

Consumption of young barley leaf extract increases fecal short-chain fatty acid levels: a before-after clinical trial

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Abstract

Short-chain fatty acids (SCFAs) bacterially produced in the intestine provide a variety of physiological effects for the host. The present before-after clinical trial was conducted to investigate the effects of young barley leaf extract (YBL) on fecal SCFA levels. For 4 weeks, female health subjects were asked to ingest two sticks (8 g) of test sample daily. Feces were collected before and after the period of treatment with YBL. Results demonstrated that YBL significantly elevated the fecal concentrations of acetate from 23.6 ± 7.5 to 36.4 ± 8.1 $\mu\text{mol/g}$ ($p < 0.001$) and propionate from 10.0 ± 5.2 to 13.6 ± 7.1 $\mu\text{mol/g}$ ($p < 0.05$) but, did not give an advantage in fecal bacterial composition. Interestingly, YBL also raised the fecal moisture by 3.9% point from the baseline ($p < 0.05$).

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1. Introduction

Intestinal microbial flora is well recognized as a mediator for health and disease. Recent evidence suggests that the microbiome may affect the host's physiological functions of energy metabolism, immune regulation, and brain function (Clemente *et al.*, 2012). There is emerging evidence that types of microbial metabolites directly influence such a variety of physiological functions (Lee and Hase, 2014). They are capable of incorporating from the intestinal environment into the blood circulation, interacting with relevant tissues. Short-chain fatty acids (SCFAs) are physiologically active byproducts primarily produced from the fermentation of soluble dietary fiber and resistant starch by commensal bacteria in the gut. Parts of bacterially produced SCFAs are utilized as an energy substrate for the host's enterocyte, another part is directly incorporated into the blood circulation, and the other part is excreted in the feces. Current literature highlight that microbiota-derived SCFAs function as signaling molecules to the host, representing a key link between diet, microbiome and health (Schroeder and Bäckhed, 2016; Alexander *et al.*, 2019).

In light of the physiological importance of SCFAs, the search for the dietary factors that increase the formation of SCFAs and/or shape intestinal microbial structure becomes the focus of scientific interest. Young

barley leaf extract (YBL), commonly referred to as "AOJIRU", is widely consumed in Japan, primarily serving as a natural source of some kinds of vitamins, minerals and dietary fibers. Our previous animal model study showed that diet treatment with YBL significantly increased the cecal levels of SCFAs and decreased the ratio of Firmicutes/Bacteroidetes, as determined by terminal restriction fragment length polymorphism (T-RFLP) patterns for fecal 16S ribosomal RNA gene, suggesting that YBL influences positively on gut health (Unno *et al.*, 2016). The next question arises to settle whether or not the consumption of YBL alters gut microbiota and the formation of SCFAs in human subjects. Therefore, we had conducted a before-after trial of investigating the effects of YBL on the microbial composition and fecal SCFA levels in female humans.

2. Materials and methods

2.1 Test sample

Test samples were supplied from Yakult Health Foods, Co., Ltd. (Tokyo, Japan). The extracting juice from young barley leaf was mixed with digestible dextrin, and then spray-dried to be a granular powder. Powdered material of 4 g was packaged in a stick of plastic bag coated with aluminum film. Subjects daily ingested 2 sticks of the test sample. Energy value and the composition of nutrients per daily intake (8 g) of test

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sample were as follows; energy, 29.6 kcal; proteins, 0.88 g; lipids, 0.14 g; carbohydrates, 6.38 g; soluble dietary fiber 0.21 g; insoluble dietary fiber 0.13 g.

2.2 Subjects and experimental design

A total of fourteen female subjects who had self-reported constipation were recruited among students from Tokyo Kasei Gakuin University. None of them was a habitual drinker of YBL. Criteria for exclusion were lactose intolerance, habitual consumption of probiotics or prebiotics, use of drugs that influence intestine motility, taking an anticoagulant, any historical or current diagnosis of large bowel disease. Subjects who enrolled in the study spent 2 weeks of a non-treatment period of time and subsequently, start a treatment period to ingest the YBL samples for 4 weeks. During the treatment period, they were daily required to consume 2 sticks of test sample suspended in water. Throughout the experimental period, they were also advised to maintain their daily physical activity, lifestyle, and sleeping habits, and were required to record drug use if any. In a single day on the final 3 days both of the non-treatment period and the treatment period, a part of fresh stools was individually collected in a plastic case (TechnoSuruga Laboratory, Co. Ltd., Shizuoka, Japan), and immediately sent to the laboratory. Stool samples were stored at -40°C until analysis. The study protocol was approved by the ethics committee of Tokyo Kasei Gakuin University (approved number 30-1). All subjects gave informed written consent before enrollment.

2.3 SCFA concentrations

The extraction of SCFAs of fecal samples was carried out according to the method of García-Villalba *et al.* (2012). The quantification of fecal SCFA (acetate, propionate, *n*-butyrate, *iso*-butyrate, *n*-valerate, *iso*-valerate and caproate) was developed by gas chromatography (GC) equipped with a flame ionization detector. Compounds were separated on a DB-WAXetr capillary column (30 m \times 0.25 mm i.d., 0.25 μm film thickness, Agilent Technology, Inc. USA) with the same type of guard column (5 m \times 0.25 mm i.d., 0.25 μm film thickness). The GC oven temperature was programmed with 50°C , firstly a $10^{\circ}\text{C}/\text{min}$ rate until 90°C , and secondly a $15^{\circ}\text{C}/\text{min}$ rate until 150°C , thirdly a $5^{\circ}\text{C}/\text{min}$ rate until 170°C , finally a $20^{\circ}\text{C}/\text{min}$ rate until 250°C , holding final temperature for 4 min. The temperature of injector rapidly increased from 50 to 250°C at a $250^{\circ}\text{C}/\text{min}$ rate. The temperature of the detector was set at 250°C . High purity helium was used as the carrier gas at 1.2 mL/min. 4-Methylvalerate was used as an internal standard.

2.4 Bacterial composition

Fecal bacterial composition was analyzed by targeting the bacterial 16S rRNA genes using a T-RFLP technique according to the procedure described by Nagashima *et al.* (2006). Fragment sizes were assigned to categories of operational taxonomic units (OTU). The OTU data were used to identify the phylotypes by matching to those predicted from various phylotypes in the literature.

2.5 Fecal moisture content

A portion of each fecal specimen was weighed and dried at a constant temperature of 80°C for 16 hrs, and then re-weighed. Fecal moisture was calculated based on the amount of weight loss through evaporation.

2.6 Diet survey

To compare the energy and nutrient intakes of an individual before and after YBL treatment, food frequency questionnaire based on food groups (FFQg) was used (Excel Eiyokun FFQg software version 8.0, Kenpakusha, Tokyo, Japan).

2.7 Statistics

The statistical analysis was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA). All data were represented as mean values and standard deviations. A comparison of characteristics of subjects between before and after treatment was performed by paired t-test. For evaluation of the difference in percentage composition of fecal microbiota, the Wilcoxon signed-rank test was adopted. Differences were considered significant at $p < 0.05$.

3. Results and discussion

During the study period, two of the enrolled subjects were excluded. One took a prescribed antibacterial drug, and the other hoped voluntarily to withdraw from the study. Accordingly statistical analyses were performed with the data of 12 subjects. Similar dietary intakes of energy, proteins, lipids, carbohydrates were observed during baseline and the intervention periods (Table 1). A significant difference was found only for insoluble dietary fiber.

The sum of acetate, propionate, *n*-butyrate, *iso*-butyrate, valerate, *iso*-valerate and caproate of the feces collected before YBL treatment averaged 48.5 ± 22.0 $\mu\text{mol}/\text{g}$ wet mass (Table 2). The mean proportion of 3 major components, namely acetate, propionate and *n*-butyrate, to total SCFAs was 52, 20 and 16%, respectively. Feces collected after 4 weeks of YBL

treatment contained higher levels of SCFAs. The total SCFA level rose to $66.3 \pm 22.3 \mu\text{mol/g}$ ($p < 0.01$). There were significant increases in fecal concentrations of acetate ($p < 0.001$) and propionate ($p < 0.05$) between before and after YBL treatment. These components contributed much to the statistical significance of the increment of the total SCFA level. Needless to say, the increments of fecal SCFAs shall be associated with the process of bacterial fermentation in the intestine. Consequently, we tried to compare the fecal bacterial composition before and after 4 weeks of YBL treatment. In spite of increasing in SCFA concentrations, the bacterial composition remained unaltered (Figure 1). Consumption of YBL for 4 weeks had little influence on the bacterial composition in feces of female subjects. Provided that SCFAs are generated from dietary fibers and indigestible carbohydrate by an extensive set of enzymes of gut bacteria, it can be potentially explained that increment of fecal SCFAs by YBL treatment was a consequence of that dietary fibers in YBL were utilized fermentatively with the production of SCFAs in the large intestine. Previous literature by Saito *et al.* (2005) showed that insoluble dietary fiber from young barley had little impact on cecal SCFA levels in a rat model. Given that the soluble type of dietary fibers generally tends to be highly fermentable, it is reasonable to say that the soluble type of dietary fibers from YBL might contribute to the increments of fecal SCFAs. The amount of dietary fibers consumed daily from the test sample was 0.34 g as measured by the enzyme-HPLC method, of which soluble type was 0.21 g. FFQg revealed daily intake of soluble fiber from the diet was 2.2 g at baseline period, implying that subjects received additional soluble dietary fibers from YBL equivalent to approximately 10% of total soluble dietary fiber from the diet. Interestingly, the daily consumption of YBL during the 4 weeks significantly also raised the fecal moisture by 3.9% point from the baseline (Table 2). The previous study by Siigur *et al.* (1994) found a positive correlation between fecal SCFAs (acetate, propionate and *n*-butyrate) and fecal moisture. In their literature, it is supposed that both the fecal SCFAs and moisture content are largely related to functions of bowel transit, with less water and SCFAs being absorbed during quicker transit, greater water and SCFAs being eventually excreted in feces. It makes sense that consumption of YBL might accelerate bowel movement, and reduced transit time, consequently, improved fecal moisture content and SCFA levels. However, in the present study, the effects of YBL on transit time and fecal bulk were not determined.

Since the majority of SCFAs produced in the intestine could be rapidly absorbed, it is unclear whether fecal SCFAs residue reflects the absolute amount of

luminal SCFA production. However, clinical trials have largely relied on fecal concentrations of SCFAs for the potential marker of healthy status (Verbeke *et al.*, 2015; Ríos-Covián *et al.*, 2016). There is emerging evidence that the diet-induced compositional changes in gut microbiota lead to variations in SCFAs. Our results presented that consumption of YBL for 4 weeks brought about significant increases in fecal SCFAs without altering the bacterial composition. It is likely to be due to soluble dietary fibers from YBL reaching the large intestine where inhabiting bacteria utilized them as fermentation substrates. On the other hand, it remains controversial that fecal excretion of SCFAs might increase as the results of reducing the rate of intestinal absorption of microbially produced SCFAs via shortening the transit time (Ikeguchi *et al.*, 2014). Indeed, there is a report documenting young barley leaf powder could shorten gastrointestinal transit time in a rat model. Shortening transit time might limit the intestinal absorption of SCFAs, and consequently, increase fecal excretion of SCFAs.

Table 1. Energy and nutrients intake of subjects

	Before	After	<i>p</i> value
Energy (kcal)	1609±314	1598±202	0.872
Water (g)	719±174	700±133	0.356
Proteins (g)	57±17	54±10	0.199
Lipids (g)	55±16	56±13	0.767
Carbohydrate (g)	208±31	207±27	0.937
Soluble dietary fiber (g)	2.2±0.5	2.3±0.4	0.072
Insoluble dietary fiber (g)	6.6±1.1	7.1±1.0	0.049
Magnesium (mg)	177±47	182±30	0.472

Values are represented mean ± SD (n = 12).

Table 2. Moisture and SCFA levels in feces collected before and after YBL treatment.

	Before	After	<i>p</i> value
Moisture (%)	70.1±7.5	74.0±6.6	0.035
SCFAs ($\mu\text{mol/g}$)			
Total	48.5±22.0	66.2±22.3	0.009
Acetate	23.6±7.5	36.4±8.1	<0.001
Propionate	10.0±5.2	13.6±7.1	0.047
<i>n</i> -Butyrate	8.4±5.8	9.9±6.3	0.295
<i>iso</i> -Butyrate	1.9±1.4	1.8±1.3	0.85
<i>n</i> -Valerate	1.6±1.3	1.8±1.6	0.607
<i>iso</i> -Valerate	2.9±2.4	2.7±2.2	0.749
Caproate	0.1±0.2	0.2±0.3	0.218

Values are represented mean ± SD (n = 12).

YBL also gain attention on its beneficial health effect on lowering plasma lipids, inhibiting LDL oxidation (Yu *et al.*, 2004) and inhibiting postprandial plasma glucose (Takano *et al.*, 2013). At the present stage, it is not known how significant changes in fecal levels of SCFAs and moisture link to the potential

physiological benefit of consuming YBL. This clinical trial is preliminary, small sample size, and not a placebo-controlled. In order to establish the efficacy of the present YBL product to exert physiological effects via the increase in SCFA production, further studies under controlled conditions are needed for the appropriate amount of YBL daily consumed, the length of the ingesting period, and the dietary habits of subjects.

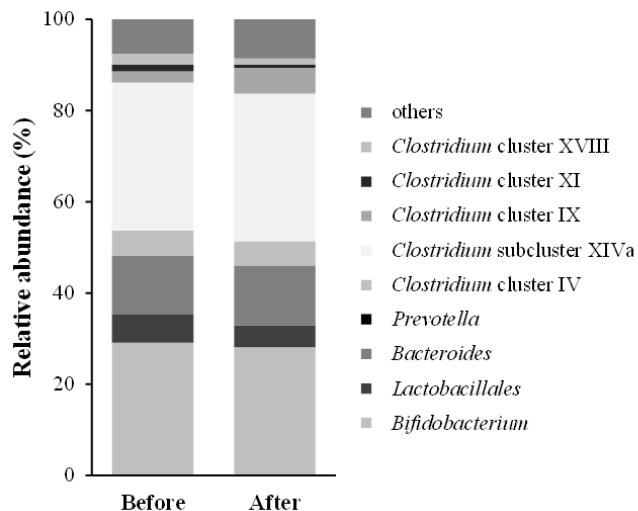


Figure 1. Relative abundance of fecal microbiota before and after YBL treatment. Fecal microbiota before and after YBL treatment was measured by a T-RFLP technique. Values are represented as mean from 12 subjects.

Conflict of Interest

This study was funded by Yakult Health Foods Co. Ltd.

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