

Bioavailability of Phytic Acid Phosphorus in Processed *Vicia faba* L. Var. Major

Mar Fernández,^{*,†} Pilar Aranda,^{†,‡} María López-Jurado,^{†,‡} M. Angeles García-Fuentes,[†] and Gloria Urbano^{†,‡}

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

An experimental animal model was used to investigate whether processing improved the nutritive utilization of phytic acid phosphorus in faba beans. Phytic acid had been considered an anti-nutritional factor because of its ability of chelate minerals and impede their absorption and because of the limited capacity of monogastric species to hydrolyze and utilize phosphorus from this molecule. This is of particular significance in legumes, where a large portion of phosphorus is in the form of phytic acid. Heating and soaking in acid solution followed by cooking led to large decreases in phytic acid. Soaking reduced phosphorus content (15.4%). Processing made part of the phytic acid phosphorus available, as suggested by our finding that absorbed P was greater than P from sources other than phytic acid. The lower phosphorus content in bone and blood in rats led to a significantly lower phosphorus balance in animals fed faba beans.

Keywords: *Faba beans; nutritive utilization; phosphorus; processing; phytic acid; inositol hexaphosphate*

INTRODUCTION

Vicia faba L. (faba bean, field bean) is an important source of minerals in human and animal nutrition. However, its use as a food is less limited by the presence of antinutritional factors that interfere with the nutritive utilization of this legume. These factors can be compensated for by appropriate processing to decrease their negative effects. In *V. faba*, phytic acid is an important antinutritional factor. Despite the high amount of total phosphorus in this legume (sufficient to satisfy the requirements of growing animals), detailed studies have shown that 40–60% of the total phosphorus is present as phytate (Griffiths and Thomas, 1981). This phosphate-rich compound is able to form insoluble chelates with several trace elements and macroelements, reducing the amount of phosphorus available for absorption in vivo. Moreover, the phosphorus content of phytate has been considered to be unavailable to the organism.

Several studies have investigated the effects of time, temperature, pH, soaking, or cooking on phytate autolysis (Chang et al., 1977; Tabekhia and Luh, 1979; Iyer et al., 1980). In the present study, we tested the effects of different types of processing on the nutritive value of phosphorus in faba beans and used biological analyses in rat tissues to determine which processes most effectively enhanced the bioavailability of phosphorus.

MATERIALS AND METHODS

Samples. Faba beans (*Vicia faba* L. var. Major) were purchased at a local market. Different batches of the seeds were processed as follows:

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

Soaking in distilled water (SF).

Soaking in 0.1% citric acid solution (AF).

Soaking in 0.07% sodium bicarbonate solution (BF).

Soaking and cooking in distilled water (SCF).

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

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

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

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

Chemical Analysis. Moisture Content. Moisture contents of the raw and processed faba beans were found by drying to a constant weight in a vacuum oven (20 mmHg, 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.

Phosphorus was determined in the diets, feces, urine, femur, longissimus dorsi muscle, blood, and plasma. We used the colorimetric method of Fiske and Subbarow (1925) to test previously ashed samples dissolved in 5 N HCl (diets, feces, femur, longissimus dorsi muscle, and blood) or untreated samples (urine and plasma).

Inositol hexaphosphate (phytic acid) was determined in diets and feces according to the colorimetric method of Latta and Eskin (1980).

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; AF = faba beans soaked in 0.1% citric acid solution; BF = faba beans soaked in 0.07% sodium bicarbonate solution; SCF = faba beans soaked and cooked in distilled water; ACF = faba beans soaked in 0.1% citric acid solution and cooked in distilled water; BCF = faba beans soaked in 0.07% sodium bicarbonate solution and cooked in distilled water.

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

† Facultad de Farmacia.

‡ Instituto de Nutrición y Tecnología de Alimentos.

Experimental Design. Rats were fed for 10 days with a faba bean diet, and the results were compared with data from a group of control animals fed the casein-methionine diet. A total of 90 rats were divided into nine groups of 10 animals each. Phosphorus intake and fecal and urinary excretion of phosphorus were determined in all rats. Phytic acid (inositol hexaphosphate) was determined in diets and feces.

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 (five male and five 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 a balance biological technique was used. A period of 3 days was allowed for adaptation to the diet, followed by a 7 day experimental period when feces and urine were collected on alternate days. Food intake was 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 of phosphorus and inositol hexaphosphate (IHP) (expressed as dry matter), apparent digestibility coefficient of phosphorus (ADC), phosphorus retention (phosphorus balance), percent phosphorus retention/phosphorus absorption, and percent transformation of inositol hexaphosphate:

$$\text{ADC} = \frac{I - F}{I} \times 100 \quad (1)$$

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

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

$$\text{IHP transform.} = \frac{\text{IHP intake} - \text{fecal IHP}}{\text{IHP intake}} \times 100 \quad (4)$$

The factors used were *I* (phosphorus intake), *F* (fecal phosphorus), *U* (urinary phosphorus), IHP intake (inositol hexaphosphate intake), and fecal IHP (inositol hexaphosphate fecal).

Statistical Methods. The data were analyzed with multifactor analysis of variance using Statgraphic Statistical Graphics 2.1 System Software (Statistical Graphics Corporation, Rockville, MD). All analyses were run on an IBM Personal System/2 Model 20 Computer (International Business Machines Corp., U.K.).

RESULTS

Chemical Analysis. Table 1 shows the percentage total mineral, phosphorus, and IHP contents in the control diet and in raw and processed faba beans. Total mineral content was similar in all diets tested here. Total phosphorus in the semisynthetic diet fed to the control group was 5.53 mg of P/g of dry matter, supplied as dibasic calcium phosphate. Total phosphorus in raw faba beans used to prepare the diets was 4.67 mg of P/g of dry matter; of this figure, 49.5% of the phosphorus was present as IHP. Dry heating to 120 °C at 1 atm for 15 min did not modify total phosphorus content. Soaking in distilled water, acid solution, or basic solution followed by cooking decreased total phosphorus by approximately 15.4% because of the solubilization of phosphorus-containing compounds.

Dry heating reduced phytic acid content of the diet by 40%, whereas soaking in acid solution followed by cooking led to a 30% reduction. Other types of processing did not modify phytic acid content, as shown by the

Table 1. Percent of Total Mineral, Total Phosphorus, and Inositol Hexaphosphate (IHP) Content of the Diets Tested^a

diets	% total mineral	total P (mg/g)	IHP (mg/g)	IHP-P (mg/g)	IHP-P % total P
C	3.0	5.53			
RF	3.6	4.67	8.11	2.31	49.5
HF	3.5	4.60	4.87	1.39	30.2
SF	3.3	3.87	8.50	2.43	62.8
AF	3.3	3.79	8.85	2.53	66.8
BF	3.2	3.78	8.28	2.36	62.4
SCF	3.1	4.17	8.14	2.32	55.6
ACF	3.1	4.08	5.77	1.65	40.4
BCF	3.2	4.02	8.58	2.45	61.0

^a C = control diet; RF = raw faba beans; HF = heated faba beans; SF = faba beans soaked in water; AF = faba beans soaked in acid medium; BF = faba beans soaked in basic medium; SCF = faba beans soaked and cooked; ACF = faba beans soaked in acid medium and cooked; BCF = faba beans soaked in basic medium and cooked.

similarities of IHP values between the raw faba bean diet and the other processes tested.

Biological Analysis. *Phosphorus and Inositol Hexaphosphate Intake.* Total phosphorus intake was significantly lower in all experimental groups in comparison with control animals fed the semisynthetic diet (Table 2). Phosphorus obtained from sources other than IHP accounted for 28% (diet SF) and 70% (diet HF) of the dietary supply in rats fed raw or processed faba beans. These values were significantly lower than the phosphorus supplied by the semisynthetic control diet.

Fecal excretion of total phosphorus (Table 3) was significantly higher in groups fed faba beans than in the control group; the highest values were found in rats given raw beans (group RF) or beans soaked in water (group SCF) or acid solution and then cooked (group ACF). Fecal amounts of IHP (expressed as milligrams/day) were very low in all groups.

The percentage of phytic acid transformation (Table 4), which reflects the breakdown of phytic acid (IHP) during digestion, was high, ranging from 94.8% after feeding with faba beans soaked in acid solution and cooked, to 99.4% in animals given beans soaked in water (group SF). The lowest value was found in rats given beans soaked in acid solution and cooked (group ACF), followed in increasing order by heating to 120 °C (group HF) and soaking in basic solution and cooking (group BCF).

Digestive Utilization of Phosphorus. In all groups fed with raw or processed faba beans, phosphorus absorption (expressed as milligrams/day, Table 4) was significantly lower than in the control group. However, in group SCF (soaking in water followed by cooking), phosphorus absorption was significantly greater than in rats fed raw beans or bean subjected to any other type of processing. The digestive utilization of phosphorus (ADC) from raw and processed beans was significantly lower than in the control group. The lowest values were found in group RF (raw beans), followed in increasing order by groups ACF, SCF, and BCF. The highest values were found in groups BF, AF, and HF, although digestive utilization did not reach the values recorded after feeding with the semisynthetic diet (Table 4).

Metabolic Utilization of Phosphorus. In rats fed raw or processed faba beans, urinary excretion of phosphorus (Table 3) was significantly higher than in the control group, with the highest value appearing in animals given raw beans. Phosphorus balance (Table 4) in this

Table 2. Food, Phosphorus, and Inositol Hexaphosphate (IHP) Intake^a

diets	food intake (g/day)	total P intake (mg/day)	IHP intake (mg/day)	P-IHP intake (mg/day)	non-IHP-P intake (mg/day)
C	12.1 ± 0.68 ^{ac}	66.8 ± 3.54			
RF	9.9 ± 0.24 ^b	46.2 ± 1.13 ^a	80.27 ± 1.96 ^a	22.91 ± 0.56 ^a	23.3 ± 0.5 ^a
HF	9.78 ± 0.34 ^b	44.5 ± 1.55 ^a	47.66 ± 1.61	13.60 ± 0.46	31.5 ± 1.07
SF	11.82 ± 0.54 ^{ac}	39.9 ± 1.84 ^b	100.43 ± 4.6 ^b	28.66 ± 1.32 ^b	11.2 ± 0.51
AF	12.71 ± 0.26 ^c	48.2 ± 1.00 ^{ac}	112.43 ± 2.3	32.11 ± 0.67	15.5 ± 0.67 ^b
BF	10.33 ± 0.40 ^b	39.1 ± 1.46 ^b	85.55 ± 3.20 ^a	24.42 ± 0.91 ^a	14.7 ± 0.55 ^b
SCF	12.93 ± 0.38 ^c	54.0 ± 1.55	105.37 ± 3.0 ^b	30.07 ± 0.86 ^b	24.0 ± 0.86 ^a
ACF	11.37 ± 0.37 ^{ac}	46.4 ± 1.50 ^{ac}	65.14 ± 2.06	18.59 ± 0.59	27.8 ± 1.59
BCF	11.28 ± 0.56 ^{ac}	45.4 ± 2.24 ^{ac}	96.11 ± 4.73 ^b	27.43 ± 1.35	18.0 ± 0.89

^a Diets same as in Table 1. The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of determinations in 10 Wistar rats.

Table 3. Excretion of Total Phosphorus, Inositol Hexaphosphate Phosphorus (IHP-P), and Non-IHP-P in Feces and Urinary Excretion of Total Phosphorus^a

diets	total P fecal (mg/day)	fecal IHP (mg/day)	fecal IHP-P (mg/day)	fecal non-IHP-P (mg/day)	urinary P (mg/day)
C	3.68 ± 0.32			3.68 ± 0.32	9.33 ± 0.59
RF	11.34 ± 0.34 ^a	1.41 ± 0.22 ^a	0.40 ± 0.06 ^a	11.01 ± 0.35 ^a	27.48 ± 1.96
HF	6.28 ± 0.56 ^b	1.83 ± 0.23 ^a	0.52 ± 0.07 ^a	5.76 ± 0.52 ^b	18.16 ± 0.60 ^a
SF	7.41 ± 0.53 ^{bc}	0.53 ± 0.10	0.15 ± 0.03	7.26 ± 0.53 ^c	19.40 ± 1.35 ^{ab}
AF	7.80 ± 0.57 ^c	2.52 ± 0.20 ^b	0.72 ± 0.06 ^b	7.08 ± 0.55 ^c	20.68 ± 1.26 ^b
BF	6.21 ± 0.50 ^b	1.67 ± 0.18 ^a	0.53 ± 0.07 ^a	5.73 ± 0.50 ^b	16.57 ± 1.44 ^{ac}
SCF	10.57 ± 0.49 ^a	1.85 ± 0.21 ^a	0.53 ± 0.07 ^a	10.05 ± 0.46 ^{ad}	20.42 ± 0.83 ^b
ACF	10.42 ± 0.52 ^a	5.34 ± 2.23	0.96 ± 0.09	9.46 ± 0.50 ^d	19.61 ± 0.80 ^{ab}
BCF	9.06 ± 0.52	2.60 ± 0.27 ^b	0.74 ± 0.08 ^b	8.32 ± 0.45	14.51 ± 0.91 ^c

^a Diets same as in Table 1. The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of determinations in 10 Wistar rats.

Table 4. Digestive and Metabolic Utilization of Phosphorus after Feeding with the Diets Tested^a

diets	P absorbed (mg/day)	ADC	P balance (mg/day)	R/A	IHP % transf
C	63.13 ± 3.66	94.49 ± 1.45	53.79 ± 2.67	85.28 ± 2.47	
RF	34.81 ± 3.42 ^a	75.22 ± 2.59 ^a	7.36 ± 4.64	21.53 ± 14.1	98.2 ± 0.97 ^a
HF	38.77 ± 3.32 ^b	86.26 ± 2.63 ^b	26.62 ± 2.71 ^a	53.13 ± 4.45 ^a	96.2 ± 1.37
SF	32.44 ± 5.16 ^a	81.34 ± 3.79 ^c	13.08 ± 7.29	38.55 ± 17.8	99.4 ± 0.43
AF	40.38 ± 2.46 ^b	83.89 ± 3.17 ^{bc}	19.80 ± 2.64 ^a	49.29 ± 7.97 ^{ab}	98.6 ± 0.70 ^a
BF	32.90 ± 4.29 ^a	84.10 ± 3.72 ^{bc}	16.33 ± 3.66 ^c	49.97 ± 10.5 ^{ab}	98.3 ± 0.84 ^a
SCF	43.47 ± 4.09	80.44 ± 2.30 ^c	23.05 ± 3.16 ^a	52.99 ± 4.65 ^{ab}	98.3 ± 0.59 ^a
ACF	35.94 ± 3.73 ^{ac}	77.54 ± 2.62 ^{ad}	16.33 ± 4.19 ^c	44.99 ± 8.19 ^b	94.8 ± 1.70
BCF	36.34 ± 5.63 ^{abc}	80.06 ± 1.46 ^{cd}	21.84 ± 4.88 ^a	59.61 ± 8.52	97.4 ± 0.51

^a Diets same as in Table 1. The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of determinations in 10 Wistar rats.

Table 5. Phosphorus Content in Plasma, Blood, *Longissimus Dorsi* Muscle, and Femur in Rats Fed the Control Diet or Raw or Processed Faba Beans^a

diets	P in plasma (mg/100 mL)	P in blood (mg/100 mL)	mg of P/g of ash (LD)	mg of P/g of ash (F)
C	5.29 ± 0.48 ^a	63.03 ± 21.4	139.22 ± 18.42 ^a	144.70 ± 18.61 ^a
RF	5.21 ± 0.76 ^{ab}	38.15 ± 8.19 ^a	120.91 ± 51.91 ^{ab}	137.42 ± 20.59 ^{ab}
HF	4.70 ± 0.56 ^b	42.46 ± 7.10 ^{ab}	133.41 ± 30.92 ^{ab}	111.90 ± 30.88 ^c
SF	4.91 ± 0.93 ^b	37.33 ± 6.76 ^{ab}	109.50 ± 32.32 ^b	140.53 ± 11.92 ^{ab}
AF	4.75 ± 1.10 ^b	32.96 ± 4.81 ^{ac}	137.59 ± 22.61 ^{ab}	120.96 ± 12.86 ^{cd}
BF	5.20 ± 1.15 ^{ab}	40.24 ± 5.93 ^{ab}	148.28 ± 43.45 ^a	137.96 ± 6.92 ^{ab}
SCF	5.05 ± 1.18 ^{ab}	32.66 ± 4.53 ^{ac}	137.88 ± 23.36 ^a	131.21 ± 10.91 ^{bd}
ACF	4.63 ± 1.27 ^b	41.02 ± 7.73 ^{ab}	107.52 ± 34.13 ^b	91.09 ± 20.66
BCF	5.22 ± 1.14 ^{ab}	35.09 ± 4.65 ^{ac}	154.88 ± 33.76 ^a	114.18 ± 22.39 ^{cd}

^a Diet same as in Table 1. The same superscript in the same column indicates no significant differences ($P \leq 0.05$). Values are means ± SEM of determinations in 10 Wistar rats.

group was approximately one-seventh the value in control animals. In comparison with rats fed raw faba beans, phosphorus retention was greater in rats given processed beans; however, in groups HF, SCF, and BCF, retention was approximately one-third the value in control animals (Table 4).

Blood and Plasma Levels of Phosphorus. All experimental groups have higher blood levels of phosphorus than the group fed the semisynthetic diet. In plasma, phosphorus levels were within the normal range for

growing rats, with no differences between any of the experimental groups (Table 5).

Phosphorus Content in *Longissimus Dorsi* Muscle and Femur. In muscle tissue there were no significant differences in phosphorus levels between groups. In bone, phosphorus (expressed as milligrams/gram of ash) was highest in the control group; feeding with faba beans led to similar values in all experimental groups. The lowest levels of phosphorus in bone were found in animals given beans soaked in acid solution and cooked.

DISCUSSION

Chemical Analysis of Phosphorus and Inositol Hexaphosphate. The amounts of total phosphorus we found in raw faba beans were within the range reported in earlier studies (Carnovale et al., 1987; Eden, 1968; Griffiths and Thomas, 1981).

Total phosphorus in faba beans was considered the sum of inorganic phosphorus, phytic acid phosphorus, phosphatids, and nucleic components (Reddy et al., 1978). Inositol hexaphosphate is the major component of phytic acid in this legume, accounting for 70–90% of the total phosphorus from this source (Sandberg and Ahderinne, 1986; Lehrfield and Morris, 1992).

All types of processing except heating alone reduced total phosphorus by 15.4% because of solubilization. This loss, similar to the figure given by Tabekhia and Luh (1980), is a drawback to processing, as it inevitably represents a reduction in the most readily utilizable fraction of total phosphorus.

Heating to 120 °C at 1 atm for 15 min reduced the phytic acid content by 40%. This may represent an effect of hydrolysis caused by heat, rather than enzymatic hydrolysis (Boland et al., 1975; Tabekhia and Luh, 1980; Duhan et al., 1989).

Soaking in acid solution followed by cooking reduced IHP by 30%, an effect similar to that reported in other legumes by Chang (1975) and Chang et al. (1977). However, we found no changes in IHP when faba beans were soaked in acid solution. The hard seed cover may have prevented the acid solution from reaching the cotyledons. In contrast, soaking followed by cooking softened the seed cover enough to allow the acid solution to penetrate. This in turn may have created a pH sufficiently acidic for endogenous phytase to act. The action of this enzyme is optimal at 50 °C, but the enzyme is destroyed at 100 °C (Lolas and Markakis, 1977; Eskin and Wiebe, 1983). This may account for the relatively small (30%) reduction in phytic acid content: the seed's phytases may have been effective only during the first stages of cooking, before the enzyme was destroyed by high temperatures.

Biological Assessment of Phosphorus. *Digestive Utilization of Phosphorus.* Under our experimental conditions, the amount of total phosphorus supplied by each of the faba bean diets was sufficient to satisfy the requirements for this ion in growing rats (AIN, 1977), despite the 15.4% reduction caused by processing (Table 2). Moreover, much of the total dietary phosphorus (50%) was present as phytic acid (an unavailable form); the remainder was supplied as inorganic phosphorus (11%), phosphatids, and nucleic components (32%) (Lolas and Markakis, 1975). These compounds must be transformed either by processing before consumption or by digestion and converted into free phosphate in order to be absorbed.

Because the amount of phosphorus absorbed was greater than the amount of nonphytic acid phosphorus, we deduce that the IHP from the raw and processed faba bean diets was transformed during digestion. This process may have resulted from the action of phytase in the legume (Pointillart et al., 1987): once in the stomach, the acid pH of the milieu may have favored enzymatic activity, leading to the transformation of IHP into inositol pentaphosphate (Sandberg and Anderson, 1988). This compound, in turn, may have been broken down by phytase from the legume together with alkaline phosphatase (Ranhotra and Loewe, 1975; Yang et al., 1991; Jany, 1993) to give inorganic phosphorus and

inositols with a lower number of phosphate groups (Sandberg and Ahderinne, 1986). Endogenous phytase present in the rat stomach may also have been involved in this transformation; Yang et al. (1991) purified and characterized an enzyme from rat intestinal mucosa, with phytase and alkaline phosphatase activity. According to these authors, this enzyme was able to hydrolyze phytic acid in the intestine.

An additional factor may have helped to create optimal conditions for phytase and alkaline phytase activity: the low calcium content of the diets (Lei et al., 1994). Enzyme activity is more efficient when phytic acid is in its free form, i.e., not bound to divalent metals. The formation of phytic acid–metal complexes is favored by the binding of calcium to phytic acid (Wise, 1983); thus, the low concentration of calcium in the faba bean diets tested here may have enhanced the availability of phytate phosphorus (Nahapetian and Young, 1980).

Phosphorus absorption via digestion (Table 4), regardless of how the diet was processed, was similar to absorption from raw faba beans despite losses due to solubilization and greater than the absorption of nonphytic acid phosphorus. This result suggests that processing not only did not reduce the bioavailability of phytic acid phosphorus but in fact ensured that at least part of this fraction was utilizable. However, despite the high degree of IHP transformation, not all of the phytic acid phosphorus was made available: the digestive utilization of this element (percent P absorbed/P ingested) after feeding with raw or processed faba beans was significantly lower than after feeding with the control diet. This finding may have resulted from the fact that IHP was degraded in the colon by bacterial action (Wise et al., 1983). This would account for our findings that IHP was absent in the feces, phosphorus absorption was impeded, and fecal excretion of phosphorus was high. If all phosphorus had been available, the apparent digestibility coefficient would have been higher than in control animals. In fact, the intake of nonphytic acid phosphorus in all experimental animals was lower than in the control diet, reflecting the organism's efforts to maximize absorption to cover nutritional requirements (Lee et al., 1979).

Metabolic Utilization of Phosphorus. The homeostatic regulation of phosphorus metabolism is known to involve variations in food intake and renal excretion.

Under our experimental conditions, the increased urinary excretion of phosphorus led to a significantly lower balance in rats fed with any of the faba bean diets than in control animals. Urinary loss of the element was favored by the effect of parathyroid hormone (PTH) on tubular reabsorption of the anion. Because the faba bean diets supplied low amounts of vitamin D, PTH synthesis and secretion were not adequately inhibited. This hormone is known to inhibit phosphate reabsorption directly in the proximal and distal tubules [adenylate cyclase (cAMP) system] and indirectly by modifying bicarbonate reabsorption in the proximal tubule. The resulting increase in pH raises the $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ ratio, which in turn impedes local phosphate reabsorption.

The increased urinary excretion of phosphorus was reflected in the lower bone and blood levels of this element in comparison with the control group; there were no significant differences in muscle and plasma levels of phosphorus. Bone levels were strongly reduced by the faba bean diets, as dietary calcium was insufficient to satisfy the recommended daily allowance for

rats, and the Ca/P ratio did not meet the requirements of this species in the exponential growth phase. Phosphorus homeostasis is regulated by renal excretion and by the levels of phosphorus-containing compounds in plasma and whole blood. We found normal levels of this element in plasma but decreased levels in whole blood because of the decline in intraerythrocyte phosphorus. This finding suggests that, as reported for magnesium (Aranda, 1984), intraerythrocyte phosphorus is a more reliable indicator of alterations in homeostasis than are tissue concentrations of the element.

Processing of faba beans increased the percentage of phosphorus retention in relation to the amount of element absorbed. However, we found no clear repercussions on phosphorus content in the erythrocyte compartment or in bone. The short duration of the experiment (10 days) may have been insufficient to allow improvements in tissue formation to be reflected in our biological assays. A longer experimental period may have revealed clearer evidence of phosphorus deficiency in rats fed with raw faba beans, as this diet resulted in a much lower R/A ratio than any of the processed diets.

Because the amount of phosphorus absorbed was greater than the amount of nonphytic acid phosphorus supplied by the raw faba bean diet, we deduce that digestive processes made part of the IHP phosphorus available to the organism. Processing the faba bean diet made more phytic acid phosphorus available and allowed the rats to compensate for losses of soluble phosphorus.

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