



Title	Intestinal absorption of zinc is promoted by low-level intake but inhibited by high-level intake of corn husk fiber in rats
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i) INTESTINAL ABSORPTION OF ZINC IS PROMOTED BY LOW-LEVEL INTAKE BUT
INHIBITED BY HIGH-LEVEL INTAKE OF CORN HUSK FIBER IN RATS

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iii) Abstract

We examined the effects of feeding several types of insoluble dietary fiber (100 g/kg diet), including cellulose (control), wheat bran (demineralized), sugar-beet fiber and corn husk fiber, on apparent zinc absorption in rats. The zinc absorption was higher after intake of a zinc-deficient diet (0.229 mmol/kg diet) containing corn husk fiber than that after intake of a cellulose-containing diet for 3 wk. Ingestion of wheat bran or sugar-beet fiber did not influence zinc absorption. We compared the effects of cellulose and demineralized- and washed-corn husk fiber at graded levels in the diets (1, 2 and 3 parts to 10 basal diet). The apparent zinc absorption in rats fed the diet with the lowest level of corn fiber was higher than that in rats fed a diet with the same level of cellulose or a fiber-free diet (control). In rats fed the diets with higher levels of corn fiber, the zinc absorption was lower than that in rats fed either the diet with the lowest level of cellulose or the fiber-free diet in the period day 9–12, however, reduced absorption that occurred upon intake of the high-fiber diet recovered in the period day 18–21. Cecal fermentation and the bulking action of corn husk fiber may not be responsible for the zinc absorption-promoting effect of the dietary fiber. In conclusion, corn husk fiber is effective to increase zinc absorption when ingested at low levels, but absorption is inhibited upon intake at higher levels in rats.

iv) Key words: zinc absorption, insoluble dietary fiber, corn husk fiber, rats

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INTRODUCTION

Zinc is essential for many physiological functions, and severe and marginal deficiencies of zinc have been defined in some people, especially in developing countries (Giugliano and Millward 1984). Even in advanced nations, zinc status during gestation was found to be suboptimal in some pregnant women (Hambidge et al. 1983) and mild zinc deficiency is a factor in nutritional failure in infants (Walravens et al. 1989). Also, in elderly subjects, mild zinc deficiency has been shown to lead to a reduced zinc concentration in immune cells (Prasad et al. 1993).

It has been reported in many studies that ingestion of insoluble dietary fiber reduces zinc absorption (Munoz 1986, Reinhold 1976). In this regard, some impurities in fiber materials, for example, phytic acid, are known to be responsible for the decrease in zinc absorption that occurs as a result of intake of dietary fiber (Hall 1989, Larsson 1996), however, the effects of the insoluble fiber itself on zinc absorption remain to be fully clarified. Our previous studies showed that cecal fermentation of dietary fiber results in an increase in calcium and magnesium absorption in the large intestine (Hara et al. 1996, Ohta et al. 1997). Intake of a fermentable and water-soluble type of fiber did not enhance zinc absorption, although the large intestine contributes to the improvement of zinc status (Hara et al. 2000).

The aims of the present study were to examine the effects of intake of widely used insoluble fiber sources with different properties on zinc absorption and to examine the contribution of cecal fermentation to the effects of insoluble dietary fiber in rats. In experiment 1, we found that apparent zinc absorption tended to be higher in rats fed a corn husk fiber-containing diet than that in rats fed a cellulose-containing diet. We further evaluated the effects of corn husk fiber intake on zinc absorption in experiment 2.

MATERIALS AND METHODS

Diets and animals

The stock diet and all test diets were sucrose-based semipurified diets containing 150 g of powdered egg white (Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) /kg diet. The basal diet used in experiments 1 and 2 contained 0.229 mmol zinc/kg diet as zinc carbonate (28.4 mg/kg diet). The stock diet for acclimation was supplemented with zinc carbonate at levels up to 66.2 mg/kg diet [35 mg (0.535 mmol) zinc/kg diet]. The mineral mixture was prepared as established by the AIN-76 Workshop held in 1989 (Reeves 1989) without zinc. The vitamin mixture was prepared in accordance with the AIN-76 mixture (AIN 1977) except that D-biotin. D-Biotin was added at 8.19 μ mol/kg diet to prevent its deficiency. Fiber sources were added to the basal diet at the expense of

the whole diet (100 g/kg diet) in experiment 1. In experiment 2, to be similar intakes in fiber-free portion in each test diet among the groups as increasing fiber sources added, test diets were prepared with adding fiber sources 1, 2 and 3 parts to 10 basal diet. Fiber sources used in experiment 1 were cellulose (Solka floc, Culter Food Science, Tokyo, Japan), wheat bran (Nissin Seihun Co., Tokyo, Japan), sugar-beet fiber (Nippon Sugar Beet Manufacturing Co., Obihiro, Japan) and corn husk fiber (Cellfer #200, Nihon Shokuhin Kako Co. Ltd., Tokyo, Japan). The wheat bran used in experiment 1 and the corn husk fiber used in experiment 2 were demineralized by treatment with 0.1 mol/l HCl (1 kg material/ 5l) three times, shaking for 1 h each time, and washed with deionized water until the pH of the washout solution reached pH 5.

Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), weighing about 50 g, were given free access to deionized water and the stock diet for 7 days to acclimate the animals. The rats were divided into several groups by a randomized block design based on body weight for two separate experiments after acclimation. The rats were housed individually in stainless-steel cages with mesh bottoms. The cages were placed in a room with controlled temperature (22-24 °C), relative humidity (40-60%) and lighting (lights on: 0800 - 2000 h). In both of the experiments, the rats were allowed free access to the assigned test diet and deionized water for 3 wk. Body weight and food intake were measured every day.

Feces were collected for 3 consecutive days during the period day 9–12 (exp. 2) or day 18–21 (exp. 1 and 2) to evaluate apparent zinc absorption and zinc intake and excretion into feces. The collected feces were freeze-dried.

At the end of the experiments, the rats were killed by withdrawal of aortic blood while under anesthesia with Nembutal (pentobarbital sodium, 50 mg/kg body wt; Abbott, Chicago, IL), and then the cecum and colon were removed without loss of their contents. The contents were collected, frozen immediately with liquid nitrogen, and stored at -40°C until subsequent analyses.

The study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Analyses

Freeze-dried feces were milled. Powdered feces were wet-ashed with a nitric acid (10 mol/l) and perchloric acid (2.3 mol/l) mixture, and zinc concentrations in these solutions were measured by atomic absorption spectrometry (AA-6400F, Shimadzu, Kyoto, Japan) after adequate dilution with water.

The cecal and colonic contents diluted with 9 volumes of deionized water were homogenized by means of a Teflon homogenizer. The total zinc in each of the homogenates was measured after the samples had been wet-ashed in the same way as in the case of the feces. Soluble zinc was assayed in the supernatant obtained upon centrifugation (30,000 \times g for 20 min) of the homogenate. Concentrations of short-chain fatty acids (SCFA) in the homogenate of the cecal contents were evaluated by the previously described method (Hara et al. 1994). Acetic, propionic and butyric acid concentrations were measured by gas-liquid chromatography [Shimadzu GC-14A with a prepacked glass column [1600 mm x 3 mm, SP-1220 + H₃PO₄ (15% + 1%) on 80-100 mesh Chromosorb W-AW DMCS, Shimadzu Corporation, Kyoto, Japan] after adding phosphoric acid (final concentration 0.67 mol/l)].

Fiber and zinc content and settling volume (Takeda and Kiriyama 1979) of the fiber materials used in both experiments were analyzed. Total dietary fiber was measured by an enzyme-gravimetric method (TDF-100A kit, Sigma Chemical Co., St. Louis, MO).

Calculations and Statistical Analyses:

Calculations were performed as follows:

$$\text{Apparent zinc absorption } (\mu\text{mol} / 3 \text{ days}) = \text{total zinc intake} - \text{fecal zinc excretion} \quad (1)$$

The results of experiment 1 were analyzed by one-way analysis of variance (ANOVA). The effects of fiber source and fiber level were analyzed by two-way ANOVA within 6 fiber-fed groups, and the results were also analyzed by one-way ANOVA in 7 groups including the fiber-free diet group (exp. 2). Duncan's multiple range test was used to determine whether mean values were significantly different ($P < 0.05$). These statistical analyses were done by the GLM procedure of SAS (SAS version 6.07, SAS Institute Inc., Cary, NC).

RESULTS

The total dietary fiber content in the case of all fiber materials was more than 75%, and the fiber content of the cellulose and demineralized corn husk fiber materials used in experiment 2 was very high (Table 1). The settling volume of each material was rather low and was similar among the types of fiber materials used.

Body weight gain and food intake during the 3-wk period of feeding the four fiber-containing diets did not differ among the diet groups in experiment 1 (Table 2). The dry weight of feces excreted during the last 3 days of the experimental period (fecal output) was higher in the cellulose and corn husk fiber groups than in the other two fiber groups. Apparent zinc absorption in rats fed the corn husk fiber was higher than in rats fed the test diet containing cellulose on the

last 3 days of the 3 wk test period (Table 3). Zinc intake did not differ among the diet groups.

The wet weights of the cecal and colonic contents were higher in the sugar-beet fiber group than in the cellulose and wheat bran groups, respectively (Table 4). The pH of the cecal contents was lower in the sugar-beet fiber group than in the cellulose and corn fiber groups. The short-chain fatty acid (SCFA, sum of acetic, propionic and butyric acids) pool in the cecum were higher in rats fed the sugar-beet fiber than in rats fed the other types of fiber .

In experiment 2, body weight gain in the 3-wk period did not differ among the rats fed cellulose or corn husk fiber at various levels (Table 5). Comparing the cellulose and corn husk fiber groups, the food intake values did not differ, however, the food intake was greater in the fiber-fed groups than in the fiber-free group. The food intake values in the case of rats fed higher levels of fiber were greater. Intake of the fiber-free portion of each of the test diets (basal values in Table 5, which were calculated from whole intake and fiber content) was similar among all of the groups. The fecal output for the 3-day period starting on day 9 and that starting on day 18 changed in a manner similar to that observed in the case of whole food intake.

Apparent zinc absorption was higher in the group fed the lowest level of corn fiber than that in the group fed the lowest level of cellulose or the fiber-free diet group in both the first and second periods (Fig. 1). In the first period, among the rats fed corn husk fiber, zinc absorption in the groups fed the higher levels was less than that in the group fed the lowest fiber level, and among the rats fed cellulose, the absorption in the group fed the highest fiber level was less than that in the lower level groups. In the second period, the absorption in the groups fed the higher levels of fiber was similar to that in the group fed the lowest level of cellulose and the fiber-free group, except for the group fed the highest level of cellulose. Zinc absorption in the group fed the highest level of cellulose was greater than that in the group fed the lowest level of cellulose.

The wet weight of the cecal contents, but not that of the colonic contents, was higher in the groups fed the corn husk diets than in the groups fed the cellulose diets at all fiber levels (Table 6). As shown by the results of two-way ANOVA, the pH of both the cecal and colonic contents was influenced by the fiber source and fiber level, that is, ingestion of corn husk fiber somewhat reduced the pH of the large intestinal contents. The soluble zinc pool was not influenced by the fiber source or the fiber level. The total zinc pool, but not the soluble pool, in the case of the group fed the fiber-free diet was extremely high compared to that of the other groups. The cecal SCFA concentration was not influenced by the fiber source or the fiber level (data not shown).

DISCUSSION

Apparent zinc absorption was higher in rats fed a diet containing a low level corn husk fiber than in rats fed a cellulose containing diet. The corn husk fiber material used in experiment 1 contained a small amount of zinc (Table 1) and possibly some low molecular weight impurities, however, that used in experiment 2 was demineralized and washed as described in Materials and Methods. Ingestion of the demineralized corn husk fiber also increased the apparent zinc absorption (Fig. 1). It is known that low molecular weight compounds, for example, phytic acid (Davies and Nightingale 1975), and chelators such as amino acids (Hempe and Cousins 1989, Wapnir and Stiel 1986), influence zinc absorption. The demineralization procedure has been shown to remove phytate from corn fiber material, if present (Rubio et al. 1994), thus it seems likely that the corn fiber material used in experiment 2 did not contain such impurities that enhance or inhibit zinc absorption. The corn husk fiber itself is probably responsible for the increase in zinc absorption observed.

There are a few possible mechanisms by which enhancement of zinc absorption occurs upon intake of corn husk fiber. The first possibility is that cecal fermentation of the fiber may promote mineral absorption. Cecal fermentation of dietary fiber has been shown to increase calcium and magnesium absorption (Hara et al. 1996, Trinidad et al. 1996, Younes et al. 1996). Fermentation of indigestible substances may solubilize zinc by lowering the pH and/or may serve to release chelated zinc from hemicellulose fractions through their degradation (Mod et al. 1981). It is not likely that both mechanisms are involved in enhancement of zinc absorption upon intake of corn fiber because the level of soluble zinc was not increased in the groups fed the low level of corn husk fiber diet, as shown in Table 6. We previously observed that cecal fermentation of ingested guar gum hydrolysate did not enhance zinc absorption (Hara et al. 2000). However, Lopez et al. (1998) reported that ingestion of resistant starch increased cecal zinc absorption. Intake of a high level of sugar-beet fiber, which is a highly fermentable type of fiber (Takahashi et al. 1994), has been shown to increase zinc absorption in rats (Fairweather-Tait and Wright 1990). In the present study, ingestion of sugar-beet fiber did not affect zinc absorption (Table 3), in spite of the greater pool of cecal SCFA and the low cecal pH in the sugar-beet fiber group. A greater extent of cecal fermentation may be necessary to increase zinc absorption. The results of experiment 1 show that corn husk fiber is a poorly fermentable type of fiber because cecal SCFA levels were low and fecal output was high in the corn fiber group as well as in the cellulose group. This was observed in the case of increasing level of these fibers (Table 5). The fecal output in the

corn husk groups was similar to that in the cellulose groups at all fiber levels. These results indicate that cecal fermentation does not contribute to the increase in zinc absorption observed upon intake of corn husk fiber.

The second possible factor affecting zinc absorption is the bulking action of the dietary fiber in the intestinal lumen. In general, the bulking effect is largely involved in the physiological actions of insoluble fiber. The settling volume, an indication of the bulking effect, of corn husk fiber was similar to that of cellulose and the other types of fiber used in experiment 1. This suggests that the bulking effect does not contribute to the promotion of zinc absorption by corn husk fiber.

The third possible mechanism by which zinc absorption is modified may involve the ion-exchange properties or chelating activity of dietary fiber, responsible for the binding of zinc to the fiber. Cation binding has been considered to be the mechanism by which dietary fiber inhibits intestinal absorption of minerals (Bagheri et al. 1985, Reinhold et al. 1981). Uronic acid residues as structural components of dietary fiber are mainly responsible for the ion-exchange or chelating properties. However, we previously found that phosphorylated dietary fiber, which also binds cations, enhanced intestinal calcium absorption in the small intestine (Watanabe et al. 2000). Cation binding by the fiber might not always inhibit mineral absorption. Corn husk fiber contains a relatively high proportion of uronic acid residues (Theander and Westerlund 1986). We speculate that zinc ions released from zinc carbonate by gastric acid remain bound to corn fiber without precipitation as insoluble salts, and the zinc bound to the fiber is absorbed after passing into the small intestine.

In experiment 2, we observed the effects of diets containing three levels of fiber on zinc absorption (Fig. 1). At the lowest level, zinc absorption was higher in the corn husk fiber group than in the cellulose group in both the first and second periods. However, zinc absorption decreased with increasing levels of corn husk fiber. The results show that high-level intake of fiber reduces zinc absorption, in agreement with previous studies (Donangelo and Eggum 1986, Ismail-Beigi et al. 1977). The decrease in zinc absorption observed after intake of a high fiber diet may not be due to the effects of impurities, for example, phytic acid, but due to the effects of the fiber itself. Corn fiber affects zinc absorption as a promoter and as an inhibitor. Hansen et al. (1996) reported that casein phosphopeptides, which have ion-exchange properties due to the presence of phosphoserine residues in their structure, enhanced zinc absorption when ingested at a low level, however, this material inhibited zinc absorption at higher levels. The dual effects of casein

phosphopeptides on zinc absorption are analogous to the effects of corn husk fiber shown in the present study. This suggests that the ion-exchange or chelating action of corn husk fiber is associated with not only the promotive but also the inhibitory effects of this fiber material.

In the second period, recovery from the decreased zinc absorption caused by intake of the higher levels of corn husk fiber or cellulose was observed. Adaptive induction of the zinc absorptive system is probably responsible for the recovery, suggesting that the rats fed diets with the higher levels of fiber were in a state of zinc deficiency in the mid-feeding stage. We previously estimated that 0.229 mmol zinc /kg diet, adopted in the present study, is near the minimum zinc requirement for normal rats (Hara et al 2000), as supported by the results of the present study. This also suggests that the increment in zinc absorption observed after corn husk fiber intake is physiologically relevant. Even so, body weight gain in the high corn fiber groups was not affected in spite of the decrease in zinc absorption to a level below that in the fiber-free control group. We previously showed that the zinc requirement for growth is considerably lower than 0.229 mmol zinc /kg diet (Hara et al. 2000). However, the immune system (Keen and Gershwin 1990) and antioxidant capacities (Bray and Bettger 1990) are defective under conditions of mild zinc deficiency.

In conclusion, a low level intake in corn husk increased zinc absorption, whereas, at higher levels intakes, zinc absorption was decreased. This may be the first report to show that intake of an insoluble dietary fiber increased zinc absorption independent of cecal fermentation.

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Table 1 Total dietary fiber and zinc, and settling volume of fiber materials used in experiments 1 and 2

	Total dietary fiber g / kg material	Zinc mmol / kg material	Settling volume ml / g material
Cellulose	910	0.004	4.9
Wheat bran			
(demineralized)	771	0.007	7.6
Sugar-beet fiber	816	0.231	9.9
Corn husk fiber	853	0.314	4.7
Corn husk fiber			
(demineralized)	896	0.012	6.1

Table 2 Body weight gain, food intake and fecal output of rats fed test diets containing four fiber sources ^{*,#}

	Body wt gain	Food intake	Fecal output
	g / days	g / days	dry g / 3 days
Cellulose	6.97	19.0	6.84 ^a
Wheat bran	7.17	18.7	5.13 ^b
Sugar-beet fiber	7.37	18.3	4.68 ^b
Corn husk fiber	7.35	19.4	6.80 ^a
Pooled SE	0.249	0.753	0.356
<i>P</i> -value	0.658	0.746	<0.001

*Values of body wt gain and food intake are means for 6 rats during an experimental feeding period of 21 days. Fecal output was measured for 3 days of the last feeding period.

#Values within a column not sharing a superscript letter are significantly different ($P<0.05$).

Table 3 Zinc intake, excretion into feces, and apparent zinc absorption in rats fed test diets containing four fiber sources at day 18-21 ^{*,#}.

	Zn intake μmol / 3 days	Zn excretion μmol / 3 days	Zn absorption μmol / 3 days
Cellulose	11.4	6.16	5.23 ^b
Wheat bran	12.0	6.11	5.92 ^{ab}
Sugar-beet fiber	12.4	6.05	5.94 ^{ab}
Corn husk fiber	12.8	6.13	6.70 ^a
Pooled SE	0.497	0.753	0.345
<i>P</i> -value	0.256	0.999	0.055

*Values are means for 6 rats measured for 3 days of the last feeding period.

#Values within a column not sharing a superscript letter are significantly different ($P<0.05$).

Table 4 Weight, pH, and concentration and pool of short-chain fatty (SCFA)in the cecal and colonic contents of rats fed test diets containing four different fiber sources for 3 wk^{*,#}

Fiber source	Wet weight of contents		pH of contents		Cecal SCFA
	Cecum	Colon	Cecum	Colon	Pool
	g / rats		μmol / g contents		μmol / contents
Cellulose	2.43 ^c	1.17 ^b	7.94 ^a	7.97 ^a	169 ^b
Wheat bran	2.35 ^c	1.17 ^b	7.45 ^{bc}	7.26 ^{bc}	220 ^b
Sugar-beet fiber	3.62 ^a	1.78 ^a	7.23 ^c	7.08 ^c	350 ^a
Corn husk fiber	3.10 ^b	1.32 ^{ab}	7.54 ^b	7.50 ^b	205 ^b
Pooled SE	0.137	0.177	0.074	0.119	17.8
ANOVA P-value	<0.001	0.075	<0.001	<0.001	<0.001

*Values are means for 6 rats.

#Values within a column not sharing a superscript letter are significantly different ($P<0.05$).

Table 5 Body weight gain, food intake and fecal output of rats fed a fiber-free diet or diets containing cellulose or corn fiber at various levels for 3 wk^{*,#}

Fiber source	Fiber level	Body wt gain	Food intake		Fecal output	
			Total	Basal (-fiber)	day 9–12	day 18–21
Fiber : basal diet g / days						
Cellulose	1:10	6.49	16.7 ^c	15.1	5.69 ^c	6.39 ^c
Corn husk fiber	1:10	6.54	16.5 ^c	15.0	5.49 ^c	5.91 ^c
Cellulose	2:10	6.78	17.9 ^b	14.9	10.5 ^b	11.2 ^b
Corn husk fiber	2:10	6.73	18.0 ^b	15.0	10.3 ^b	11.0 ^b
Cellulose	3:10	6.67	19.0 ^a	14.7	14.9 ^a	15.6 ^a
Corn husk fiber	3:10	6.97	19.0 ^a	14.6	14.3 ^a	16.4 ^a
Fiber-free	0:10	6.15	15.1 ^d	15.1	0.89 ^d	1.01 ^d
Pooled SE		0.175	0.229	0.180	0.334	0.321
1way ANOVA for 7 groups						
<i>P</i> -value		0.643	<0.001	0.574	<0.001	<0.001
2way ANOVA <i>P</i> -values for 6 fiber groups						
Fiber source (S)		0.465	0.707	0.672	0.270	0.823
Fiber level (L)		0.168	<0.001	0.068	<0.001	<0.001
S x L		0.552	0.837	0.792	0.832	0.185

*Values are means for 6 rats.

#Values within a column not sharing a superscript letter are significantly different (*P*<0.05).

Table 6 Weight and pH of the cecal and colonic contents, and the zinc pool in the cecal contents of rats fed a fiber-free diet or diets containing cellulose or corn husk fiber at various levels for 3 wk^{*,#}

Fiber source	Fiber level	Weight of contents		pH of contents		Cecal Zn pool	
		Cecum	Colon	Cecum	Colon	Total	Soluble
Fiber : basal diet		g / rat				nmol / contents	
Cellulose	1:10	2.40 ^d	1.30 ^{bc}	8.15	7.74 ^b	704 ^{bc}	37.3 ^{bc}
Corn husk fiber	1:10	2.96 ^{bc}	1.27 ^c	7.84	7.55 ^{bc}	827 ^b	34.9 ^{bc}
Cellulose	2:10	2.38 ^d	1.82 ^a	7.80	7.49 ^{bc}	383 ^{cd}	47.3 ^{ab}
Corn husk fiber	2:10	3.54 ^a	1.67 ^{abc}	7.40	7.13 ^c	636 ^{bcd}	62.8 ^a
Cellulose	3:10	2.75 ^{cd}	1.47 ^{abc}	7.70	7.49 ^{bc}	366 ^d	41.5 ^{bc}
Corn husk fiber	3:10	3.39 ^{ab}	1.75 ^{ab}	7.57	7.08 ^c	447 ^d	50.0 ^{ab}
Fiber-free	0:10	1.83 ^e	0.246 ^d	7.99	8.49 ^a	2470 ^a	30.2 ^c
Pooled SE		0.117	0.176	0.159	0.153	103	5.76
1way ANOVA for 7 groups							
<i>P</i> -value		<0.001		0.221		<0.001	
2way ANOVA <i>P</i> -values for 6 fiber groups							
Fiber source (S)		<0.001		0.823		0.014	
Fiber level (L)		0.075		0.011		<0.001	
S x L		0.172		0.559		0.607	

*Values are means for 6 rats

[#]Values within a column not sharing a superscript letter are significantly different ($P < 0.05$).

Figure legend

Fig. 1 Apparent zinc absorption in rats fed graded levels of cellulose and demineralized corn husk fiber and in rats fed a fiber-free diet, in mid (day 9–12, UPPER panel) and final (day 18–21, LOWER panel) periods within a 3-wk period of feeding the test diets (experiment 2). Each bar shown is the mean for six rats. Pooled SEs are shown. *P* values estimated by two-way ANOVA for 6 fiber-fed groups were 0.645 and 0.196 for fiber source (S), <0.001 and 0.209 for fiber level (L) and <0.001 for S × L during the mid and final period, respectively. Values not sharing a letter are significantly different between diet groups for 7 groups, as determined after one-way ANOVA for 7 groups (*P*<0.05).

