A THESIS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD AND NUTRITION

Effect of Combination of Glycerol Monolaurate, Fructooligosaccharides, and Lactic Acid Bacteria on Fecal Microflora in ICR Mouse Model

ICR 쥐 모델에서 글리세롤 모노라우레이트, 프락토올리고당 및 유산균의 조합식이 섭취가 분변미생물에 미치는 영향

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Department of Food and Nutrition
Graduate School
Seoul National University

Hyunjung Kwon

Abstract

Effect of Combination of Glycerol Monolaurate, Fructooligosaccharides, and Lactic Acid Bacteria on Fecal Microflora in ICR Mouse Model

Hyunjung Kwon

Department of Food and Nutrition

The Graduate School

Seoul National University

Because the intestinal flora is important in the maintaining the health of the host, it has gained much attention recently. Glycerol monolaurate (GML) is known to inhibit various harmful bacteria without inhibiting the growth of lactic acid bacteria (LAB) such as *Bifidobacterium* and *Lactobacillus* at the 100 ppm level while fructooligosaccharides (FOS) are prebiotic food materials which can selectively promote the growth of LAB. The objective of this study was to observe the effect of combination diets containing GML, FOS, and LAB on the composition of the fecal microflora. The experimental diets contained GML 1 mg, FOS 5 mg, and LAB (*B. bifidum* BGN4, *B. longum* BORI, *L. acidophilus* AD031 at a ratio of 3:3:4) 4 x 10^8 CFU each and their combinations per 1 g of feed. Mice (n=56) were fed the experimental diets for 2 weeks, and fecal

samples were collected once a week. Total bacteria, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides* spp., *Clostridium butyricum* and *Enterobacter* spp. & *Escherichia* spp. were analyzed with real-time PCR. After 1 week, the number of *Lactobacillus* spp. was significantly increased in the LAB, FOS, GML, LAB+FOS and LAB+GML groups ($p < 0.01$). The number of *Bifidobacterium* spp. was significantly increased in the LAB+FOS group ($p < 0.05$). The numbers of *Enterobacter* spp. & *Escherichia* spp. were significantly decreased in the FOS and LAB+FOS+GML groups. After 2 weeks, the number of *Bacteroides* spp. was significantly reduced in the LAB, FOS, FOS+GML and LAB+FOS groups. The GML alone group did not change the number of *Bacteroides* spp. and *Enterobacter* spp. & *Escherichia* spp. Noticeably, a significant increase in the number of *Bifidobacterium* spp. was observed in mice with a combination of LAB and FOS (LAB+FOS), whereas the increase was not significant in the LAB alone and FOS alone groups. Taken together, the combination of LAB and FOS, but not with GML, could be more effective than LAB or FOS alone in improving beneficial intestinal microflora.

Key words: fecal microflora, glycerol monolaurate, fructooligosaccharides, lactic acid bacteria, real-time PCR

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List of Abbreviations

GML: Glycerol monolaurate

FOS: Fructooligosaccharides

LAB: Lactic acid bacteria

ND: normal diet

L: ND with LAB

F: ND with FOS

G: ND with GML

FG: ND with FOS+GML

LF: ND with LAB+FOS

LG: ND with LAB+GML

LFG: ND with LAB+FOS+GML

1. Introduction

The intestinal microflora is composed of complex and diverse bacteria that play an important role on the maintenance of the normal functions in not only intestinal tract but also systemic body system. The microflora is related to obesity, diabetes, hypertension [1−4], immunity [5, 6], and the absorption of nutrition or drug [7]. The beneficial bacteria have a barrier effect that inhibits enteropathogenic adhesion to the enterocytes in intestinal tract. They also suppress growth of pathogens through competition in the use of nutrients by producing antimicrobial metabolite such as organic acids and various bacteriocin in intestinal tract [8, 9]. To better understand the relation between the intestinal microflora and health, analysis of microbial communities is a prerequisite.

GML, which is a derivative of lauric acid extracted from coconut oil, is formed by the glycerol monoester of lauric acid. GML is classified as generally recognized as safe (GRAS) and used in food and cosmetics [10, 11]. Food and Drug Administration (FDA) regulated GML for use at doses up to 100 mg/ml [12]. GML shows antimicrobial properties against virus, *Staphylococcus aureus* and *Bacillus anthracis* [13−16].

Prebiotics was first defined as a "non−digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health [17]." The commercially

available prebiotics such as maltooligosaccharide, fructooligosaccharide, galactooligosaccharide, and xylo-oligosaccharides are widely used in various functional foods. The prebiotic effect of these oligosaccharides takes effect through the stimulation of the activity and the promotion of growth of the useful bacteria such as *Bifdobacterium* and *Lactobacillus* [18]. The prebiotic effect of FOS have been reported in humans and experimental animals [19, 20]. FOS is composed of chains of fructose units linked by $\beta(1-2)$ glycosidic bonds. The number of units ranges from 2 to 60 and terminated with a D-glucose unit. FOS can be found from plant source such as onions, wheat, garlic, bananas, tomatoes, and honey [21]. In addition, there are many beneficial effects of FOS on the amelioration of glycemia/insulinemia, absorption of mineral, regulation of lipidic metabolism, production of short chain fatty acids, relief of the constipation, and modulation of the immune systems. Furthermore, FOS has low sweetness intensity and play a role as a soluble food fibre [22].

Probiotics are defined as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host" [23] and include commonly *Lactobacillus* and *Bifdobacterium* [24]. The common features of *Lactobacillus* and *Bifdobacterium* are gram-positive and nonmotile bacteria. While *Lactobacillus* grows in facultative anaerobic environment, the *Bifdobacterium* grows in anaerobic condition. The probiotic effects of these microorganism

are manifested through the suppression of pathogenic bacteria and improvement of irritable bowel syndrome, allergy, diarrhea, infectious diseases of the intestinal tract, lactose intolerance, and constipation [24–31]. They are frequently used in dairy products such as yoghurt and cheese, and fermented food such as kimchi and pickles. Recently, In order to maintain or modulate the balance of intestinal health, the use of supplements using probiotics and prebiotics has been increased [24, 32–34].

Whereas conventional PCR and fluorescence *in situ* hybridization (FISH) lack the capacity to precisely quantify the target bacteria, the recently developed real–time PCR is regarded to present a higher sensitivity and rapid quantitative detection for microflora assessment from fecal DNA [35–41].

The objective of this research is to observe alteration of fecal microbiology in ICR mice fed combination diets containing GML, FOS, and LAB. To analyze fecal microflora, this study optimized 16S rRNA gene–targeted specific primers for *Cl. buytiricum*, and genus primers for the *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides* spp., *Enterobacter* spp. & *Escherichia* spp. and total bacteria in the use of real–time PCR.

2. Materials and Methods

2.1. Microorganisms

The 33 experimental bacterial strains were either obtained from the Food Microbiology Laboratory at the Department and Food and Nutrition at Seoul National University or purchased from Korean Collection for Type Culture. Genomic DNAs from *L. rhamnosus* KCTC 3237, *L. acidophilus* KCTC 3168, *L. casei* KFRI 699, *L. plantarum* KFRI 708, *L. helveticus* ATCC 15009, *L. brevis* GABA 100, *L. delbrekii* KCTC 3188, *L. bulgaricus* KCTC 3186, *L. reuteri* ATCC 35608, *B. longum* RD47, *B. bifidum* BGN4, *B. longum* BORI, *B. infantis* KCTC 3249, *B. breve* KCTC 3419, *B. adolescentis* KCTC 3216, *B. catenulatum* KCTC 3221, *B. angulatum* KCTC 3236, *B. bifidum* KCTC 3418, *B. animalis* KCTC 3219, *Bac. fragils* KCTC 5013, *Bac. coprocola* KCTC 5443, *Bac. cellulosilyticus* KCTC 5800, *Bac. uniformis* KCTC 5204, *Bac. thetaiotaomicron* KCTC 5015, *E. coli* KCTC 1039, *Enterococcus faecalis* KCTC 3511, *Ruminococcus gnavus* KCTC 5920, *Ent. cloacae* subsp. *cloacae* KCTC 2361, *Prevotella intermedia* KCTC 5694, *Eubacterium rectale* KCTC 5835, *Cl. butyricum* KCTC 1871, *Cl. ramosum* KCTC 3323, *Cl. leptum* KCTC 5155 were used as positive or negative control strains for the validation of real-time PCR conditions for various primers. Among the experimental strains, six representative strains were used for the production of standard curves.

2.2. Preparation of diets and animals

FOS powder (>95%) and freeze dried strains such as *B. bifidum* BGN4, *B. longum* BORI, *L. acidophilus* AD031 were provided by Bifido Inc. (Gangwon-do, Korea). GML was purchased from Tokyo Chemical Industry CO,.LTD (Tokyo, >97%). The diets were prepared containing GML 1 mg, FOS 5 mg, 4 x 10^8 CFU LAB (*B. bifidum* BGN4, *B. longum* BORI, *L. acidophilus* AD031 at a ratio of 3:3:4) per 1 g of feed (DooYeol Biotech., Seoul, Korea). Five-week-old male ICR mice strain TAC (n=56, DooYeol Biotech., Seoul, Korea) were housed in a room where the temperature was kept at 23±3℃ with relative humidity of 50±10% and the light was maintained on a 12 h light/dark cycle. All animal studies and protocols were approved by Institutional Animal Care and Use Committee of Seoul National University. All mice (n=7 per group) were accustomed to their new environment for 1 week and consumed a commercial diet and tap water ad libitum prior to their allocation in one of eight-matched groups. After 1 week, the experimental groups consumed a normal diet (AIN-93G) and tap water ad libitum for 1 week. After 1 week, mice were fed eight different experimental diets for 2 weeks as follows: a normal diet group (ND), ND with LAB group (L), ND with FOS group (F), ND with GML group (G), ND with FOS+GML group (FG), ND with LAB+FOS group (LF), ND with LAB+GML group (LG), ND with LAB+FOS+GML group (LFG). Each feces were collected once a week during the experimental periods. Mice were sacrificed by CO_2

inhalation.

2.3. DNA extraction from bacterial cell and feces

The DNAs from 33 bacterial cells were extracted using MG Cell Genomic DNA Extraction SV kit (Doctor protein, Seoul, Korea) in triplicate and fecal genomic DNA was extracted using a QIAamp DNA minikit (QIAGEN, Hilden, Germany) in septuple. The fecal samples were stored at −80℃ until genomic DNA was extracted. All DNA samples were eluted in a final volume of 200 μl and immediately stored at −20℃until analysis.

2.4. Optimization of real−time PCR conditions for primers for fecal microflora analysis

The specificity of 11 primer pair was tested *in silico* by PCR simulation using the tools provided in the website http://insilico.ehu.es/PCR. The analysis of 11 primers was based on real−time PCR using 33 bacteria DNAs. In order to detect target bacteria, the optimization of the real−time PCR conditions for the primers for *Bacteroides* spp., *Bifidobacterium* spp., *Lactobacillus* spp., *Prevotella* spp., *Ruminococcus* spp., *Enterococcus* spp., *Enterobacter* spp. & *Escherichia* spp., *C. coccoides−E. rectale* group, *Cl. butyricum* and *Cl. leptum* subgroup were performed. The 2% agarose gel was used to measure the amplicon size (data not shown). The optimized primers are shown in Table 1 and the real−time PCR conditions are shown Table 2.

Table 1. List of primers used in this study

Group of species or species	sequence (5' - 3')	amplicon size (bp)	ref.
Total bacteria (Universal)	F: GTGSTGCAYGGYYGTCGTCA R: ACGTCRTCCMCNCCTTCCTC	147-148	[42]
Lactobacillus spp.	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	341	[43]
Bifidobacterium spp.	F: TCGCGTCYGGTGTGAAAG R: GGTGTTCTTCCCGATATCTACA	128	[42]
Bacteroides spp.	F: GAAGGTCCCCCACATTG R: CGCKACTTGGCTGGTTCAG	103	[42]
Enterobacter spp. & *Escherichia* spp.	F: GACCTCGCGAGAGCA R: CCTACTTCTTTTGCAACCCA	180	[42]
Cl. butyricum	F: GTGCCGCCGCTAACGCATTAAGTAT R: ACCATGCACCACCTGTCTTCCTGCC	205-208	[44]

Table 2. Conditions of real-time PCR for producing standard curve and fecal microflora analysis

Group of species or species	annealing temperature (℃)	annealing time	cycle for producing standard curve	cycle for fecal microflora analysis
Total bacteria (Universal)	60	30 s	40	30~40
Lactobacillus spp.	60	1 min	40	17~20
Bifidobacterium spp.	60	30 s	40	20~22
Bacteroides spp.	60	30 s	40	30
Enterobacter spp. & *Escherichia* spp.	63	30 s	40	20
Cl. butyricum	72	30 s	40	27

2.5. Standard curves for calculation of the number of target bacteria

E. coli KCTC 1039 was grown in LB broth and agar (Difco, Detroit, MI, USA) under aerobic conditions at 37℃ for 16 h. *Ent. cloacae* subsp. *cloacae* KCTC 2361 was grown in BHI broth and agar (Difco, Detroit, MI, USA) under aerobic conditions at 37℃ for 18 h. *L. acidophilus* KCTC 3168 and *B. adolescentis* KCTC 3216 were grown in MRS broth and agar (Difco, Detroit, MI, USA) under anaerobic conditions at 37℃ for 18 h. *Bac. thetaiotaomicron* KCTC 5015 and *Cl. butyricum* KCTC 1871 were grown in BHI broth and agar under anaerobic conditions at 37℃ for 18 h. The viable cell number of these bacteria was counted in the respective medium agar and genomic DNAs were extracted from the viable cells. Then, the extracted DNAs were diluted (10-fold serial dilution) in distilled water and then analyzed using real-time PCR in triplicate. The real-time PCR conditions are shown Table 2. These results were used to make standard curve (C_t=mx+b) for calculation of the number of target bacteria. C_t=mx+b described the connection between C_t and x ($\log_{10}$ CFU/ml). The m is the slope and b is y-axis intercept (Figure 1).

2.6. Fecal microflora analysis using real-time PCR

All fecal DNA samples were examined in duplicate per primer. Real-time PCR was performed in a Step one Plus and Step One (Applied Biosystems, USA). For the real-time PCR, 20 ㎕ mixture per sample was required. The mixture contained DNA 2 ㎕, 50X ROX Reference Dye 0.4 ㎕, primer pair (10 ㎕M) 0.4 ㎕, sterile distilled water 6.8 ㎕, and 2X SYBR Premix Ex Taq 10 ㎕ (Takara, Japan). The real-time PCR conditions went through an initial denaturation step at 95℃ for 30 s followed by 17~40 cycles of at 95℃ for 5 s and primer annealing at optimal temperature and time (Table 2). Real-time PCR results (Ct value) were calculated using regression equation of standard curve (Figure 1) and converted to the average of target bacteria in 1 g of feces.

2.7. Statistical analysis

Data are expressed as mean ± standard error of mean (S.E.M). The results obtained from 3 weeks were compared using a nonparametric one way ANOVA test (Kruskal-Wallis H) and Mann-Whitney U test. Differences of $p < 0.05$ or $p < 0.01$ were considered statistically significant. All data analysis was performed using IBM SPSS Statistics 22.0.

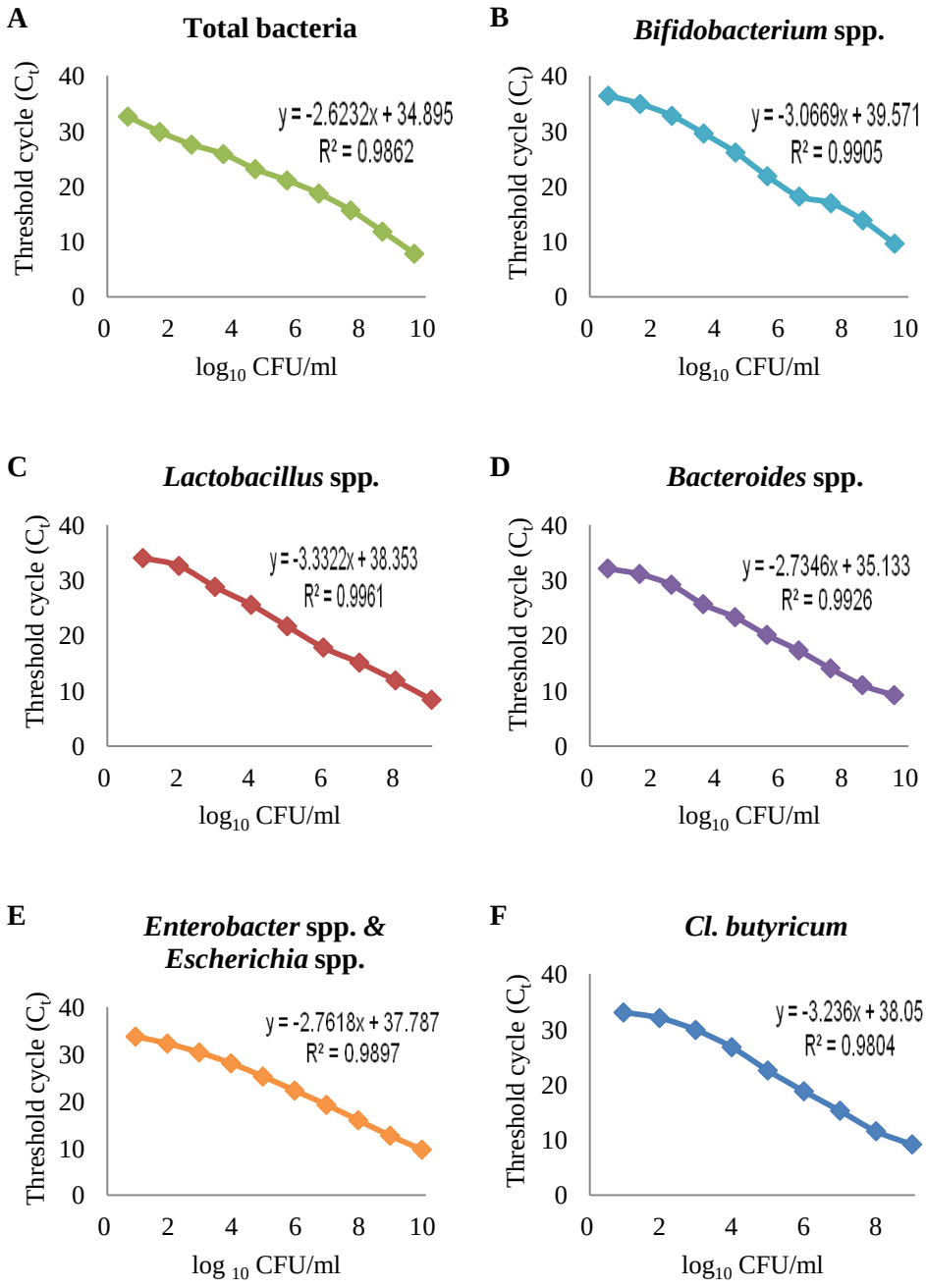

Figure 1. Standard curve for calculation of the number of target bacteria

(A) Total bacteria (Universal) (B) *Bifidobacterium* spp. (C) *Lactobacillus* spp. (D) *Bacteroides* spp. (E) *Enterobacter* spp. & *Escherichia* spp. (F) *Cl. butyricum*

3. Results

3.1. Optimization of real-time PCR conditions for primers

Validation of the real-time PCR conditions for primers was successful for 6 of the 11 primers. These validated primers included total bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* spp., *Enterobacter* spp. & *Escherichia* spp. and *Cl. butyricum*.

3.2. Alteration of fecal microflora in ICR mice

Until the end of the experiment, the number of total bacteria was about 10^{10} CFU/g for all the experimental groups (Table 3 and Figure 2), and *Cl. butyricum* was not detected in any group of the ICR mice strain TAC. The ND group, which was fed a normal diet, showed the lowest number of *Bifidobacterium* spp. and *Lactobacillus* spp. and the highest number of *Bacteroides* spp. and *Enterobacter* spp. & *Escherichia* spp. compared with the other groups.

Table 3. Changes of total bacteria in feces during the experiment

Week / Group	0 week		1 week		2 week	
	C_t value	10^{10} CFU/g	C_t value	10^{10} CFU/g	C_t value	10^{10} CFU/g
ND	8.66	5.32±0.71	8.82	4.92±1.01	9.73	2.06±0.27
L	8.09	9.26±1.74	8.16	8.15±1.12	8.16	8.39±1.32
F	8.96	4.83±1.20	8.86	4.49±0.68	8.80	9.09±2.95
G	9.53	2.36±0.12	8.85	4.78±0.96	10.36	1.45±0.32
FG	9.17	4.92±1.95	8.81	5.49±1.64	9.33	2.97±0.41
LF	9.55	2.39±0.28	8.66	6.33±1.68	9.70	2.04±0.13
LG	8.95	4.59±1.08	8.62	5.53±0.77	9.89	1.83±0.28
LFG	9.47	2.54±0.24	8.70	5.15±0.65 *	8.91	4.78±1.12

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in total bacteria for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG).

Bacterial count expressed as mean ± S.E.M. x 10^{10} CFU per gram of feces. C_t value expressed as mean.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

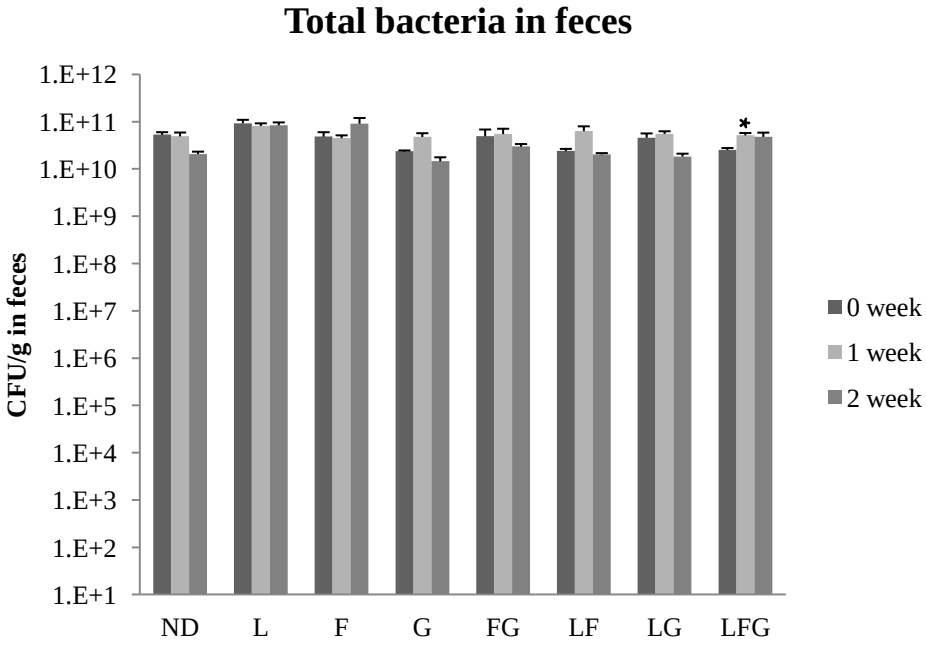

Figure 2. Changes of total bacteria in feces during the experiment

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in total bacteria for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG). The results are shown as means and error bar is S.E.M. of target bacteria in 1 g of feces.

10^n expressed E+number.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

3.2.1. Alteration of the number of *Lactobacillus* spp.

After 1 week, the number of *Lactobacillus* spp. varied from 6.64 x 10^7 to 1.51 x 10^9 CFU/g in the experimental groups. The levels of the L, F, G, LF and LG groups were significantly increased ($p < 0.01$) except for the ND, FG and LFG groups (Table 4 and Figure 3). The levels of these five groups increased 26, 5.53, 4.37, 14.61 and 8.54 fold, respectively.

After 2 weeks compared to 0 week, the number of *Lactobacillus* spp. ranged from 6.51 x 10^7 to 3.77 x 10^9 CFU/g in all the experimental groups (Table 4 and Figure 3). Although the number of *Lactobacillus* spp. was increased in the L, F, G, LF and LG groups, their levels were not significantly changed.

Table 4. Changes of *Lactobacillus* spp. in feces during the experiment

Week / Group	0 week		1 week		2 week	
	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g
ND	14.94	0.62±0.14	14.97	0.66±0.19	14.78	0.65±0.11
L	13.47	1.59±0.24	10.64	13.14±3.99 **	12.50	3.37±0.77
F	14.52	0.89±0.27	11.87	4.90±0.78 **	13.32	1.68±0.17
G	13.75	1.30±0.21	11.60	5.70±0.96 **	13.31	2.14±0.57
FG	12.02	4.34±0.78	11.75	9.21±3.32	12.54	3.04±0.48
LF	14.09	1.04±0.17	10.51	15.1±5.26 **	13.22	1.92±0.31
LG	14.25	0.93±0.15	11.11	7.93±1.10 **	13.04	2.64±0.73
LFG	12.09	4.44±0.87	11.32	6.82±0.86	12.83	3.77±2.08

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Lactobacillus* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG).

Bacterial count expressed as mean ± S.E.M. x 10^8 CFU per gram of feces. C_t value expressed as mean.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

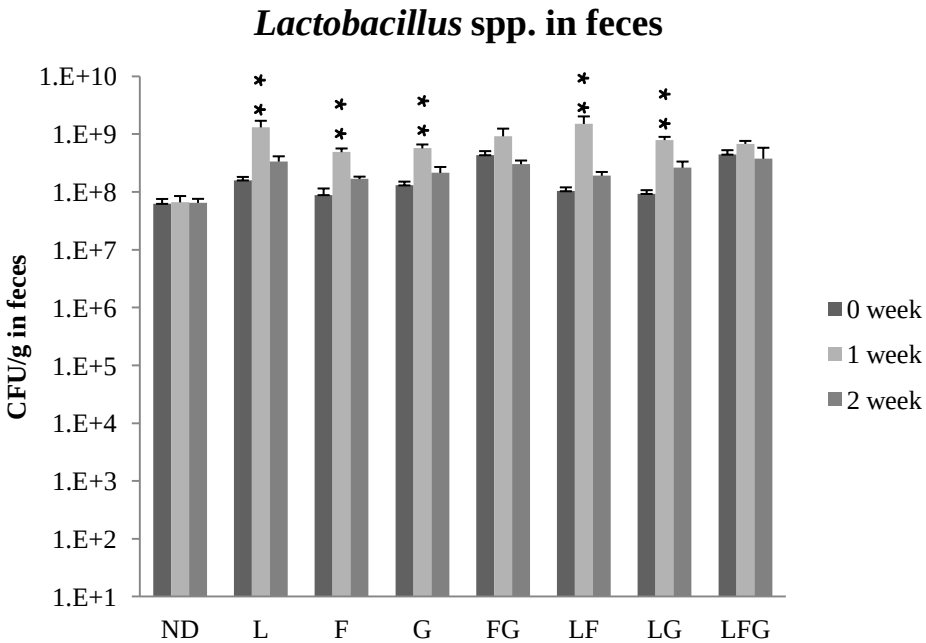

Figure 3. Changes of *Lactobacillus* spp. in feces during the experiment

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Lactobacillus* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG). The results are shown as means and error bar is S.E.M. of target bacteria in 1 g of feces. 10^n expressed E+number.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

3.2.2. Alteration of the number of *Bifidobacterium* spp.

After 1 week, the number of *Bifidobacterium* spp. varied from 1.86 x 10^8 to 1.74 x 10^9 CFU/g in all the experimental groups (Table 5 and Figure 4). Only the number of *Bifidobacterium* spp. was significantly increased in the LF group ($p < 0.05$).

After 2 weeks compared to 0 week, the number of *Bifidobacterium* spp. ranged from 2.21 x 10^8 to 1.64 x 10^9 CFU/g in the experimental groups (Table 5 and Figure 4). The number of *Bifidobacterium* spp. was increased in all the experimental groups. However, no significant increase was observed in any group.

Table 5. Changes of *Bifidobacterium* spp. in feces during the experiment

Week / Group	0 week		1 week		2 week	
	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g
ND	16.53	1.62±1.19	17.25	1.86 ±0.85	16.90	2.21±0.84
L	14.51	7.39±2.83	13.70	17.37±4.73	14.54	10.89±3.89
F	15.48	3.57±1.53	15.31	4.48±0.70	14.85	8.30±3.13
G	14.87	5.68±1.85	15.67	5.02±2.05	15.40	6.95±2.39
FG	16.69	1.44±0.75	16.75	6.53±2.18	15.39	5.16±1.51
LF	15.39	3.83±0.50	14.13	13.00±3.92 *	14.53	13.60±6.31
LG	15.34	3.97±2.02	13.75	16.47±5.22	15.42	8.51±4.49
LFG	14.34	8.42±3.06	14.09	14.24±5.59	14.26	16.42±7.22

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Bifidobacterium* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG).

Bacterial count expressed as mean ± S.E.M. x 10^8 CFU per gram of feces. C_t value expressed as mean.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

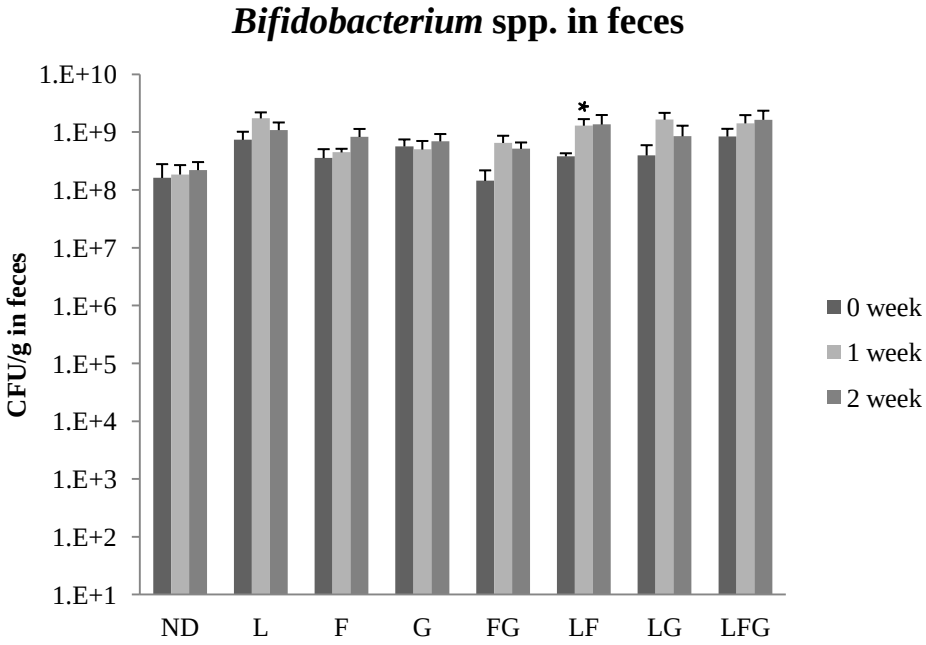

Figure 4. Changes of *Bifidobacterium* spp. in feces during the experiment

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Bifidobacterium* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG). The results are shown as means and error bar is S.E.M. of target bacteria in 1 g of feces. 10^n expressed E+number.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

3.2.3. Alteration of the number of *Bacteroides* spp.

After 1 week, the number of *Bacteroides* spp. varied from 6.92 x 10^8 to 3.50 x 10^9 CFU/g in the experimental groups (Table 6 and Figure 5), while their levels were not significantly changed.

After 2 weeks compared to 0 week, the number of *Bacteroides* spp. ranged from 3.24 x 10^8 to 1.59 x 10^9 CFU/g in the experimental groups (Table 6 and Figure 5). The levels of the L and FG groups were significantly decreased ($p < 0.01$). Second, the levels of the F and LF groups were significantly reduced ($p < 0.05$). A reduction of 45% or more of *Bacteroides* spp. was seen in the L (61%), F (49%), FG (67%) and LF (54%) groups.

Table 6. Changes of *Bacteroides* spp. in feces during the experiment

Week / Group	0 week		1 week		2 week	
	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g
ND	11.46	25.38±4.90	11.04	35.02 ±5.65	11.99	15.95±2.61
L	12.74	8.37±1.31	12.93	7.56±1.51	13.97	3.24±0.05 **
F	12.57	9.32±1.17	12.87	8.20±1.64	13.39	4.80±0.07 *
G	12.67	8.75±1.30	12.67	8.20±2.25	13.23	5.72±1.17
FG	12.42	10.78±1.48	13.02	6.92±1.33	13.76	3.52±0.06 **
LF	12.35	11.23±1.42	12.65	9.87±2.85	13.35	5.14±0.09 *
LG	12.13	15.53±4.15	12.34	11.63±1.79	12.43	11.50±2.33
LFG	12.10	14.55±2.45	12.94	8.75±3.23	12.95	7.26±1.33

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Bacteroides* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG).

Bacterial count expressed as mean ± S.E.M. x 10^8 CFU per gram of feces. C_t value expressed as mean.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

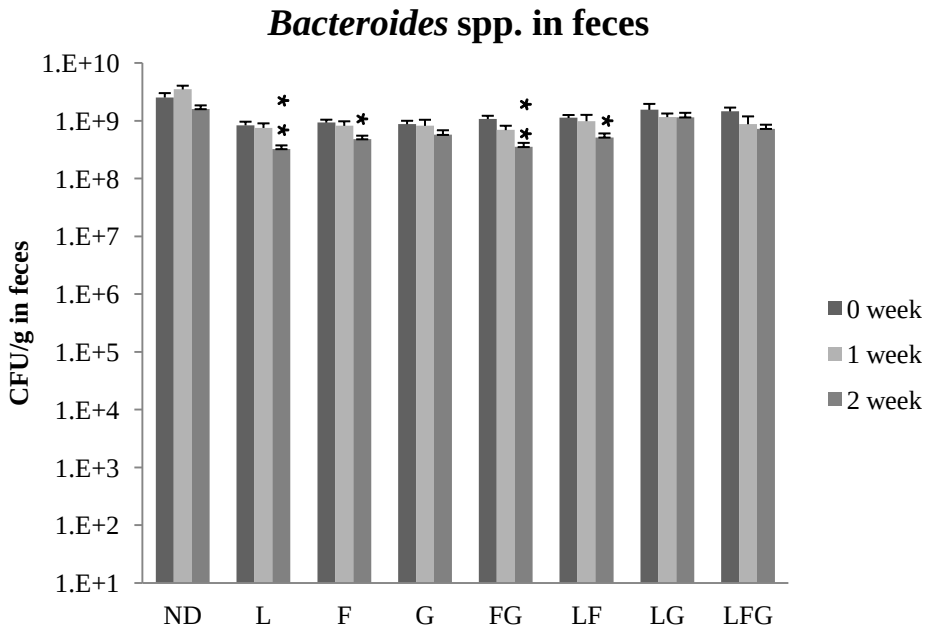

Figure 5. Changes of *Bacteroides* spp. in feces during the experiment

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Bacteroides* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG). The results are shown as means and error bar is S.E.M. of target bacteria in 1 g of feces. 10^n expressed E+number.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

3.2.4. Alteration of the number of *Enterobacter* spp. & *Escherichia* spp.

After 1 week, the number of *Enterobacter* spp. & *Escherichia* spp. varied from 1.82 x 10^8 to 3.64 x 10^9 CFU/g in the experimental groups (Table 7 and Figure 6). The levels of *Enterobacter* spp. & *Escherichia* spp. in the F and LFG groups were significantly reduced ($p < 0.01$ and $p < 0.05$, respectively). A more than 70% reduction of *Enterobacter* spp. & *Escherichia* spp. was observed in the F (70%) and LFG (75%) groups.

After 2 weeks compared to 0 week, the number of *Enterobacter* spp. & *Escherichia* spp. ranged from 2.40 x 10^8 to 4.43 x 10^9 CFU/g in all the experimental groups (Table 7 and Figure 6). A significant decrease was not observed in any group.

Table 7. Changes of *Enterobacter* spp. & *Escherichia* spp. in feces during the experiment

Week / Group	0 week		1 week		2 week	
	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g	C_t value	10^8 CFU/g
ND	15.69	10.43±6.41	15.48	36.36±31.84	14.54	44.25±27.72
L	14.78	22.09±9.95	15.11	8.11±7.48	15.72	4.88±2.23
F	14.83	10.76±1.32	16.22	3.23±1.25 **	15.88	4.26±2.28
G	16.46	2.73±0.29	16.01	3.84±1.50	16.14	3.46±1.77
FG	15.64	6.08±1.36	15.79	7.73±3.61	16.78	2.40±0.05
LF	14.77	15.54±5.49	15.34	6.68±3.17	15.00	8.88±2.47
LG	15.14	7.93±4.15	15.78	7.57±3.03	15.27	7.13±2.18
LFG	15.36	7.29±1.30	16.91	1.82±0.10 *	16.76	2.76±1.09

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Enterobacter* spp. & *Escherichia* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG). Bacterial count expressed as mean ± S.E.M. x 10^8 CFU per gram of feces. C_t value expressed as mean.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

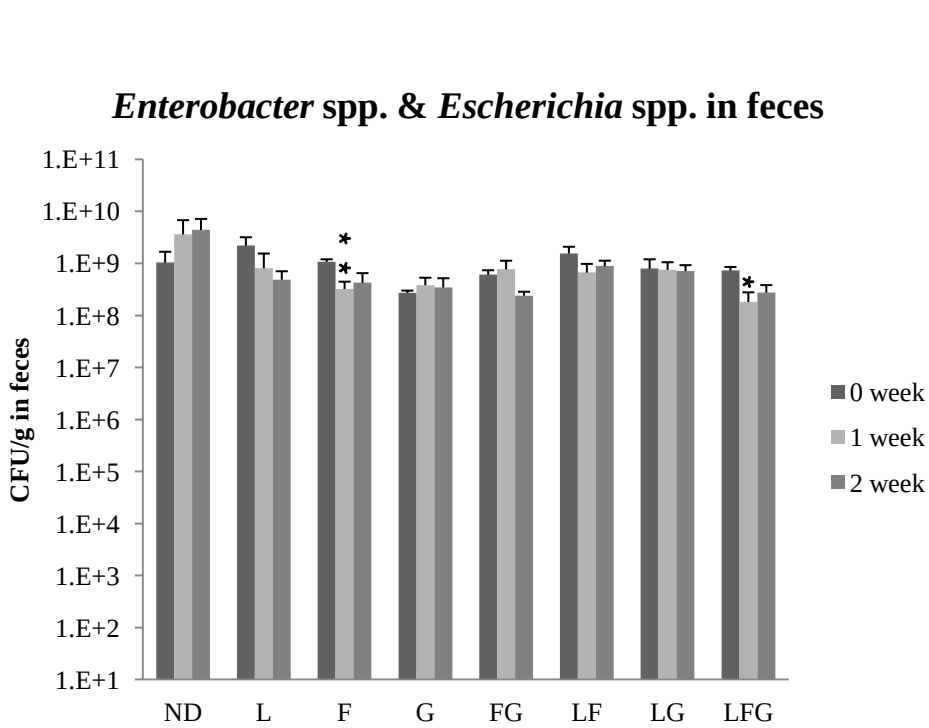

Figure 6. Changes of *Enterobacter* spp. & *Escherichia* spp. in feces during the experiment

0 week is a day which the diets of the mice were switched from the normal diet to the corresponding experimental diets. Changes in absolute number in *Enterobacter* spp. & *Escherichia* spp. for a normal diet group (ND), ND with LAB (L), ND with FOS (F), ND with GML (G), ND with FOS+GML (FG), ND with LAB+FOS (LF), ND with LAB+GML (LG), ND with LAB+FOS+GML (LFG). The results are shown as means and error bar is S.E.M. of target bacteria in 1 g of feces. 10^n expressed E+number.

* $p < 0.05$ or ** $p < 0.01$ compared with 0 week

4. Discussion

Originally, our aim was to detect the ten genera inhabiting the intestinal system. However, validation of the real-time PCR conditions for genus specific primers for *Prevotella* spp., *Ruminococcus* spp., *Enterococcus* spp., *C. coccoides-E. rectale* group, and *Cl. leptum* subgroup. was not successful due to non-specific amplification and other various reasons (data not shown). In this study, amplification with optimized real-time PCR conditions was performed to quantify total bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides* spp., *Enterobacter* spp. & *Escherichia* spp. and *Cl. butyricum*.

Even though real-time PCR for *Cl. butyricum* worked well, it was not found in any of the experimental groups. Because the murine intestinal tract is inhabited by complex and diverse microbial communities, the analysis of microflora is complex and difficult to interpret. Other studies have suggested that the composition of the microflora is different between this mouse strain and other vertebrates [35, 45, 46]. Additionally, there are genetic and life cycle as well as individual and environmental effects on the microflora [47-53]. The influence of these factors could have contributed to the inconsistent patterns in the number of *Lactobacillus* spp. and *Enterobacter* spp. & *Escherichia* spp. during the experiment (Tables 4 and 7).

Kabara and coworker [54] investigated fatty acids with high antimicrobial activity. They reported that the most effective fatty

acid against microorganisms is carbon C12 with monoglycerides. GML is a lauric acid (C12) esterified with glycerol. The distortion of the bacterial membrane lipid by GML may hinder the absorption of the nutrients, and thus suppress the growth of bacteria [55]. Despite the expected antimicrobial effects, GML had no effect on the composition of *Bacteroides* spp. and *Enterobacter* spp. & *Escherichia* spp. in this study. The relative resistance of the gram-negative bacteria to the GML because of the presence of cell wall lipopolysaccharides [56] and the reduction in the effective level of GML in the gut due to the interaction of GML with the intestinal lipid components and digestive absorption might have contributed to the presently observed ineffectiveness of GML [57].

Because FOS, which is a non-digestible oligosaccharide, can pass through the upper gut and reach the intestinal tract, this is selectively fermented by colonic bacteria and modulates the gut microflora affecting the host health [58, 59]. Previous studies used a mixture of GOS/FOS at a ratio of 9:1 and showed their health-promoting effects. [60-62]. This study used only FOS to just observe the prebiotic effect alone [63, 64]. The growth of *Bifidobacterium* was stimulated after the consumption of prebiotics at least 2 weeks [61]. The present study showed an increase in fecal bifidobacteria in the F, FG, LF and LFG groups when fed FOS. However, a significant increase in the number of *Bifidobacterium* spp. was observed only in the LF group.

When fed LAB, a significant increase in the number of

Lactobacillus spp. was observed in the L and LG groups. The numbers of *Bifidobacterium* spp. and *Lactobacillus* spp. were significant in the LF group after 1 week. In this study, multispecies probiotics were used for the beneficial improvement of the fecal microflora including two *Bifidobacterium* species and one *Lactobacillus* species. *Lactobacillus acidophilus* among the LAB is well known as a probiotics. [24, 65]. In a previous study, *B. bifidum* BGN4 revealed a noticeable adhesive capability for intestinal epithelial cells. The properties of *B. bifidum* BGN4 are suitable for a probiotic effect and is effective against inflammatory bowel disease [66]. *B. longum* BORI has an clinical effect in rotavirus-infected children [67]. Other studies have reported that multistrain were more effective than a single strain in alleviating irritable bowel syndrome symptom and antibiotic-associated diarrhea in children as well as more effective against pathogenic bacteria [65].

In the present study, the effects of combining three substances were as follows. A significant decrease in the number of *Bacteroides* spp. was observed in the L, F, FG and LF groups. The number of *Enterobacter* spp. & *Escherichia* spp. was significantly reduced in the F and LFG groups. A significant increase in the number of *Lactobacillus* spp. was observed in the L, F, G, LF and LG groups ($p < 0.01$). Meanwhile, the numbers of these bacteria did not have any significant differences between the combination diets and non-combination diets, whereas the number of *Bifidobacterium* spp. was significantly increased in the LF group ($p < 0.05$).

These results show that the combination of LAB and FOS is more effective in the promotion of *Bifidobacterium* spp. than LAB alone or FOS alone.

5. Conclusion

Taken together, the present study showed that the intake of three substances (GML, FOS and LAB) altered the fecal microflora differently. The effect of the combined LAB and FOS could be more effective than LAB or FOS alone in improving beneficial intestinal microflora. Because quantification analysis of fecal microflora in ICR mice fed combinations of different food materials (GML, FOS, and specific multistrain probiotics) is less well studied, this research contributes to providing knowledge on the effect of various combined food materials to improve intestinal microflora.

References

1. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. **444:** 1027-1131.
2. PD C, J A, MA I, M P, C K, D B, et al. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Fundam Clin Pharmacol. **56:** 1761-1772.
3. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, et al. 2010. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J. **4:** 232-241.
4. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. U.S.A. **102:** 11070-11075.
5. Pamer EG. 2007. Immune responses to commensal and environmental microbes. Nat. Immunol. **8:** 1173-1178.
6. Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. **9:** 313-323.
7. Wilson ID, Nicholson JK. 2009. The role of gut microbiota in drug response. Curr. Pharm. Des. **15:** 1519-1523.
8. Lu L, Walker WA. 2001. Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium. Am. J. Clin. Nutr. **73:** 1124S-1130S.
9. Candela M, Seibold G, Vitali B, Lachenmaier S, Eikmanns BJ, Brigidi P. 2005. Real-time PCR quantification of bacterial adhesion to Caco-2 cells: competition between bifidobacteria and

enteropathogens. Res. Microbiol. **156:** 887-895.

10. KABARA JJ. 1980. GRAS antimicrobial agents for cosmetic product. J Soc Cosmet Chem. **31:** 1-10.
11. KABARA JJ, SWIECZKOWSKI DM, CONLEY AJ, TRUANT JP. 1972. Fatty acids and derivatives as antimicrobial agents. Antimicrob. Agents Chemother. **2:** 23-28.
12. Lin YC, Schlievert PM, Anderson MJ, Fair CL, Schaefers MM, Muthyala R, Peterson ML. 2009. Glycerol monolaurate and dodecylglycerol effects on *Staphylococcus aureus* and toxic shock syndrome toxin-1 in vitro and in vivo. PLoS ONE. **4:** e7499.
13. SHIBASAKI I. 1981. Food preservation with nontraditional antimicrobial agents. J Food Saf. **4:** 35-58.
14. Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, et al. 2009. Glycerol monolaurate prevents mucosal SIV transmission. Nature. **458:** 1034-1038.
15. Zhang H, Feng F, Fu X, Du Y, Zhang L, Zheng X. 2007. Antimicrobial effect of food-grade GML microemulsions against *Staphylococcus aureus* Eur. Food Res. Technol. **226:** 281-286.
16. Vetter SM, Schlievert PM. 2005. Glycerol monolaurate inhibits virulence factor production in *Bacillus anthracis* Antimicrob. Agents Chemother. **49:** 1302-1305.
17. GR G, MB R. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. **125:** 1401-1412.
18. Roberfroid MB. 2000. Prebiotics and probiotics: are they functional foods? Am. J. Clin. Nutr. **71:** 1682-1687.
19. Tuohy KM, Kolida S, Lustenberger AM, Gibson GR. 2007. The prebiotic effects of biscuits containing partially hydrolysed guar

gum and fructo-oligosaccharides- a human volunteer study. Br. J. Nutr. 86: 341-348.

20. Bouhnik Y, Flourie B, Riottot M, Bisetti N, Gailing MF, Guibert A, et al. 1996. Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. Nutr Cancer. 26: 21-29.

21. Roberfroid MB, Delzenne NM. 1998. Dietary fructans. Annu. Rev. Nutr. 18: 117-143.

22. Sabater-Molina M, Larqué E, Torrella F, Zamora S. 2009. Dietary fructooligosaccharides and potential benefits on health. J. Physiol. Biochem. 65: 315-328.

23. FAO/WHO. 2001 Report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria www.fao.org/3/a-a0512e.pdf. ordoba, Argentina, 1-4 October.

24. Collins MD, Gibson GR. 1999. Probiotics, prebiotics, and synbiotics approaches for modulating. Am. J. Clin. Nutr. 69: 1052s-1057s.

25. Grimm V, Radulovic K, Riedel CU. 2015. Colonization of C57BL/6 mice by a potential probiotic *Bifidobacterium bifidum* strain under germ-free and specific pathogen-free conditions and during experimental colitis. PLoS ONE. 10: e0139935.

26. Yoon JS, Sohn W, Lee OY, Lee SP, Lee KN, Jun DW, et al. 2014. Effect of multispecies probiotics on irritable bowel syndrome: A randomized, double-blind, placebo-controlled trial. J. Gastroenterol. Hepatol. 29: 52-59.

27. Eskesen D, Jespersen L, Michelsen B, Whorwell PJ, Muller-Lissner S, Morberg CM. 2015. Effect of the probiotic strain

Bifidobacterium animalis subsp. *lactis*, BB-12®, on defecation frequency in healthy subjects with low defecation frequency and abdominal discomfort: a randomised, double-blind, placebo-controlled, parallel-group trial. Br. J. Nutr. **114:** 1638-1646.

28. Weston S, Halbert A, Richmond P, Prescott SL. 2005. Effects of probiotics on atopic dermatitis: a randomised controlled trial. Arch. Dis. Child. **90:** 892-897.

29. Asahara T, Shimizu K, Nomoto K, Hamabata T, Ozawa A, Takeda Y. 2004. Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing *Escherichia coli* O157: H7. Infect. Immun. **72:** 2240-2247.

30. Gareau MG, Sherman PM, Walker WA. 2010. Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol. **7:** 503-514.

31. Majamaa H, Isolauri E. 1997. Probiotics: a novel approach in the management of food allergy. J. Allergy Clin. Immunol. **99:** 179-185.

32. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al. 2007. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia. **50:** 2374-2383.

33. Bielecka M, Biedrzycka Eb, Majkowska A. 2002. Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. Food Res. Int. **35:** 125-131.

34. Hardy H, Harris J, Lyon E, Beal J, Foey AD. 2013. Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients. **5:** 1869-1912.

35. Furet JP, Firmesse O, Gourmelon M, Bridonneau C, Tap J, Mondot S, et al. 2009. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. FEMS Microbiol. Ecol. 68: 351-362.

36. Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, et al. 2002. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. Appl. Environ. Microbiol. 68: 5445-5451.

37. Rinttila T, Kassinen A, Malinen E, Krogius L, Palva A. 2004. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. J. Appl. Microbiol. 97: 1166-1177.

38. Matsumoto M, Tadenuma T, Nakamura K, Kume H, Imai T, Kihara R, et al. 2000. Effect of *Bifidobacterium lactis* LKM 512 yogurt on fecal microflora in middle to old aged persons. Microb. Ecol. Health Dis. 77- 80.

39. Gueimonde M, Tolkko S, Korpimaki T, Salminen S. 2004. New real-time quantitative PCR procedure for quantification of bifidobacteria in human fecal samples. Appl. Environ. Microbiol. 70: 4165-4169.

40. Delroisse J-M, Boulvin A-L, Parmentier I, Dauphin RD, Vandenbol M, Portetelle D. 2008. Quantification of *Bifidobacterium* spp. and *Lactobacillus* spp. in rat fecal samples by real-time PCR. Microbiol. Res. 163: 663-670.

41. Matsuki T, Watanabe K, Fujimoto J, Takada T, Tanaka R. 2004. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. Appl.

Environ. Microbiol. 70: 7220-7228.

42. Thapa. D, Louis. P, Losa. R, Zweifel. Ba, Wallace. RJ. 2015. Essential oils have different effects on human pathogenic and commensal bacteria in mixed faecal fermentations compared with pure cultures. Microbiology. 161: 441-449.

43. Malinen E, Rinttila T, Kajander K, Matto J, Kassinen A, Krogius L, et al. 2005. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. Am. J. Gastroenterol. 100: 373-382.

44. Bartosch S, Fite A, Macfarlane GT, McMurdo ME. 2004. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Appl. Environ. Microbiol. 70: 3575-3581.

45. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. 2015. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 350: 1084-1089.

46. Friswell MK, Gika H, Stratford IJ, Theodoridis G, Telfer B, Wilson ID, McBain AJ. 2010. Site and strain-specific variation in gut microbiota profiles and metabolism in experimental mice. PLoS ONE. 5: e8584.

47. BENNO Y, ENDO K, MIZUTANI T, NAMBA Y, KOMORI T, MITSUOKA T. 1989. Comparison of fecal microflora of elderly persons in rural and urban areas of Japan. Appl. Environ. Microbiol. 55: 1100-1105.

48. Weldon L, Abolins S, Lenzi L, Bourne C, Riley EM, Viney M. 2015. The gut microbiota of wild mice. PLoS ONE. 10: e0134643.

49. O'Toole PW, Claesson MJ. 2010. Gut microbiota: changes throughout the lifespan from infancy to elderly. Int. Dairy J. **20:** 281-291.

50. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, et al. 2009. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology. **137:** 1716-1724.

51. Campbell JH, Foster CM, Vishnivetskaya T, Campbell AG, Yang ZK, Wymore A, et al. 2012. Host genetic and environmental effects on mouse intestinal microbiota. ISME J. **6:** 2033-2044.

52. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci. U.S.A. **107:** 14691-14696.

53. Benson AK, Kelly SA, Legge R, Ma F, Low SJ, Kim J, et al. 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci. U.S.A. **107:** 18933-18938.

54. KABARA JJ, VRABLE R. 1977. Antimicrobial lipids: natural and synthetic fatty acids and monoglycerides. J Lipids. **12:** 753-759.

55. NIEMAN C. 1954. Influence of trace amounts of fatty acids on the growth-Fatty Acids and Monoacylglycerols. Bacteriol Rev. **18:** 147- 161.

56. Ouattara B, Simard RE, Holley RA, Piette GJ-P, Begin A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. **37:** 155-162.

57. Dayrit FM. 2014. The properties of lauric acid and their

significance in coconut oil. J Am Oil Chem Soc. **92:** 1-15.

58. Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev. **17:** 259-275.

59. Macfarlane S, Macfarlane GT, Cummings JH. 2006. Review article: prebiotics in the gastrointestinal tract. Aliment. Pharmacol. Ther. **24:** 701-714.

60. Knol J, Scholtens P, Kafka C, Steenbakkers J, Groß S, Helm K, et al. 2005. Colon microflora in infants fed formula with galacto-and fructo-oligosaccharides: more like breast-fed infants. J. Pediatr. Gastroenterol. Nutr. **40:** 36-42.

61. Moro G, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B, Boehm G. 2002. Dosage-related bifidogenic effects of galacto-and fructooligosaccharides in formula-fed term infants. J. Pediatr. Gastroenterol. Nutr. **34:** 291-295.

62. Bruzzese E, Volpicelli M, Squeglia V, Bruzzese D, Salvini F, Bisceglia M, et al. 2009. A formula containing galacto-and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. Clin Nutr ESPEN **28:** 156-161.

63. Kapiki A, Costalos C, Oikonomidou C, Triantafyllidou A, Loukatou E, Pertrohilou V. 2007. The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. Early Hum. Dev. **83:** 335-339.

64. Euler AR, Mitchell DK, Kline R, Pickering LK. 2005. Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. J. Pediatr. Gastroenterol. Nutr. **40:** 157-164.

65. Timmerman HM, Koning CJ, Mulder L, Rombouts FM, Beynen AC. 2004. Monostrain, multistrain and multispecies probiotics—a comparison of functionality and efficacy. Int. J. Food Microbiol. 96: 219-233.

66. KIM IH, PARK MS, JI GE. 2003. Characterization of adhesion of Bifidobacterium sp. BGN4 to human enterocyte-like caco-2 cells. J. Microbiol. Biotechnol. 13: 276-281.

67. Ji GE. 2005. Presented at the 2005 International Meeting of the Microbiological Society of Korea, May.

국문 초록

장내 미생물은 숙주의 건강을 유지하는데 중요하기 때문에 관련 연구가 증가하고 있다. 글리세롤 모노라우레이트 (GML)는 다양한 유해균을 저해하는 것으로 알려져 있다. 프락토올리고당 (FOS)은 프리바이오틱 효과가 있어 *Bifidobacterium*과 *Lactobacillus*와 같은 유산균의 성장을 촉진 시킬 수 있다. 유산균 (LAB)은 프로바이오틱 효과가 있어 위 3가지 물질은 장내 미생물에 영향을 미칠 수 있다.

연구 목적은 글리세롤 모노라우레이트, 프락토올리고당 및 유산균을 사료에 첨가하여 3가지 물질의 조합식이 섭취가 분변미생물에 미치는 영향을 관찰하는 것이다.

사료 1 g에 GML 1 mg, FOS 5 mg, 4 x 10^8 CFU LAB (3:3:4 비율의 *B. bifidum* BGN4, *B. longum* BORI, *L. acidophilus* AD031)를 각 군에 해당하는 물질을 첨가하여 제조하였다. 총 56마리의 ICR 쥐에게 2주 동안 각 군에 해당하는 실험식이를 섭취시켰고 분변은 일주일에 한 번씩 수집하였다. Real-time PCR의 조건을 최적화한 primer인 Total bacteria, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides* spp., *Clostridium butyricum, Enterobacter* spp. & *Escherichia* spp.에 대하여 분변미생물을 정량분석 하였다.

식이 섭취 1주일 후, *Lactobacillus* spp.는 LAB, FOS, GML, LAB+FOS 및 LAB+GML을 섭취한 군에서 유의적으로 증가했고 ($p < 0.01$) *Bifidobacterium* spp.은 LAB+FOS를 섭취한 군에서 유의적으로 증가했다 ($p < 0.05$). *Enterobacter* spp. & *Escherichia* spp.는 FOS 및 LAB+FOS+GML 섭취한 군에서 유의적으로 감소했다. 식이 섭취

2주일 후, *Bacteroides* spp.는 LAB, FOS, FOS+GML 및 LAB+FOS 성분이 포함된 군에서 유의적으로 감소했다. 단일 GML에서 *Bacteroides* spp.와 *Enterobacter* spp. & *Escherichia* spp.의 수는 변하지 않았다. 본 연구는 LAB와 FOS가 조합된 군에서 *Bifdobacterium*의 수가 유의적으로 증가한 반면 단일 LAB와 단일 FOS에서는 증가하지 않았다. GML을 제외하고 LAB와 FOS의 조합은 장내 균총 개선에 단일 FOS 또는 단일 LAB보다 더 효과적일 것이다.

주요어: 분변 균총, 글리세롤 모노라우레이트, 프락토올리고당, 유산균, real-time PCR

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