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DIGESTIBILITY OF LEGUMES
1: EFFECT OF BEANS ON ORGANS*

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ABSTRACT

While legumes are regarded as good sources of protein they may be poorly digested, so it is useful to determine their digestibility which would give a desirable objective in a breeding programme. In this respect two series of experiments were performed as follow:

(1) True digestibility of the nitrogen (TND) of cooked white haricot beans (phaseolus vulgaris) was measured by feeding it to rats at 20, 40, and 80% levels in the diet (5, 10, and 20% protein), for 10 days period. The animals used were 23 days and 63 days old. The results show TND at three mentioned dietary levels 80, 74, and 67 for young animals and 63, 55, and 51 for more mature animals.

(2) At the end of each experiment selected organs of rats dissected and weighed. The result indicated that length of small intestine was

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greater in bean fed rats. While heart and pancreas did not show any weight increment, liver's weight was 20% heavier in the 80% bean fed animals in comparison to non-protein and stock diet fed rats.

INTRODUCTION

Several factors have been suggested to be responsible for relatively low consumption of beans, such as low agricultural production, low protein digestibility, the flatulence caused by their ingestion and low acceptability of certain types (Bressani and Elias, 1974). In this respect one of the objectives suggested for a breeding programme was to increase digestibility which would correspondingly increase the amount of food available (Hulse *et al,* 1977). Low digestibility is mostly due to the presence of the toxic materials in seeds. Among the numerous toxins in raw legumes are trypsin inhibitors. Most of the work in this field has been devoted to soybeans. Liener described five trypsin inhibitors in raw soybeans (Bender 1978).

Soybean's trypsin inhibitors account for 30 to 50 per cent of the growth inhibitory effect of raw meal and for nearly all of the pacreatic hypertrophic response in rats (Rackis, 1965).

Casein showed apparent nitrogen digestibility in rats of 92% and pancreas weight 0.48 g/100 g of body weight, whereas for soybean containing 100 mg trypsin inhibitor/100 g diet, digestibility was 74% and the pancreas weight 0.68 g/100 g of body weight (Rackis & McGhee, 1975).

Digestibility was reported by Jaffe (1972) to be 52% in raw and 91% in cooked beans: from the results which were reported by Elias *et al,* (1973) for raw 42.7% and for cooked beans 74.9%. Jaffe and Flores (1975) have reported 71.2% for digestibility of autoclaved (15 psi for 30 min.) white beans in rats. Elias *et al,* (1973) showed some evidence with respect to the colour of the seed and reported the protein digestibility and animal weight gain of black beans and of white mutant (NEP2), obtained by Co-60 irradiation of black coated bean. Weight gain was 33 g/28 day for black beans and 67g/28 day for the white. Both kinds of beans were cooked in the autoclave at 121^{o}C (16 lb psi) for 30 minutes.

The present work was undertaken to examine the digestibility of legumes and if any effect has this diet to rats organs. The original method of Mitchel (1923-24) was used for determining of nitroge digestibility.

It is not clear how far the animal model is suitable for man in this respect, but the rat has been used previously by many investigators and was used here.

Legumes differ considerably in their properties, including digestibility, content of toxins, cooking time etc., but most of the work reported here was carried on one type) the canellini bean, *phaseolus vulgaris,* grown in the Argentine, purchased in London and of unknown history.

EXPERIMENT

PREPARATION OF DIETS:

White kidney beans (Canellini) *'phaseolus vulgaris'* (product of Argentine) were purchased locally in London. The beans were soaked in cold water for 14 hours and autoclaved for 30 minutes at 15 psi. The cooked beans then were dried at 60^{o}C for 20 hours. Diets were prepared with (A) 20%, (B) 40%, (C) 80% beans and a protein-free control diet (D) was included.

Table 1 shows analyses of the diets.

ANIMALS:

Twenty Lister Hooded rats (Queen Elizabeth College Colony) were weaned at 21 days and fed on stock diet for 9 days, when the average body weight was 75 $\pm$ 8 g. They were separated into 4 groups each of 5 animals, and fed on one of the four diets for 10 days. Food intake was measured every two days and faeces collected. After 10 days the animals were returned to stock diet and maintained on this for 28 days. They were now 70 days old, when the average body weight was 187 $\pm$ 12 g. They were redivided into four groups, on this occasion ensuring male and females in each group, and fed on the experimental diets for 11 days.

A faeces marker, carmine red, was added to the diets at the beginning and the end of each experimental period, and three sets of faeces collected:

(1) the first 24 hours before the marker came through (corresponding to the stock diet in the gastrointestinal tract)

(2) between faecal markers (approximately from day 1 to 10 corresponding to feeding of the experimental diet)

(3) the final 24 hours (corresponding to the last day of experimental diet.

"TABLE 1"

Analysis of Diets fed to the rats

Type of Diet		N %g of dried sample	Crude Protein * %g of dried sample	Moisture %	Ash % of dried weight
Cooked white kidney bean flour	a	3.95	24.69	9.82	4.9
	b	3.96	24.72	10.13	—
Diet A 20% beans	a	0.88	5.5	7.62	—
	b	0.88	5.52	8.21	
Diet B 40% beans	a	1.73	10.78	4.64	—
	b	1.61	10.04	5.91	
Diet C 80% beans	a	3.23	20.16	3.66	—
	b	3.08	19.22	4.85	
Diet D non-protein	a	0.04	0.23	9.53	—
	b	0.03	0.16	8.72	

* Crude protein = N x 6.25
a = Part One young anal
b = Part Two nature anal

EXPLANATORY DIAGRAM

First day of stock diet	Experimental Diet	
Day 1	Day 2 .... day 9 day 10	stock diet
Day 1	Day 2	Day 11
Faecal collection 1	Faecal collection 2	Faecal collection 3

In this way it was possible to determine the error of collecting faeces during the experimental feeding period, without the refinement of using faecal markers. This was done to estimate any errors involved in digestibility measurement made from faeces collected during the standard 10-day NPU assays. Since animals are sacrificed on the 10th day in this array, it is not possible to collect faeces corresponding to the 10th day of feeding.

True digestibility was calculated as follows:

True nitrogen digestibility % (TND) =

$$\frac{(\text{N in feed} - [\text{in faeces -metabolic N}[)}{(\text{N in feed})} \times 10$$

Apparent nitrogen digestibility % =

$$\frac{(\text{N in feed} - \text{N in faeces})}{(\text{N in feed})} \times 100$$

Apparent dry matter digestibility was calculated by subtracting from dry food intake the dry weight of faeces and dividing the difference by dry food intake.

(% apparent dry matter digestibility =

$$\frac{\text{Food intake (g)} - \text{Faecal weight(g)}}{\text{Food intake (g)}} \times 100$$

True dry matter digestibility (TDMD) was calculated by subtracting the dry weight of faeces of animals fed non-protein diet (metabolic N) from faecal weight of experimental animals.

Metabolic N is determined from the faecal N of non-protein group calculated per gramme dry weight of food eaten (since metabolic N varies with dry weight of food eaten (Mitchell 1924, and Smuts 1935).

Results and Discussion

Food intake and body weight gain :

Table 2 shows a lower food intake in Group A, 5% protein, than the other three groups in the younger, but not with the more mature rats. Otherwise food intake of all groups was similar.

In the younger rats, the 5% protein group lost weight, those fed 10% protein maintained weight and those fed 20% protein gained weight. Since this last group was fed at the abnormally high level of 80% beans in the diet and still gained weight, it would appear that this level is well tolerated and non-toxic. These beans have NPU of 47 (46, 48).

In the same animals in older age, all groups lost weight. This might be due to an inadequate food consumption or to the very low true digestibilities of nitrogen described below.

Digestibility:

Although apparent digestibility is of little value, it is a term commonly quoted and Table 3 shows that apparent nitrogen digestibility is very much lower than TND, although apparent dry matter digestibility is not so much lower than TDMD. (In the literature it is not always clear whether the authors are reporting true or apparent digestibility).

Table 4 shows TND and TDMD of different diets of beans.

"TABLE 2"

Weight gain and Food intake of young and old rats fed different amounts of the bean diet

Diets	Weight gain (g)		Food intake (g) during the 10 days assey	
	Part 1 (30-day old)	Part 2 (70-day old)	Part 1 (30-day old)	Part 2 (70-day old)
Non-protein (D)	−8.0	−25.0	78 (± 19.2)	125 (± 13)
20% beans (5% protein) (A)	−4.8	−23.0	60 (± 7.7)	123 (± 12)
40% beans (10% protein) (B)	+0.2	−21.0	72 (± 13.4)	113 (± 9)
80% beans (20% protein) (C)	+ 3.2	−12.0	76 (± 1.2)	126 (± 9)

"TABLE 3"
Apparent N and dry matter digestibility of different diets of beans of 30 (Part 1) and 70 (Part 2) day old rats (± S.D.)

Apparent N & dry matter digestibility % / Diets	Apparent N digestibility %		Apparent dry matter digestibility %	
	Part 1	Part 2	Part 1	Part 2
Group A 20% beans (5% protein)	64.5 (± 1.2)	42.9 (± 1.6)	91.0 (± 1.9)	87.9 (± 1.8)
Group B 40% beans (10% protein)	66.2 (± 1.9)	42.4 (± 1.9)	86.2 (± 1.7)	81.9 (± 2.1)
Group C 80% beans (20% protein)	62.2 (± 1.8)	44.2 (± 1.1)	76.8 (± 1.2)	72.3 (± 1.4)

"TABLE 4"
True N an dry matter digestibility of different percent beans diet using correction factors. Faecal markers — true collection

True N and dry matter digestibility % / Diets	True N digestibility %		True dry matter digestibility %	
	Part 1 (30 day old)	Part 2 (70-day old)	Part 1 (30-day old)	Part 2 (70-day old)
Group A 20% beans (5% protein)	80.10(± 1.4)	62.7 (± 1.9)	97.0 (± 1.8)	95.4 (± 1.7)
Group B 40% beans (10% protein)	74.2(± 1.3)	54.5 (± 1.2)	92.2 (± 1.6)	90.3 (± 1.7)
Group C 80% beans (20% protein)	67.1 (± 2.3)	50.5 (± 1.9)	82.8 (± 1.3)	80.7 (± 1.6)

Source of faecal nitrogen:

Faecal nitrogen comes from undigested food, bacteria, the lining of the intestinal mucosa and residues of digestive juices. True digestibility allows for the last three my measuring the total faecal nitrogen on a protein-free diet. A similar allowance is made for dry matter digestibility.

The results reported here show considerable differences between digestibilities of N and of dry matter.

TDMD	young rats	97,	92,	83%	(3 dietary levels
TND	young rats	80,	74,	67%	
TDMD	mature rats	95,	90,	81%	
TND	mature rats	63,	55,	51%	

The high dry matter digestibilities coupled with the low N digestibilities suggests that the extra faecal N is coming from one of the non-food sources. Bacterial N is effectively dietary N and so can be discounted.

Since young animals usually have a poorly developed digestive system it is surprising to find higher TND with younger compared with the older rats.

Faecal Collection:

In the normal 10 day NPU assay, faeces are collected for the same 10 days as the feeding period. The first day's collection is therefore produced from the preceding day's stock diet and the true faecec output from the tenth lay would appear on the eleventh day. As already stated, the animals are killed for analysis on the tenth day.

Table 5 shows the TND of the "true" faecal collection using the carmine marker and the 10 day collection. The differences are so small that the carmine marker appears to be unnecessary and TND calculated during the normal NPU assay, therefore, provides acceptable results.

Organ Weights:

At the end of each experiment the rats were killed (after 11 days of feeding) and selected organs dissected and weighed. The small intestine (from pylorus to caecum) was carefully separated from the mesenteric connection without stretching, and its length was measured.

It was then cut into 10-15 cm. lengths, and with the help of a syringe, was washed with cold water to remove its contents. The sections were dried between filter papers overnight and then weighed.

The caecum was weighed immediately after separation, and its outline was drawn on a paper, which was cut out and weighed for comparison of bulk.

The length and weight of the small intestine were significantly increased after 10 days feeding with beans (Table 6). The lengths of small intestine of rats fed diet C were greater than B which were greater animals (A) was greater than no-beans (D), that of 40% bean diet (B) greater still and 80% beans (C) greater.

"TABLE 5"
True digestibility of nitrogen & (TND), with and without faecal carmine marker
(70 day old rats)

Diet	TND Faecal marker (true collection) from 2nd to 11th day	TND without marker from 1st to 10th day
(A) 20% beans	62.7	63.0
(B) 40% beans	54.5	55.3
(C) 80% beans	50.5	49.8

"TABLE 6"

Weight and Length of caecum and small intestine of 70-day old rats fed 20% (A), 40% (B), 80% (C), and 0% (D) of beans in diet

Weight & Length of organs / Animals	Length of small intestine (cm)	Length per 100g of body weight (cm)	Weight of of small intestine (g)	Weight /100g body wt. (g)	Weight of full caecum (g)	Weight /100g body wt. (g)	Length of caecum (cm)	Length /100g of caecum (cm)	Relative area of caecum (mg)	Relative area/100g of body weight (mg)	Relative of 1 cm of small intestine (mg)
Group D	109.2 (± 5.4)	69.3	1.76 (± 0.47)	1.09	2.84 (± 0.26)	1.79	4.86 (± 0.17)	3.08	2.46 (± 0.13)	1.66	16
Group A	94.7 (± 5.1)	57.1	2.92 (± 0.37)	1.74	3.30 (± 0.39)	1.98	4.60 (± 0.35)	2.77	2.39 (± 0.33)	1.45	30
Group B	108.9 (± 10.5)	65.8	3.19 (± 0.56)	1.98	4.27 (±0.72)	2.63	5.32 (±0.04)	3.30	3.35 (± 0.31)	2.06	29
Group C	129.4 (± 9.0)	72.6	4.54 (± 0.42)	2.54	6.45 (± 0.93)	3.56	7.20 (±0.26)	4.03	5.55 (±0.67)	3.10	35

S. D. = (   )   n = 6

Since the animals had been fed on different levels of protein it is not clear whether these differences are due to the (poorly digested) legumes or to the differing growth rates of the animals. (In the later experiment a casein diet was used as control and showed that legume feeding caused an increase in intestinal length but not in unit (weight.)

In the caecum and the small and large intestine of the animals in Group C a lot of gas was seen. Caecal volume and weight also were significantly higher in experimental groups than the control. The results show similar enlargement of the caecum using the different kinds of measurement. Hoover and Heitmann (1972) reported an increase in caecal volume in rabbits due to dietary fibre.

The white kidney beans had no effect on the weights of heart, kidneys, spleen or pancreas, but increased the weight of the liver (Table 7). The liver weight when calculated per 100g of body weight of animals, was also higher in bean-fed animals (approximately 20% heavier in the 80%-bean fed rats than in the non-protein fed rats), but this effect may also have been due to the higher protein intake.

"TABLE 7"

Weight of liver, kidneys, heart, pancreas and spleen of 70-day old rats fed 20% (A), 40% (B), 80% (C) and 0% (D) beans in diet

Weight of organs / animals	Weight of liver (g)	Weight /100 g body wt. (g)	Weight of kidneys (g)	Weight /100 g body wt. (g)	Weight of heart (g)	Weight /100 g body wt. (g)	Weight of pancreas (g)	Weight /100 g body wt. (g)	Weight of spleen (g)	Weight /100 g body wt. (g)
Group D	7.50 (± 0.44)	4.73 (± 0.29)	1.39 (± 0.06)	0.87	0.61 (± 0.02)	0.38	0.44 (± 0.04)	0.27	0.55 (± 0.03)	0.36
Group A	8.57 (± 0.86)	5.10 (± 0.24)	1.40 (± 0.13)	0.83	0.67 (± 0.07)	0.39	0.44 (± 0.02)	0.26	0.67 (± 0.06)	0.40
Group B	9.05 (± 0.52)	5.60 (± 0.15)	1.37 (± 0.09)	0.85	0.62 (± 0.02)	0.38	0.43 (± 0.05)	0.27	0.68 (± 0.05)	0.42
Group C	10.43 (± 0.90)	5.82 (± 0.21)	1.54 (± 0.11)	0.87	0.63 (± 0.03)	0.36	0.44 (± 0.04)	0.25	0.68 (± 0.07)	0.38

n = 6 S.D. = ( )

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