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# The Effect of Fermented Food Containing Aspergillus Oryzae (Amazake or Raw-SHIOKOJI) Consumption on Abdominal Symptoms Associated with Premenstrual Syndrome in Japanese Women

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***ABSTRACT***

**Background:** Psychological and physical symptoms such as irritability, headache, abdominal pain, difficulty with bowel movements, and drowsiness that occur because of hormonal fluctuations during the menstrual cycle refer to premenstrual syndrome (PMS). The study examined whether the intake of fermented food containing Aspergillus oryza (Amazake and Raw-SHIOKOJI), a traditional Japanese food, improved the abdominal symptoms associated with PMS. **Method:** Eleven healthy Japanese collegiate women in their 20s participated in this interventional study. The study period was set to 28 days per phase, and the participants were examined over three separate phases: before, during, and after the intake of Amazake or Raw-SHIOKOJI. The participants were instructed to maintain a daily record of their food intake, bowel movements and menstruation throughout the study period. **Results:** The intake of fiber, fat, protein, and carbohydrate among 11 participants were similar, with no significant difference between the two groups. Menstrual abdominal discomfort in women in their 20s was noted during the luteal and menstrual phases. Eight participants (72.7%) experienced abdominal discomfort during the luteal phase and all participants experienced abdominal discomfort during the menstrual phase. Consuming fermented food containing Aspergillus oryzae may reduce PMS abdominal symptoms by 72.7%. **Conclusion:** This study showed that consumption of food containing Aspergillus oryzae reduced abdominal discomfort associated with the luteal phase and may improve menstruating women's health and quality of life.

## Introduction

Women have a unique biological rhythm called the menstrual cycle, which repeats every month throughout a woman's life from puberty to menopause. Previous studies have reported that the menstrual cycle has long been prevalent among women of reproductive age and

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up to 20% of women need treatment (Freeman and Sondheimer, 2003, Halbreich, 2004). The American College of Obstetricians and Gynecologists have established the diagnostic criteria for premenstrual syndrome as a symptom associated with menstruation, as follows: “Having one or more emotional symptoms and physical symptoms in the 5 days before menstruation for at least three menstrual cycles in a row. The symptoms ease within 4 days after the start of menstruation and do not recur until at least day 13 of the menstrual cycle” (American College of Obstetricians and Gynecologists, 2016). These symptoms are caused by changes in the levels of sex hormones during the menstrual cycle, and thus appear more strongly in younger women, being strongly manifested in their twenties (Mihm *et al.*, 2011, Yonkers *et al.*, 2008). Researchers have reported that gastrointestinal complaints (e.g., diarrhea, constipation) are common among women premenstrual and during pregnancy (Bernstein *et al.*, 2012, Simmons *et al.*, 1988). These symptoms often improve spontaneously at the end of menstruation and women presenting with these symptoms do not seek intervention (Tanaka *et al.*, 2013). Women tend to simply deal with the symptoms associated with menstruation, since these symptoms tend to recur and resolve every month; almost no women seek treatment for the same (Tanaka *et al.*, 2013). However, these symptoms are also present in many women. It is important to resolve these symptoms. The usefulness of progesterone as a treatment for PMS is being investigated (Dennerstein *et al.*, 1985). There is no report on the usefulness of fermented food in treating PMS. Therefore, we wondered if these symptoms could be improved on daily basis and with the help of food.

It has recently been reported that glucosylceramide contained in Amazake improves bowel movements in mice (Hamajima *et al.*, 2016), and fermented food containing *Aspergillus orizae* improves defecation (Wakana *et al.*, 2019). Consuming *Amazake* also improves constipation in humans (Mori, 2019). Thus, these findings reveal that the intake of food items containing *Aspergillus oryzae*, used in Japan since ancient times, can improve the intestinal environment. However, no studies have examined its effects on premenstrual syndrome. Therefore, this study examined whether the intake of *Amazake* and *Raw-SHIOKOJI* containing A. oryzae can improve the abdominal symptoms associated with premenstrual syndrome.

## Materials and Methods

### *Study design and sample collection*

The present study examined healthy Japanese women in their twenties. The sample size was 17 using a formula based on power and sample size calculation; hence, the target sample size was set 19. The inclusion criteria were age > 20, healthy women and no irregular menstrual cycle. The exclusion criteria were a history of laxative use for less than 1 year or taking medication. Estradiol and progesterone levels in the saliva were measured to assess the menstrual cycle and female hormone hypersecretion. The protocol is shown in **Figure 1**. One phase was 28 days and 3 phases (84 days) were performed. The first phase was performed before (baseline). In this study, the participants were examined over 3 separate phases: before, during, and after intake of *Amazake* or *Raw-SHIOKOJI*. The participants ingested *Amazake* (125 ml/day) or *Raw-SHIOKOJI* (7.5 g/day) in the second phase. Participants recorded defecation, meal, and menstrual records daily during the study by self-administration. The menstrual cycle was defined as the first day of the menstrual cycle and the end of the menstrual period. The period from the end of menstruation to the 14$^{th}$ day was the follicular phase, the 15$^{th}$ to 21$^{st}$ days were the early luteal phases, and the 22$^{nd}$ to 28$^{th}$ days were the late luteal phases. Saliva was collected from the beginning of the first phase of menstruation until the end of the second phase.

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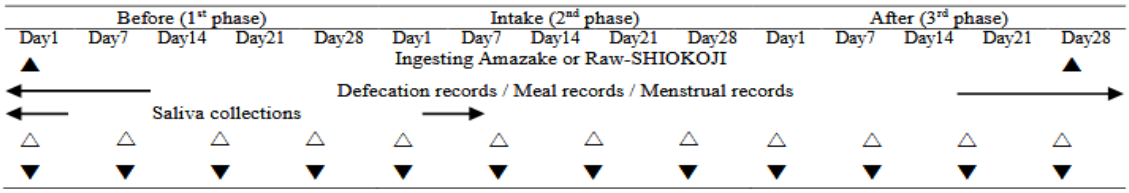


**Figure 1.** Protocol for investigating fermented food on abdominal symptoms associated with premenstrual syndrome

▲ conducted the first-day and end-of-date questionnaires.
Δ represents stool collection. One stool sample was collected during each menstrual cycle.
▼ represents body composition measurement. Body composition was measured weekly

The menstrual cycle repeats approximately every 28 days. Consuming food items containing *A. oryzae* affects premenstrual syndrome because of the constituents in the normal diet of the participants. Therefore, the study period was set to 28 days as one phase, and the participants were examined over three separate phases: before, during, and after consuming *Amazake* or *Raw-SHIKOJI* (84 days in total). The menstrual cycle in each phase was divided into four groups as follows: the period from the onset of menstruation (day 1) to the end of menstruation was defined as the menstrual period, the period from the end of menstruation to day 14 was defined as the follicular phase, days 15 to 21 were defined as the early luteal phase, and days 22 to 28 were defined as the second half of the luteal phase. The ingested food items were *Amazake* (125 ml per day) and *Raw-SHIOKOJI* (7.5 g per day); both are commercially available products from Company A, each of fermented food items are of the same amount , and the intake amount was set based on the amount recommended by the company. The participants selected either *Amazake* or *Raw-SHIKOJI*, whichever they preferred, and took the product daily throughout the second phase. During the study period, participants were prohibited from ingesting food items containing *A. oryzae*, apart from the constituents of their normal diet. On the first day of the study period, the participants completed a questionnaire on the menstrual cycle and bowel movements conducted on the first day of the study (day 1 questionnaire) and a questionnaire on the menstrual cycle and bowel movements after intake of *Amazake* or *Raw-SHIOKOJI* (post-completion questionnaire) conducted at the end of the study period. In addition, the participants' lifestyles (daily exercise habits, excessive heavy work, and sleeping hours) were asked during the questionnaire on the first day. In the day 1 questionnaire, the participants indicated whether they had daily bowel movements, the number of bowel movements per week, whether they used laxatives, and the characteristics of their bowel movements during the menstrual cycle (such as premenstrual bloating). In the post-completion questionnaire, the participants indicated whether any bowel movement discomfort associated with their menstrual cycle was alleviated after intake of *Amazake* or *Raw-SHIOKOJI* compared to the period before ingestion. The participants also indicated whether there were any changes in the characteristics of bowel movements associated with their menstrual cycle before, during, and after intake of *Amazake* or *Raw-SHIOKOJI*, as in day 1 questionnaire. In addition, the participants were instructed to maintain a daily record of all meals, including snacks. For bowel movement records, the Constipation Assessment Scale (CAS) was used to describe pain during bowel movement (McMillan and Williams, 1989), ease of bowel movement, and difficulty in bowel movement, indicating whether the participant had constipation. Stool properties were described using the Bristol stool scale (BSS) (O'Donnell *et al.*, 1990). The menstrual records showed the

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dates of menstruation in each phase (Example: ○/○ ○/○). Four stool samples were collected in each phase over the entire study period, the collected stools were immediately stored at -80 ℃, the fecal bacterial content was quantified (Bifidobacterium longum, Enterobacteriaceae and Clostridium perfringens), and pH was measured by LAQUAtwin (HORIBA, Kyoto, Japan). The moisture content in feces was measured using a Shimadzu moisture meter MOC63u after drying at 105°C for 3 h using the atmospheric drying method. Body composition was measured by Bioelectrical Impedance Analysis once a week (Yamato Seiki Co., Ltd. HA120024).

***DNA Extraction from Fecal Sample and Real-time PCR***

The number of bacteria in fecal samples was determined using QIAamp DNA Mini Kit (Qiagen Co Ltd., Tokyo, Japan). Real-time PCR amplification and detection were performed with TaKaRa TP760, and each reaction mixture was followed by the TB GreenTM Premix Ex TaqTM  (TAKARA BIO INC., Kusatsu, Japan). The following primers were used to enumerate the target bacteria in fecal samples: Forward (5´-TCG CGT CTG GTG TGA AAG-3´), Reverse (5´-CCC ACA TCC AGC ATC CA-3´) *B. longum* (Rinttila *et al.*, 2004), Forward (5´- CAT TGA CGT TAC CCG CAG AAG AAG C-3´), Reverse (5´- CTC TAC GAG ACT CAA GCT TGC-3´) *Enterobacteriaceae* (major species) (Bartosch *et al.*, 2004). Quick Primer *C. Perfringens* (TAKARA BIO INC., Kusatsu, Japan) was used for *C. perfringens*. Amplification program included an initial denaturation step at 94 ℃ for 5min, with final extension step at 95 ℃ for 15s, 60 ℃ for 30s, and 95 ℃ for 15s. *B. longum* was followed by 40 cycles of denaturation at 95℃ for 20s, primer annealing at 56 ℃ for 20s, and primer extension at 72 ℃ for 30s (Rinttila *et al.*, 2004). *Enterobacteriaceae* (major species) was followed by 40 cycles of denaturation at 95 ℃ for 15s, primer annealing at 58 ℃ for 15s, and primer extension at 72 ℃ for 30s (Bartosch *et al.*, 2004). *C. Perfringens* were followed by 40 cycles of denaturation at 95 ℃ for 15s, primer annealing at 55 ℃ for 15s, and primer extension at 72 ℃ for 20s (Bartosch *et al.*, 2004).

***Analysis of Amazake and Raw-SHIOKOJI***

Since the composition of *Amazake* and *Raw-SHIOKOJI* used in this study differed depending on the lot number, the number of oligosaccharides was analyzed by high performance liquid chromatography (Wakana *et al.*, 2019).

***Ethics approval and consent to participate***

This study was approved by the Human Research Ethics Committee at Tokyo University of Agriculture (No. 1814), and written informed consents were obtained from all participants.

***Data analysis***

In this study, participants' characteristics were presented as mean±SD, and Unpaired t-test was used for comparison between *Amazake*-intake and *Raw-SHIOKOJI*-intake groups. Fisher's exact test was used for comparison of other data between the two groups, Tukey-Kramer test was used for normal results, and Kruskal-Wallis test was used for non-normal results after an ANOVA for comparison between multiple groups. All significance levels below 5% were considered statistically significant, and the statistical analysis software SPSS 20.0 Statistics Base, Ver.20, Armonk, NY, IBM Corp.) was used for all analyses.

## Results

This study originally included 19 women in their twenties, while the analysis was conducted on 11 women with normal menstrual cycles who made no omissions in their responses to the questionnaires. They were divided into the *Amazake*-intake group (n=6) and the *Raw-SHIOKOJI*-intake group (n=5). The participants' characteristics are shown in **Table 1**.

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**Table 1.** Characteristics of participants who intake fermented food.

| Variable | Amazake (n=6) | Raw-SHIOKOJI (n=5) | P-value[b] |
|---|---|---|---|
| Height (cm) | 158.4±2.4[a] | 154.8±2.4 | 0.75 |
| Weight (kg) | 51.7±3.6 | 49.5±4.6 | 0.85 |
| Body mass index (kg/m$^2$) | 20.6±1.1 | 20.7±2.0 | 0.98 |
| Age (y) | 21.6±0.5 | 21.3±0.5 | 0.97 |
| Defecation is daily (Yes/No) | 2/4 | 3/2 | - |
| Defecation Frequency (times/week) | 7.0±2.3 | 5.6±1.7 | 0.08 |
| Laxative (Yes/No) | 0/6 | 0/5 | - |
| Discomfort symptoms of defecation with menstrual cycle (Yes/No) | 6/0 | 5/0 | - |
| Estradiol (pg/m) | 8.7±3.3 | 8.4±3.5 | 0.80 |
| Progesterone (pg/ml) | 482.6±78.7 | 517.1±94.4 | 0.40 |

*[a]: Mean±SD; [b]: Unpaired t-test.*

There were no items showing a significant difference between the two groups. The results of the diet analysis showed no significant difference between the two intake groups, and there was no significant difference in food intake during each menstrual cycle. In terms of the participants' lifestyle, there was no daily exercise habit or excessive heavy work, and sleep time was regulated. The study analyzed abdominal discomfort symptoms associated with the menstrual cycle before the intake of food items containing *A. oryzae* (**Table 2**)

The *Amazake* intake group had abdominal discomfort during the early luteal phase, latter half of the luteal phase, and during menstruation, while the *Raw-SHIOKOJI*-intake group had abdominal discomfort during the late luteal phase and menstruation. The number of women with abdominal discomfort decreased significantly from 11 to 3 (72.7%) after the intake of food items containing *A. oryzae* ($P$<0.01, before vs. after) (**Figure 2**).

Abdominal discomfort completely resolved in 4 out of 6 (66.7%) participants in Amazake-intake group and in 4 out of 5 (80.0%) participants in the *Raw-SHIOKOJI*-intake group, indicating significant reduction in the symptoms of bowel movement discomfort associated with menstrual cycle ($P$<0.01, before vs after). Subsequently, the study analyzed menstrual cycle phases in which the intake of food items containing *A. oryzae* was effective in resolving digestive symptoms (**Figure 3**). *Amazake* group showed no changes in bowel movement discomfort during the early luteal phase. Three out of five participants with abnormal bowel movements in the late luteal phase before the intervention tended to improve their symptoms after the intake of food items containing *A. oryzae*. Symptoms that improved included diarrhea (n=1) and constipation (n=1). There was a significant reduction in discomfort among the six (100.0%) participants with initial abnormal bowel movements during menstruation, with slight improvement (n=3) or moderate improvement (n=1) after the intake of food items containing A. oryzae ($P$<0.01, before vs. after). Symptoms that improved in the four participants were constipation (n=3) and diarrhea (n=1). All three women with abnormal bowel movements in the late luteal phase before intake in *Raw-SHIOKOJI*-intake group showed slight improvement after intake, and no participants experienced discomfort ($P$<0.01, before vs. after). Five participants had abnormal bowel movements during menstruation before intake, and one (20.0%) participant showed slight improvement, but the remaining four (80.0%) participants had no change in symptoms. The improvement in symptoms in one participant was a tendency for diarrhea. The investigation demonstrated that intake of food items containing *A. oryzae* improved bowel movement discomfort associated with menstrual cycle. The present study evaluated changes in difficulty of bowel movement (based on CAS) as a subjective

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indicator (subjective symptoms) for abdominal discomfort and the objective indicators of abdominal discomfort, namely stool characteristics, bowel movement frequency, fecal water content, and pH, changed with the intake of food items containing *A. oryzae* (**Table 2**). There were no significant differences in the total average CAS scores based on menstrual cycle phase or food items ingested in either *Amazake*-intake or *Raw-SHIOKOJI*-intake group. There were no significant differences in stool characteristics, bowel movement frequency, or fecal water content during menstrual cycle before, during, and after *A. oryzae* intake in either Amazake-intake group or *Raw-SHIOKOJI*-intake group. In early luteal phase in *Amazake*-intake group, fecal pH changed to alkaline after the intervention or became neutral ($P$<0.05, before vs. after), but there were no other significant differences. The *Raw-SHIOKOJI*-intake group showed no significant differences in fecal pH. The study compared each item between Amazake-intake group and Raw-SHIOKOJI-intake group. The late luteal on Bristol scale, Raw-SHIOKOJI-intake group showed significantly lower values ($P$<0.05, Amazake vs Raw-SHIOKOJI). Changes in the number of bacteria in feces following intake of food items containing *A. oryzae* are shown in **Table 3.**

There was no significant difference in the number of *B. longum* and *C. perfringens* during menstrual cycle before, during, and after the intervention in either *Amazake*-intake group or *Raw-SHIOKOJI*-intake group. In *Amazake* intake group, there was a significant reduction in *Enterobacteriaceae* during menstruation compared to the phase before intake ($P$<0.05, before vs. during). No significant difference was observed in *Enterobacteriaceae* counts in *Raw-SHIOKOJI*-intake group. In addition, detection rate of *C. perfringens* tended to decrease in both groups, while it was not significant. Since *Amazake* and *Raw-SHIOKOJI* used in this study contain oligosaccharides affecting bowel movement, the study investigated the quantity of oligosaccharides in the consumed products. It was found that *Amazake* contained 1.11g/125ml, and *Raw-SHIOKOJI* contained 0.11g/7.5g of isomalto-oligosaccharides.

## Discussion

In this study, abdominal discomfort associated with menstruation in women in their twenties was observed in luteal phase (n=8, 72.7%) and during menstruation (all participants). Both diarrhea and constipation were observed in 36.4% of participants in luteal phase, 63.6% had diarrhea tendency, and 36.4% had constipation tendency during menstruation. *Amazake* or *Raw-SHIOKOJI* intake improved discomfort in eight (72.7%) participants. Analysis by phases of menstrual cycle revealed a significant improvement in bowel movement discomfort during menstruation in *Amazake*-intake group and during late luteal phase in *Raw-SHIOKOJI*-intake group. There were no reports examining the efficacy of food for various abdominal symptoms of premenstrual syndrome. To the best of the authors' knowledge, this study is the first to investigate the usefulness of fermented food for various abdominal symptoms of premenstrual syndrome due to two possible reasons. The first is the presence of isomalto-oligosaccharides in *Amazake* and *Raw-SHIOKOJI*. Isomalto-oligosaccharides are designated as food for specified health uses in Japan. Ingesting 10 g of isomalto-oligosaccharide per day has the effect of "conditioning the abdomen". In this study, the number of isomalto-oligosaccharides was small compared to 10 g of food for specified health purposes. However, taking it daily for 28 days resulted in gradual improvement in the participants. No significant difference in detection of *C. perfringens*, but a decreasing trend was observed. The second was the consciousness of ingesting food items containing *A. oryzae*, which has an intestinal regulatory effect. Bristol scale in the late luteal showed significantly lower values in Raw-SHIOKOJI-intake group. The content of food ingested by the two groups was different. No significant difference was observed before and after each group ingested the fermented food. Further studies are required to clarify whether Amazake or Raw-SHIOKOJI is more effective.

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**Table 2.** Change in difficulty of bowel movement, stool shape, defecation frequency, moisture content, pH by intake of fermented food.

| Phase | Period | Difficulty of bowel movement | | P-value[a] | Bristol scale | | P-value | Defecation frequency (time/week) | | P-value | Moisture content (%) | | P-value | pH | | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | R | | A | R | | A | R | | A | R | | A | R | |
| Follicular | Before | 2.3±2.3[b] | 0.3±0.6 | 0.08 | 4.0±0.8 | 3.8±1.2 | 0.83 | 7.5±3.4 | 4.8±2.5 | 0.26 | 82.3±6.4 | 85.5±2.0 | 0.25 | 7.0±1.5 | 6.8±0.8 | 0.84 |
| | During | 2.2±2.4 | 0.5±0.9 | 0.23 | 4.2±0.8 | 3.7±1.0 | 0.21 | 7.3±1.8 | 4.4±1.5 | 0.05 | 79.2±5.3 | 80.7±11.4 | 0.82 | 7.3±0.6 | 6.9±0.8 | 0.47 |
| | After | 2.3±2.2 | 0.9±1.5 | 0.14 | 4.2±0.7 | 3.3±1.1 | 0.26 | 7.0±3.7 | 4.8±1.3 | 0.26 | 83.2±4.5 | 74.3±3.7 | 0.82 | 7.3±0.8 | 7.2±0.7 | 0.82 |
| Early Luteal | Before | 2.2±2.2 | 0.7±1.2 | 0.13 | 3.9±0.8 | 3.5±1.2 | 0.25 | 7.8±3.2 | 6.8±1.3 | 0.53 | 88.7±7.7 | 86.5±3.4 | 0.74 | 5.9±0.8[†] | 6.9±1.0 | 0.43 |
| | During | 2.0±1.6 | 0.7±1.3 | 0.25 | 3.9±1.0 | 3.5±1.0 | 0.15 | 7.3±3.4 | 5.0±2.2 | 0.25 | 82.3±6.7 | 83.0±3.4 | 0.44 | 6.9±0.7 | 7.1±0.3 | 0.91 |
| | After | 2.8±2.6 | 1.6±1.9 | 0.56 | 4.0±0.9 | 2.9±1.3 | 0.13 | 6.0±2.9 | 4.4±1.2 | 0.31 | 81.3±4.6 | 78.2±4.7 | 0.13 | 7.4±1.0[†] | 7.2±1.3 | 0.82 |
| Late Luteal | Before | 3.3±2.9 | 1.2±1.0 | 0.11 | 4.3±1.1 | 3.3±1.6 | 0.17 | 7.0±2.4 | 6.0±2.1 | 0.28 | 89.6±7.6 | 79.3±4.1 | 0.37 | 7.0±0.9 | 7.1±0.6 | 0.06 |
| | During | 3.8±2.6 | 1.5±1.8 | 0.18 | 4.4±1.4 | 3.1±1.3 | 0.04 | 7.5±2.2 | 5.4±2.1 | 0.18 | 84.6±7.6 | 72.9±10.2 | 0.64 | 6.5±0.8 | 7.8±0.5 | 0.23 |
| | After | 2.3±2.1 | 1.4±1.8 | 0.23 | 3.9±0.9 | 2.9±1.3 | 0.40 | 7.0±3.1 | 5.4±1.2 | 0.33 | 79.1±5.5 | 69.2±2.5 | 0.87 | 7.4±0.7 | 7.3±0.5 | 0.96 |
| Menstrual | Before | 2.6±1.8 | 1.7±1.8 | 0.26 | 3.8±1.1 | 2.8±1.5 | 0.15 | 5.8±2.0 | 5.4±3.4 | 0.83 | 84.4±3.9 | 78.3±5.7 | 0.46 | 6.7±1.0 | 6.9±0.7 | 0.06 |
| | During | 1.8±1.7 | 1.9±2.2 | 0.57 | 4.2±1.1 | 3.1±1.6 | 0.21 | 5.8±2.3 | 5.4±2.3 | 0.79 | 80.0±7.9 | 70.6±2.5 | 0.78 | 7.2±0.6 | 7.0±0.7 | 0.71 |
| | After | 2.8±2.6 | 1.4±1.5 | 0.20 | 4.0±4.0 | 3.1±1.6 | 0.27 | 5.0±2.2 | 4.4±2.9 | 0.74 | 80.7±6.1 | 74.2±7.1 | 0.31 | 7.5±0.9 | 7.2±0.8 | 0.49 |

*[a]: Unpaired t-test, Amazake vs Raw-SHIOKOJI; [b]: Mean±SD; **A**: Amazake intake group (n=6); **R**: Raw-SHIOKOJI intake group (n=5).*

**Table 3.** Fluctuation of microflora in feces by intake of fermented food

| Phase | Period | Bifidobacterium Longum subs. Longum | | P-value[a] | Enterobacteriaceae (major species) | | P-value | Clostridium perfringens | | P-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | R | | A | R | | A | R | |
| Follicular | Before | 9.7±0.7[b] | 10.3±0.3 | 0.09 | 8.6±1.0 | 8.9±1.1 | 0.15 | 8.5±0.9 | 8.8±0.9 | 0.13 |
| | During | 9.8±0.5 | 9.9±0.6 | 0.69 | 7.7±1.4 | 7.4±1.0 | 0.21 | 7.8±0.4 | 8.8±0.4 | 0.40 |
| | After | 9.8±0.6 | 10.1±0.5 | 1.00 | 8.1±1.0 | 8.8±1.6 | 0.76 | 8.6±0.9 | 8.3±1.0 | 0.14 |
| Early luteal | Before | 9.7±0.3 | 10.4±0.4 | 0.28 | 8.7±1.0 | 8.9±1.5 | 0.07 | 8.2±0.4 | 8.5±1.0 | 0.18 |
| | During | 9.7±0.5 | 10.1±0.5 | 0.99 | 7.7±1.3 | 7.9±1.3 | 0.35 | 8.5±0.8 | 8.4±0.6 | 0.24 |
| | After | 10.0±0.3 | 10.3±0.3 | 0.29 | 7.9±1.5 | 8.3±1.4 | 0.92 | 8.4±0.7 | 8.2±1.1 | 0.07 |
| Late Luteal | Before | 9.9±0.7 | 10.7±0.4 | 0.98 | 8.3±1.3 | 9.6±1.3 | 0.05 | 8.5±0.7 | 8.6±1.0 | 0.06 |
| | During | 9.1±0.7 | 10.1±0.7 | 0.62 | 7.8±1.8 | 9.0±1.6 | 0.79 | 8.5±0.3 | 8.2±0.8 | 0.42 |
| | After | 10.0±0.6 | 10.2±0.3 | 0.71 | 8.3±0.7 | 8.3±1.7 | 0.81 | 8.2±0.8 | 8.7±0.6 | 0.09 |
| Menstrual | Before | 9.4±0.7 | 10.3±0.5 | 0.11 | 8.9±0.6[†] | 8.8±1.2 | 0.07 | 8.6±0.8 | 9.1±0.9 | 0.84 |
| | During | 9.4±0.5 | 10.1±0.5 | 0.84 | 7.3±0.9[†] | 7.8±1.9 | 0.90 | 8.1±0.5 | 8.5±1.1 | 0.05 |
| | After | 10.2±0.3 | 10.2±0.5 | 0.09 | 8.1±0.9 | 8.7±0.9 | 0.43 | 8.2±0.8 | 8.1±0.7 | 0.06 |

*[a]: Unpaired t-test, Amazake vs Raw-SHIOKOJI; [b]: Mean±SD; **A**: Amazake intake group (n=6); **R**: Raw-SHIOKOJI intake group (n=5; Each bacteria unit: Log/feces g).*

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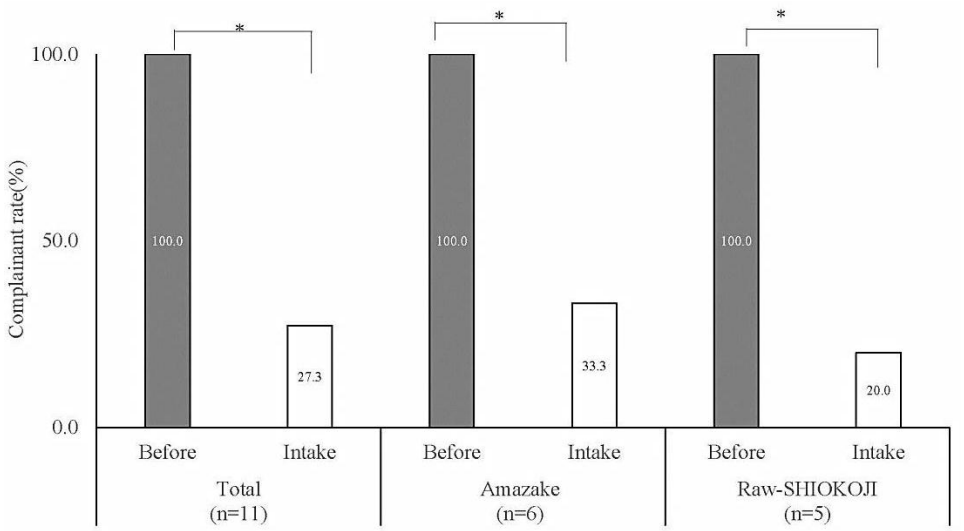


**Figure 2.** Change of abdominal discomfort of defecation by intake of fermented food.

The vertical axis represents compliance rate. The horizontal axis shows the left side before ingestion. Next, we combined *Amazake* and *Raw-SHIOKOJI* intake groups. This is the result of the *Amazake*-intake group only. The far right is the result of only the *Raw-SHIOKOJI*-intake group. Before ingestion, all participants experienced abdominal discomfort associated with their menstrual cycle. The ingestion of *Amazake* or *Raw-SHIOKOJI* reduced the complaint rate by 72.7%. *Amazake* or *Raw-SHIOKJI* alone significantly reduced the complication rate. There was no difference in the effects of the two samples.

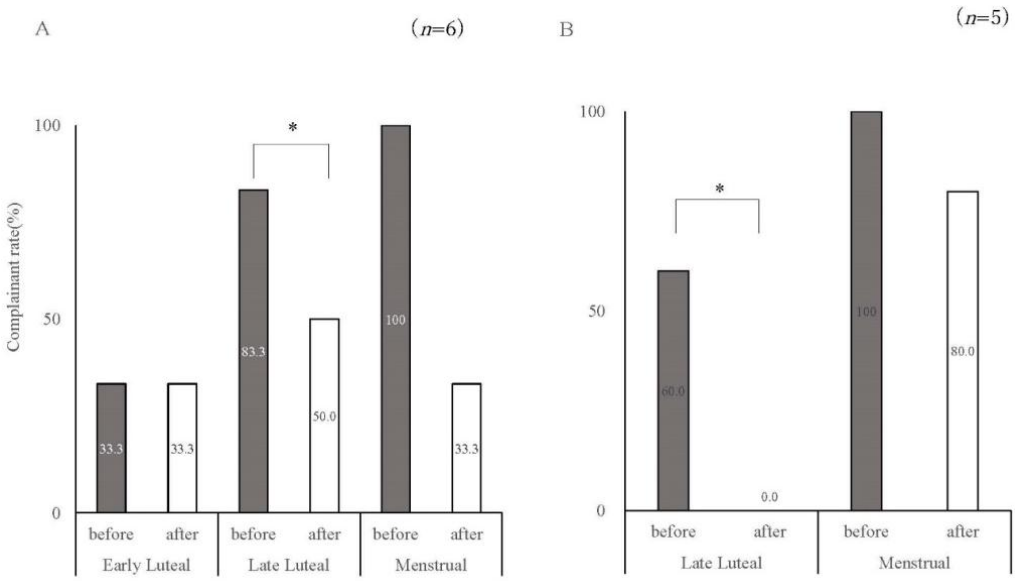


**Figure 3.** Changes in defecation discomfort associated with menstrual cycle by intake of fermented food.

Graph A show the results for Amazake-intake group (n=6), and graph B shows the results for *Raw-SHIOKOJI*-intake group (n=5). The vertical axis represents the complainant rate. The horizontal axis shows menstrual cycle.
The white column shows the incidence of abdominal discomfort before ingestion and the gray column shows the incidence of abdominal discomfort after ingestion.In Graph A, the complainant rate decreased significantly during the menstrual period. In Graph B, the complainant rate decreased significantly in the late luteal phase.
*p<0.01: Fisher's exact test, before vs. after

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Abdominal discomfort associated with menstrual cycle is thought to be related to sex hormone levels. Estrogen and progesterone, which are sex hormones related to menstrual cycle, peak in the twenties and then start to decline from the late thirties to fourth decades of life (MacGregor, 2018). Although sex hormones contribute to menstrual syndrome, a tendency to feel excessive coldness (Ko *et al.*, 2019), subjective stress, and a tendency of women to lose weight also contribute to the increase (Yamamoto *et al.*, 2009). Progesterone suppresses intestinal peristalsis and induces constipation (Bernardi *et al.*, 2017). Fluctuations in these hormones are believed to cause changes in digestive symptoms via sex steroid receptors located in the digestive tract; therefore, it is conceivable that these symptoms may be responsible for the tendency to constipation in the luteal phase and the tendency to diarrhea during menstruation (Bharadwaj *et al.*, 2015). Hormeister and Bodden stated that these symptoms of premenstrual syndrome are said to normally present about 6 days before the start of menstruation, but some reports indicate that symptoms may appear 10 days before the start of menstruation (Hofmeister and Bodden, 2016). In this study, symptoms appeared in the early luteal phase (days 15 to 21). Excess production of prostaglandins, especially F2α and F2, has been identified in women with premenstrual syndrome; this overproduction causes increased uterine tension and high-amplitude contraction, leading to menstrual pain (Bernardi *et al.*, 2017). In this study, none of the participants who indicated that they had "diarrhea tendency/loose stools" during menstruation showed significant changes in their stool properties. However, it is possible that the increased action of prostaglandins due to decreased secretion of progesterone resulted in menstrual pain and gave the woman a "diarrhea tendency/loose stool" sensation. This combined with increased intestinal peristalsis (Degen and Phillips, 1996), may have led them to experience the subjective symptom of "diarrhea tendency/loose stools". Four participants (36.4%) in this study were aware that they had a tendency to diarrhea in late luteal phase. This symptom is the opposite of the effect exerted by sex hormones. However, progesterone secretion declines before the onset of menstruation, and the action of prostaglandins begins. Therefore, menstrual pain may appear immediately before the onset of menstruation and intestinal peristalsis may enhance. Furthermore, prostaglandins were found at a higher concentration throughout the menstrual cycle in women with smooth bowel movements than in women with constipation. Therefore, intestinal peristalsis may strongly enhance (Arthur *et al.*, 1992). Bowel movement discomfort associated with the menstrual cycle improved in eight (72.7%) participants with intake of *Amazake* or *Raw-SHIKOJI*. This effect was apparent in both symptoms of "constipation tendency/hard stools" and "diarrhea tendency/loose stools". Mori et al. reported that bowel movement frequency and fecal properties improved in women with mild constipation as a result of ingesting *Amazake* (Mori, 2019). However, no participants in this study presented with daily symptoms of constipation. This is the first study to show improvement in bowel movement discomfort associated with the menstrual cycle, and improvement was seen not only in constipation but also in diarrhea. In this study, *Amazake*-intake group showed a tendency to improve symptoms, with a tendency toward constipation and diarrhea. *Raw-SHIOKOJI*-intake group showed improvement in their symptoms including tendency to constipation and diarrhea. Bowel movement discomfort associated with menstrual cycle is often overlooked as it is considered a part of women's lives. However, only limited studies have examined this topic in healthy Japanese women. Bertone-Johnson reported that in Europe and the United States, many cases of menstrual syndrome are due to obesity (Bertone-Johnson *et al.*, 2010). However, Yamamoto *et al*. (Yamamoto *et al.*, 2009) and Ko *et al*. (Ko *et al.*, 2019) reported that in Asia and some parts of Europe, it is often due to a propensity of women to be thin and a tendency to feel excessive cold. Therefore, symptoms of premenstrual syndrome vary depending on factors such as regional differences, ethnicity, and lifestyle (Sahin *et al.*, 2014). Fukuda *et al*. reported that in Japan,

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5.4% of women with premenstrual syndrome are not only unable to maintain social activities, but abnormal bowel movements in young woman also exacerbate abnormal bowel movements during menopause (Fukuda *et al.*, 2005). In this study, no dramatic fluctuations were observed after 28 d of the intake of food. However, improvement of abdominal discomfort improved. Intake of fermented food is useful for maintaining regular bowel movements (Wakana *et al.*, 2019). This study showed that fermented food items containing *A. oryzae* are useful for abdominal discomfort caused by the menstrual cycle. Therefore, it is important for women with PMS to consume food items containing *A. oryzae* on a daily basis. Improvements in food, not medicine, reduce the burden on the body caused by medicine. Therefore, this study presents a method to solve the important problem of PMS-related abdominal discomfort. The study has some limitations. The participants were asked to select *Amazake* or *Raw-SHIOKOJI* based on preference, the sample size was small, and it was not a double-blind randomized trial. Therefore, further randomized trials with larger sample sizes are required to investigate the effectiveness of food items containing *A. oryzae*.

## Conclusion

The results of this study show that ingestion of *Amazake* and *Raw-SHIOKOJI* may reduce abdominal discomfort, especially abdominal discomfort associated with the luteal phase and late menstrual cycle and may improve menstruating women's health and quality of life.

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## Authors' contributions

Wakana N, Wakana M, and Fukuyama N designed research; Inaba S, Shirai T and Homma K conducted research; Wakana M, and Tanaka E analyzed data; and Wakana N, Wakana M and Fukuyama N wrote the paper. Fukuyama N had primary responsibility for final content. All authors read and approved the final manuscript.

## Conflict of interests

The authors declare that there is no conflict of interest.

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