Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Dietary phytic acid modulates characteristics of the colonic luminal environment and reduces serum levels of proinflammatory cytokines in rats fed a high-fat diet

Yukako Okazaki[a,*], *Tetsuyuki Katayama*[b]

[a] *Faculty of Human Life Sciences, Fuji Women's University, Ishikari 061-3204, Japan*
[b] *Institution of Life Sciences and Nutrition, Kita-37, Nishi-3-1-16-308, Kita-ku, Sapporo 001-0037, Japan*

ARTICLE INFO

Article history:
Received 12 June 2014
Revised 23 September 2014
Accepted 29 September 2014

Keywords:
Phytic acid
Cecal organic acids
Microflora
Mucins
β-Glucosidase
Proinflammatory cytokines
Rats

ABSTRACT

Dietary phytic acid (PA; myo-inositol [MI] hexaphosphate) is known to inhibit colon carcinogenesis in rodents. Dietary fiber, which is a negative risk factor of colon cancer, improves characteristics of the colonic environment, such as the content of organic acids and microflora. We hypothesized that dietary PA would improve the colonic luminal environment in rats fed a high-fat diet. To test this hypothesis, rats were fed diets containing 30% beef tallow with 2.04% sodium PA, 0.4% MI, or 1.02% sodium PA + 0.2% MI for 3 weeks. Compared with the control diet, the sodium PA diet up-regulated cecal organic acids, including acetate, propionate, and *n*-butyrate; this effect was especially prominent for cecal butyrate. The sodium PA + MI diet also significantly increased cecal butyrate, although this effect was less pronounced when compared with the sodium PA diet. The cecal ratio of *Lactobacillales*, cecal and fecal mucins (an index of intestinal barrier function), and fecal β-glucosidase activity were higher in rats fed the sodium PA diet than in those fed the control diet. The sodium PA, MI, and sodium PA + MI diets decreased levels of serum tumor necrosis factor α, which is a proinflammatory cytokine. Another proinflammatory cytokine, serum interleukin-6, was also down-regulated by the sodium PA and sodium PA + MI diets. These data showed that PA may improve the composition of cecal organic acids, microflora, and mucins, and it may decrease the levels of serum proinflammatory cytokines in rats fed a high-fat, mineral-sufficient diet.

1. Introduction

Phytic acid (PA; myo-inositol [MI] hexaphosphoric acid) is an abundant plant constituent and, based on weight, comprises 1% to 5% of the edible legumes, cereals, oil seeds, and nuts that serve as major sources of human and animal sustenance [1]. Numerous studies show that PA forms insoluble complexes with polycations, due to the reactive phosphate groups attached to the inositol ring, and thereby renders them unavailable for intestinal absorption in humans and animals [1,2]. Therefore, PA has been traditionally considered an antinutrient [1]. However, since the late 1980s, several studies have indicated that PA has beneficial effects such as antioxidant, anticarcinogenic, and antidiabetic properties [2–4]. Shamsuddin

Abbreviations: ANOVA, analysis of variance; HF, high-fat; IL-6, interleukin-6; MI, myo-inositol; OUT, operational taxonomic unit; PA, phytic acid; TNF-α, tumor necrosis factor α.

* *Corresponding author.*
E-mail address: yokazaki@fujijoshi.ac.jp (Y. Okazaki).

http://dx.doi.org/10.1016/j.nutres.2014.09.012
0271-5317/

et al [5,6] reported that PA suppresses colon tumorigenesis in rats and mice. Phytic acid is degraded, only to a limited extent, in the gastrointestinal tract of monogastric animals such as humans and rodents [7,8]. When PA is administered as a dietary supplement, it is suggested that undigested PA or partially digested inositol phosphates accumulate in the gastrointestinal tract [7]. Although undigested dietary PA may have adverse nutritional consequences with respect to mineral utilization, the presence of undigested products of PA in the colon may have beneficial effects on the large bowel environment, similar to those associated with dietary fibers or oligosaccharides. In researching mineral availability, Shinoda et al [9] found that cecal weight in rats is enhanced by dietary PA but not by MI bisphosphoric acid, MI triphosphoric acid, or MI tetraphosphoric acid. However, those authors did not assess other parameters associated with the large bowel. We also observed that dietary PA, like dietary fiber, increases the weights of the cecum and cecal digesta and reduces pH of cecal digesta [10]. Accordingly, PA may act as dietary fiber and/or an oligosaccharide-like substance when under conditions associated with the development of colon cancer.

Previously, we demonstrated that dietary equimolar PA and MI concentrations down-regulate the high levels of hepatic lipids and hepatic activity of lipogenic enzymes, due to a high-sucrose diet or xenobiotic intake [11–14]. We found that supplementation with as little as 0.013% PA in the diet prevents fatty liver that is caused by a high-sucrose diet in rats [12]. Furthermore, one study indicated that MI also suppresses colon tumorigenesis in mice [6]. Sakamoto et al [15] showed that ^{3}H-PA is absorbed and distributed to various organs as inositol and inositol monophosphate. It should also be noted that in our previous study, dietary PA significantly increased the hepatic level of free MI in rats [13]. Therefore, we believe that dietary PA is partially degraded to MI in the gastrointestinal tract and thus acts as a precursor of the vitamin-like substance, MI [11–13].

Consumption of some types of dietary fiber and oligosaccharides enhances the production of intestinal organic acids, stimulates the growth of endogenous probiotic bacteria, and increases levels of intestinal mucin glycoproteins; mucins are an index of intestinal barrier function [16–20]. A high production of intestinal organic acids is associated with a lower risk of colon cancer [20,21]. Other studies also suggest that probiotics, such as *bifidobacteria* and *lactobacilli*, reduce the incidence of colonic cancer in rats [22,23]. Some types of dietary fiber and probiotics also affect the activity of intestinal bacterial enzymes, reduce intestinal and serum levels of proinflammatory cytokines, and are thought to be associated with a decreased risk of colon cancer and diabetes [24–26]. A high-fat (HF) diet decreases levels of cecal organic acids and probiotics, and it is believed to elevate the risk of colon cancer [27–29].

Because PA is regularly consumed by people throughout the world and has been for many years [1,2], it is vital to gain a thorough understanding of the nutritional implications of PA under various conditions in animals and humans. Therefore, based on the current literature, we hypothesized that in rats fed an HF diet, dietary PA would affect the colonic luminal environment in a manner similar to dietary fiber. To test our hypothesis, we assessed the effect of dietary PA and MI on colonic luminal parameters, which included composition of organic acids, microflora, and mucins; bacterial enzyme activities; and serum proinflammatory cytokines, in rats fed an HF diet.

2. Methods and materials

2.1. Rats and diets

Four-week-old male Sprague-Dawley rats were purchased from Japan SLC, Inc (Hamamatsu, Japan). They were maintained according to Fuji Women's University's "Guide for the Care and Use of Laboratory Animals," and the study was approved by the university's ethics committee. The rats were individually housed in suspended cages that were made of stainless steel (17 × 25 × 16.5 cm) and included wire screen bottoms. The cages were kept in a room with a controlled temperature (23-24°C), relative humidity (55%-65%), and a light/dark cycle (light, 0800-2000 hours).

After 6 days of acclimatization to the unpurified commercial rodent powder diet (CE-2; CLEA Japan; containing 9.3% moisture, 25.1% crude protein, 4.8% crude fat, 4.2% crude fiber, 6.7% crude ash, and 50.0% nitrogen-free extract; energy, 1.44 MJ per 100 g), the rats were randomized by weight and assigned to 4 groups with 7 to 8 rats each. Composition of the basal diet was as follows: beef tallow, 30%; casein, 20%; L-cystine, 0.3%; cellulose, 5%; sucrose, 30%; vitamin mixture [30], 1%; salt mixture [30], 3.5%; and corn starch, 10.2% (Table 1). In the sodium PA diet, 2.04% dodecasodium phytate (Sigma Chemical Co, St Louis, MO, USA) was added at the expense of corn starch (Table 1). In the MI diet, 0.4% MI (Wako Pure Chemicals, Osaka, Japan) was added at the expense of corn starch (Table 1). The molar concentration of the added PA was roughly equivalent to that of the added MI. In the sodium PA + MI diet, 1.02% dodecasodium phytate and 0.2% MI were added at the expense of corn starch (Table 1). For each experimental diet, the same amount of food was provided daily at 1900 hours (9 g for day 1, 10 g for days 2-4, 12 g for days 5-7, 14 g for days 8-

Table 1 – Ingredient composition of the experimental diets fed to rats

Ingredient	Control (%, wt/wt)	Sodium PA (%, wt/wt)	MI (%, wt/wt)	Sodium PA + MI (%, wt/wt)
Casein	20	20	20	20
L-Cystine	0.3	0.3	0.3	0.3
Beef tallow	30	30	30	30
Cellulose	5	5	5	5
Vitamin mix (AIN-93G) [a]	1	1	1	1
Mineral mix (AIN-93G) [a]	3.5	3.5	3.5	3.5
Sucrose	30	30	30	30
Corn starch	10.2	8.16	9.8	8.98
Dodecasodium phytate	–	2.04	–	1.02
MI	–	–	0.4	0.2

Sodium PA, sodium phytate; Sodium PA + MI, sodium phytate + MI.
[a] Reeves et al [30].