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Fructo-oligosaccharides enhance the mineral absorption and counteract the adverse effects of phytic acid in mice

Yu Wang, M.A.[a], Tao Zeng, M.D.[b], Shu-e Wang, M.A.[c], Wei Wang, M.A.[c], Qian Wang, M.A.[c], and Hong-Xia Yu, M.A.[c,*]

[a]*Shandong Centre for Tuberculosis Control, Shandong, Jinan, Peoples Republic of China*
[b]*Institute of Toxicology, Shandong University, Shandong, Jinan, Peoples Republic of China*
[c]*Department of Nutrition and Food Hygiene, Shandong University, Shandong, Jinan, Peoples Republic of China*

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Abstract

Objective: We explored the effects of fructo-oligosaccharides (FOS) and phytic acid (PA) on the absorption of minerals and their interaction.

Methods: A 3 × 2 factorial experiment was designed to evaluate the effects of FOS (in the presence or absence of PA) on the apparent absorption rate of minerals and the mineral status (plasma, hepatic, and bone) in mice. Sixty Kun-Ming mice were randomized into six groups: basal diet group; basal diet + 1% PA group (PA); basal diet + 0.8 g/kg of body weight FOS group (FOS1); FOS1 + 1% PA group (FOS1 + PA); basal diet + 2.5 g/kg of body weight group (FOS2); and FOS2 + 1% PA group (FOS2 + PA). The mice received FOS by gavage for consecutive 4 wk, and the PA was added in the diet. The mice were housed individually in the last week. The food intake was recorded and the feces were collected for calculation of the apparent absorption rate. Then the mice were sacrificed, the ceca were removed and weighed, and the cecum contents were used for the detection of pH and short-chain fatty acids. The blood, liver, and the left femur were collected for the measurement of the minerals.

Results: FOS supplementation resulted in the enlargement of the cecum and increased cecal acidification ($P < 0.01$). In addition, FOS effectively boosted the apparent absorption rate of calcium (FOS1, +7%; FOS2, +9%, $P < 0.05$), magnesium (FOS1, +26%; FOS2, +19%, $P < 0.05$), and iron (FOS1, +17%; FOS2, +22%, $P < 0.05$), and restored the PA-impaired magnesium and iron apparent absorption rates ($P < 0.01$). In addition, FOS significantly increased hepatic zinc levels ($P < 0.01$) and femoral magnesium levels ($P < 0.01$).

Conclusion: These data indicate that FOS effectively enhances the mineral apparent absorption rate and counteracts the deleterious effects of PA.

Keywords: Fructo-oligosaccharides; Phytic acid; mineral; Apparent absorption rate; Prebiotics

Introduction

A large number of functional foods in various forms have already been introduced into the market. Many of them contain a number of characteristic functional ingredients, e.g., dietary fiber, oligosaccharides, sugar alcohols, peptides and proteins, prebiotics and probiotics, phytochemicals, antioxidants, and polyunsaturated fatty acids [1]. Among these, prebiotics are indigestible food ingredients that stimulate the growth or activity of certain probiotics in the host and benefit health [2,3].

Fructo-oligosaccharides (FOS) are naturally occurring short- or medium-chain fructose molecules linked by a β-(2-1) glycosidic bond [4] and have been identified as prebiotics. FOS cannot be digested by small intestinal enzymes but are fermented in the large intestine to selectively stimulate the growth of probiotic-like bacteria that are part of the commensal gut microflora [3,5] and then produce short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate [6,7]. FOS are found in trace amounts as natural components in fruits, vegetables, barley, garlic, honey, onion, chicory, etc. Over recent decades, special attention has been given

*Corresponding author. Tel.: + 86-531-8838-2135; fax: + 86-531-8838-2553.

E-mail address: yuhx@sdu.edu.cn (H.-X. Yu).

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to the effect of bacterial fermentation on mineral absorption in the large intestine. The positive effect of the consumption of fructans (FOS and inulin) on mineral absorption has been broadly studied [8–16]. Several mechanisms have been proposed to elucidate the possible roles of FOS in improving mineral absorption. The main action of FOS is mainly associated with their fermentation by resident microflora [17], which converts prebiotics into biomass, SCFA, and gases [7]. SCFAs decrease luminal pH and thus create an acidic environment more favorable for mineral solubility. In addition, an increase in cecal mass has been observed in animals fed diets supplemented with FOS [18,19]. Cecal hypertrophy allows enlarging the volume where minerals from the ileum can accumulate. Consequently, there is an important increase in concentrations of soluble minerals and in concentrations of a soluble mineral pool in the cecum.

Phytic acid (PA), known as myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate, is a ubiquitous component and often exists in the form of a mixed salt (phytate or phytin) of mineral cations, including Zn^{2+} and Fe^{3+}. In plants, PA is one of the main inhibitors of the availability of divalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} [20]. Because humans lack appropriate endogenous phytate-degrading enzymes, PA is considered to be anti-nutritional, liable to cause negative effects.

Given the important role of FOS and PA in natural food as a factor influencing the absorption of minerals, their roles are diametrically opposed. As such, studies on the interaction between FOS and PA are needed. The present study was designed to investigate the influence and association of PA and FOS on the apparent absorption of minerals and the mineral status variables in mice.

Materials and methods

Animal care

Sixty male Kun-Ming mice, 4 wk old, were provided by the Laboratory Animal Center of Shandong University. The mice were maintained at approximately 22°C with a 12-h light cycle and had free access to commercial food and tap water. The composition of the diet is presented in Table 1. All procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals.

Table 1
Composition of basal diets*

Ingredient	g/kg diet
Corn flour	300
Wheat flour	300
Bean dregs	200
Wheat bran	80
Fish flour	80
Yeast powder	25
Bone meal	15
Vegetable oil	5
Sodium chloride	2
Multivitamins	0.03
Fish liver powder	0.1

* Partial components measured (g/kg): calcium 5.137, magnesium 0.897, ferrum 0.038, zinc 0.031, and phytic acid 27.021.

Experimental design

The experiment was designed as a 3×2 factorial arrangement of treatments with factors being three dietary quantities of FOS (0, 0.8, and 2.5 g/kg of body weight) in the presence or absence of 1% additive PA in diets (FOS and PA were provided by Shandong Zibo Jiyun Biotechnology Co., Ltd. (Zibo City, Shandong Province, China) and Shandong Baolingbao Biotechnology Co., Ltd (Dezhou City, Shandong Province, China), respectively). The mice were divided randomly into six groups as follows: basal diet group (BD); basal diet + 1% PA group (PA); basal diet + 0.8 g/kg of body weight FOS group (FOS1); FOS1 + 1% PA group (FOS1 + PA); basal diet + 2.5 g/kg of body weight group (FOS2); and FOS2 + 1% PA group (FOS2 + PA). FOS was given by gavage, and PA was added in diets for consecutive 4 wk. At the end of week 3, the mice were housed individually. After acclimation for 3 d, daily food intake was recorded for the total mineral consumption calculation. The feces were collected for the measurement of minerals.

Sampling procedures

At the end of week 4, the mice were anaesthetized with diethyl ether, and the blood was collected. Then the liver, femur, and cecum (including the cecum content) were quickly removed and weighed. The cecal contents were collected into Eppendorf tubes for the determination of pH and levels of SCFA. The cecal wall was flushed clean, weighed, and stored at −80°C until analysis.

Measurement of minerals (calcium, magnesium, iron, zinc)

Mineral levels were determined by using atomic absorption spectrophotometry in an acetylene–air flame at the following wavelengths: 422 nm (calcium [Ca]), 285 nm (magnesium [Mg]), 248 nm (iron [Fe]), and 214 nm (zinc [Zn]). Briefly, 250 μL of serum was diluted to 10 mL with 0.5% *n*-butyl alcohol; the dry-ashed tissues, feed, and feces (500°C for 10 h) were extracted in a digestive furnace until decoloration in HNO_3/H_2O_2 at 130°C for the determination of the Mg, Fe, and Zn, and those extracted with 5 M HCl and made up to an appropriate volume with 1 g/L of lanthanum chloride solution were used for the Ca measurement.

Apparent absorption rate was calculated by using the following equation: Ca, Mg, Fe, or Zn apparent absorption rate (%) = (total Ca, Mg, Fe, or Zn intake − Ca, Mg, Fe or Zn excretion in feces)/total Ca, Mg, or Fe intake × 100%.