Table 1. Study group setting and daily dosage

Group	Number of rats	Concentration (mg/mL)	Dosage (mL/kg)	Dosage (mg/kg)
Kumaizasa powder	8	100	30	3000
Holocellulose	8	50	30	1500
Cellulose	8	30	30	900
Control*	8	-	30	-

*Japan pharmacopoeia purified water NikP

Table 2. Summary of intervention food

	Kumaizasa powder	Holocellulose	Cellulose
Total	3000 mg	1500 mg	900 mg
Cellulose (mg)	894.0	724.5	764.1
Hemicellulose (mg)	654.0	403.5	48.6
Water (mg)	123.0	82.5	52.2

Fecal properties and amount: The fecal properties of individual rats were classified into four groups: "normal stool", "loose stool", "diarrhea stool", and "watery stool" and recorded every day from day 1 to day 14 of administration. Then individual rat was transferred to a metabolic cage and kept over a 24-hour period on the previous day of day 1 (day 0), day 7, and day 14 of administration. All the feces were collected, and the fecal wet weights were measured. In addition, the feces were dried at 55 °C for 48 hours and the dry weights were measured. The difference between the wet weight and the dry weight was calculated as the moisture content in the feces.

Other observations: From the start to day 15, visual appearance, vital sign and other data of rats were carefully observed. Body weight of individual rat was measured on day 0, 1, 3, 7, 10, 14 and 15. Further, the supplied feed amount was measured on day 1, 3, 7 and 10. The remaining amount of feed was also measured on days 3, 7, 10 and 14, respectively. The amount of food intake per day was calculated by dividing the difference between the supplied amount and the remaining amount by the number of days between measurements.

Ethics statement and management of animals: All animal experimental procedures were approved on Oct 23, 2015 (approval number: NP015-36-02) by the Institutional Animal Care and Use Committee of Safety Research Institute for Chemical Compounds Co., Ltd. (Sapporo, Japan). All the experiments were performed in compliance with Act on Welfare and Management of Animal [10] and other relevant standards.

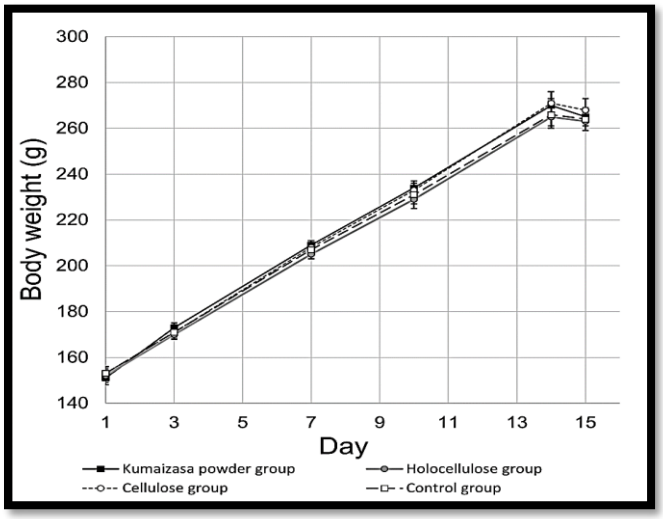
The animal experiment was conducted during Oct. 28 to Nov. 18, 2015. The rats not selected at grouping and the rats finished the experiment were euthanized by exsanguination of abdominal aortic amputation under isoflurane anesthesia.

Statistical analysis: As for fecal wet weight, fecal dry weight and fecal moisture content, a Bartlett's test was performed among the groups for each measurement point. Multiple comparisons were performed for the entire groups by the Tukey-Kramer method in the case of equal variance, and by the Steel-Dwass' test in the case of unequal variance. In addition, the measured values of each group were arranged in ascending order at each measurement point, and the values of the same rank were regarded as "corresponding data", and the control group and other groups were compared by the method of a paired t-test. The quartile deviation method was used to determine outliers.

RESULTS

Transition of weight and food intake in rats: During the experimental period, any cases of death or other abnormalities were not observed in the intervention groups nor control group. Fig. 2 shows the transition of the weight and feeding amount of the 4 groups. In each group, the rats grew normally, and their body weight increased along with food intake. In the intergroup comparison, no significant differences in body weight and feeding amount were found among all the groups.

A



B

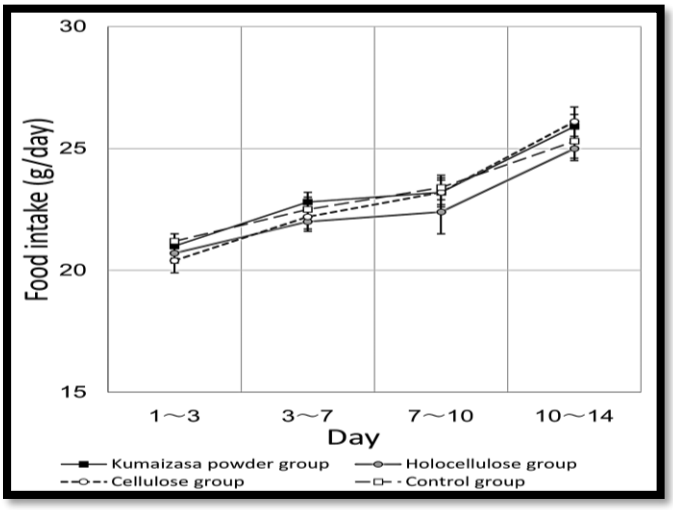


Figure 2. Transitions of body weight (A) and food intake (B) of rats

FECAL OBSERVATION

Fecal properties: In the cellulose group, a transient watery stool was found in one rat on day 2 of administration, however all other rats were observed with normal stools.

Fecal amount: In items of the fecal amount, there was one rat whose stool amount was extremely small in each intervention group. Using quartile deviation method, measurements determined to be outliers that deviated in the smaller direction were excluded from the analysis. Measurements that deviated greatly were used for analysis because the possibility of the effect of the test substance used in the intervention cannot be ruled out. Thus these 3 rats were removed from the analysis as outliers. In addition, to prevent bias among the groups, one rat that showed the lowest value in the total fecal wet weight in the control group was also excluded from the analysis. The results of changes in fecal wet weights, fecal dry weights and moisture content in feces were shown in Table 3.

Regarding multigroup comparison, the dry weight of feces collected at day 14 and total sum of those at day 0, 7 and 14 in the kumaizasa powder group significantly increased as compared with those of control group (day 14; $p<0.01$, sum of day 0, 7 and 14; $p<0.05$, by Tukey-Kramer method). These results suggest that kumaizasa powder has the effect of increasing fecal amount. Other measurements of fecal amount were not significantly different among the intervention and control groups.

Then, the ratios between the fecal amount values of intervention group and control group using each average value were calculated. We also performed a paired t-test using the corresponding data where the measured values of the same order when arranged in ascending order for each intervention group vs. control group were regarded as paired. The results were shown in Table 4. The ratios between fecal dry weights in cellulose group and control group exceeded 1 and the differences of fecal dry weights were statistically significant at day 7 ($p<0.05$), day 14 ($p<0.001$) and the total ($p<0.05$) showing that cellulose in kumaizasa powder has the effect of increasing fecal amount. Relating to moisture content in feces, the ratio between cellulose group and control group also exceeded 1 and the differences were significant at day 7 ($p<0.05$), day 14 ($p<0.05$) and the total ($p<0.001$) showing that cellulose has the effect of increasing moisture content in feces.

Regarding the effect of holocellulose, the paired t-test suggested that holocellulose had effect of increasing fecal dry weights on day 14 but no effect of increasing moisture content in feces despite that the holocellulose sample contains almost same amount of cellulose in cellulose sample as shown in Table 2. The results of kumaizasa powder on fecal dry weights coincided well with the results of the cellulose. As to the moisture content in feces, the results of kumaizasa powder coincided well with those of holocellulose.

Table 3. Transition of wet weight, dry weight, and moisture content in feces of rats

Fecal sample	Treatment group[1)]	Sampling days after treatment[2)]			
		0	7	14	Total
Fecal wet weight (g)	Kumaizasa powder	4.72±0.18	6.21±0.28	8.04±0.47	18.97±0.78
	Holocellulose	4.70±0.15	5.96±0.30	8.33±0.54	18.99±0.85
	Cellulose	4.61±0.29	6.29±0.30	8.33±0.30	19.24±0.40
	Control	4.80±0,15	5.85±0.21	7.67±0.30	18.32±0.38
Fecal dry Weight (g)	Kumaizasa powder	3.05±0.11	4.00±0.16	5.26±0.10**	12.31±0.33**
	Holocellulose	3.02±0.07	3.65±0.14	4.76±0.23	11.44±0.35
	Cellulose	2.88±0.15	3.77±0.12	4.70±0.12	11.35±0.17
	Control	3.02±0.08	3.60±0.11	4.42±0.12	11.04±0.22
Fecal moisture content (g)	Kumaizasa powder	1.67±0.09	2.21±0.13	2.78±0.45	6.66±0.58
	Holocellulose	1.68±0.11	2.31±0.18	3.57±0.32	7.56±0.53
	Cellulose	1.74±0.14	2.51±0.19	3.63±0.22	7.88±0.25
	Control	1.79±0.08	2.25±0.14	3.24±0.19	7.28±0.20

1) Data of 7 rats/group without an outlier were used.

2) Number in table 3 was shown as an average ± standard deviation.

*: <0.05, **: <0.01 (Tukey—Kramer method compared with control)

Table 4. Mean ratio of fecal wet weight, fecal dry weight and fecal moisture content compared with the control value as 1

Fecal sample	Treatment group[1)]	Days collected fecal sample			
		0	7	14	Total
Fecal wet weight[2)]	Kumaizasa powder	0.98	1.06 **	1.05	1.04
	Holocellulose	0.98	1.02	1.09 *	1.04
	Cellulose	0.96	1.08 *	1.09 *	1.05 **
Fecal dry weight[2)]	Kumaizasa powder	1.01	1.11 ***	1.19 ***	1.12 ***
	Holocellulose	1.00	1.01	1.08 *	1.04
	Cellulose	0.95	1.05 *	1.06 ***	1.03 *
Fecal moisture content[2)]	Kumaizasa powder	0.93 *	0.98	0.86	0.91
	Holocellulose	0.94	1.03	1.10	1.04
	Cellulose	0.97	1.12 *	1.12 *	1.08 ***

1) Data of 7 rats/group without outlier were used.

2) the ratio to control group

*: <0.05, **: <0.01, ***: <0.001 (Comparison with the control group by the corresponding t--test in the same rank)

DISCUSSION

Dietary plant fibers such as cellulose, hemicellulose, and pectin are resistant to digestion by human digestive enzymes and reach to human colon [11]. Dietary fiber exhibits a variety of physiological activities, including the ability to retain water in the stool and to increase specific bacteria such as genus of *Bifidobacterium* [12-13] in the human gut.

Recently, Nishihira et al. reported that the kumaizasa powder (SanSTAGE® UNIAL Co., Ltd. (Tokyo, Japan)) showed the significant increase of the stool frequency and improved the stool odor after 2-week ingestion (4.2 g/day) compared with those of the placebo (starch), in the clinical trial of a placebo-controlled, randomized, double-blinded, parallel-group comparison using 80 healthy Japanese adults (age 20 to 65) [9]. In another study, stool volume and stool frequency were also significantly improved by continuous ingestion (4.2 g/day) of kumai-zasa powder for 4 weeks in female subjects with chronic constipation [8].

In this study, the effects of the kumaizasa powder and its components, i.e., the holocellulose (hemicellulose + cellulose) and cellulose on the bowel movement in rats were evaluated to elucidate each role of holocellulose and cellulose. Multigroup comparison by the Tukey-Kramer method showed that the dry weight of feces collected at day 14 in the kumaizasa powder group significantly increased as compared with that of control group. This result confirmed that kumaizasa powder had the effect of increasing fecal amount in rats. A paired t-test between each kumaizasa intervention group and control group indicated that cellulose increased the fecal dry weights as well as moisture content in feces, while the kumaizasa powder and holocellulose increased fecal dry weights but not moisture content in the feces despite both samples containing similar amounts of cellulose. This difference seems to be attributed to hemicellulose that is rich in kumaizasa powder and the holocellulose samples but not in the cellulose sample.

The kumaizasa holocellulose is comprised of cellulose and hemicellulose and is produced by removing liposoluble components and lignin from the kumaizasa powder. The main component of cellulose is known as alpha-cellulose. Aoyama et al. reported that xylan was solubilized from kumaizasa by steaming treatment [14]. Xylan is supposedly the main component of hemicellulose in kumaizasa powder. From the results of this study, it is clear that alpha-cellulose increases the moisture content of feces due to water retention and/or swelling action. The transient watery stool observed in one rat in the cellulose group may be due to the excessive swelling action of cellulose. Xylan in holocellulose is known as a novel prebiotic derived from 5 carbon sugar xylose and is connected via beta bonds [15]. Its mechanism of action on the bowel movement is now elucidated as follows: xylan is degraded by enzymes in Bacteroides and Prevotella in the gut microbiota to yield a soluble xylooligosaccharide that Bifidobacteria can feed [16]. It is confirmed by an *in vitro* study using human feces that xylan and xylooligo-saccharides grew Bifidobacteria to produce short-chain fatty acids, such as acetic acid, that improve the bowel movement [17-18]. It is also confirmed in human trials that the consumption of xylooligosaccharides improved the bowel movement by increasing Bifidobacteria and stool frequency [19]. Moreover, Clostridium subcluster XIVa identified by terminal restriction fragment length poly-morphism (T-RELP) of 16S rRNA increased in pig flora that was fed with the kumaizasa powder [20]. Clostridium sub-cluster XIVa is known as a butyrate-producer and its reduced number is related to the decline in intestinal tract function commonly seen in elderly individuals [21-22].

Based on the present and other studies, it is indicated that the effect of kumaizasa powder on bowel movement is derived from a function of the cellulose, as well as a function of the hemicellulose whose main component is supposedly xylan, a novel prebiotic. The holocellulose meets the condition as part of the active ingredients of the kumaizasa powder.

The holocellulose derived from kumaizasa powder (SanSTAGE®) is a functional substance of Functional Food with Claims in Japan. It is a safe natural health food material that can improve bowel movement with favorable gut microbiota. Further studies are warranted to elucidate its detailed mechanism of action.

CONCLUSION

This study confirmed that kumaizasa powder had the effect of increasing fecal amount in rats. It is suggested that the cellulose increases the moisture content in the feces by swelling, while hemicellulose in the holocellulose acts as a prebiotic to regulate the moisture content in feces.

Competing interests: H.H. and T.H. are employers, and R.M. and Y.I. are employees of the UNIAL Co., Ltd., which provided financial support for this study but did not contribute in any other way to the conduct of the study.

Authors' contributions: Conceptualization, H.H., T.H. and S.O.; Methodology, H.H., R.M., Y.I. and R.Y.; Software, R.Y.; Validation, H.H., R.M. and Y.I.; Formal Analysis, R.Y.; Investigation, H.H., R.M., Y.I. and R.Y.; Resources, H.H. and T.H.; Data Curation, H.H., R.M., Y.I., R.Y. and N.I.; Writing-Original Draft Preparation, H.H., R.M., Y.I., R.Y. and N.I.; Writing-Review & Editing, H.H., R.M., Y.I., N.I. and S.O.; Visualization, H.H., R.M., Y.I. and N.I.; Supervision, H.H. and T.H.; Project Administration, H.H., R.M., and Y.I.; Funding Acquisition, H.H. and T.H.

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