

## Original Paper

# Effect of Live *Lactobacillus Paracasei* NFRI 7415 on the Preference for a Lard Diet or Fish Oil Diet in Rats

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Received: October 20, 2018      Accepted: October 31, 2018      Online Published: November 13, 2018

doi:10.22158/fsns.v2n3p57

URL: <http://dx.doi.org/10.22158/fsns.v2n3p57>

### Abstract

*This study examined the effect of live Lactobacillus paracasei NFRI 7415 on the preference of a lard diet (LD) and a fish-oil diet (FD) in rats. 4-week-old male Fischer 344 rats were fed one of four diets; LD, LD + lactic acid bacteria (LLD), FD and FD + lactic acid bacteria for 4 weeks (dietary experimental period). The LLD and FLD groups freely ingested water containing Lb. paracasei NFRI 7415 (10<sup>7</sup> cfu/ml). After 4 weeks, all rats were placed on a two-choice diet program in which they self-selected from two food cups, each containing either the LD or the FD for 5 weeks (self-selection period). After the dietary experimental period, there was no significant difference in the final body weight and total food intake among the four groups. The intake of fish-oil and live Lb. paracasei NFRI 7415 was increasing the fecal lipids excretion, and it effectively reduced plasma total cholesterol concentration (p<0.05). It was indicated that the intake of live Lb. paracasei NFRI 7415 was no influence on the preference for fat in the dietary experimental period and the self-selection period.*

### Keywords

*Lactobacillus paracasei NFRI 7415, self-selection, ratio of fish oil diet intake*

### 1. Introduction

It is estimated that there are approximately 10 million patients with diabetes and pre-diabetes in Japan (Ministry of Health Labour and Welfare 2016). According to the National Nutrition Survey in 2016, the meat intake per day for individuals in their twenties is 2.5 times the intake of fish and shellfish. Animal products such as meat contain a large quantity of saturated fatty acids and cholesterol. The risk and incidence of obesity, type-2 diabetes, atherosclerotic vascular disease, and coronary heart disease are increasing in rapid proportion to the increased intake of animal fat (Walker et al., 2009; Ota et al., 2007). Meanwhile, n - 3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and

docosahexaenoic acid (DHA) contained in fish oil are known to exhibit plasma lipid-lowering, antithrombotic, and other health-related actions (Guichardant et al., 2015; Komatsuzaki et al., 2010; Arai et al., 2009). Previously, to investigate why young people disliked fish, Sato and colleagues performed experiments using three groups of rats fed different diets: a lard diet (LD), a soybean-oil diet (SD) or a fish-oil diet (FD) (Sato et al., 2009). After 8 weeks, all rats were placed on a self-selected regimen and allowed to choose LD or FD for 3 weeks. The results showed that the rats that were initially fed an LD, i.e., rats who had ingested exclusively animal-fat and no n-3 fatty acids consumed a large amount of FD immediately after the start of the self-selection period, demonstrating that rats have the ability to compensate a deficiency of n-3 fatty acids.

Lactic acid bacteria (LAB) have been utilized as a natural health food since ancient times, and the health-promoting effects of LAB are well recognized (Elmadfa et al., 2010). Some LABs are used in fermentation; typical examples can be found in the dairy industry for the production of cheese, yogurt, and other fermented milk products (Zhao et al., 2015).

*Lactobacillus paracasei* NFRI 7415, an LAB isolated from a traditional Japanese fermented fish (funa-sushi), exhibits high  $\gamma$ -aminobutyric acid (GABA)-producing ability (Komatsuzaki et al., 2005). We previously reported that *Lb. paracasei* NFRI 7415 removed cholesterol from the plasma and liver of rats fed an ethanol-containing diet (Komatsuzaki & Shima, 2012). Oral administration of this strain may have the potential to improve intestinal conditions and immune functions in humans (Komatsuzaki et al., 2017). Our data suggest that this strain may be effectively applied as a probiotic *Lactobacillus*.

Even though fat has no taste or smell in itself, as a food component fat has a highly compelling taste for which humans show a strong preference (Fushiki et al., 2003). Similarly in rats, fatty acid has been shown to be preferred over triglyceride (Tsuruta et al., 1999). LAB have been shown to affect the lipid metabolism in intestinal cells (Tomishige et al., 2016). Thus, it is speculated that the intake of LAB may have an influence on the preference for fat.

In order to examine these assumptions, we investigated the preference for fat in rats over an LD/FD self-selection period (5 weeks) following a 4 week dietary treatment period in which they received either LD or FD either with or without *Lb. paracasei* NFRI 7415. The body weight, fat tissue weight and serum lipid concentrations of the rats were also examined and discussed in relation to their observed dietary preferences.

## 2. Materials and Methods

### 2.1 Animals and Diets

Four-week-old male Fischer 344 rats were commercially obtained from Charles River, Japan (Yokohama, Japan). They were housed individually in stainless steel cages in a room kept at a constant temperature ( $23 \pm 1$  °C) and 50% humidity and illuminated in cycles of 12 h light/12 h dark (lights on from 7:00 to 19:00). The rats were given free access to food and ion-exchanged water. They were weighed, and their food intake was measured every other day from 10:00 to 12:00. The studies were

performed in accordance with the Animal Experimentation Guidelines of the Laboratory Animal Care Committee of Seitoku University.

The composition of the experimental diet is shown in Tables 1 and 2. The experimental diet was based on the AIN-93G diet (Reeves et al., 1993). In order to avoid an n-6 FA deficiency, soybean oil (3 g/100 g diet) was added to the four diets. Casein, lard, soybean oil, and dietary components were obtained from Oriental Yeast (Tokyo). Fish oil was purchased from Nihon-Suisan (Tokyo). The fatty acid composition of the diet is shown in Table 2. The n-6/n-3 ratios of the LD and the FD were 9.9 and 0.9, respectively.

**Table 1. Composition of the Experimental Diets<sup>1</sup>**

Ingredient	(g/100g)
Casein	20.0
L-Cystine	0.3
Cornstarch	49.95
Sucrose	10.0
Soybean-oil	3.0
Fat <sup>2</sup>	7.0
Cellulose	5.0
Mineral mixture <sup>3</sup>	3.5
Vitamin mixture <sup>3</sup>	1.0
Choline bitartrate	0.25
<i>tert</i> -Butylhydroquinone	0.0014

<sup>1</sup> Diet components were purchased from Oriental Yeast.

<sup>2</sup> Fat: lard (lard diet) or fish oil (fish-oil diet).

<sup>3</sup> Mineral mixture (g/kg of mix): CaHPO<sub>4</sub>, 500.0; NaCl, 74.0; K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·H<sub>2</sub>O, 220.0; K<sub>2</sub>SO<sub>4</sub>, 52.0; MGO, 24.0; MnSO<sub>4</sub>·5H<sub>2</sub>O, 6.77; FeSO<sub>4</sub>·7H<sub>2</sub>O, 4.95; ZnCO<sub>3</sub>, 1.6; CuCO<sub>3</sub>·Cu(OH)<sub>2</sub>·H<sub>2</sub>O, 0.3; KIO<sub>3</sub>, 0.01; NaSeO<sub>3</sub>, 0.01; CrK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O, 0.55; NaF, 0.06; sucrose, 115.75. Vitamin mixture (g/kg of mix): retinol, 4.8; cholecalciferol, 0.4; thiamine, 24.0; riboflavin, 0.6; pantothenic acid, 0.6; pyridoxine, 0.7; cobalamin, 0.01; menadione, 0.05; nicotinic acid, 3.0; D-calcium pantothenic acid, 1.6; folic acid, 0.2; biotin, 0.02; para-aminobenzoic acid, 5.0; inositol, 10.0; glucose, 949.02.

**Table 2. Fatty Acid Composition of the Diets (% of Total Fatty Acids)**

Fatty acid	Lard diet	Fish oil diet
10:0	0.07	-
12:0	0.1	0.1
14:0	1.2	6.1
14:1	0.1	0.4

15:1	-	0.1
16:0	20.9	16.6
16:1	-	6.6
17:0	1.8	0.3
17:1	0.3	0.7
18:0	10.1	2.2
18:1 (n-9)	38.9	19.2
18:2 (n-6)	22.5	16.7
18:3 (n-3)	2.3	2.6
20:0	0.3	0.1
20:1 (n-9)	0.5	6.3
20:2	0.3	0.1
20:3	-	0.1
20:4 (n-6)	0.1	0.5
20:5 (n-3)	-	10
22:3	-	0.1
22:5 (n-3)	-	1.5
22:6 (n-3)	-	6.2
Unknown	0.3	3.0
Total n-6	22.8	17.9
Total n-3	2.3	20.3
n-6/n-3	9.9	0.9

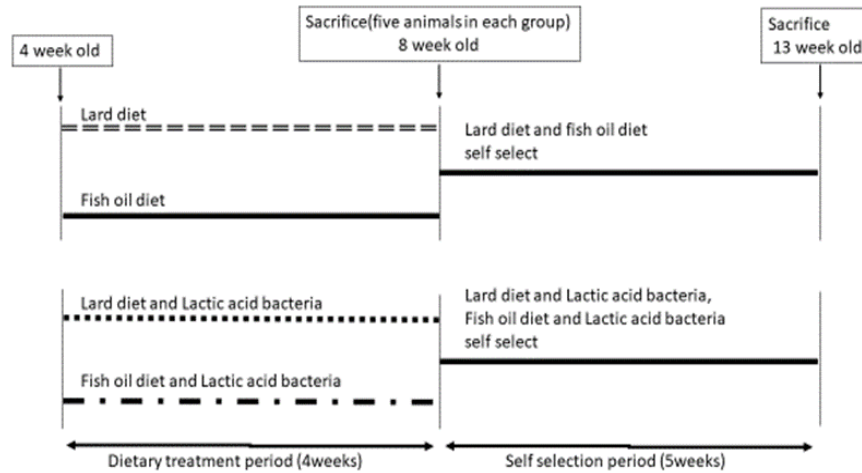
### 2.2 Preparation of Extract

A pre-culture of *Lb. paracasei* NFRI 7415 was grown to the stationary phase at 37 °C for 20 h in de Man, Rogosa, Sharpe (MRS) (Difco Laboratories, Detroit, MI) medium. The medium was separated from cells by centrifugation (5,000 rpm for 10 min at 4 °C). The cells were washed with phosphate-buffered saline (PBS; at pH 7.0) containing 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na<sub>2</sub>HPO<sub>4</sub>, and 0.24 g of KH<sub>2</sub>PO<sub>4</sub> (per liter), and then the cells were diluted (10<sup>7</sup> cfu/g) with sterilized water. The cell suspension was used in animal experiments.

### 2.3 Experimental Design

Forty-two 4-week-old male rats were divided into four groups. The groups respectively received the LD (n=10), the LD + LAB (LLD) (n=11), the FD (n=10), or the FD + LAB (FLD) (n=11) during the dietary treatment period (Figure 1).

The rats in the LLD and FLD groups freely ingested water containing *Lb. paracasei* NFRI 7415 (10<sup>7</sup> cfu/mL). After 4 weeks, half of the rats in each of the four groups were sacrificed. All rats were put on a two-choice diet program in which they self-selected from two food cups, one containing the LD and the other the FD, for 5 weeks (self-selection period). After 5 weeks, all rats were anesthetized. There was no fasting after the feeding period.



**Figure 1. Experimental Design**

The blood was centrifuged at 3,000 rpm for 10 min to separate the plasma, which was then stored at  $-80^{\circ}\text{C}$  until analysis. Liver, perirenal fat tissue and epididymal fat tissue were removed and weighed. Feces were collected for 24 h on the day before the end of the dietary treatment period and on the day before the end of the self-selection period. Feces were dried in an oven at  $105^{\circ}\text{C}$  for 24 h.

#### 2.4 Analytical Methods

Liver lipids were extracted by the methods of Folch et al. (1996), plasma triacylglycerol (TG) and total-cholesterol (T-cho) concentrations were measured, and liver extracts were similarly analyzed using test kits (Triglyceride E-test Wako and Cholesterol E-test Wako; purchased from Wako Pure Chemical Industries, Osaka, Japan).

#### 2.5 Assay of Fecal Lipids and Fecal Cholesterol

To assay the fecal lipids and cholesterol, 0.1 g of homogenized dry fecal matter was added to 4 mL of concentrated sulfuric acid in test tubes for 30 min at room temperature. Diethyl ether was added to reach 25 mL, and the solution was mixed. The diethyl-ether-containing layer was moved to a flask, and the diethyl ether was evaporated. The fecal lipid in the flask was then weighed. The T-cho concentration in fecal matter was determined in the same way as the liver T-cho concentration.

Fecal bile acids were measured by the procedure described in Iwami et al. (2002); 10 mg of the sample was mixed with 0.2 mL of 90% ethanol during vortex mixing, and incubated for 1 h at  $65^{\circ}\text{C}$ . The mixture was subjected to centrifugation at 5,000 rpm for 3 min. The supernatant was transferred to a 1.5 mL tube, and the ethanol was evaporated. Then, 0.2 mL of 90% ethanol was added to the precipitate for vortex mixing. The sample was dissolved in 1 mL 90% ethanol and measured using test kits (total bile acid test by enzyme colorimetric method; Wako Pure Chemical Industries).

#### 2.6 Statistical Analysis

Values were expressed as means  $\pm$  SD. Repeated-measures analysis of variance was used to evaluate the effects of preference group and time on food intake. Differences in mean values between groups were tested by Scheffe's multiple-range test. Differences were considered significant at  $p < 0.05$ .

### 3. Results

#### 3.1 Food Intake, Body and Organ Weight, Plasma and Liver Lipid Concentrations after the 4-Week Dietary Treatment Period

During the dietary treatment period, no significant difference in the food intake, body weight, or perirenal fat and epididymal fat tissue weights were observed among the four groups (Table 3). The liver weight of the FLD group was lower than those of the LD and LLD groups ( $p<0.05$ ) (Table 3). No significant difference was observed in LAB intake between the LLD and FLD group, based on monitoring of the drinking water levels (data not shown).

The plasma TG concentration of the FLD group was lower than that of the LD group ( $p<0.05$ ) (Table 3). The plasma T-cho concentrations of the FD and FLD groups were lower than those of the LD and LLD groups ( $p<0.05$ ) (Table 3). No differences were observed in liver TG and T-cho concentration among the four groups.

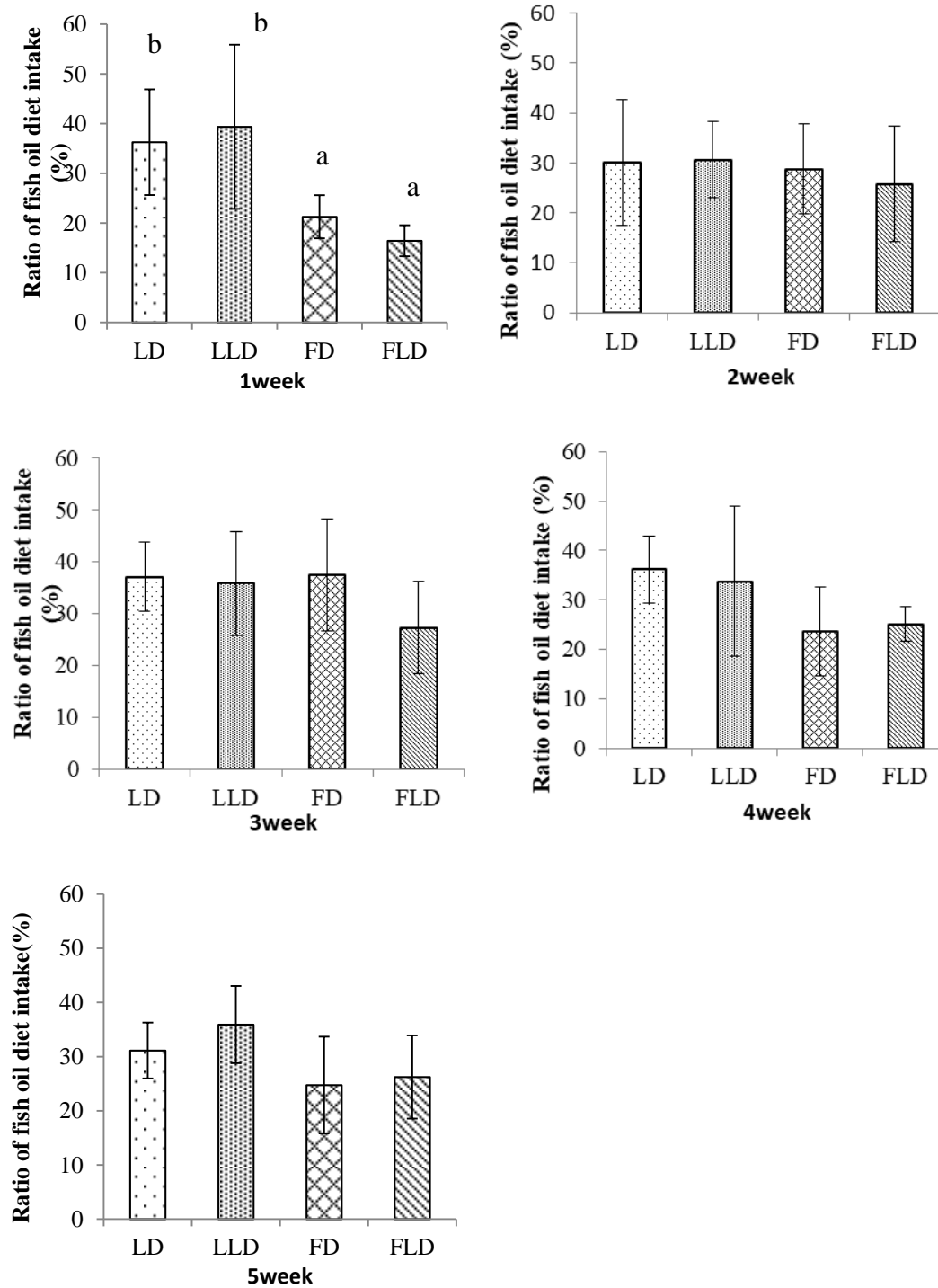
**Table 3. Food Intake, Body, Liver, and Fat Tissue Weights and Plasma and Liver Lipid Concentrations after a Dietary Treatment Period of 4 Weeks**

Group	LD (n=5)	LLD (n=5)	FD (n=5)	FLD (n=5)
Food intake (g)	212 ± 21	213 ± 21	213 ± 21	211 ± 21
Body weight (g)	194 ± 9	191 ± 17	190 ± 15	191 ± 9
Liver weight (g/100g BW)	4.12 ± 0.17 <sup>b</sup>	4.00 ± 0.24 <sup>b</sup>	3.88 ± 0.25 <sup>ab</sup>	3.56 ± 0.13 <sup>a</sup>
Perirenal fat tissue weight (g/100g BW)	0.76 ± 0.10	0.71 ± 0.05	0.76 ± 0.14	0.73 ± 0.08
Epididymis fat tissue weight (g/100g BW)	1.54 ± 0.36	1.45 ± 0.10	1.37 ± 0.15	1.41 ± 0.11
Plasma lipid (mg/dL)				
Triacylglycerol	242.5 ± 104.6 <sup>b</sup>	181.0 ± 30.2 <sup>ab</sup>	120.6 ± 94.5 <sup>ab</sup>	54.5 ± 12.7 <sup>a</sup>
T-cholesterol	78.3 ± 5.8 <sup>b</sup>	70.0 ± 4.9 <sup>b</sup>	51.6 ± 4.4 <sup>a</sup>	50.6 ± 5.6 <sup>a</sup>
Liver lipids (mg/g)				
Triacylglycerol	14.4 ± 4.2	14.5 ± 10.9	10.2 ± 3.6	7.0 ± 4.6
T-cholesterol	2.06 ± 0.60	1.01 ± 0.21	1.65 ± 0.47	2.27 ± 0.90

Values represent means ± SD. Within a row, values not sharing a common superscript letter are significantly different at  $p<0.05$ .

#### 3.2 Food Intake, Body and Organ Weight, Plasma and Liver Lipid Concentrations after the Self-Selection Period for 5 Weeks

After the self-selection period, no significant difference in the food intake, body weight, or perirenal fat and epididymal fat tissue weights were observed among the four groups (Table 4). When calculating the ratio of FD intake (fish-oil intake/total intake) over 5 weeks, it became about 30% in each of the four groups, and no significant difference was observed. Immediately after the start of the self-selection period (1 week), the ratios of FD intake of the LD and LLD groups were higher than that of the FLD group ( $p<0.05$ ) (Figure 2). However, the ratio of FD intake after 7 days of self-selection was approximately 30%; there was no significant difference among the four groups.



**Figure 2. Ratio of Fish Oil Diet Intake to Total Intake of Groups Fed a Lard Diet or Fish Oil Diet During a Self-Selection Period of 5 Weeks after Being Fed a Lard Diet or Fish Oil Diet for 4 Weeks**

Values are expressed as means  $\pm$  SD. LD and FD groups: n=5, LLD and FLD groups: n=6. Values not sharing a common superscript letter are significantly different at  $p < 0.05$ .

**Table 4. Food Intake, Body, Liver and Fat Tissue Weights and Plasma and Liver Lipid Concentrations after a Self-Selection Period of 5 Weeks**

Group	LD (n=5)	LLD (n=6)	FD (n=5)	FLD (n=6)
Food intake (g)	585 ± 20	598 ± 41	554 ± 46	595 ± 55
Lard diet intake (g)	388 ± 53	386 ± 75	433 ± 69	408 ± 49
Fish oil diet intake (g)	197 ± 39	209 ± 54	135 ± 43	142 ± 31
Ratio of fish oil diet intake (%)	34 ± 7	35 ± 10	24 ± 8	26 ± 5
n-6/n-3 ratio	2.5	2.5	3.0	3.2
Body weight (g)	297 ± 12	306 ± 17	303 ± 20	309 ± 23
Liver weight (g/100g BW)	3.26 ± 0.17	3.07 ± 0.22	2.36 ± 1.04	3.04 ± 0.15
Perirenal fat tissue weight (g/100g BW)	1.25 ± 0.24	1.25 ± 0.18	1.25 ± 0.15	1.31 ± 0.13
Epididymis fat tissue weight (g/100g BW)	2.24 ± 0.31	2.16 ± 0.32	2.38 ± 0.26	2.45 ± 0.15
Plasma lipid (mg/dL)				
Triacylglycerol	76.1 ± 21.6 <sup>a</sup>	101.7 ± 35.1 <sup>ab</sup>	122.3 ± 13.0 <sup>b</sup>	113.3 ± 15.1 <sup>ab</sup>
T-cholesterol	45.7 ± 10.2	38.7 ± 12.4	52.9 ± 9.7	56.7 ± 7.9
Liver lipid (mg/g)				
Triacylglycerol	18.7 ± 2.9	16.6 ± 7.1	10.8 ± 6.0	12.5 ± 5.8
T-cholesterol	1.08 ± 0.15	1.24 ± 0.22	1.18 ± 0.17	1.28 ± 0.27

Values represent mean ± SD. Within a row, values not sharing a common superscript letter are significantly different at  $p < 0.05$ .

After the self-selection period, the plasma TG concentration of the LD group was lower than that of the FD group ( $p < 0.05$ ) (Table 4). There were no significant differences in the plasma T-cho and liver lipid concentrations among the four groups.

### 3.3 Fecal Weight and Fecal Lipid Concentration after the Dietary Treatment Period and the Self-Selection Period

There were no significant differences in fecal weight among the four groups during the dietary treatment period and the self-selection period (Tables 5, 6). After the dietary treatment period, the fecal total fat concentration of the LD group was higher than that of the FD group ( $p < 0.05$ ) (Table 5). After the self-selection period, the fecal T-cho concentration of the FLD group was higher than those of the LD and LLD groups ( $p < 0.05$ ) (Table 6).



**Table 5. Fecal Weight and Fecal Lipids after a Dietary Treatment Period of 4 Weeks**

	LD ( n=5)	LLD ( n=5)	FD ( n=5)	FLD ( n=5)
Weight (fresh) (g)	2.21 ± 0.68	2.06 ± 0.58	2.13 ± 0.22	2.27 ± 0.36
Weight (dry) (g)	2.11 ± 0.44	1.79 ± 0.48	1.81 ± 0.24	1.91 ± 0.32
Total fat (mg/g)	50.6 ± 8.95 <sup>b</sup>	46.1 ± 4.5 <sup>ab</sup>	32.0 ± 9.34 <sup>a</sup>	40.2 ± 6.61 <sup>ab</sup>
Bile acid (mg/g)	8.40 ± 1.62	6.57 ± 0.93	7.99 ± 1.03	7.01 ± 0.76
T-cholesterol (mg/g)	0.35 ± 0.10	0.37 ± 0.10	0.32 ± 0.03	0.37 ± 0.05

Values represent mean ± SD. Within a row, values not sharing a common superscript letter are significantly different at  $p < 0.05$ .

**Table 6. Fecal Weight and Fecal Lipids after a Self-Selection Period of 5 Weeks**

	LD (n=5)	LLD (n=6)	FD (n=5)	FLD (n=6)
Weight (fresh)(g)	2.70 ± 0.31	3.10 ± 0.65	2.65 ± 0.69	2.89 ± 0.68
Weight (dry) (g)	2.02 ± 0.24	2.42 ± 0.37	2.08 ± 0.49	2.28 ± 0.54
Total lipid (mg/g)	77.0 ± 10.3	70.5 ± 15.7	87.2 ± 22.6	88.5 ± 17.9
Bile acid (mg/g)	7.42 ± 1.87	7.19 ± 1.60	8.37 ± 1.51	6.61 ± 3.07
T-cholesterol (mg/g)	0.40 ± 0.08	0.39 ± 0.10	0.47 ± 0.31	0.64 ± 0.23

Values represent mean ± SD.

#### 4. Discussion

No significant differences in total dietary intake, body weight, and perirenal fat tissue weight were observed among the four groups during the treatment period and the self-selection period. Therefore, it was suggested that a difference in ingested fats did not affect the appetite of rats. However, the liver weight and plasma TG of the FLD group were lower than those of the LD and LLD groups after the dietary treatment period ( $p < 0.05$ ) (Table 3). Previous studies have reported that *Lb.paracasei* NFRI 7415 can remove cholesterol from the plasma and liver of rats fed high-fat diets (Komatsuzaki et al., 2016). In another report, oral administration of this strain reached the intestinal tract of mice and improved enteric bacterial flora (Komatsuzaki et al., 2017). No significant differences in the liver weight and plasma TG were observed among the LD, LLD, and FD groups after the dietary treatment period (Table 3). Some LABs metabolized unsaturated fatty acids in the intestinal tract and produced conjugated fatty acid (CLA) has effect like probiotics (Kishino et al., 2011). n-3 PUFAs such as  $\gamma$ -lenolenic acid, EPA, and DHA are contained in fish oil (Table 2). It was assumed that these n-3 PUFAs were metabolized in the intestinal tract of the FD group by *Lb.paracasei* NFRI 7415. In order to clarify this assumption, it was necessary to construct an in vitro experiment to produce CLA or metabolize n-3 PUFA from this strain.

After the self-selection period, the plasma TG of the LD group was lower than that of the FD group

( $p < 0.05$ ), although no significant differences were found in the ratio of FD intake among the four groups (Table 4). The ratios of FD intake in the LD group and FD group were  $34 \pm 7$  (%) and  $24 \pm 8$  (%), respectively (Figure 2). Many reports have shown that diets enriched with fish-oil reduce the plasma TG concentration (Guichardant et al., 2015; Arai et al., 2009; Ikeda et al., 2001). Our results suggested that plasma TG concentrations in the LD group declined as a result of ingesting a large amount of FD in order to counteract the effect of n-3 fatty acids deficiency during the dietary treatment period.

Live *Lb.paracasei* NFRI 7415 has the capacity to accelerate fecal T-cho excretion, and it has been shown to effectively reduce the plasma T-cho concentration (Komatsuzaki et al., 2014). Arai et al., (2009) reported that fish-oil inhibited body weight gain and exhibited an anti-obesity effect. To investigate the cholesterol excretion effect of fish-oil and this strain, we measured the fecal lipids in the rats. Although the fecal total fat concentration of the LD group was higher than that of the FD group ( $p < 0.05$ ) (Table 5), there was no significant difference between the LLD group and the FLD group after the treatment period. The t-test analysis showed that the fecal fat concentration of the FLD group tended to be higher in the FD group ( $P = 0.06$ ). Caesar et al. (2015) showed that mice fed lard for 11 weeks have increased Bacteroides in the intestine, white adipose tissue inflammation, and reduced insulin sensitivity compared with mice fed fish oil. This phenotypic difference between the dietary groups can be partly attributed to differences in microbiota composition. More than 100 trillion intestinal bacteria inhabit the intestinal flora in the mammalian bowels, and more than 100 types of Bifidobacteria and anaerobic bacteria form intestinal flora (Mitsuoka et al., 1990). Over 99% of the bacteria in the gut in the intestinal flora of mammals are anaerobes. Among them, Streptococcus spp. and Bacteroides spp. are known as human carcinogens; further, it has been shown that the number of anaerobic bacteria exceeds that of aerobic bacteria in the feces of cancer patients (Shinohara, 1990). In this study, the intake of fish oil and live *Lb.paracasei* NFRI 7415 increased the fecal lipids excretion, and it effectively reduced the plasma T-cho concentration. At the same time, it is possible that useful bacteria such as resident LAB improve the balance of bacterial flora in the intestines.

At one week after the start of the self-selection period, the ratios of the FD intake in the LD group and LLD group were  $34.4 \pm 4.0\%$  and  $37.5 \pm 15.6\%$ , respectively (Figure 2). It was speculated that the LD and LLD groups ingested large amounts of the FD to compensate for deficient n-3 PUFA in the dietary treatment period. The plasma TG in the LD group exhibited an approximately three-fold decrease, from  $242.5 \pm 104.6$  mg/dL to  $76.1 \pm 21.6$  mg/dL, between the dietary treatment period and the self-selection period (Tables 3, 4). On the other hand, the plasma TG concentration in the FD group was  $120.6 \pm 94.5$  mg/dL in the dietary treatment period and  $122.3 \pm 13.0$  mg/dL in the self-selection period; it was nearly unchanged. After the self-selection period, the n-6/n-3 ratios of the LD, LLD, FD, and FLD groups were 2.5, 2.5, 3, and 3.2, respectively. As described in our previous paper, it was concluded that the proper n-6/n-3 ratio for the effective prevention of arteriosclerosis and cardiac diseases was 3 according to self-selection of the LD and the FD (Sato et al., 2009). Also in this study, it was suggested that the

rats had the ability to purposefully compensate for insufficient levels of essential fatty acids by consuming LD and FD.

It was previously reported that intraduodenal injection of *Lactobacillus johnsonii* La1 elevated efferent gastric vagal nerve activity, thereby increasing food intake (Horii et al., 2013). In that study, it was suggested that the intake of LAB increases the appetite. The lactic acid produced by LAB is known to have a sour taste, and a substantial proportion of young children have a preference for extremely sour tastes (Liem et al., 2004). However, in humans this preference appears to be related to the willingness to try unknown foods and a preference for intense visual stimuli; it is difficult to attribute this to a physiological need.

Because humans have preferences derived from food experiences and food culture, they are less strongly dominated by physiological needs than animals. However, it is possible to scientifically clarify preferences because humans and animals have physiological similarities (Fushiki, 2003). We previously reported that rats have the ability to compensate for a deficiency of n-3 fatty acids (Sato et al., 2009), and similar results were obtained from this study. At the same time, it was indicated that the intake of live *Lb.paracasei* NFRI 7415 had no influence on the preference for fat in the dietary treatment period or the self-selection period.

## 5. Conclusion

As in our previous study, the rats had the ability to purposefully compensate for insufficient levels of essential fatty acids in the experimental food intake period by consuming LD and FD during the self-selection period. In addition, ingestion of lactic acid bacteria did not affect this ability.

The total cholesterol concentration in feces after the self-selection period was significantly higher in the FLD group than in the FD group. By ingesting fish oil and lactic acid bacteria, the excretion effect of total cholesterol in feces of lactic acid bacteria was considered to be enhanced.

## Acknowledgments

We thank Nihon-Suisan (Tokyo) for providing the fish oil.

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