Effect of yogurt containing deep sea water on health-related serum parameters and intestinal microbiota in mice

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ABSTRACT

Deep sea water (DSW) has health benefits and is widely used as food supplement; however, its effect in fermented products has not been explored. Here, we investigated the effect of DSW-containing yogurt on health-related serum parameters and intestinal microbiota in mice. Animals were assigned to 3 feeding groups, which received water (control), normal yogurt (N-yogurt), or DSW-containing yogurt (DSW-yogurt) with a basal diet. Mice were killed at wk 4 or 8 of feeding and analyzed for serum parameters and microbial population in the small intestine. Both yogurt groups demonstrated increased populations of intestinal lactic acid bacteria compared with the control group. The activity of serum aspartate aminotransferase and alanine aminotransferase was markedly decreased in the DSW-yogurt and N-yogurt groups, and triglyceride level tended to be lower in the DSW-yogurt group compared with that in the control mice. Furthermore, the DSW-yogurt group showed a more significant decrease in the ratio of total cholesterol to high-density lipoprotein-cholesterol than did the N-yogurt group. These findings suggest that DSW supplementation of yogurt can increase its beneficial effects on lipid metabolism.
Key words: deep sea water, yogurt, serum parameter, intestinal microbiota

INTRODUCTION

Deep sea water (**DSW**) is the term for ocean layers found 200 m below the surface that are too deep to be reached by sunlight (Nakasone and Akeda, 1999). Deep sea water, comprising most of the water in the oceans on the earth, is rich in nutrients generated by the degradation of marine organisms (Nakagawa et al., 2000). Thus, DSW contains higher concentrations of Ca, Mg,

K, vanadium (V), and Zn than surface water (Hataguchi et al., 2005) and, in recent years, has been widely used in food processing, agriculture, and pharmaceutical and cosmetic industries. Deep sea water, with its unique chemical composition, has been shown to have therapeutic effects not only in the treatment of skin conditions such as atopic eczema/dermatitis syndrome, but also in the prevention of life-threatening diseases, including cancer, diabetes, obesity, hypertension, hypercholesterolemia, and atherosclerosis (Nakagawa et al., 2000; Kimata et al., 2002; Yoshioka et al., 2003; Miyamura et al., 2004; Shon et al., 2008; Hwang et al., 2009).

Some countries, including the United States, Korea, Japan, and Taiwan, have succeeded in using DSW as a natural resource for domestic industries (Nakasone and Akeda, 1999; Liu et al., 2008). Korea has commercialized the use of DSW in areas such as energy production, medicine, cosmetics, aquaculture, agriculture, and food and beverage production. In particular, the food and beverage industry uses DSW to produce salt, soybean sauce, soybean paste, bean curd, kimchi, and potable water. However, DSW has not yet been applied to the production of animal-based food such as milk products.

Yogurt is one of the major foods of probiotics that help in maintaining the healthy status of the intestinal microbiota (Ouwehand et al., 1999). It has been established that yogurt consumption can provide important health benefits, including inhibition of carcinogenesis and mutagenesis, prevention of hypertension, decrease in levels of serum total cholesterol (**TC**), low density lipoprotein-cholesterol (**LDL-C**), and triglyceride (**TG**; Matar et al., 1997; Taranto et al., 2000; Usman and Hosono, 2000; Rodríguez-Figueroa et al., 2010; Ejtahed et al., 2011). Given that mineral intake also improves lipid metabolism and thus reduces the risk of cardiovascular disease (Hines et al., 1985; Kisters et al., 1993; Bakalli et al., 1995; Jenner et al., 2007), the addition of DSW may enhance the health-improving effects of yogurt. However, there is little information on the potential health or nutritional benefits of DSW-supplemented yogurt. Therefore, this study was conducted to estimate

the effect of DSW-containing yogurt on health-related serum parameters and intestinal microbiota in mice.

MATERIALS AND METHODS

Reagents and Chemicals

Aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), TG, TC, and high-density lipoprotein-cholesterol (**HDL-C**) were assayed using commercial kits (Asan Pharmaceutical, Seoul, Korea). Phosphate-buffered saline and sodium azide were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO). Plate count agar and de Man, Rogosa, Sharpe agar (**MRS**) for the cultivation and enumeration of *Lactobacillus* spp. were purchased from Becton Dickinson (Sparks, MD).

Deep Sea Water

Experimental DSW was supplied by the Korea Water Resources Corporation (KWRC; Daejeon, Korea). Deep sea water was taken at the depth of 605 m in the East Sea (between 38.35 and 38.39 latitude and between 128.56 and 128.60 longitude), about 6 km northeast of the Deep Ocean Water Application Research Center (Goseong, Gangwon-do, Korea) and processed using a reverse osmosis membrane (SU-810, Toray Industries Inc., Tokyo, Honshu, Japan). Detailed information on DSW quality was provided by the KWRC: pH = 7.6; total dissolved solids = 51.0 g/L; electrical conductivity = 78.5 mS/cm; salinity = 53.12‰; hardness = 10.3 g of $CaCO_3$/L; chloride = 30.84 g/L. The mineral composition of DSW was as follows: Cu, 3.41 µg/L; Mn, 5.84 µg/L; Zn, 46.33 µg/L; Fe, 26.25 µg/L (Yoon et al., 2009).

Yogurt Preparation

We prepared 2 types of yogurts, normal yogurt (**N-yogurt**) and DSW-containing yogurt (**DSW-yogurt**). The N-yogurt was manufactured as follows: 70% fresh market cow milk was mixed with 3% skim milk powder (calorie, 3,550 kcal/kg; carbohydrate, 52%; fat, 1%; protein, 35%; Ca, 1.1%; Na, 0.6%; Seoul Dairy Cooperative, Seoul, Korea), 3% glucose, and 24% deionized water, sterilized at 95°C for 15 min and cooled to 40°C. Then, 0.025% (wt/wt) lactase (Ha-Lactase, Chr. Hansen A/S, Hørsholm, Denmark) and 2% (wt/wt) starter culture were added; we used salt-resistant lactic acid bacteria (**LAB**; *Lactobacillus pentosus* and *Pediococcus pentosaceus*) in consideration of the salt contained in DSW. The mixture was fermented at 38°C for 12 h.

In DSW-yogurt, 10% DSW instead of 10% deionized water was used; otherwise, the process was the same. The final products were stored at 4°C until use. The pH value of N-yogurt and DSW-yogurt was measured using a pH meter (SevenEasy pH, Mettler-Toledo AG, Zurich, Switzerland) equipped with an electrode (InLab Expert Pro, Mettler-Toledo AG). Their LAB population was determined by culture in MRS agar containing 0.02% sodium azide. Both yogurts had same pH value (4.5) and LAB count (9.1 $\log_{10}$ cfu/g).

In Vivo Experimental Design

Thirty-six outbred albino female ICR mice (BW of 20 to 26 g) were purchased from Daehan BioLink Co. Ltd. (Eumseong, Korea). For a week, the mice were acclimated to the rearing environment and fed a basal diet (CP, 20.5%; crude fat, 3.5%; crude fiber, 8%; crude ash, 8%; Ca, 0.5%; P, 0.5%; RodFeed, Daehan BioLink Co. Ltd., Eumseong, Korea). The animals were then assigned to 3 feeding groups of 12 mice each: the control group received water, and the N-yogurt and DSW-yogurt groups received the respective yogurts; all groups were fed a basal diet as well. Mice were reared in a room maintained at 23 ± 2°C and 55 ± 5% relative humidity on a 12-h light-dark cycle. At wk 4 or 8, 6 mice in each group were fasted for 12 h, anesthetized, and killed; then, blood was collected by cardiac puncture and the small intestines were harvested. Blood was left at room temperature for 30 min to coagulate. Serum was separated by centrifugation at 890 × g for 30 min.

Analyses of AST and ALT

The activities of AST (EC 2.6.1.1; L-aspartate: 2-oxoglutarate aminotransferase; glutamic-oxaloacetic transaminase) and ALT (EC 2.6.1.2; L-alanine: 2-oxoglutarate aminotransferase; glutamic-pyruvic transaminase) were analyzed as described by Reitman and Frankel (1957). For the AST assay, 200 µL of serum was mixed with 1 mL of AST substrate reagent (L-aspartate and α-ketoglutarate) and incubated at 37°C for 60 min. Samples for the ALT assay was mixed with substrate reagent (L-alanine and α-ketoglutarate) and incubated at 37°C for 30 min. Then, 1 mL of 2,4-dinitrophenylhydrazine was added, and brown-colored complex (hydrazone) was developed after incubation at room temperature for 20 min. The reaction was stopped with 10 mL of 0.4 N NaOH and the absorbance was measured at 505 nm using a UV-mini-1240 spectrophotometer (Shimadzu Corp., Tokyo, Japan). Deionized water and lithium pyruvate prepared with the same process were used as a blank and standard,

respectively. The AST (or ALT) activity was calculated as unit of AST (or ALT) in 1 L of serum using the standard curve of lithium pyruvate.

Measurement of Serum TG

Serum TG content was determined according to the method of Fossati and Prencipe (1982). Twenty microliters of serum was incubated with 3 mL of enzyme medium [150 units/mL of lipoprotein lipase, 0.075 units/mL of glycerol kinase, 2.22 units/mL of L-α-glycerolphosphate oxidase, and 1,875 units/mL of peroxidase in 0.427% N,N-bis(2-hydroxyethyl)-2-aminomethane sulfonic acid] at 37°C for 10 min. Deionized water and 0.3% glycerol (20 µL each) incubated with the enzyme medium were used as a blank and standard, respectively. The absorbance was measured at 550 nm using a UV-2401PC spectrophotometer (Shimadzu Corp.) and TG content was expressed as the amount (mg) of TG in 1 dL of serum according to the formula: {(sample absorbance − blank absorbance) ÷ (standard absorbance − blank absorbance)} × standard concentration (300 mg of glycerol/dL).

Measurement of TC

Serum TC content was determined according to the protocol of Savoldi et al. (1976), wherein 20 µL serum was first reacted with 3 mL of enzyme medium (20.5 units/mL cholesterol esterase and 10.7 units/mL cholesterol oxidase in 45 mM NaOH, 19 mM phenol, and 100 mM potassium phosphate) at 37°C for 5 min. The absorbance of the sample, blank (20 µL of deionized water), and standard (20 µL of 0.3% cholesterol) was then measured at 500 nm and TC content was calculated as the amount (mg) of TC in 1 dL of serum using the following formula: {(sample absorbance − blank absorbance) ÷ (standard absorbance − blank absorbance)} × standard concentration (300 mg of cholesterol/dL).

Measurement of HDL-C

To measure serum HDL-C content, 200 µL serum was transferred to a microtube, mixed with 200 µL of 0.5% sodium phosphotungstate-1% magnesium chloride, incubated at room temperature for 10 min, and centrifuged at 890 × g for 10 min using a Micro 17R+ centrifuge (Hanil Science Industrial, Incheon, Korea). The supernatant (100 µL) was incubated with 3 mL of enzyme medium (20.5 units/mL cholesterol esterase and 10.7 units/mL cholesterol oxidase in 45 mM NaOH, 19 mM phenol, 100 mM potassium phosphate) at 37°C for 5 min and the absorbance of the sample, standard

(100 µL of 0.05% cholesterol), and blank (100 µL of deionized water) was measured at 500 nm. The HDL-C content was calculated as the amount (mg) of HDL-C in 1 dL of serum using the following formula: {(sample absorbance − blank absorbance) ÷ (standard absorbance − blank absorbance)} × standard concentration (50 mg cholesterol/dL) × dilution factor (2).

Quantification of Total Bacteria and LAB

The contents of the small intestine (1 g) were mixed with sterile PBS for 60 s, and serially diluted 10-fold. The total bacteria (**TB**) population was determined by the pour plate technique. Each dilution was placed in an empty plate, covered with melted PCA, and incubated at 37°C for 48 h. Then, LAB were counted after incubation in MRS agar containing 0.02% sodium azide at 32°C for 72 h. Colony counts (30 to 300) were multiplied by the dilution factor and results were expressed as $\log_{10}$ cfu/g intestinal content.

Statistical Analysis

The data are expressed as the mean ± standard deviation; differences between group means were tested by ANOVA followed by Duncan's multiple range test. All statistical tests were performed using the SPSS (2011) program; $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

AST and ALT Activities

The activities of AST and ALT in blood have been found to be useful biomarkers of chronic liver diseases, including hepatitis, cirrhosis, and fatty liver disease, which may be manifestations of underlying metabolic syndromes such as diabetes mellitus, hyperlipidemia, and porphyria cutanea tarda (Jung et al., 1985; Rosenthal and Haight, 1990). At wk 4 of feeding, the DSW-yogurt and N-yogurt groups had significantly ($P < 0.05$) lower AST activity compared with that in the control mice (Figure 1); however, no statistical difference was found between the 2 yogurt groups. Yogurt also decreased serum ALT activity, which was significantly lower in the N-yogurt group at wk 8 ($P < 0.05$) and in the DSW-yogurt group at wk 4 and 8 ($P < 0.05$) of feeding compared with that in the control group; however, we found no significant difference between the yogurt groups (Figure 2). Our findings are consistent with the study of Minelli et al. (2004), who reported that feeding yogurt to rats resulted in decreases in
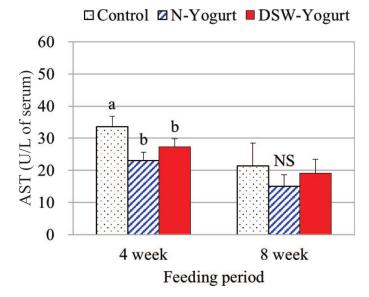
Figure 1. Effect of yogurt containing deep sea water (DSW) on serum aspartate aminotransferase (AST) activity (U/L of serum) in mice. The animals (n = 6 in each group) received water (control), normal yogurt (N-yogurt), or DSW-containing yogurt (DSW-yogurt) together with a basal diet for 4 or 8 wk. The data are presented as means ± SD. Different letters (a, b) indicate significant differences among groups ($P < 0.05$). NS = not significant ($P > 0.05$). Color version available online.

serum AST and ALT, whereas Yoshioka et al. (2003) observed that DSW supplementation decreased serum ALT activity in rabbits receiving a diet containing 1% cholesterol. The effect of DSW could be caused by its microelement components, as evidenced by previous studies showing that in rats and laying hens, dietary supplementation with Zn and Cu, respectively, resulted in a reduction of serum AST and ALT (Kechrid and Bouzerna, 2004; Güçlü et al., 2008).

TG Content

Hokanson and Austin (1996) reported that blood TG level is a risk factor for cardiovascular disease independent of HDL-C level. Our results showed that feeding N-yogurt or DSW-yogurt had insignificant effects on TG level in mice (Figure 3). However, in the DSW-yogurt group, we found a tendency for a decreasing serum TG level compared with the control group at both wk 4 and 8; in the N-yogurt mice, the TG reduction was observed at wk 8. These results are consistent with the findings that yogurt supplementation led to a small decrease of serum TG level in rats (Kawase et al., 2000) and that DSW supplementation slightly decreased serum TG in BALB/c mice (Tsuchiya et al., 2002) and rabbits fed a diet containing 0.5% cholesterol (Sheu et al., 2013). Other studies have shown that dietary mineral caused

a reduction of total lipids in tissue, including blood. Feeding Ca to goats reduced lipid content in the aorta (Hines et al., 1985), whereas Cu intake by steers decreased subcutaneous fat and saturated fatty acids in muscle (Engle et al., 2000). It has also been reported that dietary Mg supplementation decreased serum TG level in patients with hyperlipidemia (Kisters et al., 1993).

Cholesterol Content

Blood TC content is a biomarker that can predict the risk of ischemic heart disease, and it has been demonstrated that the ratio of TC to HDL-C has more predictive value than that of LDL-C to HDL-C (Lemieux et al., 2001). As shown in Figure 4, both N-yogurt and DSW-yogurt had a significant effect on serum cholesterol level in mice. Thus, DSW-yogurt decreased TC level and TC to HDL-C ratio and increased HDL-C level both at wk 4 and 8 compared with that in the control ($P < 0.05$). Furthermore, DSW-yogurt caused a more significant reduction of serum TC level at wk 8 ($P < 0.05$) and TC to HDL-C ratio at wk 4 and 8 ($P < 0.05$) compared with N-yogurt. Our findings are in agreement with previous studies showing the reduction of serum TC and LDL-C levels by yogurt in mice (Akalin et al., 1997) and by DSW in 0.25% cholesterol-fed rabbits, which also demonstrated an
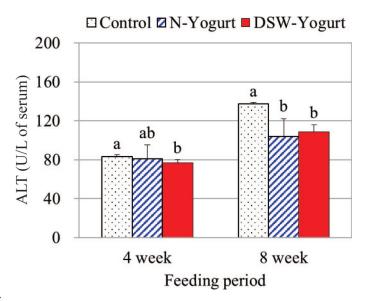

Figure 2. Effect of yogurt containing deep sea water (DSW) on serum alanine aminotransferase (ALT) activity (U/L of serum) in mice. The animals (n = 6 in each group) received water (control), normal yogurt (N-yogurt), or DSW-containing yogurt (DSW-yogurt) together with a basal diet for 4 or 8 wk. The data are presented as means ± SD. Different letters (a, b) indicate significant differences among groups ($P < 0.05$). Color version available online.
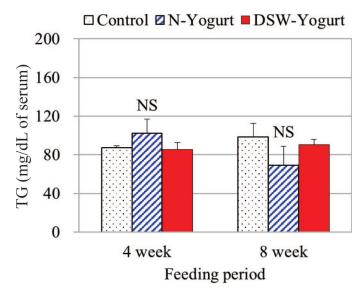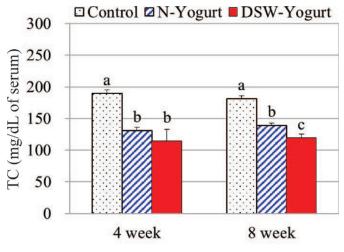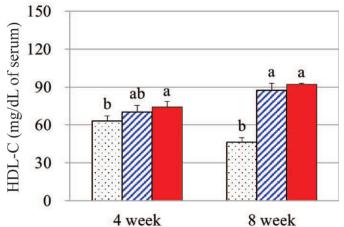
Figure 3. Effect of yogurt containing deep sea water (DSW) on serum triglyceride (TG) level (mg/dL of serum) in mice. The animals (n = 6 in each group) received water (control), normal yogurt (N-yogurt), or DSW-containing yogurt (DSW-yogurt) together with a basal diet for 4 or 8 wk. The data are presented as means ± SD. NS = not significant ($P > 0.05$). Color version available online.

increase in plasma HDL-C (Miyamura et al., 2004). Moreover, DSW decreased plasma TC level in a mouse model of type II diabetes (Hwang et al., 2009). Several studies have demonstrated the effect of dietary minerals on cholesterol metabolism in mammals. Jenner et al. (2007) reported that dietary Zn decreased TC content in the aorta of 1% cholesterol-fed rabbits, and Bakalli et al. (1995) observed that Cu supplementation sharply reduced TC in plasma and muscle of broilers. In addition, Mg consumption is thought to be beneficial for the mitigation of hyperlipidemia in patients with diabetes mellitus (Corica et al., 1994).

Microbial Population

Total bacteria and LAB counts in the small intestine were higher in mice fed DSW-yogurt and N-yogurt than in control animals at wk 4 ($P < 0.05$; Figure 5). We detected no difference between yogurt groups at either wk 4 or wk 8. Feeding DSW did not negatively affect LAB population in starter cultures or in the small intestine because the majority of LAB can survive in relatively high salinity, such as 2.5% NaCl (Orla-Jensen, 1919) and the salinity of DSW-yogurt was only about 5.31‰ (0.53%). Yogurt is a good source of probiotic bacteria, which are used as food supplements because of their beneficial effects on human health. Lactic acid bacteria help in maintaining the balance of intestinal microbiota by producing antimicrobial agents (e.g., bacteriocins)
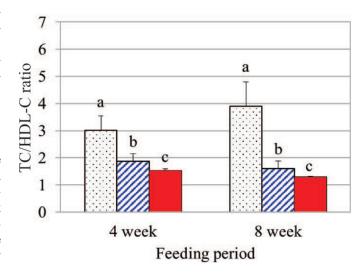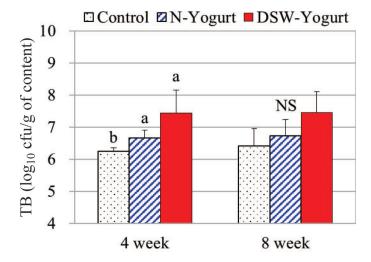

Figure 4. Effect of yogurt containing deep sea water (DSW) on serum cholesterol level (mg/dL of serum) in mice. The animals (n = 6 in each group) received water (control), normal yogurt (N-yogurt), or DSW-containing yogurt (DSW-yogurt) together with a basal diet for 4 or 8 wk. The data are presented as means ± SD. Different letters (a–c) indicate significant differences among groups ($P < 0.05$). TC = total cholesterol; HDL-C = high density lipoprotein-cholesterol. Color version available online.
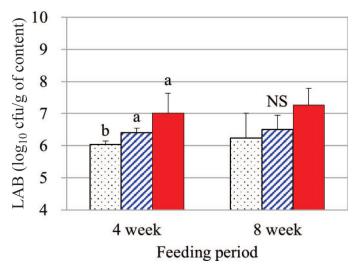
Figure 5. Effect of yogurt containing deep sea water (DSW) on microbial population ($\log_{10}$ cfu/g of content) in the small intestine. The animals (n = 6 in each group) received water (control), normal yogurt (N-yogurt), or DSW-containing yogurt (DSW-yogurt) together with a basal diet for 4 or 8 wk. The data are presented as means ± SD. Different letters (a, b) indicate significant differences among groups ($P < 0.05$). NS = not significant ($P > 0.05$). TB = total bacteria; LAB = lactic acid bacteria. Color version available online.

and inhibiting pathogenic bacteria, stimulate immune system, and play a role in the prevention of diseases such as atopic dermatitis, diabetes, obesity, and cancer (Tannock, 1998; Ouwehand et al., 1999; Taranto et al., 2000). *Lactobacillus pentosus* and *Pediococcus pentosaceus*, starter cultures in our yogurt preparations, have long been used for food fermentation and are recommended as practical probiotics for humans (EFSA, 2007; Gaggìa et al., 2010). The intake of LAB-fermented dairy products significantly increases LAB colonization of the gastrointestinal tract; however, these probiotics may not persist (Link-Amster et al., 1994; Spanhaak et al., 1998; Ouwehand et al., 1999).

CONCLUSIONS

Yogurt supplementation improved metabolic syndrome-related serum parameters and increased intestinal LAB population in mice. Deep sea water containing bioactive microelements enhanced the beneficial effects of yogurt on lipid metabolism. Further research is needed to investigate the effects of DSW-yogurt on human health.

ACKNOWLEDGMENTS

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