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Original Article

Effects of young barley leaf powder on irritable bowel syndrome in rats.

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Summary

In order to obtain an effective diet composition to relieve irritable bowel syndrome (IBS), an experiment using rats as a model was performed. Male 7 week-old Wistar rats were maintained for one week on five diets; (i) 0% cellulose (C-0), (ii) 3% cellulose (C-3), (iii) 5% cellulose (C-5), (iv) 3% young barley leaf powder (YBLP) plus 3% cellulose (Y-3+C-3), and (v) 10% YBLP (Y-10). The IBS of rat was produced by restraining a rat in a tight holder for four hrs. The degree of IBS was assessed by measuring fecal excretion frequency and serotonin concentration in serum and colonic mucosa. Under restraint stress, the number of feces in the groups of C-0 and C-3 were more than those in C-5 without (control). Restraint stress could have stimulated secretion of a large quantity of serotonin from colonic mucosa, resulting in abnormal intestinal movement. However, such fecal excretion increment was not observed in the group of Y-3+C-3, suggesting that YBLP inhibits stress-induced IBS.

Keywords: rats, young barley leaf powder, restraint stress, serotonin, feces, irritable bowel syndrome

Introduction

Typical Japanese style meals are considered to have good compositions from their nutritional viewpoints, and the life longevity of Japanese could be attributed to a high composition of dietary fiber. Because of insufficient dietary fiber intake, various health problems could be observed in many countries including Japan. Thus, it is recommended that, even in Japanese-style meals, higher amounts of dietary fibers should be taken daily. Different types of dietary fibers are contained in various foods, and some of them may be effective than others to regulate systemic hormone levels as well as their secretion from the digestive tract.

Irritable bowel syndrome (IBS) is a functional disorder accompanied with symptoms such as diarrhea and constipation without any pathological change of the digestive tract[1)]. Various stresses make the syndrome more serious, so that the quality of life may be lowered[2-4)]. Recently, the number of IBS patients in Japan is increasing[5)], especially in young people[6)]. The main morbid state of IBS is considered to be in an abnormal relationship between the brain and intestine[7, 8)]. Serotonin (5-hydroxytryptamine: 5-HT), which is distributed both in the brain and intestine, is known to take part in this relationship[8, 9)].

A number of methods to create IBS model in rats by restraint stress have been reported[10-12)]. This restraint stress may lead to abnormal fecal excretion due to stimulation of intestinal movement[10-12)]. The quantity and frequency of fecal excretion were observed as markers of abnormal movement of the digestive tract[10, 11)]. Serotonin might be involved in the stress-induced alteration of intestinal movement[7-9)].

Young barley leaf powder (YBLP) is rich in dietary fibers and it has been known as a component material of "AOJIRU, green juice". There are many papers on effects

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of YBLP on the gastrointestinal functions[13–17]. YBLP supplementation increased fecal quantity[15, 16] and defecation frequency and improved other fecal characteristics[16, 17]. In the present study, the authors intended to investigate effects of YBLP on relief-related parameters in a rat model of IBS.

Materials and Methods

1. Diets

We prepared five diets, containing cellulose or/and YBLP as follows (Table 1): (i) Diet of 0% cellulose (C-0), (ii) 3% cellulose (C-3), (iii) 5% cellulose (C-5), (iv) 3% YBLP plus 3% cellulose (Y-3+C-3), and (v) 10% YBLP (Y-10).

2. Animals

A total of 75 male Wistar rats (6 weeks old, purchased from SLC Co. Ltd., Hamamatsu, Japan) were fed on AIN-93G one week. Then, they were divided into 5 groups, each composed of fifteen rats, and fed on the respective diets (C-0, C-3, C-5, Y-3+C-3, and Y-10, Table 1) *ad libitum* for one week.

The rats were kept under 12 hrs light (7:00~19:00) – dark (19:00~7:00) cycles in feeding cages individually with a meshed bottom.

The experiments were performed under the permission of the Laboratory Animal Ethic Commission of Osaka Aoyama University (No. 14–25).

3. Restraint stress[10]

On the last day of the feeding period, restraint stress was given by holding each rat (n=10) in a tight holder made from stainless steel wires for four hrs from 7:00 to 11:00 am. Rats (n = 5) without stress were kept in a 30cm square feeding cage. During the restraint hours, neither food nor water was given to the rats.

4. Fecal excretion

The number, weight, and color of the feces excreted during the restraint period were recorded, and their individual body weights were measured before and after the restraint. The dry weights of the feces were measured after lyophilization of the wet feces.

5. Preparation of serum and colonic mucosa samples

Immediately after the restraint stress period, the rats were decapitated under light anesthesia and blood samples were collected. Serum samples were prepared by centrifugation of the blood at 3,000 rpm at 4℃ for ten minutes, and stored at −30℃ until use. The colonic mucosa was obtained by scraping with two pieces of slide glass after washing out the colonic contents with a saline solution, and stored at −30℃ until use.

6. Determination of serotonin concentrations

The colonic mucosa was homogenized in sterilized phosphate buffered saline. The serotonin concentrations in serum and in colonic mucosa were determined by Serotonin ELISA kit (Enzo Life Sciences Inc., NY, USA).

7. Protein determination

Protein in colonic mucosa was determined by the Lowry method[18].

8. Reagents

The reagents were of the JIS Special Grade or its equivalent.

Table 1 Composition of experimental diets.

Ingredients (*g/kg*) \ Diets	0% Cellulose (C-0)	3% Cellulose (C-3)	5% Cellulose (C-5)	3% YBLP + 3% Cellulose (Y-3 + C-3)	10% YBLP (Y-10)
Casein	200.0	200.0	200.0	200.0	200.0
Gelatinized corn strach	579.5	549.5	529.5	519.5	479.5
Sucrose	100.0	100.0	100.0	100.0	100.0
Soybean oil	70.0	70.0	70.0	70.0	70.0
Mineral mixture[a]	35.0	35.0	35.0	35.0	35.0
Vitamin mixture[a]	10.0	10.0	10.0	10.0	10.0
L- Cystine	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
Cellulose[b]	–	30.0	50.0	30.0	–
YBLP[c]	–	–	–	30.0	100.0

[a] Mineral mixture and vitamin mixture were prepared according to the AIN-93G formulation[23].
[b] Cellulose is Just Fiber BH-200EXT (Morimura Bros., Inc.).
[c] Young barley leaf powder (Toyo Shinyaku Co., Ltd.). The compositions (%) were as follows: water (2.1), protein (30.1), lipid (7.0), ashes (7.7), carbohydrate (8.4), dietary fiber (44.7). Dietary fiber was measured enzymatic-gravimetric method.

9. Statistical analysis

The values were expressed by means ± SD. For significant differences of the biochemical values compared with the control (C-5, without stress), Dunnett's test was used. For comparison of the biochemical values between the stress (+) and the stress (−) in the same diet group, Welch's t-test was used. Statistical analyses were performed with the software Excel 2010 (Microsoft) and Statcel 3 (OMS Publishing Co., Japan), add-in forms on Excel.

Results

1. Diet consumption and body weight changes

There were no significant differences among the body weights and daily amounts of food intake of the respective diet groups (Table 2).

2. The number and wet weight of feces

The body weight reduction and the number, weight and moisture content of the feces collected for four hrs of the restraint per are shown in Table 3. The body weight reduction was not affected by restraint stress in all groups. Under restraint stress, compared with the control, significant differences were found in C-0 and Y-10 in the number

Table 2 Body weights and food intakes.

Diet groups	Boby weight (g)		Food intake (g/day)
	Initial	Final	
C-5 (Control)	185.3 ± 7.5	212.9 ± 7.0	17.1 ± 1.1
C-0	182.0 ± 7.9	216.1 ± 8.0	18.0 ± 1.3
C-3	183.8 ± 8.1	212.1 ± 6.9	16.6 ± 0.9
Y-3 + C-3	185.3 ± 5.3	217.4 ± 4.7	17.3 ± 1.0
Y-10	182.0 ± 7.7	213.2 ± 9.2	18.8 ± 0.9

The values are expressed as mean ± SD.
For significant of differences of the biochemical values compared with the control (C-5, without stress), the Dunnett's test was used.
Diet groups; C-0: 0% Cellulose, C-3: 3% Cellulose, C-5: 5% Cellulose, Y-3 + C-3: 3% Young barley leaf powder + 3% Cellulose, Y-10: 10% Young barley leaf powder

Table 3 Effect of the restraint stress on body-weight-reguctions, fecal numbers and fecal wet weights.

	Boby weight reduction during 4 hrs (%)	Feces during 4 hrs			
		Numbers	Wet weight (g)	Wet weight / a fece	Moisture content (%)
Stress (−)					
C-5 (Control)	2.28 ± 0.42	3.40 ± 2.41	0.26 ± 0.18	0.09 ± 0.05	57.6 ± 8.9
C-0	2.39 ± 0.35	2.20 ± 1.92	0.14 ± 0.13	0.04 ± 0.02	56.5 ± 16.8
C-3	2.14 ± 0.20	2.40 ± 1.82	0.19 ± 0.07	0.07 ± 0.04	50.2 ± 11.7
Y-3 + C-3	2.75 ± 0.55	2.40 ± 2.88	0.31 ± 0.21	0.13 ± 0.15	52.9 ± 6.8
Y-10	3.16 ± 0.31	5.40 ± 5.27	0.58 ± 0.16	0.07 ± 0.03	53.6 ± 7.2
Stress (+)					
C-5	2.30 ± 1.29	6.80 ± 3.01	0.88 ± 034**†	0.16 ± 0.07	52.4 ± 13.4
C-0	2.32 ± 0.47	9.50 ± 2.84*†	0.59 ± 0.33†	0.06 ± 0.03	53.5 ± 9.6
C-3	2.79 ± 1.46	7.80 ± 3.26†	0.79 ± 0.19**†	0.12 ± 0.07	48.6 ± 15.3
Y-3 + C-3	3.68 ± 2.25	3.40 ± 1.78	0.37 ± 0.30	0.13 ± 0.06	49.2 ± 8.4
Y-10	3.05 ± 1.88	10.20 ± 4.92**	0.79 ± 0.09**	0.09 ± 0.08	51.8 ± 10.2

The values are expressed as mean ± SD.
*, **For significant of differences of the biochemical values compared with the control (C-5, without stress), the Dunnett's test was used (*$P < 0.05$, **$P < 0.01$).
†For comparison of the biochemical values between the Stress (+) and the Stress (−) in the same diet, the Welch's t-test was used ($P < 0.05$).
Body weight reduction was calculated as the ratio of the amount of the reduced weight after the restraint stress to the whole body weight before the restraint stress.
The rats with the restraint stress were designated by (+), and without the stress, (−).
Diet groups; C-0: 0% Cellulose, C-3: 3% Cellulose, C-5: 5% Cellulose, Y-3 + C-3: 3% Young barley leaf powder + 3% Cellulose, Y-10: 10% Young barley leaf powder

of feces. In C-0 and C-3 under restraint stress, higher the number of feces were observed than in C-0 or C-3 without restraint stress. On the other hand, the number of feces in C-5 and Y-3+C-3 were not affected by restraint stress. However, the effects of restraint stress seemed greatly different among the individuals, resulting no significant differences in the average values; this tendency was revealed more markedly in the diet groups of C-0, C-3, C-5 and Y-10 (Fig. 1). The fecal wet weights of C-0, C-3 and C-5 were significantly increased by restraint stress.

The water contents of feces were approximately 50% in all groups, and these were not affected by stress.

The colors of feces were as follows; C-0: black, C-3 and C-5: ochre, Y-3+C-3 and Y-10: green.

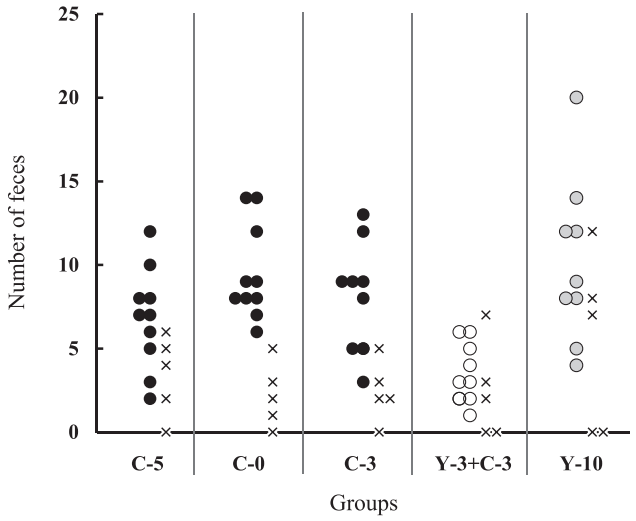

Fig. 1 Distribution of the number of feces by individual rats.
●, ○, ◍: The rats with restraint stress
×: The rats without restraint stress
Diet groups; C-0: 0% Cellulose, C-3: 3% Cellulose, C-5: 5% Cellulose, Y-3+C-3: 3% Young barley leaf powder+3% Cellulose, Y-10: 10% Young barley leaf powder

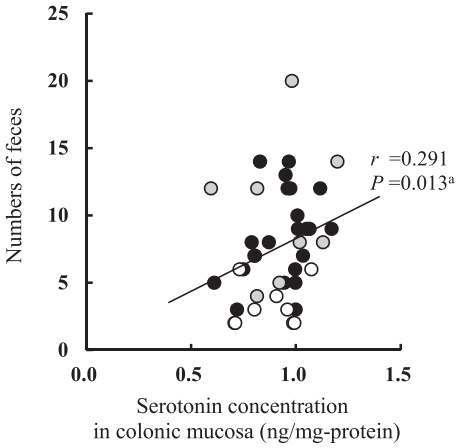

Fig. 2 Relationship between the concentration of serotonin in colonic mucosa and the number of feces in the restraint stress rats.
[a] Significance was calculated using Pearson's correlation coefficient.
●: The rats fed 5% Cellulose (C-5), 0% Cellulose (C-0) and 3% Cellulose (C-3) diets.
○: The rats fed 3% Young barley leaf powder+3% Cellulose (Y-3+C-3) diet.
◍: The rats fed 10% Young barley leaf powder diet.

Table 4 Effect of the serotonin concentration in serum and colonic mucosa.

	Serotonin concentration	
	Serum (ng/mL)	Colonic mucosa (ng/mg-protein)
Stress (−)		
C-5 (Control)	0.345 ± 0.055	0.375 ± 0.123
C-0	0.383 ± 0.025	0.302 ± 0.084
C-3	0.417 ± 0.023	0.415 ± 0.206
Y-3+C-3	0.325 ± 0.089	0.365 ± 0.104
Y-10	0.445 ± 0.038*	0.306 ± 0.089
Stress (+)		
C-5	0.478 ± 0.046**††	0.872 ± 0.119**††
C-0	0.493 ± 0.068**†	0.984 ± 0.095**††
C-3	0.479 ± 0.051**††	0.910 ± 0.187**††
Y-3+C-3	0.439 ± 0.051**	0.896 ± 0.133**††
Y-10	0.526 ± 0.023**†	0.934 ± 0.193**††

The values are expressed as mean ± SD.
**For significant of differences of the biochemical values compared with the control (C-5, without stress), the Dunnett's test was used ($P < 0.01$).
†, ††For comparison of the biochemical values between the Stress (+) and the Stress (−) in the same diet, the Welch's t-test was used (†$P < 0.05$, ††$P < 0.01$).
The rats with the restraint stress were designated by (+), and without the stress, (−).
Diet groups; C-0: 0% Cellulose, C-3: 3% Cellulose, C-5: 5% Cellulose, Y-3+C-3: 3% Young barley leaf powder+3% Cellulose, Y-10: 10% Young barley leaf powder

3. Serotonin concentration in serum and in colonic mucosa

Under restraint stress, the serotonin concentrations in serum as well as in colonic mucosa were significantly elevated compared to the control (Table 4). Y-10 without restraint stress showed a significantly higher level of serum serotonin concentration than the control.

In all groups, serotonin concentrations in colonic mucosa were significantly elevated compared to those without restraint stress. In the rats given restraint stress, there was a positive correlation between the serotonin concentrations in colonic mucosa and the number of feces (Fig. 2; $r = 0.291$, $P = 0.013$). However in Y-3+C-3, the number of feces was smaller, and the serum serotonin concentration was not influenced by restraint stress, suggesting the existence of an effective component in YBLP different from cellulose.

Discussion

As described in the introduction, the pathogenetic relation of serotonin to the symptoms of IBS seems important. In the present study we observed in rats given restraint stress: (1) increases of fecal numbers and wet weights in the groups of C-0, C-3, C-5 and Y-10, but no effect on fecal numbers and wet weights in Y-3+C-3, (2) an elevation of colonic and serum serotonin, but no effect on serum serotonin in Y-3+C-3, and (3) a positive correlation between the serotonin concentration in colonic mucosa and the number of feces. The above results suggested that the abnormal intestinal movement was caused by increased serotonin concentrations. There was no significant difference in the number of feces among the groups of C-0, C-3, C-5 (Table 3). Also in these groups, both serum and colonic serotonin concentration showed similar values. The increase in fecal numbers by stress tended to decline in the order of C-0, C-3, and C-5; this means that the fiber-free diet brought about abnormal fecal excretion. Therefore, dietary fibers seem to be essential to relieve IBS. Both the number and the wet weight of feces in Y-3+C-3 were less than those in C-5. Y-3+C-3 showed less fecal excretion than C-5, although the amount of dietary fiber in Y-3+C-3 was almost the same as in C-5.

Serotonin is considered to be strongly related to the neuronal effect on intestinal movement[7–9]. Serotonin is synthesized in the enterochromaffin (EC) cell, secreted near the target cells activating their receptors[8]. Also, the serotonin secretion is affected by intestinal bacterial flora and their products such as short chain fatty acids[12, 19]. Thus, dietary fibers, through fermentation by intestinal bacterial flora, could contribute to relieve IBS[20].

YBLP contains of minerals such as Ca, Mg, K, P, Zn, Cu, Fe, Mg, Cr and so on, proteins, lipids, vitamins such as B complexes, C, E, K, and dietary fibers[21]. Bacterial flora in the intestine and production of short chain fatty acids are likely to be affected by YBLP intake[15–17]. The extract of YBLP remaining after the removal of dietary fiber could suppress the incidence of stomach ulcer caused by stress in rats[22].

In the present study, the colonic serotonin concentration in the rats of Y-10 given restraint stress was higher than that of the control rats, and also their serotonin levels were similar to those other of groups. It is not likely that YBLP specially promoted serotonin synthesis. On the other hand, in the rats of Y-3+C-3 give restraint stress, both the number and wet weight of feces tended to be less than those in other groups given restraint stress, in spite of almost the same contents of dietary fiber as in C-5. This may suggest on effect of some components in YBLP other than pure cellulose. If YBLP suppresses neither biosynthesis nor secretion of serotonin, it might inhibit the binding of serotonin to its receptor or obstruct the signal transport from the serotonin-receptor site.

Conclusions

The rats, given restraint stress, secreted more serotonin in the colonic mucosa than the control rats. Out of these rats, the number and wet weight of feces increased in the group of the cellulose diet without YBLP. When the rats was fed the diet containing cellulose plus YBLP and given restraint stress, they showed no increment in the number and wet weight of feces, in spite of an elevation of the serotonin concentration in colonic mucosa. Therefore, some components in YBLP other than cellulose may also have contributed to relieve the symptoms of IBS such as abnormal fecal excretion occurring in restraint-stressed rats.

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References

1) Drossman DA, Corazziari E, Delvaux M, Spiller RC, Talley NJ, Thompson WG, Whitehead WE (2006) Rome III: The Function Gastrointestinal Disorders: Third Edition. *Degnon Associates*, McLean.

2) Whitehead WE, Burnett CK, Cook EW, Taub E (1996) Impact of irritable bowel syndrome on quality of life. *Dig Dis Sci* 41: 2248–2253.
3) Kaji M, Fujiwara Y, Shiba M, Kohata Y, Yamagami H, Tanigawa T, Watanabe K, Watanabe T, Tominaga K, Arakawa T (2010) Prevalence of overlaps between GERD, FD and IBS and impact on health-related quality of life. *J Gastroenterol Hepatol* 25: 1151–1156.
4) Coffin B, Dapoigny M, Cloarec D, Comet D, Dyard F (2004) Relationship between severity of symptoms and quality of life in 858 patients with irritable bowel syndrome. *Gastroenterol Clin Biol* 28: 11–15.
5) Miwa H (2008) Prevalence of irritable bowel syndrome in Japan: Internet survey using Rome III criteria. *Patient Prefer Adherence* 2: 143–147.
6) Yamamoto R, Kaneita Y, Osaki Y, Kanda H, Suzuki K, Higuchi S, Ikeda M, Kondo S, Munezawa T, Ohida T (2015) Irritable bowel syndrome among Japanese adolescents: A nationally representative survey. *J Gastroenterol Hepatol* 30: 1354–1360.
7) Fukudo S, Nomura T, Hongo M (1998) Impact of corticotropin-releasing hormone on gastrointestinal motility and adrenocorticotropic hormone in normal controls and patients with irritable bowel syndrome. *Gut* 42: 845–849.
8) Kim DY, Camilleri M (2000) Serotonin: a mediator of the brain-gut connection. *Am J Gastroenterol* 95: 2698–709.
9) Foxx-Orenstein AE, Kuemmerle JF, GriderDistinct JR (1996) 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea pig intestine. *Gastroenterology* 111: 1281–1290.
10) Kawai M (2009) A disease model mimicking the pathophysiology of irritable bowel syndrome and procedures for preparing this model. *Folia Pharmacal. Jpn* 133: 206–209. (in Japanese)
11) Miyata K, Ito H (2006) Drug discovery for irritable bowel syndrome. *Folia Pharmacal. Jpn* 128: 104–107. (in Japanese)
12) Kanauchi O, Mitsuyama K, Komiyama Y, Yagi M, Andoh A, Sata M (2010) Preventive effects of enzyme-treated rice fiber in a restraint stress-induced irritable bowel syndrome model. *Int J Mol Med* 25: 547–555.
13) Kamiya T, Takano A, Kusaba N, Takashima S, Yahiro E, Ikeguchi M, Takagaki K, Sugimura H, Sugawa-Katayama Y (2013) Suppressive effect of powdered juice containing youg barley leaf powder on postprandial blood glucose level. *Pharmacometrics* 85: 1–6. (in Japanese)
14) Ikeguchi M, Tsubaki M, Takano A, Kamiya T, Tkagi K, Ito H, Sugawa-Katayama Y, Tsuji H (2014) Effects of young barley leaf powder on gastrointestinal functions in rats and its efficacy-related physicochemical properties. *Evid Based Complement Alternat Med* Articlel ID 974840, 7 pages.
15) Ikeguchi M, Ariura Y, Takagaki K, Ishibashi Y, Inagawa A, Sugawa-katayama Y (2004) Effects of young barley leaf powder on fecal weight and fecal microflora in healthy women. *J Jpn Assoc Dietary Fiber Res* 8: 93–103. (in Japanese)
16) Ikeguchi M, Kobayashi M, Ariura Y, Mori S, Takagaki K, Ishibashi Y, Sugawa-katayama Y (2005) Effects of young barley leaf powder on defecation frequency and fecal characteristics of healthy volunteer. *J Jpn Assoc Dietary Fiber Res* 9: 12–21. (in Japanese)
17) Saito Y, Saito M, Yamamoto M, Nagao K, Yamamoto K, Matsui N, Ozeki S, Suzuki N (2005) Effects of ingestion of insoluble dietary fiber for young barley on cecal production of organic fatty acids, fecal output and organ weight in rats. *J Jpn Soc Nurt Food Sci* 58: 307–313. (in Japanese)
18) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275.
19) Fukumoto S, Tatewaki M, Yamada T, Fujimiya M, Mantyh C, Voss M, Eubanks S, Harris M, Pappas T N, Takahashi T (2003) Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am J Physiol Regul Integr Comp Physiol* 284: 1269–1276.
20) Tana C, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S (2010) Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil* 22: 512–519.
21) Yamamoto Kanpo Seiyaku. http://www.kanpo-yamamoto.com/shop/omugi_wakaba02.php (access: 2016/09/28)
22) Ohtake H, Yuasas H, Komura C, Miyaushi T, Hagiwara Y, Kubota K (1985) Studies on the constituents of green juice from young barley leaves. Antiulcer activity of fractions from barley juice. *Pharmal Society of Jpn* 105: 1046–1051. (in Japanese)
23) Reeves PG, Nielsen FH, Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 123: 1939–1951.