Edible Lily Bulb Modulates Colonic Barrier Functions, Microflora and Fermentation in Rats Fed a High-Fat Diet

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Abstract

Lily bulb (*Lilium leichtlinii* var. *maximowiczii* Baker) is traditionally consumed in Asia as both a root vegetable and a herbal medicine. The objective of this study was to determine if dietary edible lily bulbs modulated the colonic luminal environment in rats fed a high-fat (HF) diet. In experiment 1, male SD rats were fed a HF diet (30% beef tallow) with or without 5% or 10% raw lily bulb powder for 3 weeks. Dietary raw lily bulb significantly increased cecal and fecal mucins, IgA (indices of intestinal barrier and immune functions) and the cecal ratio of *Lactobacillales* and *Bifidobacterium* in a dose-dependent manner. In experiment 2, the rats were fed a HF diet with or without 7% steamed or raw lily bulb powder for 3 weeks. Cecal ratio of *Lactobacillales* was significantly increased in the HF + raw lily bulb group, with a similar trends being observed in the HF + steamed lily bulb group. Consumption of both steamed and raw lily bulb caused markedly increases in cecal and fecal mucins and cecal organic acids, including lactate, propionate, acetate, and butyrate. Unexpectedly, HF + steamed lily bulb diet caused a significant reduction in the fecal activity of mucinase, a risk factor for colon diseases, and mesenteric adipose tissue weight, whereas the HF + raw lily bulb diet did not. These results suggest a favorable effect of lily bulb on colon health by modulating colonic barrier function, microflora profile, and fermentation in rats fed a high-fat diet.

Keywords: Lily bulb; Mucins; Cecal microflora; Cecal organic acids; Rats

Introduction

The Maximowicz's lily (*Lilium leichtlinii* var. *maximowiczii* Baker) belongs to the genus *Lilium* of the family Liliaceae. Its bulbs have been traditionally consumed in Asia as a root vegetable for hundreds of years. Edible lily bulb contains physiologically active components, such as dietary fibers, flavonoids, and saponins and is also rich in nutrients including starch and protein [1-5]. Lily bulb has been reported to contain around 20% high-molecular weight (MW=230 kDa) glucomannan per dry weight, which consists of D-glucose and D-mannose units joined by β -glycosidic bound linkages [4]. Lily bulb has been also used in herbal medicine for the treatment of chronic gastritis, chronic bronchitis, pertussis and sedative, and it is assumed that lily bulb has physiological and pharmacological actions [6]. Recent *in vitro*

studies [5,6] already showed that methanol extracts of lily bulbs have anti-inflammatory and antioxidant activities. However, few studies have examined the other nutritional and physiological properties of the lily bulb.

Consumption of certain dietary fibers has been reported to increase intestinal mucins and IgA concentrations, both of which play a role in the maintenance of gut barrier function and health [7,8]. The increased production of mucins and IgA has been considered to be associated with a lower risk of colon cancer [9,10]. Consumption of dietary fiber has also been reported to reduce fecal activity of mucinase, which degrades the protective mucin coat in the gut wall, thereby exposing colonic mucosal cells to toxic and carcinogenic substances [11,12]. Several types of dietary fibers and low digestible starch are known to modulate colonic microflora profile, and promote the organic acid fermentation [13-16]. Increases in the beneficial bacteria, such as *lactobacilli* and *bifidobacteria*, and in the organic acid such as butyrate and propionate were also associated with a reduced risk of colon cancer and ulcerative colitis [17-19].

Meanwhile, consumption of a high-fat (HF) diet increases mucinase activity, alters the composition of microflora, and decreases organic acids fermentation [11,20]. Therefore, a HF diet is suggested to be a risk factor for colon diseases such as colon cancer and ulcerative colitis [21,22]. Lily bulbs contain large amounts of dietary fibers and starch, and have been used as herbal medicine for the treatment of gut inflammation. Therefore, edible lily bulb could ameliorate colonic luminal environment under the high-fat diet condition. Several recent studies examined the effect of some functional food, such as mushroom and konjac, on colon health [23,24]. In the present study, we examined if dietary supplementation with lily bulb powder could affect colonic luminal variables, including mucins, IgA, enzyme activity, microflora, and organic acids in rats fed a HF diet.

Materials and Methods

Preparation of the lily bulbs

Lily bulbs were purchased from JA Youtei (Hokkaido, Japan). The cloves of raw lily bulb were peeled and freeze-dried. This

lyophilized sample was powdered to produce raw lily bulb powder. Steamed lily bulb powder was prepared as follows: The lily bulb cloves were peeled and steamed for 25 min using an electric steamer (Groupe SEB Japan Co., Ltd., Tokyo, Japan). The steamed sample was powdered after being freeze-dried. The composition of the raw and steamed lily bulb powders is shown in Table 1. In experiment 1, the amount of dietary fibers in the raw lily bulb was measured using an enzyme-gravimetric method [25]. In experiment 2, the dietary fibers, in the raw and steamed lily bulb, including low molecular weight soluble dietary fibers were measured using the enzyme-gravimetric method in combination with HPLC (AOAC method 2001.03) [26].

Animals and diets

Four-wk old male Sprague Dawley (SD) rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Fuji Women's University and approved by the ethics committee of the same university. The rats were individually housed in an air-conditioned room at 23–24°C and 50%–60% relative humidity, with 12-h light cycle (light, 08:00–20:00 h). The rats were acclimated by feeding a nonpurified commercial rodent powder diet (CE-2; CLEA Japan, Inc., Tokyo, Japan) for 6 d, before commencing the experimental diet. In experiment 1, the rats after acclimatization were divided into three groups of 8 rats each and assigned to a HF (30%, w/w) diet or the same diet containing 5% or 10% raw lily-bulb powder. In experiment 2, the rats after acclimatization were divided into three groups of 8 rats each and assigned to a HF (30%, w/w) diet, with or without 7% raw or steamed lily bulb powder. The composition of the experimental diets is shown in Table 2. The levels of dietary carbohydrate, protein, fat, minerals, and fibers in the raw and steamed lily bulb diets were adjusted by reducing the amount of dietary corn starch, casein, beef tallow, and cellulose. To prevent difference in food intake, equal amounts of the experimental diets were provided daily in food cups at 19:00 (9 g for day 1, 10 g for days 2–4, 12 g for days 5–7, 14 g for days 8–13, and 15 g for day 14–21). The entire amount of food provided was consumed each day before the next day's food was provided. The

Table 1: Chemical composition (% w/w) of raw and steamed lily bulb powders^a.

Composition	Experiment 1	Experiment 2	
	Raw lily bulb	Raw lily bulb	Steamed lily bulb
Carbohydrate ^b	63.6	63.4	62.3
Protein ^c (N x 6.25)	9.1	8.1	8.6
Fat ^d	0.7	1.0	0.9
Dietary fibers ^e	19.8	21.9	23.3
Ash ^f	4.0	3.8	3.5

^aDuplicate analysis by The Foundation of Japan Food Research Laboratory, Chitose, Japan.

^b100 g – (protein + fat + dietary fibers + ash + water).

^cKjeldahl method.

^dSoxhlet method.

^eExperiment 1: Enzyme-gravimetric method [25]. Experiment 2: Enzyme-gravimetric-HPLC method [26].

^fDirect incineration method.

Table 2: Composition of the experimental diet.

Ingredient (%, w/w)	Experiment 1, 2	Experiment 1		Experiment 2	
	HF	HF + 5% raw lily bulb	HF + 10% raw lily bulb	HF + 7% raw lily bulb	HF + 7% steamed lily bulb
Casein	20.0	19.6	19.1	19.4	19.4
L-Cystine	0.3	0.3	0.3	0.3	0.3
Beef tallow	30.0	30.0	29.9	29.9	29.9
Cellulose Powder	5.0	4.0	3.0	3.5	3.4
Vitamin mix (AIN-93)	1.0	1.0	1.0	1.0	1.0
Mineral mix (AIN-93G)	3.5	3.5	3.5	3.5	3.5
Sucrose	30.0	30.0	30.0	30.0	30.0
Corn starch	10.2	6.7	3.2	5.4	5.5
Raw lily bulb	0	5	10	7.0	0
Steamed lily bulb	0	0	0	0	7.0
Net dietary fibers	5.0	5.0	5.0	5.0	5.0

HF = High Fat

weight of spilled diet was recorded daily and was included in the calculation of food intake. Feces were collected during the last 3 d. They were stored at -20°C and then freeze-dried and milled. At the end of the feeding period, the rats were anesthetized with sodium pentobarbital and then killed by collection of whole blood from the abdominal aorta. The serum was separated at 2,000 × g for 20 min and stored at -80°C. The cecum, liver, and adipose tissues (epididymal, perirenal and mesenteric) were removed, weighed, frozen immediately with liquid nitrogen and stored at -80°C for subsequent analysis.

Measurements

The mucins in cecal digesta and feces were extracted using the method of Bovee-Oudenhoven IM, et al. [27]. After extraction of the mucin fraction, the O-linked oligosaccharide chains were quantitated using a fluorometric assay that discriminated between O-linked glycoproteins (mucin) from N-linked glycoproteins [28]. Fecal mucinase activity was determined using previously described method [29].

Cecal and fecal IgA concentration was measured by ELISA using a Rat IgA ELISA quantitation kit (Bethyl Laboratories, Montgomery, Texas, USA) as according to the instructions of the manufacturer. Serum TNF-α, IL-6, and lipopolysaccharide-binding protein (LBP) were determined using ELISA quantitation kits (a Kit for TNF-α manufactured by Sibayagi, Gunma, Japan, a kit for IL-6 manufactured by Immuno-Biological Laboratories Co. Ltd., Gunma, Japan and a kit for LBP manufactured by Biometec GmbH, Greifswald, Germany).

The pH of cecal digesta was measured directly using a compact pH meter (B-212; Horiba Ltd., Kyoto, Japan). Cecal microflora

and organic acids were determined using the 16S rDNA based terminal restriction fragment length polymorphism (T-RFLP) analysis and HPLC method as described previously [20].

Serum triglyceride, total cholesterol, and HDL-cholesterol were quantified using enzymatic kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Hepatic lipids were extracted by the method of Folch J, et al. [30] and quantified using enzymatic kits. The hepatic activities of glucose 6-phosphate dehydrogenase and malic enzyme were determined as previously described [31]. Fecal triglyceride was measured using an enzymatic kit after lipid extraction according to the method of Folch J, et al. [30].

Statistical analyses

Data are expressed as the means $\pm$ SEM. Data that followed a normal distribution were analyzed using one-way ANOVA and data that did not follow a normal distribution were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA. The Tukey-Kramer post hoc-test was performed when a significant effect was determined by one-way ANOVA. Steel-Dwass post-hoc test was performed when a significant effect was found in the Kruskal-Wallis one-way ANOVA. The data were also analyzed using Spearman rank correlation analysis (Rs; Spearman rank correlation coefficient). All the analyses were performed using Excel Statistics 2010 for Windows (Social Survey Research Information Co., Ltd, Tokyo, Japan). Statistical significance for differences among means was estimated at $P < 0.05$.

Results and Discussions

Experiment 1

Food intake and final body weight did not differ between the HF and the HF + 5% raw lily bulb groups, but were significantly lower in the group receiving HF + 10% raw lily bulb powder than in the HF group ($P < 0.05$, Table 3). The HF + 10% raw lily bulb diet caused a significant increase in weight of pancreas, whereas the HF + 5% raw lily bulb diet did not. Raw lily bulb is known to contain a trypsin inhibitor, which induces growth and food intake depressions and pancreatic enlargement in animals [32]. Therefore, the presence of excess trypsin inhibitor in the HF + 10% raw lily bulb diet, compared with that in the HF + 5% raw lily bulb diet, may be associated with the depressions of growth and food intake, and increase in weight of the pancreas.

The cecal digesta weight was significantly higher in the group receiving HF + 10% lily bulb powder than in that of the HF group ($P < 0.05$, Table 3). In addition, the group receiving HF + 5% raw lily bulb powder had an intermediate in the cecal digesta weight. The pH of cecal digesta was significantly lower in the HF + 5% and HF + 10% raw lily bulb groups compared to the HF group ($P < 0.05$). These results suggest that dietary raw lily bulb may increase intestinal fermentation. The fecal dry weight was unaffected by dietary treatment. Dietary raw lily bulb significantly increased cecal and fecal mucin content in a dose dependent manner (Table 4). Cecal IgA markedly increased in the HF + 5% and HF + 10% raw lily bulb groups in a dose dependent manner. Fecal IgA content was also significantly higher in the HF + 5% and HF + 10% raw lily bulb groups than in the HF group (P

Table 3: Effect of dietary addition of 5% or 10% raw lily bulb on body weight, food intake, cecal and fecal weight in rats fed a HF diet¹ (Experiment 1).

	HF	HF + 5% raw lily bulb	HF + 10% raw lily bulb
Initial body weight (g)	113 $\pm$ 2	113 $\pm$ 2	113 $\pm$ 2
Final body weight (g)	265 $\pm$ 4 ^a	263 $\pm$ 3 ^a	225 $\pm$ 8 ^b
Food intake (g/3 wk)	270 $\pm$ 5 ^a	275 $\pm$ 2 ^a	235 $\pm$ 7 ^b
Pancreas weight (%)	0.23 $\pm$ 0.02 ^b	0.26 $\pm$ 0.02 ^{ab}	0.35 $\pm$ 0.03 ^a
Cecal digesta (g)	1.74 $\pm$ 0.19 ^b	2.15 $\pm$ 0.18 ^{ab}	4.07 $\pm$ 0.52 ^a
pH of cecal digesta	7.00 $\pm$ 0.12 ^a	5.91 $\pm$ 0.22 ^b	5.41 $\pm$ 0.18 ^b
Fecal dry weight (g/3 d)	3.17 $\pm$ 0.18	3.41 $\pm$ 0.10	2.70 $\pm$ 0.31

¹Values are means $\pm$ SEM, n=8. Means in a row with superscripts without a common letter (a, b) differ, $P < 0.05$ (Tukey-Kramer post-hoc test or Steel-Dwass test).

Table 4: Effect of dietary addition of 5% or 10% raw lily bulb on cecal and fecal mucins, IgA, and cecal microflora in rats fed a HF diet¹ (Experiment 1).

	HF	HF + 5% Raw lily bulb	HF + 10% Raw lily bulb
Cecal mucins (mg/ total wet cecal digesta)	0.55 $\pm$ 0.09 ^c	1.44 $\pm$ 0.20 ^b	6.10 $\pm$ 0.95 ^a
Fecal mucins (mg/3 d)	0.71 $\pm$ 0.13 ^c	5.01 $\pm$ 0.80 ^b	14.3 $\pm$ 1.5 ^a
Fecal mucinase (units/3 d)	1.68 $\pm$ 0.16	1.83 $\pm$ 0.15	1.32 $\pm$ 0.28
Cecal IgA (mg/total wet cecal digesta)	0.45 $\pm$ 0.10 ^c	1.89 $\pm$ 0.51 ^b	6.68 $\pm$ 2.44 ^a
Fecal IgA (mg/3 d)	1.07 $\pm$ 0.18 ^b	3.03 $\pm$ 0.51 ^a	7.42 $\pm$ 3.23 ^a
Cecal microflora	Occupation ratio of phylogenetic groups (%)		
<i>Lactobacillales</i>	7.18 $\pm$ 1.65 ^c	18.6 $\pm$ 3.3 ^b	32.7 $\pm$ 3.5 ^a
<i>Bifidobacterium</i>	0.089 $\pm$ 0.089 ^b	0.662 $\pm$ 0.151 ^a	1.032 $\pm$ 0.158 ^a
<i>Clostridium</i>	48.2 $\pm$ 2.8	51.7 $\pm$ 4.1	41.3 $\pm$ 4.4

¹Values are means $\pm$ SEM, n=8. Means in a row with superscripts without a common letter (a, b, c) differ, $P < 0.05$ (Tukey-Kramer post-hoc test or Steel-Dwass test).

< 0.05). These results suggest that raw lily bulbs have a favorable effect on intestinal barrier and immune functions. The fecal activity of mucinase was unaffected by dietary raw lily bulbs. The HF + 5% and HF + 10% raw lily bulb diets contained 0.99% and 1.98% dietary fibers, respectively. These levels are below those required to increase the level of IgA and mucins [7,8]. The lily bulb samples using in this study contained raw starch and saponins as well as dietary fibers. Raw starch has relatively low digestibility and it has been suggested that undigested starch may increase intestinal production of mucins and IgA [15]. Furthermore, our recent experiment [33] showed that dietary saponins increased fecal mucins and IgA levels in rats fed a HF diet. Therefore, the promoting effect of dietary lily bulb on IgA and mucins might be complexly related to its dietary fibers, undigested starch, and saponins. Further study is necessary to confirm this possibility.

The HF + 5% and HF + 10% raw lily bulb diets markedly increased the cecal ratio of *Lactobacillales* in a dose dependent manner ($P < 0.05$, Table 4). The ratio of *Bifidobacterium* in the cecum was also elevated by dietary supplementation of 5% and 10% raw lily bulb. The cecal ratio of *Clostridium* was not significantly different among the three groups. These results provide evidence that consumption of raw lily bulb has a prebiotic effect. Cecal *Lactobacillales* significantly correlated with cecal mucins ($R_s = 0.74$, $P < 0.001$), fecal mucins ($R_s = 0.71$, $P < 0.001$), cecal IgA ($R_s = 0.56$, $P < 0.005$), and fecal IgA ($R_s = 0.46$, $P < 0.05$). It has been previously reported that *lactobacilli* increases the expression of intestinal mucin and prevents attachment of pathogenic bacteria [34]. The administration of lactic acid bacteria as *Lactobacillus* has also been shown to stimulate the IgA immune response in the gut [35]. Taken together, these results suggest the possibility that higher ratio of *Lactobacillales* in the groups receiving lily bulb may at least in part contribute to increases in the levels of intestinal mucins and IgA.

Experiment 2

Because lily bulb is usually consumed after heating, we further examined whether heating treatment of lily bulb, as well as raw lily bulb, could affect colonic luminal environment in rats fed a high-fat diet. In experiment 1, the effect of HF + 10% raw lily bulb group on colonic luminal environment was prominent compared with that of the HF + 5% raw lily bulb group, whereas dietary 10 % lily bulb caused slight inhibitions of growth and food intake. Therefore, in the second experiment, the lily bulbs were added to HF diets at the level of 7% to avoid growth and food intake depressions. As expected, food intake and final body weight did not differ among the three groups (Table 5). Liver, pancreas, and fecal dry weights were also unaffected by dietary treatment. The wet weight of cecal digesta was markedly higher, and the pH of the digesta significantly lower in both the HF + raw and HF + steamed lily bulb groups than in the HF group ($P < 0.05$). Both the steamed and lily bulb diets caused markedly increased in cecal and fecal mucin content compared with that in the HF diet ($P < 0.05$, Table 6). In accordance with the experiment 1, fecal IgA was significantly increased in the HF + raw lily bulb group ($P < 0.05$), with a similar trend being observed in the HF + steamed lily bulb group. These results suggest that heating treatment of lily bulb have also a favorable effect on the intestinal barrier and its immune functions. Interestingly enough, the fecal activity of mucinase was significantly reduced in the HF + steamed lily bulb group compared with the HF group ($P < 0.05$, Table 6). The bacterial enzyme mucinase degrades the protective mucin coat in the gut wall, reduces barrier function, and therefore is considered a risk factor for colon cancer [12]. The results of our study suggest that the increase in the mucosal mucin barrier caused by the steamed lily bulb may be related, in part, to the inhibition of mucin degradation. Heat treatments of several vegetables have been reported to increase flavonoids contents and physiological activities [36-38]. Therefore, the enhanced flavonoids in steamed lily bulb might be associated with the suppression of mucinase activity. Further study is necessary to examine the molecular mechanism by which steamed lily bulb

Table 5: Effect of dietary addition of 7% raw and steamed lily bulb on body weight, food intake, cecal and fecal weight and serum inflammatory parameters in rats fed a HF diet¹ (Experiment 2).

	HF	HF + raw lily bulb	HF + steamed lily bulb
Initial body weight (g)	103 ± 3	103 ± 2	103 ± 2
Final body weight (g)	263 ± 3	259 ± 2	258 ± 6
Food intake (g/3 wk)	279 ± 0	277 ± 1	274 ± 5
Pancreas weight (%)	0.23 ± 0.02	0.26 ± 0.02	0.22 ± 0.01
Cecal digesta (g)	1.73 ± 0.09 ^b	2.51 ± 0.20 ^a	2.40 ± 0.22 ^a
pH of cecal digesta	6.90 ± 0.16 ^a	5.99 ± 0.19 ^b	6.20 ± 0.12 ^b
Fecal dry weight (g/3 d)	3.24 ± 0.13	3.23 ± 0.10	2.91 ± 0.15
Serm TNF-α (ng/L)	15.4 ± 0.8	14.0 ± 1.8	12.1 ± 1.5
Serum IL-6 (ng/L)	11.1 ± 0.3	11.8 ± 0.6	13.3 ± 1.0
Serum LBP (mg/L)	2.31 ± 0.34	2.26 ± 0.28	2.43 ± 0.30

¹Values are means ± SEM, n=8. Means in a row with superscripts without a common letter differ (a, b), $P < 0.05$ (Tukey-Kramer post-hoc test or Steel-Dwass test).

Table 6: Effect of dietary addition of 7% raw and steamed lily bulb on cecal and fecal mucins, IgA and cecal microflora in rats fed a HF diet¹ (Experiment 2).

	HF	HF + raw lily bulb	HF + steamed lily bulb
Cecal mucins (mg/total wet cecal digesta)	0.35 ± 0.03 ^b	1.09 ± 0.17 ^a	1.05 ± 0.27 ^a
Fecal mucins (mg/3 d)	0.81 ± 0.13 ^b	3.75 ± 0.72 ^a	2.63 ± 0.80 ^a
Fecal mucinase (units/g 3 d)	2.25 ± 0.15 ^a	2.13 ± 0.18 ^a	1.42 ± 0.23 ^b
Fecal IgA (mg/3 d)	0.69 ± 0.08 ^b	2.64 ± 0.34 ^a	1.69 ± 0.73 ^{ab}
Cecal microflora	Occupation ratio of phylogenetic groups (%)		
<i>Lactobacillales</i>	34.3 ± 2.0 ^b	47.1 ± 3.1 ^a	42.4 ± 3.2 ^{ab}
<i>Bifidobacterium</i>	ND	0.157 ± 0.073	0.060 ± 0.018
<i>Clostridium</i>	31.0 ± 2.0	32.5 ± 3.0	29.8 ± 2.9

¹Values are means ± SEM, n=8. Means in a row with superscripts without a common letter (a, b) differ, $P < 0.05$ (Tukey-Kramer post-hoc test or Steel-Dwass test). ND = not detectable

and its bioactive compounds suppress the enzyme activity, and to compare bacterial load in the raw and steamed lily bulbs.

The inflammatory cytokines, serum TNFα and IL-6 were both unaffected by the HF + raw and HF + steamed lily bulb diets (Table 5). The serum level of LBP, a pro-inflammatory LPS transporter used as a marker of endotoxemia [39], was also not different in the three groups. Therefore, the raw and steamed lily bulbs appear to modulate mucin and IgA levels in the gut without affecting inflammatory factors.

As in experiment 1, the HF + raw lily bulb diet significantly

increased cecal *Lactobacillales* ($P < 0.05$), with similar trend being observed in the HF + steamed lily bulb group (Table 6). *Bifidobacterium* was detected in the cecum of the groups consuming HF + raw and HF + steamed lily bulbs, whereas it was hardly detected in the HF group. The cecal content of *Clostridium* was not different among the three groups. The present study demonstrated that the heat treatment of lily bulb also can modulate the cecal microflora profile.

We have previously demonstrated that a HF diet decreases fermentation of organic acids in the rat cecum [20]. This decrease in intestinal fermentation leads to a higher risk for colon diseases [17,18]. Because the HF + raw and HF + steamed lily bulb diets increased the weight of cecal digesta and decreased the pH, we further investigated whether the bulbs affected cecal organic acids. The results demonstrated that both HF + raw and HF + steamed bulbs caused significant increases in cecal n-butyrate (+253% and +189%, respectively), succinate (+257% and +378%, respectively), lactate (+286% and +496%, respectively), and total organic acids (+169% and +174% respectively) per g wet cecal digesta ($P < 0.05$, Table 7). Dietary raw and steamed lily bulbs caused significant increase in cecal acetate (+201% and +163%, respectively), propionate (+161% and +171%, respectively), n-butyrate (+363% and +253%, respectively), lactate (+388% and +700%, respectively) and total organic acids (+242% and +245%, respectively) per total cecal digesta ($P < 0.05$). When the data were expressed relative to total cecal digesta, the increase in cecal succinate level was prominent in the HF + steamed lily bulb group (+556%). Because lactate and succinate are absorbed more slowly in the gut than other organic acids [40], the pH of cecal digesta is considerably lower, with this acidic environment favoring acid-resistant bacteria such as *lactobacilli* and *bifidobacteria*. Furthermore, organic acids such as butyrate, acetate, and propionate are also considered to play a key role in colonic health [13]. Dietary fibers and undigested starch have been reported to increase the levels of organic acids [11,13]. Kishida T, et al. [41] reported that a type of heat-moisture treated starch decreased enzymatic digestibility and increased the levels of cecal organic acids to the same degree as raw starch. Therefore, it is necessary to test whether dietary fiber and undigested starch in raw and steamed lily bulbs contribute to the observed effect on cecal organic acids.

Unexpectedly, the HF + steamed lily bulb diet caused a significant reduction in mesenteric adipose tissue weight ($P < 0.05$), whereas the HF + raw lily bulb diet did not cause any reduction (Table 8). The same tendency was observed for epididymal adipose tissue weight. Serum triglyceride, cholesterol and free fatty acids were unaffected by dietary treatment. Liver triglyceride, cholesterol, and the activities of glucose 6-phosphate dehydrogenase and malic enzyme were also unaffected. Therefore, these findings indicate that the suppressive effect of the steamed lily bulb on the adipose tissue weight cannot be explained by changes in hepatic lipogenesis. Consumption of steamed lily bulb significantly increased fecal excretion of triglyceride and neutral sterol ($P < 0.05$), but the HF + raw lily bulb diet did not. It is therefore possible that the reduction in the adipose tissue weight

Table 7: Effect of dietary addition of 7% raw and steamed lily bulb on cecal organic acids in rats fed a HF diet¹ (Experiment 2).

	HF	HF + raw lily bulb	HF + steamed lily bulb
Organic acids	(μmol/g wet cecal digesta)		
Acetate	31.3 ± 2.7 ^b	43.4 ± 2.0 ^a	37.5 ± 4.5 ^{ab}
Propionate	9.04 ± 0.93	10.06 ± 0.92	10.76 ± 1.06
n-butyrate	6.80 ± 1.07 ^b	17.18 ± 2.12 ^a	12.88 ± 1.50 ^a
Succinate	8.1 ± 1.5 ^b	20.8 ± 4.8 ^a	30.6 ± 6.4 ^a
Lactate	1.37 ± 0.27 ^b	3.92 ± 0.95 ^a	6.79 ± 1.91 ^a
Total organic acids	56.6 ± 4.7 ^b	95.4 ± 4.3 ^a	98.6 ± 5.9 ^a
	(μmol/total wet cecal digesta)		
Acetate	53.4 ± 4.9 ^b	107.3 ± 7.6 ^a	87.2 ± 9.3 ^a
Propionate	15.3 ± 1.3 ^b	24.7 ± 2.5 ^a	26.1 ± 3.3 ^a
n-butyrate	11.7 ± 1.8 ^b	42.5 ± 5.6 ^a	29.6 ± 3.3 ^a
Succinate	14.2 ± 3.0 ^b	51.5 ± 11.2 ^{ab}	78.9 ± 20.8 ^a
Lactate	2.28 ± 0.35 ^b	8.85 ± 1.40 ^a	15.96 ± 5.10 ^a
Total organic acids	97 ± 9 ^b	235 ± 14 ^a	238 ± 27 ^a

¹Values are means ± SEM, n=8. Means in a row with superscripts without a common letter (a, b) differ, $P < 0.05$ (Tukey-Kramer post-hoc test or Steel-Dwass test).

Table 8: Effect of dietary addition of 7% raw and steamed lily bulb on lipid status in rats fed a HF diet¹ (Experiment 2).

	HF	HF + raw lily bulb	HF + steamed lily bulb
Serum			
Triglyceride (mmol/L)	2.73 ± 0.38	2.47 ± 0.52	2.68 ± 0.98
Total cholesterol (mmol/L)	1.91 ± 0.07	1.87 ± 0.07	1.87 ± 0.10
HDL-cholesterol (mmol/L)	0.80 ± 0.03	0.84 ± 0.02	0.80 ± 0.03
VLDL + IDL + LDL-cholesterol ² (mmol/L)	1.08 ± 0.06	1.02 ± 0.01	1.04 ± 0.02
Atherogenic index ³	1.35 ± 0.08	1.22 ± 0.08	1.24 ± 0.13
Liver			
Triglyceride (μmol/g tissue)	208 ± 37	190 ± 34	232 ± 46
Cholesterol (μmol /g tissue)	26.6 ± 0.8	24.0 ± 1.0	23.0 ± 1.81
G6PD activity (units/g tissue)	2.54 ± 0.26	2.98 ± 0.36	2.58 ± 0.24
ME activity (units/g tissue)	3.36 ± 0.31	3.74 ± 0.32	3.80 ± 0.39
Adipose tissue weight (%)			
Mesenteric adipose tissue	1.21 ± 0.04 ^a	1.19 ± 0.04 ^{ab}	1.05 ± 0.05 ^b
Epididymal adipose tissue	1.41 ± 0.06 ^{ab}	1.58 ± 0.09 ^a	1.26 ± 0.09 ^b
Feces			
Triglyceride (μmol /3 d)	3.15 ± 0.29 ^b	5.11 ± 0.64 ^{ab}	5.35 ± 0.66 ^a
Neutral sterol (μmol /3 d)	29.2 ± 3.6 ^b	31.5 ± 2.1 ^{ab}	38.8 ± 3.4 ^a

¹Values are means ± SEM, n=8. Means in a row with superscripts without a common letter (a, b) differ, $P < 0.05$ (Tukey-Kramer post-hoc test).

²VLDL + IDL + LDL-cholesterol were calculated as total cholesterol minus HDL-cholesterol.

³Atherogenic index was calculated as (total-cholesterol minus HDL-cholesterol) /HDL-cholesterol

G6PD = Glucose 6-Phosphate Dehydrogenase; ME = Malic Enzyme.

associated with the dietary steamed lily bulb may at least be mediated by a reduction in lipid absorption. Several studies have reported that heat treatments enhance flavonoids contents in vegetables [36,37]. Dietary flavonoids are known to suppress fat accumulation in obese animal [42]. Therefore, it is of interest to test the possibility that steam treatment may increase flavonoids contents in lily bulb, and the increase may be associated with the reduction of adipose tissue weight.

It has been recently suggested that obesity is related to dysbiosis of the gut microbiota [43]. Several studies [44,45] have shown a higher ratio of *Firmicutes* and reduction in the ratio of *Bacteroidetes* in overweight and obese animals than among lean animals. Further study is in progress in rats fed a HF diet to test whether dietary supplementation with steamed lily bulbs modulates the intestinal ratios of *Firmicutes* and *Bacteroidetes*, and whether this modulation is related to a decrease in the adipose tissue weight.

Conclusion

This study provides first evidence that dietary lily bulb increases the levels of intestinal mucin, IgA, cecal *Lactobacillales* and organic acid fermentation. An interesting finding in this study of rats fed a HF diet is that steam treatment of the lily bulbs significantly reduced mesenteric adipose tissue weight and fecal mucinase activity, a risk factor for colon cancer. These findings suggest that lily bulb can be considered as a novel functional food for colon health. Further studies are in progress to isolate the dietary fiber, low digestible starch, flavonoids, and saponins from lily bulb that may be responsible for the functions described in this study.

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