JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Different Soaking Solutions on Nutritive Utilization of Minerals (Calcium, Phosphorus, and Magnesium) from Cooked Beans (*Phaseolus vulgaris* L.) in Growing Rats

TERESA NESTARES,† MERCEDES BARRIONUEVO,† MAGDALENA LÓPEZ-FRÍAS,*,†
CONCEPCIÓN VIDAL,‡ AND GLORIA URBANO†

Department of Physiology, School of Pharmacy and Institute of Nutrition and Food Technology, University of Granada, E-18071 Granada, Spain, and Industrial Fermentation Institute, CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain

The effects of the commonly used processing techniques of soaking (at different pH values) and cooking on the digestive and nutritive utilization of calcium, phosphorus, and magnesium from common beans (*Phaseolus vulgaris* L.) were studied. Before the cooking step, the beans were soaked in solutions of acid (2.6 and 5.3) or basic (8.4) pH. Chemical and biological methods were used to determine nutritional parameters in growing rats, and the fiber content of the beans was established. As the pH of the soaking solution increased, so did mineral absorption and the apparent digestibility coefficient, which reached suitable values for growing rats, due to the reduced losses of soluble minerals and the increased food intake. Metabolic utilization also improved with increased pH of the soaking solution, although the values were, in general, low as a result of urinary losses under the experimental conditions. For the experimental period of 10 days, the femur and the muscle seem to be good metabolic indicators for calcium, but not for phosphorus or magnesium. The increased amount of cellulose in the soaked seed did not have a negative effect on the digestive utilization of minerals.

KEYWORDS: Common bean; nutritive utilization; *Phaseolus vulgaris*; processing techniques; pH; minerals, fiber

INTRODUCTION

Due to the relationship between a diet low in dietary fiber and the development of illnesses such as hypertension, arteriosclerosis, and diabetes, an increase in the intake of complex carbohydrates and dietary fiber is recommended for diets in the West. The role played by fiber in influencing mineral bioavailability, however, has yet to be clarified (*1*, *2*).

The common bean (*Phaseolus vulgaris* L.) contains high levels of minerals and fiber and is the third most widely consumed legume in Spain (*3*). Mineral bioavailability, however, is influenced directly or indirectly by other components of the legume such as protein (*4*−*6*), phytic acid (*7*), antinutritive factors (*8*), other minerals (*2*, *9*), and fiber (*10*). In fact, the traditionally accepted capacity of dietary fiber to bind polyvalent mineral ions may also have a negative effect on the bioavailability of some nutrients, fundamentally mineral elements, and thus modify the balance of these cations. Moreover, the processing stage, which is necessary before the legume can be consumed, affects the above-mentioned factors, and so the bioavailability of minerals may be enhanced or reduced (*1*, *11*−*15*), varying depending on the techniques and experimental conditions (*15*−*18*).

The mineral content and nutritional value of bean seeds, together with the effects of processing, have been investigated in detail (*7*, *19*, *20*). However, recent work has brought to light the importance of the pH of the soaking or cooking solution, in view of the effect of pH on other seed components such as antinutritional factors (*21*), protein (*22*), starch (*23*, *24*), and fiber (*15*, *25*), because modifications in these factors may affect the nutritive utilization of legume minerals. In addition, as occurs with protein, mineral solubility varies under different pH conditions, and this might be a major cause of the poor digestibility of this legume (*26*−*28*).

The present study was designed to evaluate how the pH of the soaking solution, a processing technique applied before the legume is cooked, affects the nutritive quality of the main minerals in a common bean grown in southern Spain. The pH values tested were chosen to reproduce, as closely as possible, the methods commonly used in Spanish homes: soaking in water alone, in water with bicarbonate (baking soda), or in water with citric acid (lemon juice). The aim of the study was to determine whether adequate soaking—a simple and cheap process—enables beans to be prepared for consumption without damaging their mineral quality (and possibly improving it).

* Address correspondence to this author at Depto. de Fisiología, Facultad de Farmacia, Campus de Cartuja, Universidad de Granada, E-18071 Granada, Spain (telephone +34-58-243879; fax +34-58-248959; e-mail maglopez@ugr.es).
† University of Granada.
‡ CSIC.

10.1021/jf020693y CCC: $25.00
Published on Web 12/12/2002

MATERIALS AND METHODS

Samples. Raw, dried common beans (R) (*P. vulgaris* L.) were grown in Andalusia (southern Spain). The seeds were subjected to three different treatments, characterized by the pH of the soaking solution: sA, soaking in strongly acid solution (pH 2.6) and cooking; mA, soaking in moderately acid solution (pH 5.3) and cooking; B, soaking in basic solution (pH 8.4) and cooking.

Processing Techniques. *Soaking.* Raw seeds were soaked at room temperature for 9 h in a citric acid solution (0.1%, pH 2.6), in distilled water (pH 5.3), or in a sodium bicarbonate solution (0.07%, pH 8.4). The seed-to-solution ratio was 1:3 (w/v). The soaking liquid was drained off, and the seeds were blended and lyophilized.

Cooking. The soaked common beans were cooked by boiling in distilled water for 35 min, at a seed-to-water ratio of 1:6.67 (w/v). The cooking water was drained off, and the seeds were crushed and lyophilized.

Analytical Techniques. The moisture content of the samples was determined by using AOAC method 925.10 (*29*).

Calcium, magnesium, and phosphorus were determined in aliquots of bean diets and the feces, femur, and longissimus dorsi muscle from rats, reduced to ash in a muffle furnace at 450 °C, and then dissolved in 6 N HCl for analysis. Urine samples were measured as such. Lanthanum chloride (1−0.1%) was added to avoid interferences during the analysis. The magnesium and calcium content was measured by atomic absorption spectrometry (Perkin-Elmer 1100-B apparatus). Inorganic phosphorus was determined by visible spectrophotometry with the colorimetric technique of Fiske and Subbarow (*30*).

The method of Van Soest and Wine (*31*) as modified by McQueen and Nicholson (*32*) was used to determine neutral detergent fiber (NDF), cellulose (CL), hemicellulose (HMC), and lignin (LN). To remove starch, the samples were incubated overnight with a solution of 0.5% bacterial α-amylase (*33*).

Biological Methods. *Experimental Design and Diet.* For nutritional evaluation, a biological balance technique was used, by which food intake and changes in body weight were recorded, and the intake and the fecal and urinary excretion of minerals (calcium, magnesium, and phosphorus) were calculated.

Three experiments were carried out, in which processed common beans were the only source of food, provided to the following groups: sA, beans soaked in strongly acid solution and cooked; mA, beans soaked in moderately acid solution and cooked; and B, beans soaked in basic solution and cooked. Each experiment lasted 10 days. The diet and demineralized water (Milli-Q, Ultrapure Water System, Millipore, Bedfore, MA) were available ad libitum throughout the experimental period. During the first 3 days the rats were allowed to adapt to the diet and experimental conditions, and the main experimental period comprised the next 7 days, during which body weight and food intake were recorded and feces and urine were collected for subsequent analysis. Feces were dried, weighed, and homogenized. Urine was collected on 0.5% HCl (v/v), filtered (Whatman filter paper no. 40, Whatman, Maidstone, U.K.,) and diluted.

The diets had a protein content of 211.2−214.2 g kg^{-1} (dry matter basis), which was not significantly modified by the processing method used (*22*).

Animals. In each experiment 10 young albino Wistar rats were used (5 males, 5 females), bred by the University of Granada Animal Services Laboratory. The growing animals (recently weaned), with an initial body weight of 54.39 ± 0.18 g, were housed in individual metabolic cages kept in a thermoregulated room (22 ± 1 °C) with a controlled 12-h light/dark period (lights on at 9:00 a.m.).

Biological Indices. The following indices and parameters were determined for each group, according to the formulas given below: apparent digestibility coefficient (ADC) (eq 1); retention (balance) (eq 2), and percent retention/absorption (% R/A) (eq 3), for calcium, magnesium, and phosphorus:

$$\mathrm{ADC} = [(I - F)/I] \times 100 \quad (1)$$

$$\mathrm{balance} = I - (F + U) \quad (2)$$

$$\%\ \mathrm{R/A} = \{[I - (F + U)]/(I - F)\} \times 100 \quad (3)$$

Table 1. Composition of Ash, Calcium, Phosphorus, and Magnesium in Raw and Processed Beans in Dry Matter and Loss (Percent with Respect to Raw State) Caused by Processing

	ash[a]		calcium[a]		phosphorus[a]		magnesium[a]	
diet	% diet	% loss	mg/100 g of diet	% loss	mg/100 g of diet	% loss	mg/100 g of diet	% loss
R[b]	4.15		165.5		384.6		161.7	
sA	2.96	28.7	139.0	15.8	326.7	15.1	117.8	27.2
mA	2.97	28.4	155.8	5.5	322.6	16.1	121.0	25.3
B	3.22	22.4	161.4	2.4	349.2	9.4	133.0	17.9

[a] Values are means ± SEM from five replications. Standard errors ranged from ±0.01 to ±0.001. [b] R, raw beans.

Table 2. Composition of Fiber in Raw and Processed Beans in Dry Matter (Grams per 100 g of Diet)

diet	NDF	CL	HMC	LN
R[a]	22.59 ± 0.52	6.22 ± 0.57	10.62 ± 0.92	5.76 ± 0.37
sA	20.77 ± 0.25	12.60 ± 0.21	2.21 ± 0.71	5.96 ± 0.16
mA	21.86 ± 0.02	14.50 ± 0.15	2.53 ± 0.05	4.83 ± 0.24
B	24.40 ± 0.62	16.10 ± 0.10	2.13 ± 0.40	6.17 ± 0.65

[a] R, raw beans. Values are means ± SEM from five replications

In accordance with the formulas recommended by the National Research Council (*34*), the factors used were *I* (mineral intake), *F* (fecal mineral), and *U* (urinary mineral). Mineral intakes are expressed as milligrams per rat per day.

Statistical Methods. Data were tested statistically by one-way analysis of variance (ANOVA) using Statgraphic Statistical Graphics 2.1 System software (Statistical Graphics Corp., Rockville, MD) with an IBM Personal System/2 model 20 computer. Comparisons between the means were determined by Duncan's multiple-range test. Differences were considered to be significant when $P < 0.05$.

RESULTS

Chemical Analysis. Table 1 gives the values for ash, calcium, phosphorus, and magnesium content in the raw and processed beans diets and also the percentage lost due to processing. Raw bean contained 1.65 g of calcium, 3.85 g of phosphorus, and 1.62 g of magnesium/kg of sample. Soaking followed by cooking decreased the mineral content by 2.5−16% for calcium, by 9−16% for phosphorus, and by 18−27% for magnesium. In general, mineral loss fell as the pH of the soaking solution increased.

Table 2 shows the fiber content (NDF, CL, HMC, and LN) of raw and processed beans. Raw beans contained 23% NDF; most of this amount (10.6%) was HMC. Soaking followed by cooking significantly decreased the HMC content and increased that of CL. Lignin was not significantly affected by any of the treatments.

Biological Analysis. *Biological Analysis of Calcium.* Calcium intake increased significantly as the pH of the soaking solution rose. The digestive utilization of calcium, calculated as ADC (**Table 3**), also improved, and differences between the highest and lowest pH values were statistically significant.

Calcium retention (**Table 3**) was generally low, although it increased significantly with higher pH values of the soaking solution. The metabolic utilization of calcium, assessed as the ratio of retained to absorbed calcium (**Table 3**), also improved with respect to digestive utilization, that is, as the pH of the soaking solution rose, and differences between the highest and lowest pH values were statistically significant.

Biological Analysis of Phosphorus. Phosphorus intake was significantly greater among the animals given the diet that was

Table 3. Digestive and Metabolic Utilization of Calcium from Processed Beans[a]

group	Ca intake (mg/rat/day)	absorbed Ca (mg/rat/day)	ADC	balance	% R/A
sA	9.45 ± 0.23	5.22 ± 0.90	54.54 ± 9.07a	4.01 ± 0.82	68.07 ± 8.12a
mA	11.15 ± 0.23	7.68 ± 0.36	72.68 ± 4.57ab	6.38 ± 0.33	84.08 ± 1.44ab
B	12.44 ± 0.34	9.74 ± 0.69	78.38 ± 5.23b	8.53 ± 0.87	85.94 ± 4.65b

[a] Balance = Ca intake − (fecal Ca + urinary Ca); % R/A = [balance/(Ca intake − fecal Ca] × 100. The same letter in the same column indicates no significant differences ($P < 0.05$). Values are means ± SEM of 10 Wistar rats.

Table 4. Digestive and Metabolic Utilization of Phosphorus from Processed Beans[a]

group	P intake (mg/rat/day)	absorbed P (mg/rat/day)	ADC	balance	% R/A
sA	21.90 ± 0.60a	15.20 ± 0.20	69.23 ± 1.69a	7.63 ± 0.80a	68.07 ± 8.12a
mA	23.09 ± 0.55a	17.17 ± 0.24	74.40 ± 0.64b	7.49 ± 0.36a	83.08 ± 1.44ab
B	26.92 ± 0.74	19.30 ± 1.71	71.87 ± 1.60ab	9.40 ± 0.36	85.94 ± 4.65b

[a] Balance = P intake − (fecal P + urinary P); % R/A = [balance/(P intake − fecal P] × 100. The same letter in the same column indicates no significant differences ($P < 0.05$). Values are means ± SEM of 10 Wistar rats.

Table 5. Digestive and Metabolic Utilization of Magnesium from Processed Beans[a]

group	Mg intake (mg/rat/day)	absorbed Mg (mg/rat/day)	ADC	balance
sA	7.96 ± 0.63	1.02 ± 0.20	12.47 ± 4.13	
mA	8.67 ± 0.65	2.60 ± 0.21a	30.31 ± 3.64a	
B	10.26 ± 0.89	3.55 ± 0.28a	34.80 ± 3.73a	

[a] Balance = Mg intake − (fecal Mg + urinary Mg); % R/A = [balance/(Mg intake − fecal Mg] × 100. The same letter in the same column indicates no significant differences ($P < 0.05$). Values are means ± SEM of 10 Wistar rats.

soaked in a basic solution (B) (**Table 4**) with respect to those consuming the diets based on soaking in an acid solution (sA and mA). The digestive utilization of phosphorus, assessed as the ADC, was highest for the mA group, although this was only statistically significant with respect to the other acid medium (sA).

Total phosphorus retention (**Table 4**) significantly improved for group B, and there were no differences between the two groups with acid media. However, expressing metabolic utilization as the percentage of amount retained with respect to that absorbed, the basic medium (B) was significantly better than only the most acid group (sA); this was also true for calcium.

Biological Analysis of Magnesium. Magnesium intake (**Table 5**) increased significantly as the pH of the soaking solution rose. The digestive utilization of magnesium, calculated as the ADC, was very low for the sA group, but was spectacularly higher in the other two groups, with a higher pH.

Magnesium retention (**Table 5**) was negative in all experimental groups.

Mineral Content in Femur and Muscle. No significant differences were found in muscle ash content between the animals given the three experimental bean diets (**Table 6**); nor were there any significant differences in the calcium and magnesium contents. The only difference was for phosphorus, the mA group presenting higher values than the other two groups.

The femur (**Table 6**) of the animals in group sA had a muscle ash content and levels of phosphorus that were significantly lower than those of the other two groups. However, the mA group presented lower levels of calcium than the other two groups, whereas the magnesium contents in the femur were the same in all three groups.

DISCUSSION

Chemical Analyses. The mineral (calcium, phosphorus, and magnesium) and ash contents of the raw bean variety examined in this study were within the ranges reported by other Spanish researchers (*35*−*37*). Soaking followed by cooking, as expected, reduced the ash content, due to solubilization. Mineral losses were reduced as the pH of the soaking solution increased, on the contrary to what happens when the chickpea (*Cicer arietinum* L.) is processed in the same way (*15*, *38*). This interesting protective effect against mineral loss, when beans are soaked at higher pH values, was previously observed by Minka et al. (*39*), who studied the effects of cooking the beans in an alkaline medium and found reduced losses of soluble compounds in the cooking medium.

The largest mineral losses in quantitative terms were of magnesium, although the quantity retained by the seed is still very high, and so the bean that is processed in this way may still be considered an excellent source of magnesium. Of the minerals studied, calcium was most retained within the soaked seed, and the amount lost in the basic solution was insignificant.

The fiber content of the bean variety assayed in this study was within the range reported by other authors for various varieties grown in Spain (*37*, *40*). Soaking followed by cooking decreased HMC due to solubilization, regardless of the pH employed. However, the increase in CL varied depending on the pH; it was higher with a basic pH, which is of great biological importance, as discussed below, and implies a greater consumption of insoluble fiber by the animals given the B diet. Values of LN were not significantly affected by processing.

Biological Analysis of Calcium. Calcium intake increased significantly as the pH of the soaking solution rose. This correlated positively with the animals' food intake and with the calcium content of the beans consumed. Both the diet consumed and its calcium content (**Table 1**) increased as the pH of the soaking solution rose. Thus, the rats given the beans soaked in a very acid solution (sA), a moderately acid solution (mA), or a basic solution (B) consumed, respectively, 6.8 ± 0.17, 7.16 ± 0.17, and 7.71 ± 0.21 mg/rat/day of dry substance (*22*).

The quantity of calcium consumed and absorbed, in absolute values, by the rats given the three types of bean assayed did not meet the nutritional requirements of growing rats (*34*). Nevertheless, in the present study CDA values for calcium were close to the 84% found with the standard caseine−methionine diet (*41*), and so the absorption of calcium with the bean diets may be considered to be sufficient, under our experimental conditions. This result is due to the fact that the processing of the legume modifies other components that affect calcium absorption. Thus, the improvement in the protein quality of the bean by soaking the seed in a basic pH solution (*22*) favors calcium absorption (*5*). Moreover, as the pH of the soaking solution increases, less soluble calcium is lost, and digestive and metabolic utilization of the mineral improve, as described by Kaup et al. (*42*).

Under our experimental conditions, the greater intake of cellulose in the diet did not affect calcium absorption, as the two values rose with increased pH of the soaking solution, and so processing the legume in this way is of considerable nutritional interest. Previously, it was assumed that fiber negatively affected calcium balance, either by physical entrap-

Table 6. Calcium, Phosphorus, and Magnesium Content in Muscle (Longissimus Dorsi) and Bone (Femur)[a]

	muscle				bone			
group	mg of ash/g	mg of Ca/g of ash	mg of P/g of ash	mg of Mg/g of ash	mg of ash/g	mg of Ca/g of ash	mg of P/g of ash	mg of Mg/g of ash
sA	58.78 ± 1.15a	13.00 ± 2.70a	155.99 ± 8.42a	19.42 ± 1.09a	387.39 ± 8.93	364.67 ± 4.59a	159.50 ± 17.57	8.57 ± 0.35a
mA	56.10 ± 0.74a	7.38 ± 2.16a	167.88 ± 5.31	19.23 ± 0.45a	415.22 ± 7.82a	338.67 ± 9.17	179.19 ± 11.56a	8.34 ± 0.61a
B	57.37 ± 0.77a	6.97 ± 1.40a	156.16 ± 7.18a	18.65 ± 0.39a	412.93 ± 4.48a	381.03 ± 5.46a	176.72 ± 9.29a	8.36 ± 0.50a

[a] The same letter in the same column indicates no significant differences ($P < 0.05$). Values are means ± SEM of 10 Wistar rats.

ment or by cationic binding with uronic acid residues (*43*); however, it is more likely that the phytic acid associated with fiber-rich products such as beans is the component that affects calcium absorption (*44*). In almost all of the studies made of single sources of fiber, such as cellulose, no harmful effects on mineral balance were found (*1*, *10*). Furthermore, during digestion, inositol hexaphosphate [which accounts for 90% of the total phytic acid in legumes (*45*)] is transformed into inorganic phosphorus and inositols, which contain fewer phosphate groups because of the action of phytase in the legume itself (*45*−*48*) and that of endogenous phytase in the rat (*47*). This fact, which has been confirmed with other legumes processed in the same way (*38*, *49*), could explain the absence of the chelating effect of phytic acid on bean calcium, under our experimental conditions.

The metabolic utilization of bean calcium as a function of the calcium balance (**Table 3**) was low because of the rates of urinary excretion and net absorption of the cation. However, there was a high correlation between calcium absorption and retention, which indicates that calcium levels are fundamentally regulated in the digestive tract. High concentrations of parathyroid hormone (as a result of the low calcium intake) probably increase calcium retention in a manner directly proportional to calcium absorption.

Biological Analysis of Phosphorus. Phosphorus intake (**Table 4**) was significantly higher among the animals that consumed diet B than among those given the two acid media (sA and mA). This is explained, as with calcium, by the greater food intake by the animals in group B (*22*), together with the higher phosphorus content of this diet (**Table 1**).

The digestive utilization of phosphorus, assessed as the ADC (**Table 4**), was in general acceptable for growing rats. It was significantly better with the moderately acid pH (mA) than with the strongly acid solution (sA), despite the fact that the food intake of the two groups was not significantly different. This improvement might be because the pH used in the mA diet (5.6) has been described as optimum for the activity of the phytase in the legume (*45*−*48*), and so the phytase (as described above) affected the availability of the anion, derived from phytic acid, in the intestinal lumen and thus produced a greater amount of absorbable phosphorus.

Phosphorus retention (**Table 4**), estimated as a function of the balance, was very low. The homeostatic regulation of the phosphorus metabolism is known to involve variations in food intake and renal excretion. Under our experimental conditions, the high rate of urinary excretion (because of the low calcium retention) and the relatively low rate of phosphorus absorption led to a lower metabolic utilization of phosphorus. This balance significantly improved with the B diet, probably because of the better metabolic utilization of calcium by this group. As with calcium, a strong correlation was observed between phosphorus absorption and retention.

Biological Analysis of Magnesium. In all three diets tested, the magnesium content remained above the level required for growing rats (40 mg/100 g of diet) (*34*) despite the losses in mineral content caused by processing. Magnesium intake increases with higher pH of the soaking medium, as does that of calcium, due to the greater food intake (*22*) and the reduced loss of the cation from the seed.

The digestive utilization of magnesium, expressed as the ADC (**Table 5**) was very low for all assayed diets, despite the substantial improvement obtained by the mA and B diets with respect to the sA diet. This was expected in view of the high magnesium intakes of the diets, 20−40% higher than the intake with a standard caseine−methionine diet (*41*). This effect was previously observed in other magnesium-rich legumes (*15*, *50*). The lower protein quality in the bean diets (*22*) may also contribute to the lesser digestive utilization of the magnesium (*4*), which would explain the worse ADC values obtained for the sA diet, in which the excess of magnesium was reduced but in which the protein quality was worse. Moreover, the vitamin D deficiency in the bean (*40*) would be expected to reduce the active transport of magnesium (*51*) and thus produce lower absorption of the mineral.

Despite the low ADC for magnesium in growing rats fed bean diets, net magnesium absorption was high, and in the case of the mA and B diets it exceeded the 1.91 ± 0.22 mg/rat/day of magnesium absorbed with a caseine−methionine diet (*50*). The quantity of magnesium absorbed depends both on its concentration in the diet and on the presence of other nutritional ingredients (favoring or inhibiting the process) (*2*). The large amount of magnesium absorbed in the case of the beans assayed in this study, despite the low digestive utilization, was due to the fact that a high quantity was consumed (*52*). Because the bean diet consumed by the rats is calcium-deficient, there is no interaction between calcium and magnesium at the digestive level, which favors absorption of the latter (*9*).

Under our experimental conditions, the fecal excretion of magnesium bore no relation to the intake of fiber, whether HMC or CL, as was also seen with calcium.

Despite the high net absorption of magnesium in bean diets, the high rate of urinary excretion led to a negative magnesium balance (**Table 5**), and so the kidneys play an essential role in the homeostasis of magnesium. Thus, a high intake of magnesium not only reduces its digestive absorption but also increases its urinary excretion (*2*, *53*). Moreover, the low protein quality of the beans assayed (*22*) raised proteinuria and inhibited the tubular reabsorption of the cation, thus increasing urinary losses of the latter (*6*).

Fecal Excretion. Interestingly, the increase in fiber consumption (mainly cellulose) observed as the pH of the soaking medium rose, which was a consequence of the increase in food intake together with the higher quantity of fiber in the diet, did not give rise to greater fecal excretion; there were no significant differences among the weights of the feces (mg/rat/day, dry substance) (**Table 5**) in the three diets tested. This was because the fecal mass of the groups that consumed less fiber (mA and, especially, sA) contained, in addition to this fiber, the remains

of nutrients (hydrocarbons and proteins) unused by the animal. This was deduced from the worse results obtained by these groups with regard to the nutritive utilization of the protein, carbohydrates (*22*), and minerals assayed.

Mineral Content in Muscle and Femur. The total mineral content in the muscle did not vary significantly among the three experimental groups (**Table 6**). The values found for the minerals in this organ show that, despite the balances recorded (low for calcium and phosphorus and negative for magnesium), muscular depletion occurred only in the case of calcium, compared to the normal values for growing rats. This fact is of great biological interest, confirming similar results obtained with other legumes (*15*, *38*). It shows the longissimus dorsi muscle to be a good short-term metabolic indicator for calcium, although not for phosphorus or magnesium.

As with the muscle, under our experimental conditions there was a correlation between the low calcium balance and the low content of this mineral in the femur, compared to the normal values for growing rats (*28*). This mobilization was not found for phosphorus or for magnesium. In the latter case, if the experimental period had been longer than 10 days, mobilization at the level of the muscle, and possibly of the femur, would probably have been found, as was the case of longer experimental periods with negative magnesium balances (unpublished data).

In conclusion, soaking the bean in a basic pH solution seems to have a protective effect regarding mineral losses (calcium, phosphorus, and magnesium) by solubilization. Thus, the reduced mineral loss in the soluble (more absorbable) form of the mineral, together with its greater intake (as the content is greater), favors the digestive utilization of the minerals. Moreover, the increase in the cellulose in the seed does not have a negative effect on the digestive utilization of the minerals contained, which means the bean is a nutritionally important food. Specifically, soaking beans in a bicarbonate salt solution and then cooking them in fresh water can thus be recommended to enhance the nutritional value of common beans.

ABBREVIATIONS USED

mA, soaking in moderately acid solution and cooking; sA, soaking in strongly acid solution and cooking; B, soaking in moderately basic solution and cooking; ADC, apparent digestibility coefficient; % R/A, percent ratio of mineral retention to mineral absorption; NDF, neutral detergent fiber; CL, cellulose; HMC, hemicellulose; LN, lignin.

LITERATURE CITED

(1) Torre, M.; Rodriguez, A. R.; Saura-Calixto, F. Effects of dietary fibre and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* **1991**, *1*, 1−22.

(2) Shils, M. E. Magnesium. In *Conocimientos Actuales sobre Nutrición*; Ziegler, E. E., Filer, L. J., Jr., Eds.; ILSI: Washington, DC, 1997; p 273.

(3) Varela, G.; Moreiras, O.; Carbajal, A.; Campo, M. *National Study in Nutrition and Alimentation 1990/91*; Publicaciones del Instituto Nacional de Estadística: Madrid, Spain, 1995.

(4) Aikawa, J. K. Biochemistry and physiology of magnesium. *World Rev. Nutr. Diet.* **1978**, *28*, 112−142.

(5) Khan, N.; Chakrabarti, C. H. Effect of different levels of pulse proteins (*Bengal gram* and *Lentil*) on calcium and phosphorus balance and calcium and phosphorus contents of different tissues and serum. *Indian J. Nutr. Diet.* **1978**, *15*, 371−376.

(6) Brink, E. J.; Dekker, P. R.; Van Berestejin, E. C. H.; Beynen, A. C. Inhibitory effect of dietary soybean protein vs casein on magnesium absorption in rats. *J. Nutr.* **1991**, *121*, 1374−1381.

(7) Lombardi-Boccia, G.; Lucarini, M.; Di Lullo, G.; Del Puppo, E.; Ferrari, A.; Carnovale, E. Dialysable, soluble and fermentable calcium from beans (*Phaseolus vulgaris* L.) as model for *in vitro* assessment of the potential calcium availability. *Food Chem.* **1998**, *61*, 167−171.

(8) Van der Poel, T. F. B.; Blonk, J.; Van Zuilichem, D. J.; Van Oort, M. G. Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*Phaseolus vulgaris*) and effects on protein quality. *J. Sci. Food Agric.* **1990**, *53*, 215−228.

(9) Hardwick, L. L.; Jones, M. R.; Brautbar, N. Magnesium absoption: mechanisms and the influence of vitamin D, calcium and phosphate. *J. Nutr.* **1991**, *121*, 13−23.

(10) Torre, M.; Rodriguez, A. R.; Saura-Calixto, F. Study of the interactions of calcium ions with lignin, cellulose, and pectin. *J. Agric. Food Chem.* **1992**, *40*, 1762−1766.

(11) Kumar, K. G.; Venkataraman, L. V.; Jaya, T. V.; Krishnamurthy, K. S. Cooking characteristics of some germinated legumes: changes in phytins, calcium and pectins. *J. Food Sci.* **1978**, *43*, 85.

(12) Rao, P. S.; Deosthale, Y. G. Tannin content of pulses, varietal differences and effects of germination and cooking. *J. Sci. Food Agric.* **1982**, *33*, 1013−1016.

(13) Jood, S.; Chauhan, B. M.; Kapoor, A. C. Polyphenols of chickpea and blackgram as affected by domestic processing and cooking methods. *J. Sci. Food Agric.* **1987**, *39*, 145−149.

(14) Duhan, A.; Chauhan, B. M.; Punia, D.; Kapoor, C. A. Phytic acid content of chickpea (*Cicer arietinum* L) and black gram (*Vigna mung*): varietal differences and effect of domestic processing and cooking methods. *J. Sci. Food Agric.* **1989**, *49*, 449−455.

(15) Nestares, T.; Urbano, G.; López-Frías, M.; Barrionuevo, M. Nutritional assessment of magnesium from raw and processed chickpea (*Cicer arietinum* L.) in growing rats. *J. Agric. Food Chem.* **1997**, *45*, 3138−3142.

(16) Singh, U. Nutritional quality of chickpea (*Cicer arietinum* L): current status and future research needs. *Qual. Plant Plant Foods Hum. Nutr.* **1985**, *35*, 339−351.

(17) Vidal-Valverde, C.; Frías, J. Legume processing effects on dietary fibre components. *J. Food Sci.* **1991**, *56*, 1350−1352.

(18) Nestares, T.; López-Frías, M.; Barrionuevo, M.; Urbano, G. Nutritional assessment of raw and processed chickpea (*Cicer arietinum* L.) protein in growing rats. *J. Agric. Food Chem.* **1996**, *44*, 2760−2765.

(19) Martincabrejas, M. A.; Esteban, R. M.; Waldron, K. W.; Maina, K. W.; Grant. G.; Bardocz, S.; Pusztai, A. Hard to cook phenomenon in beans. Changes in antinutrient factors and nitrogenous compounds during storage. *J. Sci. Food Agric.* **1995**, *69*, 429−435.

(20) Hughes, J. S.; Acevedo, E.; Bressani, R.; Swanson, B. G. Effects of dietary fibre and tannins on protein utilization in dry beans (*Phaseolus vulgaris*). *Food Res. Int.* **1996**, *29*, 331−338.

(21) Carvalho, M. R. B.; Sgarbieri, V. C. Heat treatment and inactivation of trypsin-chymotrypsin inhibitors and lectins from beans (*Phaseolus vulgaris* L.). *J. Food Biochem.* **1997**, *21*, 219−233.

(22) Nestares, T.; Barrionuevo, M.; Urbano, G.; López-Frías, M. Nutritional assessment of protein from beans (*Phaseolus vulgaris* L) processed at different pH values, in growing rats. *J. Sci. Food Agric.* **2001**, *81*, 1522−1529.

(23) Tovar, J. Bioavailability of carbohydrates in legumes. Digestible and indigestible fractions. *Arch. Latinoam. Nutr.* **1994**, *44* (Suppl. 4), 36S−40S.

(24) Pusztai, A.; Grant, G.; Duguid, T.; Brown, D. S.; Peumans, W. J.; Vandamme, E. J. M.; Bardocz, S. Inhibition of starch digestion by alpha-amylase inhibitor reduces the efficiency of utilization of dietary proteins and lipids and retards the growth of rats. *J. Nutr.* **1995**, *125*, 1554−1562.

(25) Vidal-Valverde, C.; Frias, J.; Valverde, S. Changes in the carbohydrate composition of legume after soaking and cooking. *J. Am. Diet. Assoc.* **1993**, *93*, 547−550.

(26) Marquez, U. M. L.; Lajolo, F. M. Composition and digestibility of albumin, globulins and glutelins from *Phaseolus vulgaris*. *J. Agric. Food Chem.* **1981**, *29*, 1068−1074.
(27) Carbonaro, M.; Cappelloni, M.; Nicoli, S.; Lucarini, M.; Carnovale, E. Solubility−digestibility relationship of legume proteins. *J. Agric. Food Chem*. **1997**, *45*, 3387−3394.
(28) Bronner, F. Calcium. In *Handbook of Nutritionally Essential Minerals Elements*; O'Dell, B. L., Sunde, R. A., Eds.; Dekker: New York, 1997.
(29) AOAC. *Official Methods of Analysis*; Helrich, K., Ed.; Association of Official Analytical Chemists: Arlington, VA, 1990.
(30) Fiske, C. H.; Subbarow, Y. The colorimetric determination of phosphorus. *J. Biol. Chem.* **1925**, *66*, 375−400.
(31) Van Soest, P. J.; Wine, R. H. Determination of lignin and cellullose in acid detergent fibre with permanganate. *J. Assoc. Off. Anal. Chem.* **1968**, *51*, 780.
(32) McQueen, R. E.; Nicholson, J. W. G. Modification of the neutral detergent fibre procedure for cereals and vegetables by using α-amylase. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 676.
(33) Vidal-Valverde, C.; Frías, J.; Esteban, E. Dietary fibre in processed lentils. *J. Food Sci.* **1992**, *57*, 11−16.
(34) National Research Council, *Nutrient Requirements of Laboratory Animals*, 4th revised ed.; National Academy of Sciences: Washington, DC, 1990.
(35) Moro, R.; Fernández, M. T.; Vargas, A.; Cuesta, M. J. Evaluación de cuatro variedades de judías cultivadas en Asturias. *Alimentaria* **1997**, *280*, 65−67.
(36) Moro, R.; Cuesta, M. J.; Fernández, M. T.; Vargas, A. Composición y valor nutricional de la faba en la variedad granja asturiana. *Alimentaria* **1997**, *279*, 23−26.
(37) Casanas, F.; Bosch, L.; Pujola, M.; Sanchez, E.; Sorribas, X.; Baldi, M.; Nuez, F. Characteristics of a common bean ladrance (*Phaseolus vulgaris* L.) of great culinary value and selection of a commercial inbred line. *J. Sci. Food Agric.* **1999**, *79*, 693−698.
(38) Nestares, T.; Barronuevo, M.; Urbano, G.; López-Frías, M. Effect of processing methods on the calcium, phosphorus and phytic acid contents and nutritive utilization of chickpea (*Cicer arietinum* L.). *J. Agric. Food Chem.* **1999**, *47*, 2807−2812.
(39) Minka, S. R.; Mbofung, C. M. F.; Gandon, C.; Bruneteau, M. The effect of cooking with kanwa alkaline salt on the chemical composition of black beans (*Phaseolus vulgaris*). *Food Chem.* **1999**, *64*, 145−148.
(40) Mataix, J.; Mañas, M.; Llopis, J.; Martínez de Victoria, E. Alimentos ricos en nutrientes específicos. *Tabla de Composición de Alimentos Españoles*; Servicio de Publicaciones de la Universidad de Granada: Granada, Spain, 1998; pp 259−287.
(41) Urbano, G.; López-Jurado, M.; Fernández, M.; Moreu, M. C.; Porres-Foulquie, J.; Frías, J.; Vidal-Valverde, C. Ca and P bioavailability of processed lentils as affected by dietary fibre and phytic acid content. *Nutr. Res.* **1999**, *19*, 49−64.
(42) Kaup, A.; Behling, A. R.; Choquette, L.; Greger, J. L. Calcium and magnesium utilization in rats: effect of dietary butterfat and calcium and of age. *J. Nutr.* **1990**, *120*, 266−273.
(43) James, W. P. T.; Branch, W. J.; Southgate, D. A. T. Calcium binding by dietary fibre. *Lancet* **1978**, *1*, 638−639.
(44) Weaver, C. M.; Heaney, R. P. Calcium. In *Modern Nutrition in Health and Disease*, 9th ed.; Shils, M. E., Olson, J. A., Shike, M., Ross, A. C., Eds.; Lippincott Williams and Wilkins: Baltimore, MD, 1999; p 147.
(45) Sandberg, A. S.; Ahderinne, R. HPLC methods for determination of inositol tri-, tetra-, penta- and hexaphosphates in foods and intestinal contents. *J. Food Sci.* **1986**, *51*, 547−550.
(46) Ranhotra, G. S.; Loewe, R. J. Effect of phytase of wheat on dietary phytic acid. *J. Food Sci.* **1975**, *40*, 940−942.
(47) Yang, W.; Matsuda, Y.; Sano, S.; Nakagawa, H. Purification and characterization of phytase from rat intestinal mucosa. *Biochim. Biophys. Acta* **1991**, *10*, 75−82.
(48) Jany, K. L.-D. Mechanism of degradation of inositol phosphates in the gut. In *Proceedings of Bioavailability '93, Nutritional, Chemical and Food processing Implications of Nutrient Availability*; Schlemmer, S., Ed.; Berichte der Bundesforschungsanstalt für Ernährung: Ettlingen, Germany, 1993; Part 2, p 58.
(49) Fernández, M.; Aranda, P.; López-Jurado, M.; García-Fuentes, M. A.; Urbano, G. Bioavailability of phytic acid phosphorus in processed *Vicia faba* L. Var. *Major*. *J. Agric. Food Chem.* **1997**, *45*, 4367−4371.
(50) Moreu, M.; Fernández, M. M.; Urbano, G.; Aranda, P.; López-Jurado, M. Bioavailability of magnesium in raw and processed faba beans. *Proceedings of the 9th World Congress of Food Science and Technology*; July 30−Aug 4, 1995, Budapest, Hungary; Hungarian Scientific Society for Food Industry (MÉTE): Budapest, 1995; Vol. II, p 132.
(51) Ebel, H.; Gunther, T. Magnesium metabolism: a review. *J. Clin. Chem. Clin. Biochem.* **1980**, *18*, 257−270.
(52) Fine, K. D.; Santa Ana, C. A.; Porter, J. L. Intestinal absorption of magnesium from food and supplements. *J. Clin. Invest.* **1991**, *88*, 396−402.
(53) Sutton, R. A. L.; Domrongkitchaiporn, S. Abnormal renal magnesium handling. *Miner. Electrolyte Metab.* **1993**, 232−240.

Received for review June 20, 2002. Revised manuscript received October 8, 2002. Accepted October 8, 2002.

JF020693Y