

Original article

Evaluation of zinc and magnesium bioavailability from pea (*Pisum sativum*, L.) sprouts. Effect of illumination and different germination periods

Gloria Urbano,* María López-Jurado, Carlos Aranda, Antonio Vilchez, Lydia Cabrera, Jesus M. Porres & Pilar Aranda

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

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Summary The effect of sprouting pea seeds (*Pisum sativum*, L) for 2, 4 and 6 days, with and without light, on the content of zinc (Zn) and magnesium (Mg) and their nutritive utilisation by growing rats was studied. Soaking of pea seeds prior to germination caused a 49% reduction in Zn content followed by minor losses during germination. The content of Mg decreased by 6% as a result of the soaking process, and by 20–28% during germination. Sprouting for 2 and 4 days improved the bioavailability of Zn and Mg from pea seeds (from 32.2 to 88.6–108.0 µg retained Zn per day, and from 1.64 to 2.97–4.79 mg retained Mg per day in raw and sprouted pea flour diets, respectively), outweighing the reductions in Zn and Mg content because of seed soaking. The presence or absence of light during the germination process did not affect the results. We conclude that sprouting of peas for 4 days was the most effective treatment to improve the bioavailability of Zn and Mg in pea seeds.

Keywords Bioavailability, germination, growing rats, magnesium, *Pisum sativum*, sprouts, zinc.

Introduction

The pea is a legume with great nutritional potential because of its high content of protein (27.8%), complex carbohydrates (42.65%), vitamins, minerals, dietary fibre and antioxidant compounds (Urbano *et al.*, 2003). Nevertheless, its nutritional importance may be limited by the presence of non-nutritional components that have a negative effect on the nutritional value of this legume. These non-nutritional components include trypsin inhibitors (TIA), lectins, α -galactoside oligosaccharides, polyphenols and phytic acid (Savage & Deo, 1989; Urbano *et al.*, 2003). With the aim of improving the nutritive value of legumes, preparation techniques including germination have been developed to increase the bioavailability of their nutrients.

Germination is a complex metabolic process that oxidises the lipids and carbohydrates within the seed and breaks down storage proteins in order to obtain the energy and amino acids necessary for the plant's development (Ferreira *et al.*, 1995; Jachmanián *et al.*,

1995; Ziegler, 1995). The reduction of non-nutritional components such as phytic acid and polyphenols during germination has been extensively described (Mbithi-Mwikya *et al.*, 2001; Egli *et al.*, 2002; Vidal-Valverde *et al.*, 2002). The antinutritional effect of phytic acid is due to its ability to form insoluble complexes with di- and trivalent cations at the physiological pH conditions of the small intestine of monogastric animals, rendering them unavailable for absorption (Zhou *et al.*, 1992). It is important to establish the degree of phytate hydrolysis during germination, because inositol polyphosphates with a lower degree of phosphorylation may still retain some of their inhibitory effect on mineral absorption in the small intestine (Persson *et al.*, 1998; Sandberg *et al.*, 1999).

On the contrary, it has been reported that microbiota present in the caecum and colon of rats and rabbits can hydrolyse phytic acid, thus releasing the complexed nutrients and making them potentially more available for absorption in certain segments of the large intestine (Wise & Gilbert, 1987; Marounek *et al.*, 2003).

The effect of dietary fibre on the nutritive utilisation of minerals is not clear. Some authors suggest that it can interfere with mineral absorption (Torre *et al.*, 1992;

*Correspondent: Fax: 34 958 248 959;
e-mail: gurbano@ugr.es

Nestares *et al.*, 1997), whereas others have found no effect (Kanauchi *et al.*, 2000; Vaquero *et al.*, 2000). Martín-Cabrejas *et al.* (2003) have reported time-dependent changes in the content of total, insoluble, and soluble dietary fibre during germination.

The presence of light may advance the metabolic changes that take place during the different stages of germination (Martín-Cabrejas *et al.*, 2003), and so it is necessary to evaluate how these changes influence the bioavailability of zinc (Zn) and magnesium (Mg).

The aim of this work was to study how increasing periods of germination and different illumination conditions could affect the bioavailability of Zn and Mg from pea sprouts in growing rats.

Materials and methods

Legume

Pisum sativum, L. var. Arvense cv. Esla from germplasm collection of Valladolid (Spain). For analysis, the pea seeds were crushed, milled to a fine powder (0.18 mm sieve), and then lyophilised.

Sprouting

The process was carried out in a semi-pilot scale. Pea seeds (500 g) were sterilised by soaking in 2500 mL of 9.4 mM sodium hypochlorite solution for 30 min at room temperature. Seeds were then drained and washed to neutral pH, and then soaked in distilled water for 5.5 h. Finally, imbibed seeds were germinated at a pilot scale by layering over them a moistened filter paper continuously watered by capillarity in a seed germinator (G-120 Snijders, The Netherlands) for 2, 4, and 6 days with or without light (G2DL, G2DNL, G4DL, G4DNL, G6DL, G6DNL) at 20 °C, 99% relative humidity. Sprouts were freeze-dried and ground to pass 0.18 mm sieve for chemical and biological analysis. Germination processes were adjusted in order to standardise preparation time of the pea diets. Olive oil (40 g kg⁻¹ of diet) was added to all the experimental diets prior to being fed to the animals in powder form.

A control diet of casein-methionine (C + M; 20% protein) was formulated according to the recommendations of the American Institute of Nutrition (Reeves *et al.*, 1993) in order to meet the nutrient requirements for rats.

Analysis

The moisture content of the different pea diets was determined by drying to constant weight in an oven at 105 ± 1 °C. Zn and Mg were determined in diets, faeces, femur and longissimus dorsi muscle after samples were ashed in a muffle furnace at 450 °C,

and then dissolved in 6 N HCl for analysis. Regarding urine, Zn and Mg were measured samples that had not been previously ashed. Lanthanum chloride (0.1–1%) was added to avoid interferences with phosphate ions during the analysis of Mg. The Zn and Mg content was measured by atomic absorption spectrometry (Perkin-Elmer 1100-B, Perkin Elmer, Norwalk, CT, USA).

Biological methods

Experimental design and diet

We used a biological balance technique, recording changes in body weight and food intake and then calculating Zn and Mg intake and faecal and urinary excretion of minerals (Zn and Mg). Seven experiments of 10-days duration, in which raw or germinated pea flour was the only food source, were conducted. During the first 3 day of experiments, 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 faeces and urine were collected for analysis.

Animals

For each experimental diet we used ten young albino Wistar rats (a total of seventy animals). The growing animals (recently weaned), with an initial body weight of 75.9 ± 1.0 g, were housed from day zero of the experiment in individual stainless steel metabolic cages designed to minimise food spoilage and avoid mixing of food with drinking water, and for separate collection of faeces and urine; the cages were located in a room with a 12 h light/dark period, at a temperature of 21 ± 2 °C, fitted with an appropriate ventilation system. Throughout the experimental period all rats had free access to double distilled water and the diet was consumed *ad libitum*. At the end of the experimental period the animals were anaesthetised with CO₂ and killed by decapitation. The femur and the longissimus dorsi muscle were collected for analysis. All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (ECC).

Biological indices

The following indices and parameters were determined for each group according to the formulas given below: intake expressed as dry weight in grams per day (determined by weighing the amounts of diet given, refused, and spilled, refusals were measured daily), body weight gain (g day⁻¹), apparent digestibility coefficient (ADC); retention (balance), and percentage of retention/absorption (%R/A), for Zn and Mg:

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

$$\text{Balance} = I - (F + U)$$

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

where I = intake, F = faecal excretion, and U = urinary excretion. The amount of ingested insoluble dietary fibre (IDF), soluble dietary fibre (SDF), Lignin, Inositol hexaphosphate (IP6), inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), and inositol triphosphate (IP3) was calculated from daily food intake and composition data for the raw and germinated pea flours used in the present study that has been previously published by Martin-Cabrejas *et al.* (2003), and Vidal-Valverde *et al.* (2002). These authors have reported significant increments in the content of SDF and IDF, and a large reduction in the content of inositol phosphates with increasing germination periods.

Statistical methods

One-way analysis of variance was applied to the data with the use of Statgraphic Statistical Graphics 2.1 System Software (Statistical Graphics Corporation, Rockville, MD, USA). Differences between means were compared with Duncan's multiple-range test. The level of significance was set at $P < 0.05$. Faecal excretion of Zn and Mg was adjusted to a multiple linear regression model with dietary intake of IP6, IP5, IP6 + IP5, SDF, IDF, and lignin as regressors. The regression model was adjusted stepwise with the aim of maximising the fit of the model at each step.

Results

Chemical analysis

Total mineral content of the raw pea flour (RP) used for the present study was 3.00 g (100 g DM)⁻¹ (Table 1), and the content of Zn and Mg was 8.4 and 115 mg (100 g DM)⁻¹, respectively. The germination process decreased the content of Zn and Mg. In the case of Mg, the proportion of mineral lost into the soaking solution as a result of germination was attenuated by increased germination periods. After 2 days' germination, 20% of Mg was lost compared with RP, an additional 5% was lost from 2 to 4 days' germination and a further 2.8% was lost from 4 to 6 days' germination. In the case of Zn, the percentage of mineral lost during the first 2 days of germination was high (55%), but did not subsequently rise. No major differences were observed in the amount of Zn or Mg lost as a result of the presence or absence of light during the germination process.

Biological analysis

The dietary intake of IDF, lignin, SDF, inositols hexa-, penta-, tetra-, and triphosphate (IP6, IP5, IP4 and IP3, respectively; Table 2), Zn, and Mg (Table 3) was a reflection of daily food intake (Table 2), the chemical composition of the diets, and the changes in chemical composition caused by different germination periods (Table 1). Daily food intake expressed as gram per rat per day increased significantly in the groups of animals fed the diets of peas germinated for 2 or 4 days compared with the group of animals fed the raw pea

Table 1 Chemical composition of raw and sprouted pea flours in dry matter

	Ash		Zn			Mg		
	[g (100 g) ⁻¹]	% Loss	[mg (100 g) ⁻¹]	Loss (mg)	% Loss	[mg (100 g) ⁻¹]	Loss (mg)	% Loss
Casein + methionine	3.00		3.9 ^a			68 ^a		
Raw pea flour	3.00		8.4 ^b			115 ^b		
Seed after imbibition period	2.90	3.5	4.3 ^c	4.1	49	109 ^b	6	5
Germination								
2 Days								
No light	2.94	2.0	3.8 ^a	4.6	55	91 ^c	24	21
Light	2.95	1.7	3.8 ^a	4.6	55	91 ^c	24	21
4 Days								
No light	2.96	1.3	3.8 ^a	4.6	55	86 ^{cd}	29	26
Light	2.97	1.0	3.8 ^a	4.6	55	86 ^{cd}	29	26
6 Days								
No light	2.99	0.3	3.9 ^a	4.5	54	82 ^d	33	28
Light	2.97	1.0	3.8 ^a	4.6	55	82 ^d	33	28
Pooled SEM	0.015		0.15			3.4		

The same superscript letter in the same column indicates no significant difference ($P < 0.05$). Values are mean values from four replications.

Table 2 Dietary fibre and phytic acid intake by rats fed raw and sprouted pea flour diets

Diet	Food intake (g day ⁻¹)	IDF intake (g day ⁻¹)	Lignin intake (mg day ⁻¹)	SDF intake (g day ⁻¹)	IP6 intake (mg day ⁻¹)	IP5 intake (mg day ⁻¹)	IP4 intake (mg day ⁻¹)	IP3 intake (mg day ⁻¹)
C + M	12.53 ± 0.62 ^a	1.00 ± 0.05 ^a	–	–	–	–	–	–
Raw pea flour	9.23 ± 0.33 ^b	0.90 ± 0.03 ^a	6.54 ± 0.24 ^a	0.52 ± 0.018 ^a	32.12 ± 1.16 ^a	4.71 ± 0.17 ^a	–	–
Germination								
2 Days								
No light	11.85 ± 0.18 ^c	1.02 ± 0.015 ^a	4.18 ± 0.06 ^b	0.82 ± 0.012 ^b	18.84 ± 0.28 ^b	4.74 ± 0.04 ^a	0.59 ± 0.010 ^a	0.59 ± 0.010 ^a
Light	10.71 ± 0.33 ^c	1.07 ± 0.033 ^a	4.50 ± 0.14 ^b	1.02 ± 0.031 ^c	18.31 ± 0.56 ^b	4.71 ± 0.14 ^a	0.43 ± 0.013 ^b	0.54 ± 0.016 ^b
4 Days								
No light	10.84 ± 0.28 ^c	1.27 ± 0.032 ^b	7.86 ± 0.20 ^c	1.38 ± 0.035 ^d	13.33 ± 0.34 ^c	2.71 ± 0.07 ^b	0.65 ± 0.017 ^c	0.76 ± 0.019 ^{bc}
Light	10.17 ± 0.32 ^c	1.08 ± 0.033 ^a	2.48 ± 0.08 ^d	0.93 ± 0.029 ^e	11.80 ± 0.37 ^d	2.64 ± 0.08 ^b	0.61 ± 0.019 ^a	0.71 ± 0.022 ^c
6 Days								
No light	7.11 ± 0.21 ^d	0.94 ± 0.028 ^a	8.73 ± 0.26 ^e	0.98 ± 0.029 ^{ce}	6.40 ± 0.19 ^e	0.99 ± 0.03 ^c	0.57 ± 0.017 ^a	0.85 ± 0.025 ^d
Light	7.99 ± 0.18 ^d	0.92 ± 0.021 ^a	2.02 ± 0.05 ^d	0.74 ± 0.016 ^f	7.04 ± 0.16 ^e	0.96 ± 0.02 ^c	0.64 ± 0.014 ^c	0.96 ± 0.021 ^e

The same superscript letter in the same column indicates no significant difference ($P < 0.05$). Values are mean ± SEM ($n = 10$).

IDF, insoluble dietary fibre; SDF, soluble dietary fibre; IP6, inositol hexaphosphate; IP5, inositol pentaphosphate; IP4, inositol tetraphosphate; IP3, inositol triphosphate.

Table 3 Digestive and metabolic utilization of zinc and magnesium from raw and sprouted pea flours

	Raw peas	2-Day germination		4-Day germination		6-Day germination		C + M
		No light	Light	No light	Light	No light	Light	
Zinc								
Zn intake (µg day ⁻¹)	776.2 ± 28.1 ^a	453.9 ± 6.9 ^{bc}	409.2 ± 12.7 ^{cd}	413.0 ± 10.6 ^{cd}	390.5 ± 10.9 ^{cd}	276.0 ± 8.1 ^d	311.9 ± 7.0 ^d	483.8 ± 23.7 ^b
Zn absorbed (µg day ⁻¹)	86.9 ± 14.5 ^a	125.6 ± 2.4 ^{bc}	115.7 ± 4.6 ^c	142.8 ± 3.8 ^b	136.4 ± 6.1 ^b	108.5 ± 3.6 ^c	123.1 ± 2.5 ^{bc}	205.9 ± 11.1 ^d
Zn ADC (%)	11.0 ± 1.6 ^a	27.7 ± 0.3 ^b	28.2 ± 0.4 ^b	34.7 ± 1.1 ^c	35.0 ± 1.4 ^c	39.3 ± 0.3 ^d	39.5 ± 0.3 ^d	42.6 ± 1.2 ^e
Zn balance (µg day ⁻¹)	32.2 ± 8.9 ^a	96.6 ± 1.9 ^{bcd}	88.6 ± 3.5 ^d	108.0 ± 2.2 ^b	104.2 ± 3.8 ^{bc}	93.6 ± 3.6 ^{cd}	107.1 ± 2.1 ^b	165.8 ± 9.3 ^e
Zn %R/A	32.8 ± 4.1 ^a	76.9 ± 0.4 ^b	76.6 ± 0.3 ^b	75.9 ± 1.5 ^b	76.9 ± 2.2 ^b	86.2 ± 0.9 ^c	87.0 ± 0.8 ^c	80.5 ± 1.3 ^b
Magnesium								
Mg intake (mg day ⁻¹)	10.62 ± 0.39 ^a	10.96 ± 0.20 ^a	9.78 ± 0.30 ^b	10.15 ± 0.25 ^{ab}	9.61 ± 0.20 ^b	5.85 ± 0.17 ^c	6.58 ± 0.15 ^c	8.55 ± 0.42 ^d
Mg absorbed (mg day ⁻¹)	4.82 ± 0.20 ^a	5.79 ± 0.16 ^b	5.38 ± 0.19 ^b	7.02 ± 0.14 ^c	6.73 ± 0.13 ^c	3.73 ± 0.12 ^d	4.16 ± 0.14 ^d	5.84 ± 0.32 ^b
Mg ADC (%)	45.5 ± 1.3 ^a	52.9 ± 1.5 ^b	55.1 ± 1.1 ^b	69.4 ± 1.4 ^c	70.1 ± 0.8 ^c	63.8 ± 1.2 ^d	63.2 ± 1.1 ^d	68.2 ± 1.04 ^c
Mg balance (mg day ⁻¹)	1.64 ± 0.08 ^a	2.97 ± 0.19 ^b	2.97 ± 0.09 ^b	4.79 ± 0.13 ^c	4.60 ± 0.14 ^c	1.36 ± 0.01 ^a	1.59 ± 0.10 ^a	3.11 ± 0.17 ^b
Mg %R/A	34.2 ± 1.0 ^a	50.9 ± 2.6 ^b	55.7 ± 2.3 ^c	68.2 ± 0.8 ^d	68.3 ± 1.5 ^d	36.7 ± 1.3 ^a	37.9 ± 1.2 ^a	53.4 ± 1.2 ^{bc}

The same superscript letter in the same row indicates no significant difference ($P < 0.05$). Values are mean ± SEM ($n = 10$).

flour diet, although it was still lower than the daily food intake of the casein–methionine control group ($P < 0.05$). No significant differences were found among the animals fed the diets of peas germinated for 2 or 4 days. Daily food intake of rats fed the 6-day germinated pea flour diets (with or without light) was significantly lower than the raw pea flour diet. The intake of IDF did not vary substantially among the animals fed the casein–methionine control diet, and raw or germinated pea flour diets. The highest intake of IDF corresponded to the animals fed the 4-day-germinated pea flour diet ($P < 0.05$). The dietary intake of SDF increased significantly in all the experimental groups fed germinated pea flour (2, 4 or 6 days, with or without light) compared with the animals fed the raw pea flour diet, and the highest dietary intake was for the animals

fed the 4-day-germinated pea flour diet group ($P < 0.05$). The dietary intake of IP6 and IP5 decreased significantly from 2 to 6 days' germination.

Digestive and metabolic utilisation of Zn and Mg

The animals fed the raw pea flour diet and diets of peas germinated for 2 or 4 days had a higher dietary intake of Mg and Zn than those fed the 6-day-germinated pea flour diets (Table 3). The highest dietary intake of Zn corresponded to the group of animals fed the raw pea flour diet ($P < 0.05$). The amount of Zn ingested daily by the animals fed the casein–methionine control diet was lower than that consumed by the group of animals fed the raw pea flour diet, but higher than the experimental groups fed germinated pea flours, whereas

daily Mg intake was lower in the casein–methionine control group than in the animals fed raw and germinated pea flour diets with the exception of groups fed 6-day-germinated pea flour diets.

The digestive utilisation of Zn and Mg from the raw pea flour diet, calculated as ADC (Table 3), was significantly lower than that of germinated peas and the casein–methionine control diet. The best ADC index for Mg was obtained for the animals fed the 4-day-germinated pea flour and casein–methionine control diets, followed by 6-day-germinated and finally 2-day-germinated pea flour diets. For Zn, the best digestive utilisation was observed in the group of animals fed the 6-day-germinated pea flour, followed by the casein–methionine control diet, 4-day-germinated, and finally 2-day-germinated pea flour diets. No significant differences in digestive utilisation as a result of the presence or absence of light during the germination period were observed in the two minerals studied.

Net Zn absorption was higher in the casein–methionine control group than in the rest of experimental groups studied ($P < 0.05$). In case of Mg, net absorption of this mineral by the casein–methionine control group was significantly higher than that found in the animals fed raw or 6-day-germinated pea flour diets, similar to the animals fed the 2-day-germinated, and significantly lower than the animals fed the 4-day-germinated pea flour diets. Among the experimental groups that consumed pea flour diets, the highest net absorption of Zn and Mg corresponded to the groups of rats fed the diets of 4-day-germinated pea flour ($P < 0.05$).

The multiple linear regression model applied (Table 4) showed a strong direct correlation between the faecal

excretion of Zn or Mg and the dietary intake of IP6 and IP5. Regarding dietary fibre, an inverse correlation was found between the dietary intake of SDF and the faecal excretion of both minerals, whereas a direct correlation was found between the dietary intake of IDF and lignin and the faecal excretion of Zn or Mg.

The balance of Zn in all the animals fed the different pea flour diets was significantly lower than that obtained for the casein–methionine control group. The Zn %R/A of such control group was significantly higher than the group of animals fed the raw pea flour diet, similar to the animals fed the 2- and 4-day-germinated pea flour diets, and lower than the experimental groups fed the 6-day-germinated pea flour diets. Mg balance in the casein–methionine control group was higher than in the groups fed raw, 2 and 6-day germinated pea flour diets, but inferior to the groups fed the 4-day-germinated pea flour diets. Metabolic utilisation of Mg expressed as %R/A (Table 3) was higher in the casein–methionine control group than in the groups of animals fed the raw and 6-day-germinated pea flour diets, similar to the groups of animals fed the 2-day-germinated pea flour diets, and lower than the groups of animals fed the 4-day-germinated pea flour diets.

The metabolic utilisation of Zn and Mg assessed as balance or %R/A was significantly improved by the germination of peas for 2 and 4 days, compared with the raw pea flour diet. The Mg balance in the groups of rats fed the 6-day-germinated pea flour diets was similar to that of the group fed the raw pea flour diet, whereas the Zn balance was similar to that of the 2- and 4-day-germinated groups, and the %R/A was significantly higher than that of the other experimental diets studied.

Table 4 Relationship between faecal excretion of magnesium and dietary intake of IP6, IP5, IP6 + IP5, SDF, IDF, and lignin

Variable	Partial r^2	Model r^2	C_p	F-value	$P > F$
Faecal excretion of Zn*					
IP6 + IP5	0.8730	0.8730	104.282	467.64	<0.0001
SDF	0.0233	0.8963	75.0738	15.03	0.0002
Lignin	0.0270	0.9233	40.8690	23.23	<0.0001
IDF	0.0282	0.9515	5.000	37.87	<0.0001
Faecal excretion of Mg ⁻¹					
IP6	0.8287	0.8287	43.9487	328.98	<0.0001
IP5	0.0611	0.8898	6.7283	37.15	<0.0001
SDF	0.0055	0.8954	5.1698	3.50	0.0659
Lignin	0.0034	0.8987	5.000	2.17	0.1456
IDF	–	–	–	–	NS

*Faecal excretion of Zn = $-107.12 + 9.59(IP6 + IP5) - 566.66(SDF) + 622.27(Lignin) + 625.97(IDF)$; $P < 0.0001$, $r^2 = 0.9515$.

†Faecal excretion of Mg = $1.68 + 0.050(IP6) + 0.57(IP5) - 0.85(SDF) + 0.05(Lignin)$; $P < 0.0001$, $r^2 = 0.8987$.

IP6, inositol hexaphosphate; IP5, inositol pentaphosphate; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; C_p , Mallow's C_p statistic; NS, not significant.

Weight gain and Zn and Mg content in plasma, femur and muscle

The greatest weight gain of all the experimental groups studied was found for the casein–methionine control diet ($P < 0.05$). Daily weight gain ($g\ day^{-1}$) was significantly higher among the rats fed the diets of 2- and 4-day-germinated pea flour than that of those fed the raw pea flour diet, with no significant differences between the four germinated pea groups (Table 5). Germination for 6 days caused a smaller weight gain and significant differences were found not only with the groups fed the diets of 2- and 4-day-germinated pea flour, but also with the group fed the raw pea flour diet.

The content of Zn in plasma did not vary significantly as a result of the different germination periods. In contrast, Zn content in the femur and longissimus dorsi muscle increased significantly in response to germination of peas for 2, 4 or 6 days, reaching similar values to the casein–methionine control group. Mg content in plasma was significantly higher among the animals that consumed the diets of 2- and 4-day-germinated pea

Table 5 Magnesium and zinc content in plasma, bone (femur) and muscle (longissimus dorsi)

Diet	Weight gain (g day ⁻¹)	Zn plasma [$\mu\text{g (100 mL)}^{-1}$]	Zn femur ($\mu\text{g g}^{-1}$ ash)	Zn muscle ($\mu\text{g g}^{-1}$ ash)	Mg plasma [mg (100 mL) ⁻¹]	Mg femur (mg g ⁻¹ ash)	Mg muscle (mg g ⁻¹ ash)
C + M	5.65 ± 0.34 ^a	10.86 ± 0.20 ^a	240 ± 11 ^{ab}	63 ± 2.13 ^a	2.86 ± 0.21 ^a	6.9 ± 0.32 ^a	12.8 ± 1.15 ^a
Raw pea flour	2.10 ± 0.10 ^b	10.50 ± 0.30 ^a	220 ± 15 ^a	43 ± 2.27 ^b	2.29 ± 0.14 ^b	7.89 ± 0.45 ^b	14.6 ± 0.37 ^b
Germination							
2 Days							
No light	4.05 ± 0.15 ^c	10.47 ± 0.25 ^a	260 ± 13 ^b	60 ± 2.03 ^a	2.90 ± 0.21 ^a	6.84 ± 0.30 ^a	12.4 ± 2.0 ^a
Light	3.77 ± 0.07 ^c	10.27 ± 0.27 ^a	252 ± 17 ^b	62 ± 3.01 ^a	2.93 ± 0.30 ^a	6.29 ± 0.25 ^a	12.1 ± 1.20 ^a
4 Days							
No light	4.30 ± 0.2 ^c	10.50 ± 0.22 ^a	248 ± 11 ^b	68 ± 3.18 ^a	2.95 ± 0.20 ^a	6.64 ± 0.20 ^a	11.6 ± 0.83 ^a
Light	4.11 ± 0.27 ^c	10.32 ± 0.28 ^a	262 ± 12 ^b	66 ± 2.14 ^a	2.96 ± 0.28 ^a	7.01 ± 0.30 ^a	12.1 ± 0.40 ^a
6 Days							
No light	1.25 ± 0.15 ^d	9.96 ± 0.35 ^a	294 ± 30 ^b	65 ± 5.30 ^a	2.15 ± 0.10 ^b	8.29 ± 0.60 ^b	15.6 ± 1.60 ^b
Light	1.67 ± 0.09 ^d	10.20 ± 0.26 ^a	311 ± 28 ^b	62 ± 5.85 ^a	2.20 ± 0.42 ^b	7.93 ± 0.40 ^b	15.3 ± 1.52 ^b

The same superscript letter in the same column indicates no significant difference ($P < 0.05$). Values are mean ± SEM ($n = 10$).

flour, when compared with the animals fed the raw pea flour diet, reaching similar values to those of the casein–methionine control group. The lowest results were found in the groups that consumed the raw and 6-day-germinated pea flour diets. Mg content in the femur and longissimus dorsi muscle, expressed as milligram per gram ash, was significantly lower in the rats fed the 2- and 4-day-germinated pea flour diets, compared with the animals fed the raw and 6-day-germinated pea flour diets. The results obtained for the experimental groups fed the 2- and 4-day-germinated pea flour diets were similar to those of the casein–methionine control group.

Discussion

Chemical analysis

Total mineral and Mg content of the pea variety used for the present experiment fell within the range of values found in the literature (Alonso *et al.*, 2001; Ereifej & Haddad, 2001). In contrast, the content of Zn was higher than the values usually described, and could be attributed to different soil conditions.

The sharp loss of Zn to the soaking solution used prior to the germination process can be attributed to the leaching of this mineral in free form and/or in the form of small soluble complexes (Welch *et al.*, 1974) or associated with phytic acid and polyphenols that are solubilised during the soaking process (López-Amorós, 2000). Perlas & Gibson (2002) and El-Adawy *et al.* (2000) have observed appreciable losses of Zn in soybean, common bean, lupin or mung bean after different soaking periods in water or sodium bicarbonate. Zn losses did not increase further as a result of germination for 2, 4 or 6 days, with or without light, in spite of the further reduction in phytic acid content (Vidal-Valverde *et al.*, 2002). This indicates that the

experimental conditions used for the germination process were optimum and allowed the release of Zn from phytic acid without leaching out of the seed as was the case with Mg. These results are in agreement with those obtained by Lee & Karunanithy (1990), Khalil & Mansour (1995) and Khalil (2001), who did not find any appreciable reduction in the levels of Zn in beans and faba beans as a result of the germination process.

Magnesium losses during the soaking process prior to germination [$6 \text{ mg (100 g DM)}^{-1}$] are due to the high solubility of this cation, which leaches out of the seed in free form and/or complexed by phytic acid or associated with phenolic compounds that are significantly reduced by the germination process in this same legume (López-Amorós, 2000; Vidal-Valverde *et al.*, 2002) and other legumes such as lentils and faba beans (Abou-Samaha *et al.*, 1985; El-Adawy *et al.*, 2000). During the process of germination for 2, 4, and 6 days with or without light, Mg losses continue, albeit in descending order (16, 6.5, and 3.8% during 0–2, 2–4, and 4–6 days of germination, respectively), which suggests that the amount of soluble Mg liable to leach out of the seed decreases with increasing germination periods.

Biological analysis

Digestive utilisation of Zn

Digestive utilisation of Zn (ADC) from the raw pea flour diet was 3.9-fold lower than that from the casein–methionine control diet with similar protein content (20%). Under our experimental conditions, this lower digestive utilisation may have been caused by factors such as the lower methionine content of the raw pea flour diet, given the importance of this amino acid for Zn absorption (House *et al.*, 1997). On the contrary, the high dietary intake of Zn by the group of rats fed the raw pea flour diet (1.6-fold higher than the casein–

methionine control diet) may have contributed to decrease the digestive utilisation of this cation. Nevertheless, these two factors do not seem to be the only ones involved, given that the net absorption of Zn in the group of animals fed the raw pea flour diet was 2.4-fold lower than that of those consuming the casein–methionine control diet, and 1.25–1.65-fold lower than that for the experimental groups fed the diets of germinated peas with a methionine content similar to the raw pea flour diet. Under our experimental conditions, the presence of inositol phosphates (0.4%) in the raw pea flour diet (Vidal-Valverde *et al.*, 2002), with great affinity for Zn, may have severely impaired the absorption of this mineral in the small intestine. Under the gastric pH conditions, Zn-phytate complexes are hydrolysed, thus releasing the cation. However, in the fairly neutral pH environment of the small intestine, phytic acid becomes negatively charged and is able to complex exogenous dietary Zn and endogenous Zn from the digestive secretions, interfering with the absorption and reabsorption of this mineral (Zhou *et al.*, 1992; Oberleas, 1996).

The ability of the caecum and colon to absorb limited amounts of Zn has been observed in *in vitro* and *in vivo* experiments using intact or caecocolonectomised rats (Hara *et al.*, 2000; Condomina *et al.*, 2002). However, the contribution of the large intestine to Zn absorption from the raw pea flour diet under our experimental conditions did not seem to be very efficient. It would be expected that upon reaching the large intestine, phytate-Zn complexes would be hydrolysed by the bacterial microbiota, releasing free Zn. Furthermore, the presence in the raw pea flour diet of SDF (Martín-Cabrejas *et al.*, 2003), susceptible to being fermented in the large intestine, would decrease the pH of intestinal lumen and improve the solubility of Zn and thus its absorption (Goodlad & Mathers, 1992; Lopez *et al.*, 1998; Yonekura *et al.*, 2004). Nevertheless, net absorption of the cation was very low.

In general, our results indicate that several dietary factors (phytic acid, SDF, IDF, lignin) may play a role in the digestive utilisation of Zn. Nevertheless, phytic acid was the main factor involved whereas the contribution of the other dietary factors was lower, as is shown by the multiple linear regression model applied (Table 4). A 45.8% reduction in the inositol phosphates content of 2-day-germinated pea flour diets (Vidal-Valverde *et al.*, 2002) gave rise to a 2.5-fold increase in the digestive utilisation of Zn (Table 3). This improvement continued in the diets of 4- and 6-day germinated pea flour, reaching ADC values that were close to those of the casein–methionine control diet.

Metabolic utilisation of Zn

Under our experimental conditions and with a diet of raw pea flour that provided a high dietary intake of Zn

but induced a low net absorption of the mineral, no visible renal regulation of this mineral was observed. The animals fed the raw pea flour diet retained only 32% of absorbed Zn in contrast to the experimental groups fed the different germinated pea diets or the casein–methionine control diet, in which Zn %R/A ranged from 76% to 87%. This reduced metabolic utilisation can be attributed to the fact that metabolic regulation of Zn is carried out within certain limits of mineral absorption. In the groups of animals fed the germinated pea flours, mineral absorption improved significantly. This improvement led to balances of Zn that were significantly higher than those found in rats fed the raw pea flour diet, but lower than in those fed the casein–methionine control diet. The metabolic utilisation of Zn expressed as %R/A was similar in the germinated pea diets and the casein–methionine control diet, which indicates that, at the levels of absorption found for the animals fed the germinated pea diets, a renal regulation was evident, with a similar amount of Zn being excreted in the urine by all the experimental groups fed the germinated pea diets, an amount inferior to that of the casein–methionine control group. On the contrary, it should be pointed out that despite the lower Zn balances found in the experimental groups that consumed germinated pea flours, compared with the casein–methionine control diet, no major differences were observed in the content of Zn in plasma, femur or longissimus dorsi muscle.

Digestive and metabolic utilisation of Mg

Magnesium homeostasis when high amounts of this mineral are consumed is achieved by efficient regulation at intestinal and renal level. The digestive utilisation of Mg assessed as ADC was lower in the animals fed the raw pea flour diet than in the casein–methionine control group. The lower digestive utilisation of Mg can be attributed to the high amount of Mg provided by the raw pea flour. This effect has been observed previously in other Mg-rich legumes (chickpeas, common beans) (Nestares *et al.*, 1997, 2003). Under the experimental conditions of the present study, dietary factors that have been reported to influence Mg digestibility such as the levels of vitamin D, the calcium-Mg interaction and the protein quality of pea diets did not appear to play an important role in the absorption of this cation. This is supported by the fact that digestive utilisation was significantly improved in the rats fed the 6-day-germinated pea flour diets with respect to the animals fed the raw pea flour diet, with values that were similar to those for the casein–methionine control diet, in spite of the lower metabolic utilisation of protein found in the germinated pea diets compared with the casein–methionine control group (54% in casein–methionine vs. 37% and 39% in G6DNL and G6DL, respectively), and the lack of

differences in vitamin D and calcium content with respect to the raw pea flour diet.

The increase in Mg digestibility as a result of germination for 2 and 4 days was mainly because of the reduction in the levels of total inositol phosphates (Vidal-Valverde *et al.*, 2002). Under our experimental conditions, the lower intake of IP6 and IP5 as a result of germination was responsible for the improved digestive utilisation of Mg, as suggested by the high correlation between the daily intake of these inositol phosphates and the faecal excretion of Mg (Table 4). The influence of IP4 and IP3 on this improved digestibility was not relevant in view of the low levels of these compounds present in germinated peas (Vidal-Valverde *et al.*, 2002) and their lower binding strength for different minerals (Sandström & Sandberg, 1992; Persson *et al.*, 1998).

Another factor that may have affected and improved the digestive utilisation of Mg is the transformation experienced by dietary fibre during the course of germination. There is no consensus in the literature concerning the effect of dietary fibre in legume seeds, and its transformation as a result of the germination process, on the digestive utilisation of Mg. Under our experimental conditions, IP6 and IP5 seemed to be the main factors related to the digestive utilisation of Mg (Table 4), whereas the contribution of SDF and lignin was lower.

Magnesium balance among rats fed the raw and 6-day-germinated pea flour diets was significantly lower than that obtained in the group of animals fed the casein-methionine control diet. These lower balances are due to the high urinary excretion of Mg in the pea diets compared with the control group, given that net absorption was similar among the four groups. Nevertheless, the Mg balance was positive, in contrast to what has been observed for beans and chickpeas, in which Mg balance was almost null despite a similar net absorption of the mineral (Nestares *et al.*, 1997, 2003).

Germination for 2 days improved the Mg balance and %R/A in relation to the raw pea flour diet. This improvement was more pronounced in the groups of rats fed the diets of peas germinated for 4 days. In these groups, the Mg balance and %R/A were much higher than in the casein-methionine control group. Under our experimental conditions, the nutritive utilisation of Mg was correlated with the weight gain of the animals and oriented towards metabolic processes involved in structural development.

The balance of Mg among rats fed the diets of germinated peas was related to the amount of plasma Mg in a similar way to what has been observed in other legumes (Nestares *et al.*, 1997, 2003). In contrast, the content of Mg in the femur and longissimus dorsi muscle was dependent on the size of the animal, and, therefore, its weight gain.

Despite the reported changes in chemical composition of sprouted peas as a result of the presence or absence of light during germination process (Vidal-Valverde *et al.*, 2002; Martín-Cabrejas *et al.*, 2003), no major changes could be detected in any of the biological indices and parameters studied in response to the different illumination conditions used during germination in the present experiment.

Conclusions

The soaking process used prior to seed germination was responsible for the losses of Zn and Mg, and Mg continued leaching from the seed in small quantities during germination. We recommend the use of peas germinated for short periods of 2–4 days, with or without light, because of the substantial improvement in the palatability of the meal and in the bioavailability of Zn and Mg as a result of the reduction in total inositol phosphates and changes in the SDF content produced by the germination process. This increment in the bioavailability of Zn and Mg more than compensated for their loss during seed soaking. The presence or absence of light during the germination process did not affect the results achieved.

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