines were performed with growing dogs. The animals were fed white, red and black cooked beans as the sole dietary source (20) at a level of nitrogen intake of about 287 mg/kg/day. Table 10 summarizes some of the results. It shows the percentage soluble and insoluble nitrogen of the cooked beans fed. The results

indicate that the lower the digestibility obtained, the higher the soluble nitrogen fraction, and that the soluble nitrogen fraction itself was poorly digested as compared to the insoluble nitrogen fraction of the cooked beans.

VI. THE EFFECT OF POLYPHENOLS

A final problem under study is the role polyphenolic compounds may have on protein digestibility (16, 21). Table 11 summarizes the polyphenolic content of cooked beans as fed, expressed as tannic acid and as catechin, as well as the percentage digestibility in adult humans. It is evident that higher intakes of phenolic compounds going from white to black decrease digestibility at intake levels of nitrogen which remain relatively constant among the beans fed.

Regression equations between tannic acid and catechin to fecal nitrogen were calculated and these are shown in Table 12. The correlations are negative and statistically significant, confirming therefore, that polyphenolic compounds are factors also responsible for the low digestibility of the protein in beans.

VII. SUMMARY

The evidence available indicates that cooking increases protein digestibility, and the extent of the increase is affected by the processing conditions such as period of soaking and cooking time. However, if the conditions of processing are not exceeded the effects are small, and in the order of about 5-6%. Apparently, there is a protein fraction soluble in salt solutions which could be responsible to some extent in limiting higher digestibility, however, the effect of the fraction may be the same as that due to processing. Finally, polyphenolic compounds also play a role in affecting protein digestibility. The estimation is that these compounds may decrease

protein digestibility of beans about 10-12%. Adding all of this together the protein digestibility would be of the order to about 85%, leaving some 15% protein unaccounted for, when digestibility is expressed as apparent and the endogenous losses have not been considered. Protein digestibility of beans is an important question which must be solved in order to produce beans of greater nutritional value.

VIII. REFERENCES

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TABLE 1. AVERAGE WEIGHT GAIN OF THE ANIMALS FED WITH DIFFERENT FLOURS AND DIFFERENT LEVELS OF BEANS WITHOUT METHIONINE

Level of beans in diet	cassava	plantain	corn starch
0	-13.5	-11.6	-14.1
10	- 6.6	- 1.2	- 5.8
20	9.1	10.9	2.0
30	22.0	34.2	21.6
40	34.4	58.8	46.5

Average 8 animals.

TABLE 2. APPARENT PROTEIN DIGESTIBILITY OF BLACK BEANS (Phaseolus vulgaris) FED WITH STARCHY FOODS AND CEREAL GRAIN IN HUMAN SUBJECTS

Food mixture	Nitrogen intake mg/kg/day	Apparent protein digestibility, %
Beans/Starch	115.6 ± 0.9	60.0 ± 2.2
Beans/Plantain	117.4 ± 0.6	52.5 ± 4.0
Beans/Cassava	105.7 ± 0.2	55.7 ± 2.0
Beans/Corn	127.6 ± 0.5	61.0 ± 9.0
Beans/Rice	102.5 ± 1.1	59.1 ± 7.4

TABLE 3. APPARENT PROTEIN DIGESTIBILITY OF BLACK BEANS (Phaseolus vulgaris) FED WITH RICE AND MILK IN HUMAN SUBJECTS

Food Mixture	Nitrogen intake mg/kg/day	Apparent protein digestibility, %
Beans/Rice (45 kcal)	102.5 ± 1.1	59.1 ± 7.4
(50 kcal)	102.4 ± 1.1	59.6 ± 6.4
Beans/Rice/Milk (45 kcal)	104.5 ± 2.9	65.3 ± 7.6
(50 kcal)	104.4 ± 3.0	64.6 ± 10.2
Milk	101.7 ± 1.2	75.6 ± 2.0

TABLE 4. APPARENT PROTEIN DIGESTIBILITY OF LIME-COOKED CORN AND RICE IN HUMAN SUBJECTS

Cereal grain	Nitrogen intake mg/kg/day	Apparent protein digestibility, %
Lime cooked corn	239	79.2
	326	76.4
Rice	249	82.0
	326	78.7

TABLE 5. APPARENT PROTEIN DIGESTIBILITY AND NPR OF COMMON BEANS (Phaseolus vulgaris) OF DIFFERENT SEED COAT COLOR

Color of seed coat	No. of samples	Apparent protein Digestibility, %	N.P.R.
White	10	76.6	2.31
Red	23	72.4	1.80
Black	21	71.5	1.79
Brown	3	70.7	1.94
All	57	72.7	1.89

TABLE 6. REGRESSION EQUATIONS BETWEEN APPARENT PROTEIN DIGESTIBILITY AND NPR

Color of seed coat	NPR = a + b (PD)	r
White	5.13 - 0.037	-0.50 (NS)
Red	1.72 - 0.001	0.01 (NS)
Black	4.52 - 0.038	-0.46 (NS)
All	1.30 - 0.008	0.09 (NS)

TABLE 7. APPARENT PROTEIN DIGESTIBILITY OF BEANS OF DIFFERENT COLOR IN ADULT HUMANS

Bean color	No. of subjects	Nitrogen intake mg/kg/day	Apparent protein Digestibility, %
White	12	109.6 ± 0.5	62.1 ± 2.9
Red	12	106.7 ± 0.3	55.7 ± 4.6
Black (I)	12	108.1 ± 0.5	43.4 ± 2.1
50% W/50% B	12	108.4 ± 0.3	57.4 ± 2.6
Black (J)	12	106.8 ± 0.5	49.6 ± 2.9
Cheese	12	110.1 ± 0.6	76.2 ± 1.4

TABLE 8. FRACTIONATION OF FECAL NITROGEN FROM HUMAN SUBJECTS FED COMMON BEANS, CHEESE AND A NITROGEN-FREE (LOW) DIET

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

TABLE 9. EFFECT OF SOAKING TIME AND OF COOKING TIME OF DIFFERENT PROTEIN DIGESTIBILITY (%) OF COMMON BEANS*

Cooking time	Soaking time, hrs			Effect of cooking time
	0	12	24	
15	73.2	71.1	70.1	72.4
30	72.2	67.8	69.2	70.6
45	70.8	68.9	68.1	70.1
60	71.5	68.7	67.3	68.9
Effect of soaking time	71.9	69.1	68.7	-

TABLE 10. CONTENT OF SOLUBLE AND INSOLUBLE NITROGEN IN COOKED BEANS AND ITS RELATION TO THE PROTEIN DIGESTIBILITY OF THE WHOLE BEAN NITROGEN*

Color of bean	In cooked beans		N. intake	Apparent Prot.	Apparent Digest.	
	Soluble Nitr. %	Insoluble Nitr. %	mg/kg/day	digestibil. %	Soluble Nitr. %	Insoluble Nitr. %
White	23.3	76.6	290	59.3 ± 1.8	41.1 ± 7.9	78.7 ± 2.5
Red	17.1	82.8	287	64.8 ± 5.6	14.2 ± 2.5	64.5 ± 5.6
Black	15.2	84.8	285	68.8 ± 4.3	36.4 ± 10.2	79.7 ± 0.9

* Young dogs.

TABLE 11. ABSOLUTE INTAKE OF TANNIC ACID AND OF CATECHIN ON THE APPARENT PROTEIN DIGESTIBILITY OF BEANS IN HUMAN ADULTS

Color of bean	Absolute intake (mg/day)		Apparent protein Digestibility,%
	Catechin	Tannic acid	
White	10 ± 0.2	380 ± 15	62.0
White/Black 50/50	34 ± 1	817 ± 29	57.4
Red	41 ± 3	1246 ± 24	55.7
Black (I)	80 ± 2	1365 ± 41	53.4
Black (J)	90 ± 2	1634 ± 37	49.6

FIGURE 1

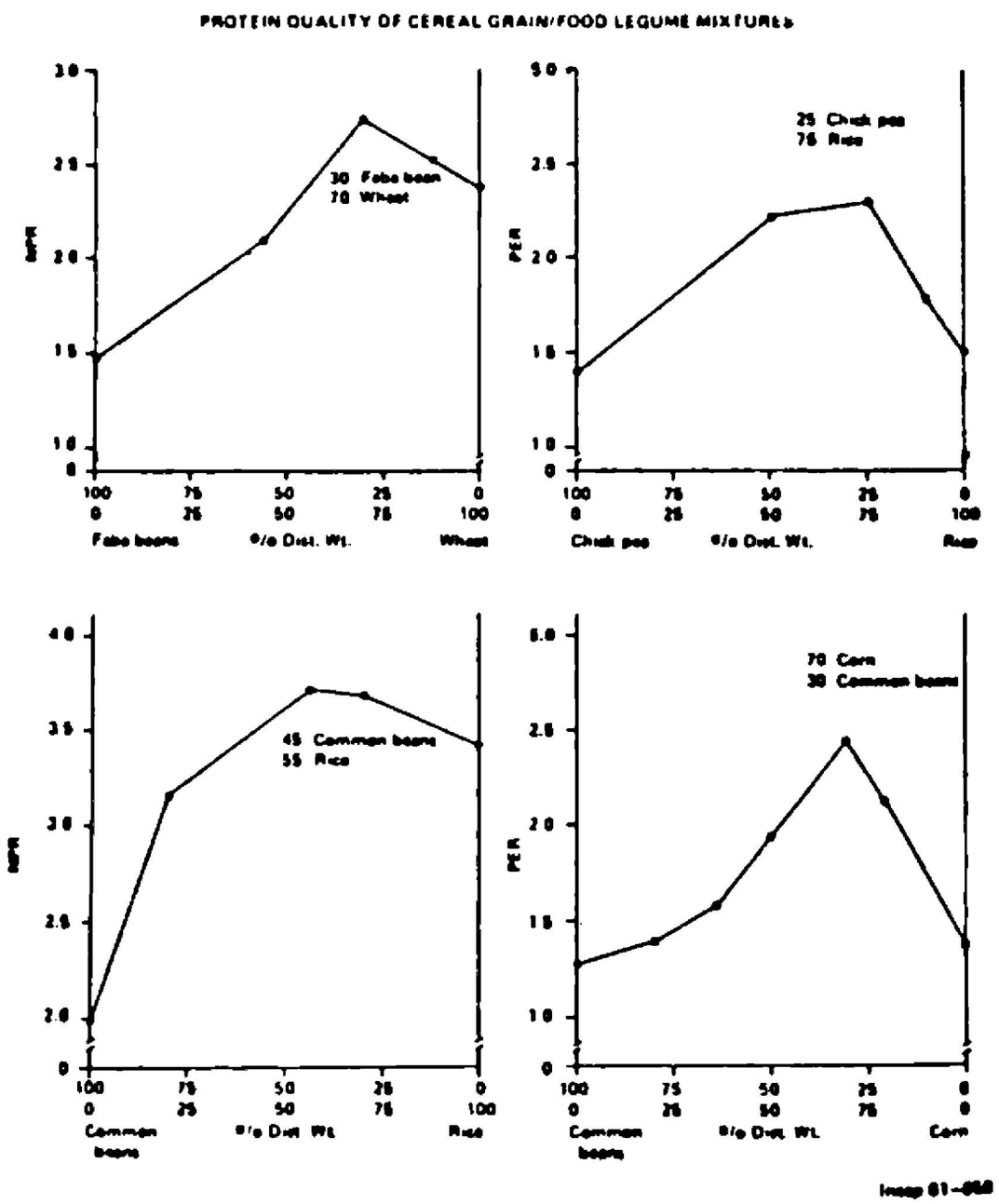
PROTEIN QUALITY OF CEREAL GRAIN/FOOD LEGUME MIXTURES
30 Faba bean
70 Wheat
25 Chick pea
75 Rice
45 Common beans
55 Rice
70 Corn
30 Common beans
PER
Faba beans
% Dict. Wt.
Wheat
Chick pea
% Diet. Wt.
Rice
Common beans
% Diet Wt
Rice
Common beans
% Diet Wt.
Corn

FIGURE 2

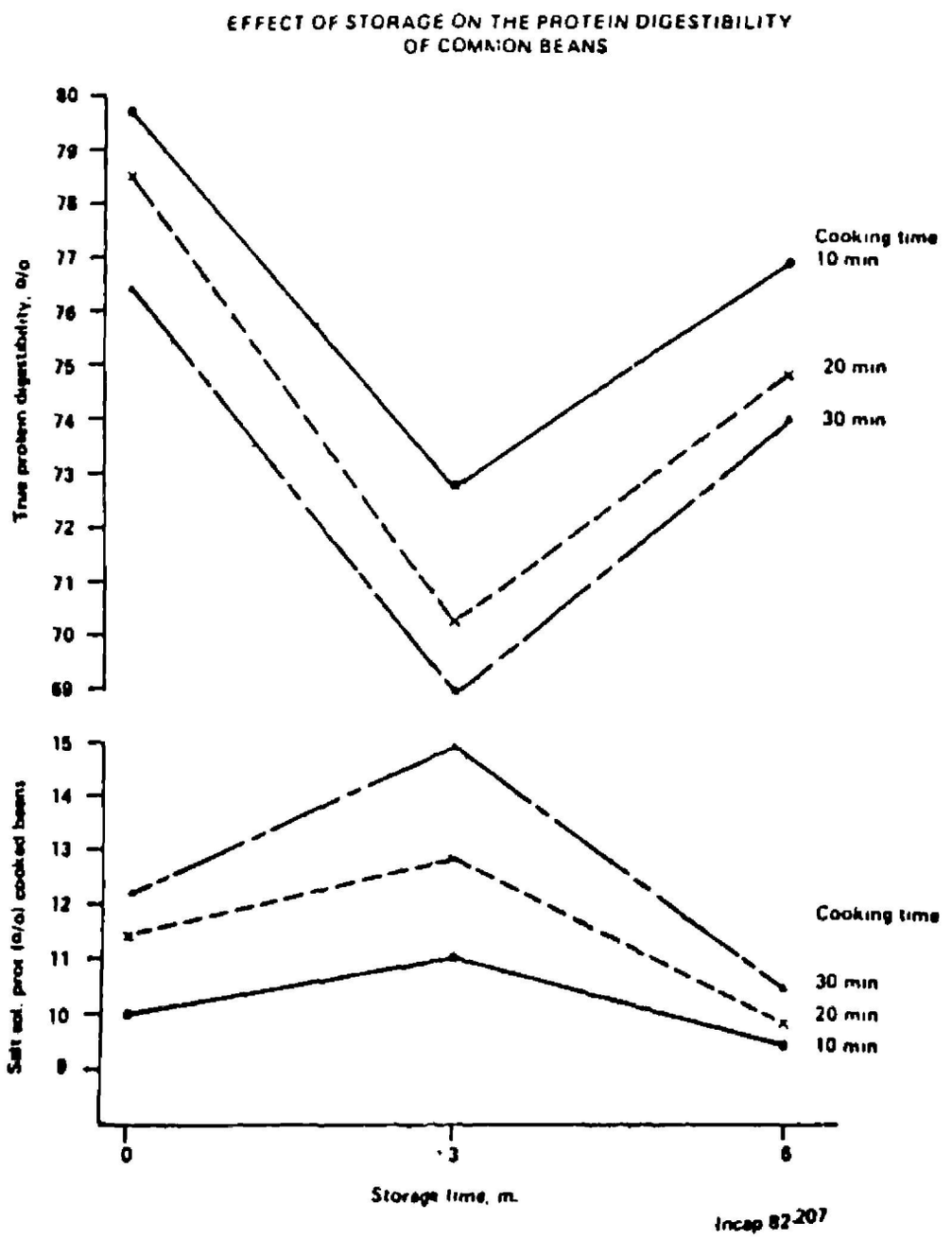
EFFECT OF STORAGE ON THE PROTEIN DIGESTIBILITY OF COMMON BEANS
True protein digestibility, %
80
79
78
77
76
75
74
73
72
71
70
69
Cooking time
10 min
20 min
30 min
Salt sol. prot (%) cooked beans
15
14
13
12
11
10
9
Cooking time
30 min
20 min
10 min
0
3
6
Storage time, m.
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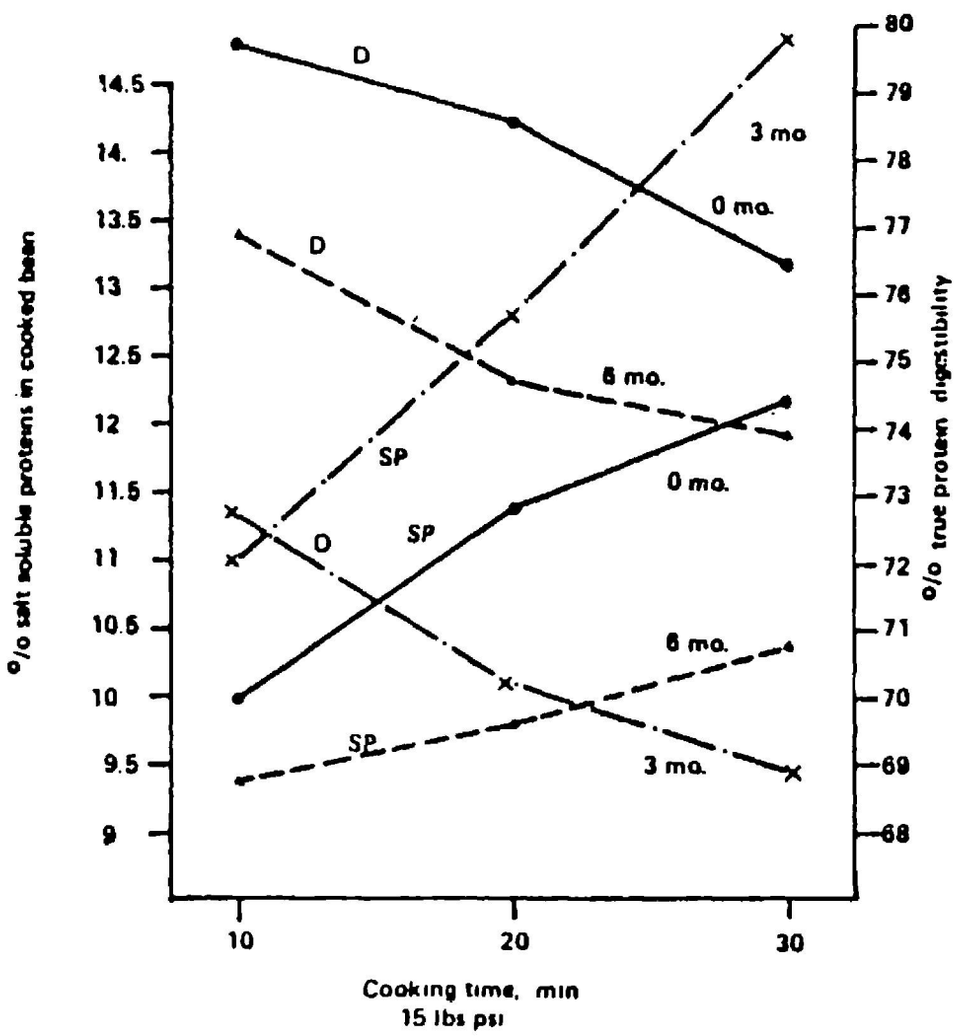
FIGURE 3



SP NaCl soluble protein
0, 3, 6 mo.: storage time.

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EFFECT OF COOKING TIME OF BEANS STORED FOR 0, 3, 6 MONTHS ON SALT SOLUBLE PROTEIN IN COOKED BEANS AND ON TRUE PROTEIN DIGESTIBILITY

FIGURE 4

RELATIONSHIP BETWEEN SALT SOLUBLE PROTEINS IN COOKED BEANS AND THEIR TRUE PROTEIN DIGESTIBILITY