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Phytate from an alternative dietary supplement has no effect on the calcium, iron and zinc status in undernourished rats

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SUMMARY. A mixture of cereal bran, eggshells and cassava leaf powder, known as multimixture (MM), has been widely used in developing countries as a dietary supplement to combat malnutrition in children. The introduction of phytate from cereal bran in infant diets has generated serious controversy about MM due to the mineral chelating effect of phytate. This paper reports on a study to investigate the bioavailability of calcium, iron and zinc in rats fed with a deficient diet supplemented with MM. Undernourished rats were treated with a deficient diet (DD) to which MM containing different phytate and mineral concentrations was added. Body weight gains, Ca, Fe, Zn and phytate balances, blood hemoglobin concentration and the mineral content of tissue were determined. DD supplemented with 5% and 25% of MM increased the rats' hemoglobin blood concentration, fur regrowth, Ca concentration in the femur and promoted body weight gain 40 times higher than did the DD. Extra calcium, iron and zinc added to the diet with 25% of MM did not increase the rats' growth rates. Both the addition of NaCl, KF and KI in MM and the use of dephytinized bran in the MM composition led to a significant increase in the rats' growth ($P < 0.0001$); however, these changes failed to increase Ca, Fe and Zn bioavailability. Our findings suggest that the Ca, Fe and Zn bioavailability was not affected by the MM phytate content or by the concentrations of NaCl, KF and KI in the diet.
Key words: Cereal bran, phytate, bioavailability, minerals.

RESUMEN. El fitato de una dieta suplementaria alternativa, no tiene efecto sobre el estatus del calcio, hierro y zinc en ratas subnutridas. Una mezcla de salvado de cereal, cáscara de huevo y hoja de mandioca en polvo, conocida como multi-mezcla (MM), ha sido ampliamente utilizada en países sub-desarrollados como suplemento de la dieta para combatir la desnutrición en niños. La introducción de fitato proveniente de salvado de cereal en dietas para bebés ha generado serias controversias acerca de la MM debido al efecto quelante de minerales por parte del fitato. Este artículo describe un estudio llevado a cabo para investigar la bio-disponibilidad de calcio, hierro, y zinc en ratas alimentadas con una dieta deficiente suplementada con MM. Ratas sub-alimentadas se trataron com una dieta deficiente (DD) a la cual se le agregó una MM conteniendo diversas concentraciones de fitato y minerales. Se determinó el aumento de peso corporal, Ca, Fe, Zn, balance de fitato, concentración de hemoglobina en la sangre y el contenido mineral de los tejidos. La DD suplementada con 5% y 25% de MM aumentó la concentración de hemoglobina en la sangre de las ratas,- crecimiento del pelo, concentración de Ca en el fémur y promovió un aumento de peso corporal 40 veces mayor que con DD. Extra calcio, hierro y zinc agregados a la dieta con 25% de MM, no aumentó la velocidad de crecimiento de las ratas. Tanto la adición de NaCl, KF y KI en la MM así como el uso de salvado sin fitato en la composición de la MM produjeron un aumento significativo en el crecimiento de los animales ($P>0,0001$); sin embargo, estos cambios fallaron en aumentar la bio-disponibilidad del Ca, Fe y Zn. Nuestros resultados sugieren que la bio-disponibilidad del Ca, Fe y Zn no fue afectada por el contenido de fitato en la MM o por las concentraciones de NaCl, KF y KI en la dieta.
Palabras clave: Salvado de cereal, fitato, bio-disponibilidad, minerales.

INTRODUCTION

Malnutrition in Brazil among children under 5 years of age dropped by over 60% between 1975 and 1989 (1). However, this drop was less accentuated in low income communities in the rural areas of the north and northeast due to their remoteness from the agricultural productive areas. An alternative program to fight malnutrition has therefore been proposed. This program consists of using byproducts such as cereal bran, powdered cassava leaves, eggshells, sesame and squash seeds as dietary supplements, known as multimixture (MM). Due to its rich chemical composition, the MM has been considered a potential source of both vitamins (thiamin, riboflavin, A, E) and minerals (Ca, Fe and Zn). Reduced infantile malnutrition has been reported in communities that received MM (2); however, no scientific study has yet been made demonstrating nutritional improvements promoted by MM supplementation or by other factors simultaneously introduced with MM, such as improved sanitary conditions or/and educational campaigns

focusing on mothers. Moreover, the presence of phytate in the composition of bran has given rise to doubts about the bioavailability of minerals in MM (3). Studies are needed to confirm the nutritional benefit of MM (4,5). Phytate, a highly charged inositol molecule, is a powerful multivalent metal ion chelator, which may impair the absorption of minerals in diets (6). However, phytate may be cleaved by phytase present in plants, microorganisms or the intestines of some animals (7,8), producing lower phosphoric esters and, thus, eliminating its metal chelating action (7). Considering that the supplementation of diets with MM increases both mineral and phytate intake, we proposed to investigate the bioavailability of calcium, iron and zinc from the MM in rats.

MATERIALS AND METHODS

Animals: Forty-two male Wistar rats at 21 d, body weight mean 36.3±1.8g, from Bioplan, Brasília, Brazil, were separated into seven groups and housed in individual stainless steel cages, under a 12:00 h light:dark cycle, at 22 ± 2°C. Feces were collected using a nylon net at the bottom of the cages to allow the urine to become separated from the feces. The animal protocol was approved by the Universidade de Brasília's Ethical Committee for the use of Animals. **Diets**: The deficient diet (DD) was prepared with cooked polished rice with soybean oil (50g per kg of rice). The multimixture (MM) was prepared with (g/kg): 350 wheat bran, 350 rice bran, 210 cassava leaf powder, and 90 eggshell powder, the proportions and main constituents of MM used by children's Affairs in Brazil (Pastoral da Criança - CNBB) to combat malnutrition. The six test diets contained DD supplemented with MM added in different proportions of minerals and phytate (g/kg): (5) 50 MM; (25) 250 MM; (25-PS) 250 MM, 112 $CaCO_3$, 1.2 $FeSO_4.7H_2O$, 1.0 $ZnSO_4.7H_2O$; (25-AS) 250 MM, 1.3 NaCl, $0.198x10^{-3}$ KI and 0.003 KF, these elements was added to diet 25 due to their low level found in MM constituents as well as in the DD; (25-LPC) 250 MM with low phytate content. The AIN-93G (9) was used as a reference diet (RD). **Treatment:** All the rats were fed the deficient diet for 15 d to induce malnutrition, which was characterized by a significantly lower body weight gain (4g per 15 d), partial loss of fur, associated to apathy when compared to reference rats (27g per 15 d). The undernourished rats were divided into seven groups (6 rats/group) and allowed free access to one of the experimental diets and distilled water for 28 d. Daily food consumption and fecal excretion were recorded twice a week and body weight once a week. The rats were anesthetized and blood was collected from the heart to determine blood hemoglobin concentrations and hematocritic values. The pancreas and both femurs were removed, weighed and frozen at -70°C for subsequent determination of Ca, Fe and Zn contents. **Phytate reduction process:** Commercial rice and wheat bran (315g of each) were soaked for 24 hours in 3.78 L of an aqueous solution of sodium acetate buffer (50mmol/L, pH 5.15) at 55°C, with simultaneous stirring (44 rpm). This process reduced 79,5% of the concentration of native phytate in the bran. **Analytical methods:** Total protein contents were determined by the Kjeldahl method (10) while total lipids by the Soxhlet method (11). Minerals were determined by inductively coupled plasma atomic emission spectrometry (ICP – AES / Spectro, Kleve, Germany). The calibration curves standardized for Titrisol (Merck) were linear in the ranges of 0-250 mmol/L, 0-17.9 mmol/L, 0-15.3 mmol/L and 0-9.7 mmol/L for Ca, Fe, Zn and P, respectively. The precision of this method was better than 2% of the mean. Diet and feces samples were prepared by Jorhem's method (12), pancreas samples by the method of Hartiti et al. (13) and femur samples by the method of Franz et al. (14).. The phytate content in the diet and feces was measured according to Latta & Eskin (15) Total reducing sugars were determined by the dinitrosalicylic acid method (16). Hematocrit and hemoglobin were determined using a cell counter (COULTER T-890 from Coulter Corporation, Miami, Florida). The precision of this method was better than 2% of the mean. **Statistical Analysis:** The results are expressed as mean ± SEM, and statistical differences were determined by ANOVA with Bonferroni correction (17), using the Stats 95 program. Data that did not meet the assumption of equal variance were log-transformed before statistical analysis and reconverted to antilogarithms to recover the original units.

RESULTS

Diets composition

All the test diets contained about 16 MJ/kg and were protein deficient. 5% of MM added to the deficient diet increased the calcium, iron and zinc contents; however, these nutrients recommended for rats (AIN-93G) were supplied with a total of only 25% of MM (Table 1). Molar phytate:calcium (PA:Ca), phytate:iron (PA:Fe) and phytate:zinc (PA:Zn) ratios were calculated for each test diet. All test diets had molar ratios lower than reported critical values for PA:Ca and PA:Fe (19, 20). Molar ratio higher than reported critical values for PA:Zn (20) was found in 25 and 25-AS diets.

TABLE 1
Composition of diets and nutrient recommendations of the American Institute of Nutrition, for rats (AIN-93G)

Diet	DD	5	25	25-PS	25-AS	25-LPC	MM	AIN-93G recommended
				(Unit/kg)				
Energy (MJ)*	16.6	16.6	16.6	16.6	16.6	16.6	20.0	-
Lipids (g) †	56	63	71	71	71	58	196	70
Protein (g) †	72	75	88	88	88	88	137	200
Ca (g) †	0.2	2.7	12.5	23.7	12.5	12.4	49.4	5
Fe^2 (mg) †	11	26	85	147	85	78	307	35
Zn^2 (mg) †	22	27	47	106	47	37	121	30
Na (mg) *	166	162	145	145	695	145	87	1,019
P (g) †‡	1.0	2.6	8.5	8.5	8.5	6.15	31	3.0
Phytate(g) †	-	1.1	4.8	4.8	4.8	0.98	19.3	-

*Content was calculated considering data cited by Madruga (18).
† Analysis determined in the present study.
‡ Nonphytate P, calculated by the difference between total P minus phytate P.
(DD) Deficient Diet; Test diets contained DD supplemented (g/100g): (5) MM 5; (25) MM 25; (25-PS) MM 25, $CaCO_3$, $FeSO_4$ and $ZnSO_4$; (25-AS) MM 25, NaCl, KI and KF; (25-LPC) dephytinized MM 25; (RD) Reference Diet.

Mineral and phytate balance

Mineral and phytate balance (21) was defined in this study as the difference between intake and fecal excretion (I-FE difference) in a period of 24:00 h. Group DD excreted about 50% of the calcium intake and showed a negative balance of iron and zinc (Table 2). All the test groups showed higher a ($P < 0.048$) calcium, iron and zinc balance than group DD. The relative calcium balance (I-FE/I) in Group 5 was not different from the other test groups, while the relative balance of iron and zinc were lower ($P< 0.002$). The increased calcium, iron and zinc balance found in groups 25-PS, 25-AS and 25-LPC in comparison to group 25 was proportional to the amount of dietary intake or to the dietary elements contents, as confirmed by their relative balances shown in Table 2. Group 5 excreted less than 8% of the phytate intake while the other groups excreted over 44% in the two fortnights of the study (Table 3).

TABLE 2
Daily ingestion (I), excretion (FE) and balance (I-FE) of A) calcium, B) iron and C) zinc in undernourished rats fed DD supplemented with multimixture containing different phytate and mineral concentrations*

Diets	I		FE		I-FE		I-FE/I	
			(mg/d)				(%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A) Calcium balance								
DD	0.78 ^{d}	0.09	0.34 ^{c}	0.08	0.44 ^{d}	0.08	56.41 ^{c}	8.50
5	18.87 ^{c}	3.73	0.94 ^{c}	0.76	17.93 ^{c}	3.86	95.02 ^{a}	5.00
25	104.10 ^{b}	19.34	16.32 ^{b}	8.48	87.78 ^{b}	16.06	84.32 ^{ab}	6.70
25-PS	165.95 ^{a}	50.53	38.07 ^{a}	18.57	127.88 ^{a}	52.18	77.06 ^{bc}	22.80
25-AS	145.15 ^{a}	29.95	25.91 ^{ab}	21.08	119.24 ^{a}	21.83	82.15 ^{ab}	11.80
25-LPC	154.11 ^{a}	26.27	34.12 ^{ab}	27.42	119.99 ^{a}	26.34	77.86 ^{ab}	15.10
Significance	(P<0.001)		(P<0.036)		(P<0.021)		(P<0.006)	
B) Iron balance								
DD	0.05 ^{d}	0.01	0.18 ^{ab}	0.08	-0.13 ^{d}	0.08	-	-
5	0.20 ^{c}	0.03	0.09 ^{b}	0.02	0.11 ^{c}	0.04	55.00 ^{b}	11.69
25	0.72 ^{b}	0.15	0.19 ^{a}	0.07	0.53 ^{b}	0.15	73.61 ^{a}	9.37
25-PS	1.09 ^{a}	0.30	0.19 ^{a}	0.08	0.90 ^{a}	0.31	82.57 ^{a}	9.25
25-AS	0.95 ^{a}	0.22	0.14 ^{ab}	0.08	0.81 ^{a}	0.21	85.26 ^{a}	7.42
25-LPC	0.98 ^{a}	0.14	0.25 ^{a}	0.13	0.73 ^{a}	0.13	74.49 ^{a}	11.93
Significance	(P<0.014)		(P<0.010)		(P<0.023)		(P<0.0001)	
C) Zinc balance								
DD	0.08 ^{c}	0.01	0.20 ^{ab}	0.03	-0.12 ^{d}	0.03	-	-
5	0.19 ^{d}	0.04	0.09 ^{d}	0.03	0.10 ^{c}	0.06	52.63 ^{b}	22.23
25	0.36 ^{c}	0.04	0.11 ^{cd}	0.03	0.25 ^{b}	0.03	69.44 ^{a}	5.85
25-PS	0.72 ^{a}	0.17	0.21 ^{a}	0.05	0.51 ^{a}	0.18	70.83 ^{a}	11.02
25-AS	0.51 ^{b}	0.11	0.12 ^{cd}	0.02	0.39 ^{a}	0.09	76.47 ^{a}	4.38
25-LPC	0.50 ^{b}	0.05	0.15 ^{bc}	0.06	0.35 ^{a}	0.08	70.00 ^{a}	13.35
Significance	(P<0.047)		(P<0.006)		(P<0.048)		(P<0.002)	

*Average and standard errors for n = 48 (six rats per group, eight records per rat). Column not sharing a common superscript are significantly different. Data were log-transformed before statistical analysis. Please refer to Table 1 for abbreviations

TABLE 3
Phytate ingestion (I), excretion (FE) and balance (I-FE) of undernourished rats fed DD added multimixture containing different phytate and mineral concentrations *

	Fortnight 1								Fortnight 2							
	I		FE (mg/d)		I – FE		I – FE/I (%)		I		FE mg/d		I – FE		I – FE/I (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
5	7.4[b]	0.6	0.2[c]	0.06	7.2[b]	0.6	97.6[a]	0.7	8.6[d]	0.8	0.6[e]	0.1	8.0[b]	1.2	92.4[a]	4.1
25	38.2[a]	5.8	22.3[a]	4.3	15.9[a]	3.1	41.5[c]	5.8	43.2[b]	11.7	25.7[b]	5.1	17.5[a]	11.5	40.4[b]	13.3
25-PS	36.4[a]	13.9	16.4[a]	8.5	20.0[a]	7.2	54.9[c]	9.9	35.6[b]	9.6	18.9[c]	4.4	16.7[ab]	10.1	47.0[b]	17.2
25-AS	41.1[a]	8.7	19.9[a]	8.0	21.2[a]	6.3	51.6[c]	14.1	67.8[a]	8.2	34.5[a]	1.4	33.3[a]	11.8	46.1[b]	9.2
25-LPC	9.1[b]	0.8	4.5[b]	1.0	4.6[c]	1.3	50.7[c]	12.4	13.3[c]	1.4	8.5[d]	2.4	4.8[b]	2.5	36.4[b]	15.9
Significance	(P<0.000)		(P<0.025)		(P<0.043)		(P<0.025)		(P<0.009)		(P<0.043)		(P<0.044)		(P<0.02)	

Average and standard errors for n = 24 (six rats per group, four records per rat). Column not sharing a common superscript are significantly different. Data were log-transformed before statistical analysis. Please refer to Table 1 for abbreviations

Mineral bioavailability

All the rats fed test diets showed significantly higher body weight gains than did group DD (P< 0.0001); no difference in body weight gain was observed between groups 5 and 25 (Figure 1). The addition of calcium, iron and zinc to the supplemented diet (group 25-PS) failed to promote any body weight increase. In fact, the rats fed this diet showed a smaller body weight gain than group 25 (P< 0.0001). The addition of NaCl, KF and KI to the supplemented diet (group 25-AS), however, resulted in a 2-fold increase in body weight in relation to the rats fed diet 25. The diet containing a low phytate concentration (group 25-LPC) also provided a significant increase in weight gain (P< 0.0001) compared to the diet containing a native phytate concentration (group 25). The rats from groups 25-AS and 25-LPC reached, respectively, 50% and 57% of the body weight gain displayed by the reference group (Figure 1).

The rats suffered a partial loss of fur during the malnutrition induction period. All the test diets promoted fur regrowth, although the rats fed with the 5, 25 and 25-PS diets showed a lower regrowth rate than the others, while group DD continued to lose fur until the end of study.

The test groups showed femur calcium contents at least 3 times higher than group DD (Figure 2). The femur calcium concentrations found in groups 25-AS, 25-LPC and 25-PS did not differ significantly from group 25, although the femur weight of the first two groups was higher (Figure 1). The highest femur zinc concentration was found in group 25-PS (P< 0.026). The femur zinc concentration found in groups 25-AS and 25-LPC was not significantly different from group DD and showed the lowest values (Figure 2), while the pancreas zinc concentration found in these two test groups was significantly lower than that of group DD (P< 0.001) (Figure 3).

FIGURE 1
Body weight gain (□) and Femur weight (■) of undernourished rats fed Deficient Diet with or without supplementation, for 28 days. (DD) Deficient Diet; Test diets contained DD supplemented (g/100g): (5) MM 5; (25) MM 25; (25-PS) MM 25, $CaCO_3$, $FeSO_4$ and $ZnSO_4$; (25-AS) MM 25, NaCl, KI and KF; (25-LPC) dephytinized MM 25; (RD) Reference Diet. Columns sharing the same letter are not significantly different (P< 0.012 for weight gain and P< 0.049 for femur weight). Average and standard errors for six rats. Data were log-transformed before statistical analysis.

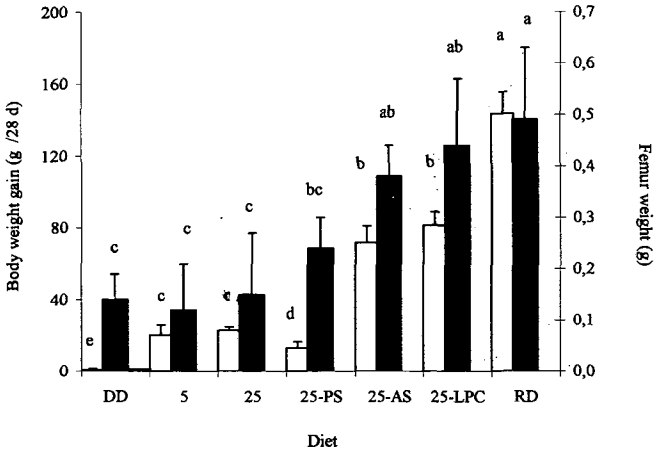


FIGURE 2
Calcium (□) and zinc ■ content in femurs of undernourished rats fed the Deficient Diet with or without supplementation, for 28 days. (DD) Deficient Diet; Test diets contained DD supplemented with (g/100g): (5) MM 5; (25) MM 25; (25-PS) MM 25, $CaCO_3$, $FeSO_4$ and $ZnSO_4$; (25-AS) MM 25, NaCl, KI and KF; (25-LPC) dephytinized MM 25; (RD) Reference Diet. Columns sharing the same letter are not significantly different ($P< 0.026$). Average and standard errors for six rats. Data were log-transformed before statistical analysis.

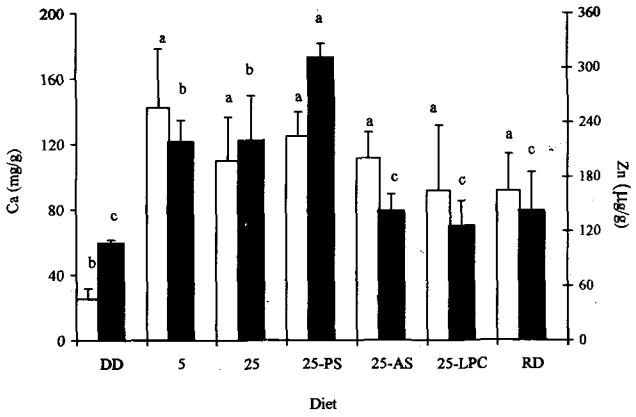
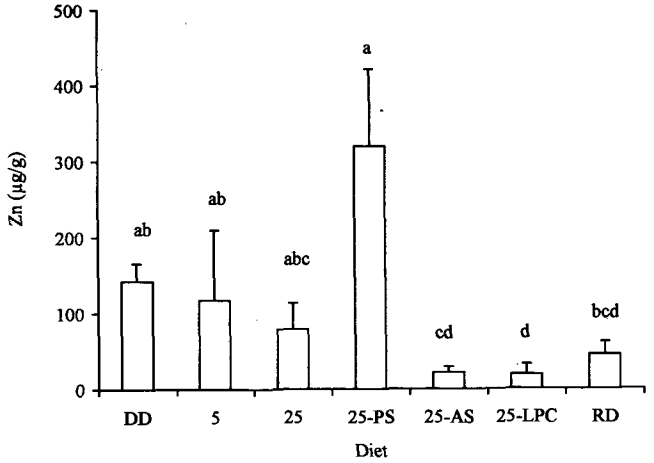


FIGURE 3
Zinc content in pancreas of undernourished rats fed Deficient Diet with or without supplementation for 28 days. (DD) Deficient Diet; Test diets contained DD supplemented (g/100g): (5) MM 5; (25) MM 25; (25-PS) MM 25, $CaCO_3$, $FeSO_4$ and $ZnSO_4$; (25-AS) MM 25, NaCl, KI and KF; (25-LPC) dephytinized MM 25; (RD) Reference Diet. Columns sharing the same letter are not significantly different ($P< 0.029$). Average and standard errors for six rats. Data were log-transformed before statistical analysis.



A comparative analysis of the rats' hemoglobin values showed that, with the exception of group 5, all were anemic in relation to group RD. Although groups 25 and 25-PS were anemic, their hemoglobin concentration was no different from group 5 ($P< 0.028$). All the experimental groups showed hematocrit values lower ($P < 0.026$) than those of the RD group. The hemoglobin and hematocrit values of groups 25-AS and 25-LPC were the lowest.

DISCUSSION

Molar phytate:calcium (PA:Ca), phytate:iron (PA:Fe) and phytate:zinc (PA:Zn) ratios have been considered as bioavailability indicators of these elements in diets. The critical molar ratio, above which ion absorption may be impaired, has been determined by several authors at PA:Ca >1.56, PA:Fe >14 and PA:Zn >10 (19, 20). More recently, the concept of bioavailability has included not only the element's absorption but also its final effect on the organism (22). Therefore, body weight gains, the regrowth of rat fur, hemoglobin concentrations and the Ca, Fe and Zn content in tissues were also used in this study as indicators of the bioavailability of these cations in MM. The addition of a 5% MM supplement in the mineral, protein and vitamin deficient diet promoted a significant increase in the rats' growth rate and aided fur recovery. However, group 25, which was fed four times more MM than group 5, showed a similar growth rate.

The reduction of PA:Ca, PA:Fe and PA:Zn by the addition of Ca, Fe and Zn (diet 25-PS) did not increase the relative balance of the elements nor did it promote any increase in femur Ca and hemoglobin concentration, in body weight or in fur regrowth in relation to diet 25. These results suggest that the reduction in the PA:Ca, PA:Fe and PA:Zn molar ratios were not a relevant factor in the bioavailability of these three ions in the supplemented diet.

Contrary to our expectations, the use of dephytinized wheat and rice bran in the MM (group 25-LPC), which also reduced the phytate:element molar ratio, increased neither the relative calcium, iron and zinc balances nor the Ca, Fe and Zn tissue contents in comparison to group 25. Moreover, the rats fed the 25-LPC diet showed an even lower hemoglobin count and femur Zn concentrations than did group 25 (Table 4, Figure 2). Our results suggest that the extra weight gain (Figure 1) and the total fur recovery shown by group 25-LPC were not a consequence of an improvement in the element bioavailability but were, in fact, due to the increase in food consumption. Development of anemia was also reported in children during the malnutrition treatment (23), Refino and Dallman (24) found also that rate of iron repair deficiency anemia or blood loss was lower in younger rats. The severe anemia found in the rats with the highest growth rate (groups 25-LPC and 25-AS) may have been caused by the fact that

the iron content in the diet was insufficient to simultaneously maintain iron homeostasis and promote hematopoiesis in the rapidly growing animals. A longer period of treatment with those diets might reverse the anemia in undernourished rats.

TABLE 4

Blood hemoglobin concentration (HGB) and hematocrit values (HCT) of undernourished rats fed DD supplemented with multimixture containing different phytate and mineral concentrations for 28 days *

Diet	HGB (g/L)		HCT l	
	Mean	SE	Mean	SE
DD	149 ^{cd}	4.0	0.45 ^{d}	0.01
5	163 ^{ab}	5.0	0.50 ^{b}	0.01
25	152 ^{bc}	3.3	0.47 ^{cd}	0.01
25-PS	159 ^{bc}	4.5	0.48 ^{c}	0.02
25-AS	132 ^{e}	6.1	0.42 ^{e}	0.02
25-LPC	136 ^{de}	6.3	0.42 ^{e}	0.01
RD†	173 ^{a}	14	0.63 ^{a}	0.06
Significance	(P<0.028)		(P<0.026)	

Average and standard errors for n = 6. Column not sharing a common superscript are significantly different.
† Normal values were considered those obtained for Wistar rats weaned at 21 d and fed the reference diet during 28 d.
Please refer to Table 1 for abbreviations.

Investigations of dephytinized diets in humans and rats have shown an increase in ion bioavailability, which has been attributed to the reduction of phytate concentration (19,25,26). However, a more recent study concluded that wholewheat flour, rich in phytic acid and minerals, caused no negative effect on mineral absorption but, on the contrary, improved the bioavailability of some minerals in rats (27). Most phytate reduction methods have employed either acid-salt washing or enzymatic hydrolysis processes. In the former, the protein conformations must be considered since the ion-binding sites in proteins may be irreversibly destroyed during the acid-salt washing process, possibly rendering the minerals more available for absorption. Hurrell et al. (25) working with low phytate soy protein isolates, concluded that, even after the removal of virtually all phytate, soy protein itself is still inhibitory to iron absorption.

Several endogenous enzymes may be activated during the hydrolytic action of phytase, resulting in the hydrolysis of several nutrients such as polysaccharides or even proteins and releasing products that are more easily absorbed by the body. The effect of complex carbohydrate fermentation on the bioavailability of trace elements has been reported by other authors (28). Our results showed that the concentration of reducing sugars in diet 25-LPC was 28.0 ± 3.4 g/100g, while diet 25 showed 18.9 ± 1.24g /100g. These data support the above hypothesis and may also explain the increase of food consumption of the 25 LPC diet by the rats, which was responsible for their increased growth rate.

Diets 5 and 25-LPC contained similar phytate concentrations (Table 1); however, the former group showed a higher relative phytate balance (Table 3). A possible explanation for the unusually high phytate balance found in group 5 may be the adaptive mechanism described by Moore and Veum (29), according to which the digestibility of phytate increases in phosphorus-deprived rats. Although this induction mechanism has yet to be characterized, an increase in bacterial alkaline phosphatase activity and an induced extracellular fungal phytase have been observed in microbial fermentation under limited inorganic phosphorus (8). Therefore, as the phosphorus content in diet 5 was lower than that in diet 25-LPC (Table1), we suggest that the phosphatase in the rats' microflora was induced, resulting in enhanced phytate hydrolysis.

Mineral salts, which are present in MM in low concentrations, were added to the 25 diet to verify the possible effects of some essential anions on the bioavailability of calcium, iron and zinc. Although the addition of KI, KF and NaCl salts (group 25-AS) increased the rats' growth rate (Figure 1), the relative balance of calcium, iron and zinc (Table 2) and the concentration of Ca and Zn in the tissues (Figures 2 and 3) were no higher than in the rats that received diet 25. These results suggest that MM was deficient in KI, KF and NaCl and that these minerals did not affect the bioavailability of Ca, Fe and Zn in diet 25-AS.

The low zinc concentrations in the pancreas (Figure 3) of the rats with the largest weight gain (groups 25-AS and 25-LPC) is apparently correlated with growth rates and zinc requirements. Zinc requirements for undernourished rats with high growth rates should be higher than the RNN, both for recovery of the normal zinc levels in the body and to supply the zinc requirements for growth. Because the pancreas is responsible for homeostasis of the body's zinc (30,31), dietary zinc during growth would not accumulate in the pancreas but instead, would be distributed to priority tissues involved in the rats' growth. On the other hand, the distribution role of the pancreas in rats showing moderate growth (groups 5, 25 and 25-PS) would be diminished and hence, surplus dietary zinc might be stored in the femur (Figure 2).

To conclude, the addition of up to 25% MM to a deficient diet aided the recovery of undernourished rats, although the lack of some mineral salts restricted the potential of MM as a complete dietary supplement. Reduction of molar PA:(Ca, Fe or Zn) ratios by the addition of these ions did not increase the Ca, Fe and Zn bioavailability of the MM. The dephytinization process and the addition of KI, KF and NaCl

to the supplemented diet increased its food consumption, thus being responsible for the rats' incremental growth. However, the above procedures increased neither the relative balance of Ca, Fe and Zn in undernourished rats nor these ion concentrations in the target tissues. Our findings suggest that the bran phytate content does not impair Ca, Fe and Zn bioavailability in deficient diets supplemented with up to 25% of MM. Considering that phytase activity in rats is higher than in humans (32) and that this fact may have influenced our results, it is recommended that studies be carried out on human subjects before feeding MM to undernourished children.

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