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The Food Safety of Superfine Saengshik Processed by Top-down Technique in Mice

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Abstract

Saengshik is an uncooked and powdered functional food composed of various edible plants, and has been consumed widely due to its health benefits and convenient uptake. Recently, superfine ground saengshik, which contains a certain extent of nanoscale particles, has been commercialized to enhance efficacy, but its safety has not been determined. This study was conducted to evaluate the food safety of superfine saengshik (SS) through general toxicity examination after oral uptake in mice compared to conventional fine saengshik (FS). The SS particle size distribution was 0.479-26.303 μm in diameter, with about 68.92% of particles with a diameter <0.955 μm. From our safety evaluation, the number of white blood cells (WBCs) and biochemical values in the serum fell into the normal range, and the weight of organs showed no significant difference between FS and SS groups. Histological observation of the liver, small intestine and large intestine did not show any abnormal or pathological findings under light microscopy. Our results suggest that oral intake of SS is not harmful to mice in terms of general toxicity.

Keywords: Nanomill, Food safety, Saengshik, Superfine

Uncooked formulated food (called saengshik in Korea) is a representative functional food in Korea. It is composed of various vegetal ingredients in dried and pulverized forms including grains, vegetables, fruits, soy beans, mushrooms and marine plants. Compared to heating processed foods, saengshik preserves bioactive substances such as vitamins, enzymes and various phytochemicals due to its non-heating process[1]. Saengshik intake has been known to have antioxidative effects[2], anti-cancer effects[3,4], anti-diabetic effects[5], and improvements in hyperlipemia[6]. Moreover, saengshik provides better health effects than single ingredients through a synergistic combination of various phytochemicals[7]. Due to these health benefits and the convenience of ingestion, saengshik traditionally has been consumed by people in Korea for a long time. Various types of saengshik products have been manufactured as functional foods to promote health, prevent disease, and substitute regular meals for the past two decades.

Fine saengshik (FS), which is pulverized by a conventional mill technique, has been empirically proven by consumers to be safe for humans. However, FS involves the possibility of microbial hazards by contamination of pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens*. This is related to its unheated manufacturing process, which is used for the preservation of heat-unstable nutrients, as well as unsanitary control of pulverizing equipments and insufficient washing and sterilization of raw materials[8]. In the context of saengshik food safety, Kim *et al.* suggested some hazard analysis critical control points (HACCPs): a washing process for controlling microbial contamination, a freeze-drying process for controlling moisture content to prevent the deterioration and growth of microorganisms, and a pulverization process for controlling

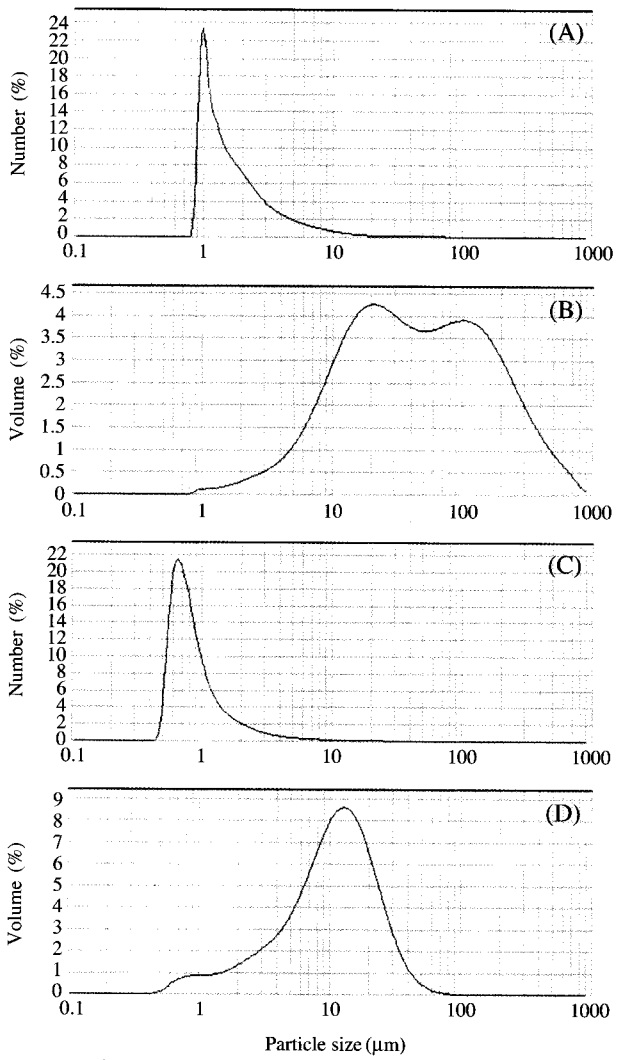

Figure 1. The particle size distribution in number and volume. A and B : fine saengshik, C and D : superfine saengshik.

the contamination of foreign substances such as metals[9]. Until now, however, food risk management, such as processing standards and performance standards, of saengshik has not been adopted by the Korean government.

Currently, the use of nanotechnology is increasing dramatically in the area of food, and food and nutrition products containing nano-scale materials are already commercially available. The top-down approach in the food area involves size reduction by enhanced forces such as compression, impact and shear. Impact and shear forces are especially important in producing nanoparticles for food application. Due to the development of the dry mill equipment, particle sizes of saengshik powder are getting smaller. Superfine saengshik (SS) produced by the top-down approach contains considerable nano-scale particles. The degree of particle size reduction influences the properties of the processed food materials and usually relates to the functionality of food. A smaller size leads to a larger surface area, which improves the absorptive capacity and bioavailability of the nutritive components[10,11]. However, the reduced particle size of SS can act as a new hazardous factor in spite of its various health benefits, and its influence on the human body has not yet been identified. New processing techniques for food requires new risk analyses for consumer health protection and societal trust.

SS containing nano- and micro-particles is important to the physiological and histological aspect due to its uptake in large quantities and also due to direct contact with gastrointestinal tract. Therefore, we studied the food safety of SS produced by the top-down technique, focusing on general toxicity after oral intake in mice compared to FS.

Table 1. Composition of experimental diets, saengshik.

Contents	%
Barley	40.0
Corn	24.0
Red bean	11.5
Fructooligosaccharide	4.0
Buckwheat	3.0
Black bean	2.5
Brown rice	2.0
Sorghum	2.0
Acorn	2.0
Quince	2.0
Mushroom (*Agaricus bisporus*)	1.5
Potato	1.5
Millet	1.4
Perilla seed	1.0
Salt	0.6
Black sesame	0.5
Glutinous rice	0.5
Total	100.0

Particle Size Distribution and Surface Area of Superfine and Fine Saengshik

The size distributions of FS and SS particles less than 0.955 μm in diameter were 7.02% and 68.92%, respectively (Figure 1). The result of particle diameter measured at 50% of distribution ($d_{(0.5)}$) showed that SS decreased about 2-fold in number distribution and 4-fold in volume distribution (Table 3). From surface area analysis, SS (1.18 m^2/g) increased 2.7-fold compared to FS (0.436 m^2/g).

Food Intake, Organ Weight and Body Weight

In terms of food intake, the FS group (5.1 ± 0.9 g/day,

$P<0.01$) and SS group (5.3±1.2 g/day, $P<0.05$) decreased compared to the N group (6.6±0.1 g/day). However, the body weight of mice in the SS group (30.1±2.0 g) did not show a significant difference from that of the N group (31.8±1.2 g), while the body weight of mice in the FS group (29.1±1.6 g, $P<0.01$) decreased compared to that of the N group. The weight of organs showed no statistical difference among groups (Table 4).

Table 2. Ingredients and nutrient contents of saengshik.

Component	Contents
Calories (cal/100 g)	400.0
Carbohydrates (g/100 g)	74.7
Fat (g/100 g)	5.1
Protein (g/100 g)	6.3
Total dietary fiber (g/100 g)	7.2
Total sugars (g/100 g)	10.0
Moisture (g/100 g)	3.8
Ash, total (g/100 g)	2.3
Cholesterol (mg/100 g)	ND
Vitamin A (IU/100 g)	193.0
Vitamin C (mg/100 g)	ND
Sodium (mg/100 g)	78.0
Calcium (mg/100 g)	71.3
Iron (mg/100 g)	6.6

Table 3. Particle diameter at 10%, 50% and 90% in number and volume distribution.

Particle diameter (μm)	Distribution in number		Distribution in volume	
	FS	SS	FS	SS
d(0.1)[a]	0.973	0.579	8.627	2.729
d(0.5)[b]	1.413	0.782	44.059	10.950
d(0.9)[c]	3.980	1.645	242.082	25.077

[a]particle diameter at 10% of the number (or volume) distribution
[b]particle diameter at 50% of the number (or volume) distribution
[c]particle diameter at 90% of the number (or volume) distribution
FS : fine saengshik, SS : superfine saengshik

Hematological and Biochemical Test

In hematological testing, the number of total WBC, neutrophils, lymphocytes, monocytes, eosinophils, and basophils in the FS and SS groups had no statistical differences from those of the N group (Table 5). Upon biochemical examination using the serum, Na, K, Cl, Ca, inorganic phosphate, albumin, total cholesterol, creatinine and BUN, the FS and SS groups had no significant differences compared to the N group (Tables 6 and 7). However, the value of alanine amino transferase (ALT) in the FS group ($P<0.001$) and SS group ($P<0.05$) showed a significant decrease compared to those of the N group (29.6±5.6) (Table 7).

Histopathological Examination

Upon histopathological examination, the livers of mice in all groups did not show histopathological changes, including necrosis, fibrosis and the infiltration of inflammatory cells. Small and large intestines of mice also did not show any abnormal findings in any of the groups (Figure 2).

Discussion

People ideationally prefer natural entities over those that are produced with human intervention, especially regarding food. According to a report by Rozin *et al.* there is a substantial preference for natural products

Table 4. The weight of organs (g).

Group	Heart	Liver	Stomach	Kidney	Spleen	SI	LI
N	0.15±0.02	1.05±0.07	0.28±0.06	0.35±0.03	0.07±0.02	1.07±0.19	0.38±0.04
FS	0.13±0.02	1.28±0.13	0.31±0.06	0.34±0.02	0.07±0.02	0.94±0.11	0.37±0.10
SS	0.11±0.01	1.03±0.10	0.26±0.07	0.30±0.01	0.06±0.01	0.82±0.11	0.32±0.08

All values are expressed as means±S.D. (n=8 for each group)
SI : small intestine, LI : large intestine

Table 5. WBC numbers.

Group	Total WBC	NE	LY	MO	EO	BA
N	8.23±3.16	2.28±1.09	5.16±1.85	0.55±0.21	0.16±0.10	0.03±0.02
FS	7.72±2.10	2.29±0.65	4.87±1.34	0.58±0.19	0.15±0.11	0.03±0.02
SS	7.77±2.87	2.34±1.07	4.81±1.62	0.64±0.24	0.12±0.12	0.03±0.03

All values are expressed as means±S.D. (n=8 for each group)
NE : neutrophil, LY : lymphocyte, MO : monocyte, EO : eosinophil, BA : basophil

and that preference is stronger for foods than for medicine, although the healthfulness or effectiveness of food and medicine is specified as equivalent[12]. This survey reflects the thought that natural food materials may be safer for the body than artificial materials. Saengshik is composed entirely of natural plant materials without artificial additives. By this account, saengshik satisfies consumers' preferences for natural foods and their consumption is somewhat based on consumer trust. In many countries, including Korea, various saengshik products as functional foods have been consumed popularly to promote health and treat disease, and the saengshik market is growing rapidly. FS pulverized with conventional mill techniques has generally been recognized to be a safe food by scientific and empirical evidence. However, FS products have some problems with food safety, such as microbial hazards or contamination of foreign substances, which may be caused by issues with the the saengshik manufacturing process, such as unheated steps and unsanitary equipment, but not by the saengshik material itself.

Recently through the development of new pulverizing techniques, SS containing a certain extent of nanoscale particles was commercialized. As a result, the necessity of a food safety evaluation due to the reduced particle size was suggested. Consumers are deeply concerned about food-related hazards from the development of a diverse group of food products through new technology. Frewer *et al.* reported that people want to be provided with *information* about food risk *uncertainty* as soon as the uncertainty is identified[13]. In addition, Wandel and Fagerli also found that consumers felt a need for more dependable information on food safety issues[14]. Therefore, hazardous properties of SS products from new processing technology should be identified in the early stages of commercialization for consumer protection and public confidence. Until now, the dry mill process of plant materials had not reached the nanotechnology level, neither in shape nor the state of the particle surface, due to technical limitations. However, superfine processing is important for improving functional properties and as a precursor to nano-scale processing.

In terms of particle size distribution, FS and SS showed marked differences in the percentage of particles < 1 μm in diameter, with 7.02% and 68.92% respectively. The surface area of SS increased about 2-fold compared to that of FS. From hematological and biochemical examination, the FS and SS groups were fed FS and SS respectively, and did not show any abnormal findings compared to the normal (N) group. Moreover, the representative liver enzyme ALT was significantly decreased in the FS and SS groups. ALT is used as an indirect diagnosis of liver function. Serum ALT is measured at low levels in the normal state but released into the blood in large quantities after injury of hepatocytic membranes. Therefore, a decrease of serum ALT indirectly indicates improved liver function as well as the non-toxicity of SS. The relationship between food intake and body weight is also explained in the same context. Food intake by mice in the SS group was decreased ($P < 0.05$) compared to the N group; however, body weight showed no statistical change. These results suggest that the reduction of particle size improves the bioavailability of saengshik.

Some kinds of engineered nanoparticles (size < 100 nm) were reported to affect the body through various pathways[15,16]. Researchers have reported that particulates within the micrometer range were transferred across the mucosal barrier of the small intestine (persorption) after oral administration and then transmitted to secondary organs of the body[15,17]. Volkheimer demonstrated that starch, spores, pollen and powdered lobster shells as well as starch granules and cellulose fibers between 15 and 75 μm were found in body fluids, in the chyle taken from the thoracic duct, in blood, in bile, and in the urinary bladder two hours after eso-

Table 6. The level of electrolytes in serum.

Group	Na	K	Cl	Ca	IP
N	147.9±2.4	5.6±0.5	112.9±2.3	9.3±0.4	8.9±0.8
FS	145.8±1.5	5.2±0.6	111.6±1.8	9.6±0.3	9.1±0.7
SS	147.6±2.7	5.6±0.3	113.5±2.7	9.7±0.3	8.4±1.4

All values are expressed as means±S.D. (n=8 for each group)
IP: inorganic phosphate

Table 7. The level of biochemical parameters in serum.

Group	AST	ALT	Albumin	TC	Creatinine	BUN
N	85.2±10.8	29.6±5.6	2.4±0.1	149.1±40.1	0.4±0.0	22.9±2.6
FS	88.0±22.3	18.5±3.4***	2.5±0.2	128.4±24.0	0.4±0.0	20.6±3.7
NS	78.3±9.6	21.0±4.1*	2.3±0.1	127.4±23.0	0.4±0.0	22.5±2.9

All values are expressed as means±S.D. (n=8 for each group)
* and *** represent significant difference from N group at $P < 0.05$ and $P < 0.001$, respectively
TC: total cholesterol, BUN: blood urea nitrogen, AST: aspartate amino transferase, ALT: alanine amino transferase

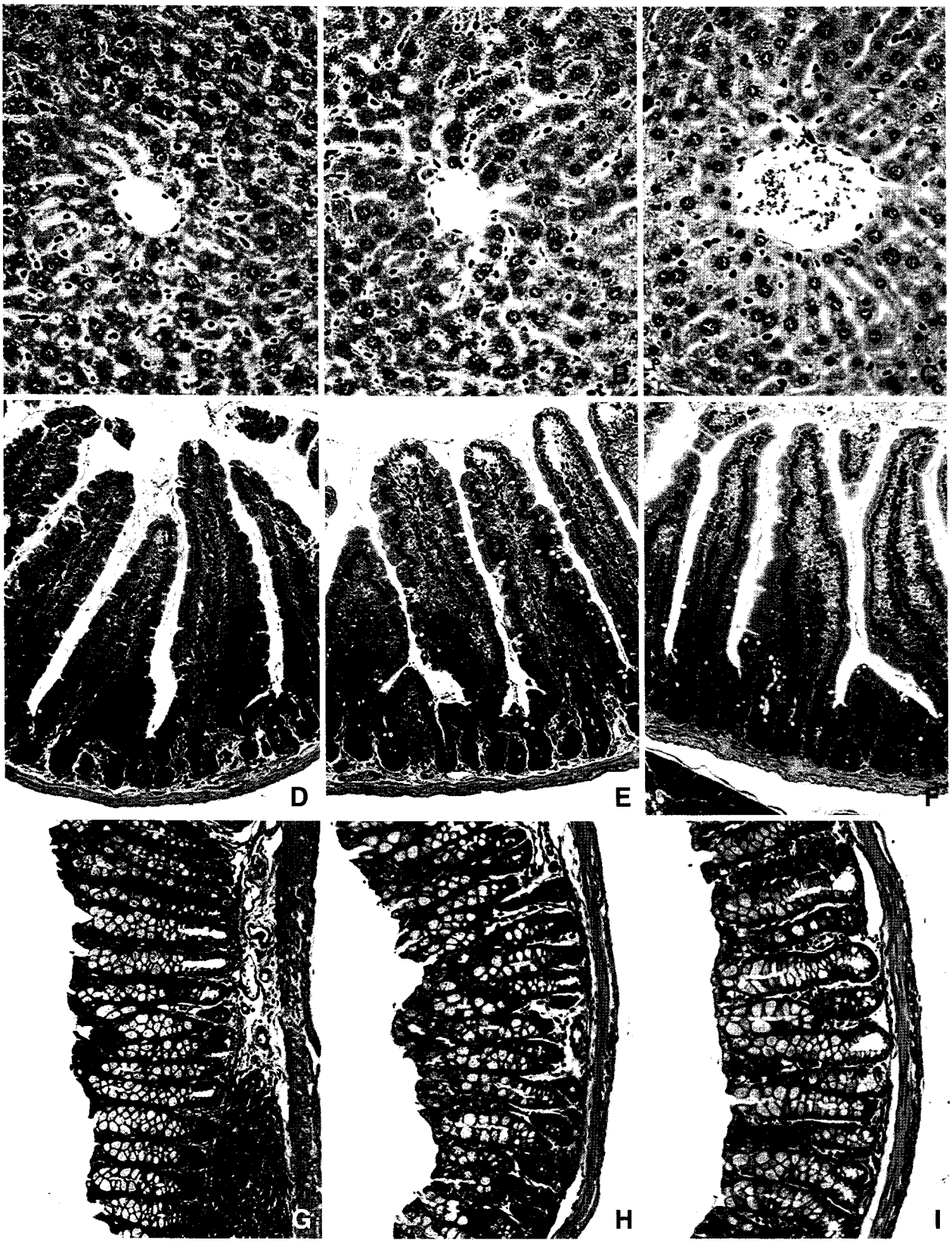

Figure 2. The micrographs of liver (A, B and C), small intestine (D, E and F) and large intestine (G, H and I). All groups showed normal finding. A, D and G : N group; B, E and H : FS group; C, F and I : SS group; H-E stain.

phageal or rectal administration by tube[18]. He also reported that in the lymphatic fluid of a patient with chylothorax, cellulose particles and starch granules were found after the intake of uncooked cereals, but boiled starch granules lost their perorbability[19]. These results suggest that large particulates in uncooked SS can influence the body histopathologically and physiologically. According to our particle size analysis, the particle size of SS was in the range of 0.479-26.303 μm and 77.51% of particles were < 1 μm in diameter. SS also contains nondigestible microparticles such as resistant starch, cellulose, lignin and lectin. Our histological results did not show any pathological and morphological changes in the liver, small intestine and large intestine as well as physiological abnormalities by the oral uptake of SS. Such results are relevant to the elimination mechanism of the particles absorbed and perorbed in the tissue. Particulates passed through the epithelial layer are eliminated by defense mechanisms such as phagocytosis, enzymatic degradation, and by the urinary and biliary systems. The residual time of particles in the body is dependent on the size, type, incoming route, particle dose, status of the GI tract, diet type (solid or liquid), etc.[17,18,20] In this context, although our histological results showed negative findings, there is a need to identify the potential risks of SS intake through long-term monitoring and to develop effective methods to trace the particulates in the body.

SS applied by a top-down approach has been known to lead to more improved health benefits than conventional FS, and has been consumed popularly for health promotion and disease prevention. In this study, we screened general risk factors of SS relevant to the reduction in particle size. Consequently, oral uptake of SS was confirmed to be nontoxic to mice through hematological, biochemical, and histological examination. Our data will provide basic information to establish the safety of SS. However, further study is needed to identify the potential risks of SS intake by tracing the absorbed and perorbed particulates in the body after uptake. The development of new dry-mill techniques is expected to create food products with much smaller particles, so there is a need to develop new risk assessments to evaluate newly developed food products and to monitor food safety continuously.

Materials & Methods

Animals

The experimental animals were five-week-old male ICR mice with weights of 25 ± 2 g (Jungang Lab. Animal Inc., Seoul, Korea). They were acclimated for one week and reared under room temperature conditions of 23 ± 2°C and 12 h light/dark cycles. The experimental animals were divided into three groups of eight; the normal (N) group was fed commercial food for mice (Formula M07, Feedlab Korea Co., Ltd., Korea), the FS group was fed a FS diet, and the SS group was fed a SS diet. Enough food and water was supplied for six days. Animal experiments were conducted in accordance with the animal use and care guidelines established by The Ethics Review Committee of Yonsei Wonju College of Medicine, Wonju, Gangwon, Korea.

Pulverization of Saengshik, and Determination of Particle Size Distribution and Surface Area

FS powder was produced by a general pulverizer (KFM-10, Koreamedi. Co., Ltd., Korea) through impact, compression and shear forces at 1,000 rpm. The temperature of the milling chamber was kept below 40°C. SS was processed by Turbo Mill HKP-05 (Korea Energy Technology Co., Ltd., Korea) which had the same principles as KFM-10. The rotor speed was 10,500 rpm, and the grinding media diameter was 210 mm. The temperature of the body was kept at -20°C and the temperature of the milled products was kept at 25-30°C by a cooling device in the Turbo Mill using cooling media R-22. Also, classification of particles was performed at the same time as pulverization by centrifugal and dragging forces in the Turbo Mill. The entire process was carried out under the in-line circuit system. Particle size distribution (%) and surface area (m^2/g) were measured by Mastersize 2000 (Malvern instruments Co., Ltd., UK).

Production of Experimental Foods

The commercial solid feed (Formula M07, Feedlab Korea Co., Ltd., Korea) fed to the N group was composed of protein 22.1%, fat 3.5%, fiber 5.0%, ash 6.54%, moisture 8.22%, calcium 0.6%, and phosphorus 0.4%. Raw ingredients for the saengshik diet were offered by Ohaeng Saengshik Co., Ltd. (Taean-Gun, Korea). The composition of the experimental diets are presented in Table 1. Ingredients and nutrient contents of saengshik were analyzed by Microbac Laboratories, Inc. (CA, USA) and are presented in Table 2. Solid experimental foods were produced with 100% FS and SS powder. The powder was mixed with distilled water, made into the size and shape of commercial feeds, and dried in the oven at 60°C.

Determining Food Intake, Organ Weight and Body Weight

Food intake was measured once daily at the same

time for each group during the 6 day experimental period. The body weight of each mouse was measured twice, once on the first day of the experiment and once on the sixth day before sacrifice of the mouse. The weights of the organs (heart, liver, stomach, kidney, spleen, small intestine, and large intestine) were measured after anesthesia with ether and the mice were sacrificed at the end of the experiment.

Hematological and Biochemical Examination

The mice were anesthetized by ether and the blood sample was collected from the orbital plexus on the sixth day. Hematological parameters included the number of total WBC, neutrophils, lymphocytes, monocytes, eosinophils and basophils, and were analyzed by an automated blood analyzer (HEMAVET HV950 FS, Drew Scientific Inc., USA). For biochemical tests, Na, K, Cl, Ca, inorganic phosphate, aspartate amino transferase (AST), alanine amino transferase (ALT), total cholesterol, albumin, creatinine, and blood urea nitrogen (BUN) were determined with an automated serum analyzer (Fuji Dry/Chem 3500, Fuji Photo Film Co., Ltd., Japan).

Histological Examination

The liver, small intestine and large intestine were taken from the experimental animals after anesthesia with ether. The tissue samples were fixed with 10% formalin for 24 hr, dehydrated with ethyl alcohol, cleared with xylene and embedded with paraffin according to the routine method. They were sectioned with a thickness of 2 μm, deparaffinized, hydrated and then stained with hematoxylin and eosin solution (Merck, Germany). The tissues were observed under an optical microscope after dehydration, clearing and mounting.

Statistical Analysis

Values are represented as the mean ± standard deviation. Statistical analysis was carried out using ANOVA (non-parametric) with significance level set at $P < 0.05$. Differences among groups were evaluated with Dunn's multiple comparison tests using the Prism 4.0 (Graphicpad Software Inc., USA) statistic program.

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