Decreased number of days of fever detection and duration of fever with continuous intake of a fermented milk drink: a randomized, double-blind, placebo-controlled study of elderly nursing home residents

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We conducted a randomized, double-blind, placebo-controlled parallel study to investigate the effects of a fermented milk on elderly nursing home residents. Eighty-eight participants each drank one bottle of fermented milk containing *Lactobacillus casei* strain Shirota, or a placebo, on a daily basis for 6 months in winter. Peripheral blood, saliva, fecal samples, and clinical data were analyzed to assess the milk's efficacy. Fermented milk consumption was associated with a significant decrease in the number of days on which fever was detected and the mean duration of fever compared with these values in the placebo group. No significant differences were observed in other biological parameters. Continuous intake of this fermented milk could be beneficial for the elderly in terms of suppressing the number of days of detection of fever and the duration of fever, which usually increase in winter.
Key words: probiotics, elderly, fever, fermented milk drink

INTRODUCTION

The elderly are susceptible to infection, and infections in residents in an aged-care facility easily spread to other residents and staff. As the immune response becomes compromised with age, infections can become refractory and symptom severe [1]. Pyrexia is an indicator of infectious disease, and regular observation for fever results in prompt medical care. It is important in the elderly to decrease the incidence and duration of fever because persistent fever leads to dehydration and more serious complications [2, 3].

There have been several reports of the ability of a fermented milk containing *Lactobacillus casei* strain Shirota (LcS) to prevent infectious disease [4–7] and modulate immune function (e.g., natural killer cell activity) [8, 9]. Consumption of fermented milk also has beneficial effects on bowel movements and helps alleviate the symptoms of constipation, which can be a problem in the elderly [10].

Here, we examined the efficacy of fermented milk consumption in suppressing fever, alleviating constipation, improving peripheral blood and salivary secretory IgA (sIgA) parameters, and modulating fecal microbiota.

MATERIALS AND METHODS

Subjects

The study subjects were residents of the Morinoie Katori nursing home (MK; Chiba, Japan) or the Morinoie Yashio nursing home (MY; Saitama, Japan). The inclusion criteria were as follows: elderly person (over 60 years old) living in MK or MY, able to take a test drink for 6 months, and able to give written informed consent either directly or through their family. The exclusion criteria were inability to ingest the test drink, deterioration of health status during the study inclusion period, milk allergy or lactose intolerance, habitual intake of fermented milk products or inability to discontinue taking them during the test period, or judgment by the principal medical doctor of the respective nursing home as inappropriate for trial participation. The trial was approved by the Ethics Committee of Kanagawa Institute of Technology (Kanagawa, Japan) and performed in compliance with the principles of the Helsinki Declaration. Details of the trial (including purpose, risks, and human rights protection) were explained to subjects or their families, and written informed consent was obtained from 88 subjects or their families before inclusion in the trial.

Test drinks

The test drinks were a fermented milk product and a placebo. Each 65-ml bottle of the fermented milk drink contained at least 1.5×10^{10} cfu of *Lactobacillus casei* strain Shirota (LcS; our collection strain number is YIT 9029), sugar, skimmed milk, high-fructose corn syrup, and flavor.

The nutrient composition of one bottle was 50 kcal of energy, 0.8 g of protein, 0.1 g of lipid, 11.5 g of carbohydrate, and 12 mg of sodium. The placebo drink contained the same components as the fermented milk drink but did not contain any bacteria. The taste of the placebo drink was made similar to that of the fermented milk drink by adding lactic acid. The taste, flavor, and external appearance of the placebo were indistinguishable from those of the fermented milk drink. The fermented milk drink and the placebo drink were both manufactured and distributed by Yakult Honsha Co., Ltd (Tokyo, Japan). They were refrigerated until ingestion.

Study design

The study was a randomized, double-blind, placebo-controlled parallel group trial. The participants lived in blocks in each facility; each block contained 5 to 14 residents. Residents of each block gathered in the block's dining room to eat. Because the subjects were instructed to have the test drink during breakfast, each block was allocated to a fermented milk-drink ingestion group (LcS group) or a placebo drink ingestion group (placebo group). Allocation lists were compiled by a person who was not in charge of sample or data analysis and were not been revealed until all the data had been processed. Nursing care level [11], age, and gender did not differ significantly between the two groups.

During the 6-month ingestion period, subjects consumed one bottle of the test drink on a daily basis. Before and at the end of the ingestion period, peripheral blood, saliva, and fecal samples were taken. For operational reasons, the ingestion period started in October 2013 and ended in March 2014 at MK, whereas it started in November 2013 and ended in April 2014 at MY. Clinical data on fever, bowel movements, diarrhea, and suppository and enema use were gathered from each subject's nursing care diary. Pyrexia was considered to be present when the body temperature was 37°C or more. Basically body temperature was measured once a day; however, in the event of pyrexia, body temperature was measured several times a day. The duration of fever was counted from the day when the body temperature was 37°C or more to the day when the body temperature decreased to less than 37°C. Baseline data for the pre-ingestion period were obtained from patient diaries for the 6-month period before the ingestion period (from April 2013 to September 2013 at MK and from May 2013 to October 2013 at MY).

Peripheral blood analysis

Ten milliliters of peripheral blood was taken before and at the end of the ingestion period. Serum concentrations of iron, copper, zinc, and albumin were measured by LSI Medience Corporation (Hino, Japan). Transformation testing of lymphocytes stimulated by phytohemagglutinin (PHA) was also done by LSI Medience Corporation. The stimulation index (SI) in the lymphocyte transformation test was calculated by dividing the amount of incorporated ^{3}H-thymidine (count per minute, cpm) upon PHA stimulation by the amount of incorporated ^{3}H-thymidine (cpm) without stimulation. We were unable to collect enough blood from some participants to measure all of the analytical items. In these cases, some items were not analyzed.

Fecal sample analysis

Fecal samples were collected before and at the end of the ingestion period. About 1 g of fecal sample was taken by facility staff and suspended in RNAlater solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) for microbiota analysis. Another approximately 1 g of fecal sample was placed into an empty tube for the measurement of fecal organic acid concentration. Fecal samples were transported to Yakult Central Institute (Tokyo, Japan), where RNA was extracted and counts for *Bifidobacterium*, *Clostridium perfringens,* and *Enterobacteriaceae* were determined by reverse transcription-quantitative PCR [5, 12]. The sequences of PCR primers and PCR conditions were described previously [12]. Organic acid was extracted from fecal samples and analyzed by HPLC [13]. Total organic acid was the sum of lactic acid, formic acid, acetic acid, propionic acid, butyric acid, iso-butyric acid, succinic acid, valeric acid, and iso-valeric acid. Fecal sample analysis was done by Yakult Central Institute. We were unable to analyze fecal samples for some participants because they did not allow facility staff to collect fecal samples.

Salivary sIgA analysis

Saliva samples were collected before and at the end of the ingestion period by facility staff and transported to and analyzed by LSI Medience Corporation. We were unable to collect saliva samples from some participants because they did not open their mouths wide enough for saliva collection.

Nutrient survey of meals

Nutrient analysis of food intake for 3 consecutive days before and at the end of the ingestion period was performed by weighing all dishes before and after eating and calculating the ingested nutrient components on the basis of the menu. This survey was done by Kanagawa Institute of Technology.

Statistical analysis

Data are expressed as individual values or means ± standard deviation. Statistical differences between the two groups were determined by ANCOVA using baseline values as covariates. The Wilcoxon's signed-rank test was performed to compare parameters before and at the end of the ingestion period, and Fisher's exact test was performed to compare categorical values. As a *post hoc* analysis, stratified analysis by median age (86 years) and nursing care level (groups 1 to 3 and groups 4 and 5) [11] was done. A nursing care level of 4 or more means that an elderly person is not able to perform almost all ordinary activities without help. All tests were performed at the 5% significance level. Statistical analysis was done using the SPSS software (version 22, IBM Japan, Tokyo, Japan) by Kanagawa Institute of Technology.

RESULTS

Participant characteristics

Eighty-eight participants (73 women and 15 men) were enrolled in the trial. The mean age was 86.3 ± 7.8 years, and the mean nursing care level was 3.7 ± 1.1. Forty-four participants were allocated to the LcS group (22 residents of MK and 22 residents of MY), and 44 participants were allocated to the placebo group (22 residents of MK and 22 residents of MY).

Sixty-eight participants (57 women and 11 men) finished the trial (Table 1). Of the 20 who did not complete the trial, 15 died before trial completion and 5 withdrew. Ingestion of the test drink was not related to cause of death as judged by the principal medical doctors. Thirty-five participants (17 residents of MK and 18 residents of MY) in the LcS group and 33 participants (16 residents of MK and 17 residents of MY) in the placebo group finished the trial. There were no significant differences between the two groups in terms of gender ratio, number in each facility, age, or nursing care level (Table 1).

Data analysis of fever, bowel movements, diarrhea, suppository use, and enema use

In the pre-ingestion and ingestion periods, no records on pyrexia were available for 5 participants, and no records of bowel movements, diarrhea, or suppository and enema use were available for 8 participants. The LcS group developed fever on an average of 0.90 days/person in the 6-month pre-ingestion period and 0.92 days/person in the 6-month ingestion period. The placebo group developed fever on an average of 1.22 days/person in the pre-ingestion period and as many as 2.89 days/person in the ingestion period (Table 2). The increase in the number of days on which fever was recorded was significantly greater in the placebo group than in the LcS group (p=0.026). In the LcS group, the mean duration of fever in the pre-ingestion period was almost the same as that in the ingestion period. In contrast, in the placebo group, the duration of fever in the ingestion period was double that in the pre-ingestion period (Table 2). The increase in the mean duration of fever was also significantly greater in the placebo group than in the LcS group (p=0.022). In the placebo group, the number of instances of fever in the ingestion period was significantly higher than that in the pre-ingestion period (p=0.001). In contrast, there was no difference in this number between the two periods in the LcS group (p=0.967). The increase in the number of fever instances was not significantly greater in the placebo group than in the LcS group (p=0.051).

For number of days per month on which bowel movements were recorded and for the number of bouts of diarrhea and number of uses of suppositories or enemas per 6-month period, the changes from the pre-ingestion period to the ingestion period were not significantly different between the two groups (Table 2).

Table 1. Characteristics of the participants who completed the trial

	LcS group (n=35)	Placebo group (n=33)	p value
Women	28	29	0.514
Men	7	4	
MK residents	17	16	1.000
MY residents	18	17	
Age (mean ± SD)	84.9 ± 8.5	86.6 ± 7.7	0.393
Nursing care level (mean ± SD)	3.8 ± 1.1	3.6 ± 1.1	0.610

Fisher's exact test was performed for categorical data. An unpaired t-test was performed for numerical data.

Analysis of data on peripheral blood and saliva sIgA

Before or at the end of the ingestion period, no records of serum iron and copper levels were available for 8 participants, no records of serum zinc levels were available for 10 participants, no records of SI and serum CD4/CD8 levels were available for 4 participants, and no records of salivary sIgA were available for 10 participants. The serum iron level in the LcS group and the serum zinc levels in both groups at the end of the ingestion period were higher than those before the ingestion period, but the changes between before and at the end of the ingestion period in these parameters were not significantly different between the two groups (Table 2). SI at the end of the ingestion period in the placebo group was significantly higher than that before the ingestion period (p=0.002), but when the changes between the two periods were compared, there was no significant difference between the two groups. The salivary sIgA concentration did not differ significantly between the two groups when the changes between the two periods were compared.

Analysis of data on organic acid concentrations and microbiota in fecal samples

Before or at the end of the ingestion period, no records of fecal organic acid content were available for 6 participants, and no records of fecal microbiota content were available for 7 participants. Acetic acid, propionic acid, and butyric acid were major components of organic acid in the fecal samples. The concentrations of total organic acid, acetic acid, propionic acid, and butyric acid in the fecal samples did not differ significantly between the two groups (Table 3). The number of *Bifidobacterium* cells at the end of the ingestion period in the LcS group was significantly higher than that before the ingestion period, and the numbers of *Enterobacteriaceae* at the end of ingestion period in both groups were significantly lower than those before the ingestion period, but when the changes between two periods were compared, there were no significant differences between the two groups (Table 3).

Analysis of data for weight, body mass index, and nutrient content of meals

We performed a data analysis on the data collected for weight, body mass index (BMI), and nutrient content of meals

Table 2. Data for fever, bowel habits, peripheral blood parameters, and saliva sIgA

Item	Group	N	Pre-ingestion period (mean ± SD)	Ingestion period (mean ± SD)	p value *	p value #
Days of fever (days/6 months)	LcS	32	0.90 ± 1.50	0.92 ± 1.88	0.966	0.026
	Placebo	31	1.22 ± 2.72	2.89 ± 4.87	0.001	
Duration of fever days (days)	LcS	32	0.45 ± 0.73	0.45 ± 0.78	0.984	0.022
	Placebo	31	0.52 ± 0.81	1.17 ± 1.57	0.090	
Fever incidences (incidences/6 months)	LcS	32	0.62 ± 0.97	0.64 ± 1.28	0.967	0.051
	Placebo	31	0.85 ± 1.81	1.65 ± 2.81	0.001	
Number of bowel movements (times/month)	LcS	31	29.83 ± 18.10	30.86 ± 19.49	0.804	0.507
	Placebo	29	28.87 ± 14.69	28.57 ± 12.47	0.875	
Days of bowel movements (days/month)	LcS	31	18.50 ± 6.41	18.60 ± 6.61	0.789	0.574
	Placebo	29	18.85 ± 6.26	18.46 ± 5.64	0.356	
Number of diarrhea episodes (times/6 months)	LcS	31	24.38 ± 58.85	19.64 ± 33.83	0.673	0.197
	Placebo	29	27.44 ± 30.49	29.88 ± 34.78	0.561	
Number of suppositories (times/6 months)	LcS	31	1.28 ± 2.75	1.01 ± 2.45	0.878	0.787
	Placebo	29	0.97 ± 2.89	0.94 ± 2.65	0.731	
Number of enemas (times/6 months)	LcS	31	1.48 ± 7.69	1.65 ± 8.08	0.078	0.808
	Placebo	29	0.07 ± 0.26	0.14 ± 0.44	0.250	
Albumin (g/dL)	LcS	31	3.52 ± 0.44	3.58 ± 0.49	0.192	0.124
	Placebo	29	3.64 ± 0.43	3.56 ± 0.52	0.257	
Iron (µg/dL)	LcS	31	69.1 ± 19.9	75.6 ± 26.2	0.018	0.415
	Placebo	29	68.7 ± 25.7	71.3 ± 28.6	0.723	
Copper (µg/dL)	LcS	31	95.4 ± 27.1	100.0 ± 29.4	0.054	0.850
	Placebo	29	106.7 ± 19.5	109.5 ± 19.5	0.117	
Zinc (µg/dL)	LcS	31	64.1 ± 10.8	76.5 ± 11.0	< 0.001	0.543
	Placebo	27	67.3 ± 9.6	77.5 ± 10.4	< 0.001	
Stimulation index	LcS	34	192.82 ± 81.79	210.56 ± 87.55	0.657	0.102
	Placebo	30	161.61 ± 84.18	250.04 ± 113.76	0.002	
CD4/CD8	LcS	33	1.652 ± 0.937	1.786 ± 1.109	0.707	0.818
	Placebo	30	1.324 ± 0.741	1.541 ± 0.976	0.082	
Saliva sIgA (µg/mL)	LcS	30	494.2 ± 515.3	455.6 ± 537.4	0.507	0.423
	Placebo	28	400.3 ± 324.1	526.4 ± 382.0	0.071	

*Wilcoxon signed-rank test was performed. #ANCOVA was performd. LcS: *Lactobacillus casei* strain Shirota.

Table 3. Data for fecal organic acid concentrations and microbiota

Item	Group	N	Pre-ingestion period (mean ± SD)	Ingestion period (mean ± SD)	p value *	p value #
Acetic acid (mM)	LcS	31	68.80 ± 29.45	61.85 ± 35.84	0.425	0.604
	Placebo	31	65.78 ± 24.34	63.28 ± 25.78	0.431	
Propionic acid (mM)	LcS	31	16.89 ± 9.91	14.59 ± 10.58	0.133	0.687
	Placebo	31	18.48 ± 10.31	16.37 ± 9.50	0.350	
Butyric acid (mM)	LcS	31	9.68 ± 7.28	8.33 ± 9.51	0.231	0.794
	Placebo	31	14.23 ± 10.88	12.28 ± 10.64	0.174	
Total organic acids (mM)	LcS	31	102.93 ± 48.13	91.64 ± 55.51	0.223	0.511
	Placebo	31	107.05 ± 48.03	101.40 ± 50.03	0.326	
Bifidobacterium ($\log_{10}$ cells/g feces)	LcS	30	8.42 ± 1.20	8.76 ± 1.07	0.027	0.712
	Placebo	31	8.64 ± 1.01	8.81 ± 1.18	0.203	
C. perfringens ($\log_{10}$ cells/g feces)	LcS	30	6.24 ± 2.13	6.15 ± 2.26	0.761	0.876
	Placebo	31	5.70 ± 2.64	5.65 ± 2.77	0.275	
Enterobacteriaceae ($\log_{10}$ cells/g feces)	LcS	30	7.73 ± 1.32	6.78 ± 2.01	0.004	0.623
	Placebo	31	7.08 ± 1.34	6.44 ± 1.87	0.013	

*Wilcoxon signed-rank test was performed. #ANCOVA was performd. LcS: *Lactobacillus casei* strain Shirota.

(Table 4). Calcium intake at the end of the ingestion period was significantly lower than before the ingestion period in both groups. Weight, BMI, and nutrient intake did not differ significantly between the two groups when the changes between two periods were compared.

Post hoc stratified analysis

Because the number of days on which fever was detected differed significantly between the two groups, we stratified the subjects by median age and nursing care level to elucidate a group in which the fermented milk drink was more efficient (Table 5). The change in the number of days on which fever was detected between the pre-ingestion period and the ingestion period was significantly higher in members of the placebo group aged ≥86 years than in members of the LcS group in the same age bracket (p=0.008). In contrast, there was no significant difference in the subgroup of members aged <86 years (p=0.678). In subjects whose nursing care level was 4 or 5, the increase in the number of days on which fever was detected between the two periods was significantly higher in the placebo group than in the LcS group (p=0.016), but in subjects whose nursing care level was 1 to 3, no significant difference was observed (p=0.535).

DISCUSSION

Our findings showed that continuous intake of the fermented milk drink reduced the number of days on which fever was detected and the duration of fever, both of which usually increase in winter. These results were consistent with those of previous studies [4–7]. In the LcS group, the number of days on which fever was detected and the duration of fever during the ingestion period were almost the same as those in the pre-ingestion period. On the other hand, in the ingestion period in the placebo group, the values of these parameters were more than double those in the pre-ingestion period. In the elderly, it is beneficial to shorten the duration of fever because persistent fever can lead to dehydration and more severe complications [2, 3]. Frequent pyrexia or long-term duration of pyrexia would worsen the quality of life of elderly subjects and lead to deterioration of the exercise ability. This study showed that ingestion of the fermented milk drink, especially in winter, would maintain the health condition of elderly subjects. The study design was based on a previous trial [7]. The previous study was a randomized, double-blind, placebo-controlled parallel study performed to investigate the effects of a fermented milk drink containing LcS. The ingestion period of the previous study was 6 months, and the number of randomized elderly subjects was 88; 72 subjects finished the trial. The results of the study revealed that the LcS group showed lower incidence of fever and improved bowel movements compared with the placebo group [7]. The ingestion period of this study was set to 6 months, and 88 subjects were randomized to the study groups. Sixty-eight subjects of the 88 subjects completed the trial. The number of withdrawn subjects in this study was comparable to that of the

previous study. In Table 2, the baseline data for days of fever and duration of fever days in the placebo group was higher than those in the LcS group. We applied ANCOVA analysis using baseline values as covariates to evaluate the effects of the fermented milk drink; therefore, the higher baseline value in the placebo group compared with the LcS group would not affect the results.

By analyzing the peripheral blood, saliva sIgA, and microbiota, we expected that we would be able to elucidate the mechanism by which the fermented milk drink helped to prevent infectious disease. However, mineral levels, immune parameters such as SI and saliva sIgA, and microbiota of the LcS group in the ingestion period were not significantly improved compared with those of the placebo group. Takeda et al. showed that NK cell activity increased significantly after ingestion of a fermented milk drink containing LcS for 3 weeks [8]. The study design was cross-over trial using a fermented milk drink and a placebo drink with 10 elderly subjects (aged 69–97 years). On the other hand, we could not detect significant immune modulation activity of LcS in this study. The ingestion period of this study was 6 months and we collected blood samples before and at the end of the ingestion period but could not collect them periodically (e.g., monthly or every 3 months) because frequent blood sampling is a burden on the elderly. We might have missed the times when the immune modulation was activated by LcS. In addition, the nursing care levels of the subjects in this study were high, and it might be difficult to detect significant differences in immune modulation activity in these subjects. These differences might contribute to failing to detect immune modulation activity in this trial. Based on our examination of several parameters, we could not clarify the mechanism by which LcS had a health benefit in the elderly. In the case of a trial with elderly subjects, surrogate markers that can be obtained noninvasively and periodically are prerequisite to elucidate the mechanism of the effects of the fermented milk drink.

Our stratified analysis revealed that, in the placebo group, subjects aged ≥86 years or at nursing care level 4 or 5 developed fever more frequently in the ingestion period than in the pre-ingestion period (p=0.002 and 0.020, respectively, Table 5). These results showed that older persons and those who needed more care were more vulnerable to winter infections. In contrast, in both of these subgroups in the LcS group, the number of days on which fever was detected tended to be lower during the ingestion period than in the pre-ingestion period. The number of subjects aged ≥86 years in the placebo group was 19, and that in the LcS group was 15. We considered that this difference would not affect the results because the difference between the ingestion period and the pre-ingestion period of each subject was evaluated by ANCOVA using the baseline values as covariates.

The mean daily energy intake in the two groups was around 1,000 kcal, which is less than the recommendation of the Japanese Ministry of Health, Labor and Welfare [14]. However, mean body weight was constant during the study period (Table 4). Therefore, energy intake and energy

Table 4. Data for weight, body mass index, and nutrient content of meals

Item	Group	N	Pre-ingestion period (mean ± SD)	Ingestion period (mean ± SD)	p value*	p value#
Weight (kg)	LcS	35	43.77 ± 10.07	42.73 ± 10.88	0.194	0.311
	Placebo	33	44.73 ± 8.22	44.59 ± 8.08	0.937	
BMI (kg/m^2)	LcS	35	20.43 ± 3.43	19.93 ± 3.74	0.183	0.187
	Placebo	33	21.05 ± 3.21	21.10 ± 3.18	0.773	
Energy (kcal)	LcS	35	1,060.8 ± 256.7	1,026.3 ± 246.5	0.309	0.800
	Placebo	33	1,053.0 ± 267.9	1,034.7 ± 273.7	0.979	
Protein (g)	LcS	35	38.84 ± 9.05	36.97 ± 9.72	0.106	0.940
	Placebo	33	37.90 ± 13.94	36.36 ± 9.44	0.787	
Fat (g)	LcS	35	26.63 ± 6.93	25.12 ± 7.42	0.082	0.368
	Placebo	33	25.80 ± 8.54	25.90 ± 6.52	0.668	
Carbohydrate (g)	LcS	35	160.60 ± 47.61	157.27 ± 44.30	0.574	0.965
	Placebo	33	162.11 ± 46.87	158.49 ± 46.65	0.958	
Sodium (mg)	LcS	35	2,284.4 ± 775.3	2,209.5 ± 783.6	0.286	0.950
	Placebo	33	2,155.5 ± 883.0	2,125.4 ± 744.1	0.760	
Potassium (mg)	LcS	35	1,383.8 ± 385.0	1,288.8 ± 384.6	0.172	0.078
	Placebo	33	1,249.7 ± 418.1	1,332.4 ± 455.8	0.175	
Calcium (mg)	LcS	35	396.2 ± 132.7	329.7 ± 131.6	<0.001	0.853
	Placebo	33	395.7 ± 115.3	323.8 ± 142.8	0.023	
Magnesium (mg)	LcS	35	166.1 ± 49.1	155.5 ± 48.4	0.085	0.921
	Placebo	33	145.0 ± 57.0	145.0 ± 47.1	0.747	
Soluble fiber (g)	LcS	35	2.92 ± 1.14	2.91 ± 1.09	0.885	0.538
	Placebo	33	2.81 ± 1.34	3.01 ± 1.29	0.189	
Insoluble fiber (g)	LcS	35	6.22 ± 2.15	6.28 ± 2.25	0.677	0.821
	Placebo	33	5.89 ± 2.27	5.96 ± 2.33	0.728	
Total fiber (g)	LcS	35	9.57 ± 3.03	9.51 ± 2.96	0.791	0.863
	Placebo	33	9.65 ± 3.38	9.65 ± 2.81	0.924	
Sodium chloride equivalent (g)	LcS	35	5.79 ± 1.96	5.62 ± 2.01	0.333	0.828
	Placebo	33	5.46 ± 2.27	5.44 ± 1.91	0.937	

*Wilcoxon signed-rank test was performed. #ANCOVA was performd. BMI: body mass index; LcS: *Lactobacillus casei* strain Shirota.

Table 5. Stratified analysis of the number of days on which fever was detected by age and nursing care level

Item	Stratified value	Group	N	Pre-ingestion period (days / 6 months)	Ingestion period (days / 6 months)	p value*	p value#
Age	<86	LcS	17	0.94 ± 1.55	1.43 ± 2.31	0.432	0.678
		Placebo	12	1.08 ± 1.50	1.84 ± 2.60	0.473	
	≥86	LcS	15	0.86 ± 1.50	0.33 ± 1.05	0.625	0.008
		Placebo	19	1.31 ± 3.31	3.55 ± 5.85	0.002	
Nursing care level	1 to 3	LcS	10	1.00 ± 1.48	1.83 ± 2.83	0.563	0.535
		Placebo	12	2.08 ± 3.98	4.35 ± 7.16	0.078	
	4 and 5	LcS	22	0.86 ± 1.55	0.50 ± 1.11	0.633	0.016
		Placebo	19	0.68 ± 1.37	1.97 ± 2.44	0.020	

*Wilcoxon signed-rank test was performed. #ANCOVA was performd. LcS: *Lactobacillus casei* strain Shirota.

consumption were well balanced, and participants were considered to have sufficient energy for their activities. The estimated mean necessary protein intake for elderly people, based on a pooled analysis of five studies, is 0.85 g/kg weight/day, as proposed by Japanese Ministry of Health, Labor and Welfare [14]. The mean body weight of the participants was around 44 kg; therefore, a protein intake of 37 g would have been well balanced. Mean total fiber intake in the two groups during the study was less than 10 g, which is about half the recommendation of the Japanese Ministry of Health, Labor

and Welfare [14]. As shown in Table 2, bowel movements, enema administration, and diarrhea frequency during the ingestion period in the LcS group were not improved compared with those in the pre-ingestion period. Suboptimal fiber intake was likely one of the reasons for the lack of improvement in bowel habits. Not only drinking the fermented milk drink but also consuming sufficient fiber may be necessary to improve bowel habits. We calculated the frequency of diarrhea but did not record the severity of diarrhea. The severity of diarrhea is one of the important parameters to evaluate the effect of the fermented milk drink. We are planning to record the severity of diarrhea in the next trial.

After ingestion of the fermented milk drink, the number of *Bifidobacterium* cells increased, and the number of *Enterobacteriaceae* decreased (Table 3). These results were consistent with those of previous reports [4, 5, 7]. In a report by Nagata *et al.*, the number of *Bifidobacterium* cells did not increase and the number of *Enterobacteriaceae* did not decrease, rather increased, during the ingestion period in the placebo group, leading to a significant difference between the LcS group and the placebo group [7]. In this study, although the number of *Bifidobacterium* cells increased and the number of *Enterobacteriaecea* decreased as a result of ingesting the fermented milk, we could not detect any significant differences between the two groups because similar trends were detected in the placebo group. *Bifidobacterium* cells were detected at the level of 10^8 per g feces in the two groups throughout the trial period. These values were at the same level as in previous reports [4, 5, 7]. In the case of *Enterobacteriaceae*, the numbers of cells in the pre-ingestion period were at the level of 10^7 in both groups, and these values were about 10 times lower than in previous reports [4, 5, 7]. We could not explain why fewer *Enterobacteriaceae* cells were detected in the subjects of this study. We did not measure the cfu of LcS in feces in this study. It would have been necessary to collect feces into another sampling tube, store them in a refrigerator, and transport them within several days in order to measure live LcS in feces. These procedures would have overloaded the nursing staff; therefore, we decided not to measure LcS in this study. In previous studies, LcS were detected at the level of 10^8 cfu/g feces [4, 5].

In conclusion, continuous intake of a fermented milk drink by the elderly may reduce the number of days on which fever is detected and the duration of fever. Further studies are required to clarify the mechanism by which the fermented milk drink exerts these prophylactic effects.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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