Effect of the Fermented Soybean Product "Natto" on the Composition and Metabolic Activity of the Human Fecal Flora

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The effects of the fermented soybean product "Natto" on the composition and metabolic activity were studied in seven healthy volunteers (22-49 years of age) who ingested 50 g of Natto/day for 14 days. During Natto consumption, the counts of *Bacillus subtilis* (*B. natto*; $p < 0.001$) and *Bifidobacterium* ($p < 0.05$) were significantly increased, except for the numbers of *Bifidobacterium* in two volunteers, whereas the counts and the frequency of occurrence of lecithinase-positive clostridia ($p < 0.05$), including *Clostridium perfringens* were significantly decreased, when compared to the values before the consumption. The decreased tendency in the counts of *Enterobacteriaceae* and the increased tendency in the detection rate of *B. subtilis* were observed during the consumption, compared to the values before the consumption. No detectable changes occurred in the counts of other organisms throughout the experimental periods.

The amounts of fecal acetic acid ($p < 0.05$) during the consumption, and total organic acids and succinic acid ($p < 0.05$) on day 14 of the consumption were significantly increased when compared to the values before and after the consumption. Fecal concentrations of phenol, ethylphenol, and skatol ($p < 0.05$) were significantly decreased during the consumption. Fecal ammonia and cresol ($p < 0.05$) were significantly decreased on day 14 of the consumption. Fecal pH values ($p < 0.05$) were significantly decreased on day 14 of the consumption. The odor of the feces was slightly reduced during the consumption.

Key words: Fecal flora, Fecal metabolic products, Natto (The fermented soybean product)

Introduction

Recently, functional foods such as probiotics, prebiotics and biogenics are being actively developed, and were recognized in 153 foods by the Japanese Ministry of Welfare on July 23, 1999. "Natto" is a traditional Japanese food made from soybeans by the fermentation of *Bacillus subtilis* ("*B. natto*"), and has both probiotic and biogenic actions. Soybeans have various oligosaccharides such as raffinose and stachyose¹¹⁾, isoflavones such as genistin and genistein⁵⁾, dietary fibre^{4, 20)} and high protein¹²⁾. On the other hand, Natto has various ingredients such as amino acids³⁴⁾, vitamin B³³⁾ and vitamin K²⁵⁾, and oligosaccharides such as melibiose and manninotriose¹¹⁾ that are increased by fermentation.

Various effects of Natto as a medical food are known, including the prevention of high blood pressure and heart disease with nattokinase²⁴⁾, the lowering of cholesterol with soybean protein¹²⁾, antibacterial activity against *Escherichia coli* with dipikorin acids²³⁾, the inhibition of cancer with isoflavones¹⁾, the induction of interferon with toxin of gram-positive bacteria²⁰⁾ and the reduction of soybean allergenicity with substances produced in the fermentation process of Natto³⁵⁾.

Hara *et al.*⁷⁾ showed that raffinose and soybean oligosaccharides selectively enhanced the growth of *Bifidobacterium*, except for *Bifidobacterium bifidum in vitro* as those in stachyose⁹⁾. Minami *et al.*¹³⁾ observed that raffinose is utilized by 49% of 37 *Enterobacteriaceae* strains, and stachyose by 19% of the strains. Tsuchihashi *et al.*³³⁾ demonstrated that the addition of 0.5% *Bacillus* selectively

Table 1. Experimental schedule

	Before consumption	During consumption		After consumption
	Day 0	Day 7	Day 14	Day 7
Week	←2→	←1→	←1→	←1→
Natto intake (g/day)	50	50	50	50
Sampling day	0	7	14	7
Item for analysis;				
Water content (%)	*	*	*	*
pH	*	*	*	*
Fecal weight (g)	*	*	*	*
Fecal flora	*	*	*	*
SCFA (mg/g)	*	*	*	*
Ammonia (μg/g)	*	*	*	*
Sulfide (μg/g)	*	*	*	*
Phenols (μg/g)	*	*	*	*
Indole (μg/g)	*	*	*	*

*: Items examined.

SCFA: Short chain fatty acids.

Table 2. The media and culture methods for comprehensive investigation of intestinal flora

Medium	Organisms enumerated	Incubation method	Incubation at 37°C
Non-selective media			
EG agar	Anaerobes	Steel wool method replaced air with CO ₂	3 days
BL agar	Anaerobes		
Trypticase soy blood agar	Aerobes	Air	1-2 days
Selective media			
BS agar	Bifidobacteria	Steel wool method replaced air with CO ₂	3 days
ES agar	Eubacteria		
NBGT agar	<i>Bacteroidaceae</i>		
Neomycin Nagler agar	Clostridia		
VS agar	Veillonellae and Peptostreptococci		
LBS agar (modified)	Lactobacilli	Replaced air with CO ₂	2 days
DHL agar	<i>Enterobacteriaceae</i>	Air	1 day
TATAC agar	Streptococci		2 days
PEES agar	Staphylococci		
NAC agar	<i>Pseudomonas</i>		
Potato dextrose agar	Yeasts and molds		
Media after heat treatment			
BL agar and CW agar	Clostridia	Steel wool method replaced air with CO ₂	2 days

enhanced the growth of *Bifidobacterium* in rat ceca. Some oligosaccharides in human were also observed to have an enhancing effect on *Bifidobacterium*^{2, 8, 9, 18)} and a deodorizing effect on fecal odor^{7, 9, 29)}.

The present study was carried out to evaluate the effects of Natto on fecal flora, fecal concentrations of putrefactive products and short chain fatty acids (SCFA), fecal pH, weight and moisture, all of which are related to the health promotion and the offensive odor of feces in humans.

Materials and Methods

Subjects and diet

Seven subjects were chosen from healthy male volunteers (aged 22-49 y). None of the subjects received medication and foods with abundant viable cultures for 14 days prior to or during the experiment.

Natto prepared for this study was a commercially-prepared boiled soybean product fermented by *B. subtilis* and its compositions was *B. subtilis* 4.9 × 10⁹/g, energy 84.0 kcal, protein 7.8 g, fat 4.6

Table 3. Effect of Natto consumption on anaerobic fecal flora of seven human volunteers

Organism	Before consumption	During consumption		After consumption
	Day 0	Day 7	Day 14	Day 7
Total anaerobes	10.65±0.14 ^a	10.64±0.11	10.64±0.20	10.53±0.22
<i>Bifidobacterium</i>	9.84±0.27 (100) ^b	10.21±0.20*	10.24±0.27*	9.85±0.24 (100)
<i>Bacteroidaceae</i>	10.52±0.10 (100)	10.40±0.14 (100)	10.38±0.14 (100)	10.38±0.22 (100)
<i>Eubacterium</i>	9.45±0.48 (100)	9.17±0.32 (100)	9.12±0.44 (100)	8.92±0.62 (100)
<i>Peptococcaceae</i>	8.92±0.93 (100)	8.82±0.91 (100)	8.72±0.45 (100)	8.58±0.74 (100)
<i>Megasphaera</i>	8.63±0.50 (57)	8.47±0.57 (43)	8.59±0.29 (43)	8.38±0.49 (43)
<i>Veillonella</i>	6.40±0.70 (43)	6.63±0.32 (43)	6.70±0.33 (43)	6.50±0.80 (57)
<i>Clostridium</i>				
Lecithinase-positive	5.09±0.53 (100)	4.15±0.32* (43)*	3.29±1.07* (43)*	3.95±1.21 (57)
Lecithinase-negative	8.80±0.64 (86)	8.18±0.52 (57)	8.42±0.55 (57)	9.02±0.52 (86)
<i>Lactobacillus</i>	6.61±1.14 (86)	7.43±1.19 (100)	7.49±0.65 (100)	7.29±1.64 (100)

^a: Data expressed as mean log number per gram feces±S. D.

^b: Figures in parentheses are frequency of occurrence (%).

Significant difference from the counts before consumption; * $p < 0.05$, ** $p < 0.001$.

Significant difference from frequency of occurrence before consumption; * $p < 0.05$.

g, carbohydrate 3.4 g, sodium 1 mg, and moisture 29.5 g. The volunteers consumed 50 g/day of Natto for 14 days during the experiment, as shown in Table 1. This work was performed in accordance with the Helsinki Declaration as updated in Tokyo, 1975.

Collections of specimens

Freshly voided fecal specimens were collected on days 0 before the consumption, day 7 and 14 during the consumption, and day 7 after the consumption. The specimens were immediately transported at 4°C to the laboratory for analysis. The fecal flora, bacterial metabolites, weight, moisture content and pH value were analyzed within 3 hr. The remainder of the samples were frozen at -80°C for later analysis of bacterial metabolites.

Analysis of specimens

Analysis of fecal microflora was carried out by using the methods and media of Mitsuoka *et al.*¹⁶⁾ and the heat treatment of Terada *et al.*²⁸⁾ as shown in Table 2. The bacterial count per gram wet weight of fecal material was calculated and converted to a logarithmic equivalent.

Fecal amounts of SCFA were analyzed by high-performance liquid chromatography using the methods of Hara *et al.*⁷⁾ Fecal concentrations of ammonia and sulfide were determined by potentiometer ILO-30 (DKK Co., Ltd., Tokyo) with an ammonia gas-sensing electrode (DKK Co., Ltd., Tokyo) and with a sulfide electrode 7100 (DKK Co., Ltd., Tokyo) using the methods of Terada *et al.*³²⁾, respectively. Fecal indole and phenols were examined by gas chromatography using the methods of Yoshihara³⁶⁾.

Fecal pH values were measured with a flat glass-electrode (DKK Co., Ltd., Tokyo). Fecal water content were determined using 1 g samples, which were weighed before and after drying in a vacuum oven at 105°C for 2 hr by infrared moisture gauge FD-230 type (Ketto Science Lab., Tokyo). The weight of fecal output for 24 hr was measured for 3 consecutive days, including the days of sampling for the bacterial analyses.

Table 4. Effect of Natto consumption on aerobic fecal flora of seven human volunteers

Organism	Before consumption	During consumption		After consumption
	Day 0	Day 7	Day 14	Day 7
Total aerobes	8.21±0.43	8.68±0.33	8.33±0.28	8.19±0.46
<i>Enterobacteriaceae</i>	8.12±0.69 (100)	7.41±0.55 (100)	7.57±0.20 (100)	7.80±0.31 (100)
<i>Streptococcus</i>	7.48±0.84 (100)	7.78±0.86 (100)	7.31±0.67 (100)	7.96±0.63 (100)
<i>Staphylococcus</i>	3.69±0.80 (100)	3.42±1.04 (100)	3.53±0.97 (100)	3.85±0.93 (100)
<i>Bacillus</i>	2.72±0.25 (57)	8.60±0.36** (100)	8.20±0.70** (100)	3.08±0.11 (57)
Yeasts	4.17±0.41 (43)	3.46±0.46 (43)	3.49±1.01 (71)	4.25±0.85 (43)

^a: Data expressed as mean log number per gram feces±S.D.

^b: Figures in parentheses are frequency of occurrence (%).

Significant difference from the counts before consumption; * $p < 0.05$, ** $p < 0.001$.

Significant difference from frequency of occurrence before consumption; * $p < 0.05$.

Fig. 1. Changes in fecal flora by Natto consumption.

Statistical analysis of data

The paired *t*-test and chi-square test were used for statistical analysis of the fecal flora. The paired *t*-test was used for analysis of bacterial metabolites, pH values, and water content.

Results

Fecal flora analysis

The effects of Natto consumption on the composition of fecal flora in seven volunteers are shown in Tables 3 and 4, and Fig. 1. The levels of *B. subtilis* ($p < 0.001$) and *Bifidobacterium* ($p < 0.05$) were significantly increased during the consumption, except for the counts of *Bifidobacterium* in two volunteers (Figs. 2, 3), while the numbers and the frequency of occurrence of lecithinase-positive clostridia ($p < 0.05$), including *Clostridium perfringens*, were significantly decreased during the consumption (Fig. 4). The counts of *Enterobacteriaceae* showed a tendency to decrease during the consumption, except for *Enterobacteriaceae* in three volunteers (Fig. 5), whereas the frequency of occurrence of *B. subtilis* demonstrated an increased tendency during the consumption, when

Fig. 2. Change in the levels of *Bacillus subtilis* during Natto consumption by 7 human volunteers.

Fig. 3. Change in the levels of *Bifidobacterium* during Natto consumption by 7 human volunteers.

Fig. 4. Change in the levels of lecithinase-positive clostridia during Natto consumption by 7 human volunteers.

compared to the values before the consumption.

No detectable changes occurred in the counts of other organisms or in the total bacteria throughout the experimental periods.

Fig. 5. Change in the levels of *Enterobacteriaceae* during Natto consumption by 7 human volunteers.

Fig. 6. Change in the concentrations of fecal short chain fatty acids during Natto consumption. Graphs show the mean for 7 human volunteers. Significant difference from the values before consumption; * $p < 0.05$.

Fecal organic acids

The concentrations of fecal acetic acid during the period and total organic acids and succinic acid on day 14 of the consumption showed a significant increase ($p < 0.05$), as shown in Fig. 6. No significant changes were shown in other fecal SCFA during the consumption.

Fecal putrefactive products

A significant decrease ($p < 0.05$) in the amounts of fecal indole, skatol and ethylphenol during the consumption, and fecal ammonia and *p*-cresol on day 14 of the consumption were observed when compared to those before the consumption (Figs. 7 and 8). Offensive odor of feces decreased slightly during the consumption.

Fecal water content and pH value

Fecal pH values were significantly decreased on day 14 of the consumption compared to those

Fig. 7. Change in the concentrations of ammonia and sulfide during Natto consumption. Graphs show the mean $\pm$ S.E. for 7 human volunteers. Significant difference from the values before consumption; * $p < 0.05$.

Fig. 8. Change in the concentrations of fecal metabolites during Natto consumption. Putrefactive products are expressed as the mean for 7 human volunteers. Significant difference from the values before consumption; * $p < 0.05$.

Table 5. Effect of Natto consumption on fecal pH, weight and water content of seven human volunteers

Item	Before consumption	During consumption		After consumption
	Day 0	Day 7	Day 14	Day 7
pH	6.61 $\pm$ 0.16	6.43 $\pm$ 0.30	6.36 $\pm$ 0.29*	6.69 $\pm$ 0.37
Fecal weight (g)	138.00 $\pm$ 12.20	150.74 $\pm$ 24.94	144.56 $\pm$ 21.80	141.69 $\pm$ 11.31
Water content (%)	73.63 $\pm$ 3.41	75.77 $\pm$ 2.66	75.29 $\pm$ 5.83	74.26 $\pm$ 2.65

^a: Data expressed as mean $\pm$ S.D.

Significant difference from the values before consumption; * $p < 0.05$.

before and after the consumption (Table 5). The water content and weight were slightly increased during the consumption.

Discussion

The intestinal flora and its metabolic activities are known to play an important role in the host's health^{6, 14, 15)}. *Bifidobacterium* is composed of a member of predominant flora of humans¹⁵⁾. The increase of *Bifidobacterium* in the intestine brings about a beneficial effect for the host by lowering the pH in the gut, inhibiting the growth of potential pathogens and stimulating the host's immune systems^{6, 15, 20)}. The amounts of *Bifidobacterium* in the feces should be above 30% for the host's health as the results in oligosaccharides^{7, 29)}. On the other hand, a the decrease in the number of lecithinase-positive clostridia, including *C. perfringens* is relative to the reduction of the offensive odor of the feces as is the case with probiotics^{17, 31)} and prebiotics^{9, 29, 30)}.

The effects of Natto consumption on the intestinal flora in rats are conflicting. Watanabe *et al.*³⁴⁾ showed that Natto consumption enhances the growth of *Bacillus*, *Streptococcus*, and *Lactobacillus*, and reduce *E. coli* in rat ceca. In contrast, Isshiki *et al.*¹⁰⁾ also found a significant increase of *Enterobacteriaceae* and *Bacteroidaceae*, and a decrease of bifidobacteria in rat ceca after Natto consumption. The reported results of the intestinal flora in rats are thought to change by various factors such as weaning, diets, antibiotics, pathogens, environmental stressors, and examination method. The stable microbial flora in the intestine of animals is supposed to be effectively maintained by diet and environmental hygiene. In the present study, a significant increase in *Bifidobacterium* and a decrease in the counts and the detection rate for lecithinase-positive clostridia in humans during Natto consumption were similar to the results in humans administered soybean oligosaccharides⁸⁾ and raffinose²⁾. Major increases in the counts of *B. subtilis* are found during the consumption, which may be considered to mean that there is no effect of acid on the strain in the stomach.

The end-products of the bacterial metabolites such as the SCFA, fermentation gases, and energy are thought to play an important role in intestinal disorders²⁰⁾. Cummings *et al.*⁴⁾ described that little change in fecal SCFA occurred with variation in diet, and the total SCFA increased parallel to fecal weight. In this study, the increase of fecal acetic acid, succinic acid and total SCFA on Natto consumption was similar to those in humans given lactosucrose⁷⁾. Fecal weight did not increase the reason that the amount of dietary fiber in Natto decreased during fermentation²⁶⁾.

Potential toxic substances such as ammonia, sulfide, amines and phenols that are potentially harmful to the host are considered to be important factors in lifestyle-related diseases²²⁾. In the present study, fecal concentrations of ammonia, phenol, skatol and cresol were decreased in Natto consumption, as has been noted in humans fed yoghurt¹⁷⁾. These results might be related to the reduction in lecithinase-positive clostridia and *Enterobacteriaceae* in the large intestine, as noted by Bone *et al.*³⁾. It seems that Natto consumption inhibits the metabolic activity of the intestinal flora, resulting in reduction of the effects of aging and causation of lifestyle-related diseases.

Pietroiusti *et al.*¹⁹⁾ noted that the fecal pH of humans is not affected by fecal weight, age, sex, or diet of the subjects. Some oligosaccharides^{7, 29)}, alginate³⁰⁾, chitosan³²⁾, and yoghurt¹⁷⁾ have been described as effective pH-demoting factors. The level of lowering of fecal pH values during Natto consumption is 0.25, which is weaker than oligosaccharides^{7, 29)} and yoghurt¹⁷⁾ consumption.

It may be concluded that Natto consumption contributes to improvement of both the composition and the metabolites of the intestinal flora, resulting in a relative deodorization of human feces.

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納豆摂取がヒト腸内フローラおよび腐敗産物に及ぼす影響

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健康成人 7 名に納豆 (50 g/日) を 2 週間摂取させ、腸内フローラおよび腐敗産物に及ぼす影響を検討した。腸内フローラでは納豆摂取中に *Bifidobacterium* ($p < 0.05$), *Bacillus subtilis* (*B. natto*; $p < 0.001$) は有意に増加し、レシチナーゼ陽性 clostridia ($p < 0.05$) の菌数と検出率は有意に減少した。また、納豆摂取中に *Enterobacteriaceae* は減少傾向を示し、*Bacillus subtilis* の検出率は増加傾向を示した。その他の細菌群の変動は認められなかった。

短鎖脂肪酸では納豆摂取中に酢酸 ($p < 0.05$)、摂取 2 週目に総有機酸 ($p < 0.05$) とコハク酸 ($p < 0.05$) は有意に増加した。腐敗産物では納豆摂取中にフェノール、エチルフェノール、スカトール ($p < 0.05$) は有意に減少し、摂取 2 週目ではアンモニア、クレゾール ($p < 0.05$) が有意に減少した。pH ($p < 0.05$) は納豆摂取 2 週目に有意に低下した。

以上より、納豆摂取は腸内フローラの構成と代謝活性によって、腸内環境の改善と便の脱臭効果が示唆された。