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Changes in Microbiota and Short-Chain Fatty Acids Following 3-Month Pilot Intervention Study Feeding Brown Rice Ball (Omusubi) to Healthy Volunteers

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Abstract

Background: The health effects of brown rice are still in debate. From September to December 2019, 30 employees in the Ministry of Agriculture, Forestry, and Fisheries conducted the pilot intervention study on bowel movement, intestinal microbiota, fecal short-chain fatty acids, and inflammatory biomarkers to see the health effects.

Subjects and Methods: Brown rice genmai onigiri (rice cake) was provided 5/week as a business lunch for 12 weeks. Participants practiced the pre- and post-questionnaires, daily life records, monthly blood pressure measurements, and body composition. Before and after the intervention, the fecal samples were used for the simultaneous measurement of intestinal microbiota and short-chain fatty acids. Biochemical data involving IL-6, CRP, and TNFa were obtained for correlation analysis with microbiota changes.

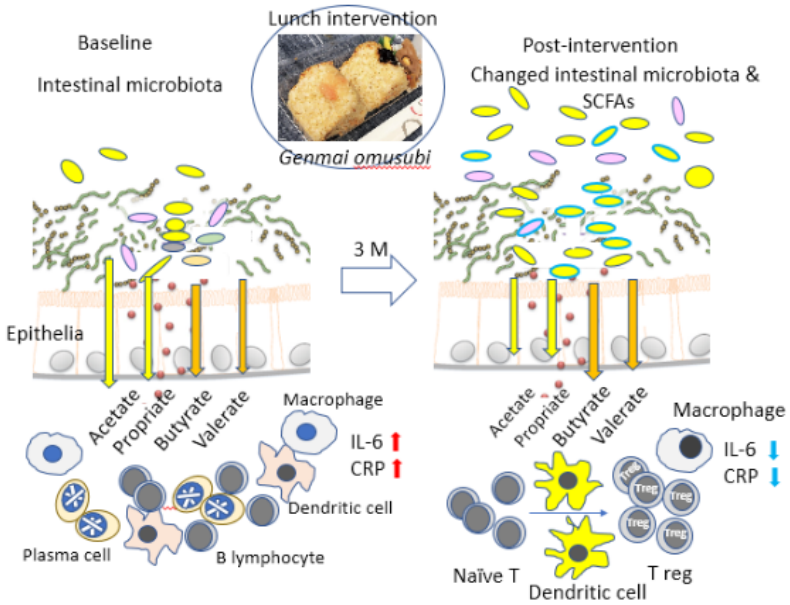
Results and Discussion: The body weight decreased in about half the participants, and bowel movements and stool status improved significantly. Dominant microbiota were Firmicutes (around 65%), Actinobacteria (15-17%), Bacteroidetes (5-7%), and less than 1% of Proteobacteria, Verrucomicrobia, and Fusobacteria. Significant microbiota change was an increase in Actinobacteria and a decrease in Proteobacteria. Verrucomicrobia and Fusobacteria also tended to decrease. In short-chain fatty acids, acetate and propionate grew to decline, while n-butyrate and i-valerate slightly increased.

Acetate, propionate positively correlated with IL-6, and n-butyrate, and n-valerate showed a positive correlation with IL6 and CRP. Isobutyrate and isovalerate negatively correlated TNFα. The upper tertial of genmai eaters showed beneficial effects.

Conclusion: Replacement of one meal per day to brown rice omusubi showed health benefits in more than half of the participants. The relationship between bacterial species and short-chain fatty acids suggested the holistic control of SCFA and inflammatory biomarkers.

Keywords: Brown Rice; Intervention; Microbiota; Blautia; Short-Chain Fatty Acid; IL-6; CRP

Graphical Abstract



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We conducted an intervention study on the effects of eating brown rice ball (Genmai omusubi) for 3 months, and examined changes of bowel movement, intestinal microbiota, fecal short chain fatty acids (SCFA), and inflammatory biomarkers, simultaneously. After 3-month intervention, Blautia wexlerae became dominant population, and individual species correlated with SCFA positively or negatively. Acetate and propriate decreased, and relative increase of butyrate and valerate may stimulate Treg proliferation, which may calm down the hyper immune response like cytokine storm. Yellow bacilli are Firmicutes, pink Actinobacteria, and blue circled bacilli show suppressive correlation. Inflammatory marker, IL-6 and CRP decreased corresponding to above changes.

Key Messages

• Pilot intervention study by Genmai (brown rice) omusubi lunch for three months improved bowel movement and body weight.

• Firmicutes remained dominant in pre- and post-intervention. Actinobacteria significantly increased, while Proteobacterium decreased. Groups of species specifically correlated with short-chain fatty acids (SCFAs) to represent an intestinal ecosystem.

• Fecal acetate and propionate tended to decrease, but other SCFAs were stable according to the intervention. Capronate detection increased from 4 to 10 participants.

• Firmicutes showed a negative correlation with CRP, while Verrucomicrobia showed a positive correlation with TNFa.

• Acetate, propionate, n-butyrate, and n-valerate correlated with IL6 and CRP, while i-butyrate and i-valerate negatively correlated with TNFa.

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Introduction

Current epidemiological data are inconclusive about the effects of traditional brown rice on health [1,2]. We previously carried out a cross-sectional study of 1100 participants who consumed brown rice daily [2]. Brown rice eaters preferred to eat Japanese foods and traditional vegetables, avoiding meat, dairy products, and western foods [3]. Brown rice eaters showed a lower BMI in men and women of all ages. We found that eating brown rice (Genmai) is beneficial for maintaining proper body weight. It was challenging to find habitual genmai eaters in the previous epidemiological studies because white rice is softer and tastier and fits with any side dish.

Kenzo Futaki founded the Japan Society of Integrative Medicine in 1953, advocated the "20 virtues of Genmai," and tried to spread the brown rice diet. Dietary fiber, which brown rice is rich in, positively influences bowel movement and creates a beneficial intestinal environment by maintaining bacterial flora [4-7]. Recent intestinal bacterial research has found that people who ingest dietary fiber show increased bacteria producing citric acid, propionic acid, and butyric acid. Bifidobacteria are predominant in the microbiota of these people. Blautia wexlerae and Blautia luti were also common in Japanese [8]. The bowel movements of those on a brown rice diet can induce an excellent intestinal environment, as indicated by banana-shaped stools and defecation more than once a day.

The Medical Rice Association of Japan advocates brown rice consumption based on accumulating scientific evidence about its health benefits. As part of the "Brown Rice Taste Contest G1 Grand Prix", sponsored by the medical rice association, 54 kinds of organic brown rice were proven to contain dietary fiber, γ-oryzanol, high antioxidant activity, GABA, vitamins, and minerals [9]. These ingredients are the functional basis of brown rice.

Volunteer members of The Ministry of Agriculture, Forestry, and Fisheries attempted to confirm the relationship between brown rice eating and health by eating genmai omusubi for lunch for 12 weeks [10]. Previous cross-sectional studies have strongly suggested that eating brown rice improves bowel movement through the ideal symbiosis with intestinal microbiota and the production of short-chain fatty acids (SCFAs). These cause various health effects on obesity, blood pressure, and healthy feeling [11,12].

This project investigated simultaneous changes in the gut microbiota and short-chain fatty acids before and after brown rice ball (omusubi) intervention.

Ethical Issues

The Ethics Review Board in the Life Science Promoting Association approved this study (No. 003 in 2018). Each participant was free to withdraw at any time during the course. The research group stored confidential information separately in a locked container, and the analysis was done using anonymous data.

Subjects and Method

Subjects

Thirty participants were recruited from the Ministry of Agriculture, Forestry, and Fisheries employees and voluntarily participated in the study, providing written informed consent before the examination. Eighteen men and 12 women participated in the study [10]. They were all healthy workers except for seven males who showed mild hypertension. They completed pre- and post-intervention questionnaires, daily records of genmai (brown rice) consumption and bowel movements, monthly health check-ups, and collected feces before and after the intervention.

Questionnaire

Each participant completed an 8-page questionnaire before the intervention [10]. It included questions about age, height, current weight, BMI, weight at 20 years old, maximal weight and age in the past, kind of staple rice, food preferences and eating habits, dietary awareness, food intake status (sFFQ), meal sketches, lifestyle and habits, current health condition, bowel movements, and stool features, health

history of themselves and their parents and siblings, chief complaints, changes in health condition since the previous year, healthy habits, liquor, tobacco, eating out, supplements, fasting experience, physical activities, lifestyle, occupations, education, income, stress, and life creed (religion). In women's health, we also asked questions about menarche, menstruation, hormone use, delivery history, childcare history, etc. After the intervention, participants completed a shorter, four-page questionnaire to discover diet, health condition, and feeling changes.

Intervention

The intervention was carried out from October 2019 to December 2019 [10]. The participants ate a rice ball lunch box made from brown rice "omusubi" on weekdays for at least four days a week. Twenty-five participants ate brown rice 45 or more times in the three months, and 7 participants ate brown rice 57 times or more. Bread and noodle intake decreased under increasing rice consumption. Before and after the survey, stools were collected to analyze the intestinal flora and SCFAs simultaneously. Biochemical data were obtained from 9 ml of blood samples collected at the end of the study. During the intervention, the participants measured body weight, blood pressure, and body composition using impedance body composition monitors. Participants reported the number of bowel movements and stool shapes (modified Bristol stool scale) every day. There was no intervention for breakfast, dinner, and snacks.

Intestinal Bacteria and Short-Chain Fatty Acids

Thirty participants provided stool samples in dry tubes. Fresh fecal samples were collected from 3 points on the stools and immediately frozen in a home refrigerator. Fecal samples (approximately 50-100 mg) were sent to Techno Suruga Laboratory, Shizuoka, in a dry ice container for sequence amplicon analysis [12-14]. The stools were separated for microbiota analysis and short-chain fatty acids measurement.

An 0.8 ml sample of the suspension was homogenized with zirconia beads using a FastPrep24 Instrument (MP Biomedicals, Santa Ana, CA). DNA was extracted from the suspension using an extractor (Precision System Science, Chiba, Japan). MagDEA DNA 200 (GC) (Precision System Science) was used for automatic nucleic acid extraction. The V3-V4 region of 16S rDNA was amplified using a mixture of the forward primer and reverse primer.

PCR was performed using the GeneAmp PCR system 9700 (ABI, Foster City, CA). The PCR reaction and preparation of the amplicon pool were performed.

More than 30,000 determined 16S rDNA sequences from each sample were subjected to a homology search using Metagenome@Kim software (World Fusion Co., Ltd., Tokyo, Japan) against the Techno-Suruga Lab Microbial Identification Database DB-BA10.0 (Techno-Suruga Laboratory).

Short-chain fatty acids (SCFAs) in the feces were determined by a modified method, as previously described by García-Villalba R, et al. (2012) [15]. For the determination of SCFA, 0.1 g of feces was put in a 2.0 ml tube with zirconia beads and suspended with 0.9 mL 0.5% phosphoric acid. Each sample was heated at 85°C for 15 min, vortexed at 5 m/s for 45 s using FastPrep 24 (MP Biomedicals, CA, USA), and centrifuged at 14,000 rpm for 10 min. Then, 0.4 ml of the supernatant was transferred to a 1.5 ml tube, mixed with 0.4 ml ethyl acetate, shaken for 30 min, and centrifuged at 14,000 rpm for 10 min. Finally, 0.2 ml of the supernatant was mixed with 1 mM 4-methyl valeric acid as an internal standard.

SCFAs were measured by gas chromatography with a flame ionization detector (7890B, Agilent Technologies, USA) and a capillary column DB-WAXetr (30 m, 0.25 mm id, 0.25 μm film thickness, Agilent Technologies, USA). Helium was used as the carrier gas at 1.2 mL/min. The detector temperature was kept at 250°C. The oven temperature program was as follows: 50°C; then 10°C/min to 90°C; 15°C /min to 150°C; 5°C /min to 170°C; 20°C/min to a final temperature of 250°C, held for 4 min. One microliter of the extract was injected in the splitless mode. Acetate, propionate, n-butyrate, i-butyrate, n-valerate, i-valerate, and n-caproate were measured.

Statistical Analysis

All data were stored in an Excel database and transferred to IBM-SPSS ver. 24 for statistical analyses [16]. Parametric analysis for continuous variables and non-parametric analysis for categorical data were carried out. Data were examined using an unpaired t-test or the two-sided Mann-Whitney test and Fisher's X^2 test for categorical variables. Spearman's correlation analysis was carried out between microbiota profiles at the phylum level and species level. A paired t-test was used to examine the changes between pre- and post-intervention. The principal component analysis was performed to detect groups of coexistence and interaction. Bacterial profiles of more than 0.1% of the total composition on average or max profiles of more than 1.0% were selected for analysis. Most tables listed mean, sd, or median, because the distribution of bacilli was irregular, mostly where the population was small, and the profile was variable.

P values less than 0.05 were marked and $p < 0.05$ was considered significant. The statistical significance was shown as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Results

Anthropometric Data and Lifestyle

Of the 30 research volunteers in this study, the male participants were most commonly in their 40s and 50s, and female participants were most commonly in their 40s. There were nine obese participants with a BMI greater than 25-eight males and one female-while there were four thin participants with BMI less than 18-one male and three females [10].

According to the questionnaire, 15 men and 11 women (87%) ate white rice before starting the study. In terms of eating habits for breakfast, 17 (57%) were "eating daily," and 7 (24%) ate breakfast less than twice a week. Total genmai omusubi intake during the three months was 59.6±25.8 (median 54) in males and 50.8±8.8 (median 51) in females. The median intake for both males and females were 52. The tertial ranges for the number of onigiri eaten were: up to 35, 35 to 56, and more than 56. These yielded Genmai omusubi consumption category.

Following brown rice intervention, weight loss occurred in 11 males (61%) and 5 females (42%). Body weight (kg) decreased from 65.3±15.3 to 64.8±15.2 (p=0.063), BMI (kg/m^2) 23.2±4.0 to 23.0±4.0 (p=0.034) and BMR ($kcal/m^2$) 1399.8±305.0 to 1374.7±289.6 (p=0.004).

According to the questionnaire completed before the start of the survey, the exercise intensities reported by participants were as follows: 43% reported "almost sedentary"; 40%, "occasionally walking"; and 17%, "outside working". Reported sleeping times were 53%, "6 hours"; 43%, "7 hours"; and 3.3%, "8 hours".

The intervention did not cause significant changes in the lifestyle of the participants.

In terms of recent medical history, there were seven males with hypertension, mostly in their 40s. Fifteen healthy participants (50%) did not have any symptoms. As for smoking, 21 (70%) were non-smokers, 6 (20%) smoked in the past, and 3 (10%) quit smoking. There was one female smoker.

Changes in Bowel Movements and Stool Properties

According to the questionnaire before the start of the survey, bowel movements of less than 3 times a week was high in females (p=0.023). Once a day was the most frequent answer given, with five men (29%) reporting movements more than twice a day. As for stool shape, males tended to have diarrhea, and females tended to have hard stools (p=0.028). The banana shape was present by 13 (43%) of participants. Three months later, men had more frequent bowel movements and improved shape (p=0.02). The number reporting banana shape, which corresponds to Bristol type 4, increased from 11 to 18 (p=0.03). Two women alternated between diarrhea and hard stools, which improved after 3 months by eating brown rice for lunch. People who had more frequent brown rice meals tended to have more bowel movements (p=0.07).

Changes of Microbiota Profiles and Short-Chain Fatty Acids

The dominant bacterial profiles (%) at the phylum level and short-chain fatty acids in the same feces are present in Tables 1a and 1b. All people had Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria before and after the intervention. Correlation analysis among microbiota profiles at baseline showed a reverse correlation between Firmicutes and Actinobacteria and a positive correlation between Verrucomicrobia, Fusobacterium, and Synergistetes.

The order of abundance was Firmicutes (65%), Actinobacteria (16%), then Bacteroidetes (6%) at baseline. Actinobacteria significantly increased at the end of the intervention (21%), while Proteobacteria (1.03%) decreased significantly. Bacteroidetes also tend to fall. All participants had above four phyla, but Fusobacterium's positive rate was 44% in males and 58% in females at baseline and three months later. The positive rate of Verrucomicrobia decreased from 61% to 39% in males and from 50% to 42% in females following the intervention. The positive rate of Synergistetes was variable, ranging from 11% to 17% in males and from 17% to 8% in females.

In terms of SCFAs, acetate and propionate tended to decrease, and other SCFA were stable or slightly increased (Table 1b). The total concentration of SCFAs decreased from 59.7±23.7 μmole/g to 54.7±15.7 μmole/g at post-intervention without statistical significance (p=0.21).

Acetic acid always showed a positive correlation with propionate and n-butyrate and negative correlation with i-butyrate, n- and i-valerate at baseline and post-intervention (Figure 1). Isovaleric acid was found in 27 participants, while n-valeric acid was present in 21 participants, caproic acid was only detected in 4 participants at baseline, which increased to 10 at post-intervention. Isobutyric acid and isovaleric acid had a high coefficient, reflecting a close metabolic pathway. The number of correlations increased at post-intervention, and the negative association between acetate and i-valerate became significant.

Changes of Microbiota at the Species Level

In the comparison between pre- and post-intervention microbiota profiles at the species level, the increased proportion of species in the phylum Firmicutes is noteworthy. The top 25 species made up 58% of the microbiota, of which 82.2% were Firmicutes, at baseline, while it occupied 63%, of which 80% belonged to Firmicutes, at the end of intervention with increased variety.

Blautia wexlerae occupied the top component (13.7±9.2%), then Collinsella aerofacience (7.9±6.7%) and Eubacterium halii (median

Table 1a: Comparison of phyla microbiota before and after omisubi intervention.

	Pre intervention						Post intervention						
Phyla	n	mean		sd	median	max	n	mean		sd	median	max	*p*
Firmicutes	30	66.43	±	13.70	65.61	98.59	30	65.25	±	13.66	66.29	91.19	0.54
Actinobacteria	30	15.99	±	12.53	11.39	54.72	30	21.30	±	15.26	15.98	61.37	0.006
Bacteroidetes	30	5.95	±	7.33	2.88	30.70	30	3.37	±	4.24	1.70	19.85	0.089
Proteobacteria	30	1.03	±	1.35	0.48	5.43	30	0.56	±	1.15	0.16	5.88	0.037
Verrucomicrobia	17	0.81	±	1.43	0.03	4.44	15	0.64	±	1.21	0.02	4.22	0.248
Fusobacteria	15	0.20	±	0.38	0.06	1.49	18	0.08	±	0.12	0.04	0.45	0.351
Synergistetes	4	0.03	±	0.02	0.03	0.05	5	0.02	±	0.02	0.00	0.05	0.506
Euryarchaeota	2	0.27	±	0.22	0.27	0.43	4	0.01	±	0.01	0.01	0.03	0.273

n; number of positive participants, mean, median, max; percent profiles.

Table 1b: Concentration of Short chain fatty acids.

	Pre intervention						Post intervention						
SCFA	n	mean		sd	median	max	n	mean		sd	median	max	*paired t*
acetate	30	36.15	±	14.31	35.75	64.40	30	31.73	±	9.57	32.23	60.40	0.085
propionate	29	13.15	±	7.49	11.60	34.30	30	11.17	±	4.80	9.82	21.40	0.107
n_butyrate	29	7.79	±	4.73	7.30	18.80	30	7.94	±	4.53	6.98	21.00	0.641
i_butyrate	26	0.95	±	0.60	1.10	2.10	29	0.97	±	0.59	1.01	2.30	0.423
n_valerate	21	1.19	±	1.19	1.50	5.30	24	1.19	±	0.99	1.39	3.30	0.925
i_valerate	27	1.21	±	0.84	1.20	3.00	30	1.42	±	0.94	1.46	3.40	0.224
n_capronate	4	0.04	±	0.11	0.30	0.40	10	0.09	±	0.24	0.32	1.20	0.04
		59.81	±	23.64	57.8	99.9		54.71	±	15.68	51.667	108.99	0.208

Units of SCFA; (*μ* mole/g feces)

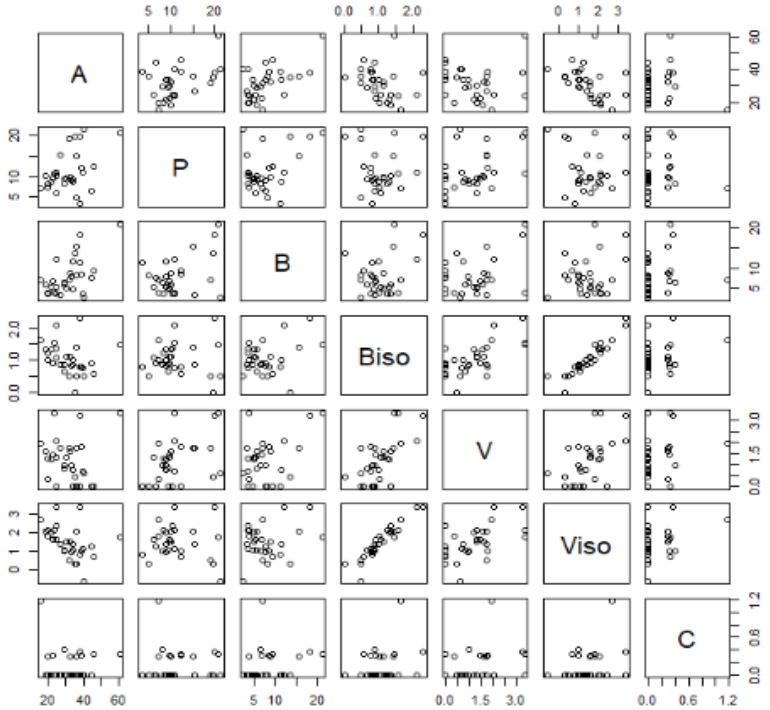

Figure 1: Correlation among SCFA after the intervention. Acetate shows positive correlation with n-butyrate, while negative correation is present with i-butyrate, n-varalate and isovalerate. Isobutyrate shows positive correlation with n-valerate and iro-valerate. Number of caproate was only 4, so the correlation data is not reliable. A; acetate, P; propriate, B; n-butyrate, Biso; i-butyrate, V; n-valerate, Viso; i-varelate, C; copronate. Unit is μ mole/g feces.

1.8%), Fusicateribacterium, Bifidobacterium, Blautia luti also increased without statistical significance. Blautia obeum, Faecalibacterium prausnitzii, and Ruminococcus gnavus decreased with marginal value (p=0.06) (Table 2).

The complete profile of genus Blautia increased from 15.99% to 20.58% (Figure 2).

Faecalibacterium prausnitzii showed a significant correlation with Blautia, Ruminococcus, Bacteroides, Parabacteroides, Roseburia, Eubacterium, Alistipes, Hesperia, etc., while Blautia wexlerae correlated only with Slackia butyricocons and Clostridium glycyrrhizinilyticum positively and Roseburia intestinalis negatively.

Overall changes of species significantly correlated with SCFA

Table 2: Order of species at pre and post-intervention.

order	Pro	mean		sd	median	Post	mean		sd	median	*pairedT*
1	Blautia wexlerae	10.10	±	5.91	9.62	Blautia wexlerae	13.74	±	9.22	10.33	*0.043*
2	Fusicatenibacter saccharivorans	4.50	±	4.86	2.54	Collinsella aerofaciens	7.90	±	6.70	6.76	*0.001*
3	Faecalibacterium prausnitzii	4.49	±	4.80	2.01	Fusicatenibacter saccharivorans	5.25	±	4.74	3.65	*0.281*
4	Collinsella aerofaciens	4.55	±	4.74	4.19	Blautia luti	3.88	±	5.63	2.33	*0.217*
5	Ruminococcus gnavus	3.50	±	5.33	0.74	Bifidobacterium longum	3.85	±	7.10	2.11	*0.230*
6	Ruminococcus faecis	3.08	±	4.43	0.91	Faecalibacterium prausnitzii	3.11	±	4.12	1.19	*0.100*
7	Blautia luti	2.96	±	3.38	1.84	Eubacterium hallii	2.71	±	3.32	1.80	*0.007*
8	Bifidobacterium longum	2.74	±	2.83	1.94	Ruminococcus faecis	2.62	±	3.04	1.27	*0.239*
9	Ruminococcus bromii	2.32	±	4.71	0.00	Ruminococcus bromii	2.25	±	3.71	0.00	*0.918*
10	Megamonas funiformis	2.30	±	6.99	0.00	Subdoligranulum variabile	2.42	±	3.50	0.29	*0.074*
11	Agathobacter rectalis	1.99	±	3.22	0.03	Blautia obeum	2.09	±	2.36	1.46	*0.063*
12	Subdoligranulum variabile	1.67	±	2.39	0.47	Ruminococcus gnavus	2.26	±	2.99	0.52	*0.065*
13	Blautia obeum	1.55	±	2.45	0.78	Streptococcus salivarius	1.87	±	3.81	0.95	*0.085*
14	Eubacterium hallii	1.55	±	1.80	1.12	Agathobacter rectalis	1.48	±	2.33	0.01	*0.176*
15	Enterococcus faecalis	1.23	±	6.68	0.00	Dorea longicatena	1.36	±	1.77	0.44	*0.442*
16	Blautia caecimuris	1.38	±	3.11	0.21	Streptococcus thermophilus	1.01	±	3.89	0.03	*0.581*
17	Dorea longicatena	1.26	±	1.85	0.22	Romboutsia ilealis	1.13	±	2.40	0.19	*0.992*
18	Romboutsia ilealis	1.12	±	2.19	0.41	Total	58.93	±	11.13	33.33	*0.021*
19	Streptococcus salivarius	1.13	±	2.45	0.39						
	Total	53.41	±	9.22	27.41						

Unit of mean, median; % of profile; Yellow; increasing Firmicutes, blue; decreasing Firmicultes, Pink;

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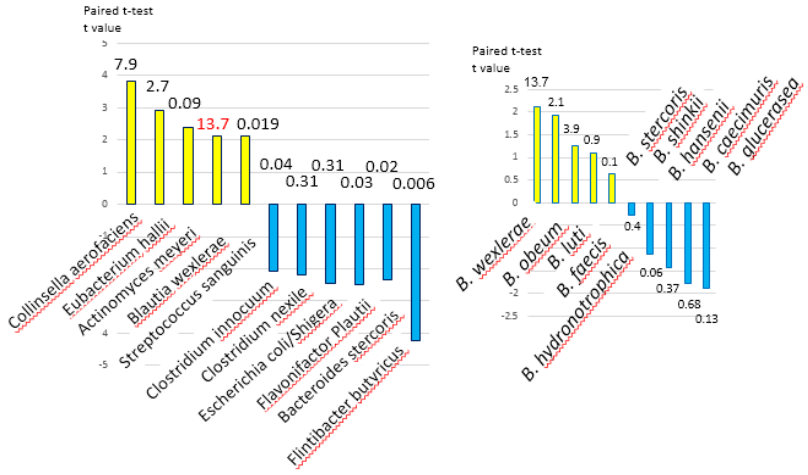

Figure 2: A. Species showing significant change between pre- and post-intervention, and changes of genus *Blautia*. Species correlated with SCFA significantly increased by *Genmai omusubi* intervention. *Blautia wexlerae* and *Collinsella aerofaciens* made a big polulation, 13.7% and 7.9%, respectively. Blue columns are significantly decreased but the occupancies are small (left). B. Increased and decreased Blautia species by *Genmai omusubi* intervention. Number of each column is profiles of Blautia. Positive and negative number seemed to be balanced, but positive and negative population is 20.7% vs. 1.64%, respectively, and the ratio is 12.6. Number on and below the columns is % profile of each species (right).

positively or negatively by Genmai omusubi intervention are present in Table 3.

Each SCFA seemed to be controlled individually by both positively or negatively correlated species. The number of species with significant correlation was 37 at baseline, which increased to 54 at

Table 3: Species that have significant correlation with short chain fatty acids at pre-(left column-A) and post-(righ column-B) intervention.

Table 3a

no	name pre	%	acetate	propionate	n_butyrate	i_butyrate	n_valerate	i_valerate	capronate
1	Parabacteroides johnsonii	0.03	-.446*	0.089	-0.185	0.242	-0.1	0.285	-0.3
2	Blautia stercoris	0.37	-.418*	-0.279	-0.061	0.244	-0	0.273	0.203
3	**Fusobacterium mortiferum**	0.06	-.490**	-.409*	-0.038	0.194	-0.2	0.243	0.221
4	**Blautia obeum**	**1.55**	0.119	.409*	0.279	0.25	0.2	0.135	-0.06
5	Alistipes putredinis	0.14	0.111	.396*	0.163	-0.041	0.1	-0.12	0.265
6	**Butyricicoccus faecihominis**	0.13	0.004	.558**	0.165	0.052	0.4	0.017	0.279
7	Acutalibacter muris	0.02	0.174	.495**	-0.035	0.187	0.1	0.058	-0.19
8	Clostridium nexile	0.64	-0.294	-.484**	-0.255	0.007	-0	0.077	-0.16
9	**Bacteroides plebeius**	0.41	-0.249	-.444*	-0.002	0.052	-0.2	-0	-0.03
10	Shigella boydii	0.01	-0.134	-.369*	0.091	-0.002	0.2	-0	0.529
11	Enterobacter kobei	0.01	-0.303	-.455*	-0.257	0.053	-0.1	0.089	0.411
12	**Agathobacter rectalis**	**1.99**	0.179	0.185	.391*	0.002	0.1	-0.09	0.118
13	**Dorea longicatena**	**1.26**	0.036	0.154	.452*	0.189	.504*	0.157	0.362
14	Roseburia faecis	0.51	0.074	0.236	.396*	0.09	0.1	0.04	-0.48
15	**Dorea formicigenerans**	0.30	-0.025	0.038	.485**	0.358	0.3	0.293	0.337
16	Methanobrevibacter smithii	0.02	0.154	0.31	.370*	0.203	-0.1	-0.09	-0.41
17	Streptococcus lactarius	0.01	0.097	0.113	.373*	0.005	-0.1	-0.11	.
18	**Hespellia porcina**	0.08	0.04	0.141	.498**	.516**	0.3	.380*	-0.12
19	**Sellimonas intestinalis**	0.29	-0.162	-0.082	-.455*	-0.223	-0.1	-0.15	-0.11
20	**Enterococcus gallinarum**	0.01	-0.262	0.142	-.459*	0.124	0.1	0.187	.
21	Anaerostipes butyraticus	0.01	-0.178	0.114	-.497**	0.062	-0.2	0.131	-0.41
22	Eubacterium fissicatena	0.03	-0.055	-0.086	-.389*	-.390*	-0.3	-0.31	-0.32
23	Gordonibacter faecihominis	0.02	-0.141	-0.103	0.027	.369*	0.2	0.339	-0.61
24	Blautia faecis	0.70	0.117	0.217	0.35	.388*	.416*	0.27	0.092
25	Clostridium spiroforme	0.09	-0.285	-0.143	0.051	.402*	0.3	.366*	-0.04
26	Roseburia hominis	0.03	-0.164	0.173	-0.127	.429*	0.2	.417*	-0.09
27	Bifidobacterium bifidum	0.19	0.248	-0.043	-0.058	-.473**	-0.2	-0.33	-0.18
28	**Lactobacillus delbrueckii**	0.01	0.243	0.024	0.055	-.507**	-0.1	-.383*	-0.42
29	Blautia hansenii	**0.55**	-0.273	-0.074	-0.035	0.104	.442*	0.13	0.203
30	Coprobacter fastidiosus	0.13	-0.257	0.049	-0.208	0.068	-.463*	0.022	0.038
31	Ruminococcus torques	0.38	-0.234	0.119	-0.127	0.175	0.3	0.149	.730*
32	Roseburia intestinalis	0.19	-0.331	-0.211	0.044	0.276	-0	0.268	-.831**
33	**Eubacterium eligens**	0.06	-0.264	-0.165	-0.007	0.184	-0.1	0.185	-.793**
34	Klebsiella oxytoca	0.03	-0.055	-0.149	0.099	-0.026	0.1	-0.04	.657*
35	**Megasphaera massiliensis**	0.03	-0.171	-0.313	0.183	0.022	0.1	-0.02	.692*
36	Enterobacter tabaci	0.02	-0.054	-0.219	-0.071	-0.089	0.2	-0.06	.677*
37	Lactobacillus gasseri	0.02	0.006	-0.238	-0.062	-0.111	0	-0.05	.692*

Citation: Kikuchi K, Watanabe S, Matsuo M, et al. (2021) Changes in Microbiota and Short-Chain Fatty Acids Following 3-Month Pilot Intervention Study Feeding Brown Rice Ball (Omusubi) to Healthy Volunteers. Prensa Med Argent, Volume 107:2. 315. DOI: https://doi.org/10.47275/0032-745X-315.

Table 3b

no	name post	%	acetate	propionate	n_butyrate	i_butyrate	n_valerate	i_valerate	capronate
1	**Blautia wexlerae**	**13.74**	.406*	-0.059	0.018	-.442*	-0.4	-.402*	-0.31
2	Alistipesonder donkii	0.05	.474**	.459*	.496**	0.057	0.1	-0.18	0.13
3	Bacteroides vulgatus	0.42	-.395*	-0.068	-0.145	0.317	-0.1	0.266	0.478
4	**Fusobacterium mortiferum**	0.03	-.556**	-0.106	-0.069	0.091	-0	0.227	0.137
5	Holdemania filiformis	0.53	0.11	.533**	0.256	0.076	0.2	0.013	-0.22
6	Bacteroides dorei	0.51	0.224	.370*	.439*	0.012	0.3	-0.02	-0.44
7	Bacteroides uniformis	0.47	0.185	.402*	0.302	0.16	0.1	0.029	0.018
8	Turicibacters anguinis	0.45	-0.227	-.368*	-0.182	-0.202	-0.2	-0.15	-0.1
9	Bacteroides plebeius	0.17	-0.138	-.434*	-0.013	-0.047	-0.1	-0.03	0.2
10	**Butyricicoccus faecihominis**	0.16	0.119	.625**	0.285	0.07	0.3	0.005	-0.03
11	Ruminococcus callidus	0.15	0.108	.372*	0.224	0.074	0	-0.08	0.279
12	Veillonella tobetsuensis	0.04	-0.174	-.421*	-0.218	-0.08	-0.4	-0.04	0.113
13	Flavonifractor plautii	0.03	0.153	.381*	0.004	0.087	0.1	0.075	-0.19
14	Bacteroides eggerthii	0.03	0.049	.435*	0.253	0.249	.475*	0.268	0.27
15	Parasutterellaex crementihominis	0.02	0.317	.441*	.657**	0.204	0.2	0.045	-0.33
16	Coprobacilluscateniformis	0.01	0.28	.372*	0.111	-0.155	0.2	-0.1	-0.37
17	Clostridium aldenense	0.01	-0.096	.387*	-0.105	0.056	0.3	0.084	-0.17
18	Clostridium lavalense	0.00	0.035	.430*	-0.06	0.257	0.2	.366*	-0.03
19	**Blautia obeum**	**2.09**	0.334	0.217	.387*	0	0.1	-0.13	-0.13
20	**Agathobacter rectalis**	**1.48**	0.183	0.23	.361*	-0.044	0	-0.11	-0.28
21	**Dorea longicatena**	**1.36**	0.053	0.216	.369*	0.066	.435*	0.043	0.337
22	**Dorea formicigenerans**	0.41	0.172	0.235	.654**	0.237	0.2	0.089	-0.26
23	**Sellimonas intestinalis**	0.43	-0.081	-0.085	-.468**	-0.302	-0.1	-0.19	-0.46
24	**Eggerthella lenta**	0.33	0.04	0.204	-.477**	-0.182	0	-0.04	0.055
25	Eubacterium ramulus	0.17	0.073	0.136	.441*	.408*	0.3	0.294	-0.41
26	**Hespellia porcina**	0.08	-0.033	0.252	.440*	.478**	.411*	.402*	-0.21
27	Parabacteroides merdae	0.07	0.174	0.259	.369*	0.313	0	0.079	-0.06
28	Blautia schinkii	0.06	0.215	-0.06	.441*	0.043	0.3	-0.11	0.246
29	Clostridium innocuum	0.04	0.008	-0.022	-.417*	-0.276	-0	-0.15	0.227
30	Enterococcus avium	0.03	-0.031	-0.165	-.421*	-0.104	-0.3	0.032	-0.41
31	Bacteroides faecis	0.02	0.223	0.303	.431*	0.34	0.2	0.165	-0.1
32	Alistipes shahii	0.02	0.279	0.325	.370*	0.133	0	-0.12	-0.13
33	Clostridium saccharogumia	0.01	0.339	0.288	-.416*	-0.356	-0.4	-0.27	.
34	Solobacterium moorei	0.01	-0.019	-0.246	.432*	0.1	-0	-0.04	0.234
35	**Enterococcus gallinarum**	0.01	-0.015	0.044	-.447*	-0.092	-0.1	0.057	.
36	**Odoribacter laneus**	0.00	0.2	0.322	.401*	.371*	0.3	0.321	-0.14
37	Peptoniphilus gorbachii	0.00	-0.297	-0.129	-.368*	0.228	-0	0.277	0.018
38	Roseburia intestinalis	0.05	-0.095	0.168	-0.016	.450*	-0	0.356	-0.33
39	Streptococcus cristatus	0.02	-0.3	-0.261	-0.126	.408*	0.1	.436*	0.481
40	Lachnospira pectinoschiza	0.01	-0.217	0.212	-0.16	.422*	0.2	0.343	0.098
41	Streptococcus thermophilus	**1.01**	0.015	-0.117	-0.192	-.378*	-.579**	-0.35	-0.21
42	Anaerostipes caccae	0.03	0.151	0.176	-0.277	-.410*	-0	-0.34	0.082
43	**Lactobacillus delbrueckii**	0.01	0.15	0.072	-0.216	-.425*	-0.3	-0.32	0.377
44	Veillonella parvula	0.01	0.02	-0.358	-0.023	-0.215	-.410*	-0.17	-0.41
45	Bacteroides finegoldii	0.00	-0.052	0.159	-0.029	-0.26	-.498*	-0.35	0.061
46	Clostridium asparagiforme	0.00	-0.083	-0.132	-0.13	0.093	-.440*	0.06	-0.26
47	Acidaminococcus intestini	0.01	-0.214	0.072	0.024	0.291	0.1	.386*	0.294
48	Actinomyces viscosus	0.00	-0.311	-0.157	-0.354	0.302	-0.1	.371*	-0.23
49	Megamonas funiformis	0.33	0.11	-0.027	0.097	-0.324	-0.2	-.379*	-0.05
50	**Megasphaeram assiliensis**	0.07	-0.276	-0.215	0.151	0.194	0.1	0.151	.709*
51	Eubacterium sulci	0.00	-0.005	-0.29	0.007	0.113	-0	0.151	.823**
52	Phascolarctobacterium faecium	0.07	-0.003	0.162	0.075	-0.058	0.1	0.042	-.634*
53	**Eubacterium eligens**	0.04	-0.147	0.192	-0.025	0.201	-0.3	0.031	-.793**
54	Prevotella histicola	0.01	-0.113	0.114	-0.075	-0.208	0	-0.15	-.910**

post-intervention, in which 13 species were common in pre- and post-intervention (Figure 3).

N-butyrate-producing species, like Blautia obeum and genus Dorea increased, but the suppressive correlation was also increased, such as Sellimonas intestinalis, Eggerthalla lenta, etc. The effects of individual species on SCFA levels could be grouped by characteristic correlation. Blautia wexlerae had negative correlation coefficients with i-butyrate and i-valerate. Collinsella aerofaciens is well-known acetate, lactate, formate, and ethanol producer, but it did not positively correlate with acetate in feces [17]. Bifidobacterium showed a positive relationship

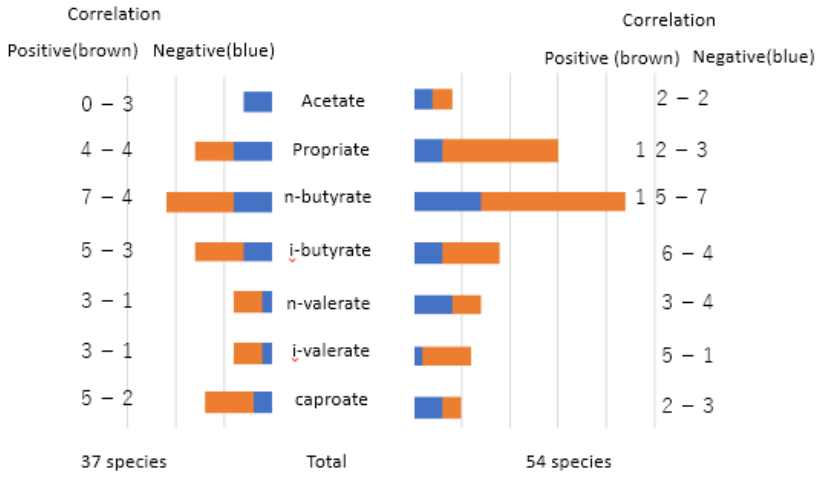

Figure 3: Number of species showing positive or negative correlation with short chain fatty acids. It increased 37 at baseline to 54 at post intervention by either positively or negatively correlated with SCFA. These species form a network to keep concentration of SCFA.

with n-valerate but a weak negative relationship with butyrate and propionate.

Relationship Among Microbiota, SCFA, and Inflammatory Biomarker

In terms of inflammatory biomarkers, CRP and IL-6 were positively correlated, while TNF-α was independent. As for the correlation between microbiota and inflammatory markers, the phylum Firmicutes was negatively associated with CRP, and Verrucomicrobia positively correlated with TNF-α. In terms of SCFAs, acetate, propionate positively associated with IL-6, and n-butyrate and n-valerate were positively associated with IL-6 and CRP. Isobutyrate and i-valerate were negatively associated with TNF- α (Table 4).

Table 4: Spearman's correlation coefficient between microbiota, SCFA and inflammatory markers.

	IL6	CRP	hsTNFα
Firmicutes	-0.086	-.375*	0.35
Bacteroidetes	-0.011	0.359	-0.325
Actinobacteria	0.078	-0.034	0.136
Proteobacteria	0.339	-0.218	0.05
Fusobacteria	-0.028	-0.125	0.01
Verrucomicrobia	0.032	-0.006	0.27*
Euryarchaeota	-0.214	0.051	-0.084
Synergistetes	0.026	-0.075	0.065
Acetate	.484**	0.108	0.016
Propionate	.412*	0.222	0.051
n-butyrate	.449*	.374*	0.006
iso-butyrate	0.019	0.339	-.464**
n-valerate	.457*	.432*	-0.237
iso-valerate	-0.095	0.263	-.408*
IL6	1	.386*	0.125
CRP	.386*	1	-0.174
hsTNFα	0.125	-0.174	1
*p<0.05, **p<0.01			

Comparison of Microbiota, SCFA and Inflammatory Marker by Genmai Consumption Category

As the number of Genmai omusubi eating group was small, statistical significance was not present except for only a few. Still, trends of changing microbiota and SCFA by genmai consumption showed the difference in box plot graphs (Figure 4). The tertial ranges for the number of onigiri eaten were; up to 35, 35 to 56, and more than 56; Group 1, Group 2, and Group 3.

Low Actinobacteria and Fusobacterium and high Firmicutes and

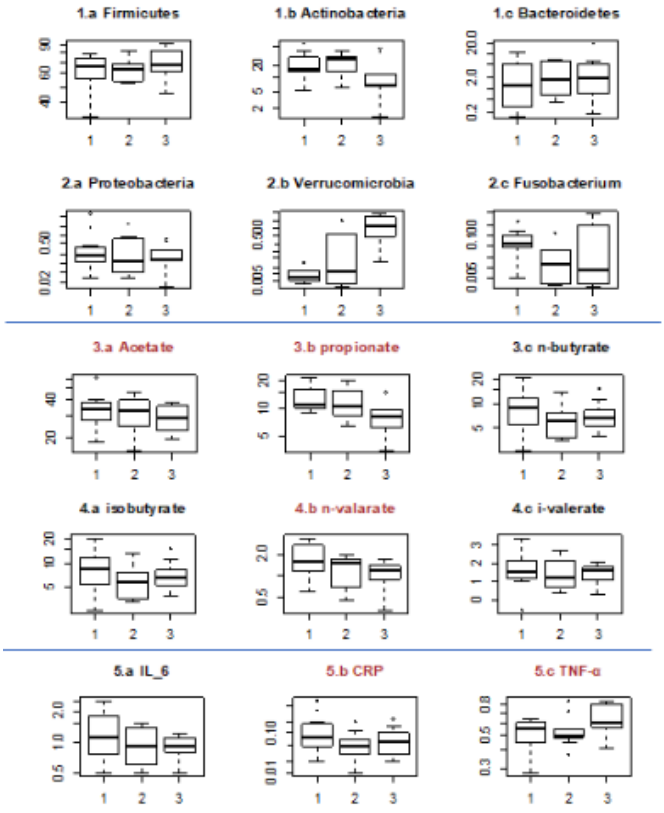

Figure 4: The tertial ranges for number of *onigiri* eaten (Group 1; low, Group 2; med, Group 3; high) were; up to 35, 35 to 56, and more than 56. High *Firmicutes* and *Verrucomicrobia* are present in group 3, in while low *Actinobacteria* and *Fusobacterium* are noticed. In SCFAs, acetate, propionate and n-valerate showed low concentration, but in general, SCFAs tended to be low compared to the less *omusubi* consumption group 1. CRP and IL6 seemed to be parallel to SCFAs. TNF-α is high in group 3.

Verrucomicrobia were present in group 3. Most Blautia species showed higher concentrations in Group 3.

In SCFAs, acetate, propionate, and n-valerate showed low in Group 3, but in general, SCFAs tended to be low compared to the less omusubi consumption group 1. IL-6 and CRP and IL-6 tended to be low in Group 3 compared to the low consumption group. TNFa was elevated in Group 3.

Discussion

There have been many reports on the health effects of Genmai (brown rice), but the mechanism has not been clarified yet [1,2]. Dietary fiber and γ-oryzanol in genmai are candidates for its functional factors [6,9, and 18]. Dietary fibers, mostly indigestible, water-insoluble fibers, stimulate fermentation by the microbiota in the colon. It produces SCFAs, such as acetic acid, propionic acid, butyric acid, and valeric acid, causing various health effects [7,19]. Different dietary fibers led to different responses. Generally, brown rice eaters' intestinal bacterial profiles maintained their diversity and balance of bacteria [13].

We found that genmai eating helped maintain healthy body weight, BMI, and right bowel movements [2]. As an additional benefit, brown rice eaters preferred to eat plant-based Japanese foods, avoiding meat and dairy products [3]. They disliked oily and spicy flavors, and their selection was based upon factors such as whether the food was fresh, organic, without additives or genetically modified foodstuffs, and domestically produced.

In animal experiments using experimental mice, γ-oryzanol works on the brain through the blood-brain barrier to suppress animal fat intake [6].

Numerous animal models and human studies have consistently demonstrated that gut microbiota can modulate host health [4,7].

There is no research on whether brown rice should be eaten in every meal or replaced some of it. To understand the dose-effect of brown rice eating, we adopted the method of merely changing lunch to brown rice balls (omusubi) 5 days a week. Most participants ate genmai only for lunch, but it caused specific microbiota profiles and improved the intestinal environment. They significantly improved bowel movements and stool conditions.

When the number of meals with brown rice for lunch was divided into three, and the composition of intestinal bacteria in each was examined, the top one third had higher Firmicutes and Actinobacteria levels, Bacterioidetes and Proteobacteria decreased.

Short-chain fatty acids implicate the maintenance of the host's ecological homeostasis not only as an energy source but also through G protein-coupled receptors on the cell membrane, such as GRP41, GRP43, GRP109, and Olfr78 [17,20-25]. Butyrate is the ligand of GRP109, which suppresses the expression of IL-6 from macrophages and dendritic cells in the large intestine, enhances the production of IL-10 and retinoic acid, which allows the maintenance of regulatory T cell (Treg) homeostasis. In particular, butyrate enhances histone acetylation in the gene promoter region and enhancer region of Foxp3, the master gene of Treg, by inhibiting histone deacetylase and causing differentiation of naive T cells to Treg cells. The increase of Treg cells reduces the excess intestinal inflammatory response.

The above phenomenon contributes to host eco-regulation as a whole. The two most dominant bacterial species in the human colon with a significant contribution to butyrate production are Faecalibacterium prausnitzii and Eubacterium rectale [26,27]. In the present study, genus Blautia and Ruminococcus increased, but Faecalibacterium and Bifidobacteria were decreased or slightly increased. Brown rice eaters showed additional benefits, such as the low prevalence of Fusobacterium.

In about 200 microbiota profiles, 8 out of 30 regression factors showed a significant correlation with SCFAs. Each component factor had a 1:1 correspondence with a specific SCFA, and some showed a correlation with a few SCFAs. Each bacterial species was positive or negative concerning a specific SCFA. Acetate has a negative relationship with most bacteria, indicating that it may inhibit many bacteria. Blautia wexlerae showed a positive relationship with acetate but a negative relationship with i-butyrate and i-valerate. Large populations like Blautia may have a strong influence, but a deficient proportion of minor bacteria at a 0.01% level could also influence the network.

We used to believe that the more SCFAs, the better immunity of the subject. However, a negative correlation seemed to be more important to maintain a stable environment [28,29]. A pathogenic bacterium, like Escherichia coli/Shigera was suppressed to very low concentration under the predominant growth of Firmicutes.

All of these components contribute to stabilizing the innate immunity of a subject.

Acetate, propionate, n-butyrate, and n-valerate were all significantly correlated with IL-6 and CRP inflammatory biomarkers. Those who consumed brown rice at a high rate showed higher levels of Blautia wexlerae and low acetate, propionate, and n-valerate levels. They also tended to show low IL-6 and CRP levels. Páez A, et al. (2020) [29] suggested that the depletion of B. luti and B. wexlerae species in the gut ecosystem may occur in obesity cases and contribute to metabolic inflammation leading to insulin resistance. Changes in the microbiota caused by brown rice eating should strengthen innate immunity by suppressing SCFAs. The negative relationship between COVID-19 pneumonia and rice consumption by countries may reflect such innate immunity [30].

Our study's weakness is that our study was a comparison of pre- and post-intervention data and not a randomized clinical study. However, in nutritional intervention studies for healthy people, individual variation in the control population is always a problem. Even though a crossover design was planned, it could not exclude the effect of waiting time. Therefore, pre- and post-intervention comparison seemed to be the most cost-effective design to determine the complex microbiota and SCFA profiles' alterations. High adherence to the monthly interview and the writing of daily records of consumption of genmai omusubi and bowel movements by participants guaranteed the high reliability of the data. However, the possibility of selection bias nevertheless remained. Although fecal samples may not represent all intestinal events, the microbiota compositions in this study were suitable for comparison between individuals. Changes in the microbiota and SCFAs were limited to what could be detected in the feces, but this may still reflect the colonic environment. The interaction of the intestinal flora-composing bacteria group involves various factors such as nutrition, cross-feeding, optimal environment (pH, acidity, etc.), and antibacterial substances. In the intestinal flora, an autonomous balance-maintaining mechanism of the intestinal microbial ecosystem has been advocated, and our research was also conducted in that category.

Repeating this intervention study with other populations could allow generalization of the results.

In summary, implementing a brown rice diet for lunch yielded benefits by changing the microbiota and SCFAs to improve bowel movement and shape of stools. Blautia wexlerae was the dominant bacterium found at the species level. There were special groups of microbiota that maintained the levels of SCFAs. The group that consumed more significant amounts of genmai lunch showed a high prevalence of Firmicutes, increased Actinobacteria, and decreased Proteobacteria and Fusobacteria. These changes lowered SCFA levels, and inflammatory markers, such as IL-6 and CPR, suggested an inverse correlation with the intake number of genmai lunches. Proliferation and suppression of microbiota to maintain the gut ecosystem seemed essential for anti-inflammation activity, in which Blautia wexlerae would play a vital role.

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Declaration of Interest

None.

Credit Roles

S.W, K.K: Conceptualization, Formal analysis, Project administration, Writing review & editing,

K.K, M.M, T.E: Recruit of participants, interview and clinical measurement, original draft,

S.M: Statistical calculation and judgment,

T.H, T.K, J.M: Measure of microbiota and SCFA, and data processing.

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