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Effect of Bean Broth on the Nutritive Value and Digestibility of Beans

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Black, red and white beans were cooked (1:3, water: beans) at 120°C and 16 lb in^{-2} for 20 min. Chemical analysis of the broth showed that it contained low levels of protein, ether extract, and carbohydrates and high levels of ash and polyphenols. The effect of the addition of bean broth, on the PER and digestibility of beans was studied in rats. PER values and protein digestibility decreased as the amount of bean broth increased in the diet. White beans showed highest values of PER, weight gain and protein digestibility. The latter was lower in the groups supplemented with methionine than in those not supplemented; there was no interaction beween level of methionine and amount of bean broth in the diet on digestibility. The effect of methionine was not related to either metabolic nitrogen in faeces or amount of food consumed. A high proportion of the faeces nitrogen was soluble in NaOH. When rats from the different groups were fed diets containing 18% bean protein and killed at 6 weeks of age they showed no difference in pancreas weight that could be related to the amount of broth in the diet, nor were the levels of trypsin in the pancreas or the amount of PABA excreted upon ingestion of BT-PABA related to the amount of broth in the diet.

Keywords: Bean broth; methionine supplementation; digestibility.

1. Introduction

Common beans (*Phaseolus vulgaris*) in any of their colours and varieties are one of the main sources of protein in the Central American diet. The digestibility of beans is, however, low compared with other legumes and other sources of vegetable protein.[1-3]

Aside from the fact that beans, as most legumes, contain trypsin inhibitors and haemagglutinins—factors which interfere with the digestion of raw beans—the low digestibility of cooked beans has been ascribed to their tannin content,[4] which may bind protein and make it unavailable for enzymic digestion, or to the tertiary structure of the protein itself or fractions thereof, which are equally resistant to enzymic hydrolysis.

Methods of cooking beans vary from country to country but all involve boiling the beans until they are soft.[5] Cooking not only destroys antiphysiological factors but also renders the protein more digestible. Some studies have shown that when beans are consumed together with the cooking liquor (bean broth) their digestibility is lower than when they are consumed alone,[1] a fact which may be accounted for by the amount of tannins in the broth, by the water soluble protein extracted—which may be the most undigestible fraction—or by the pattern of amino acids present in the broth which may alter total nitrogen digestibility. In some studies an increase in the size of the pancreas of rats fed cooked beans has been reported.[6]

The question of the low digestibility of bean broth is of primary importance because this is the first bean preparation that is offered to the child during weaning and for some time afterwards,[5] and beans *per se* are allowed as a food only after the first year of age.

The present study was designed to gather information on the composition of bean broth and its effect on bean digestibility, and to determine the effect, if any, on the pancreas of albino rats.

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2. Materials and methods

2.1. Materials

White (navy), red (kidney) and black (Tamazulapa variety) beans were cleaned and cooked in a 3 : 1, water : bean ratio for 20 min at 16 lb in^{-2} and 121°C. The cooking water (bean broth) was separated from the beans, dried at 60°C in a convection oven and analysed chemically. Bean samples dried with and without broth, and with two and three times the amount of broth added were analysed for nitrogen by the Kjeldahl procedure[7] and tested biologically.

2.2. Chemical analyses

Proximate chemical composition was determined by the methods of the AOAC;[7] tannins by the method of Folin-Denis;[8] trypsin inhibitors by the methodology outlined by Kakade *et al*;[9] calcium by the AOAC method; phosphorus by the procedure of Lowry and López[10] and haemagglutinins by the technique of Jaffé and Bruchner.[11]

2.3. Biological assays

2.3.1. PER and digestibility studies

Weaning rats of the Wistar strain from the INCAP colony were used in all assays. They were distributed by weight into homogeneous groups of four male and four female rats each. The animals were housed in individual cages with raised screen bottoms in an air conditioned room and fed *ad libitum* water and the experimental diets. The latter contained 10% protein from the different samples of beans (beans without broth, with broth, with twice the amount of broth and with three times the amount of broth, unsupplemented or supplemented with 0.3% DL-methionine) and all nutrients in adequate amounts as reported elsewhere.[1] Weekly records of weight gain and food consumed were kept for a 28-day period, at the end of which PER values were calculated. During the last week of the experiment faeces were collected, dried, weighed and analysed for nitrogen and apparent digestibility calculated from nitrogen intake and excretion. To calculate true digestibility, an additional group was fed a nitrogen-free diet. In all cases casein was used as the standard protein.

A pair-feeding experiment was carried out with diets containing black beans, following the experimental design outlined in the preceding paragraphs. The animals fed the methionine supplemented diets were fed the amount consumed *ad libitum* the previous day by their respective unsupplemented groups.

2.3.2. Pancreas studies

Five 49-day-old rats from the previous PER experiments were placed in metabolic cages, allowed to familiarise themselves with the cage for 2 days, starved for 24 h and fed early the next day on 2.5 g of a diet[12] containing 250 mg. kg^{-1} body weight of the peptide N-benzoyl-L-tyrosine-p-aminobenzoic acid (BT-PABA) dissolved in 5% sucrose solution of pH 8.5. All animals consumed the food within 30 min, and urine was collected for the next 7 h, measured, frozen and analysed for p-aminobenzoic acid by the procedure of Yamato and Kinoshita.[13] The amount of PABA excreted was calculated as a percentage of the amount ingested. The rats were then killed and the pancreas and liver removed, weighed and frozen. Pancreases were analysed for their trypsin content by the method of Erlanger *et al.*[14] after converting all trypsinogen into trypsin by the addition of enterokinase followed by 1 h incubation at 37°C.

Statistical evaluation of the data was carried out as described by Snedecor and Cochran.[15]

3. Results

Table 1 shows the chemical composition of the broth from black, red and white beans. Composition was similar for the three beans except that the tannin content was, as expected, lower in white beans. Most of the protein was soluble in 0.1M NaOH. Trypsin inhibitors and haemagglutinins were absent due to the heat treatment to which beans were subjected.

Table 1. Chemical composition of black, red and white bean broth (figures on a dry weight basis)

Component	Black bean broth	Red bean broth	White bean broth
Moisture (g/100 g)	11.2	10.2	12.7
Protein (g/100 g)	14.1	19.8	17.8
Ash (g/100 g)	15.2	14.7	18.1
Ether extract (g/100 g)	0.7	0.7	0.5
Crude fibre (g/100 g)	0.3	0.6	0.3
Carbohydrates (g/100 g)	58.5	54.0	50.6
Tannins (as tannic acid) (g/100 g)	3.32	2.87	0.81
Starch (g/100 g)	19.5	22.0	18.1
Trypsin inhibitors (TUI/ml)	0	0	0
Haemagglutinins	0	0	0
Calcium (mg/100 g)	176.0	133.76	265.89
Phosphorus (mg/100 g)	644.7	649.1	415.1
Soluble protein in NaOH (g/100 g)	83.6	78.8	96.8
Non protein soluble N (g/100 g)	1.6	1.2	2.0

Table 2. PER and weight gain in rats fed different varieties of beans

	PER			Weight gain (g)		
Treatment	Black beans	Red beans	White beans	Black beans	Red beans	White beans
Beans without broth	1.13 ± 0.19^{d}	0.90 ± 0.29^{c}	1.48 ± 0.20^{b}	28 ± 9^{c}	24 ± 12^{d}	45 ± 11^{c}
Beans with broth	0.80 ± 0.14^{e}	0.66 ± 0.16^{cd}	1.25 ± 0.16^{b}	17 ± 3^{c}	16 ± 4^{de}	34 ± 9^{c}
Beans+2×broth	0.59 ± 0.20^{e}	0.53 ± 0.25^{cd}	1.23 ± 0.16^{b}	11 ± 4^{c}	11 ± 6^{de}	32 ± 7^{c}
Beans+3×broth	0.49 ± 0.16^{e}	0.37 ± 0.16^{d}	1.14 ± 0.19^{b}	9 ± 4^{c}	7 ± 3^{e}	30 ± 10^{c}
Beans without broth+0.3% methionine	2.61 ± 0.35^{ab}	2.28 ± 0.16^{a}	2.95 ± 0.21^{a}	115.21^{a}	109 ± 14^{a}	130 ± 21^{a}
Beans with broth+0.3% methionine	2.28 ± 0.17^{bc}	2.28 ± 0.28^{a}	2.81 ± 0.18^{a}	117 ± 15^{a}	89 ± 12^{b}	122 ± 17^{ab}
Beans+2×broth+0.3% methionine	2.03 ± 0.15^{c}	1.86 ± 0.18^{b}	2.63 ± 0.23^{a}	82 ± 12^{b}	87 ± 10^{bc}	107 ± 16^{b}
Beans+3×broth+0.3% methionine	2.12 ± 0.16^{c}	$1.78\pm0.,34^{b}$	2.74 ± 0.22^{a}	83 ± 13^{b}	71 ± 7^{c}	107 ± 13^{b}
Casein	2.87 ± 0.31^{a}	2.65 ± 0.31^{a}	2.85 ± 0.15^{a}	90 ± 13^{b}	110 ± 13^{a}	114 ± 13^{ab}

All means without a common superscript differ significantly ($P<0.05$).

Table 3. Apparent and true digestibility of several bean varieties

	Apparent digestibility (%)			True digestibility (%)		
Treatment	Black beans	Red beans	White beans	Black beans	Red beans	White beans
Beans without broth	64.14 ± 3.10^{b}	69.22 ± 3.08^{bc}	74.31 ± 5.14^{b}	81.97 ± 2.22^{bc}	84.80 ± 0.93^{b}	86.44 ± 6.70^{b}
Beans with broth	61.97 ± 2.48^{bc}	71.76 ± 2.33^{b}	72.66 ± 5.08^{b}	80.52 ± 2.28^{bcd}	84.63 ± 1.94^{b}	86.68 ± 2.45^{b}
Beans+2×broth	60.53 ± 2.86^{bc}	66.80 ± 4.28^{bc}	73.14 ± 4.22^{b}	78.45 ± 1.88^{d}	82.05 ± 2.36^{b}	86.18 ± 2.46^{b}
Beans+3×broth	59.68 ± 4.42^{bc}	66.59 ± 4.26^{bc}	78.86 ± 2.60^{b}	78.95 ± 2.85^{cd}	82.87 ± 2.74^{b}	86.18 ± 1.81^{b}
Beans without broth+0.3% methionine	62.10 ± 2.67^{b}	69.50 ± 2.76^{bc}	74.54 ± 3.20^{b}	82.69 ± 2.25^{b}	85.53 ± 3.89^{b}	87.65 ± 3.37^{b}
Beans with broth+0.3% methionine	60.44 ± 0.89^{bc}	63.27 ± 9.25^{c}	75.73 ± 4.94^{b}	81.44 ± 1.38^{bcd}	82.13 ± 5.17^{b}	88.30 ± 2.74^{b}
Beans+2×broth+0.3% methionine	59.08 ± 1.62^{bc}	64.92 ± 2.78^{bc}	70.70 ± 2.08^{b}	78.95 ± 1.42^{cd}	82.80 ± 1.72^{b}	84.87 ± 1.69^{b}
Beans+3×broth+0.3% methionine	56.64 ± 1.92^{c}	65.97 ± 9.39^{bc}	71.60 ± 4.30^{b}	78.98 ± 1.35^{cd}	83.00 ± 4.52^{b}	87.03 ± 2.66^{b}
Casein	90.21 ± 1.48^{a}	91.15 ± 2.66^{a}	90.43 ± 1.70^{a}	94.68 ± 0.93^{a}	94.08 ± 1.63^{a}	93.80 ± 3.34^{a}

All means without a common superscript differ significantly ($P<0.05$).

Table 2 shows the PER and weight gain of rats fed the three beans studied. Methionine, of course, increased PER values significantly. There was in all cases a significant ($P<0.05$) decrease in PER as the amount of bean broth increased in the diet, whether methionine was added or not. When PER values were corrected for digestibility the same tendency was observed in this parameter to decrease as bean broth was increased in the diet. Red beans resulted in the lowest PER and white beans in the highest with black beans between. This same tendency was observed with weight gain figures, and it is noteworthy that white beans resulted in the highest PER values and that weight gain with these beans was twice as high as that obtained with the other varieties.

Table 3 shows the results for apparent and true digestibility. When the three varieties of beans were analysed together there was a significant difference ($P<0.05$) in apparent digestibility for the different levels of broth and methionine addition; the effects were additive; that is, there was no interaction between broth and methionine addition on apparent digestibility. The effect of methionine in lowering digestibility values was unexpected. Table 4 shows the consumption of tannins from the different diets. As increasing levels of bean broth were added to the diet there was a corresponding increase in tannin consumption for all varieties. When methionine was added there was an increase in food consumption and corresponding increase in tannin intake. There was a

Table 4. Tannin consumption by rats from different varieties of beans

	Tannin consumption (g)		
Treatment	Black beans	Red beans	White beans
Beans without broth	0.4317±0.0799^{c}	0.7444±0.1445^{b}	0.2296±0.0306^{c}
Beans with broth	0.6775±0.1002^{d}	1.0012±0.1362^{b}	0.2862±0.0548^{bc}
Beans+2×broth	0.6306±0.0658^{d}	1.1155±0.1756^{b}	0.2966±0.0347^{bc}
Beans+3×broth	0.9344±0.0948^{c}	0.8191±0.0898^{b}	0.3062±0.0490^{b}
Beans without broth+0.3% methionine	0.7690±0.0969^{b}	1.3326±0.1395^{b}	0.3358±0.0390^{b}
Beans with broth+0.3% methionine	1.4275±0.1874^{cd}	1.2809±0.1085^{b}	0.4772±0.0540^{a}
Beans+2×broth+0.3% methionine	1.3214±0.1121^{b}	2.1342±0.2678^{a}	0.4526±0.0602^{a}
Beans+3×broth+0.3% methionine	1.9188±0.2043^{a}	2.0352±0.3172^{a}	0.4560±0.0601^{a}
Casein	—	—	—

All means without a common superscript differ significantly ($P<0.05$).

Table 5. Apparent and true digestibility of black beans in rats (pair feeding)

Treatment	Weight gain (g)	Apparent digestibility (%)	True digestibility (%)
Beans without broth	20±8de	67.70±5.63^{b}	82.22±4.31^{b}
Beans with broth	18±10de	64.42±5.06^{bc}	80.30±3.18^{b}
Beans with 2×broth	10±4^{e}	61.72±4.33^{bc}	78.67±4.23^{b}
Beans with 3×broth	8±5^{e}	58.75±4.09^{c}	77.20±3.46^{bc}
Beans without broth+0.3% Met.	44±7^{b}	63.69±6.63^{bc}	81.54±3.82^{b}
Beans with broth+0.3% Met.	40±6^{bc}	58.56±5.93^{c}	77.66±3.75^{bc}
Beans+2×broth+0.3% Met.	29±2^{cd}	49.71±6.10^{d}	72.73±5.24^{c}
Beans+3×broth+0.3% Met.	24±6^{d}	48.58±3.21^{d}	72.25±1.39^{c}
Casein	119±16^{a}	91.65±0.81^{a}	95.58±0.87^{a}

All means without a common superscript differ significantly ($P<0.05$).

Table 6. Weight of pancreas and liver of rats fed different varieties of beans

	Pancreas (% of body weight)			Liver (% of body weight)		
Treatment	Black beans	Red beans	White beans	Black beans	Red beans	White beans
Beans, 18% protein	0.25±0.03	0.15±0.02	0.15±0.03	4.85±0.38	5.19±0.71	4.35±0.45
Beans, 18% protein +0.53% methionine	0.25±0.04	0.15±0.01	0.19±0.05	4.80±0.26	5.17±0.66	4.02±0.52
Beans+3×broth, 18% protein	0.26±0.04	0.16±0.03	0.19±0.02	4.89±0.39	6.48±0.68	4.77±0.34
Beans+3×broth+ 0.53% methionine	0.28±0.02	0.15±0.02	0.17±0.06	4.61±0.23	4.53±0.42	3.98±0.28
Casein	0.23±0.04	0.17±0.04	0.16±0.03	5.08±0.32	5.86±0.32	4.06±0.26

Pancreas (% body weight): F significant ($P<0.01$); LSD=0.06.
Liver (% body weight): F significant ($P<0.05$); LSD=0.86.

significant ($P<0.01$) negative correlation ($r=-0.64$) between tannin intake with and without methionine and apparent digestibility.

Table 5 shows the results for weight gain and apparent and true digestibility for the pair feeding experiment. Weight gain decreased with increasing levels of bean broth both in the groups fed *ad libitum* (without methionine) and in those with restricted feeding (with methionine). As in the previous experiments, apparent and true digestibility decreased with increasing levels of bean broth, and the addition of methionine resulted again in a further decrease in digestibility values in spite of the restriction in food intake.

Table 6 shows the weight of pancreas and liver expressed as percentage of live weight. Results for pancreas weight were significantly different between black beans and either red or white ones, while no significant difference was found between the latter two. Addition of bean broth did not have any significant effect on pancreas weight. Liver weight was significantly higher in animals fed red beans as compared to those fed black or white beans. The nutritional significance of these findings, however, should be considered with caution on the basis of the results observed with the respective case in controls which would suggest the difference to be due to the group of rats used rather than to bean colour. Table 7 shows the results for trypsin content in pancreas and for excretion of PABA. Both parameters varied significantly among the three varieties of beans, but there was no discernible pattern that could be related to the presence or absence of bean broth and methionine. The low levels of PABA excretion observed in animals consuming red beans are probably due to the fact that rats in these groups passed very little urine during the 7-h experimental period.

Table 7. Trypsin in pancreas and excretion of PABA in rats fed different varities of beans

	Trypsin in pancreas ($U^a\ g^{-1}$)			Excretion of PABA (%)		
Treatment	Black beans	Red beans	White beans	Black beans	Red beans	White beans
Beans, 18% protein	3900±965	12 161±2026	6846±2743	40.94±16.65	11.19±4.62	51.91±22.99
Beans, 18% protein +0.53% methionine	4577±2538	4578±1881	4106±2219	62.44±5.11	13.26±5.85	59.53±6.95
Beans+3×broth, 18% protein	5124±1190	7871±2682	4131±1095	25.20±16.59	9.19±3.79	38.69±19.20
Beans+3×broth, 18% protein 0.53% methionine	8269±2220	3419±2031	5384±3885	68.31±12.80	10.10±3.27	65.00±10.80
Casein	7008±2616	1287±651	2448±746	39.40±10.52	10.89±2.63	40.55±4.28

[a]Unit of activity. One unit is described as an absorbance change of 0.001 absorbance unit under the conditions specified (25°C, 10 min).

Table 8. Soluble nitrogen in water and in 0.02M NaOH in the faeces of rats fed black bean diets

	Nitrogen soluble in H_2O in faeces (% of total N)	Nitrogen soluble in 0.02 M NaOH in faeces (% of total N)	N soluble in NaOH in faeces, (% N Sol. ingested)	Apparent digestibility (%)
Beans without broth	25.8±4.1[a]	66.21±3.33[ab]	33.15±4.05[cd]	65.24±3.70[bc]
Beans with broth	20.7±3.5[ab]	67.36±2.05[a]	41.40±5.49[ab]	56.76±5.84[e]
Beans+2×broth	20.9±1.3[ab]	63.32±1.89[ab]	38.51±3.83[abc]	62.58±3.04[cd]
Beans+3×broth	21.1±3.5[ab]	63.62±3.26[a]	37.94±3.00[bc]	61.70±2.68[cd]
Beans+0.3% methionine	20.9±2.4[ab]	68.77±3.41[a]	30.82±3.32[d]	68.62±2.28[b]
Beans+broth+0.3% methionine	18.0±1.6[b]	65.66±1.30[b]	35.31±3.29[cd]	63.86±3.26[cd]
Beans+2×broth+0.3% methionine	21.2±3.3[ab]	68.62±6.46[ab]	42.29±4.29[ab]	61.96±3.14[cd]
Beans+3×broth+0.3% methionine	22.3±2.5[ab]	68.52±2.36[a]	43.86±1.71[a]	59.91±1.23[de]
Casein	25.5±8.7[a]	58.87±4.81[ab]	5.10±1.10[e]	91.52±1.44[a]

All means without a common superscript differ significantly ($P<0.05$).

Finally, Table 8 shows the amount of water and 0.02M NaOH soluble nitrogen in the faeces of rats fed black bean diets. As can be seen, water soluble nitrogen in faeces was about 20% for all groups, casein included. NaOH soluble nitrogen was higher for animals consuming bean diets than for those fed casein, and the amount of NaOH soluble nitrogen in faeces expressed as a percentage of the NaOH soluble nitrogen ingested was, on average, 38% in animals consuming bean diets and only 5.1% for animals consuming casein. Apparent digestibility followed the same pattern as in previous experiments with black beans.

4. Discussion

Bean broth as consumed in Central America contains about 7% dry matter.[5] On the basis of the data in Table 1 it would contain about the same amount of protein as human milk but much less ether extract and carbohydrates. The tannin content is quite high as are the ash, calcium and phosphorus contents. The methionine content is about half that of milk, and most of the protein is soluble in NaOH which, according to the results presented in this study, is the undigestible fraction found in faeces of animals fed beans with or without broth. On this basis, bean broth appears to be a nutritionally poor substitute for milk, especially when the high amount of tannins present in this food preparation are considered.

This biological trial showed that, in the first place, the bean varieties studied differ, with white beans being superior in both PER and digestibility. Black beans were intermediate and red beans had the lowest values. This is important for bean consuming areas since beans should not be treated as one single homogenous food. Colour of variety must be taken into consideration. In Central America, black beans are preferred in the northern part of the Isthmus and red in the southern part. It is an interesting observation that although white beans are grown through out the area they are consumed only sporadically, in spite of their evident higher nutritional quality.

In all cases, the addition of bean broth resulted in a decrease in PER and digestibility, whether supplemented with methionine or not. The component in bean broth responsible for this appears to be the tannin as shown by the significant negative correlation obtained between tannin intake and digestibility. Tannin intake, however, cannot account for the decrease in digestibility observed when supplemented with methionine. A possible explanation could be the amount of metabolic nitrogen which could be higher in animals fed bean diets supplemented with methionine. However, when 49-day-old rats from the PER studies were placed on a nitrogen-free diet for 5 days and faeces collected for 5 additional days and analysed for nitrogen, no significant differences were found between the metabolic nitrogen from rats that had consumed bean diets with or without methionine, or between rats that had been previously fed on bean or casein diets, which does not support the contention that metabolic nitrogen may vary with the bean diets used in this study.

The other explanation could be the possibility that the higher food intake in animals fed the methionine-supplemented diets could account for the decrease in digestibility. The pair feeding experiment, however, demonstrated clearly that the methionine effect was not related to the amount of food ingested and that, if anything, restricting food intake enhanced the methionine effect on digestibility. So far there is no explanation for this effect of methionine but studies are underway to clarify it.

Regarding pancreas weight, no effect of bean consumption on the size of the pancreas was detected, in spite of the fact that the animals consumed diets containing 18% protein from beans (about 65% bean flour in the diet). This is in contradiction with the results of Elias *et al.*[6] who reported an increased pancreas weight in rats fed bean diets. Age of the rats may have a bearing in explaining this discrepancy, since Lee and Liener[12] reported that the effect of feeding raw or heated soyabeans on the size of the pancreas disappeared as the rats grew older, and the rats in these experiments were killed when they were 3 weeks older than those from the experiments by Elias *et al.*

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