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Effects of Mannooligosaccharides from Coffee Mannan on Fecal Microflora and Defecation in Healthy Volunteers

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Mannooligosaccharides (MOS) were purified from the thermally hydrolyzed mannan in spent coffee grounds, with the aim of achieving the full utilization of natural unused food material. The effects of MOS on fecal microflora and the defecating conditions in eight healthy volunteers were investigated. The subjects were administered MOS at 1.0 g/day and 3.0 g/day for 2 weeks. A 2 week interval was observed between the two dose intake periods. The content of *Bifidobacterium* significantly increased during the two periods ($p<0.05$). It appeared that MOS intake had a tendency to increase the content of *Bifidobacterium* in fecal microflora depending on the dosage level. Defecating conditions were also improved at both dosage levels ($p<0.05$). These results suggested that MOS ingestion caused *Bifidobacterium* to be the predominant bacteria in the intestine and improved defecation.

Keywords: coffee, mannooligosaccharides, *Bifidobacterium*, intestinal microflora

Recently the increasing amount of coffee beverage consumption has led to an increase in the discharge of spent coffee grounds. Spent coffee grounds are disposed of as industrial waste, however, these grounds contain mannan. The authors obtained mannooligosaccharides (MOS) from mannan in spent coffee grounds by thermal hydrolysis. It is quite meaningful to fully utilize the by-products produced in the food industry. It is also important to evaluate the physiological functions of newly extracted oligosaccharides for effective application to food products.

In a previous *in vitro* study, fractionated MOS from hydrolyzed coffee mannan was used by *Bifidobacterium adolescentis*, *Lactobacillus acidophilus* and *L. gasseri*, but not used by harmful bacteria such as *Clostridium perfringens* and *Escherichia coli* (Asano *et al*., 2001). Furthermore, MOS were resistant to digestive enzymes and were fermented by human fecal bacteria. The fermentation products were short chain fatty acids (SCFA) (Asano *et al*., 2003). These results suggested that MOS were indigestible saccharides, which were selectively utilized by beneficial bacteria and converted to SCFA in the human large intestine. Short chain fatty acids are thought to lower the pH in the human intestine and to thereby prevent the growth of harmful bacteria like *Cl. perfringens*, and to cause greater constipation. However, it was not clear whether the administration of MOS influenced the intestinal microflora and defecation frequency.

In this paper, we investigated the effects of MOS on human fecal microflora *in vivo* and the defecation of eight healthy volunteers, using two different doses (1.0 g/day and 3.0 g/day).

Materials and Methods

Preparation of mannooligosaccharides (Asano et al., 2003) The thermally hydrolyzed product from spent coffee grounds was decolorized using active carbon powder (Umehachi; Taihei Chemicals, Osaka) and desalted using cation exchange resin (SKIB; Mitsubishi Chemicals) and anion exchange resin (WA30; Mitsubishi Chemicals, Tokyo). One hundred grams of a mixture of monosaccharides and MOS mixture were obtained from 300 g of the hydrolyzed products of spent coffee grounds. Monosaccharides were eliminated using active carbon chromatography with a stepwise gradient of water and 10.0% (v/v) ethanol. The purified solution was concentrated in an evaporator and freeze-dried. The MOS mixture (mannose; 1.0%, mannobiose; 35.4%, mannotriose; 25.9%, mannotetraose; 18.6%, mannopentaose; 10.0%, more than mannohexaose; 5.2% and moisture 3.9%) was used for human clinical tests.

Intake schedule Healthy volunteers consisting of two men and six women (from 18 to 45 years old, average 36.1 years) were assigned as subjects to test the effects of MOS on fecal analysis and defecation frequency. The MOS intake schedule is shown in Fig. 1. After a 2 week observation period, each volunteer was administered 3.0 g of MOS per day for 2 weeks. After a 2 week no-dose interval period, each subject was then administered 1.0 g of MOS per day for 2 weeks. The volunteers dissolved the MOS in an appropriate volume of drinking water or a beverage such as green tea and drank it every day during the intake period. The intake time was not mandated. Throughout the entire examination period, the subjects had their meals in the ordinary way and recorded details of the meals. However, they were restricted from consuming foods and medicine that influenced intestinal microflora, such as fermented milk, indigestible oligosaccharide products, natto (fermented soybeans), antibiotic medicine and purgative medicine. The subjects collected whole parts of freshly voided feces in vinyl pouches at the end of each period. Fecal samples in the pouches were packed in aluminum-coated plastic bags with an Anaero-Pack (Mitsubishi Gas Chemical Co., Inc., Tokyo) and sent to the authors for keeping in cold

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storage until analysis. This analysis of fecal microflora and pH was performed within 12 h after excretion. Specimens for SCFA analysis were kept at −20°C and thawed just before analysis. This study, which was in accordance with the guidelines of the Helsinki Declaration, was approved by the Ethical Review Board of Ajinomoto General Foods, Inc.

Determination of fecal microflora Determination of the fecal microflora was carried out using the modified Mitsuoka method (Mitsuoka, 1982). After the collected feces were made uniform by mashing them, 5 g of each fecal sample was dissolved in 45 ml of a diluent solution. One milliliter of 10-fold fecal solution was mixed with 9 ml of the diluent and then a series of 10-fold dilutions was prepared from 10^2 to 10^9. A 0.05 ml sample of each dilution was inoculated on media. Three kinds of de-selective media (EG agar, BL agar and TS Blood agar) and ten kinds of selective media (BS agar, ES agar, *Bacteroides* agar, CW agar, Clostridia Count agar, Modified LBS agar, DHL agar, TATAC agar, PEES agar and P agar) were used for bacterial analyses (Table 1).

Determination of moisture, pH and SCFA in feces The moisture content was determined by drying 4 g of feces under reduced pressure in a vacuum oven at 70°C. Fecal pH was directly measured by inserting a glass electrode into the feces. For SCFA analysis, 0.5 g of feces was mixed with 4.5 ml of 0.05 N H_2SO_4 and homogenized. The 10-fold fecal solutions were centrifuged at 10,000 rpm for 10 min. The supernatant was used for HPLC analysis after being filtered through a 0.45 μm membrane filter (Advantec, Tokyo). HPLC was performed with a Shimadzu LC-10 device and a refractive index detector (Erma EC-7512, Erma, Tokyo). A Merck-packed PolyspherOA KC column was used for anion exchange chromatography with 0.012 N H_2SO_4 elution. The flow rate was maintained at 0.4 ml/min, and the sensitivity of detection was 1 μg/ml.

Defecation frequency and visual fecal characteristics The volunteers observed their defecation (frequency and volume) and visual fecal characteristics and completed a questionnaire every day throughout the examination period. Fecal volume was determined as the approximate number of ping-pong balls equal to the volume of the feces (Inaki *et al*., 1999). Visual fecal characteristics (color and shape) and smell were determined and scored by comparing the shape (Okada, 1985) and color against standard color sheets (Inaki *et al*., 1999). Shape was scored between one (Firm) and six (Soft & watery). Color was scored between one (Yellow) and six (Dark brown). Odor was scored between one (Weak) and five (Very strong). We delivered a ping-pong ball and a standard score sheet that showed fecal color and shape to the subjects. The subjects determined and scored their fecal volume, color and shape by comparison with the standard sheet; in addition, they were trained to determine their fecal volume and visual fecal characteristics for one week before the examination.

Statistical analysis The results of the feces analysis were presented as mean values with standard errors. The data were analyzed using Bartlett test and this was followed by Fisher's Protected Least Significant Difference test. For the analysis of the defecation questionnaire, the data of the last week in each period, when the intestinal microflora had changed, were collected. The results of the defecation conditions were calculated as the mean values and standard errors of the last week. The data

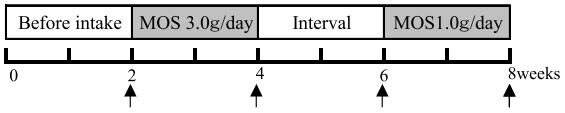

Fig. 1. Schedule of mannooligosaccharides (MOS) intake. ↑ means fecal sampling.

Table 1. The culture media and methods.

Media	Microorganisms mainly enumerated	Dilutions to be plated
Aerobic incubation		
TS Blood agar	Predominant aerobes	$10^{-5,-6,-7,-8}$
DHL agar	Enterobacteriaceae	$10^{-1,-3,-5,-7}$
TATAC agar	*Streptococcus*	
PEES agar	*Staphylococcus*	
P agar	Yeasts and molds	
Anaerobic incubation		
EG agar	Predominant anaerobes	$10^{-6,-7,-8,-9}$
BL agar	Predominant anaerobes	
BS agar	*Bifidobacterium*	$10^{-1,-3,-5,-7}$
ES agar	*Eubacterium*	
Bacteroides agar	Bacteroides	
Clostridia Count agar	*Clostridium*	
CW agar	*Cl. perfringens*	
Modified LBS agar	*Lactobacillus*	

The all cultures were incubated at 37°C for 48 h.

Table 2. Influence of MOS intake on fecal microflora.

	Before intake		MOS 3 g/day intake		Interval period		MOS 1 g/day intake	
Total bacterial counts	10.60[a)]±0.10	8/8[b)]	10.02±0.13*	8/8	10.39±0.10	8/8	10.54±0.03	8/8
Bifidobacterium	9.54±0.18	8/8	9.55±0.14	8/8	9.61±0.28	8/8	10.01±0.05*	8/8
Lactobacillus	6.87±0.46	4/8	6.13±0.55	7/8	5.74±0.53	7/8	4.83±0.44	6/8
Eubacterium	8.42±0.16	8/8	7.45±0.42*	8/8	8.77±0.25	8/8	8.85±0.14*	8/8
Bacteroides	9.77±0.11	8/8	9.41±0.41	8/8	9.88±0.12	8/8	9.74±0.11	8/8
Clostridium	8.82±0.20	8/8	7.84±0.36*	8/8	8.32±0.10	8/8	8.20 ±0.06	8/8
Cl. perfringens[c)]	5.01±0.32	7/8	5.41±0.20	4/8	5.86±0.01	5/8	5.83±0.18	5/8
Streptococcus	6.62±0.28	8/8	8.18±0.51*	8/8	7.69±0.46	8/8	8.02±0.37	8/8
Enterobacteriaceae	7.73±0.33	8/8	7.54±0.25	8/8	8.02±0.42	8/8	7.40±0.33	8/8
Staphylococcus	N.D.	0/8	4.88	1/8	3.26	1/8	4.08	1/8
Yeasts	3.81	1/8	3.54	2/8	2.35	1/8	2.33	1/8

[a)] Bacterial counts were expressed as mean±S.E. of $\log_{10}$ per gram of wet feces (n=8).
[b)] Frequency of occurrence was expressed as no. of subjects yielding the organism/no. of subjects examined.
[c)] Lecithinase positve.
* Statistically significant from the before intake (p<0.05).

were analyzed for multiple differences using the Kruskal-Wallis test and this was followed by the Wilcoxon matched paired test. Statistical significance was accepted at a probability level of $p<0.05$.

Results

Influence of fecal microflora The changes in fecal microflora caused by the administration of MOS are shown in Table 2. Total bacteria, *Eubacterium* and *Clostridium* in feces were decreased, while *Streptococcus* was increased by a MOS intake of 3.0 g/day for 2 weeks ($p<0.05$). The frequency of *Cl. perfringens* in feces was decreased from 7/8 to 4/8 and the frequency of *Lactobacillus* was increased from 4/8 to 7/8 by the administration of 3.0 g of MOS for 2 weeks. No significant difference was observed in concentration of each bacterium in the feces before the intake period or during the interval period. Total bacteria counts, *Eubacterium* and *Clostridium* in feces were increased up to the original state by stopping the MOS 3.0 g/day intake, and the fecal microflora was changed again by reinstatement of the MOS administration. However, the fecal microflora in MOS 1.0 g/day differed from that of MOS 3.0 g/day. *Bifidobacterium* and *Eubacterium* in feces were significantly increased. The influence of MOS administration on the content of *Bifidobacterium* is depicted in Fig. 2; it significantly increased in both the MOS 3.0 g/day and 1.0 g/day administration periods ($p<0.05$). No significant difference was observed between the MOS 3.0 g/day and MOS 1.0 g/day trials.

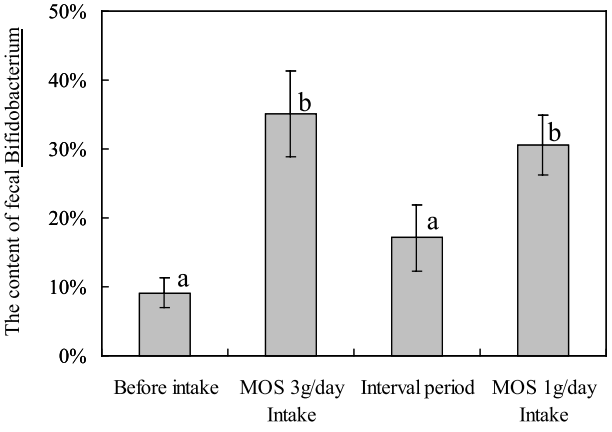

Fig. 2. Influence of MOS intake on the content of fecal *Bifidobacterium*. Mean±S.E. of the content of *Bifidobacterium* ($n=8$). *Different superscripts are significantly different according to Fisher's PLSD test ($p<0.05$).

Influences of moisture, pH and SCFA in feces Changes in moisture, pH and SCFA in feces are shown in Table 3. Fecal moisture showed a slight tendency to increase during the MOS administration periods, while no significant differences among the four periods were noted in fecal pH and SCFA.

Influence of defecating conditions No abnormal symptoms related to physical condition of the eight volunteers was observed during any of the examination periods for MOS intake. Table 4 shows the results of the defecating conditions. The data presented represents the mean value per week for each subject. The defecation days and defecation frequency during the MOS 3.0 g/day intake period were significantly higher than before this period ($p<0.05$). The defecation days during the MOS 1.0 g/day intake period also rose significantly compared with that before the intake ($p<0.05$). However, no significant difference in defecation frequency between before the intake period and the MOS 1.0 g/day intake period was noted, nor were any significant changes in fecal volume or visual fecal characteristics observed.

Discussion

Extensive studies using clinical experiments have shown that various indigestible oligosaccharides affected fecal microflora and defecation conditions (Hidaka *et al*., 1986; Benno *et al*., 1987; Ito *et al*., 1990; Wada *et al*., 1991). However, clinical experiments using MOS had never been reported. Tokunaga *et al*. (1993) administered three doses of fructooligosaccharides

Table 3. Changes in fecal pH, moisture and short chain fatty acids (SCFA) by MOS intake.

	Before intake	MOS 3 g/day intake	Interval period	MOS 1 g/day intake
Moisture (wt%)	70.3±3.3	72.2±2.2	71.1±2.1	74.4±1.5
Fecal pH	6.5±0.1	6.7±0.1	6.5±0.2	6.7±0.3
Total SCFA (mg/g feces)	7.58±0.94	7.92±0.58	6.92±0.9	6.91±0.72
Acetate	3.12±0.33	3.63±0.27	3.03±0.48	3.12±0.33
Propionate	1.70±0.26	1.53±0.15	1.52±0.23	1.29±0.20
n-Butyrate	1.75±0.31	1.67±0.23	1.51±0.21	1.41±0.25
iso-Butyrate	0.38±0.03	0.38±0.03	0.35±0.04	0.35±0.04
Valerate	0.26±0.05	0.27±0.04	0.15±0.06	0.29±0.03
iso-Valerate	0.31±0.06	0.36±0.05	0.35±0.07	0.33±0.06

Values presented as means and standard errors for the eight subjects.

Table 4. Effects of MOS administration on the defecating conditions.

	Before intake	MOS 3 g/day intake	MOS 1 g/day intake
Defecation days per week	4.5±0.6	6.0±0.5*	5.5±0.3
Defecation frequency per week	4.9±0.7	8.0±0.7*	6.8±0.6*
Volume (determined as no. of ping-pong balls)	15.8±1.7	16.3±2.1	19.4±2.4
Shape (1: firm to 6: soft & watery)	2.8±0.2	2.7±0.2	2.9±0.2
Color (1: yellow to 6: dark brown)	3.1±0.1	3.3±0.2	3.2±0.2
Odor (1: weak to 5: very strong)	3.2±0.2	2.8±0.1	2.8±0.2

Values presented as means and standard errors for the eight subjects.
* Statistically significant from the before intake ($p<0.05$).

(FOS), 1.0 g/day, 3.0 g/day and 5.0 g/day, to healthy volunteers and investigated fecal microflora and defecation. In the present study, the effects of MOS intake on fecal microflora and defecation in healthy volunteers were investigated by administering 2 doses, 3.0 g/day and 1.0 g/day. As the minimum effective dose of MOS is not known, the authors began with administration of a high dose (3.0 g/day) thinking this probably more effective. After confirming the effectiveness of the MOS administration, a smaller dose was administered. After a 2 week interval, fecal microflora had returned to the same state as before any intake. Fecal microflora and defecation were compared before and after the MOS intake in an open test.

In this research, we first confirmed that the MOS 3.0 g/day intake caused a significant increase in the content of *Bifidobacterium* in feces (from 9.1% to 35.1%, $p<0.05$). This significant increase was due to a significant decrease of total bacteria and no change in *Bifidobacterium* by the MOS intake of 3.0 g/day. The frequency of *Lactobacillus* increased from 4/8 to 7/8. On the other hand, the frequency of *Cl. perfringens* that are typically harmful bacteria decreased from 7/8 to 4/8. This result seemed to suggest that the MOS intake changed the intestinal environment in which putrefactive bacteria do not proliferate. The population of *Bifidobacterium* during the MOS 3.0 g/day intake showed some variation, so that the mean seemed not to be increased by the intake. In the clinical test using FOS by Mitsuoka *et al.* (1987), the populations of *Bifidobacterium* in feces were widely varied before the FOS intake depending on the subject, however, FOS intake caused the scatter to become much smaller. FOS are utilized by many kinds of *Bifidobacterium* spp. such as *B. adolescentis*, *B. breve*, *B. infantis* and *B. longum* (Mitsuoka *et. al.*, 1987). Therefore, *Bifidobacterium* spp. seems to be increased by the FOS intake without individual variation. On the other hand, MOS are used by a limited number of *Bifidobacterium* spp., *B. adolescentis* (Asano *et al.*, 2001), so that there might be few *Bifidobacterium* spp. that can utilize MOS in the intestine before the first MOS intake. It appears that the wide variation of *Bifidobacterium* in feces was caused by the difference of individual intestinal microflora, namely the difference in *Bifidobacterium* constituents. After the 2 week interval period, fecal microflora had statistically returned to their original state. The content of *Bifidobacterium* was significantly decreased due to increase of total bacteria but there was no change by interruption of the MOS intake. The increment of total bacteria seemed to be caused by proliferation of bacteria that do not utilize MOS. However, the frequency of *Lactobacillus* and *Cl. perfringens* did not completely return to the initial, pre-intake state. The first high dose intake may have influenced the frequency of those bacteria. The administration of MOS 1.0 g/day for 2 weeks statistically increased the content of *Bifidobacterium* from 17.1% to 30.5% ($p<0.05$) and the overall population of *Bifidobacterium* ($p<0.05$). It appears that a dose of MOS of 1.0 g/day also effectively increases the proliferation of *Bifidobacterium*. Moreover, *Bifidobacterium* content tended to increase with the MOS dose level. *Bifidobacterium* variation became much smaller during the MOS 1.0 g/day intake. This might suggest that the constituents of *Bifidobacterium* spp. changed during the first MOS intake, namely the percentage of the spp. that can utilize MOS increased, and the constituents were retained during the interval period. *Bifidobacterium* might significantly increase at even a small dose level when MOS intake is resumed.

Indigestible oligosaccharides escape digestion and encourage the proliferation of beneficial bacteria like *Bifidobacterium* in the intestine. These bacteria produce lactic acid or SCFA to lower the pH in the intestine and thereby inhibit the growth of harmful bacteria like *Cl. perfringens* (Hidaka *et al.*, 1986). A decrease in the frequency of *Cl. perfringens* suggests that the intestinal pH was lowered by the MOS administration. However, compared with prior to the intake significant increases of pH and SCFA in the feces were not observed in either MOS intake period. An intake of 1.0 g, 3.0 g, or 5.0 g/day of fructooligosaccharides significantly increased *Bifidobacterium* in the intestine but the pH of the feces did not fall (Tokunaga *et al.*, 1993), while an intake of 8.0 g/day lowered fecal pH (Mitsuoka *et al.*, 1987). Tamai *et al.* (1992) reported that the fecal pH did not change with a 2.0 g/day intake of galactooligosaccharides, although an 8.0 g/day intake lowered the pH. They suggested that for a small dose of galactooligosaccharides insufficient amounts of organic acids were produced and thus the fecal pH was unaffected. On the other hand, Engelhardt (1995) reported that over 95% of SCFA produced in the large intestine were absorbed into the large intestinal epithelium cells. This suggested that the SCFA produced by the administration of small doses were absorbed in the large intestine. Therefore, it was theorized from this study that lack of a significant drop in the pH is due to the fact that most of the SCFA does not remain in feces to lower pH, even though intestinal pH is lowered by the SCFA produced from the MOS.

The volunteers recorded their daily defecation throughout the test period. The defecating days and times of defecation weekly were significantly increased under the administration of the high dose, compared to before the MOS intake ($p<0.05$). The weekly defecation frequency for the administration of 1.0 g/day of MOS was greater than before the intake ($p<0.05$). Dose dependence on defecation times was identified. However, MOS ingestion did not affect fecal volume or fecal characteristics in one week. The volunteers assigned to this study defecated 4.5 days/week before the MOS intake. This suggested that they did not suffer from obstinate constipation and were healthy at least in regard to defecation. Their scores for feces volume, visual and smell characteristics were in the normal range before MOS ingestion. Therefore, it seems that MOS administration did not cause marked improvement in feces volume or characteristics. Furthermore, this examination was not carried out with a completely controlled menu, and the fecal volume was not observed by actual weight measurement. The lack of effect of MOS intake on fecal volume might be due to these experimental conditions and method. In order to confirm the exact effects on fecal volume or characteristics by MOS intake, a clinical experiment under which the complete menu and the subjects are controlled is required, and actual feces weight should be measured.

In conclusion, *Bifidobacterium* became the predominant bacteria in feces with the ingestion of MOS 1.0 g/day and 3.0 g/day. Defecating conditions were improved for both dosages. These results suggest that the MOS escaped digestion and was selectively utilized by *Bifidobacterium* in the large intestine. Under the continual intake of MOS it is expected that *Bifidobacterium* will become the dominant strain in the large intestine and defecation will be improved. Moreover, depending on the dosage level MOS intake had a tendency to improve the content of *Bifidobac-*

terium and thus the defecating conditions. Small dose administration studies are required to identify the minimum effective dose of MOS on *Bifidobacterium* increments in the large intestine.

References

Asano, I., Nakamura, Y., Hoshino, H., Aoki, K., Fujii, S., Imura, N. and Iino, H. (2001). Use of mannooligosaccharides from coffee mannan by intestinal bacteria. *Nippon Nogeikagaku Kaishi*, **75**, 1077–1083 (in Japanese).

Asano, I., Hamaguchi, K., Fujii, S. and Iino, H. (2003). *In vitro* digestibility and fermentation of mannooligosaccharides from coffee mannan. *Food Sci. Technol. Res.*, **9**, 62–66.

Benno, Y., Endo, K., Shiragami, N., Sayama, K. and Mitsuoka, T. (1987). Effects of raffinose intake on human fecal microflora. *Bifidobacteria Microflora*, **6**, 59–63.

Engelhardt, W. (1995). Absorption of short-chain fatty acids from large intestine. In "Physiological and Clinical Aspects of Short Chain Fatty Acids." ed. by Cummings, J.H., Rombeau, J.L. & Sakata, T. Cambridge University Press, Cambridge, pp. 149–170.

Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T. and Tashiro, Y. (1986). Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora*, **5**, 37–50.

Inaki, M., Fujii, S. and Iino, H. (1999). Effect of the administration of powdered soft drink containing indigestible dextrin on defecation frequency and fecal characteristics of Japanese healthy female volunteers. *Kenko Eiyou Shokuhin Kenkyu*, **2**, 44–51 (in Japanese).

Ito, M., Deguchi, Y., Miyamori, A., Matsumoto, K., Kikuchi, H. and Matsumoto, K. (1990). Effects of administration of galactooligosaccharides on the human fecal microflora, stool weight and abdominal sensation. *Microb. Ecol. Health Dis.*, **3**, 285–292.

Mitsuoka, T. (1982). A Color Atlas of Anaerobic Bacteria, Sobunsha, Tokyo (in Japanese).

Mitsuoka, T., Hidaka, H. and Eida, T. (1987). Effect of fructooligosaccharides on intestinal microflora. *Die Nahrung*, **31**, 423–436.

Okada, J. (1985). Health conditions and feces. *Chiryou-gaku*, **14**, 650–654 (in Japanese).

Tamai, S., Ohtsuka, K., Ozawa, O. and Uchida, T. (1992). Effect of a small amount of galactooligosaccharide on fecal Bifidobacterium. *Nihon Eiyou Shokuryou Gakkaishi*, **45**, 456–460 (in Japanese).

Tokunaga, T., Nakada, Y., Tashiro, Y., Hirayama, M. and Hidaka, H. (1993). Effects of fructooligosaccharides intake on the intestinal microflora and defecation in healthy volunteers. *Bifidus*, **6**, 143–150 (in Japanese).

Wada, K., Mizutani, J., Watabe, J., Suzuki, H. and Sirayanagi, S. (1991). Effects of soybean oligosaccharides intake on fecal microflora. *Bifidus*, **5**, 51–54 (in Japanese).