Food Sci. Biotechnol. Vol. 18, No. 1, pp. 213 ~ 217 (2009)

Food Science and Biotechnology

Morphological Characteristics of Intestine in Rats Fed Acidified Small Black Soybean

Chang-Hyun Lee[1], Byung-Moon Ko[1], Geun-Seoup Song[2], Hyun-Il Jun, and Young-Soo Kim*

Faculty of Biotechnology, Institute of Agricultural Science and Technology, Chonbuk National University, Jeonju, Jeonbuk 561-756, Korea

[1]Department of Anatomy, College of Oriental Medicine, Woosuk University, Samrye, Jeonbuk 565-701, Korea

[2]Division of Biotechnology, College of Environmental and Bioresource Science, Chonbuk National University, Iksan, Jeonbuk 570-752, Korea

Abstract In this study, the effects of processed small black soybeans on the intestinal morphological characteristics of rats were examined. Adult male rats were fed diets containing raw, cooked, or acidified small black soybean powders for 4 weeks. The total short chain fatty acid (SCFA) level was significantly higher in the acidified small black soybean supplemented group than in the raw and cooked soybean diet groups. The major SCFAs found in the experimental groups were acetate, followed by propionate and butyrate. The duodenal villus height and colonic mucosal thickness were also significantly higher in the acidified small black soybean supplemented group than in the raw and cooked soybean diet groups. The acidified small black soybean supplemented group showed the densest colonic mucosa by staining with alcian blue (AB), as compared to the raw and cooked soybean diet groups. The acidified small black soybean supplemented group exhibited strongly stained $CD4^+$ in the mucosal lamina propria, while cooked and acidified diet groups were more strongly stained $CD8^+$ in the submucosal lamina propria than the raw diet group. These results suggest that acidified small black soybeans may help improve intestinal function.

Keywords: small black soybean, short chain fatty acid, goblet cell, $CD4^+$, $CD8^+$, intestinal function

Introduction

There is an increasing public interest in functional and medicinal foods that prevent or treat chronic diseases. Soybeans, in particular, have taken their place as a functional food of interest for human health because they contain many physiologically active compounds such as phytates, phenolics, isoflavones, and tannins (1,2). Previous studies have shown that soybean consumption has beneficial effects against hypercholesterolemia, cardiovascular diseases, osteoporosis, cancer, obesity, diabetes, and menopausal symptoms (3-5), resulting in an increased consumption of soybean-based products.

Black soybeans, which are one type of soybean with a black seed coat, have been used as food and as a material for folk medicine (6). Several studies have demonstrated that black soybeans contain considerable amounts of bioactive compounds, including anthocyanins and isoflavones, and have many important physiological functions (7-11). In a recent study, Lee *et al.* (9) reported that cooked and germinated black soybean consumption prevented the retardation of fecal excretion in experimental normal and constipation model rats via the acceleration of bowel evacuation. In addition, Shinomiya *et al.* (10) reported that the intake of seed coat extract from black soybeans enhanced long-term memory and learning ability in rats. So far, however, few studies have reported on the effects of black soybeans on physiological functions.

Therefore, in the present study, we examined the effects of processed small black soybeans on intestinal functions such as changes in fecal short chain fatty acids (SCFAs), duodenal villus heights, and colonic mucosal thickness, changes in colonic goblet cells and immunohistochemical changes in $CD4^+/CD8^+$ lymphocytes in rat colons after feeding processed black soybean supplemented diets.

*Corresponding author: Tel: +82-63-270-2569; Fax: +82-63-270-2572
E-mail: ykim@chonbuk.ac.kr
Received July 23, 2008; Revised September 20, 2008;
Accepted September 22, 2008

Materials and Methods

Chemicals The reagents for analysis such as formic acid, propionic acid (C3), isobutyric acid (i-C4), isovaleric acid (i-C5), *n*-valeric acid (C5), and 2-ethylbutyric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid (C2), *n*-butyric acid (C4), and *n*-caproic acid (C6) were obtained from Aldrich (Milwaukee, WI, USA). All chemicals used in this study were reagent grade.

Preparation of small black soybean powders The small black soybeans were purchased from a local agricultural cooperative market located at Imsil (Jeonbuk, Korea). The soybeans were soaked in distilled water (1:4, w/v) at room temperature for 12 hr. The water was drained off and the seeds were frozen, freeze-dried, and ground to pass through a 300-μm sieve to yield the raw small black soybeans (9). The cooked small black soybeans were prepared by boiling the soaked soybeans at 100°C for 30 min. The seeds were cooled, frozen, freeze-dried, and then ground to pass through a 300-μm sieve (9). The acidified black soybeans were prepared by soaking in persimmon vinegar (1:4, w/v) at room temperature for 3 weeks. The vinegar was drained off

and the seeds were frozen, freeze-dried, and then ground to pass through a 300-μm sieve.

Animals and diets Male Sprague-Dawley rats weighing 150-160 g were fed a nonpurified diet for 5 days, and then the experimental diet was started. The rats were maintained in a room at a 22±2°C, 60±5% relative humidity, and a 12-hr light:dark cycle, and were given free access to food and water *ad libitum*. The rats were divided into 3 groups of 6 rats each. For 4 weeks they were fed diets containing 10% raw small black soybean powder, 10% cooked small black soybean powder, and acidified small black soybean powder. The diets used in this study consisted of the following ingredients in g/100 g of diet: 20 g casein, 0.3 g DL-methionine, 55 g sucrose, 5 g corn oil, 3.5 g AIN-76 mineral (American Institute of Nutrition), 1 g AIN-76 vitamin mix, 0.2 g choline bitartate, 5 g cellulose, and 10 g test substances. The test substances included the raw, cooked, and acidified small black soybean powders.

Measurement of SCFA in feces Fresh fecal material was frozen immediately after collection and stored at −20 °C until use. The fecal homogenate was prepared using a modified version of the method by Tangerman and Nagengast (12). Ten g of fecal material were suspended in 50 mL of water (HPLC grade) and homogenized with a blender at 10,000 rpm for 3 min. Some of the homogeneous suspension was transferred into a centrifuge tube and centrifuged for 20 min at 30,000×g. The supernatant (1 mL) and a solution (100 μL) of 2-ethylbutyric acid (18.75 mM, internal standard) in formic acid were added to an Eppendorf tube. The suspension was centrifuged for 1 min at 10,000×g and the resulting supernatant was injected into a gas chromatograph for analysis. The gas chromatogrph was a HP 5890 series II plus, equipped with a flame ionization detector and a HP 6890 series injector. The injection port of the chromatograph was installed with a split liner with wool and FS beads (RESTEK). The SCFAs were separated on an HP-FFAP capillary column (25 m×0.32 mm i.d.×0.5 μm). Nitrogen was used as a carrier gas at a constant flow rate of 2.30 mL/min. The initial oven temperature (80°C) was maintained for 1 min following injection (1 μL), and then increased to 120°C at 20°C/min, and to 205°C at 6°C/min. This final temperature was maintained for 2 min. The temperatures of the injector and detector were held at 260°C.

Morphological characteristics and immunohistochemical method Morphological characteristics were measured as described by Choi *et al.* (13). Gastrointestinal tract of rat was dissected and cut into proximal duodenum and descending colon (1 cm) after sacrificing them. The segments were fixed in 10% neutral buffered formalin for 24 hr after washing with physiological saline and embedded in paraffin using a dehydration process. Hematoxylin (Sigma-Aldrich) and eosin (Sigma-Aldrich) staining were applied to the embedded test piece. Alcian blue (AB, Sigma-Aldrich) and periodic acid-Schiff reagent (PAS) staining were also applied to observe changes in mucous cells. Villus height and mucous layer thickness were measured using a graticule (1×1 mm) attached to a microscope.

Immunohistochemical staining was measured as described by Hsu *et al.* (14). The tissue sections were deparaffinized, and then hydrated with xylene and a graded alcohol series. Because certain antigenic determinants are masked by formalin fixation, the tissue slides were placed in a container and covered with a 10 mM sodium citrate buffer heated to 95°C for 5 min. The slides were allowed to cool in the buffer for 20 min and were then washed in distilled water 3 times for 2 min each. To quench endogenous peroxidase activity, the tissue sections were incubated for 10 min in 1% H_2O_2 in phosphate buffered saline (PBS), and then incubated for 1 hr in 5% normal goat serum in PBS. Next, some sections were incubated overnight at 4°C in rat anti-CD4 and anti-CD8 (diluted 1:50, Santa Cruze Technology, Santa Cruze, CA, USA) in PBS containing 0.3% Triton X-100 and 2% bovine serum albumin (BSA). After washing the specimens 2 times for 10 min using PBS, the sections were incubated sequentially in biotinylated anti-rat IgG (Vector Lab., Burlingame, CA, USA), and diluted 1:200 in the same solution as the primary antiserum for 2 hr. These specimens were incubated with an avidin-biotin enzyme (Vector Lab.) for 1 hr, and then washed again in PBS. The sections were visualized with 3,3'-diaminobenzidine (DAB, Sigma-Aldrich) in a 0.1 M Tris buffer and mounted on gelatin-coated slides. The immunoreactions were observed under an Axioscope microscope (Carl Zeiss, Oberkochen, Germany), and the immunoreactive density was determined by comparing the stained intensity of each group after observing 10 slides/sample.

Immunoreactive cell counts The immunoreactive cells were counted using an objective lens with magnification of 40× and 10× ocular lens. The optical visual field was 0.96 mm with 10 slides/sample (15).

Statistical analysis All data are expressed as mean± standard deviation (SD). Data were analyzed by analysis od variance (ANOVA) using the SAS statistical analysis system (SAS Institute Inc., Cary, NC, USA). Differences among the samples were analyzed using Duncan's multiple-range tests ($p<0.05$).

Results and Discussion

SCFA changes in feces The fecal changes in SCFAs after administering the raw, cooked, and acidified small black soybean supplemented diets are shown in Table 1. The total SCFA level was significantly higher in the acidified small black soybean supplemented group than in the raw and cooked groups. The major SCFAs in the experimental groups were acetate, followed by propionate and butyrate. The acetate concentration in each group was the highest, ranging from 32.39±2.11 μmol/g for the raw group to 62.65±2.06 μmol/g for the acidified group. The propionate (9.51±0.12 μmol/g) and butyrate (5.36±0.19 μmol/g) concentrations in the acidified small black soybean diet group were significantly higher than those of the raw (8.12±0.47 and 3.43±0.23 μmol/g, respectively) and cooked diet (8.03±0.24 and 3.88±0.48 μmol/g, respectively) groups. However, the concentrations of isovalerate and isobutyrate were very low compared to the other 3 major SCFAs mentioned above, and their concentrations were not significantly different in the cooked and acidified small black soybean diet groups.

Table 1. Short-chain fatty acids contents in feces of rats after feeding processed small black soybean powder (SBSP) supplemented diets for 4 weeks[1)]

Dietary group	Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Isovaleric acid	Valeric acid	Total
Raw SBSP diet	32.39±2.11^{c}	8.12±0.47^{b}	3.43±0.23^{b}	0.66±0.04^{a}	1.28±0.05^{b}	0.63±0.01^{ab}	46.23±1.79^{c}
Cooked SBSP diet	49.46±2.22^{b}	8.03±0.24^{b}	3.88±0.48^{b}	0.70±0.06^{a}	1.45±0.01^{a}	0.71±0.01^{a}	64.23±2.99^{b}
Acidified SBSP diet	62.65±2.06^{a}	9.51±0.12^{a}	5.36±0.19^{a}	0.77±0.05^{a}	1.42±0.02^{a}	0.59±0.07^{b}	80.30±2.24^{a}

[1)]μmol/g; Determined in duplicate (mean±SD); Different letters within columns are significantly different at $p<0.05$ by Duncan's multiple tests.

Table 2. Duodenal villus heights and colonic mucosal thickness in rats after feeding processed small black soybean powder (SBSP) supplemented diets for 4 weeks[1)]

Dietary group	Duodenum	Colon
	Villus height (μm)	Mucosal thickness (μm)
Raw SBSP diet	916.7±50.0^{b}	234.2±27.8^{c}
Cooked SBSP diet	1080.0±43.3^{a}	245.8±12.8^{bc}
Acidified SBSP diet	1093.3±22.2^{a}	300.8±30.8^{a}

[1)]Values are mean±SD, $n=8$; Different letters within columns are significantly different $p<0.01$ by Duncan's multiple tests.

Table 3. Histochemical densities of goblet cells in rat colon after feeding processed small black soybean powder (SBSP) supplemented diets for 4 weeks

Dietary group	Colon[1)]
	AB/PASAB-PAS
Raw SBSP diet	++/++/++
Cooked SBSP diet	++/+/+
Acidified SBSP diet	+++/+/++

[1)]AB, alcian blue; PAS, periodic acid-Schiff reagents; AB-PAS, alcian blue-periodic acid-Schiff reagents; Staining density: +, weak; ++, medium; +++, strong.

Acetate, propionate, and butyrate, which are major products of intestinal microbial metabolism as well as prevalent anions in the intestinal lumen (16,17), are used as an energy source by colonic epithelial cells (18), and thus promote the health of the colonic mucosa (19). In particular, butyrate is of interest because it is a preferred energy source for the colonic epithelial cells, resulting in the stimulation of cell proliferation (20) and colonic mucus secretion (21). In addition, a high butyrate concentration in the distal colon has been suggested to prevent colon cancer (22). Therefore, the high increase in butyrate concentration that occurred with the acidified small black soybean diet suggests that acidified small black soybeans could help maintain intestinal (colonic) health.

Duodenal villus height and colonic goblet cells The villus height measurements for the duodenum as well as the mucosal thickness for the colon in rats after feeding raw, cooked, and acidified small black soybean supplemented diets for 4 weeks are presented in Table 2. The villus heights in the duodenum of rats in the cooked and acidified small black soybeans supplemented diet groups were 1,080.0±43.3 and 1,093.3±22.2 μm, respectively, which was significantly higher than that in the raw diet group (916.7±50.0 μm, $p<0.01$), indicating more developed villi than that of the raw diet. The mucosal thickness of the colon in acidified small black soybean supplemented diet group (300.8±30.8 μm) was also significantly increased as compared to the raw and cooked diet groups (234.2±27.8 μm and 245.8±12.8 μm, respectively, $p<0.01$). The significant increases in duodenal villus height and colonic mucosal thickness of acidified small black soybeans supplemented diet group might induce an increase in enterocytes, resulting in enhancing the function of the intestinal villi (23).

The goblet cell changes in rat colons for the rats fed the raw, cooked, and acidified small black soybean supplemented diets for 4 weeks, were measured using AB, PAS, and AB-PAS staining methods; the results are shown in Table 3 and Fig. 1. The acidified small black soybean supplemented diet showed the heaviest density compared to the other experimental groups, and thus, stimulated a higher production of acid mucin than the raw and cooked black soybean powder supplemented groups. However, the density

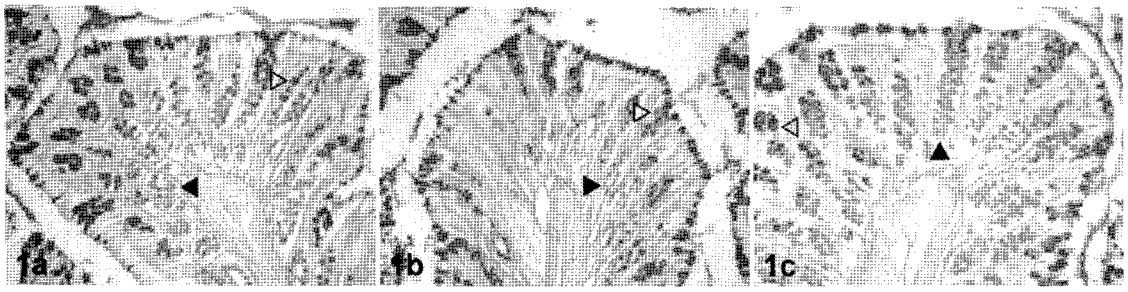

Fig. 1. Microphotographic observation of rat colon after raw (1a, 100×), cooked (1b, 100×), and acidified (1c, 100×) small black soybean powder diet fed for 4 weeks by alcian blue (black arrow head) and periodic acid-Schiff reagent (white arrow head) staining method.

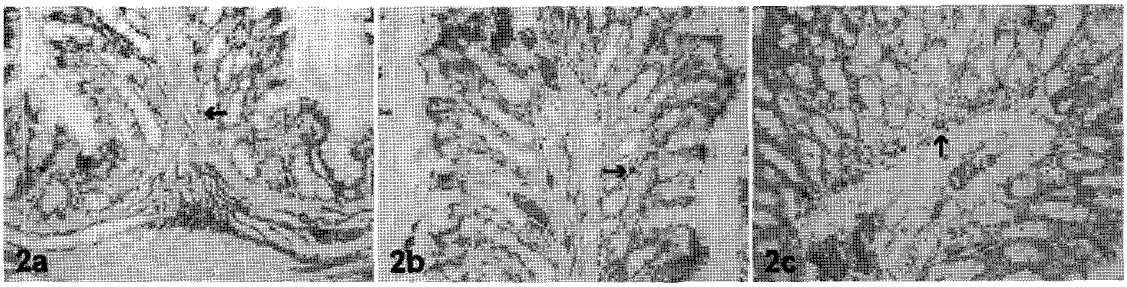

Fig. 2. $CD4^+$ labeled cells (arrow) in rat colon after raw (2a, 100×), cooked (2b, 100×), and acidified (2c, 100×) small black soybean powder diet fed for 4 weeks by immunohistochemical method.

Table 4. Immunohistochemical densities of $CD4^+/CD8^+$ lymphocytes in rat colon after feeding processed small black soybean powder (SBSP) supplemented diets for 4 weeks[1)]

Dietary group	Mucosa		Submucosa	
	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
Raw SBSP diet	++	+	+++	++
Cooked SBSP diet	++	+	+++	+++
Acidified SBSP diet	+++	+	+++	+++

[1)]Immunoreactive density: +, weak; ++, medium; +++, strong.

of the colonic mucosa stained with PAS and AB-PAS decreased or not changed in cooked and acidified diet groups compared to the raw diet group, indicating there were little productions of alkaline or neutral mucin in both diet groups.

Mucus forms a protective layer over the surface of the gastrointestinal mucosa, resulting in the protection of the underlying epithelium from mechanical damage as well as substances such as gastric acid and digestive enzymes in the lumen (24,25). Previous studies (13,26,27) demonstrated that dietary fibers contributed to an increase in colon mucus release. In particular, SCFAs are produced from dietary fibers via microflora metabolism in the large intestine, which stimulates mucus secretion through chemosensitive receptors that are connected to the cholinergic nerves or located directly on mucus-producing cells (21), resulting in contributing to the health of colon. Accordingly, the consumption of acidified small black soybeans could be a good means for producing high mucus levels in the colon, contributing to the protection of the mucosa from mechanical damage and harmful external materials (28).

$CD4^+$ and $CD8^+$ immunoreactive cells in rat colon The results of the immunohistochemical densities of the $CD4^+$/$CD8^+$ lymphocytes in rat colons after feeding the raw, cooked, and acidified small black soybean supplemented diets for 4 weeks are shown in Table 4. The acidified small black soybean supplemented group exhibited more strongly stained $CD4^+$ than raw and cooked diet groups (Fig. 2), but weakly stained $CD8^+$ immunohistochemical densities in the mucosal lamina propria. In the submucosa, the cooked and acidified small black soybean supplemented diet groups exhibited more strongly stained $CD8^+$ immunoreactive cells than raw diet group.

Intraepithelial lymphocytes (IELs) play important roles in immune action against foreign antigens (29,30), as well as in the maintenance of intestinal epithelial homeostasis (31). In particular, SCFAs, which are produced by bacterial fermentation of dietary fibers within the large intestine, were reported to play a significant role in the luminal contents of the large intestine (31). According to Ishizuka and Tanaka (31), sugar beef fiber, a fermentable fiber, promoted an increase in $CD8^+$ IEL populations in the epithelial layers of rat large intestines, indicating that increases in luminal SCFAs produced from dietary fiber might activate epithelial chemokine secretion to stimulate $CD8^+$ IEL homing. Consequently, the significant increases in the immunoreactive $CD4^+/CD8^+$ cells in the mucosa and submucosa of the colon that occurred by feeding the acidified small black soybean supplemented diet, could modulate immune responses in the colon.

In conclusion, ingestion of acidified small black soybean had an influence on intestinal characteristics in rat, contributed to an increase of SCFAs, development of duodenal villus height and colonic mucosal thickness, stainability of acid mucin in the colon, and strong staining $CD4^+$ and $CD8^+$ in the mucosal lamina propria and the submucosal lamina propria. These results suggest that acidified small black soybeans could play an important role in improving intestinal function.

Acknowledgment

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (the Center for Healthcare Technology Development, Chonbuk National University, Korea).

References

1. Messina M, Persky VP, Barnes S. Soy intake and cancer risk- a review of the *in vitro* and *in vivo* data. Nutr. Cancer 21: 260-268 (1994)
2. Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. Obstet. Gynecol. 87: 897-904 (1996)
3. Ali AA, Velasquez MT, Hansen CT, Mohamed AI, Bhathena SJ. Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. J. Nutr. Biochem. 15: 583-590 (2004)
4. Anderson JW, Smith BM, Washnock CS. Cardiovascular and renal benefits of dry bean and soybean intake. Am. J. Clin. Nutr. 70: 464S-474S (1990)
5. Tham DM, Gardner CD, Haskell WL. Potential health benefits of dietary phytoestrogens: A review of the clinical, epidemiological, and mechanistic evidence. J. Clin. Endocrinol. Metab. 83: 2223-

2235 (1998)
6. Kim JS, Kim JG, Kim WJ. Changes of isoflavone contents in soybean cultivars pickled in persimmon vinegar. Korean J. Food Sci. Technol. 36: 833-836 (2004)
7. Kim JA, Hong SB, Jung WS, Yu CY, Ma KH, Gwag JG, Chung IM. Composition of isoflavones composition in seed, embryo, cotyledon, and seed coat of cooked-with-rice and vegetable soybean (*Glycine max* L.) vatieties. Food Chem. 102: 738-744 (2006)
8. Choung MG, Baek IY, Kang ST, Han WY, Shin DC, Moon HP, Kang KH. Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* L. Merr.). J. Agr. Food Chem. 49: 5848-5851 (2001)
9. Lee CH, Oh SH, Yang EJ, Kim YS. Effects of raw, cooked, and germinated small black soybean powders on dietary fiber content and gastrointestinal functions. Food Sci. Biotechnol. 15: 635-638 (2006)
10. Shinomiya K, Tokunaga S, Shigemoto Y, Kamei C. Effect of seed coat extract from black soybeans on radial maze performance in rats. Clin. Exp. Pharmacol. P. 32: 757-760 (2005)
11. Takahashi R, Ohmori R, Kiyose C, Momiyama Y, Ohsuzu F, Kondo K. Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. J. Agr. Food Chem. 53: 4578-4582 (2005)
12. Tangerman A, Nagengast FM. A gas chromatographic analysis of fecal short-chain fatty acids, using the direct injection method. Anal. Biochem. 236: 1-8 (1996)
13. Choi YK, Lee CH, Lee MW, Kwon J, Song GS, Kim YS. Effect of alcohol insoluble residues from stem and root barks of *Ulmus davidiana* on intestinal characteristics in rats. Food Sci. Biotechnol. 15: 380-384 (2006)
14. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29: 577-580 (1981)
15. Lee CH, Yang EI, Song GS, Chai OH, Kim YS. *Cheonggukjang* mucilage stimulates immunohistochemical activities of gastrointestinal tract in rats. Food Sci. Biotechnol. 14: 813-817 (2005)
16. Cummings JH. Short chain fatty acids in the human colon. Gut 22: 763-779 (1981)
17. Engelhardt WV, Ronnau K, Rechkemmer G, Sakata T. Absorption of short-chain fatty acids and their role in the hindgut of monogastric animals. Anim. Feed Sci. Tech. 23: 43-53 (1989)
18. Davidson MH, McDonald A. Fiber: Forms and functions. Nutr. Res. 18: 617-624 (1998)
19. Schneeman BO. Fiber, inulin, and oligofructose: Similarits and differences. J. Nutr. 129: 1424S-1427S (1999)
20. Sakata T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: A possible explanation for for trophic effects of fermentable fiber, gut microbes, and luminal trophic factors. Brit. J. Nutr. 58: 95-103 (1987)
21. Shimotoyome A, Meguro S, Hase T, Tokimitsu I, Sakata T. Short chain fatty acids but lactate or succinate stimulate mucus release in the rat colon. Comp. Biochem. Phys. A 125: 525-531 (2000)
22. Kim YS, Tsao D, Siddiqui B, Whitehead JS, Arnstein P, Bennet J, Hicks J. Effects of sodium butyrate and dimethylsulfoxide on biochemical properties of human colon cancer cell. Cancer 45: 1185-1192 (1980)
23. Samanya M, Yamauchi K. Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. *natto*. Comp. Biochem. Phys. A 133: 95-104 (2002)
24. Cross CE, Halliwell B, Allen A. Antioxidant protection- a function of tracheo-bronchial and gastrointestinal mucus. Lancet 323: 1328-1330 (1984)
25. Strugaka V, Allen A, Dettmar PW, Pearson JP. Colonic mucin: Methods of measuring mucus thickness. P. Nutr. Soc. 62: 237-243 (2003)
26. Satchithanandam S, Vargofcak-Apker M, Calvert RJ, Leeds AR, Cassidy MM. Alteration of gastrointestinal mucine by fiber feeding in rats. J. Nutr. 120: 1179-1184 (1990)
27. Shimotoyodome A, Meguro S, Hase T, Tokimitsu I, Sakata T. Sulfated polysaccharides, but not cellulose, increase colonic mucus in rats with loperomide-induced constipation. Digest. Dis. Sci. 46: 1482-1489 (2001)
28. Matsuo K, Ota H, Akamatsu T, Sugiyama A, Katsuyama T. Histochemistry of the surface mucosal gel layer of the human colon. Gut 40: 782-789 (1997)
29. Camerini V, Panwala C, Kronenberg M. Regional specialization of the mucosal immune system. J. Immunol. 151: 1765-1776 (1993)
30. Nagai T, Ishizuka S, Hara H, Aoyama Y. Dietary sugar beet fiber prevents the increase in aberrant crypt foci induced by γ-irradiation in the colorectum of rats treated with an immunosuppressant. J. Nutr. 130: 1682-1687 (2000)
31. Ishizuka S, Tanaka S. Modulation of $CD8^+$ intraepithelial lymphocyte distribution by dietary fiber in the rat large intestine. Exp. Biol. Med. 227: 1017-1021 (2002)