Research Note

Consumption of Lily Bulb Modulates Fecal Ratios of Firmicutes and Bacteroidetes Phyla in Rats Fed a High-Fat Diet

Yukako Okazaki*, Ayaka Sekita, Hideyuki Chiji, and Norihisa Kato¹

Faculty of Human Life Sciences, Fuji Women's University, 4-5 Hanakawa Minami, Ishikari, 061-3204, Japan

¹Graduate School of Biosphere Science, Hiroshima University, 1-4-4, Higashi-Hiroshima, 739-8528, Japan

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*Corresponding Author
Tel: +81-133-74-7498
Fax: +81-133-74-7498
E-mail: yokazaki@fujijoshi.ac.jp

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Abstract It has been recently suggested a high ratio of fecal Firmicutes and reduction in the Bacteroidetes in obese animals. This study investigated the effect of dietary lily bulb (LB) on fecal Firmicutes and Bacteroidetes in rats fed a high-fat (HF) diet. In experiment 1, rats were fed an HF diet with or without 7% raw (R) or steamed (S) LB. In experiment 2, rats were fed the HF diet with or without 7% RLB, 0.9% ethanol extract of LB, or 6.1% ethanol extract residue of LB. In experiment 1, fecal Firmicutes was reduced and Bacteroidetes was increased in both the RLB and SLB groups. In experiment 2, the fecal Firmicutes/Bacteroidetes ratio was not affected by the ethanol extract or ethanol extract residue of LB. These results suggest that LB in its entirety modulates colonic microflora, regardless of heat treatment.

Keywords: lily bulb, high-fat diet, fecal microflora, colonic environment, rats

Introduction

Maximowicz's lily (*Lilium leichtlinii* var. *maximowiczii* Baker) belongs to the genus *Lilium* of the family Liliaceae, and its bulbs have been traditionally consumed in Asia. Edible lily bulb (LB) contains dietary fibers, flavonoids, and saponins and is also rich in nutrients such as starch and protein (1-5). Of particular note is that LB has been reported to contain approximately 20% high-molecular weight (230 kDa) glucomannan per dry weight (1). We have recently observed that dietary LB considerably increased the levels of colonic immunoglobulin A, mucin (indices of intestinal immune and barrier functions), cecal organic acids such as lactate, propionate, and butyrate, and the ratios of Lactobacillales and *Bifidobacterium* in high-fat (HF) diet-fed rats (6), suggesting that LB is beneficial for colon health.

There is accumulating evidence to support the notion that intestinal microbiota play a significant role in the maintenance of health (7-9). It has been reported that an HF diet induces obesity and is associated with the modulation of the composition of gut microbiota (10). An HF diet is said to reduce fecal Bacteroidetes, increase the Firmicutes, and modify the relative proportion of Firmicutes to Bacteroidetes (11). Studies (11,12) have shown a higher ratio of Firmicutes and reduction in the ratio of Bacteroidetes in overweight and obese animals than among lean animals. Our previous study demonstrated that the consumption of LB, in addition to the beneficial effect on colon health, reduces adipose tissue weight in rats fed an HF diet (6).

In the present study, we assessed the effect of dietary LB on the fecal ratio of Firmicutes to Bacteroidetes phyla in rats fed an HF diet.

Materials and Methods

Preparation of the LB LBs were purchased from JA Youtei (Hokkaido, Japan). The cloves of raw (R) LB were peeled and freeze dried. In experiment 1, this lyophilized sample was powdered. Steamed (S) LB powder was prepared as follows: the LB cloves were peeled and steamed for 25 min using an electric steamer (T-fal Simply Smart; Groupe SEB Japan Co., Ltd., Tokyo, Japan). The steamed sample was powdered after being freeze dried. In experiment 2, RLB powder was extracted using 70% ethanol. The supernatant of ethanol extract was concentrated and freeze dried. The extract residue was also dried. The composition of the LB and ethanol extract residue powders is shown in Table 1.

Animals and diets Four-week-old male Sprague Dawley 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 12/12 h light/dark cycle (light, 08:00-20:00 h). The rats were acclimated by feeding a nonpurified commercial rodent powder diet

Table 1. Chemical composition of LB powder (Unit: %, w/w)

Composition	LB powder	Ethanol extract residue powder
Carbohydrate ²⁾	62.3 ¹⁾	60.8
Resistant starch	1.7	5.6
Protein ³⁾ (Nx6.25)	8.6	8.4
Fat ⁴⁾	0.9	0.5
Dietary fibers ⁵⁾	23.3	24.8
Fructan	0.3	0.1
Ash ⁶⁾	3.5	3.9

¹⁾Duplicate analysis by The Foundation of Japan Food Research Laboratory (Chitose, Japan).

²⁾100 g–(protein+fat+dietary fibers+ash+water).

³⁾Kjeldahl method.

⁴⁾Soxhlet method.

⁵⁾Enzyme-gravimetric-HPLC method (AOAC, Method 2001.03)

⁶⁾Direct incineration method

(CE-2; CLEA Japan Inc., Tokyo, Japan) for 6 days before commencing the experimental diet. Composition of the basal diet was as follows: 30% beef tallow, 20% casein, 0.3% L-cystine, 5% cellulose, 30% sucrose, 1% vitamin mixture (13), 3.5% salt mixture (12), and 10.2 % corn starch. In experiment 1, after acclimatization, the rats were divided into three groups of eight rats each and assigned to an HF (30%, w/w) diet, with or without 7% RLB or SLB powder. In experiment 2, after acclimatization, the rats were divided into four groups of eight rats each and assigned to an HF (30%, w/w) diet with or without 7% RLB, 0.9% ethanol extract of RLB, or 6.1% ethanol extract residue of LB. The levels of dietary carbohydrate, protein, fat, minerals, and fibers in the LB diets were adjusted by reducing the amount of dietary corn starch, casein, beef tallow, and cellulose. To prevent differences 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 days 14–21) (14). The entire amount of food provided was consumed each day before the next day's food was provided. The weight of spilled diet was recorded daily and included in the calculation of food intake. Feces were collected for 3 days at the end of weeks 1, 2, and 3. The feces were stored at –20°C and then freeze dried and milled. At the end of the feeding period, the rats were anesthetized using sodium pentobarbital and then killed by exsanguination through the abdominal aorta. The cecum and colon were removed, weighed, frozen immediately with liquid nitrogen, and stored at –80°C.

Measurements Bacterial genomic DNA was isolated from the feces using the QIAamp DNA Stool Mini Kit (Quiagen, Venlo, the Netherlands). Phylum-level analysis was performed on Bacteroidetes, Firmicutes, and all bacteria combined using a slight modification of the method described by Guo *et al.* (15). Total DNA was diluted 100-fold to reduce polymerase chain reaction (PCR) inhibition. The relative ratio of Bacteroidetes and Firmicutes to all bacteria was quantified based on the amplification of genomic DNA coding 16S ribosomal RNA (rRNA) using real-time quantitative PCR (qPCR) with a Light Cycler

480 System II (Roche Applied Science, Penzberg, Germany) and group-specific primers (14). For the analysis of Firmicutes, the primers Firm934F (5'-GGAGYATGTGGTTTAATTCGAAGCA-3') and Firm1060R (5'-AGCTGACGACAACCATGCAC-3') were used. For the analysis of Bacteroidetes, we used the primers Bact934F (5'-GGARCATGTGGTTTAATTCGATGAT-3') and Bact1060R (5'-AGCTGACGACAACCATGCAG-3'). For the analysis of all bacteria, forward (5'-ACTCCTACGGGAGG CAGCAGT-3') and reverse (5'-GTATTACCGCGGCTGCTGGCAC-3') primers were used. Real-time qPCR was performed in a reaction volume of 20 µL containing 10 µL SYBR Premix Ex Taq II (Takara Bio Inc., Shiga, Japan), 300 nM each of the forward and reverse primers, and 2 µL DNA samples. After initial denaturation at 95°C for 30 s, 40 PCR cycles were conducted with denaturation at 95°C for 5 s, annealing at 60°C for 30 s, and extension at 72°C for 15 s. Melting curve analysis was performed after amplification to distinguish between the targeted and non-targeted PCR products. Data were analyzed by the second derivative maximum method of the LightCycler 480 Basic Software. The relative abundances of the microbial populations are expressed as the proportions of total bacterial 16S rDNA gene.

Statistical analyses Data were expressed as means±SEM. For data that followed normal distribution, we used one-way analysis of variance (ANOVA). Data that did not follow the normal distribution were analyzed using the nonparametric Kruskal-Wallis one-way ANOVA. The Tukey post hoc test was performed when a significant effect was detected using one-way ANOVA. The Steel-Dwass post hoc test was performed when a significant effect was detected using the Kruskal-Wallis one-way ANOVA. Data analysis was performed using the Excel statistics 2012 software for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan). *P* values (<0.05) were considered statistically significant.

Results and Discussion

In experiment 1, mean final body weights of the control group, RLB group, and SLB group were 263±3, 259±2, and 258±6 g, respectively. The body weight and food intake (data not shown) did not differ among the three groups of rats. Cecal weight was significantly increased by the RLB and SLB diets (data not shown). Because LB is usually consumed after heating, we examined whether SLB as well as RLB could affect fecal microflora in rats fed an HF diet. Fecal ratio of Firmicutes was significantly reduced in the RLB group compared with the control group in weeks 1 and 2 (*p*<0.05, Table 2), and a similar trend was observed in the SLB group. These decreasing effects of dietary RLB and SLB on fecal Firmicutes were most evident in week 3 (*p*<0.05). Fecal ratio of Bacteroidetes was significantly increased by SLB intake in weeks 1 and 2 (*p*<0.05) and a similar result was observed in the RLB group. In week 3, these increasing effects of dietary LB on fecal Bacteroidetes were most evident (*p*<0.05). Fecal relative proportion of Firmicutes to Bacteroidetes is an indicator of

Table 2. Effects of dietary 7% RLB or SLB powder on the profile of fecal microflora in rats fed an HF diet for 3 weeks (Experiment 1)

	Control	RLB ²⁾	SLB
Fecal microflora			
week 1			
Firmicutes (%)	52.4±1.5 ^{a1)}	44.6±1.6 ^b	47.9±1.5 ^{ab}
Bacteroidetes (%)	6.99±0.88 ^b	8.97±1.59 ^{ab}	15.94±3.41 ^a
Firmicutes/Bacteroidetes	8.82±1.79	6.06±0.96	4.50±1.04
week 2			
Firmicutes (%)	57.7±1.5 ^a	45.4±1.6 ^b	49.7±1.8 ^{ab}
Bacteroidetes (%)	6.93±0.76 ^b	10.19±1.11 ^{ab}	12.83±2.66 ^a
Firmicutes/Bacteroidetes	8.18±0.75 ^a	5.14±0.96 ^{ab}	5.05±0.86 ^b
week 3			
Firmicutes (%)	58.4±1.1 ^a	49.5±1.8 ^b	43.3±3.6 ^b
Bacteroidetes (%)	5.83±0.36 ^b	11.75±2.01 ^a	21.38±3.06 ^a
Firmicutes/Bacteroidetes	10.40±0.91 ^a	5.27±0.98 ^b	2.86±0.97 ^b

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

²⁾RLB, raw lily bulb; SLB, steamed lily bulb.

major changes in microflora constitution and is known to increase based on HF diet and obesity (12). It has been reported that obese leptin-deficient (ob/ob) mice, which are efficient at harvesting energy from food, showed a 50% reduction in the Bacteroidetes and an increase in Firmicutes (11). In this study, the fecal ratio of Firmicutes to Bacteroidetes in HF-fed rats was significantly reduced by RLB and SLB diets in week 3 ($p<0.05$). In addition, we have previously demonstrated that the consumption of LB suppressed the accumulation of fat tissue in rats fed an HF diet (6). Further study is necessary to examine the association between the modulating effect of LB on gut microflora and the attenuation of fat accumulation.

We previously found that the consumption of LB elevated the cecal ratio of Lactobacillales in rats fed an HF diet (6). Lactobacillales belong to the Firmicutes phylum, and several Lactobacillus species are reported to be effective in improving lipid metabolism (16). Therefore, these studies raise a question of whether the reduced fecal Firmicutes by dietary LB is associated with a decrease in other bacteria, except for Lactobacillales. Metagenomics methods should be used in the future to examine the composition of Firmicutes in detail.

Table 3. Effects of dietary 7% RLB powder, 0.9% the ethanol extracts, and 6.1% the ethanol extracts residue of RLB on body weight, food intake, and the profile of fecal microflora in rats fed an HF diet for 3 weeks (Experiment 2)

	Control	RLB ³⁾	Ethanol extract of RLB	Ethanol extract residue of RLB
Final body wt (g)	260±2 ¹⁾	262±1	261±2	262±2
Food intake (g/3 weeks)	279±0	279±0	279±0	279±0
Fecal microflora				
Firmicutes (%)	50.5±2.7	47.7±2.0	51.1±2.3	48.4±2.3
Bacteroidetes (%)	9.68±1.95 ^{b2)}	20.1±1.8 ^a	11.3±1.8 ^b	14.0±1.7 ^{ab}
Firmicutes/Bacteroidetes	7.24±1.51 ^a	2.26±0.24 ^b	5.48±0.91 ^a	4.10±0.88 ^{ab}

¹⁾Values are means±SEM (n=8).

²⁾Means in a row with superscript without a common letter differ, $p<0.05$ (Tukey-Kramer post-hoc test or Steel-Dwass test).

³⁾RLB, raw lily bulb.

In experiment 2, final body weight and food intake did not differ among the four groups of rats (Table 3). Fecal Firmicutes did not differ among the groups. The fecal Bacteroidetes ratio was significantly elevated in the LB groups but not in the ethanol extract or the ethanol extract residue of LB group. Relative proportion of Firmicutes to Bacteroidetes in feces was significantly lower in the LB group than in the control group ($p<0.05$), although this proportion was not significantly affected by the ethanol extract or ethanol extract residue of LB. The powdered ethanol extract of LB contains mainly flavonoids and saponin (2,5) and the residue contains high amounts of glucomannan. Prebiotic fibers are known to alter Firmicutes and Bacteroidetes in obese mice (17). Therefore, we can speculate that the effect of LB on the fecal Firmicutes/Bacteroidetes ratio is mainly because of the multiple effects of the ethanol extract and the residue fractions, not because of a single integrant.

In conclusion, this study provides first evidence that dietary LB, as a whole food, may modulate colonic microflora in rats fed an HF diet, regardless of heat treatment. Further study is in progress to investigate the effect of a practical level of dietary LB on gut microflora in rats fed an HF diet for a longer period.

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References

- Wozniowski T, Blaschek W, Franz G. Physiologically active polysaccharides from *Lilium testaceum*: Isolation and structural investigation. *Planta Med.* 55: 638 (1989)
- Satou T, Mimaki Y, Kuroda M, Sashida Y, Hatakeyama Y. A pyrrolidine glucoside ester and steroidal saponins from *Lilium martagon*. *Phytochemistry* 41: 1225-1230 (1996)
- Mullin WJ, Peacock S, Loewen DC, Turner NJ. Macronutrients content of Yellow Glacier Lily and Balsamroot; Root vegetables used by indigenous peoples of northwestern North America. *Food Res. Int.* 30: 769-775 (1997)
- Shin KS, Chakrabarty D, Paek KY. Sprouting rate, change of carbohydrate contents and related enzymes during cold treatment of lily bulb regenerated *in vitro*. *Sci. Hortic.* 96: 195-204 (2002)
- Jin L, Zhang Y, Yan L, Guo Y, Niu L. Phenolic compounds and antioxidant activity of bulb extracts of six *Lilium* species native to China. *Molecules* 17: 9361-9378 (2012)

6. Okazaki Y, Fukuda E, Chiji H, Kato N. Edible lily bulb modulates colonic barrier functions, microflora and fermentation in rats fed a high-fat diet. *J. Nutrit. Health Food Sci.* 2: 1-7 (2014)
7. Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: New insights into the gut microbiota. *Curr. Opin. Pharmacol.* 9: 737-743 (2009)
8. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* 125: 1401-1412 (1995)
9. Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Macé K, Chou CJ. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J.* 22: 2416-2426 (2008)
10. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 299: 440-448 (2010)
11. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *P. Natl. Acad. Sci. USA* 102: 11070-11075 (2005)
12. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: Human gut microbes associated with obesity. *Nature* 444: 1022-1023 (2006)
13. Reeves PG, Nielsen FH, Fahey GC Jr. 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.* 123: 1939-1951 (1993)
14. Okazaki Y, Tomotake H, Tsujimoto K, Sasaki M, Kato N. Consumption of a resistant protein, sericin, elevates fecal immunoglobulin A, mucins, and fecal organic acids in rats fed a high-fat diet. *J. Nutr.* 141: 1975-1981 (2011)
15. Guo X, Xia X, Tang R, Zhou J, Zhao H, Wang K. Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. *Lett. Appl. Microbiol.* 47: 367-373 (2008)
16. Ritze Y, Bardos G, Claus A, Ehrmann V, Bergheim I, Schwierz A, Bischoff SC. *Lactobacillus rhamnosus* GG protects against non-alcoholic fatty liver disease in mice. *PLoS ONE* 9: e80169 (2014)
17. Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR: LA-cp rats. *Brit. J. Nutr.* 107: 601-613 (2012)