Supplemental fermented plant product (‘Manda Koso’) reduces succinate and deoxycholate, as well as elevates IgA and mucin levels, in rats fed a high-fat diet

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Abstract. ‘Manda Koso’ is a commercial fermented plant product (FPP) made from 53 types of fruits and vegetables that have been fermented for >3 years and 3 months. We hypothesized that FPP intake improves the luminal environment of rats fed a high-fat diet. Thus, the present study examined the effects of consumption of 5% FPP diet for 3 weeks on colonic luminal parameters in rats fed a 30% beef tallow diet. Food intake and body weight gain were unaffected.

Consumption of the FPP diet did not influence the proportions of Bifidobacterium, Lactobacillus, Bacteroides, Prevotella or Clostridium in cecal contents. However, the FPP diet caused a significant reduction (~88%) in the level of cecal succinate, a putative inflammatory signal (P<0.01), but did not affect the levels of n-butyrate, propionate, acetate and lactate. The fecal levels of deoxycholate and hyodeoxycholate, which are toxic bile acids, were also significantly reduced by the FPP diet (P<0.05). The FPP diet significantly increased fecal immunoglobulin A and mucins responsible for intestinal immune and barrier functions (P<0.05). The results suggest that the consumption of FPP is beneficial for the colonic luminal environment in rats fed a high-fat diet.

Introduction

‘Manda Koso’ (Manda Fermentation Co., Ltd., Onomichi, Japan) is a fermented plant product (FPP) made of naturally fermented fruits, plant roots, cereals, marine algae and kokuto, a type of non-antifungal cane sugar. The raw ingredients are crushed and fermented by Lactobacillus and yeast generated spontaneously from raw materials at room temperature for 3 years and 3 months. The product is a well-known natural health food that is consumed in Japan. The FPP is a sweet, black-brown, paste-like substance comprising 36.9% water, 2.4% proteins and amino acids, 3.7% dietary fibers, 55.2% carbohydrates and 1.8% ash. The consumption of FPP is reported to reduce the fat content without affecting bone weight or strength in ovariectomized rats (1). The FPP also exhibits free radical scavenging activity (2). The consumption of FPP in fish decreases thiobarbituric-acid reactive substance levels in their tissues (3). Additionally, FPP intake has been recently suggested to improve feed efficiency and the intestinal histological status in broilers (4).

The consumption of certain dietary fibers, including inulin and oligosaccharides, increases the concentrations of intestinal immunoglobulin A (IgA) and mucins, which have roles in the maintenance of gut barrier function (5,6). Colon IgA levels are decreased in patients with ulcerative colitis (7). IgA production was recently suggested to be associated with a decreased incidence of colon cancer (8). The intestinal fermentation of dietary fibers and oligosaccharides is associated with the enhanced intestinal production of n-butyrate (9). Elevated intestinal production of n-butyrate by fermentation is associated with decreased risks of colon cancer and ulcerative colitis (10,11). Certain fibers and polyphenols are reported to reduce fecal secondary bile acids, such as deoxycholate and lithocholate; secondary bile acids, which are the highly cytotoxic intestinal microbial metabolites of primary bile acid that promote colon cancer development (12,13). A high-fat diet increases fecal secondary bile acids and the production of succinate, a putative pro-inflammatory signal, and decreases n-butyrate production (14-16). These alterations are believed to be associated with the increased risks of colon cancer and ulcerative colitis.

Due to the favorable effect of FPP intake on the intestinal histological status in broilers, as mentioned above (4), we hypothesized that FPP intake improves the colonic luminal environment of rats fed a high-fat diet. Therefore, the effects
of FPP consumption was investigated on intestinal luminal variables, including microflora, fermentation, secondary bile acids, IgA, mucins and harmful enzymes in rats fed a high-fat diet.

Materials and methods

Materials. The FPP was obtained from Manda Fermentation Co., Ltd., and the chemical composition is shown in Table I.

Animals. Male Sprague-Dawley rats (3-week-old) were purchased from Hiroshima Laboratory Animal Centre (Hiroshima, Japan) and maintained according to the ‘Guide for the Care and Use of Laboratory Animals’ established by Hiroshima University; the study protocol was approved by the University Ethics Committee. The rats were individually housed in an air-conditioned room at 23-24°C with a 12-h light cycle (light, from 08:00 a.m. to 8:00 p.m.). Following acclimatization and feeding with a non-purified commercial rodent diet (moderate fat; Oriental Yeast Co., Ltd., Tokyo, Japan) for 7 days, 13 rats (mean body weight, 105 g) were divided into 2 groups with 6 or 7 rats in each. The compositions of the experimental diets are shown in Table II. The FPP was added to the diet at 7.9% (w/w) (5% on dry weight basis). The levels of dietary minerals and fibers in the FPP diet were adjusted by reducing the salt mixture and cellulose, respectively. The amounts of dietary fibers in the FPP were measured using the AOAC 2001.03 enzyme-gravimetric method in combination with high-performance liquid chromatography (17). Equal amounts of each experimental diet were incorporated daily into food cups at 7:00 p.m. (9, 10, 12, 14 and 15 g for days 1, 2-4, 5-7, 8-13 and 14-21, respectively) to ensure a standardized food intake. All the diet was consumed each day until the diet was served on the following day. The weight of the spilled diet was recorded daily and accounted for in the calculation of food intake. Feces were collected during the last 3 days. At the end of the 21-day feeding period, the rats were sacrificed by decapitation under diethyl ether anaesthesia. The liver, epididymal and perirenal adipose tissues and gastrocnemius muscle were excised rapidly and weighed. The cecum was excised, and its contents were immediately collected, weighed, and stored at -70°C until analysis.

Quantification analyses. Bacterial genomic DNA was extracted from cecal digesta using an Isofecal DNA extraction kit (Nippon Gene, Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. The cecal microflora was analyzed using a terminal restriction fragment length polymorphism method as described previously (18). Cecal organic acids were measured as described previously (19). Fecal acidic sterols were analyzed using an internal standard (nor-deoxycholic acid; Steraloids, Wilton, NY, USA) by gas chromatography as described previously. The total IgA concentration in feces was measured using an ELISA quantitation kit (Bethyl Laboratories Inc., Montgomery, TX, USA). Mucins were extracted according to the method of Bovee-Oudenhoven et al (20) and quantitated using a fluorometric assay (21). The activities of harmful fecal enzymes, such as tryptophanase, β-glucuronidase and β-glucosidase, were determined as described previously (22).

Table I. Chemical composition of FPP.

| Composition per 100 g FPP | FPP |
|--------------------------|-----|
| Nitrogen x 6.25, g        | 2.4 |
| Carbohydrates, g         | 55.2|
| Glucose, g               | 18.6|
| Fructose, g              | 15.8|
| Maltose, g               | 0.23|
| Isomaltose, g            | 0.8 |
| Dietary fibers, g        | 3.7 |
| Ash, g                   | 1.8 |
| K, mg                    | 530 |
| Ca, mg                   | 130 |
| Mg, mg                   | 54  |
| Na, mg                   | 49  |
| P, mg                    | 47  |
| Fe, mg                   | 3.2 |
| Zn, mg                   | 0.6 |
| Water, g                 | 36.9|
| Ile, mg                  | 77  |
| Leu, mg                  | 142 |
| Lys, mg                  | 43  |
| Met, mg                  | 23  |
| Phe, mg                  | 83  |
| Tyr, mg                  | 44  |
| Thr, mg                  | 63  |
| Try, mg                  | 12  |
| Val, mg                  | 99  |
| His, mg                  | 23  |
| Arg, mg                  | 37  |
| Ala, mg                  | 86  |
| Asp, mg                  | 238 |
| Glu, mg                  | 336 |
| Gly, mg                  | 62  |
| Pro, mg                  | 115 |
| Ser, mg                  | 73  |
| Vitamin B1, mg           | 0.01|
| Vitamin B2, mg           | 0.02|
| Vitamin B6, mg           | 0.16|
| Vitamin K1, μg           | 2   |
| Folic acid, μg           | 11.5|
| Niacin, mg               | 0.73|
| Retinol, μg              | 7   |
| α-Carotene, μg           | 5   |
| β-Carotene, μg           | 85  |
| Soy isoflavone, mg       | 1.3 |
| Total polyphenols, g     | 0.48|
| Lactate, g               | 1.2 |
| Acetate, g               | 0.3 |
| Tartarate, g             | 0.01|
| Succinate, g             | 0.03|
| Gluconate, g             | 0.72|

FPP, fermented plant product.
Statistical analysis. Data are expressed as mean ± standard error. Statistical analysis was performed by Student’s t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics. Final body weight, total food intake, weights of tissues and fecal weight did not differ significantly between the groups (Table III). The data of cecal microflora and organic acids are shown in Table IV. The proportions of the cecal microflora examined were unaffected. The cecal level of succinate was markedly reduced in the FPP diet group (-88%, P<0.01), while the levels of other organic acids did not differ significantly between groups.

The fecal contents of deoxycholate and hyodeoxycholate acid were significantly lower in the FPP diet group (-50 and -56%, respectively, P<0.05, Table V), while those of cholate and lithocholate were not significantly different. Cecal levels of IgA and mucins were 1.9- and 3.2-fold significantly greater in the FPP diet group (+91 and +219%, respectively, P<0.05). Furthermore, the activity of fecal β-glucuronidase tended to be lower in the FPP diet group (-23%, P=0.073). The activities of the other enzymes did not differ significantly between the groups.

Discussion

Notably, the cecal succinate level was markedly reduced by the FPP diet in the present study, whereas other organic acids...
were unaffected. To the best of our knowledge, this is the first evidence of the marked reduction of colonic succinate by dietary factor(s). A high-fat diet was recently found to increase colonic succinate production and decrease butyrate production together with low-grade inflammation (16). Succinate is considered an inflammatory and hypoxic signal; it stabilizes the transcription factor hypoxia-inducible factor-1α in specific tumors and activated macrophages, and stimulates dendritic cells via succinate receptor GPR91 (23). Succinate, produced abundantly by members of the family Bacteroidaceae, particularly B. caccae, is considered the ulcerogenic agent, and reduces fecal deoxycholic acid and adipose tissue by which the FPP exerts such effects and to identify the active compounds responsible.

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