

Nutritional Assessment of Raw and Processed Faba Bean (*Vicia faba* L.) Cultivar Major in Growing Rats

Mar Fernández, María López-Jurado, Pilar Aranda, and Gloria Urbano*

Departamento de Fisiología, Facultad de Farmacia, and Instituto de Nutrición y Tecnología de Alimentos, Universidad de Granada, Campus Universitario de Cartuja s/n, 18071 Granada, Spain

We tested the effects of several processing methods on the nutritional value of faba beans. Nutritional assessment was based on chemical analyses of legume protein content and on the digestive and metabolic utilization in rats. Protein content in *Vicia faba* (*V. faba*) was 28%, and the supply of essential amino acids, except for sulfur-containing acids, was sufficient to cover the rat's nutritional requirements. Food intake was significantly lower in animals fed with raw faba beans than in the control group given the casein diet. We attribute this to the deficient levels of some amino acids and the presence of antinutritional factors (α -galactosides and tannins) in faba beans. All processing methods except heating under pressure increased food intake. The digestive utilization of *V. faba* protein was high, although lower than that of the casein diet. This may be related to the presence of protease inhibitors and tannins in faba beans. Nitrogen retention was similar in control animals and rats fed with raw beans; however, growth was faster in the former group, probably because of the better balance in amino acids supplied by this diet. Soaking in basic medium with or without subsequent cooking greatly increased growth, probably as a result of greater nitrogen retention and utilization of carbohydrates, which may have been stored as fat.

Keywords: *Faba beans; nutritive utilization; processing; digestive utilization; metabolic utilization*

INTRODUCTION

Legumes are an important source of protein and minerals in human and animal nutrition. Because of recent recommendations to reduce meat and increase legume consumption, the latter have received greater interest as human foodstuff in developing countries. Detailed knowledge of the nutritional utilization of this plant food is therefore needed.

The protein content of *Vicia faba* (*V. faba*; faba bean) ranges from 26 to 41% (Picard, 1977). The quality of this protein, although superior to that of other legumes tested in our laboratory (Nestares et al., 1993; Urbano et al., 1993), appears to be limited by the low content in sulfur-containing amino acids and in tryptophan, valine, isoleucine, and threonine (Baudet and Mose, 1980).

The usefulness of faba beans as a human and animal food is limited by antinutritional factors that curtail their nutritional utilization. However, these factors can be controlled with appropriate processing aimed at decreasing their damaging effects. α -Galactosides cause flatulence, and digestive function may be altered (Price et al., 1988) if endogenous α -galactosidase is lacking in the digestive tract. Trypsin inhibitors limit the activity of the digestive enzyme trypsin, reducing the utilization of dietary amino acids. These factors also interfere with trypsin synthesis and production in the pancreas, an alteration which can lead to pancreatic hypertrophy (Liener, 1979). The antinutritional effect of tannins is due to their affinity for certain nutrients, especially proteins (Kuman and Singh, 1984). Consequently, they can inhibit the activity of digestive enzymes (Bartolomé et al., 1994).

The purpose of the present study was to test the effects of several processing methods on the nutritional

value of faba beans, which we measured with chemical analyses of the legume's protein content. We then tested the digestive and metabolic utilization of faba beans in rats and compared the results to findings in a control group fed with a diet recommended by the American Institute of Nutrition (AIN, 1977).

MATERIALS AND METHODS

Samples. Faba beans, *V. faba* L. var. Major, were purchased at a local market. The seeds were submitted to the following processes:

Heating. Raw ground faba beans were dry-heated under pressure at 120 °C, 1 atm for 15 min (HF).

Soaking in distilled water (SF).

Soaking in 0.1% citric acid solution (SAF).

Soaking in 0.07% sodium bicarbonate solution (SBF).

Soaking and cooking in distilled water (SCF).

Soaking in 0.1% citric acid solution and cooking in distilled water (SACF).

Soaking in 0.07% sodium bicarbonate solution and cooking in distilled water (SBCF).

In processes SF, SAF, and SBF the beans were soaked for 9 h. The proportion of beans to soaking solution was 1/3 (wt: vol). After the beans were soaked, the solution was drained and the seeds were blended and lyophilized. The cooking processes (SCF, SACF, SBCF) were carried out with the beans previously soaked as above. Beans were cooked in bidistilled water (1/6.7, wt:vol) for 35 min. The beans were then strained, crushed, and lyophilized.

The findings in these experiments were compared with those obtained after feeding with a *control diet* that contained 20% casein and 0.3% methionine (AIN, 1977).

Chemical Analysis. Nitrogen was determined according to the Kjeldahl method. The protein conversion factor was 6.25.

Amino Acids. The amino acid composition of the faba beans was determined by high-performance liquid chromatography (Pico-Tag method) of acid-digested samples (except for tryptophan); cysteine and methionine were analyzed after performic acid oxidation.

* Author to whom correspondence should be addressed (telephone +34-58-243885; fax +34-58-243879).

Moisture Content. Moisture contents of the raw and processed faba beans were obtained by drying to constant weight in a vacuum oven (20 mm/Hg, 35 °C).

Ether extraction was performed by gravimetry of the ethyl ether extract.

Ash was measured by calcination at 500 °C to a constant weight.

Biological Methods. *Diets.* Nine diets were studied: C = control diet of casein (20%) + methionine (0.3%); RF = raw faba beans; HF = dry-heated faba beans; SF = faba beans soaked in distilled water; SAF = faba beans soaked in 0.1% citric acid solution; SBF = faba beans soaked in 0.07% sodium bicarbonate solution; SCF = faba beans soaked and cooked in distilled water; SACF = faba beans soaked in 0.1% citric acid solution and cooked in distilled water; SBCF = faba beans soaked in 0.07% sodium bicarbonate solution and cooked in distilled water.

Experimental Design. We studied the influence of faba beans given, raw or subjected to different treatments, on the intake of diet and the digestive and metabolic utilization of nitrogen. Rats were fed for 10 days with the faba bean diets, and the results were compared with data from a group of control animals fed a casein-methionine diet. A total of 90 rats were divided into nine groups of 10 animals each. Food intake, body weight, change in body weight, nitrogen intake, and fecal and urinary excretion of nitrogen were determined in all rats.

Animals. The animals were 4-week-old (recently weaned) albino Wistar rats with an initial body weight of 55 ± 5 g, reared in the University of Granada Laboratory Animal Services. The animals were divided into groups of 10 rats each (5 male, 5 female), which were housed from day 0 of the experiment in individual metabolic cages designed for the separate collection of feces and urine. The cages were located in a well-ventilated, thermostatically controlled room (21 ± 2 °C) with a 12 h light/dark period (lights on at 9:00 am). In all experiments the Thomas-Mitchell biological technique was used (Mitchell, 1923). A period of 3 days was allowed for adaptation to the diet, followed by a 7 days experimental period when feces and urine were collected on alternate days. Body weight and food intake (the total amount consumed daily by each rat was determined by weighing the amounts of diet given, refused, and spilled) were recorded at the beginning and at the end of the experimental period, that is on days 4 and 10 of all experiments. Throughout the experimental period all rats had free access to double-distilled water. The diet was consumed *ad libitum*.

Biological Indices. The following indices and parameters were determined for each group: intake (expressed as dry matter), body weight, protein efficiency ratio (PER), food transformation index (TI), apparent digestibility coefficient (ADC), nitrogen retention (nitrogen balance), and percent nitrogen retention/nitrogen absorption:

$$\text{PER} = \frac{\text{weight gained ((g/rat)/day)}}{\text{protein intake ((g/rat)/day)}} \quad (1)$$

$$\text{TI} = \frac{\text{food intake ((g/rat)/day)}}{\text{weight gained ((g/rat)/day)}} \quad (2)$$

$$\text{ADC} = \frac{I - F}{I} \times 100 \quad (3)$$

$$\text{balance} = I - (F + U) \quad (4)$$

$$\%R/A = \frac{I - (F + U)}{I - F} \times 100 \quad (5)$$

In accordance with the formulas recommended by the FAO/WHO (1966), the factors used were *I* (nitrogen intake), *F* (fecal nitrogen), and *U* (urinary nitrogen). Body weight and protein intakes were expressed as grams per rat per day.

Statistical Methods. Multifactor analysis of variance was applied to the data with Statgraphic Statistical Graphics 2.1 System Software (Statistical Graphics Corporation, Rockville,

Table 1. Composition of Nitrogen in the Control Diet and in Raw and Processed Fabia Beans (g/100 g of Dry Matter)^a

| diet | nitrogen | protein |
|------|----------|---------|
| C | 3.20 | 20.00 |
| RF | 4.52 | 28.25 |
| HF | 4.12 | 25.75 |
| SF | 4.19 | 26.19 |
| SAF | 4.21 | 26.31 |
| SBF | 4.51 | 28.19 |
| SCF | 4.52 | 28.25 |
| SACF | 4.51 | 28.19 |
| SBCF | 4.52 | 28.25 |

^a C, control diet; RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked.

MD); all analyses were run on an IBM Personal System/2 Model 20 Computer (International Business Machines Corporation, U.K.).

RESULTS

Chemical Analysis. Table 1 summarizes the nitrogen and protein content in the control diet and in raw and processed faba beans. The control diet was adjusted to contain 20% protein (casein + 0.3% DL-methionine), in accordance with the recommendations of the American Institute of Nutrition (AIN, 1977). Nitrogen content in the control diet was 3.2 mg/100 g of dry matter. Nitrogen content in all of the faba bean diets was similar and ranged from 4.12 to 4.52 mg/100 g of dry matter.

The essential amino acid composition of raw and processed faba beans is shown in Table 2. The nonessential amino acid composition of raw and processed beans is given in Table 3. When we calculated the chemical score by comparing the relative amount of each amino acid with a reference protein FAO/WHO (1973), we found that the major limiting amino acids in raw and processed beans were the sulfur-containing amino acids cysteine and methionine (Table 4).

Biological Analysis. In rats fed with dried faba beans as the only source of food, intake expressed as grams of dry matter per rat per day was significantly lower than in control animals given the casein diet. The intake of dry-heated beans and beans soaked in basic medium was similar to that of raw beans, although slightly greater for basic-soaked beans. All other processes increased intake to control values. Body weight increase expressed as grams per day was greatest in the control (casein) group. Raw and dry-heated faba beans, for which intakes were lowest, led to significantly smaller weight gains than in the other groups. All other processes led to significantly greater weight gains, although no group reached the control value (Table 5).

Protein intake was similar in controls and the groups fed with raw or dry-heated beans. All other processes led to significantly higher values of protein intake (Table 5).

The protein efficiency ratio (PER) revealed no relationship between protein intake and weight gain. The PER was greatest in animals fed with the control diet; intermediate values were found for the groups given raw, dry-heated, soaked, acid-soaked, basic-soaked, or cooked beans, and the PER was significantly lower in the group given soaked + cooked beans. In comparison with all other experimental diets, basic-soaked and basic-soaked + cooked beans led to a significantly higher PER, although these groups did not reach control

Table 2. Essential Amino Acid (AA) Composition of the Diets (g/16 g of Nitrogen)^a

| diet | for given AA | | | | | | | | |
|------|--------------|------|------|------|------|------|------|------|-----|
| | Phe | Tyr | Ile | Leu | Lys | Met | Cis | Val | Thr |
| C | 5.42 | 4.31 | 4.33 | 7.36 | 7.17 | 7.65 | 0.96 | 4.85 | 4.0 |
| RF | 3.19 | 2.15 | 2.70 | 7.43 | 4.72 | 0.79 | 0.34 | 3.84 | 3.9 |
| HF | 4.14 | 2.52 | 3.96 | 9.10 | 5.55 | 1.16 | 1.10 | 5.01 | 4.5 |
| SF | 3.03 | 2.32 | 2.95 | 7.51 | 4.37 | 0.92 | 0.59 | 4.10 | 3.9 |
| SAF | 4.22 | 2.58 | 3.80 | 9.02 | 6.24 | 1.07 | 0.74 | 4.81 | 4.1 |
| SBF | 4.73 | 3.65 | 3.87 | 10.1 | 6.03 | 1.10 | 0.99 | 4.34 | 4.3 |
| SCF | 3.62 | 2.22 | 3.86 | 8.70 | 5.18 | 1.07 | 0.84 | 4.80 | 4.4 |
| SACF | 3.38 | 2.11 | 3.68 | 8.08 | 4.90 | 0.93 | 0.58 | 5.00 | 4.0 |
| SBCF | 3.16 | 2.11 | 3.46 | 7.61 | 4.75 | 0.88 | 0.42 | 4.84 | 4.4 |

^a C, control diet; RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked.

Table 3. Nonessential Amino Acid (AA) Composition of the Diets (g/16 g of Nitrogen)^a

| diet | for given AA | | | | | | | |
|------|--------------|-------|------|------|------|------|------|------|
| | Asp | Glu | Ser | Gly | His | Ala | Arg | Pro |
| C | 6.32 | 21.71 | 5.33 | 1.74 | 3.42 | 2.98 | 3.43 | 7.28 |
| RF | 8.26 | 16.80 | 5.40 | 3.06 | 2.62 | 4.33 | 6.39 | 3.72 |
| HF | 12.78 | 21.41 | 6.04 | 4.15 | 3.02 | 5.65 | 8.00 | 4.86 |
| SF | 8.90 | 17.87 | 5.51 | 3.27 | 2.56 | 4.63 | 6.96 | 3.68 |
| SAF | 10.22 | 19.35 | 5.57 | 4.14 | 2.90 | 5.21 | 7.99 | 5.26 |
| SBF | 8.27 | 19.23 | 6.81 | 3.74 | 2.42 | 4.24 | 7.33 | 5.10 |
| SCF | 9.34 | 20.14 | 5.45 | 3.54 | 2.74 | 5.51 | 7.84 | 4.23 |
| SACF | 7.68 | 17.92 | 5.25 | 2.98 | 2.59 | 4.87 | 7.10 | 3.79 |
| SBCF | 7.21 | 17.34 | 6.01 | 2.88 | 2.64 | 4.63 | 7.23 | 3.54 |

^a C, control diet; RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked.

Table 4. Chemical Score in Raw and Processed Faba Beans^a

| diet | for given AA | | | | | | | | |
|------|--------------|------|------|-----|------|------|------|------|-----|
| | Phe | Tyr | Ile | Leu | Lys | Met | Cys | Val | Thr |
| RF | 89.0 | 89.0 | 67.4 | 106 | 85.9 | 32.3 | 32.3 | 76.8 | 98 |
| HF | 111 | 111 | 99.0 | 130 | 101 | 64.6 | 64.6 | 100 | 113 |
| SF | 89.2 | 89.2 | 73.7 | 107 | 79.5 | 43.1 | 43.1 | 82.0 | 98 |
| SAF | 113 | 113 | 95.0 | 129 | 114 | 51.7 | 51.7 | 96.2 | 103 |
| SBF | 140 | 140 | 96.6 | 145 | 110 | 59.7 | 59.7 | 86.8 | 108 |
| SCF | 97.3 | 97.3 | 96.6 | 124 | 94.2 | 54.6 | 54.6 | 96.0 | 110 |
| SACF | 91.5 | 91.5 | 92.0 | 115 | 89.1 | 43.1 | 43.1 | 100 | 100 |
| SBCF | 87.8 | 87.8 | 86.6 | 109 | 83.6 | 37.1 | 37.1 | 96.0 | 110 |

^a RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked.

Table 5. Food Intake and Weight Change in Rats Fed with Control Diet and with Raw and Processed Faba Beans^a

| diet | Δ wt ((g/rat)/day) | dry matter intake ((g/rat)/day) | protein intake ((g/rat)/day) | PER ^b | TI ^c |
|------|---------------------------|---------------------------------|------------------------------|---------------------------|--------------------------|
| C | 5.3 ± 0.19 | 12.1 ± 0.22 ^a | 2.5 ± 0.04 ^a | 2.10 ± 0.05 | 2.31 ± 0.19 ^a |
| RF | 3.0 ± 0.19 ^a | 9.9 ± 0.24 ^b | 2.6 ± 0.06 ^a | 1.18 ± 0.07 ^a | 3.38 ± 0.62 ^b |
| HF | 2.8 ± 0.12 ^a | 9.8 ± 0.33 ^b | 2.5 ± 0.09 ^a | 1.09 ± 0.03 ^{ab} | 3.58 ± 0.37 ^b |
| SF | 3.5 ± 0.20 ^b | 11.8 ± 0.54 ^a | 3.3 ± 0.15 ^b | 1.05 ± 0.06 ^{bc} | 3.48 ± 0.66 ^b |
| SAF | 4.3 ± 0.18 ^c | 12.7 ± 0.26 ^a | 3.6 ± 0.07 ^c | 1.15 ± 0.07 ^{ac} | 3.19 ± 0.66 ^c |
| SBF | 4.5 ± 0.19 ^{cd} | 10.3 ± 0.39 ^b | 2.9 ± 0.11 | 1.55 ± 0.06 ^d | 2.31 ± 0.23 ^a |
| SCF | 3.3 ± 0.16 ^{ab} | 12.9 ± 0.37 ^a | 3.6 ± 0.10 ^c | 0.92 ± 0.04 | 3.96 ± 0.61 |
| SACF | 3.8 ± 0.21 ^b | 11.4 ± 0.37 ^a | 3.2 ± 0.10 ^b | 0.92 ± 0.04 | 3.07 ± 0.40 ^c |
| SBCF | 4.7 ± 0.22 ^{cd} | 11.3 ± 0.56 ^a | 3.2 ± 0.16 ^b | 1.48 ± 0.08 ^d | 2.44 ± 0.39 ^a |

^a C, control diet; RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked. The same superscript letter in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM in 10 Wistar rats. ^b PER = [wt gained ((g/rat)/day)]/[protein intake ((g/rat)/day)]. ^c TI = [food intake ((g/rat)/day)]/[wt gained ((g/rat)/day)].

values. The TI (total intake in grams of dry matter per day per increase in body weight in grams per rat per day) varied depending on the diet, but in the opposite direction from PER (Table 5).

Nitrogen intake is a reflection of food intake and the amount of nitrogen present in the diet. The highest nitrogen intakes were found in animals fed with raw, soaked, or soaked + cooked faba beans. In the control

group, fecal nitrogen excretion was much lower than in any of the experimental groups. Fecal excretion of nitrogen was significantly lower in rats fed raw or dry-heated beans than in the other groups. Nitrogen absorption was significantly higher in animals given basic-soaked, water-soaked + cooked, basic-soaked + cooked, and acid-soaked + cooked beans than in rats fed with raw beans (Table 6).

Table 6. Digestive Utilization of Nitrogen^a

| diet | daily nitrogen intake ((mg/rat)/day) | daily total fecal nitrogen ((mg/rat)/day) | dialy absorbed nitrogen ((mg/rat)/day) | ADC ^b |
|------|--------------------------------------|---|--|--------------------------|
| C | 399.8 ± 48.9 ^a | 32.9 ± 14.5 | 366.90 ± 29.6 ^a | 91.7 ± 3.8 |
| RF | 445.1 ± 23.2 ^b | 79.5 ± 6.0 ^a | 365.60 ± 29.9 ^a | 82.1 ± 1.0 ^a |
| HF | 403.8 ± 23.2 ^a | 74.1 ± 16.8 ^a | 329.77 ± 29.5 | 81.8 ± 2.5 ^a |
| SF | 487.5 ± 71.1 ^c | 119.5 ± 24.0 ^b | 367.97 ± 54.5 ^a | 75.5 ± 2.9 ^b |
| SAF | 524.3 ± 34.4 ^d | 123.6 ± 15.9 ^b | 400.50 ± 25.2 ^b | 76.4 ± 2.2 ^b |
| SBF | 455.8 ± 55.4 ^{bc} | 95.6 ± 13.2 ^c | 371.24 ± 45.2 ^a | 79.5 ± 2.6 ^c |
| SCF | 582.1 ± 48.9 | 116.8 ± 14.9 ^b | 465.29 ± 39.8 | 79.9 ± 1.8 ^c |
| SACF | 515.5 ± 52.9 ^{c-e} | 102.9 ± 8.6 ^c | 412.64 ± 48.0 ^b | 79.9 ± 1.5 ^c |
| SBCF | 519.5 ± 62.3 ^e | 99.9 ± 18.1 ^c | 419.52 ± 46.5 ^b | 80.8 ± 1.6 ^{ac} |

^a C, control diet; RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked. The same superscript letter in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM in 10 Wistar rats. ^b ADC = [nitrogen intake - fecal nitrogen]/[nitrogen intake × 100].

Table 7. Metabolic Utilization of Nitrogen^a

| diet | total urinary nitrogen ((mg/rat)/day) | balance ^b | %R/A ^c |
|------|---------------------------------------|------------------------------|----------------------------|
| C | 169.40 ± 38.93 ^{ab} | 197.50 ± 22.96 ^a | 54.18 ± 7.64 ^a |
| RF | 153.21 ± 33.17 ^a | 212.39 ± 44.54 ^a | 57.82 ± 9.77 ^a |
| HF | 129.63 ± 19.97 ^c | 200.14 ± 40.42 ^a | 60.24 ± 8.09 ^{ab} |
| SF | 161.30 ± 32.35 ^a | 206.67 ± 50.35 ^a | 55.73 ± 8.85 ^{ab} |
| SAF | 146.57 ± 32.60 ^{ab} | 252.93 ± 26.15 ^b | 63.48 ± 7.50 ^b |
| SBF | 113.52 ± 26.89 | 256.62 ± 38.72 ^{bc} | 69.49 ± 5.95 |
| SCF | 204.40 ± 28.03 | 260.89 ± 38.80 ^b | 55.97 ± 5.54 ^{ab} |
| SACF | 182.99 ± 26.08 ^b | 229.65 ± 55.83 ^{ab} | 55.11 ± 7.72 ^a |
| SBCF | 169.22 ± 31.59 ^{ab} | 250.30 ± 32.98 ^c | 59.75 ± 5.65 ^{ab} |

^a C, control diet; RF, raw faba beans; HF, heated faba beans; SF, soaked faba beans; SAF, faba beans soaked in acid medium; SBF, faba beans soaked in basic medium; SCF, faba beans soaked and cooked; SACF, faba beans soaked in acid medium and cooked; SBCF, faba beans soaked in basic medium and cooked. The same superscript letter in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM in 10 Wistar rats. ^b Balance = nitrogen intake - (fecal nitrogen + urinary nitrogen). ^c %R/A = (balance)/[nitrogen intake - fecal nitrogen] × 100.

The ADC of the casein diet was significantly higher than in all experimental groups. For raw and dry-heated beans, the ADC was higher than for water-soaked or acid-soaked beans. Although we obtained higher ADC in basic-soaked, water-soaked + cooked, basic-soaked + cooked, and acid-soaked + cooked beans than in water-soaked or acid-soaked beans, the only process that yielded an ADC as high as that of raw beans was basic-soaking + cooking. Nitrogen balance after feeding with raw, dry-heated, soaked, and basic-soaked + cooked beans was similar to that in the control group. Acid-soaking, basic-soaking, water-soaking + cooking, and basic-soaking + cooking led to significantly higher nitrogen balances.

Percent nitrogen retention/nitrogen absorption (%R/A) was similar in the control groups and in rats given raw beans. Only acid-soaking and basic-soaking yielded %R/A values above control figures; after all other processes, %R/A was similar to that in rats fed with raw beans (Table 7).

DISCUSSION

Chemical Analysis of Protein. The amino acid composition of the faba bean diets we tested is within the range reported for *V. faba* (Nitsan, 1971; Marquardt

et al., 1975; Kaldy and Kasting, 1974; Palmer and Thompson, 1975; Hove et al., 1978). Comparisons of our analytical data with values recommended by the National Research Council (1978) show that both the raw and processed faba beans provided sufficient amounts of essential amino acids (except for cysteine and methionine) to ensure normal development in growing rats.

In our experimental diets the proportion of essential amino acids was within 36–40%, that of nonessential acids ranging from 60 to 64%. According to Santidrian (1987), proteins should contain an optimum proportion of about 33% essential and 66% nonessential amino acids, whereas in low-quality protein the proportion approached 25%/75%. On the basis of these figures, the diets we tested can be considered of high nutritional value. However, our methods of chemical analysis do not take into account amino acid imbalances (Munro, 1987) or differences in the rate of absorption between amino acids. Moreover, faba beans are deficient in some essential amino acids, and the total or partial lack of an essential amino acid can lead to weight loss and alterations in growth (Sanchez, 1990). The *E/N* ratio (ratio between essential (indispensable) and non-essential (dispensable) amino acids) was below 1 in all of our diets. According to Stuki and Harper (1962), optimum growth in weanling rats is obtained with an *E/N* of 4/1; if this figure is valid, our diets should have led to limited growth. Rats fed with any of the faba bean diets would therefore have been expected to show less efficient growth than the animals given the control diet.

Biological Analysis of Protein. Intake. The significantly lower food intake in rats fed with raw faba beans than in control rats was probably due in part to the difference between the diets in protein quality and to the effects of antinutritional factors in this species of legume. As earlier studies have pointed out, faba beans are deficient in sulfur-containing amino acids and in isoleucine, valine, and probably tryptophan (Baudet and Mose, 1980). The amino acid balance in our experimental diets was therefore inappropriate and was probably responsible for alterations in food intake.

Another factor that may account for decreased food intake in rats given raw or processed faba beans is this species' high α -galactoside content. *V. faba* is rich in verbascose and stachyose and also contains a considerable amount of raffinose. Total α -galactoside content in raw beans was 3.7%; the major carbohydrate was verbascose (2.3%), followed by stachyose (1.1%) and raffinose (0.3%) (Fernández et al., 1973). These α -galactosides cause flatulence (i.e., the production of large amounts of hydrogen and carbon dioxide and small amounts of methane gas (Reddy et al., 1980; Andersen et al., 1979) in humans and animals, and the presence of these sugars in faba beans therefore makes them less desirable as a food.

Tannins are another quantitatively important antinutritional factor in faba beans. Our experimental diets contained large proportions of these compounds, which ranged from 1.83 to 4.51 mg/100 g of dry weight (Fernández et al., 1993). These values are within the range given by Chavan et al. (1989) in an earlier study. During chewing and swallowing, soluble tannins can bind to precipitate proteins in the saliva. This reduces the saliva's lubricating properties and causes sensations of dryness of the mouth, which interferes with swallowing (Mole, 1989), and thus reduces food intake.

Processed faba beans were usually consumed in larger

amounts than raw beans. Of the antinutritional factors in this legume, α -galactosides were significantly reduced (by 39–44%) by all processing methods tested here. This result may also be related to the larger food intakes, despite the fact that processing increased the total amount of tannins and decreased that of palatable low molecular weight sugars (Fernández et al., 1993). Fructose was significantly lower, whereas the disaccharide saccharose was slightly lower after processing. In contrast, the monosaccharide glucose, which was not detected in raw faba beans, made up 0.3–0.4% by dry weight of processed beans. This increase may have made these diets more palatable and may thus account for part of the greater food intake in comparison with rats fed with raw beans.

In a study by Abdel-Gawad (1993), the proportion of α -galactoside carbohydrates (41–62%) eliminated by processing was larger than in our experiments. This may be related with differences in experimental conditions, which used a higher seed/liquid ratio to cook the beans.

Dry-heating at 120 °C and 1 atm led to the greatest reduction in α -galactoside content (from 3.62 to 1.55 mg/100 g of dry weight) (Fernandez et al., 1993). However, this treatment did not increase food intake in comparison with the group fed with raw beans. Dry-heating may have denatured proteins (Bressani et al., 1974), an effect that may have partially offset the benefits of removing α -galactosides.

Digestive Utilization of Protein. The ADC of protein, which we considered an indicator of protein absorption, showed that nitrogen absorption was high in rats fed with the control diet (91.7), as expected from the excellent nutritional quality of casein.

The ADC of raw faba beans was higher (82.1) than the figures published in earlier studies (Moseley and Griffiths, 1979; Rani and Hira, 1993). In our experiments faba beans were the only source of food, whereas these authors tested diets in which 10% of the protein was provided by this legume. Nonetheless, our ADC for raw beans is lower than the figure obtained in the control group.

Protease inhibitors and tannins may account for the decrease in digestive utilization. The concentration of trypsin inhibitor differs notably between cultivars of *V. faba*. Our values (2.62 ± 0.17) (Fernández et al., 1993) are within the range given in an earlier study by Bhatti (1977). These substances inhibit the proteolytic action of proteases, which leads to incomplete protein digestion, with lower amounts of amino acids becoming available for growth.

The concentrations of tannins (1.82 mg/100 g) and catechins (0.29 mg/100 g) (Fernández et al., 1993) in the cultivar we tested are similar to those found in an earlier study by Chavan et al. (1989). These compounds increase fecal nitrogen excretion and may be responsible for part of the decrease in ADC for protein.

The ADC of faba beans that were autoclaved at 120 °C for 15 min did not differ from that of raw beans, although nitrogen intake was significantly lower in the former group. This was because fecal nitrogen excretion was similar in the two groups. The decrease in trypsin inhibitor activity (TIA) (70%) (Fernández et al., 1993) as a consequence of heat treatment would have been expected to reduce fecal nitrogen excretion; the fact that no such decrease occurred may have been due to the increase in tannin content as a result of heating, together with the effect of protein denaturation. Heat

treatment causes isopeptides to form (Hurrell and Carpenter, 1975; Mauron, 1985; Bender, 1978a; Dutson et al., 1984; Kirk, 1984; Gomez Piñol, 1989); this further reduces the nutritional value of heated beans, as isopeptides are not hydrolyzed in the intestine, are resistant to proteolytic enzymes, and are thus excreted in feces. As a result, digestibility and bioavailability of some amino acids are reduced (Dutson et al., 1984; Kirk, 1984). Because of the low fat and water contents of faba beans, the protective effect of these substances against heat-caused damage to proteins (Varela et al., 1967) was impaired.

The other processes used to treat faba beans significantly decreased ADC in comparison with raw beans. Although processing led to greater food intakes, there was also a nonproportional increase in fecal nitrogen excretion.

The decreased TIA found in rats given processed beans did not improve protein digestibility. Soaking in water or basic medium decreased TIA (Fernandez et al., 1993) by 20% and 24%, respectively, although soaking in a citric acid solution had no such effect, because of the stability of the TIA at acid pH values (Chavan et al., 1989). Heating caused a sharp decrease in TIA (70–83% of the activity disappeared), a finding that confirms the sensitivity of TIA in *V. faba* to the effects of heat (Griffiths, 1984; Hamza et al., 1986).

Tannins appear to be the antinutritional factor most clearly associated with decreased protein absorption. Processing made *V. faba* tannins more accessible: the tannin/catechin ratio (an indicator of tannin polymerization) decreased, suggesting that heating, soaking, and cooking condensed these compounds. When we compared tannin contents with the results of our biological analyses, we found no relationship between nitrogen intake and tannin content. However, fecal nitrogen excretion correlated significantly ($P < 0.05$) with dietary tannin content, a finding in agreement with results published by Butler (1989).

In rats fed basic-soaked and cooked faba beans, the ADC was close to that found in animals fed with raw beans. This treatment clearly improved the digestive utilization of protein. Although fecal nitrogen excretion was high, nitrogen intake from this diet was significantly higher than from the raw bean diet; consequently, net nitrogen absorption was favored. Basic-soaking followed by cooking led to the greatest reduction in raffinose (38%) (Fernandez et al., 1993). This result is in consonance with the findings of Jood et al. (1986), who obtained the greatest reduction in soluble carbohydrates when sodium bicarbonate was added to the soaking solution. The reduction in raffinose clearly improved food intake in rats fed with the basic-soaked + cooked diet and led to a better digestive utilization of protein.

Metabolic Utilization of Protein. We studied the metabolic utilization of nitrogen as nitrogen balance after the consumption ad libitum of different experimental diets. The quality of dietary protein was one of the factors that most clearly affects the retention of dietary nitrogen by the organism. This characteristic reflects the affinity between protein composition and the amino acid profile in tissue, i.e., mainly muscle tissue in growing animals. Nitrogen balance should be considered in conjunction with other indices such as PER and TI. When the dietary protein is of poor quality, TI is increased and PER decreases (Okamura and Mori, 1979; Prieto, 1979; Boorman, 1980). In a recent com-

parison of diets that contain proteins of differing quality, Nieto (1994) noted that when the TI or PER of the diets differed by more than 10%, this was associated with notable differences in growth.

Under our experimental conditions, the control group, which was given the highest quality protein (casein + methionine), had the best PER and the lowest TI. Nitrogen retention in this group was the same as in the group fed with dried faba beans: in both groups nitrogen absorption and urinary nitrogen excretion were similar. These findings show that, in control animals, the protein retained—which contained all essential amino acids—led to greater weight gains than did dried faba beans. In other words, rats that received dietary casein were able to take fuller advantage of the nitrogen they retained to favor growth, probably as a result of the more balanced supply of amino acids provided by this diet. In rats fed with raw faba beans, part of the nitrogen retained may have been rerouted toward the synthesis of digestive enzymes such as trypsin and chymotrypsin (Liener, 1976, 1979) to offset the effects of the trypsin inhibitors present in *V. faba*.

Much of the cysteine and methionine supplied by the diet was used to synthesize pancreatic enzymes. This exacerbated the deficiency in sulfur-containing amino acids in faba beans and was manifested as a lower production of body tissues. Rerouting of retained nitrogen in the group fed with raw beans may explain why PER in this group was significantly lower than in controls despite the fact that nitrogen balance was similar in these two groups.

When we compared nitrogen balance between the experimental groups, we found that this index improved when the beans were soaked in solutions of acid or basic pH regardless of whether they were cooked afterwards. However, the increase in nitrogen balance was accompanied by better body growth (higher PER and lower TI) only in animals given basic-soaked beans or basic-soaked + cooked beans. The TI in these two groups differed from that in the group fed with raw beans by more than 10%, suggesting that, in accordance with the findings of Nieto (1994), body growth was favored.

Nitrogen retention was also high in rats fed acid-soaked beans, water-soaked + cooked beans, and acid-soaked + cooked beans, although gains in body weight in these groups did not reach the values found in rats fed with basic-soaked or basic-soaked + cooked beans. The efficient utilization of faba bean carbohydrates, which may have been deposited as fat, may have contributed to the good body growth in the groups fed with basic-soaked or basic-soaked + cooked beans. In a similar experiment with lentils, we found that soaking and cooking increased the usable starch/total starch ratio, probably because of changes in the branched structure of the starch molecule, which made it more accessible to enzymatic activity and thus enhanced availability (Frías, 1992).

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