Research Article Open Access

Effects of 4-week continuous ingestion of champignon extract on bowel movements and intestinal putrefaction products: A randomized, placebo-controlled, double-blinded, parallel-group comparative trial

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Submission Date: April 6th, 2018, **Acceptance Date:** May 27th, 2018, **Publication Date:** May 30th, 2018

Citation: Nishihira J., Nishimura M., Tanaka A., Kagami-Katsuyama H., Yamaguchi A., Taira T., Effects of 4-week continuous ingestion of champignon extract on bowel movements and intestinal putrefaction products: a randomized, placebo-controlled, double-blinded, parallel-group comparative trial. *Functional Foods in Health and Disease* 2018; 8(5): 280-291. DOI: https://doi.org/10.31989/ffhd.v8i5.517

ABSTRACT

Background: The aim of this study was to analyze the putrefaction products in the feces of subjects from a previous study (age range 50–79 years) which assessed the improvement of breath, body, and fecal odor after ingesting champignon extract.

Methods: The study was designed as a randomized, placebo-controlled, double-blinded, and parallel-group comparative trial. Subjects were divided into four groups, including the placebo (n=20), champignon extract at 50 mg/day (n=20), champignon extract at 500 mg/day (n=20), and champignon extract at 1000 mg/day (n=20) for 4 weeks.

Results: The results revealed significant reduction in ammonia and p-cresol levels (both of which are intestinal putrefaction products) among subjects who ingested 50, 500, and 1000 mg of champignon extract per day compared with subjects in the placebo group. Additionally, a significant difference was observed in indole levels in the group that consumed 500 mg/day of the extract compared to the placebo group.

Conclusions: The re-analysis of bowel movement in each test group revealed that the extract improved the number of days with bowel movement, number of bowel movements, and stool volume, which suggests the intestinal environment was improved.

Clinical trial registration: UMIN000014256

Keywords: ammonia, champignon, fecal odor, p-cresol, putrefaction

BACKGROUND

Intestinal bacteria produce various intestinal putrefaction products using nutrients that we ingest daily through our food [1]. Ammonia, indole, skatole, and p-cresol are some of the components produced by intestinal bacteria [2, 3]. Additionally, intestinal putrefaction products account for odor specific to the stool and are toxic substances that damage the intestinal tract and worsen the intestinal environment [4], which has to the assumption that decreasing intestinal putrefaction products to sustain a good intestinal environment is effective for maintaining intestinal health.

Previously, a study investigating the decrease in intestinal putrefaction products [5] illustrated a method of attempting to reduce the ammonia levels by ingesting *Bifidobacteria* and dietary fibers. *Bifidobacteria* are known to reduce the ammonia levels. A study on dietary fiber suggested that consuming a large quantity of dietary fiber facilitates partial absorption of intestinal putrefaction products.

While it has been demonstrated that intestinal putrefaction could be reduced by the consumption of champignon extract (Ricom Co., Ltd) the result was only obtained by an open-labelled study [6]. Consequently, we needed to confirm these results using more reliable methods. The purpose of this study was to elucidate the effects of ingesting the champignon extract to decrease intestinal putrefaction products and improve bowel movement through a randomized, placebo-controlled, double-blinded, and parallel-group comparative trial.

Champignon extract contains polyphenols, amino acids, polysaccharides, flavonoids, vitamins, and minerals. Champignon is produced by mixing the extract obtained from indoor-

grown mushrooms (*Agaricus bisporus*; Japanese name, *tsukuritake*) with hot water, dextrin, and spray which dries the mixture into a powder [7]. Currently, the product has a patent and is commercially available in Japan, Korea, the United States, Canada, the United Kingdom, France, Germany, Switzerland, Spain, and Sweden.

Many trials have been conducted to investigate the putrefaction product-decreasing functions of champignon extracts. A study including 14 hospitalized elderly patients who ingested the extract for 4 weeks found that the subjects had lower levels of intestinal putrefaction products, such as ammonia, methyl mercaptan, amines, and hydrogen sulfide compared to those at the baseline [6].

Furthermore, 2-week ingestion of champignon extract in 9 residents and staff of an intensive-care old-age home significantly lowered their levels of ammonia, phenol, cresol, indole, and other intestinal putrefaction products compared with the baseline. Changes in intestinal flora, such as a significant increase of *Bifidobaceria* and decrease of lecithinase-positive *Clostridium*, *Escherichia coli*, and *Staphylococcus* were also observed, suggesting that consumption of champignon extract improved the intestinal environment [8].

Administering champignon extract to dairy calves was reported to decrease diarrhea compared with the control group which suggests it was helpful for intestinal health [9]. Administering champignon extract to domestic rabbits was reported to significantly decrease the blood concentrations of indole and tryptamine, which are generated in the intestines and transferred to the blood stream, compared with the control group [10].

The most recent study consisted of a placebo-controlled, double-blinded, and parallel-group comparative trial on males and females aged 50–79 years with halitosis and body and fecal odor. Ingesting champignon extract for 4 consecutive weeks at 50, 500, and 1000 mg/day significantly decreased halitosis and body and fecal odor compared with the placebo group. The evaluation was conducted using the visual analog scale, with significant differences among the test groups [11].

This study analyzed the feces collected from subjects who participated in the previous study [11] which examined the effects of champignon extract on the levels of fecal putrefaction products, such as ammonia, p-cresol, and indole between the test and placebo groups.

METHODS

Study design

The study was designed as a randomized, placebo-controlled, double-blinded, and parallel-group comparative trial. Subjects were divided into four groups: those who consumed (1) the placebo (placebo group, 20 subjects); (2) champignon extract at 50 mg/day (50 mg/day group, 20 subjects); (3) champignon extract at 500 mg/day (500 mg/day group, 20 subjects); and (4) champignon

extract at 1000 mg/day (1000 mg/day group, 20 subjects) for 4 weeks. The test food comprised of one package (2.0 g/day) but no restrictions were placed on when or how to ingest the test food. From the three varieties (BX50FPD, BX100FPD, and BX150FPD) of champignon extract products based on the concentration, we used BX100FPD in this study. Furthermore, the test schedule comprised of medical interviews and blood tests of all subjects at the baseline and after 2 and 4 weeks of ingesting the test food. A washout period was implemented for 1 week before starting the ingestion of the test food.

Study procedures

This study was approved by the Hokkaido Information University's Committee of Bioethics. Written informed consent was obtained from all participants as per the Declaration of Helsinki after comprehensively explaining (both in writing and orally) the free nature of participation in this study. For screening, individuals who provided their consent to participate in this study were evaluated to assess whether they fulfilled the inclusion and exclusion criteria. The principal investigator considered 80 participants eligible for participation in this study. The staff of a third-party data center (Media Educational Center, Hokkaido Institute of Information Technology, Ebetsu city, Hokkaido) was responsible for assigning the subjects by stratified randomization to groups with the consideration for age, sex ratio, and points on the survey form by referring to the subject list. Furthermore, the staff stored documents associated with the group assignment, including documents comprising subjects' personal information, in a secure place. Next the date, time, and place of the clinical trial were communicated to the subjects. Three subjects withdrew before initiation of the trial due to personal reasons, so the final sample size of our study cohort was 77.

All subjects were asked to continuously ingest one package of the test food comprising either 2.0 g of the champignon extract or placebo food every day for 4 weeks from the day of the study initiation. All subjects were instructed to come to the laboratory on the first day and after 2 and 4 weeks of ingesting the test food for examination of the specified items. In this study, all the tests were conducted at the Health Center, Hokkaido Information University, Ebetsu. Additionally, all subjects were asked to maintain a diary to record their daily parameters. For example, physical status, whether they consumed the test food, and bowel movement for approximately 5 weeks starting 1 week before initiating the test food ingestion until after the completion of feces collection. Subjects were also asked to submit these records on each test day and on the day of feces submission.

The assignment list was disclosed by the staff in charge of the group assignment after all the test results and analyzed data pertaining to this study were collected. The ingestion rate was calculated using the following equation:

Ingestion rate (%) = (Actual number of test foods consumed) / (Scheduled number of test foods to be consumed) × 100

Test items

Bowel movement and daily life diaries

In this study, all subjects maintained a daily life diary throughout the study period. The bowel movement diary comprised of the following items: (a) number of bowel movements; (b) stool quantity (measured by visual inspection, with reference to the size of the stool collection container); (c) stool form; (d) stool color; (e) odor; and (f) sense of complete evacuation of stool. Subjects were asked to keep records of these items every day. The evaluation period was the week during the pre-observation period (non-ingestion period) and 4 weeks of the test food ingestion period. With days where subjects experienced multiple bowel movements, subjects were only asked to record items (b)–(f) on the first bowel movement. In the daily life diary, subjects were instructed to record what they ate, physical status, and whether they consumed the test food every day.

Measurements and blood tests

A physician conducted a medical interview and checked the following parameters at the baseline (the first day of ingestion) and 4 weeks after ingestion: physical measurements (height, weight, body mass index, and body fat percentage), vital signs (blood pressure, heart rate, and body temperature on arrival at the laboratory), general blood tests (white blood cell counts, red blood cell counts, hemoglobin level, hematocrit level, and platelet count), liver function (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, and lactate dehydrogenase levels), renal function (blood urea niterogen, carbapenem-resistant enterobacteriaceae, and urea levels), blood lipids (total cholesterol, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol levels), and blood sugar (blood sugar and hemoglobin A1c levels).

Analysis: Water and putrefaction products in stool

Water content

The mass lost through drying for 30 h at 80°C was considered the water content (percentage).

Measurement of ammonia levels

We suspended 0.2 g of stool in sterile water, sterilized for 15 min at 85°C, and filtered the solution. The remaining liquid was used as the test liquid. All measurements were obtained using ion chromatography system (ICS-1000; DIONEX, California, US) with IonPac CS12A columns (4 mm × 250 mm) using 10.7 mmo1/L H_2SO_4 as eluent at a temperature of 35°C and injection volume of 25 μL using an electric conductivity detector (with suppressor).

Measurement of p-cresol and indole levels

After suspending 0.2 g of stool in phosphate buffer solution, it was sterilized for 15 min at 85°C, cooled, and extracted with acetonitrile. The test liquid comprised of the extract processed in a solid-phase cartridge. All measurements were obtained using a standard gas chromatograph-mass spectrometer (GC/MS-QP-2010, Shimadzu Corporation, Kyoto, Japan). Furthermore, all measurements were obtained under the following conditions: column, inert cap WAX (30 m × 0.25 mm × 0.25 μm); column temperature, 70°C (maintained for 2 min), 200°C (20°C/min, maintained for 3 min), 240°C (10°C/min, maintained for 15.5 min); and carrier gas, helium.

Selection: Subjects for analysis

This study assessed fecal samples obtained from subjects who participated in a previous study [11]. For the subjects who fulfilled the above criteria with the amount of ammonia, the numbers of subjects were 9, 8, 9, and 11 for the groups of the placebo, which were 50 mg champignon, 500 mg champignon, and 1000 mg champignon respectively. As for p-cresol, the respective numbers of subjects were 6, 6, 8, and 11 for the placebo, 50 mg champignon, 500 mg champignon, and 1000 mg champignon. Similarly, for indole the numbers of subjects were 10, 8, 11, and 13 subjects for the placebo, 50 mg champignon, 500 mg champignon and 1000 mg champignon respectively. From these results, only subjects who fulfilled the following criteria were included in the analysis: (1) no major change (≥10%) in water content of the feces; (2) concentrations of ammonia ≥0.5 mg/g, that of p-cresol ≥10 μg/g, and that of indole ≥10 μg/g before collection; (3) being within standard values during the study period for the Smirnov–Grubbs test and Thompson rejection test results. In particular, subjects who exhibited ammonia levels ≥1.7 mg/g, p-cresol levels ≥100 μg/g, or indole levels ≥70 μg/g were excluded.

Statistical analysis

Data are presented as the mean ± the standard deviation. The amount of changes from before ingestion to after ingestion were classified as increasing or decreasing. Then the frequency distribution tables were obtained. We did not use internal standards for quantification. Appropriate subjects were selected instead based on the numerical value of fecal metabolites without correction. In statistical analysis, we did not examine whether normal distribution or not and thereby performed Wilcoxon rank-sum test as discrete data. In order to explore the distribution of the effect we used a chi-square test.

RESULTS

Analyses of intestinal putrefaction products and bowel movement

Table 1 summarizes the analysis results (mean, standard deviation) of stool putrefaction products, including ammonia, p-cresol, and indole.

Ammonia levels declined after 2 weeks in the 50 and 1000 mg/day groups with a significant difference compared with the placebo group. Additionally, ammonia levels significantly decreased after 4 weeks in the 50 and 1000 mg/day groups compared with the placebo group.

Furthermore, p-cresol levels significantly decreased after 2 weeks in the 50 and 1000 mg/day groups compared with the placebo group. Moreover, the p-cresol levels significantly decreased after 4 weeks in the 500 mg/day group compared with the placebo group. Additionally, the levels significantly decreased after 4 weeks in the 1000 mg/day group compared with the placebo group Indole levels significantly changed after 4 weeks in the 500 mg/day group compared with the placebo group.

Explicitly, the number of days with bowel movements (<7 per week) increased significantly in the 50 mg/day group after 2 and 4 weeks ($p<0.05$) and in the 1000 mg/day group after 2 weeks ($p<0.05$). Additionally, there was increase in all the test groups (50, 500, and 1000 mg/day) after 2 and 4 weeks ($p<0.05$). The number of bowel movements (<7 per week) increased significantly after 4 weeks in the 500 and 1000 mg/day group ($p<0.05$). Furthermore, there was significant increase after 2 weeks in all the test groups (50, 500, and 1000 mg/day), with a significant difference compared with the placebo group ($p<0.05$). Furthermore, the stool volume (<2 units/day) significantly increased after 4 weeks in the 50, 500, and 1000 mg/day groups; there was significant increase compared with the placebo group in the 50 and 500 mg/day groups ($p<0.05$). Moreover, we observed a significant increase after 4 weeks in all the test groups (50, 500, and 1000 mg/day) ($p<0.05$).

Table 1. Changes in intestinal putrefaction products

	Ammonia(mg/g)				*p*-cresol				indole			
	n	before ingestion	after 2 weeks of ingestion	after 4 weeks of ingestion	n	before ingestion	after 2 weeks of ingestion	after 4 weeks of ingestion	n	before ingestion	after 2 weeks of ingestion	after 4 weeks of ingestion
placebo group	9	0.79±0.19	1.00±0.29	0.78±0.23	6	39.88±15.14	44.76±32.11	49.31±32.03	10	22.19±14.59	22.13±14.05	24.02±13.92
amount of change			0.21±0.24	-0.01±0.23			4.88±25.68	9.43±25.70			-0.06±10.84	1.83±9.04
50 mg/day champignon ingestion group	8	0.87±0.40	0.74±0.22	0.83±0.35	6	47.08±35.82	37.95±26.39	48.49±34.38	8	28.91±17.68	27.40±11.40	35.96±17.64
amount of change			-0.13±0.24†	-0.04±0.38			-9.13±29.08†	1.41±43.22			-1.51±10.94	7.05±17.99
500 mg/day champignon ingestion group	9	0.85±0.19	0.87±0.30	0.75±0.28	8	46.05±27.05	48.23±30.59	49.89±32.09	11	25.82±12.31	26.91±15.36	26.05±11.23
amount of change			0.02±0.26	-0.10±0.17†			2.18±26.76	3.84±25.57†			1.09±7.96	0.23±9.44†
1000 mg/day champignon ingestion group	11	0.85±0.23	0.62±0.24*	0.81±0.30	11	46.63±24.27	32.14±25.50	26.27±28.16	13	25.89±11.19	24.54±10.49	24.47±12.51
amount of change			-0.23±0.27†	-0.04±0.26†			-14.49±39.95†	-20.36±35.58†			-1.35±13.84	-1.42±6.87

∗ statistically significant vs before taking test foods or placebo (p<0.05,Wilcoxon)

† statistically significant compared with the placebo (p<0.05,Chi-square)

DISCUSSION

Previously, some studies reported a correlation between the decrease in stool putrefaction products and improvement in bowel movement (stool volume, number of bowel movements) for a variety of foods [12, 13]. Based on these findings, a placebo-controlled, double-blinded, and parallel group comparative trial was conducted on 80 males and females aged 50–79 years to assess the effects of consuming champignon extract (50, 500, and 1000 mg/day) for 4 weeks on the improvement of intestinal environment with the following markers: levels of putrefaction products (ammonia, indole, and p-cresol), number of days with bowel movement, number of bowel movements, and stool volume.

The results demonstrated significant differences between the 50 mg/day and placebo groups after 2 weeks and between the 500 mg/day and placebo groups after 4 weeks in terms of levels of ammonia and p-cresol. Furthermore, compared with the placebo group, there was a significant difference in terms of both ammonia and p-cresol levels after consuming 1000 mg/day for 2 and 4 weeks. Additionally, indole levels also changed, with a significant difference between the placebo and 500 mg/day groups after 4 weeks. Polyphenol combines with a phenolic hydroxyl group of polyphenol to form a complex compound which also shows inclusion effect toward pores inside the three-dimensional structure formed by bonding polyphenols [14].

The decreases in these intestinal putrefaction products could be attributed to the formation of complexes due to polyphenols in the champignon extract or due to the inclusion effect. The colon environment also improved, resulting in the increase of the number of days with bowel movement, number of bowel movements, and stool volumes. As the previous study [11] was a trial on healthy subjects, no subject had outlier values for any of the data associated with bowel movement (number of days with bowel movement, number of bowel movements, and stool volumes). As a result, the improvement in parameters indicating bowel movement in the test group was not conclusive, as the subjects who ingested the champignon extract already had regular, healthy bowel movement. However, in the previous study a significant increase was still observed compared with the placebo in the number of bowel movements and stool volume after 2 and 4 weeks of ingesting the test food at 1000 mg/day.

In this study, we selected subjects with relatively slow bowel movements (e.g., those with <7 bowel movements per week, ≤7 bowel movements per week, <2 units of stool volume/day at the baseline) and who demonstrated improved bowel movement in the test group for our analysis. No changes were observed in the placebo group. Significantly, improvement in defecation was only

observed in subjects with poor bowel movement. In contrast, there was no change in subjects with fair condition of defecation during the course of the study.

In a previous study, we demonstrated improvements in stool color, sense of complete evacuation of stool, and stool odor [11], in which stool odor significantly decreased after 4 weeks in all the test groups (50, 500, and 1000 mg/day) compared with the placebo group. This report is consistent with the current results shown in this study.

This study suggested that ingesting champignon extract exerted a positive effect on the intestinal environment, as demonstrated by markers of the number of days with bowel movement, number of bowel movements, stool volume, stool color, and stool odor due to decreased levels of intestinal putrefaction products. Additionally, we elucidated the safety of champignon extract through acute and subacute oral toxicity testing and mutagenicity testing, which exhibited no abnormal results. In this study, no major differences existed between the 50, 500, and 1000 mg/day and placebo groups in terms of parameters of vital signs, blood components, liver and renal function, lipid metabolism, blood sugar, and body composition. As most factors remained within normal values, no findings indicated problems with the safety of champignon extract.

CONCLUSION

This trial indicated that consuming champignon extract (BX100FPD) at 50–1000 mg/day is effective for decreasing intestinal putrefaction products and improving the intestinal environment. The generation of intestinal putrefaction products is associated with the occurrence of diarrhea or constipation [15]. Consequently, this study demonstrated how champignon extract improved the intestinal environment and its effects tended to intensify in a dose-dependent manner.

Competing interests:-There are no conflicts of interest to declare.

Authors' contributions: JN, MN, AY, and TT designed and conducted the research. HK-K. performed statistical analyses. MN, AT, and JN wrote the manuscript. JN had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

Acknowledgments and Funding: We are grateful to the members of Hokkaido Information University, Center of Health Information Science (Y. Fukuda, T. Saito, Y. Anzai, N. Shima, N. Ito)

for their technical assistance during the course of the clinical trial. This study was conducted with research funds under contract with Ricom Co., Ltd (Tokyo), and supported in part by Grants-in-Aid for Regional R&D Proposal-Based Program from Northern Advancement Center for Science & Technology of Hokkaido Japan.

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