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ORIGINAL CONTRIBUTION

Lina Yonekura
Hiroo Suzuki

Effects of dietary zinc levels, phytic acid and resistant starch on zinc bioavailability in rats

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L. Yonekura · H. Suzuki
Dept. of Biochemistry and Food Science
Kagawa University
Kagawa, Japan

L. Yonekura (✉)
Present address:
Lipid Laboratory
National Food Research Institute
Kannodai 2-1-12
Tsukuba
Ikaraki 305-8642, Japan
E-Mail: linayonekura@yahoo.co.jp

H. Suzuki
Present address:
Dept. of Food and Nutrition
Kochi Gakuen College
Asahitenjin 292-26
Kochi, Japan

Summary *Background* Owing to its fermentability, it has been advocated that resistant starch (RS) has a positive effect on the absorption of minerals by increasing their solubility in the hindgut. In marginally zinc-deficient rats, the enhancement of zinc bioavailability by RS occurs mostly when the diet contains phytic acid. *Aim of the study* This study aims to investigate the effect of dietary zinc level and phytic acid on the cecal zinc pools and zinc bioavailability of rats fed RS. *Methods* Wistar rats (male, 3wk old) were divided into eight groups (n = 6), and fed diets containing either 5 % cellulose (control fiber: insoluble and low fermentable) or 20 % RS (test fiber: soluble and fermentable), with or without the addition of 1 % sodium phytate, at the 10 and 30 mg/kg dietary zinc levels, for 21 days. *Results* At 10 mg Zn/kg, RS increased femur zinc concentration only in the group receiving the phytate-containing diet, while at 30 mg Zn/kg it increased femur zinc concentration in rats fed both phytate-free and phytate-containing diets. The total content of zinc in the cecum was increased by the higher dietary zinc level and tended to be increased by the addition of phytate, which is assumed to impair zinc absorption in the small intestine. Feeding RS lowered cecal pH values, which correlated with increasing values of zinc solubility ($r = -0.3471$; $P < 0.05$). The later was, in turn, directly associated with zinc apparent absorption ($r = 0.3739$; $P < 0.05$). *Conclusions* The increase in zinc bioavailability by RS occurs when dietary zinc levels are adequate and/or zinc absorption is impaired in the small intestine, increasing the influx of unabsorbed zinc into the cecum and favoring the increase of zinc bioavailability when RS fermentation lowers the cecal pH.

Key words zinc bioavailability – phytic acid – resistant starch – fermentation – magnesium – rat

Abbreviations

AAS	Atomic absorption spectrophotometry
phy	Phytic acid
SCFA	Short chain fatty acids
RS	Resistant starch (from raw potato starch)

Introduction

Resistant starch (RS) is known to be fermented in the rat cecum [1–3] and in the human large bowel [4], producing SCFA (short chain fatty acids) that lower the luminal pH. Owing to that property it has been advocated that RS has a positive effect on the absorption of minerals by increasing their solubility in the cecum [5–7]. However, in contrast with the numerous studies on the effects of RS

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fermentation on magnesium and calcium bioavailability [6–8], few studies have been done on zinc, and its relationship with cecal fermentation is not fully understood. Our previous studies, using marginally zinc-deficient diets, have detected the enhancement of zinc bioavailability by RS mostly when the diet contained phytic acid [9, 10]. From those findings we suggest that phytic acid might have increased the cecal zinc pool and, therefore, the amount of zinc that could be solubilized when pH was lowered by SCFA.

Phytic acid forms insoluble chelates with zinc and other essential minerals, reducing their bioavailability [11, 12]. *In vitro* [13, 14] and *in vivo* [15] studies support that phytic acid forms complexes with zinc and inhibits its absorption mainly in the small intestine; thus, larger amounts of zinc might reach the cecum when the diet contains phytic acid. When the dietary zinc level is limited (i. e. marginally deficient), only a small amount of this mineral is likely to reach the cecum. Therefore, increasing the dietary zinc level would also increase the cecal zinc pools and, consequently, a larger amount of soluble zinc would be formed when cecal pH is lowered.

Magnesium absorption is largely increased by decreases in the cecal pH, while zinc absorption is less favored [10]. In the present study, magnesium apparent absorption and its solubility in the cecum will be used to assess the efficiency of cecal fermentation in increasing the bioavailability of this mineral. Moreover, the rationale of the presence or the absence of the same promoting effects of RS fermentation on zinc availability will be discussed.

The objective of this study is to investigate the effect of phytic acid and the increase of zinc level in diets containing RS, on the cecal zinc pool size and zinc bioavailability of rats fed those diets. A higher zinc intake and the presence of phytic acid in the diet are expected to enlarge the cecal zinc pools and promote an enhancement of zinc solubility and absorption when RS fermentation reduces the cecal pH.

Materials and methods

Forty-eight male, three week-old, Wistar rats (SLC, Hamamatsu, Japan) were housed individually in wire bottomed stainless-steel cages, and provided with a commercial pelleted diet (CE-2, Clea Japan Inc., Tokyo, Japan) and water, *ad libitum*, until their body weights reached approximately 80 g. The concentration of RS in test diets was set to 20 %, as this was the concentration that significantly increased zinc bioavailability in previous experiments [10]. Animals were fed test diets containing either 5 % cellulose (control fiber: insoluble and low fermentable) or 20 % RS (test fiber: soluble and fermentable) as the sole dietary fiber source, with or without the addition of 1 % sodium phytate, at two dietary zinc levels: 10 and 30 mg/kg diet. Dietary groups were named as follows: 10Zn Cellulose, 10Zn RS, 10Zn Cellulose + phy, 10Zn RS + phy, 30Zn Cellulose, 30Zn RS, 30Zn Cellulose + phy and 30Zn RS + phy. Experimental diets (Table 1) and deionized water were provided *ad libitum* for 21 days (temperature, 24 ± 1 °C; relative humidity, 40 ~ 70 %, photoperiod from 8:00 to 20:00 h). Food intake and body weight were measured daily at 9:30~10:00 h. This experiment was conducted in accordance with the Kagawa University's guidelines for the use and care of animals.

Feces were collected during the last four days of the

Table 1 Composition of test diets (g/kg diet)

Component	Dietary groups							
	10Zn Cellulose	10Zn RS	10Zn Cellulose + phy	10Zn RS + phy	30Zn Cellulose	30Zn RS	30Zn Cellulose + phy	30Zn RS + phy
Fixed components[1]	346.9	346.9	346.9	346.9	346.9	346.9	346.9	346.9
Phytic acid[2]	–	–	10	10	–	–	10	10
Cellulose[3]	50	–	50	–	50	–	50	–
Raw potato starch (RS)[4]	–	200	–	200	–	200	–	200
Pre-gelatinized potato starch	603.1	453.1	593.1	443.1	603.1	453.1	593.1	443.1
Zinc (as $Zn_2SO_4 \cdot 7H_2O$) (mg/kg)	10	10	10	10	30	30	30	30

[1] Fixed components consisted of: casein (Sigma-Aldrich Japan K. K., Tokyo, Japan) demineralized as described by Daijoh et al. [16] and calculated to provide 200 g/kg of crude protein; 50 g/kg of corn oil; 1.5 g/kg of choline chloride; 50 g/kg of Zn-free mineral mixture (each kg of mixture contained: $CaHPO_4 \cdot 2H_2O$, 4.3 g; KH_2PO_4, 343.1 g; NaCl, 250.6 g; Fe-citrate 6.23 g; $MgSO_4$, 48.764 g; $MnSO_4 \cdot 4{\sim}5H_2O$, 1.21 g; $CuSO_4 \cdot 5H_2O$, 1.56 g; KI, 0.005 g; $CaCO_3$, 292.9 g; $(NH_4)6Mo_7O_{24} \cdot 4H_2O$, 0.025 g; cellulose powder 41.306 g); and 8.5 g/kg of vitamin mixture (each kg of mixture containing: menadione, 60 mg; thiamine hydrochloride, 590 mg; riboflavin, 590 mg; pyridoxine hydrochloride, 290 mg; cyanocobalamin, 2 mg; ascorbic acid, 5880 mg; D-biotin, 10 mg; folic acid, 20 mg; calcium pantotenate, 2350 mg; nicotinic acid, 2940 mg; inositol, 11760 mg; lactose, q. s. to 1kg) . Retinyl acetate, calciferol and tocopherol acetate were added to diets to final concentrations of 6000 IU/kg, 600 IU/kg and 100 mg/kg, respectively.
[2] Phytic acid (inositol hexaphosphoric acid) dodecasodium salt, from rice. Sigma Chemical Co., St Louis, USA.
[3] Avicel FD 101. Asahikasei Co., Tokyo, Japan.
[4] Potato starch. Wako Pure Chemical Industries Ltd., Tokyo, Japan

experimental period, and Zn and Mg were determined by flame atomic absorption spectrophotometry (AAS, Model Z-5000, Hitachi, Japan) after dry-ashing at 550 °C, as described elsewhere [9]. The apparent absorption ratios of minerals were calculated by using the following equation: apparent absorption (%) = 100 x {[mineral intake (17~20d) – mineral excretion (18~21d)]/mineral intake (17~20d)}. At the end of the experimental period, rats were sacrificed by heart puncture under ether anesthesia. Blood, right femur and testis were collected. The concentration of zinc in serum was determined by flame AAS after a suitable dilution with 0.1 M HNO_3. Femur and testes were wet-ashed (HNO_3/H_2O_2) and diluted with 0.1 M HNO_3 prior to analysis by flame AAS. The cecum of each animal was excised, weighed and emptied. The cecal wall was washed, blotted on filter paper and weighed. The pH and SCFA + succinic acid concentration of the cecal content were measured as previously described [10]. A sample of the cecal content was 9-fold diluted with distilled water and centrifuged (25000 rpm, 25 min, 10 °C). Aliquots of the total cecal content and the supernatant were dry-ashed at 550 °C for 12 hours, followed by the addition of 0.3 mL of concentrated HNO_3, dried-up on a hot plate and ashed for 5 hours at 550 °C. Ashes were subsequently dissolved in a suitable volume of 0.5 M HNO_3 and the concentrations of total and soluble Zn and Mg were measured by flame AAS.

Statistical analyses were performed by ANOVAs (Analyses of Variance) with a 2^3 factorial design, using the StatView® software for Macintosh (SAS Institute, Cary, NC, USA). When the ANOVA revealed effects with P values < 0.05, differences between means were assessed by the Tukey's post hoc multiple range test [17], with a confidence interval of 95 %.

Results

Body weight gain, feed intake and efficiency, zinc apparent absorption and retention

At the 10 mg/kg dietary zinc level, feeding RS increased femur zinc concentration only when the diet contained phytic acid (10Zn RS + phy group). At 30 mg/kg of dietary zinc, RS increased femur zinc concentration in rats fed both phytate-free and phytate-containing diets (Table 2). In agreement with that, significant interactions were observed between the effects of fiber and dietary zinc ($P = 0.002$) and phytate ($P = 0.003$) on femur zinc concentration (Table 2). However, feeding RS did not improve zinc concentrations in the serum and testes of rats (Table 2). There was no significant effect of feeding RS on body weight gain, feed intake and feed efficiency (Table 2).

One rat of the 10Zn RS group, 4 rats of the 10Zn RS + phy, 3 of the 30Zn RS group and 2 of the 30Zn RS + phy group had loose stools, making it impossible to collect total fecal output for those animals. Therefore, the apparent absorption data were obtained only from the animals with normal stools. Femur zinc concentrations were strongly correlated with zinc apparent absorption (Fig. 1; $r = 0.8229$; $n = 38$; $P < 0.01$), confirming

Table 2 Effects of dietary zinc level and phytic acid on the concentration of zinc in the femur, testis and serum, body weight gain, feed intake and feed efficiency of rats fed diets containing cellulose (control) or RS as dietary fiber[1]

	Femur Zn (µg/g dry wt)	Testis Zn (µg/g)	Serum Zn (µg/mL)	Body weight gain (g)	Feed intake (g/21 days)	Feed efficiency (g BW gain/g intake)
10Zn Cellulose	179.8 ± 1.0^{c}	21.69 ± 0.31^{a}	1.461 ± 0.084^{a}	100.5 ± 2.9^{a}	284.1 ± 7.1^{a}	0.3538 ± 0.0057^{a}
10Zn RS	182.4 ± 2.2^{c}	21.63 ± 0.58^{a}	1.397 ± 0.089^{a}	92.3 ± 2.1^{a}	$277.5 \pm 5.7^{a,b}$	0.3330 ± 0.0060^{a}
10Zn Cellulose + phy	74.5 ± 0.2^{f}	19.27 ± 0.27^{b}	0.526 ± 0.051^{b}	54.7 ± 2.7^{c}	216.3 ± 6.9^{c}	0.2524 ± 0.0070^{c}
10Zn RS + phy	93.7 ± 1.2^{e}	$20.22 \pm 0.75^{a,b}$	0.700 ± 0.075^{b}	$67.8 \pm 8.1^{b,c}$	$241.3 \pm 7.7^{b,c}$	$0.2781 \pm 0.0261^{b,c}$
30Zn Cellulose	204.4 ± 1.4^{b}	21.83 ± 0.18^{a}	1.525 ± 0.075^{a}	101.0 ± 3.2^{a}	286.6 ± 10.2^{a}	0.3529 ± 0.0056^{a}
30Zn RS	224.8 ± 1.0^{a}	21.52 ± 0.44^{a}	1.601 ± 0.067^{a}	$85.0 \pm 5.0^{a,b}$	$270.5 \pm 10.6^{a,b}$	$0.3133 \pm 0.0087^{a,b}$
30Zn Cellulose + phy	134.3 ± 0.5^{d}	21.59 ± 0.37^{a}	1.237 ± 0.083^{a}	$86.7 \pm 3.7^{a,b}$	$271.0 \pm 7.9^{a,b}$	$0.3195 \pm 0.0073^{a,b}$
30Zn RS + phy	171.1 ± 2.2^{c}	21.74 ± 0.32^{a}	1.383 ± 0.123^{a}	$83.7 \pm 5.0^{a,b}$	$263.4 \pm 8.1^{a,b}$	$0.3168 \pm 0.0131^{a,b}$
ANOVA[2]						
A (Zn)	< 0.001	0.003	< 0.001	0.024	0.003	0.015
B (Phytate)	< 0.001	0.004	< 0.001	< 0.001	< 0.001	< 0.001
C (Fiber)	< 0.001	NS	NS	NS	NS	NS
AB	< 0.001	0.004	< 0.001	< 0.001	0.001	0.001
AC	0.002	NS	NS	NS	NS	NS
BC	0.003	NS	NS	0.010	NS	0.017
ABC	NS	NS	NS	NS	NS	NS

[1] Values are means ± SE of six rats. Means in a column not sharing a superscript letter are significantly different ($p < 0.05$, Tukey's multiple range test)
[2] *NS* not significant. P values are shown when < 0.05

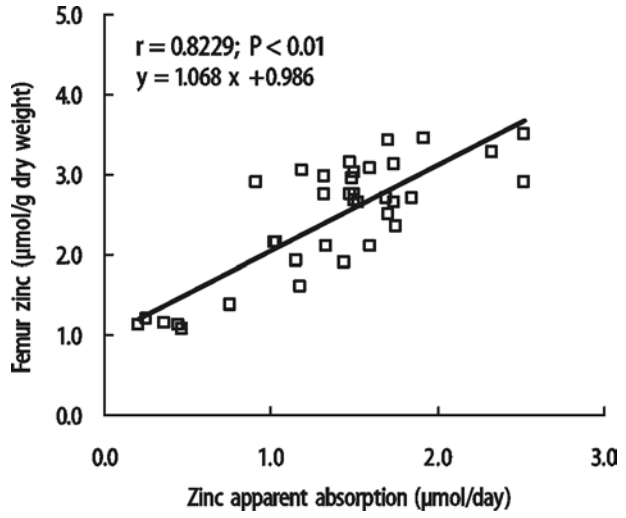

Fig. 1 Correlation between mean femur zinc concentration (μmol/g) and zinc apparent absorption (μmol/day) of rats fed either phytate-free or phytate-containing diets, containing different dietary fibers (cellulose or RS) and zinc levels (10 or 30 mg/kg).

that femur zinc is a good parameter to assess zinc bioavailability.

Table 3 Effects of dietary zinc level and phytic acid on the cecal pH and the cecal concentrations of SCFA, succinic acid and SCFA + succinic acid of rats fed diets containing cellulose (control) or RS as dietary fiber[1]

	Cecal pH	SCFA + Succinic Acid (μmol/g)
10Zn Cellulose	7.13 ± 0.076^{a}	$61.7 \pm 5.4^{c,d}$
10Zn RS	5.55 ± 0.120^{b}	138.3 ± 11.1^{a}
10Zn Cellulose + phy	7.16 ± 0.079^{a}	44.7 ± 5.4^{d}
10Zn RS + phy	6.04 ± 0.320^{b}	$106.0 \pm 18.6^{a,b}$
30Zn Cellulose	7.18 ± 0.126^{a}	$60.6 \pm 7.5^{c,d}$
30Zn RS	6.04 ± 0.228^{b}	$89.6 \pm 10.8^{b,c}$
30Zn Cellulose + phy	7.12 ± 0.104^{a}	$53.9 \pm 2.5^{c,d}$
30Zn RS + phy	5.54 ± 0.141^{b}	$124.1 \pm 5.4^{a,b}$
ANOVA[2]		
A (Zn)	NS	NS
B (Phytate)	NS	NS
C (Fiber)	< 0.001	< 0.001
AB	0.028	0.007
AC	NS	NS
BC	NS	NS
ABC	NS	0.044

[1] Values are means ± SE of six rats. Means in a column not sharing a superscript letter are significantly different ($p < 0.05$, Tukey's multiple range test)
[2] *NS* not significant. P values are shown when < 0.05

Cecal fermentation parameters, cecal pools and apparent absorption of zinc and magnesium

All groups fed RS had significantly lower cecal pH compared to the respective Cellulose-fed controls (Table 3). RS-fed groups generally had higher cecal SCFA + succinic acid concentration than their respective Cellulose-fed controls (Table 3). In agreement with the observations above, the cecal SCFA + succinic acid concentration was strongly and inversely correlated with the cecal pH ($r = -0.8536$; $n = 48$; $P < 0.01$).

The increase of dietary zinc level from 10 to 30 mg/kg enlarged the cecal total zinc pool size ($P < 0.001$, Table 4). Accordingly, within the groups fed RS-containing diets, the increase in dietary zinc level (from 10 to 30 mg/kg) significantly increased the cecal total zinc pool size in rats fed phytate-free diets, and tended to increase that of rats fed phytate-containing diets (Table 4). Feeding RS-containing diets significantly increased the cecal content weight (Table 4). A tendency for larger cecal total zinc pools was also observed in the groups fed RS, except for the 10Zn RS group (fed the phytate-free, 10 mg/kg Zn diet). Feeding RS also tended to increased cecal soluble zinc pools and percentages, as compared to those of rats fed cellulose, independently of dietary phytate or zinc level (Table 4).

Feeding RS lowered the cecal magnesium concentration (data not shown), probably due to a dilution caused by the increase of cecal content in the groups fed RS (Table 4). No effects of either phytate or RS were observed on the cecal total magnesium pool size (Table 4). Feeding RS-containing diets generally increased the cecal soluble magnesium pools ($P < 0.001$) and the percentages of soluble magnesium ($P < 0.001$). The cecal soluble magnesium pools in the 10Zn RS and 30Zn RS + phy groups, and the percentage of soluble magnesium in the 10Zn RS, 30Zn RS and 30Zn RS + phy groups were significantly higher than those in the corresponding cellulose-fed groups (Table 4). In line with those observations, cecal pH was inversely correlated with the concentrations of soluble magnesium in the cecum (Fig. 2; $r = -0.7457$; $P < 0.01$), and the soluble magnesium pool size was directly and strongly correlated with magnesium apparent absorption (μmol/day) (Fig. 3, $r = 0.7932$; $P < 0.01$). Regarding zinc, the correlation between cecal pH and cecal soluble zinc (Fig. 2; $r = -0.3471$; $P < 0.05$) was significant, but weaker than that of magnesium. Cecal soluble zinc pools were related to zinc apparent absorption by a natural logarithmic curve (Fig. 3, $r = 0.3739$; $P < 0.05$).

Discussion

RS enhances the bioavailability of zinc and other minerals by cecal fermentation, reducing the luminal pH and increasing zinc solubility in the cecum [5, 8]. Consequently, the cecal zinc pool size is likely to influence the

Table 4 Effects of dietary zinc level and phytic acid on the cecal content weight, the total and soluble cecal pools and the solubility percentage of zinc and magnesium in rats fed diets containing cellulose (control) or RS as dietary fiber[1]

	Cecal content weight (g)	Total Mg (μmol/cecum)	Soluble Mg (μmol/cecum)	Soluble Mg (%)	Total Zn (μmol/cecum)	Soluble Zn (μmol/cecum)	Soluble Zn (%)
10Zn Cellulose	2.54 ± 0.19^{c}	107.5 ± 9.0	6.38 ± 2.48^{b}	5.81 ± 2.22^{c}	0.414 ± 0.045^{b}	0.016 ± 0.008^{b}	$3.45 \pm 1.55^{a,b}$
10Zn RS	8.72 ± 0.63^{a}	163.4 ± 28.6	67.98 ± 9.76^{a}	$45.16 \pm 6.19^{a,b}$	1.675 ± 0.417^{b}	0.133 ± 0.023^{a}	15.58 ± 6.56^{a}
10Zn Cellulose + phy	2.17 ± 0.08^{c}	126.9 ± 4.3	3.84 ± 0.70^{b}	3.09 ± 0.61^{c}	1.035 ± 0.042^{b}	0.009 ± 0.004^{b}	0.93 ± 0.42^{b}
10Zn RS + phy	5.56 ± 1.15^{b}	115.1 ± 32.9	$30.04 \pm 10.35^{a,b}$	$24.52 \pm 3.64^{b,c}$	$2.331 \pm 0.976^{a,b}$	$0.082 \pm 0.047^{a,b}$	$4.51 \pm 2.30^{a,b}$
30Zn Cellulose	2.52 ± 0.22^{c}	119.6 ± 10.4	5.93 ± 1.69^{b}	5.00 ± 1.40^{c}	$2.362 \pm 0.398^{a,b}$	$0.038 \pm 0.016^{a,b}$	1.53 ± 0.57^{b}
30Zn RS	5.49 ± 0.81^{b}	143.5 ± 13.2	$39.11 \pm 9.19^{a,b}$	$29.01 \pm 7.67^{a,b}$	5.482 ± 1.242^{a}	0.149 ± 0.047^{a}	$3.87 \pm 1.85^{a,b}$
30Zn Cellulose + phy	2.48 ± 0.11^{c}	147.9 ± 9.3	4.45 ± 0.62^{b}	3.04 ± 0.47^{c}	$3.909 \pm 0.229^{a,b}$	0.019 ± 0.006^{b}	0.50 ± 0.18^{b}
30Zn RS + phy	$6.69 \pm 0.52^{a,b}$	155.8 ± 44.4	68.45 ± 24.72^{a}	49.83 ± 11.89^{a}	6.684 ± 3.442^{a}	$0.112 \pm 0.035^{a,b}$	$6.30 \pm 4.78^{a,b}$
ANOVA[2]							
A (Zn)	NS	NS	NS	NS	< 0.001	NS	NS
B (Phytate)	NS	NS	NS	NS	0.049	NS	NS
C (Fiber)	< 0.001	NS	< 0.001	< 0.001	0.004	0.048	0.007
AB	0.028	NS	0.011	0.006	NS	NS	NS
AC	NS	NS	NS	NS	0.068	NS	NS
BC	NS	NS	NS	NS	NS	NS	NS
ABC	0.044	NS	0.014	0.007	NS	NS	NS

[1] Values are means ± SE. Due to limited sample amounts, the number of replicates in each group are as follows: 10 Cellulose, n = 5;10 RS, n = 6; 10 Cellulose + phy, n = 5; 10 RS + phy, n = 5; 30 Cellulose, n = 6; 30 RS, n = 5; 30 Cellulose + phy, n = 6; 30 RS + phy, n = 4. Means in a column not sharing a superscript letter are significantly different ($p < 0.05$, Tukey's multiple range test) .

[2] *NS* not significant. P values are shown when < 0.05

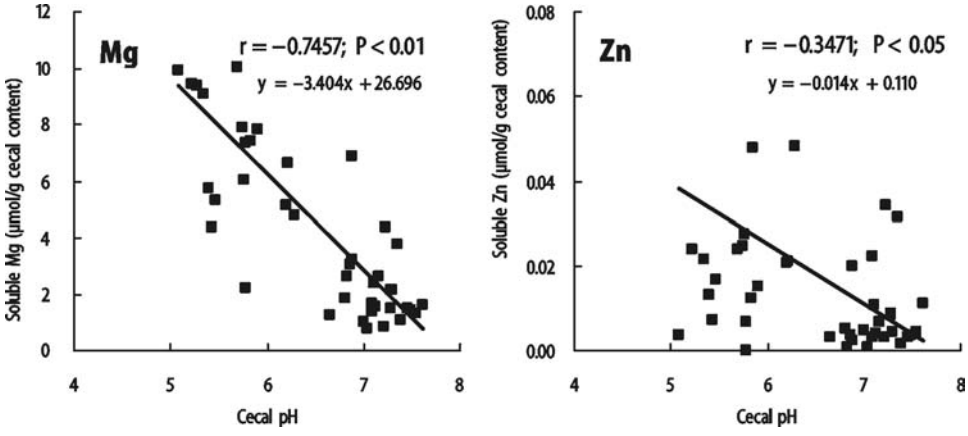

Fig. 2 Correlations between pH and mineral (Mg and Zn) solubility in the cecum of rats fed either phytate-free or phytate-containing diets, containing different dietary fibers (cellulose or RS) and zinc levels (10 or 30 mg/kg).

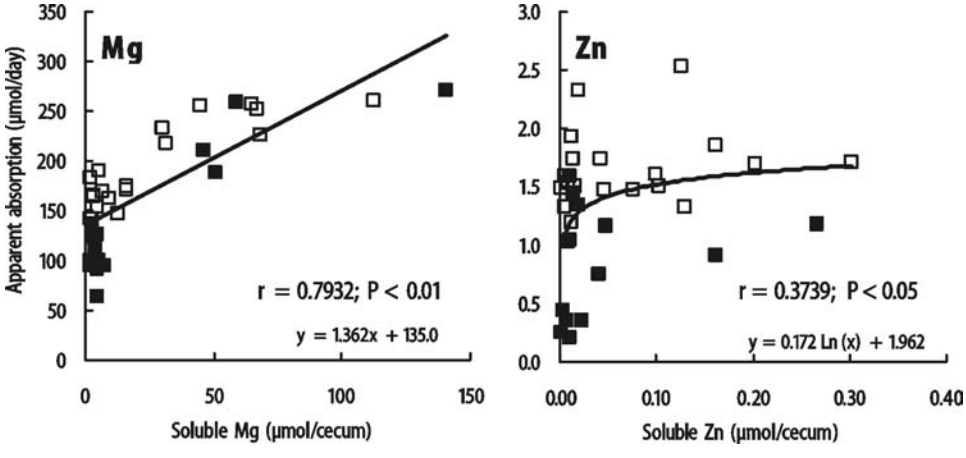

Fig. 3 Correlations between the soluble Mg and Zn pools and the apparent absorption of these minerals in rats fed either phytate-free (□) or phytate-containing diets (■), containing different dietary fibers (cellulose or RS) and zinc levels (10 or 30 mg/kg).

effect of RS on zinc bioavailability. In the present study, we investigated: 1) the possible increase of the cecal total zinc pool size by increasing the dietary zinc level or by adding phytic acid to the diet and, 2) the implications of the cecal zinc pool size on the enhancement of zinc bioavailability by RS. Zinc retention in the femur was used to assess zinc bioavailability.

Dietary zinc level and cecal zinc pools

This experiment showed that an increment of dietary zinc from 10 to 30 mg/kg increased the cecal total zinc pools of rats. In agreement with that, RS increased zinc retention in rats receiving phytate-free diets only at the 30 mg/kg dietary zinc level. Similarly, López et al. [5] observed an increase of zinc apparent absorption by RS at the 50 mg/kg dietary zinc level (regardless of the presence or the absence of dietary phytate). However, a separate study by Hara et al. [18], using a highly fermentable fiber (guar gum hydrolysate), at the 10 mg/kg dietary zinc level did not detect any enhancement of zinc absorption, in spite of the low cecal pH. We suggest that the enhancement of zinc absorption by a fermentable fiber is modulated by dietary zinc level, probably through the changes caused in the cecal total zinc pool size.

Dietary phytic acid and cecal zinc pools

The addition of sodium phytate to the diets containing 10 mgZn/kg reduced growth, feed intake, feed efficiency and zinc concentrations in the femur, serum and testis, and tended to enlarge total cecal zinc pools. Phytic acid reduces solubility mainly in the small intestine [15], the main site of zinc uptake. Thus, at the 10 mg/kg dietary zinc level, dietary phytic acid may have increased the total cecal zinc pools by inhibiting the absorption of this element in the small intestine. Supporting that postulation, our results showed that, at the 10 mg/kg dietary zinc level, RS increased zinc retention in the femur only when phytic acid was included in the diet (i. e., when cecal total zinc pools tended to be larger). Similarly, Hara et al. [18] showed that the cecum and colon do not show sufficient zinc absorptive efficiency, but were able to compensate for the impaired zinc absorption in the upper GI tract induced by omeprazole, a proton pump inhibitor that suppresses gastric acid secretion. At the 30 mg/kg dietary zinc level, however, RS increased zinc retention in the femur independently of dietary phytic acid. At this dietary zinc level, zinc absorption ratio is lower and the total cecal zinc pools are larger than at 10 mgZn/kg. We assumed that, at the 30 mg/kg dietary zinc level, phytic acid did not affect the zinc uptake and the total cecal zinc pool size as much as when dietary zinc was at 10 mg/kg. In fact, the addition of sodium phytate to the diet containing 30 mgZn/kg reduced zinc concentration only in the femur, which is considered to be a low-priority tissue and one of the first tissues affected by the onset of zinc deficiency [19, 20].

Cecal fermentation of RS and its effects on zinc and magnesium absorption

Feeding RS markedly increased cecal concentrations of succinic acid and SCFA. The strong and inverse correlation between SCFA + succinic acid concentrations and the cecal pH ($r = -0.8536$; $n = 48$; $P < 0.01$) reinforces the existence of pH-lowering effects of both succinic acid and SCFA, as previously reported [10].

Despite limited information about the effects of RS fermentation on zinc bioavailability, the increase in cecal solubility and absorption of magnesium has been observed in a number of studies with rats [6–8]. Our results also support the favorable absorption of magnesium when cecal pH is lowered. There were strong correlations between cecal pH and soluble magnesium concentration (Fig. 2; $r = -0.7457$; $P < 0.01$) and between the cecal soluble magnesium pool and apparent magnesium absorption (Fig. 3, $r = 0.8307$; $P < 0.01$). These results strongly suggest that a large ratio of magnesium is absorbed in the cecum of rats; thus, the increase in magnesium solubility in the cecum directly enhances the absorption of this element. In agreement with our results, there is much evidence that magnesium absorption takes place mainly in the colon and in the distal small intestine [21, 22]. In contrast, zinc absorption is believed to occur chiefly in the small intestine [18, 23]. Thus, the significant, but weak, correlation between the cecal pH, cecal zinc solubility and zinc apparent absorption is consonant with the smaller ratio of zinc being absorbed in the cecum.

The mechanisms of magnesium uptake in the intestinal tract involve passive diffusion, solvent drag and carrier-mediated transport. However, at adequate magnesium intakes, most of the magnesium is absorbed by passive diffusion and solvent drag [21]. As a result, the concentration of soluble magnesium at the luminal site is the major factor controlling magnesium absorption [21]. In our experiment, the soluble magnesium pools in the cecum were directly and linearly related to magnesium apparent absorption (Fig. 3; $r = 0.7932$; $P < 0.01$), in agreement with the previous explanation of a predominantly passive mechanism of magnesium absorption. In contrast with magnesium, zinc absorption takes place mostly in the small intestine and by a carrier-mediated process, which is also likely to be the chief mechanism of zinc uptake in the colon [23]. Consonant with that, the cecal pools of soluble zinc were correlated to zinc apparent absorption by a natural logarithmic curve (Fig. 3; $r = 0.3739$; $P < 0.05$). However, the low correlation coeffi-

cient does not allow a definite confirmation of the passive, carrier-mediated, nature of zinc absorption in the cecum.

RS was able to increase zinc retention in rats fed phytate-containing diets. This suggests that the decrease in cecal pH by RS could increase the solubility of highly insoluble zinc complexes, like the ones formed with phytic acid.

Apart from the decrease in cecal pH, fermentation may result in enhanced phytate hydrolysis, owing to microbial phytase [24]. We did not measure microbial phytase activity or the degree of phytate degradation in this experiment. However, it is possible that phytate degradation by microbial phytase took place in the cecum, releasing part of the minerals bound to phytate.

Femur zinc concentration as an indicator of zinc retention in the body

A strong correlation between zinc apparent absorption and the concentration of zinc in the femur (Fig. 1; $r = 0.8229$, $n = 38$; $P < 0.01$) was observed in the present study. A separate study by Larsen and Sandström [25] also showed a significant ($P < 0.001$) correlation between zinc absorption and femur zinc concentrations. Femur zinc is known as a very sensitive indicator of zinc status of rats [19, 20], reflecting the total body zinc concentration [26]. The apparent absorption of minerals could not be obtained from some animals, which had loose stools during the period of feces collection, thus reducing the power of this parameter for the assessment of zinc bioavailability in this study. Moreover, the apparent absorption is calculated during a limited period (four days), while femur zinc concentrations reflect the changes occurred during the whole experimental period.

Hara et al. [18] reported a linear increase of femur zinc concentrations in rats fed increasing levels of zinc, in phytate-free diets. Based on that relation, we estimated that phytate-containing diets with 10 mg Zn/kg corresponded to a phytate-free diet containing 4 mg Zn/kg, when the fiber source was cellulose. Feeding RS under the same conditions increased femur zinc concentration to 93.7 μg/g, which is a concentration expected for rats fed 8 mg Zn/kg diet.

In conclusion, the increase of zinc bioavailability by RS occurs when dietary zinc levels are adequate and/or zinc absorption is impaired in the small intestine, increasing the influx of unabsorbed zinc in the cecum. A low dietary supply of zinc and its effective absorption in the small intestine may restrict the amount of zinc reaching the cecum, thus limiting the enhancement of zinc uptake when cecal pH is lowered by RS fermentation. Moreover, the effects of the decreases in pH on zinc solubility and absorption are not as strong as on magnesium.

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