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The Functional Food Containing Lactobacillus and Dietary Fiber for Amelioration of Bowel Movement in Healthy Japanese:

A Randomized, Double-blind, Placebo-controlled Study

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● Abstract

Objectives: The objective of this study is to examine how the ingestion of the functional food containing *Enterococcus faecalis* (EC- BabyM®) and indigestible dextrin (dietary fiber), "Dietary fiber Nuusankin 5,000 oku-ko" ("DFN") contributes to improve bowel movement.
Methods: A randomized, placebo-controlled, double-blind study was conducted to elucidate an effect of food. In this study we measured frequency of bowel movement, days of defecation, volume of stool, fecal properties, and intestinal flora as the primary outcome. We also evaluated blood biochemical parameters as the secondary outcome, and adverse events were collected by means of a written questionnaire during the study.
Results: From all of 63 applicants, 8 were eliminated due to not meeting inclusion criteria. 50 subjects were randomly assigned to an intervention group and made a start with ingestion. 22 subjects discontinued due to personal reasons, and the remaining 28 subjects completed the study. 1 was eliminated because of inadequacy of the records of bowel movement, thus data obtained with 27 subjects (DFN: 15, Placebo: 12) was used for the analysis of efficacy. After 12-weeks of ingestion, the intergroup analysis showed significant differences in "Frequency of bowel movement", "Days of defecation", and "*Bifidobacterium*" share in the intestinal flora compared to the Placebo. No adverse events were observed after the ingestion of the test product.
Conclusion: We found out that the ingestion of the functional food containing *Enterococcus faecalis* (EC-BabyM®) and indigestible dextrin (dietary fiber) for 12 weeks improved bowel movement. In addition, no safety-related matter occurred during the 12-week test period.

Key Words: indigestible dextrin, dietary fiber, lactobacillus, *Enterococcus faecalis*, EC-BabyM®, constipation, bowel movement

1. INTRODUCTION

Improving the intestinal environment is considered to be a key element in our healthcare. A number of studies indicate that the intestinal flora is involved in pathogenesis of gastrointestinal disorders such as inflammatory bowel disease, metabolic diseases such as obesity and diabetes mellitus, as well as neuronal disorders including multiple sclerosis. On the other hand, according to the Comprehensive Survey of Living Conditions conducted by Ministry of Health, Labor and Welfare in 2016, constipation is prevalent in 16.4% of males and females of 65 years and over[1]. Needless to say, it is important to change the lifestyle habits, increase the amount of exercise, or reduce stress, in order to improve bowel movement. In addition, it is also effective to actively ingest dietary fiber (recent Japanese are said to lack this) or the nutrition which stimulates the intestinal functions. Moreover to supply non-viable microbial ingredients as well as live one in gastrointestinal disorders has been illustrated in many studies[2)-5)].

In this study, we conducted a randomized, placebo-controlled, double-blind study to verify the efficacy and the safety of the functional food containing *Enterococcus faecalis* (EC- BabyM®) and indigestible dextrin (dietary fiber) for the amelioration of bowel movement in healthy Japanese.

2. METHODS

2.1.Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from M. Foods Corporation (Tokyo) at Japan Clinical Trial Association (JACTA, Tokyo). 12 days pre-observation period was carried out, followed by a 12 weeks intervention (from June 17th to September 10th, 2017). This study was conducted in accordance with the ethical principles of the declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study protocol was approved by the Institutional

1) JACTA (Japan Clinical Trial Association)
2) Nihonbashi M's Clinic
3) M. Foods Corporation

Table 1 Ingredients (per 12 g / daily intake)

Item	DFN	Placebo
Enterococcus faecalis (EC-BabyM®)	505 billion (number)	−
indigestible dextrin (dietary fiber)	7 g	−
whole milk powder	○	−
Isomaltooligosaccharide	○	−
fructose	○	−
maltodextrin	−	○

Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all Subjects. This trial was registered at UMIN Clinical Trial Registry (Trial ID: UMIN 000027526). The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of TRIBELATE CORPORATION (Tokyo), recruited from April through May, 2017.

2.2.1. Inclusion criteria

(1) Japanese aged between 20 and 60 years;
(2) Japanese within normal level of defecation or with a tendency toward constipation.

2.2.2. Exclusion criteria

(1) Subjects with chronic constipation;
(2) Subjects with food allergies;
(3) Subjects who are pregnant or lactating;
(4) Subjects who consume medicinal products which may influence the outcome of the study;
(5) Subjects who consume food which may influence the outcome of the study;
(6) Subjects who are judged as unsuitable for the study by the principle investigator.

2.3. Randomization

From all 63 applicants, 8 were declined and 5 were eliminated. The inclusion/ exclusion criteria was judged by the principle investigator. All subjects were sequentially allocated to Group A (n = 25, M; 12, F; 13) and Group B (n = 25, M; 12, F; 13) using a random number table. In the process of subject assignment, background factors such as gender, age, and frequency of bowel movement were taken into consideration to avoid biased distribution. Subjects in Group A ingested placebo and subjects in Group B ingested the test sample, "DFN" for 12 weeks.

2.4. Description of test foods and blinding

The test product was "Dietary fiber Nyuusankin 5,000 oku-ko" ("DFN") containing *Enterococcus faecalis* (EC-BabyM®) and indigestible dextrin (dietary fiber), prepared by M. Foods Corporation. The amount of daily intake was 3 spoonfuls of powdery DFN (weighing 12 g). The placebo ("Placebo") does not include indigestible dextrin (dietary fiber) and *Enterococcus faecalis* (EC-BabyM®). **Table 1** shows the ingredients of the sample. Both powders were indistinguishable in color, flavor, or taste. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 3 spoonfuls of powder directly, or drinking by dissolving it in water after every dinner for 12 weeks. Subjects were instructed as follows: to take the assigned concoction; to maintain their usual lifestyles and habits; to avoid taking other supplements; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to avoid excessive intake of lactobacillus food (such as yogurt, lactobacillus beverage, and Natto), oligosaccharide, or dietary fiber which may influence fecal flora; to maintain a daily record of one's bowel movement (frequency and volume of stool) and fecal properties (scent, sensation, color, and texture); and to send the diary to the study coordinator.

2.5.2. Outcome

The objective of this study is to elucidate the change for the improvement of bowel movement by taking the food containing *Enterococcus faecalis* (EC-BabyM®) and indigestible dextrin (dietary fiber). To evaluate this objective, frequency of bowel movement, days of defecation, volume of stool, fecal properties, and intestinal flora were measured as the primary outcome. As the secondary outcome, blood biochemical parameters were recorded to evaluate the safety of DFN. Furthermore, adverse events were collected by means of a written questionnaire during the study. According to the schedule shown in **Figure 1**, we measured parameters on efficacy and safety. These assessments were conducted upon pre- and post- intervention.

2.5.2.1. Bowel movement

Dairy reporting of "Frequency of bowel movement", "Days of defecation", and "Volume of stool" were calculated. "Frequency of bowel movement" is the amount of times one defecated during 1 week. "Days of defecation" is the number of days in which defecation occurred during 1 week. Regarding "Volume of stool", subjects compared volume to a 5 x 2.5 cm cylindrical

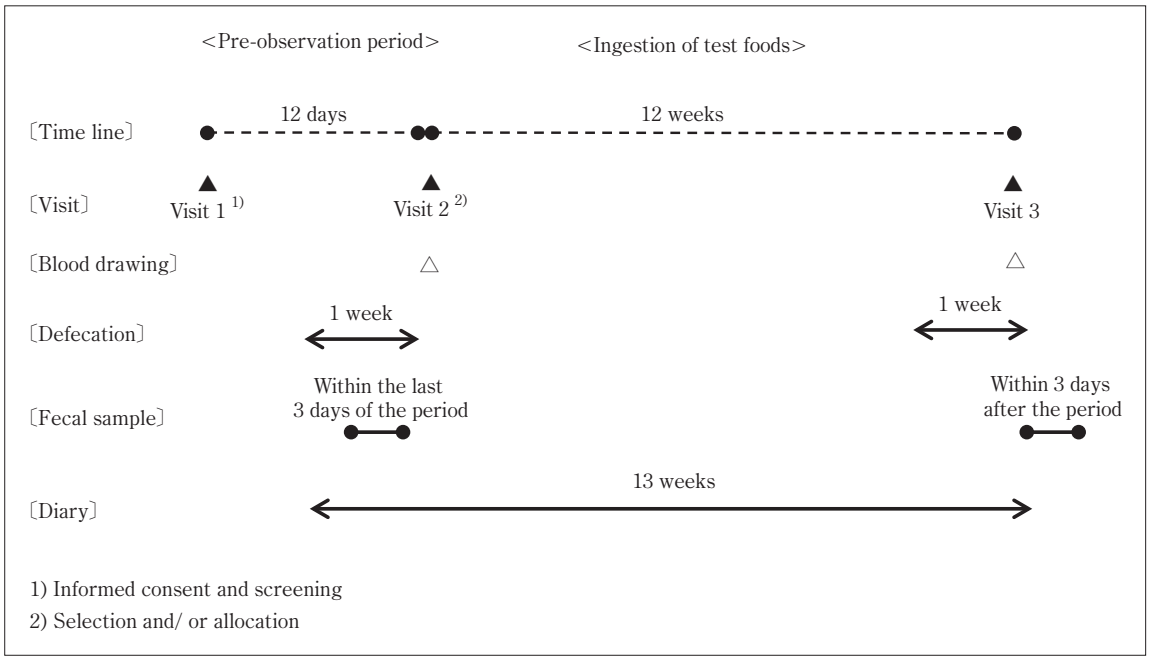

Figure 1　Schedule for the study

Table 2　Questionnaire about fecal properties

Item	Choices and score	Unit	Valuation method
Scent of stool	1; Very strong, 2; Strong, 3; A little strong, 4; Odorless	Score/ 1 defecation	Average score at 1 defecation for a week
Color of stool	1; Yellow, 2; Ocher, 3; Light brown, 4; brown, 5; Dark brown, 6; Blackish brown	Score/ 1 defecation	Average score at 1 defecation for a week
Texture of stool	1; Watery, no solid pieces, 2; Mushy, 3; Soft blobs with clear-cut edges, 4: Like a banana, smooth and soft, 5; Lumpy or cracks in the surface, 6; Separate hard lumps, like a nut	Score/ 1 defecation	Average score at 1 defecation for a week

object and recorded every time of defecation, then totaled the amount of 1 week.

2.5.2.2. Fecal properties

As for fecal properties, subjects rated 3 items; "Scent of stool", "Color of stool", and "Texture of stool" every time of defecation on an ordinal scale, which are illustrated in **Table 2**. The subjects' assessment of "Color of stool" used a color chart. In the way of "Texture of stool", we used "Bristol stool form scale"[6] and modified to Japanese. Then the average score was calculated during a week.

2.5.2.3. Intestinal flora

Subjects collected their fecal by the brush-type of feces sampling kit containing preservative solution twice just before starting to intake (at the end of pre-observation period) and just after 12 weeks of intake period. We sampled feces excreted within 72 hours just before and after the test period, for the reason that it is considered that it takes 36-72 hours for ingested food to be excreted as feces[7], and subjects with constipation defecate once every few or three days[8] (**Figure 1**).

A fecal analysis was carried out in the Techno Suruga Laboratory Co., Ltd, by means of Terminal restriction fragement length polymorphism (the Nagashima method of T-RLFP)[9][10], and the ratio to the total number of bacteria (occupancy) of *Bifidobacterium*, Lactobacillales, *Bacteroides*, *Prevotella*, *Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, *Clostridium* cluster XVIII, *Clostridium* cluster XVIII, and others were measured.

2.5.2.4. Biochemical blood test

At visit 2 (0-week) and visit 3 (12-week), the subjects had their blood taken. 12 hours prior to the visit, the subjects were not allowed to eat or drink (except water). The subjects took a 10 minute rest before proceeding with blood drawing to check blood biochemical parameters.

2.6. Data analysis

Per protocol set (PPS) was adopted in the study and no sample size design was used. All statistics were

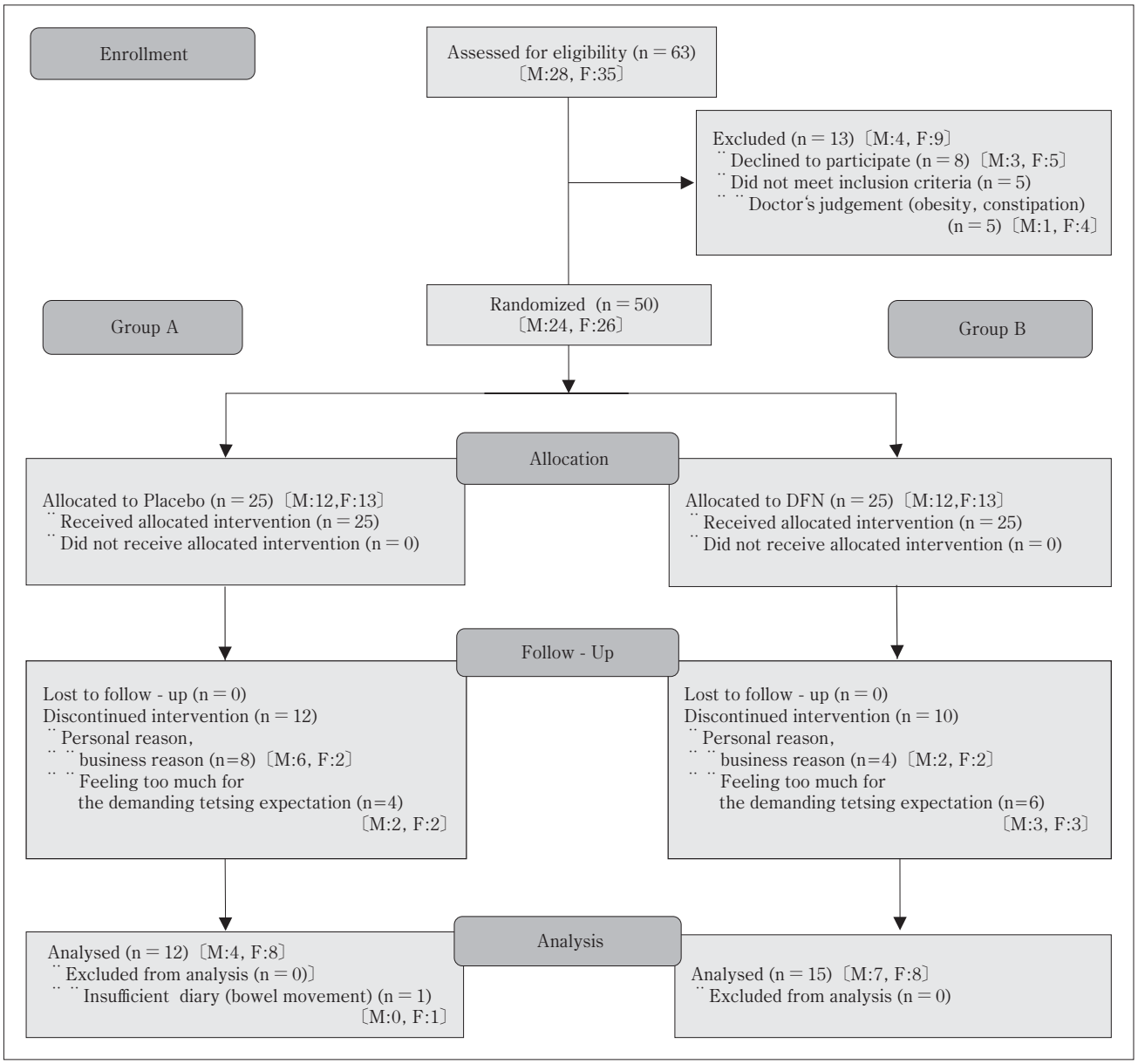

Figure 2 Flow diagram of subject disposition

Table 3 Subject demographics

Item	Unit	DFN	Placebo
Subjects	numbers	15	12
Male: Female *	numbers	7:8	4:8
Age *	years	41.7 ± 11.9	49.3 ± 5.5
Frequency of bowel movement *	times/ a week	10.5 ± 3.8	7.4 ± 4.6

mean ± SD

* No significant difference

expressed as mean ± standard deviation (SD). For the frequency of bowel movement, days of defecation, volume of stool, fecal properties, intestinal flora, and blood biochemical parameters, Student's t-test was used for intergroup comparisons, and paired t-test was used for intragroup analysis. A chi-square test and Student's t-test were used to compare subject backgrounds between groups. Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. Statistical analyses were performed using Statcel 4 (Yanai, 2015) and Excel Tokei 2015 (SSRI). The results were considered significant at a

Table 4　Results of bowel movement

Item	Unit	Time point	DFN (n = 15)	Placebo (n = 12)
Frequency of bowel movement	number/ 1 wk	0-week	10.5 ± 3.8	7.4 ± 4.6
		12-week	11.9 ± 4.3	8.3 ± 4.8 #
Days of defecation	day/ 1 wk	0-week	6.4 ± 0.9	5.3 ± 1.5 #
		12-week	6.4 ± 0.8	4.9 ± 1.7 ##
Volume of stool	piece/ 1 wk	0-week	27.5 ± 11.8	24.6 ± 19.3
		12-week	38.0 ± 10.4 **	30.9 ± 14.1

Values are expressed as the mean ± SD.
** $p < 0.01$ against 0-week.
# $p < 0.05$, $p < 0.01$ between-group difference in measured value.

Table 5　Results of fecal properties

Item	Unit	Time point	DFN (n = 15)	Placebo (n = 12)
Scent of stool	score/ 1 defecation	0-week	2.7 ± 0.4	2.9 ± 0.6
		12-week	2.9 ± 0.6	2.8 ± 0.6
Color of stool	score/ 1 defecation	0-week	4.1 ± 0.4	4.1 ± 0.5
		12-week	4.0 ± 0.4	3.9 ± 0.6
Texture of stool	score/ 1 defecation	0-week	3.4 ± 0.5	3.5 ± 0.6
		12-week	3.7 ± 0.4	4.1 ± 0.7 *

Values are expressed as the mean ± SD.
* $p < 0.05$ against 0-week.

< 5% level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 50 subjects were randomly assigned to an intervention group and made a start with ingestion. 22 subjects were withdrawn due to personal reason (business reasons:12, or feeling too much for the demanding testing expectation:10), and the remaining 28 subjects completed the study. Due to inadequacy of the records of bowel movement, 1 (Placebo) was eliminated, thus data obtained with 27 subjects (DFN: 15 <M; 7, F; 8> , Placebo: 12 <M; 4, F; 8>) was used for the analysis of efficacy (**Figure 2**). The subject's age range was 23-58 years old (mean age 45.1±10.1 y.o.). There was no significant difference in gender, age, and frequency of bowel movement between groups (**Table 3**). The test sample intake rates were 100% and 100% , respectively.

3.2. Bowel movement

Table 4 depicts the results of bowel movement. As for "Frequency of bowel movement", a significant difference was yielded in the intergroup analysis at week 12, without intragroup analysis. In Regard to "Days of defecation", a significant difference was observed at the baseline. At week 12, DFN maintained almost the same consistency. In contrast Placebo decreased, which realized the between-group difference in measured value. In "Volume of stool", DFN increased significantly after 12-weeks of ingestion, whereas the Placebo showed no change.

3.3. Fecal properties

Table 5 depicts the results of the state of stool. All items showed no significant difference, whereas "Texture of stool" indicated a tendency between two groups. As for "Texture of stool", DFN as well as Placebo were evaluated between type 3 (Soft blobs with clear-cut edges) and type 4 (Like a banana, smooth and soft) at baseline. At week 12, Placebo showed a significant hardness.

3.4. Intestinal flora

Table 6 shows changes of compositions of the intestinal flora. "*Bifidobacterium*" share of DFN was significantly increased after 12-weeks of ingestion, so that significant difference was yielded in the intergroup analysis. As for "Lactobacillales", Placebo showed decreased tendency, meanwhile DFN stayed about the same as pre-intake. Other parameters did not show any significant difference.

3.5. Biochemical blood test

Though an abnormal value was detected on CK (CPK) of pre-ingestion of DFN, the principle investigator judged it as the range of physiological variation, because of returning to its normal state after 12-weeks of ingestion. Moreover, DFN showed a significant difference in Glucose, Neutral fat (TG), Sodium, Chloride, and

Table 6 Results of intestinal flora

Item	Time point	DFN (n = 15)	Placebo (n = 12)
Bifidobacterium	0-week	8.4 ± 9.0	9.4 ± 6.7
	12-week	10.0 ± 9.7	6.1 ± 5.0*
	⊿ 0 -12 w	1.6 ± 3.4	− 3.3 ± 5.6#
Lactobacillales	0-week	2.2 ± 2.9	3.0 ± 2.8
	12-week	2.3 ± 2.3	1.4 ± 1.9
	⊿ 0 -12 w	0.1 ± 3.4	− 1.6 ± 3.2
Bacteroides	0-week	43.5 ± 15.5	40.6 ± 11.3
	12-week	45.4 ± 17.1	42.9 ± 16.8
	⊿ 0 -12 w	1.9 ± 8.9	2.3 ± 17.9
Prevotella	0-week	6.5 ± 14.8	8.8 ± 15.7
	12-week	5.5 ± 12.6	9.5 ± 15.2
	⊿ 0 -12 w	− 0.9 ± 4.7	0.6 ± 3.5
Clostridium cluster IV	0-week	6.5 ± 3.9	5.5 ± 3.6
	12-week	6.3 ± 6.3	4.6 ± 3.3
	⊿ 0 -12 w	− 0.2 ± 3.3	− 0.9 ± 4.7
Clostridium subcluster XIVa	0-week	15.9 ± 7.3	16.4 ± 8.4
	12-week	15.0 ± 6.7	19.0 ± 14.8
	⊿ 0 -12 w	− 0.9 ± 3.9	2.6 ± 14.4
Clostridium cluster IX	0-week	8.6 ± 8.5	6.8 ± 6.4
	12-week	8.1 ± 7.8	8.1 ± 9.1
	⊿ 0 -12 w	− 0.5 ± 7.3	1.3 ± 6.3
Clostridium cluster XI	0-week	0.5 ± 1.1	0.4 ± 0.7
	12-week	0.3 ± 0.6	0.2 ± 0.5
	⊿ 0 -12 w	− 0.3 ± 0.9	− 0.2 ± 0.7
Clostridium cluster XVIII	0-week	1.2 ± 0.7	1.6 ± 1.7
	12-week	1.0 ± 0.6	1.7 ± 1.7
	⊿ 0 -12 w	− 0.3 ± 0.6	0.1 ± 2.1
Others	0-week	6.8 ± 2.4	7.4 ± 4.2
	12-week	6.3 ± 3.1	6.6 ± 3.8
	⊿ 0 -12 w	− 0.5 ± 2.5	− 0.8 ± 3.3

Unit; %, Values are expressed as the mean ± SD.

* $p < 0.05$ against 0-week.

# $p < 0.05$ between-group difference in change from 0-week.

Calcium. In either case, since the difference was a minor one, the principle investigator judged it clinically safe. There was no significant difference in change between two groups (data not shown).

3.6. Safety

No adverse events associated with the test product were observed in the course of the reporting.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study for examining the effect of food containing *Enterococcus faecalis* (EC- BabyM®) and indigestible dextrin (dietary fiber) on bowel movement. As the primary outcome, the intergroup analysis showed significant differences in "Frequency of bowel movement", "Days of defecation", and "*Bifidobacterium*" share in the intestinal flora between the groups, after 12-weeks of ingestion. As the secondary outcome, it proved that no abnormal change was triggered by the ingestion of the test product.

Main Findings

This study examined the efficacy of ingesting food containing *Enterococcus faecalis* (EC- BabyM®), a species of lactobacillus, and indigestible dextrin, a type of water soluble dietary fiber. After 12-weeks of ingestion, DFN showed a significant difference in "Frequency of bowel movement", "Days of defecation", and "*Bifidobacterium*" share in the intestinal flora compared

to Placebo.

The Japanese society of Gastroenterology developed an evidence-based clinical practice guideline for irritable bowel syndrome[11], which followed Roma Ⅲ[12] (currently updated Rome Ⅳ[13]). This guideline covers chronic abdominal pain, abdominal discomfort, abnormal bowel movement. In this guideline, diet therapy and behavioral modification play important roles as step Ⅰ therapy. These considerations should be highly referred to in the intestinal environment improvement study of Japanese within normal level of defecation or with a tendency toward constipation.

The Food and Agricultural Organization of the United Nations and the World Health Organization defined probiotics as "living microorganisms, which when administered in adequate amounts confer health benefits on the host"[14]. Nobel laureate Elie Metchnikoff linked the longevity of Bulgarians with consumption of fermented milk products containing viable Lactobacilli, and introduced the concept of probiotics to the scientific community. Since then, probiotics had been widely consumed, mostly as functional foods or dietary supplement. Recently, increasing evidences have showed that not only viable Lactobacilli but also non-viable Lactobacilli is sufficient to modify biological responses[15]. Dietary nutrients may be converted into metabolites by intestinal microbes that serve as biologically active molecules affecting regulatory functions in the host. They could improve the intestinal environment, namely, manipulation of intestinal microbial communities, suppression of pathogens, immunomodulation, stimulation of epithelial cell proliferation and differentiation and fortification of the intestinal barrier[16][17].

Enterococcus faecalis is a spherical bacteria that is part of the normal flora of humans, and is commonly found in the vaginal and intestinal regions of about 40 to 80% of individuals. EC-BabyM® is one of the non-viable *Enterococcus faecalis* isolated from newborn Japanese babies. The orally administered non-viable *Enterococcus faecalis* cell wall preparations stimulate the gut immune system[18]. Moreover EC-BabyM® is a kind of strain, for which the trade mark is registered, and recorded as "*Enterococcus faecalis* EC-BabyM" in NITE Patent Microorganisms Depositary (NPMD), National Institute of Technology and Evaluation (NITE).

In the contrast, the indigestible dextrin is a water-soluble dietary fiber taken out and prepared from the indigestible components in the resulting mush. Dextrin is considered a prebiotic, compounds that feed probiotics or "good" bacteria[19]. It has been shown to aid lactobacilli, a powerful beneficial bacteria responsible for supporting digestion[20]. As the good bacteria continues to proliferate throughout the digestive system, a balanced ratio of good to bad bacteria is established.

"Prebiotics" are indigestible dietary fibers that probiotic bacteria use as a source of fuel to flourish. The discovery of prebiotics and supporting theories is relatively recent and the term of prebiotics was defined first in 1995[21]. Since then, numerous researches have shown that adequate prebiotic intake has many potential benefits. Microecology of the gastrointestinal tract is the physiologic basis for the effect of dietary fiber. The ecology consists in the intestines, the foods that are fed into the tract, and the flora living within. Within this ecology, normal flora and ferment dietary fiber produce a short chain fatty acids, which is very effective for the treatment of gastrointestinal disorders[22].

In the present study it was observed that the share of *Bifidobacterium* in the stool was improved, moreover the ingestion of the DFN increased the stool frequency and the number of days with bowel movement. This observation suggests that *Enterococcus faecalis* (EC-BabyM®) can make feeds for intestinal bacteria to reach the intestine, followed by indigestible dextrin (dietary fiber) in DFN.

Secondary Findings

In this study we examined the safety of the test product by blood test. Although both groups showed significant differences in the levels of glucose, natural fat, sodium, chloride, and potassium after 12 weeks, the differences were minor and the principle investigator judged them as the range of physiological variation (or clinically safe). During the test period 22 subjects discontinued the test. The reasons for discontinuance were personal circumstances such a business trip. A subject was withdrawn from analysis due to inadequacy of the records of bowel movement, and had nothing to do with the ingestion of the test product.

Therefore, based upon the blood test and the diaries of the subjects, we observed no harmful influence against biochemical and/or physiological matters of the subjects, and this result indicated the safety of the ingestion of the test product for 12 weeks of test period.

General Information

Roma Ⅲ[12] defined Functional Constipation Diagnostic criteria as follows [Criteria fulfilled for the last 6 months with symptom onset at least 6 months prior to diagnosis] ; 1. Must include *two or more* of the following: a. Straining during at least 25% of defecations, b. Lumpy or hard stools in at least 25% of defecations, c. Sensation of incomplete evacuation for at least 25% of defecations, d. Sensation of anorectal obstruction/blockage for at least 25% of defecations, e. Manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor), f. Fewer than three defecations per week; 2. Loose stools are rarely present without the use of laxatives; 3. Insufficient criteria for irritable bowel syndrome. On the other hand, according to The Japanese Society of Internal Medicine, constipation is "a case where the number of times of defecation is significantly reduced and/or exhibits difficulty in defecation". Decrease in stool frequency is defined as frequency of stool less

than 3 times per week, and difficulty in defecating means "straining during defecation", "feeling of incomplete evacuation", "cooped-up feeling of anal (uncomfortable feeling in perineum)". Many constipation patients mainly complain about difficulty in defecating rather than frequency of stool[23]. Accordingly, the thought that bowel movement is an essential part of QOL enhancement can be supported. While Japanese tend to suffer from irregular life in modern stress-sick society, it can be difficult to change one's lifestyle. On the other hand, habits such as taking supplements do not require them to change their lifestyle substantially, and therefore they are a much easier way for them to continue for a long period. Moreover, DFN is a powder which might be ingested soluble in drinks or foods, so we are able to take DFN divided several times without any damage of the taste. DFN is expected to be a convenient health supplement, and is easy to ingest by people with no constipation depending on the effect of supporting good bacteria.

Limitations

In this study we examined the effect of the food containing *Enterococcus faecalis* (EC- BabyM®) and indigestible dextrin (dietary fiber) by evaluating frequency of bowel movement, days of defecation, volume of stool, fecal properties, and intestinal flora. The screening was performed to choose subjects with normal level of defecation or with a tendency toward constipation. Meanwhile the principle investigator excluded the subjects with chronic constipation by an interview, because subjects were not able to be diagnosed with constipation judging only by the number of defecations. It is thought that many subjects with normal level of defecation were included by reason that the number of defecation for a week (at baseline) of all subjects (n 27) was 9.1±4.3 times. Moreover, it is assumed that at the baseline many subjects were normal in fecal properties such as scent of stool, color of stool, and texture of stool. Due to these conditions, the effect of DFN observed in this study should be applied not only for subjects with a constipation tendency but also subjects with normal level of defecation. In addition, regarding days of defecation, a significant difference between two groups was found at the baseline. Because of this, the effect of DFN for days of defecation would have limited.

5. CONCLUSION

In conclusion, we found out that the ingestion of "Dietary fiber Nyuusankin 5,000 oku-ko" containing *Enterococcus faecalis* (EC- BabyM®) and indigestible dextrin (dietary fiber) for 12 weeks improved bowel movement. In addition, no safety- related matter occurred during the test period.

CONFLICT OF INTEREST

All parts of this study were funded by M. Foods Corporation. Kimitoshi Yamaguchi is the CEO. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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