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# VARYING DIETARY CONCENTRATIONS OF FRUCTOOLIGOSACCHARIDES AFFECT APPARENT ABSORPTION AND BALANCE OF MINERALS IN GROWING RATS

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## ABSTRACT

Carbohydrates that bypass digestion in the small intestine and are fermented in the large intestine may affect the absorption of certain minerals. A study was conducted to determine the effect of varying dietary concentrations of fructooligosaccharides (FOS) on the apparent digestibility and balance of certain macro and trace minerals. Forty growing male Sprague-Dawley rats were assigned one of four treatments: 1) purified diet (control); 2) purified diet + 1% FOS; 3) purified diet + 3% FOS; or 4) purified diet + 5% FOS. Rats were acclimated to their metabolic cages for 5 d, after which they were subjected to a 10-d growth period, followed by a 7-d balance period in which diets were restricted to 90% *ad libitum* intake of the lowest consuming rat. Cecal pH decreased ($P < 0.01$) as dietary FOS concentration increased, reflecting an increase in cecal short-chain fatty acid concentration. In addition, FOS supplementation increased ($P < 0.01$) fecal N excretion, although N balance was similar among groups. Apparent absorption of Mg increased linearly ($P < 0.01$) as FOS concentration increased in the diet; however, Mg balance (mg/d) was similar ($P > 0.10$) across treatments. The macrominerals (Ca, P, Mg, Na, Cl, K) and trace minerals (Cu, Fe, Mn, Zn) were in a positive balance for all experimental treatments. However, Cu absorption decreased with increasing FOS concentration. This may be explained by an increase in fecal microbial mass which would contain a relatively significant amount of Cu or by an increase in hepatic bile Cu excretion. These results suggest that dietary FOS alters acute Cu metabolism in growing rats.
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KEY WORDS: Rat, Fructooligosaccharides, Apparent Mineral Digestion and Balance

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## INTRODUCTION

Over the past decade, the role of dietary fiber in a "healthy" diet has come into fruition. Certain dietary fibers have been suggested to: provide bulk to the stool, decrease intestinal transit time (e.g., relieve constipation), attenuate glycemic response, improve cholesterol and lipid metabolism, and reduce the risk of colon cancer. The Life Sciences Research Office, Federation of the American Societies for Experimental Biology (1) recommends that we consume between 20 to 35 g of total dietary fiber daily of which 70 to 75% should be insoluble and 25 to 30%, soluble. According to Cummings (2), the total amount of carbohydrate available for fermentation in the colon ranges from 21 to 76 g/d. In order to supply the carbohydrate to the colon, as recommended in a healthy diet, dietary fiber must be incorporated into the diet. Many fibrous food ingredients that serve as a source of fermentable carbohydrate (e.g., dietary fiber) in prepared foods are not very palatable. Therefore, novel fiber ingredients should be identified to meet consumers' needs. One such ingredient is fructooligosaccharides [FOS; a mixture of 1-kestose, nystose, and $1^F$-β-fructofuranosyl nystose], which are indigestible by human enzymes in the small intestine but extensively fermented almost exclusively by bifidobacteria that inhabit the large bowel (3, 4). Using healthy human subjects fed 20.1 g FOS/d, Molis et al. (5) determined that the FOS reaching the colon was completely fermented by the colonic microflora.

It has been reported that the large bowel (cecum and colon) plays a role in mineral absorption. Ohta et al. (6, 7) found that FOS supplemented at 5% of the diet increased the absorption of Ca and Mg in normal rats; however, FOS increased the absorption of Mg but not Ca in cecectomized rats. In a Mg-deficient rat model, Ohta et al. (8) found a significant increase in the apparent absorption of Mg, but not Ca or P, for animals fed diets containing 5% FOS. Furthermore, in a Fe-deficient, anemic rat model (9), dietary supplementation with 5% FOS improved absorption of Fe, Ca, and Mg. It is not known if lower dietary concentrations of FOS have similar beneficial effects. The present study was conducted to evaluate the effect of FOS incorporated at several dietary concentrations (0, 1, 3 and 5 g/100 g) on apparent mineral digestion and balance in growing rats. The primary objective was to determine the dose response effect of FOS on apparent digestion and balance of Ca, P, Mg, Na, Cl, K, Cu, Fe, Mn and Zn.

## MATERIALS AND METHODS

### Animals

Forty growing male Sprague-Dawley rats (5 wk old; 104 ± 4.4 g, mean ± SD) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Rats were individually housed in metabolic nalgene-stainless steel cages in an environmentally controlled room (25°C and 12:12 light-dark cycle) and allowed free access to double distilled, deionized water. To avoid mineral contamination, all materials (e.g., cages, collection devices, etc.) were washed with double distilled, deionized water daily. The animal use protocol was reviewed and approved by The Ohio State University Animal Care Committee.

*Diets*

Rats were assigned among one of four treatments (10 rats/treatment). Treatments included: 1) purified diet (control; AIN-93G powder); 2) purified diet + 1% FOS; 3) purified diet + 3% FOS; or 4) purified diet + 5% FOS. Fructooligosaccharides were added to replace sucrose in the control diet. The overall composition of the experimental diets are shown in Table 1. Diets were manufactured by Harlan Teklad (Madison, WI). Fructooligosaccharides were obtained in powder form, > 95% FOS (Golden Technologies Company, Inc., Westminster, CO).

TABLE 1

Composition of Experimental Diets (g/kg)

| | Dietary concentration of FOS*, % | | | |
|---|---|---|---|---|
| Ingredient | 0 | 1 | 3 | 5 |
| Casein | 200 | 200 | 200 | 200 |
| L-cystine | 3 | 3 | 3 | 3 |
| Corn starch | 397.486 | 397.486 | 397.486 | 397.486 |
| Maltodextrin | 132 | 132 | 132 | 132 |
| Sucrose | 100 | 90 | 70 | 50 |
| Soybean oil | 70 | 70 | 70 | 70 |
| Cellulose | 50 | 50 | 50 | 50 |
| Mineral mix (AIN-93G-MX) | 35 | 35 | 35 | 35 |
| Vitamin mix (AIN-93-VX) | 10 | 10 | 10 | 10 |
| Choline bitartrate | 2.5 | 2.5 | 2.5 | 2.5 |
| Tert-butylhydroquinone | 0.014 | 0.014 | 0.014 | 0.014 |
| FOS* | 0 | 10 | 30 | 50 |

*FOS = fructooligosaccharides.

*Experimental design*

The duration of the study was 27 d, during which rats had free access to double distilled, deionized water. Feed intake, water intake, and body weight was determined daily throughout the experiment. Rats were acclimated to their metabolic cages for 5 d. During this time they had free access to the control diet. On d 6, animals were weighed and assigned to dietary treatments such that each group had a similar mean initial body weight and weight distribution. During d 6 through 17, rats were given free access to their treatment diets. During d 18 through 27, rats received diets at 90% of the *ad libitum* intake of the lowest consuming rat in the previous phase

(average of d 13, 14 and 15). This was conducted to insure dietary intake would be similar among rats. Total feces and urine were collected daily (d 20 through 27; balance phase), stored in a refrigerator and pooled separately among individual animals for analysis. Urine was collected in plastic bottles containing 1 mL of 2 g/L sulfuric acid, to minimize bacterial growth and ammonia loss. Feed, feces and urine were analyzed for dry matter (except urine), Kjeldahl N (10), and mineral composition. Apparent digestibilities of dry matter, N and minerals and balance of N and minerals were calculated. On d 27, rats were killed by carbon dioxide asphyxiation. Cecal contents were immediately removed and pH measured by electrode. Sample pH was determined on wet cecal contents following the preparation of a 1:5 dilution with double distilled, deionized water.

*Mineral analysis*

Minerals (Ca, P, Mg, Na, K, Cu, Fe, Mn, Zn) were analyzed using an Inductively Coupled Plasma (ICP) spectrometry method (The Ohio State University Research-Extension Analytical Laboratory, Ohio Agricultural Research and Development Center, Wooster, OH) after dry ashing according to AOAC (10). Urine was analyzed directly on ICP. Chloride was determined by a titration method (11). Trace minerals (Cu, Fe, Mn, Zn) in feed samples only were analyzed by ICP at Ross Products Division (Columbus, OH). Apparent mineral absorption and balance were calculated by the following formulas:

Apparent mineral absorption, % =
[(mineral intake - fecal mineral excretion) / mineral intake] x 100%

Apparent mineral balance, unit/d =
 (mineral intake - fecal mineral excretion) - urinary mineral excretion

Apparent mineral balance, % absorption =
[apparent mineral balance in unit·d$^{-1}$ / (apparent mineral absorption x mineral intake)] x 100%

Apparent mineral balance data are also presented as a percent of absorption. This expression allows the evaluation of the rat's response to the absorbed mineral (e.g., if the apparent intestinal absorption is low, does the body respond by decreasing urinary excretion?).

*Statistical methods*

All data were analyzed as a completely randomized design by analysis of variance using the General Linear Models procedure of SAS (12). Treatment effects were tested using the following contrasts: 1) linear, 2) quadratic, and 3) control versus the average of 1, 3, and 5% FOS. Coefficients for the linear and quadratic contrasts were adjusted for unequally spaced treatments according to Steel and Torrie (13).

# RESULTS

*Dietary composition*

To verify the level of FOS in dietary treatments, diet samples were collected and sent to the University of Illinois (Champaign-Urbana) for analysis using ion chromatography (14). Percentage FOS for treatment 1, 2, 3, and 4 were 0, 0.86, 3.30, and 4.93, respectively. These values conform well with the target values of 0, 1, 3, and 5%. Dietary N and mineral compositions are presented in Table 2. The Cu and Fe concentration of diets (dry matter basis) were slightly below the estimated minimal nutrient composition of AIN-93G as determined by Reeves et al. (15); however, all other minerals met or exceeded this estimate.

TABLE 2

Analyzed Nitrogen and Mineral Composition of Diets Fed to Growing Rats

| Nutrient | Dietary concentration of FOS*, % | | | |
|---|---|---|---|---|
| | 0 | 1 | 3 | 5 |
| | unit/g diet dry matter | | | |
| N, mg | 29.28 | 29.42 | 29.31 | 28.33 |
| Ca, mg | 5.88 | 5.67 | 5.73 | 5.70 |
| P, mg | 3.49 | 3.51 | 3.51 | 3.52 |
| Mg, mg | 0.66 | 0.65 | 0.65 | 0.64 |
| Na, mg | 1.38 | 1.38 | 1.38 | 1.34 |
| Cl, mg | 1.89 | 1.89 | 1.89 | 1.81 |
| K, mg | 3.96 | 4.12 | 3.97 | 3.95 |
| Cu, μg | 6.35 | 5.64 | 5.86 | 6.40 |
| Fe, μg | 46.3 | 44.8 | 49.9 | 46.6 |
| Mn, μg | 11.5 | 11.5 | 11.6 | 12.0 |
| Zn, μg | 44.3 | 43.2 | 45.8 | 45.2 |

*FOS = fructooligosaccharides.

*Performance*

Dry matter intake, water intake, and average daily gain did not differ ($P \geq 0.10$) among treatment groups during the *ad libitum* intake phase (d 6 to 17; Table 3). However, feed

efficiency was affected quadratically ($P = 0.02$), primarily because of a lower feed efficiency for rats consuming 5% FOS.

TABLE 3

Performance and Dry Matter and N Metabolism by Rats Fed Varying Concentrations of Fructooligosaccharides (FOS)*

| Item | Dietary FOS concentration, % | | | | | Probability of contrasts | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | SEM | Linear | Quadratic | Control vs. FOS |
| 6 to 17 d, *ad libitum* feed intake | | | | | | | | |
| Dry matter intake, g/d | 16.2 | 16.2 | 16.3 | 17.0 | 0.4 | NS | NS | NS |
| Average daily gain, g | 7.29 | 7.34 | 7.34 | 6.94 | 0.15 | 0.10 | NS | NS |
| Feed efficiency, g/g | 0.450 | 0.452 | 0.451 | 0.412 | 0.008 | <0.01 | 0.02 | NS |
| Water intake, mL/d | 22.4 | 21.8 | 21.6 | 22.0 | 0.8 | NS | NS | NS |
| | | | | | | | | |
| 18 to 27 d, restricted feed intake | | | | | | | | |
| Dry matter intake, g/d | 13.3 | 13.3 | 13.4 | 13.5 | 0.1 | 0.03 | NS | NS |
| Average daily gain, g | 2.40 | 2.79 | 2.67 | 2.64 | 0.12 | NS | NS | 0.04 |
| Feed efficiency, g/g | 0.180 | 0.209 | 0.199 | 0.195 | 0.009 | NS | NS | 0.05 |
| | | | | | | | | |
| 20 to 27 d, balance phase | | | | | | | | |
| Dry matter intake, g/d | 13.6 | 13.6 | 13.4 | 13.4 | 0.1 | 0.02 | NS | NS |
| Dry matter digestion, % | 93.1 | 93.1 | 92.4 | 92.4 | 0.1 | <0.01 | NS | <0.01 |
| Fecal dry matter, g/d | 0.94 | 0.94 | 1.02 | 1.02 | 0.01 | <0.01 | NS | <0.01 |
| N intake, mg/d | 399 | 401 | 392 | 379 | 3 | <0.01 | 0.06 | <0.01 |
| Fecal N, mg/d | 18.6 | 21.7 | 27.7 | 29.4 | 0.5 | <0.01 | <0.01 | <0.01 |
| Urinary N, mg/d | 204 | 191 | 196 | 181 | 7 | 0.04 | NS | 0.06 |
| N balance, mg/d | 176 | 189 | 169 | 169 | 6 | 0.10 | NS | NS |
| N absorption, % | 95.3 | 94.6 | 92.9 | 92.2 | 0.1 | <0.01 | <0.01 | <0.01 |
| N balance, % absorption | 46.3 | 49.7 | 46.3 | 48.2 | 1.7 | NS | NS | NS |
| Water intake, mL/d | 26.9 | 24.5 | 25.9 | 25.0 | 1.3 | NS | NS | NS |
| Cecal pH | 7.49 | 7.06 | 6.63 | 6.38 | 0.05 | <0.01 | <0.01 | <0.01 |

*Values are means, n = 10. NS = not significant, $P > 0.10$.

During the restricted-feeding phase (d 18 to 27), rats fed FOS-containing diets had a higher average daily gain and improved feed efficiency compared to controls (Table 3). In addition, there was a linear increase in dry matter intake as FOS concentration increased. In direct contrast, during the balance phase (d 20 to 27), there was a linear decrease in dry matter intake as FOS concentration in the diet increased. This discrepancy suggests that this statistical difference in dry matter intake was an artifact of the extremely low variance and, therefore, is not of biological importance.

Apparent dry matter digestibility decreased ($P < 0.01$) linearly as the concentration of dietary FOS increased, resulting in a lower ($P < 0.01$) apparent dry matter digestibility for rats consuming the FOS-containing diets (Table 3). In addition, N intake decreased ($P < 0.01$) linearly as FOS concentration increased, again probably an artifact of the low variance and disparity in diet fortification. Excretion of N in the feces showed a quadratic effect ($P < 0.01$) with the largest increase from 1 to 3% FOS. In contrast, urinary N excretion decreased ($P = 0.04$) linearly as FOS concentration increased, resulting in N balance (expressed as % of absorption and mg/d) not differing ($P \geq 0.10$) among groups. Apparent absorption of N was affected quadratically ($P < 0.01$), generally showing that rats consuming 3 and 5% FOS diets had lower absorption than rats fed the control diet.

The pH of cecal contents was lower ($P < 0.01$) for rats consuming the diets containing FOS (Table 3). The quadratic response was due to a decreased rate of pH decline with increasing FOS. In addition, as dietary level of FOS increased, water concentration of the cecal contents and cecum size appeared to increase (visual observation).

*Macromineral absorption*

The metabolism of macrominerals by rats fed varying levels of FOS is presented in Table 4. Calcium intake was lower ($P < 0.01$) for rats consuming FOS-containing diets and also showed a quadratic ($P = 0.01$) effect. Apparent absorption and balance (mg/d) of Ca did not differ ($P > 0.10$) among groups; however, apparent Ca balance expressed as % of absorption was lower ($P < 0.01$) for rats consuming FOS-containing diets compared to the control diet and also showed a quadratic ($P = 0.04$) effect.

Phosphorus intake did not differ ($P > 0.10$) among groups. There was a linear effect ($P < 0.01$) on apparent P absorption generally showing a reduction in absorption as FOS concentration in the diet increased. Apparent P balance (mg/d) did not differ ($P > 0.10$) among groups. However, when apparent P balance was expressed as % of absorption, it was higher ($P < 0.01$) for rats consuming FOS-containing diets and increased ($P < 0.01$) linearly with the increasing concentration of dietary FOS.

Magnesium intake did not differ ($P > 0.05$) among groups. Apparent absorption of Mg increased linearly ($P < 0.01$) as FOS concentration in the diet increased, resulting in a higher ($P < 0.01$) apparent Mg absorption for rats consuming FOS-containing diets. Apparent Mg balance (mg/d) did not differ ($P > 0.10$) among groups. However, when apparent Mg balance was expressed as % of absorption, it was lower ($P = 0.03$) for rats consuming FOS-containing diets and decreased ($P < 0.01$) linearly with the increasing concentration of dietary FOS.

TABLE 4

Macromineral Intake and Metabolism by Rats Fed Varying Concentrations of
Fructooligosaccharides (FOS)*

| | Dietary FOS concentration, % | | | | | Probability of contrasts | | |
|---|---|---|---|---|---|---|---|---|
| Item | 0 | 1 | 3 | 5 | SEM | Linear | Quadratic | Control vs. FOS |
| Ca intake, mg/d | 80.1 | 77.3 | 76.8 | 76.4 | 0.5 | <0.01 | 0.01 | <0.01 |
| Ca absorption, % | 61.8 | 65.1 | 62.2 | 64.3 | 1.1 | NS | NS | NS |
| Ca balance, mg/d | 48.7 | 49.4 | 46.7 | 47.3 | 1.0 | NS | NS | NS |
| Ca balance, % absorption | 98.5 | 98.2 | 97.9 | 96.4 | 0.2 | <0.01 | 0.04 | <0.01 |
| P intake, mg/d | 47.5 | 47.9 | 47.0 | 47.2 | 0.3 | NS | NS | NS |
| P absorption, % | 70.5 | 72.1 | 68.3 | 67.1 | 1.2 | <0.01 | NS | NS |
| P balance, mg/d | 27.2 | 28.1 | 27.7 | 28.2 | 0.7 | NS | NS | NS |
| P balance, % absorption | 81.0 | 81.2 | 86.2 | 89.1 | 1.2 | <0.01 | NS | <0.01 |
| Mg intake, mg/d | 8.83 | 8.75 | 8.66 | 8.69 | 0.06 | 0.08 | NS | 0.06 |
| Mg absorption, % | 70.0 | 72.9 | 73.8 | 77.4 | 1.0 | <0.01 | NS | <0.01 |
| Mg balance, mg/d | 2.74 | 2.83 | 2.52 | 2.61 | 0.11 | NS | NS | NS |
| Mg balance, % absorption | 44.1 | 44.3 | 39.3 | 38.7 | 1.2 | <0.01 | NS | 0.03 |
| Na intake, mg/d | 18.8 | 18.8 | 18.5 | 17.9 | 0.1 | <0.01 | NS | 0.01 |
| Na absorption, % | 96.7 | 97.1 | 96.5 | 95.1 | 0.3 | <0.01 | 0.07 | NS |
| Na balance, mg/d | 5.84 | 5.75 | 5.53 | 4.74 | 0.33 | 0.02 | NS | NS |
| Na balance, % absorption | 32.2 | 31.5 | 31.0 | 27.7 | 1.8 | 0.09 | NS | NS |
| Cl intake, mg/d | 25.8 | 25.8 | 25.3 | 24.2 | 0.2 | <0.01 | 0.03 | <0.01 |
| Cl absorption, % | 97.7 | 98.0 | 97.7 | 98.0 | 0.2 | NS | NS | NS |
| Cl balance, mg/d | 5.64 | 5.42 | 5.20 | 4.08 | 0.44 | 0.01 | NS | NS |
| Cl balance, % absorption | 22.4 | 21.5 | 21.0 | 17.2 | 1.8 | 0.04 | NS | NS |
| K intake, mg/d | 54.0 | 56.1 | 53.1 | 52.9 | 0.4 | <0.01 | NS | NS |
| K absorption, % | 96.6 | 95.7 | 93.0 | 90.9 | 0.4 | <0.01 | NS | <0.01 |
| K balance, mg/d | 19.0 | 20.4 | 17.6 | 17.5 | 0.8 | 0.03 | NS | NS |
| K balance, % absorption | 36.5 | 38.0 | 35.6 | 36.3 | 1.5 | NS | NS | NS |

*Values are means, n = 10. NS = not significant, $P > 0.10$.

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Sodium intake decreased ($P < 0.01$) linearly as FOS concentration increased in the diet, resulting in a lower ($P < 0.01$) Na intake for rats consuming FOS-containing diets compared to

the control diet. Apparent Na balance and absorption (mg/d) decreased linearly ($P = 0.02$ and $P < 0.01$, respectively) as the dietary concentration of FOS increased; however, apparent Na balance (expressed as % of absorption) did not differ ($P \geq 0.09$) among groups.

Chloride intake was affected quadratically ($P = 0.03$), generally showing that rats consuming the 5% FOS diet had the lowest intake. Apparent Cl absorption did not differ ($P > 0.10$) among groups. There was a linear decrease in apparent Cl balance, expressed as mg/d or as % of absorption ($P = 0.01$ and $P = 0.04$, respectively), as FOS concentration increased in the diets.

Potassium intake decreased ($P < 0.01$) linearly as dietary FOS concentration increased. Apparent K absorption decreased ($P < 0.01$) linearly as FOS concentration increased in the diet, resulting in a lower ($P < 0.01$) apparent K absorption for rats consuming FOS-containing diets compared to the control diet. This resulted in a linear decrease ($P = 0.03$) in apparent K balance (expressed in mg/d) as FOS concentration increased in the diet; however, apparent K balance (expressed as % of absorption) did not differ ($P > 0.10$) among groups.

*Trace mineral absorption*

The metabolism of trace minerals in rats fed varying concentrations of FOS is presented in Table 5. There was a quadratic effect ($P < 0.01$) of FOS on Cu intake, generally showing lower ($P < 0.01$) intakes for rats fed diets containing FOS compared to the control diet. Apparent absorption of Cu showed a quadratic effect ($P < 0.01$), with rats consuming FOS-containing diets having a lower ($P < 0.01$) apparent absorption compared to controls. Similarly, apparent balance of Cu (expressed as mg/d and as % of absorption) had quadratic effects ($P < 0.01$ and $P = 0.04$, respectively), showing a reduced ($P < 0.01$) apparent Cu balance in rats consuming FOS-containing diets compared to the control diet.

Iron intake was affected quadratically ($P < 0.01$) by dietary FOS concentration. Similarly, apparent absorption and balance (expressed in mg/d) were affected quadratically ($P = 0.01$ and $P < 0.01$, respectively), generally showing a lower apparent absorption and balance for rats consuming diets containing 5% FOS. Apparent Fe balance (expressed as % of absorption) did not differ ($P > 0.10$) among groups.

Intake of Mn was affected quadratically ($P = 0.02$) by FOS concentration. However, apparent absorption and balance did not differ ($P > 0.10$) among groups. A linear effect ($P = 0.05$) on Zn intake was found, but apparent absorption and balance did not differ ($P > 0.10$) among groups.

B.W. WOLF et al.

TABLE 5

Trace Mineral Intake and Metabolism in Rats Fed Varying Concentrations of
Fructooligosaccharides (FOS)*

| | Dietary FOS concentration, % | | | | | Probability of contrasts | | |
|---|---|---|---|---|---|---|---|---|
| Item | 0 | 1 | 3 | 5 | SEM | Linear | Quadratic | Control vs. FOS |
| Cu intake, μg/d | 89 | 82 | 79 | 80 | 1 | <0.01 | <0.01 | <0.01 |
| Cu absorption, % | 29.7 | 22.8 | 17.1 | 17.6 · | 1.2 | <0.01 | <0.01 | <0.01 |
| Cu balance, μg/d | 23 | 15 | 10 | 10 | 1 | <0.01 | <0.01 | <0.01 |
| Cu balance, % absorption | 85.6 | 80.0 | 70.5 | 69.4 | 2.1 | <0.01 | 0.04 | <0.01 |
| Fe intake, μg/d | 631 | 611 | 668 | 625 | 4 | 0.03 | <0.01 | NS |
| Fe absorption, % | 32.1 | 33.1 | 33.5 | 26.5 | 1.4 | <0.01 | 0.01 | NS |
| Fe balance, μg/d | 191 | 189 | 217 | 160 | 9 | 0.08 | <0.01 | NS |
| Fe balance, % absorption | 94.2 | 93.8 | 96.9 | 96.6 | 1.6 | NS | NS | NS |
| Mn intake, μg/d | 157 | 157 | 156 | 161 | 1 | 0.03 | 0.02 | NS |
| Mn absorption, % | 6.1 | 9.2 | 7.0 | 11.0 | 1.6 | NS | NS | NS |
| Mn balance, μg/d | 9 | 14 | 11 | 16 | 3 | NS | NS | NS |
| Mn balance, % absorption | 104.1 | 92.5 | 95.6 | 109.5 | 7.3 | NS | NS | NS |
| Zn intake, μg/d | 604 | 589 | 613 | 606 | 4 | 0.05 | NS | NS |
| Zn absorption, % | 26.6 | 29.6 | 28.9 | 28.4 | 1.5 | NS | NS | NS |
| Zn balance, μg/d | 153 | 167 | 169 | 163 | 9 | NS | NS | NS |
| Zn balance, % absorption | 95.1 | 95.3 | 95.5 | 94.6 | 0.4 | NS | NS | NS |

*Values are means, n = 10. NS = not significant, $P > 0.10$.


DISCUSSION


Because of the beneficial relationship between mineral supplementation and health (e.g., Ca and osteoporosis), researchers have evaluated the effects of dietary fiber on mineral absorption. Historically, dietary fiber was considered to be detrimental to mineral absorption (16). It may be hypothesized that highly charged fibers such as pectin (via uronic acids), which bind cations like Ca, decrease mineral bioavailability. However, more recently, investigators have documented that certain dietary fibers and indigestible oligosaccharides (e.g., FOS) improve mineral absorption (6-9). For the purpose of this manuscript, we have defined indigestible oligosaccharide as a small carbohydrate moiety (degree of polymerization < 20) that is resistant to endogenous digestion in the upper digestive tract of nonruminant animals; however, it may be extensively fermented in the large bowel. The fermentation of these

carbohydrates results in the production of short-chain fatty acids, which decrease the pH within the lower bowel (17) which may ultimately displace cations from the digesta matrix (i.e., increase cation solubility).

Trinidad et al. (18) evaluated the direct effect of the short-chain fatty acids, acetate and propionate, on Ca absorption from the distal colon of humans. Calcium absorption improved when it was infused with acetate and propionate into the distal colon. Propionate, however, was more effective than acetate. The authors suggested that propionate (being more lipid-soluble than acetate) would be more readily absorbed by direct diffusion. However, for this to occur, the short-chain fatty acid must be in the protonated (undissociated) form. Once intracellular, H ions would dissociate from the short-chain fatty acids because the $pK_a$ values for short-chain fatty acids ($\sim 4.8$) are lower than the intracellular pH (between 6 to 7). These H ions would then be secreted from the mucosal cell (enterocyte) in exchange for free cations such as Ca in the lower bowel, allowing for the transfer of Ca across the enterocyte.

Chonan and Watanuki (19) noted an increase in Ca absorption when rats were fed diets containing galactooligosaccharides [i.e., transgalactosylated oligosaccharides (TOS)]. These oligosaccharides, like FOS, are indigestible by the mammalian enzymes of the small intestine but are readily fermented in the large bowel. These researchers found that growing rats fed diets containing TOS (5 or 10%) had improved apparent Ca absorption and balance. They hypothesized that this stimulatory effect was due to the increase in the solubility of Ca (increasing its bioavailability) via a lower pH in the large bowel. They also addressed the work of Bronner (20), who postulated that an increase in fluid content of the gastrointestinal tract (the body's response to maintain isotonicity due to the presence of osmotically active sugars and short-chain fatty acids) might increase the passive paracellular absorption of Ca by increasing the permeability of the intercellular junctions between enterocytes.

Rat performance during the *ad libitum* intake phase (d 6 to 17) did not differ among groups with the exception of a lower feed efficiency for rats consuming diets containing 5% FOS (Table 3). This may be because FOS (an indigestible sugar in the small intestine) replaced sucrose (a highly digestible sugar) in the diets, thus diluting the available energy density of the diet. This conclusion is supported by the work of Hosoya et al. (21), who, utilizing radioactive FOS, estimated the metabolizable energy value of FOS to be 6.28 kJ/g (1.5 kcal/g). Small improvements in average daily gain and feed efficiency were noted during the restricted-feeding phase (d 18 to 27) for rats consuming FOS containing diets.

During the balance phase, a slight decrease in apparent dry matter digestibility was noted in the rats fed diets containing 3 and 5% FOS. This may be explained by the high fermentability of FOS. Theoretically, as the percentage of FOS increased in the diet (and was ultimately fermented), the amount of microbial mass would increase and be excreted in the feces, thus resulting in a lower apparent digestibility of N. This reasoning is supported by the observation that fecal N excretion increased (due to microbial protein) as the concentration of FOS in the diet increased, even though N intake decreased. Overall, N balance was similar among treatments. The inclusion of dietary FOS led to a slight shift in N excretion from urine to feces. This may not be a result of decreased true N digestibility but, rather, may be due to the presence of highly ureolytic bacteria in the cecum, which favored a net transfer of urea into the cecal lumen (22).

Bacteria within the cecal lumen produce ammonia (via urease) for bacterial protein synthesis, thus trapping nitrogen for elimination in the feces as microbial protein. Ultimately, fecal N excretion calculated as a percentage of total N excretion was 8.3 in the control versus 14 in rats fed 5% FOS. This shift in N excretion has been noted by others who fed 7.5% FOS to rats (22).

Cecal pH, which is an indirect measure of the production of short-chain fatty acids, decreased with increasing dietary FOS concentration. This result was as expected; however, the absolute pH was higher than expected and may be attributed to a high buffering capacity of the AIN-93G diet. The inability of our experiment to show improved mineral absorption may be attributed to this higher pH. Chonan and Watanuki (19) found that animals fed supplemental TOS had a cecal pH of 5.3 to 5.6 with a concomitant improvement in Ca absorption. Ohta et al. (6, 9) also showed improved Ca absorption in rats fed supplemental FOS and documented a cecal pH of 5.4 and 5.9 to 6.3, respectively. More recently, Younes et al. (23) documented that the fermentation of resistant starch (cecal pH 5.05 to 5.72) improved the absorption of Ca and Mg, especially when dietary Ca levels were low.

In the present study, we attempted to verify the benefits and to titrate the effects of FOS on apparent mineral absorption and balance of selected macro and trace minerals. Overall, the macrominerals and trace minerals were in a positive balance (positive retention, mg/d) for all experimental treatments as would be expected in growing animals (Tables 4 and 5). Generally, the macromineral and trace mineral intakes during the balance phase decreased with increasing levels of FOS, even though we attempted to limit-feed the animals so that dietary intake would be similar across treatments. This slight decrease in mineral intake corresponds well with the variation in dietary mineral composition (Table 2) and the slight decrease in dry matter intake.

The only biologically significant change in macromineral absorption was for Mg. The present experiment documented a linear increase in apparent Mg absorption as FOS concentration in the diet increased. There was a 10.6% increase in apparent Mg absorption for rats consuming 5% FOS-containing diets compared to rats consuming the control diet. However, apparent Mg balance was not improved in direct contrast to previous work (6-9).

In contrast with minor changes in most macrominerals, Cu absorption was decreased by 41% in animals fed 5% FOS. This, like the N data, may be explained by the increase in fecal microbial mass, which would contain a relatively significant amount of Cu (24). On the other hand, the reduced apparent digestibility of Cu also may have been an indirect result of the fermentation of FOS. Rodent diets supplemented with fermentable substrates like FOS have been shown to increase cecal total weight and wall weight (17, 22). This effect may be mediated by short-chain fatty acids (specifically butyrate). Roediger (25) found that more than 70% of the oxygen consumed by colonocytes from the ascending and descending colon was due to butyrate oxidation in isolated human colonocytes. In addition, in vivo studies with rats have documented the trophic effects of short-chain fatty acids on epithelial cell proliferation (26-28). The trophic effects of fermentable carbohydrates may increase the intestinal Cu pool. Moundras et al. (29) recently reported that the bile acid pool in the small intestine and cecum was significantly increased by feeding the soluble, fermentable fiber, guar gum. In the present study, FOS probably increased the size of the cecum, which may have increased the cecal pool of bile-Cu (the primary excretory route of absorbed Cu). In addition, Moundras et al. (29) saw an increase

in the bile acid flux from the liver to the small intestine. Considering this, the decrease in Cu apparent digestibility may be due to an increase in the hepatic excretion of Cu into the intestine. Aoyagi and Baker (30) determined that the bioavailability of Cu from bile is negligible in chickens. In the present study, FOS, via fermentation to SCFA, may have increased the intestinal pool of bile-excreted Cu and/or increased the biliary bile influx of Cu into the intestine where it would ultimately be excreted in the feces, thereby decreasing apparent Cu digestibility. The effects of FOS on long-term Cu status, and its clinical significance, are not known. However, extensive toxicological evaluations of FOS give no indication that FOS possesses any dose related effects on survival, growth, hematology, blood chemistry, organ weights or nonneoplastic lesions (31).

A quadratic affect on apparent Fe absorption was found, showing a 17% lower absorption of Fe in rats consuming the 5% FOS diet. Again, this may be explained by the increase in fecal microbial mass, which would contain a relatively significant amount of Fe (24).

This experiment documented an improved apparent absorption of Mg but not Ca, P or Fe, as noted by other researchers. However, apparent Cu absorption decreased with increasing dietary FOS concentration. The long-term effects of FOS consumption on Cu metabolism is unknown and should be considered in future research. In addition, this information should be considered when formulating milk-replacers or infant foods supplemented with fermentable carbohydrates. Furthermore, this study verified the reduction of urinary N excretion by inclusion of dietary FOS. Future research should evaluate the role of the diet's buffering capacity when evaluating the effects of fermentable carbohydrates on mineral metabolism.

## ACKNOWLEDGMENTS

## REFERENCES

1.    Federation of American Societies for Experimental Biology. Physiological Effects and Health Consequences of Dietary Fiber. Bethesda: Life Sciences Research Office, 1987.

2.    Cummings JH. Production and metabolism of short-chain fatty acids in humans, In: Roche AF, ed. Short-Chain Fatty Acids: Metabolism and Clinical Importance, Report of

the Tenth Ross Conference on Medical Research, Columbus, Ohio, Ross Laboratories, 1991: 11-7.

3.    Hidaka H, Eida T, Takizawa T, Tokunaga T, Tashiro Y. Effects of fructooligosaccharides on intestinal flora and human health. Bifidobact Microflora 1986; 5:37-50.

4.    Mitsuoka T, Hidaka H, Eida T. Effect of fructo-oligosaccharides on intestinal microflora. Die Nahrung 1987; 31:427-36.

5.    Molis C, Flourié B, Ouarne F, Gailing M-F, Lartigue S, Guibert A, Bornet F, Galmiche J-P. Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. Am J Clin Nutr 1996; 64:324-8.

6.    Ohta A, Ohtuki M, Takizawa T, Inaba H, Adachi T, Kimura S. Effects of fructooligosaccharides on the absorption of magnesium and calcium by cecectomized rats. Internat J Vit Nutr Res 1994; 64:316-23.

7.    Ohta A, Ohtsuki M, Baba S, Adachi T, Sakata T, Sakaguchi E. Calcium and magnesium absorption from the colon and rectum are increased in rats fed fructooligosaccharides. J Nutr 1995; 125:2417-24.

8.    Ohta A, Baba S, Takizawa T, Adachi T. Effects of fructooligosaccharides on the absorption of magnesium in the magnesium-deficient rat model. J Nutr Sci Vitaminol 1994; 40:171-80.

9.    Ohta A, Ohtsuki M, Baba S, Takizawa T, Adachi T, Kimura S. Effects of fructooligosaccharides on the absorption of iron, calcium and magnesium in iron-deficient anemic rats. J Nutr Sci Vitaminol 1995; 41:281-91.

10.   Association of the Official Analytical Chemists. Official Methods of Analysis, 15th ed. AOAC, Arlington, VA. 1990.

11.   Association of the Official Analytical Chemists. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA. 1995.

12.   SAS Institute Inc. SAS User's Guide: Statistics, Cary, NC: SAS Institute, 1992.

13.   Steel RGD, Torrie JH. (1980) Principles and Procedures of Statistics. 2nd Edition. New York: McGraw Hill, 1980: 463-6.

14.   Campbell JM, Bauer LL, Fahey GC Jr, Hogarth AJCL, Wolf BW, Hunter DE. Selected fructooligosaccharide (1-kestose, nystose, $1^F$-β-fructofuranosylnystose) composition of foods and feeds. J Agric Food Chem 1997; 45: 3076-82.

15.    Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993; 123:1939-51.

16.    Gordon DT, Stoops D, Ratliff V. Dietary fiber and mineral nutrition. In: Kritchevsky D, Bonfield C. eds. Dietary Fiber in Health and Disease, St. Paul, MN: Eagan Press, 1995: 267-93.

17.    Campbell JM, Fahey GC Jr, Wolf BW. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J Nutr 1997; 127:130-6.

18.    Trinidad TP, Wolever TMS, Thompson LU. Effect of acetate and propionate on calcium absorption form the rectum and distal colon of humans. Am J Clin Nutr 1996; 63:574-8.

19.    Chonan O, Watanuki M. Effect of galactooligosaccharides on calcium absorption in rats. J Nutr Sci Vitaminol 1995; 41:95-104.

20.    Bronner F. Intestinal calcium absorption: mechanisms and applications. J Nutr 1987; 117:1347-82.

21.    Hosoya N, Dhorranintra B, Hidaka H. Utilization of [U-$^{14}$C]fructooligosaccharides in man as energy resources. J Clin Biochem Nutr 1988; 5:67-74.

22.    Younes H, Garleb K, Behr S, Rémésy C, Demigné C. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the rat cecum. J Nutr 1995; 125:1010-6.

23.    Younes H, Demigné C, Rémésy C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. Br J Nutr 1996; 75:301-14.

24.    Durand M, Kawashima R. Influence of minerals in rumen microbial digestion. In: Ruckebusch Y, Thivend P, eds. Digestive Physiology and Metabolism in Ruminants, Westport, CT: AVI Pub Co, Inc, 1980: 375-408.

25.    Roediger WEW. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 1980; 21:712-5.

26.    Frankel WL, Zhang W, Singh A, Klurfeld DM, Don S, Sakata T, Modlin I, Rombeau JL. Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. Gastroenterol 1994; 106:375-80.

27.    Koruda MJ, Rolandelli RH, Settle RG. Effect of parenteral nutrition supplemented with short-chain fatty acids on adaption to massive small bowel resection. Gastroenterol 1988; 95:715-20.

28.     Kripke SA, Fox AD, Berman JM, Settle RG, Rombeau JL. Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. J Parent Enteral Nutr 1989; 13:109-16.

29.     Moundras C, Behr SR, Rémésy C, Demigné C. Fecal losses of sterols and bile acids induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. J Nutr 1997; 127:1068-76.

30.     Aoyagi S, Baker DH. Biological efficacy of copper in chicken bile. J Nutr 1993; 123:870-5.

31.     Clevenger MA, Turnbull D, Inoue H, Enomoto M, Allen J, Henderson L, Jones E. Toxicological evaluation of neosugar: Genotoxicity, carcinogenicity, and chronic toxicity. J Amer College Toxicology 1988; 7:643-62.