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Hypocholesterolaemic effect of probiotic yogurt enriched with barley β-glucan in rats fed on a high-cholesterol diet

Rushdy Mohamed Ahmed[a], Rafaat M. Elsanhoty[b,c], Mahmoud Abd-Alhameid Al-Saman[c] and Mohamed Fawzy Ramadan[d,e,*]

[a]*Department of Special food and nutrition, Food Technology Research Institute, Agriculture Research Center, Cairo, Egypt*
[b]*Max-Rubner Institute, Federal Research Institute of Nutrition and Food, Detmold, Germany*
[c]*Industrial Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat, Egypt*
[d]*Scientific Research Deanship, Umm Al-Qura University, Makkah, Saudi Arabia*
[e]*Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt*

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Abstract.
BACKGROUND: Barley, which is rich in β-glucans, is known to exhibit hypocholesterolemic effect.
OBJECTIVE: The study aimed to investigate the hypocholesterolemic effect of yogurt containing barley β-glucan (BBG) and probiotic bacteria in rats fed on a cholesterol-enriched diet.
METHODS: The methodology was based on adding of 0.75% BBG to skim milk (SM) powder. Four treatments of yogurt were formulated, wherein the first treatment was produced from SM without the addition of BBG and fermented by yogurts starter (YS). The second treatment was produced from SM with the addition of 0.75% BBG, and fermented by YS. The third treatment was produced from SM without the addition of 0.75% BBG, and fermented by *Bifidobacterium lactis* plus *Lactobacillus acidophilus*. The fourth treatment was produced from SM with addition of 0.75% BBG, and fermented by *Bifidobacterium lactis* plus *Lactobacillus acidophilus*. All formulations were evaluated for their effect on plasma lipids, liver lipids, lipid peroxidation, and the fecal excretion of bile acids in rats.
RESULTS: The results indicated that yogurt containing probiotic bacteria and BBG was more effective in lowering of plasma and liver cholesterol levels than other treatments. The fecal excretions of bile acids and lipid peroxidation were markedly promoted in yogurt formulated with BBG and probiotic bacteria compared with the positive control group. The results showed an inverse relationship between the fecal excretions of bile acids and the levels of total cholesterol in the plasma from rats fed on a high-cholesterol diet.

*Corresponding author: Prof. Dr. Mohamed Fawzy Ramadan, Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt. Tel.: +2 055 2320282/055 2313940; Fax: +2 055 2287567/055 2345452; E-mail: hassanienmohamed@yahoo.com.

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CONCLUSION: The inclusion of BBG and probiotic bacteria in the diet of rats fed on high-cholesterol diet had health-promoting impacts on the levels of plasma and liver lipids. Yogurt with *Bifidobacterium* plus *L. acidophilus* and supplemented with BBG were effective in lowering the levels of cholesterol in plasma and liver lipids, while the excretion of bile acids in the feces was enhanced. These hypocholesterolemic effects of yogurt preparations containing BBG and probiotic bacteria could create an effective and economic contribution in treating hypercholesterolaemia.

Keywords: Barley, bile acids, cholesterol, health-promoting effect, dairy products, functional foods

1. Introduction

There is an increasing interest in the popularity of functional foods due to the increase in consumer awareness and demand [1]. The applications of probiotics in food products have been well established throughout generations. The interest in the microorganisms in the recent years emanated from the discovery of their salubrious impact in lowering plasma cholesterol. The amount and type of fat consumed are important to the etiology of several chronic diseases, such as obesity, cardiovascular diseases, and cancer [2–4]. The serum cholesterol level is widely considered as a contributory risk factor for the development of cardiovascular diseases (CVD) such as atherosclerosis, CHD and stroke. The World Health Organization (WHO) has predicted that by 2030, CVD will remain the leading causes of death and affect approximately 23.6 million people globally [5].

The reported health promoting ability of lactic acid bacteria (LAB) in humans and livestock include cholesterol-lowering effect [6], inhibition of pathogenic microorganisms [7], immune-modulation [8], and neutralization of food mutagens produced in the colon and halting of intestinal dysfunction [9]. Some bacterial species excrete bile salt hydrolase, leading to increased bile excretion in feces [10]. However, other reports are contradictory and fail to show hypocholesterolemic effects of probiotics [11–13]. Consequently, this area remains controversial.

β-Glucan is a functional bioactive ingredient comprise a group of β-D-glucose polysaccharides found in the cell walls of cereals, yeast, bacteria, and fungi, with different properties dependent on the source [4]. Food and Drug Administration (FDA) has approved β-glucan (3 g/day) to qualify for the coronary heart disease (CHD) claim FDA [54]. Fortification of foods with β-glucan is of great interest. Attempts to fortify foods with β-glucan including pasta, tea cakes [14], muffins [15], bread [16], and beverages have been reported [17–20]. Worrasinchai et al. [21] stated that β-glucans is poorly absorbed in the human digestion tract and, therefore, β-glucans could be used as a non-caloric food and can be used in foods as a thickener, water retention, or oil bending agent and an emulsion stabilizer [2, 22–26].

β-glucans is known to exhibit hypocholesterolemic impact. Increased intestinal viscosity is thought to be crucial for cholesterol lowering. Concentration, structure, and molecular weight, play important roles in β-glucan functionality [27]. β-glucans decreased serum total cholesterol (18.9%), and LDL-cholesterol (24.3%) in Sprague-Dawley rats [28]. Addition of β-glucan also resulted in greater bile acid excretions. It was suggested that the hypocholesterolemic effects of β-glucan might be due to the enhancement of CYP7A1 expression resulting from increased fecal excretion of bile acids. On the other hand, β-glucan extracted from oat and barley did not affect cholesterol metabolism in young healthy adults [27].

Yogurt is traditionally made from fermenting milk. The belief in the beneficial influence of yogurt on human health and nutrition has existed in many civilizations over a long period of time [29]. Milk fat plays an important role in the texture, flavor and color development of dairy products. Although the manufacture of low-or non-fat dairy products was carried out for many years, the use of fat replacers in the manufacture of dairy products is still novel. β-glucans use in food is interesting, espe-cially in yogurt wherein non-fat yogurt enriched with β-glucan could be used as health promoter for many people suffering from diseases. There are limited reports on the fortification of yogurt with barely β-glucan (BβG). Sahan et al. [30] studied the effects of adding β-glucans

on yogurt properties but they used very low levels of β-glucans (0.05%) from a hydrocolloidal composite of β-glucans. Vasiljevic et al. [55] studied the growth and metabolic activity of probiotic organisms in β-glucans enriched yogurt and reported that addition of oat β-glucans resulted in improved probiotic viability and stability. Brennan and Tudorica [31] found that β-glucans (0.5%) addition improved serum retention and viscoelastic nature of yogurt.

There is little information about the hypocholesterolemic effects of yogurt fortified with probiotic bacteria and BBG as a fat replacer. This study was undertaken to provide such information on the hypercholesterolemic effect of yogurt containing probiotic bacteria and BBG in rats fed on a high-cholesterol diet.

2. Materials and methods

2.1. Materials

Spray-dried skim milk (SM) powder (type low heat, grade A) was obtained from local market (Giza, Egypt). Hull-less barley was obtained from Institute of Field Crop Research (Agricultural Research Centre, Giza, Egypt).

2.2. Extraction of barley β-glucan (BBG)

BBG was extracted from hull-less barely flour according to Benito-Román et al. [32]. Hull less barley flour was weighted and added to an Erlenmeyer flask, then water was added wherein the liquid: solid ratio was 10 : 1 and pH of water was 6. Erlenmeyer flask transferred to a water bath, incubated for 3 h at 55°C and the flour suspended at the high stirring rate. The clear supernatant was collected by centrifugation at 5500 rpm and 4°C for 10 min. BBG was extracted from the clear supernatant after adjusting the concentration with absolute ethyl alcohol to 30%. The precipitated BBG was freeze-dried (Christ BETA 2–16, Osterode am Harz, Germany). The final product was packed in a plastic container and keep at –20°C until used.

2.3. Bacterial strains and culture preparation

Yogurt cultures of *Streptococcus salivarius* sub sp. *thermophilus,* and *Lactobacillius dulbrueekii* sub sp. *bulgaricus* were obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Centre (MIRCEN, Faculty of Agriculture, Ain Shams University, Egypt). *Bifidobacterium lactis* Bb-12, and *Lactobacillus acidophilus* LA-5 (freeze-dried) were obtained from Chr. Hansen laboratories (Copenhagen, Denmark). Strains were cultured on deMan, Rogosa and Sharpe (MRS, Difico Laboratories) agar plates. Anaerobic strains were kept in an anaerobic jar (Anaerogen, Oxoid). Fully grown colonies were stored on plates at 4°C until needed with sub culturing on a monthly basis. For long-term conservation of strains, spore or cell suspensions were kept in cry vials at –80°C with 90% glycerol as cryoprotectant. *Lactobacilli* were cultivated in MRS broth and *bifidobacteria* were grown in MRS broth with some supplementation with cysteine and incubated for 24 h at the suitable growth temperature. An appropriate volume of overnight culture was used to inoculate 150 mL cultures and incubated for 24 h at 37°C in an anaerobic jar (Anaerogen, Oxoid). The culture was prepared by adding a few mg of the subculture to 100 mL of previously reconstituted and sterile (121°C/2 min) skimmed milk (10% total solids). This mixture was incubated at 42°C until the onset of gelatin. Two mL of culture from this passage were transferred into 100 mL of sterile SM at 42°C and the culture was incubated until a gel had just formed. This second culture was used for the propagation of a bulk culture (1 L) for inoculation of the different treatments. Bulk cultures were prepared 24 h before the production of yogurt.

2.4. Preparation of yogurt

2.4.1. Preliminary studies

Preliminary studies were carried out to select the suitable concentrations of BBG that can be used in the production of low-fat yogurt. Different amounts (0.25%, 0.5%, 0.75%, and 1%) of BBG were incorporated in the yogurt mixtures to substitute fat in milk. The yogurt samples were sensory evaluated and the results indicated that the 0.75% BBG had the highest score. Based on the preliminary studies, 0.75% of BBG was added to SM powder to produce low-fat yogurt in the further investigation.

2.4.2. Yogurt formulation

Fresh whole cow's milk was obtained from the Dina company (Giza, Egypt). Yogurt was produced as control samples according to Singh et al. [29]. The yogurt was prepared by blending with 13% (w/v) non-fat milk solids and water at 50°C. The mixture was divided into four portions. The first portion was heated at 80°C for 15 min and cooled to 45°C, then inoculated with 3% (v/v) of *Streptococcus salivarius* sub sp. *thermophilus* plus *Lactobacillus dulbrueekii* sub sp. *bulgaricus* and abbreviated as YSM (treatment I). The second portion was mixed with 0.75% BBG, heated at 80°C for 15 min and cooled to 45°C, then inoculated with 3% (v/v) of *Streptococcus salivarius* sub sp. *thermophilus* plus *Lactobacillus dulbrueekii* sub sp. *bulgaricus* and abbreviated as YSMBBG (treatment II). The third portion was heated at 80°C for 15 min and cooled to 45°C, then inoculated with 3% (v/v) of probiotic bacteria (*Bifidobacterium lactis* Bb-12 plus *Lactobacillus acidophilus* LA-5) and abbreviated as PYSM (treatment III). The forth portion was mixed with 0.75% BBG, heated at 80°C for 15 min and cooled to 45 °C, inoculated with 3% (v/v) from probiotic bacteria (*Bifidobacterium lactis* Bb-12 plus *Lactobacillus acidophilus* LA-5) and abbreviated as BYSMBBG (treatment IV). All treatments were incubated at 42°C for 4 h until the pH reached 4.5. The yogurt samples were stored in the refrigerator at 5°C till further analysis.

2.5. Counts of yogurt cultures, Lactobacillus acidophilus and Bifidobacterium lactis

Streptococcus thermophilus was enumerated using of M17 agar according to Ravula and Shah [33]. *L. delbrueckii* subsp. *bulgaricus* was enumerated using of MRS agar. Enumeration of *Lactobacillus acidophilus* and *Bifidobacterium lactic* Bb 12 was performed using selective MRS agar (Himedia) under anaerobic incubation at 37°C for 3 days. Plates were incubated an aerobically at 37°C for 48 h. Table 1 indicates the viable count yogurt cultures, *Lactobacillus* and *bifidobacteria* in the experimental products.

Table 1
The viable count of yogurt cultures and probiotic bacteria in the experimental treatments[a]

Treatment	Microbial count (10^7 cfumL^{-1})			
	Yoghurt culture		Probiotic culture	
	S. thermophilus	*L. bulgaricus*	*B. Bifidium* Bb 12	*L. acidophilus* LA5
III	44.6	41.9	ND[b]	ND
IV	46.9	43.8	ND	ND
V	ND	ND	2.9	3.2
VI	ND	ND	3.8	4.5

[a]The data presented are the means of three replicate trials. [b]ND: not determined.

Table 2
Experimental groups of rats and diets used in the feeding study

Diet	Diet encoder	Formulation
Basel diet (cholesterol-free diet)*	Negative control (I)	100 g basel diet+50 mL water
Basel diet and cholesterol-enriched diet	Positive control (II)	99.5 g basal diet+0.5 g cholesterol+50 mL water
Cholesterol-enriched diet+YS (YSSM)**	III	99.5 g basal diet+0.5 g cholesterol+50 g (YSSM) yogurt
Cholesterol-enriched diet+(YSSM+BBG)	IV	99.5 g basal diet+0.5 g cholesterol+50 g (YSSM+BBG) yogurt
Cholesterol-enriched diet+probiotic yogurt (PYSMM)	V	99.5 g basal diet+0.5 g cholesterol+50 g (PYSMM) yogurt
Cholesterol-enriched diet+probiotic yogurt with BBG (PYSMBBG)	VI	99.5 g basal diet+0.5 g cholesterol+50 g (PYSMBBG) yogurt

*Basel diet composition according to Reeves et al. [34]. **Addition of yogurt to diets according to Abd Al-Gawad et al. [6].

2.6. Feeding study

2.6.1. Experimental conditions

Forty-eight weaning male albino rats (average weight 65–80 g) were obtained from Institute of Ophthalmology Research (Giza, Egypt). The animals were housed individually in well aerated cages with screen bottoms and fed on a basal diet as described by Reeves et al. [34]. All the experiments using animals were performed under the permission of Ethical Committee of ARC (protocol 2014-15, 25 March 2014). Salt and vitamin mixtures were prepared as described in AOAC [35]. Temperature and humidity were maintained at 25°C and 60%, respectively wherein food and water were provided. The animals were randomly sorted into six groups each group contain 8 rats. Group one was fed on control diet according to Reeves et al. [34], and considered as negative control group. Group two was fed on control diet with addition of 0.5 g cholesterol for each 100 g diet plus 50 mL water and considered as positive control group. Group three fed on control diet with addition of 0.5 g cholesterol for each 100 g diet plus 50 mL yogurt starter (YS). Group four fed on control diet with addition of 0.5 g cholesterol for each 100 g diet plus 50 mL yogurt enriched with 0.75% BBG (YSSMBBG). Group five was fed on the control diet with addition of 0.5 g cholesterol for each 100 g diet plus 50 mL from yogurt fermented by *Bifidobacterium lactis* plus *Lactobacilus acidophilus* (PYSM). Group six feed on control diet with addition of 0.5 g cholesterol for each 100 g diet plus 50 mL from yogurt fermented by *Bifidobacterium lactis* plus *Lactobacillus acidophilus* and included 0.75% BBG (PYSMBBG). The composition of diets and animal groups is presented in Table 2. During the experimental period (7 weeks) rats were kept separately in well-aerated cages. The diet consumed and body weight, organs weight were recorded at the end of the experimental period.

2.6.2. Blood sampling and serum analysis

All feed was removed and blood samples were collected from rats within different treated groups from the orbital venous plexuses by a capillary tube at the end of experimental period. Blood serum was separated by centrifugation at 3000 rpm for 15 min to obtain plasma, which was kept frozen at –20°C until analysis. Total cholesterol (TC) in serum, high density lipoprotein cholesterol (HDL), and triglycerides (TG) were determined according to Trinder [56]. Low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) were calculated as follows: VLDL+LDL cholesterol = TC – HDL. TC and TG were extracted from the liver according to Fernandez and McGregor [36], and measured by the method of Trinder (1969).

2.6.3. Determination of lipid peroxidation

Malondialdehyde (MDA) level in plasma was determined as an index of lipid peroxidation because MDA is an end-product of unsaturated fatty acid peroxidation and can react with thiobarbituric acid to form a colored complex called the thiobarbituric acid reactive substance (TBARS). TBA reactivity was assayed by the method of Erdincler et al. [37].

2.7. Feces collection and determination of bile acids

Feces were collected on the last 2 days of the feeding period and kept frozen (–25°C) until analyzed for bile acids. The rats were sacrificed and the liver, heart, kidney and spleen were excised and weighed. The liver was washed with an ice-cold saline solution and stored at 25°C. Total bile acids (TBA) value was determined according to Onning et al. [38]. TC of feces was determined as described by Rudel and Morris [39]. To extract bile salts, 1.0 g of feces were suspended in chloroform:methanol (1 : 1, v/v), then extracted at 60°C for 60 h. The extract was evaporated and dissolved in methanol to measure TBA.

2.8. Statistical analysis

Statistical analysis was performed by running student *t*-test using Stat view 512 software (1986). Chi-square was performed to compare between the controls and experimental yogurt. Significant effects were declared $p < 0.05$.

3. Results and discussion

3.1. Body weight and food intake

Data in Table 3 presents the total food intake by the animal groups at the end of the experiment. Data indicated that there was a lower food intake in rats that fed on each yogurt supplement compared to the control groups. The groups fed on the diet supplemented with yogurt (group III), yogurt plus BBG (group IV), yogurt with probiotic bacteria (group V) and probiotic bacteria plus BBG (group VI) showed a significant increase ($p < 0.05$) in body weight. All groups showed also differences in food efficiency (Table 3). The rats fed on the yogurt-supplemented diets demonstrated a lower food intakes compared to the control groups. However, the body weights of the rats treated with the yogurt were significantly higher than those of the control, due to the ingestion of yogurt, resulting in a decrease in food intake and an increase in body weight. The results were in agreement with the results obtained by Abd El-Gawad et al. [6]; Al-Sheraji et al. [40]; Mahrous et al. [25], and Ogunremi et al. [41].

3.2. Body weight gain and organs weight

Data in Table 4 presents the effects of feeding on diet contained yogurt with BBG and probiotic bacteria on rats fed on a cholesterol-high diet. The group fed on cholesterol-high diet had the highest liver weight (6.985 g), while basal diet group had the least (3.925 g). There were significant differences in the liver weight between the group fed basal diet and the respective groups fed on cholesterol-high diet. There was a decrease in the liver weight for groups fed on cholesterol-high diet and supplemented with yogurt containing YS, probiotic bacteria and BBG. However, there is no significant difference was recorded. There were no significant differences in the weight of the heart for the six animal groups. There was a significant difference in kidney weight between rats fed the positive control diet and other groups. Similar results were reported by Abd-Al-Gawad et al. [6]; Al-Sheraji et al. [40]; Mahrous et al. [25]; Sharifuzzaman et al. [42] who reported that the incorporation of oat β-glucan and other fibers improve the body weight gain of animals due to the improve in the growth and performance. High

Table 3
Body weight gain, total food intake, food efficiency of rats fed a high-cholesterol diet and different yogurt formualtions after 7 weeks

Treatment/animal group	Initial weight (g)	Weight (g)	Body weight gain (g)	Food intake (g)	Food efficiency (%)*
Negative control (I)	73.6 ± 3.8	223.7 ± 13.7	150.1 ± 18.1	743.0 ± 23.5	20.2 ± 1.8
Positive control (II)	72.7 ± 2.9	238.9 ± 12.8	166.2 ± 21.7	789.8 ± 23.9	21.1 ± 3.2
III	74.5 ± 4.4	239.7 ± 11.7	164.3 ± 15.2	689.5 ± 21.8	23.81 ± 2.7
IV	72.4 ± 4.1	243.6 ± 9.67	171.2 ± 20.4	667.8 ± 25.7	25.6 ± 2.1
V	71.9 ± 3.9	292.8 ± 11.2	220.9 ± 13.7	654.0 ± 19.5	33.7 ± 1.9
VI	72.6 ± 4.6	298.8 ± 10.4	226.2 ± 17.5	634.0 ± .21.7	35.6 ± 2.2

*Food efficiency (%) = (body weight gain/food intake) × 100.

Table 4
Body weight gain and organ weight of rats fed on experimental diets for seven weeks

Treatment/animal group	Weight after 7 weeks (g)	Body weight gain (g)	Organ weight (g)			
			Liver	Kidney	Heart	Spleen
Basel diet (I)	223.7 ± 13.7	150.1 ± 18.1	3.92 ± 0.2	0.90 ± 0.1	0.72 ± 0.0	0.42 ± 0.1
II	238.9 ± 12.8	166.2 ± 21.7	6.98 ± 0.6	1.4 ± 0.1	0.74 ± 0.1	0.44 ± 0.1
III	239.7 ± 11.7	164.3 ± 15.2	4.88 ± 0.4	1.1 ± 0.1	0.72 ± 0.0	0.41 ± 0.2
IV	243.6 ± 9.67	171.2 ± 20.4	4.67 ± 0.5	0.99 ± 0.1	0.73 ± 0.1	0.42 ± 0.1
V	292.8 ± 11.2	220.9 ± 13.7	4.62 ± 0.4	1.0 ± 0.0	0.71 ± 0.1	0.43 ± 0.1
VI	298.8 ± 10.4	226.2 ± 17.5	4.73 ± 0.6	1.0 ± 0.1	0.72 ± 0.1	0.44 ± 0.0

cholesterol in diets induced an increase in liver weight suggested to be due to the accumulation of lipid masses in liver cells.

3.3. Effect of feeding on the levels of plasma and liver lipids

Table 5 presents the effect of feeding different yogurt preparations on the levels of plasma and liver lipids in rats. The feeding of cholesterol-high diet for 7 weeks significantly increased the levels of serum TC, TG and LDL. Cholesterol-high diet in groups fed on probiotic bacteria and BBG had lower TC, TG and LDL. The serum TC levels in the groups fed yogurt supplemented with BBG (groups II, III, IV, V and VI) were significantly different from the positive control group with mean values of 145.7, 126.6, 131.8 and 120.7 mg/100, respectively. The cholesterol-high diet group had a serum TC content of 264.8 mg/100 mL. The serum TG and LDL-C levels in the respective groups also showed similar trends as serum TC. The control group that fed on the basal diet had serum TG and LDL values of 112.9 and 82.9 mg/100 mL, respectively. The data indicated that the diet contained yogurt supplemented with BBG and probiotic bacteria had effects on reduction of TC, TG and LDL. On the other hand, there were no significant differences in the plasma HDL levels between the negative control group and other experimental groups at the end of the experimental period. The data in Table 5 shows that the content of liver TC in the positive control group (7.36 mg/g) was significantly higher than that in the negative control group (2.93 mg/g). There were significant differences between groups fed on the positive control diet and those fed on basal diet and yogurt with supplemented with BBG. The basal diet supplemented with yogurt contained probiotic bacteria and BBG (groups III, IV, V and VI) were more effective in lowering the levels of liver TC than other diets. The obtained results agree with the results obtained by Abd Al-Gawad et al. [6], who found

Table 5
Effect of feeding different yogurt formulations on the levels of plasma and liver lipids of rats*

Treatment/ animal group	Plasma				Liver	
	TC (mg/100 mL)	TG (mg/100 mL)	HDL-cholesterol (mg/100 mL)	VLDL+LDL-cholesterol (mg100/mL)	Cholesterol (mg/g)	Triglycerides (mg/g)
Basel diet (I)	121.4 ± 8.90	44.60 ± 4.6	38.5 ± 1.9	82.90 ± 1.8	2.93 ± 0.1	16.87 ± 0.4
II	264.8 ± 12.7	112.9 ± 11.8	23.9 ± 2.4	240,9 ± 2.7	7.36 ± 0.0	25.20 ± 0.9
III	145.7 ± 11.4	84.23 ± 3.9	17.4 ± 1.4	128.3 ± 3.6	2.45 ± 0.1	16.40 ± 1.0
IV	126.6 ± 10.5	68.90 ± 6.7	18.6 ± 2.1	108.0 ± 1.2	2.40 ± 0.2	15.90 ± 1.2
V	131.8 ± 9.25	73.70 ± 6.7	16.7 ± 2.1	115.1 ± 1.6	2.34 ± 0.1	16.80 ± 1.1
VI	120.7 ± 8.55	61.05 ± 3.6	15.3 ± 2.3	105.4 ± 0.9	2.30 ± 0.1	16.65 ± 0.5

*Values are given as means ± SD from eight rats in each group.

that yogurt or soy yogurt containing probiotic bacteria was more effective in the lowering of plasma and liver TC levels. Probiotic *lactobacillus* strains have also been reported to have hypocholesterolemic effect in rats [43] Singh et al. [57]. The content of liver TG was significantly higher in rats fed on the positive control diet than those fed the negative control diet (Table 5). In contrast, the levels of liver TG in rats fed the experimental diet groups were significantly lower than in rats fed on the positive control diet. These results are in agreement with those reported by Kheadr et al. [44] and Xie et al. [45] who suggested that the yogurt fermented by *Bifidobacterium lactis* Bb-12 plus *Lactobacillus acidophilus* LA-5 and supplemented with BBG reduced TC in rat liver tissues.

3.4. Effect of feeding on fecal bile acid excretion

Data in Table 6 show the effect of feeding on different yogurt preparations on the fecal bile acid excretion in rats. There was no significant difference in the levels of total bile acids between rats fed the negative control and positive control diets. The rat groups fed on yogurt supplemented with BBG and probiotic bacteria excreted significantly higher levels of bile acids than either the positive or negative control diets. The excretion of bile acids in rats fed on the yogurt supplemented with BBG and probiotic bacteria were significantly higher than those fed on yogurt with YS diets. These results demonstrated an inverse relationship between the level of bile acids excreted in the feces and the total levels of TC, LDL and VLDL in rats treated with diet contained probiotic bacteria and BBG. The results obtained in this study suggest that hepatic cholesterol metabolism could be altered to supply more cholesterol for bile acid synthesis. Several studies reported that *Bifidobacterium* spp. and *Lactobacillus* spp. enhanced both the secretion of bile acids and the activity of cholesterol 7-α-hydroxylase, a rate-limiting enzyme in the synthesis of bile acids [40] (Singh et al., 2015). Moreover, an *in vitro* study conducted by Tahri et al. [46] demonstrated that bifidobacteria cells can eliminate cholesterol from the growth culture medium through both assimilation and coprecipitation with deconjugated bile salts. In this way, bifidobacteria increases the fecal excretion of bile salts. Similar results were obtained by Mahrous et al. [25], and Ivey et al. [47] who found that the incorporation of oat β-glucan with bifidobacteria and *Lactobacillus* in yogurt and synbiotic products increased the excretion of fecal bile acids in rats. This finding and our results suggest that hepatic cholesterol metabolism could change in order to provide more cholesterol for synthesis of bile acids.

3.5. Lipid peroxidation

Table 7 presents the effect of feeding different yogurt formulations on lipid peroxidation in rats. Plasma MDA level was significantly ($p < 0.05$) increased in hypercholesterolaemic animals of the positive control group as an expression of the oxidative stress caused by a cholesterol-rich diet. Nagao et al. [48] has suggested that the

Table 6
Effect of feeding different yogurt formulations on the excretion of bile acids in feces of rats*

Treatment/animal group	Bile acid				
	Cholic acid (mg/100 g)	Chenodeoxycholic acid (mg/100 g)	Deoxycholic acid (mg/100 g)	Lithocholic acid (mg/100 g)	Total bile acids (mg/100 g)
Basel diet (I)	204,6 ± 2.9	35.6 ± 1.8	20.7 ± 0.9	0.9 ± 16.8	277.7
II	210.3 ± 5.7	34.5 ± 3.2	19.1 ± 1.3	16.5 ± 5.2	280.4
III	233.7 ± 5.3	37.6 ± 6.8	21.6 ± 4.2	18.8 ± 4.2	311.7
IV	254.9 ± 4.8	37.7 ± 5.3	25.2 ± 2.8	21.8 ± 1.9	339.6
V	267.7 ± 2.5	38.9 ± 1.7	26.3 ± 3.6	23.9 ± 3.8	356.8
VI	283.4 ± 4.9	44.4 ± 6.2	29.6 ± 2.9	26.2 ± 2.8	383.6

*Values are given as means ± SD from eight rats in each group.

Table 7
Effect of feeding different yoghurt formuations on lipid peroxidation in rats*

Treatment/animal group	Blood MDA (μmol/L)	Liver MDA (pmol/mg protein)
Basel diet (I)	9.12 ± 0.4	9.43 ± 1.0
II	13.7 ± 1.1	12.9 ± 0.5
III	7.62 ± 0.6	8.50 ± 0.6
IV	7.20 ± 0.2	6.74 ± 1.1
V	6.30 ± 0.4	6.30 ± 0.7
VI	6.00 ± 0.1	5.80 ± 1.2

*Values are given as means ± SD from eight rats in each group.

accumulation of fat in the body might result in a high susceptibility to lipid peroxidation. The plasma MDA decreased significantly in all other groups that contained yogurt culture, BBG and probiotic bacteria (treatments III, IV, V and VI). Similar results were obtained by Ou et al. [49]; Uskova and Kravchenko, [50]; Harisa et al. [51] and Singroh et al. [13] who found that the fermented dairy drink containing *L. casei* 114001 reduced MDA content in the blood plasma of Wistar rats. *L. acidophilus* has been shown to significantly decrease the elevation of MDA in diabetic rats wherein the inhibitory percentages of *S. salivarius* ssp. *thermophilous* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 on plasma and liver lipid peroxidation were 57% and 41%, respectively. Lin and Chang [52] found that the inhibitory percentages of *B. longum* ATCC 15708 and *L. acidophilus* ATCC 4356 on plasma lipid peroxidation were 34% and 29%, respectively. The obtained results were agree with the results that obtained by Lin and Chang [52] and Abubakr et al. [53] who reported that LAB and probiotic bacterial strains possess highly antioxidant properties. Therefore, it could be said that the yogurt with probiotic and LAB supplemented with BBG has antioxidant properties.

4. Conclusion

Our results demonstrated that the inclusion of BBG and probiotic bacteria in the diet of rats fed on high-cholesterol diet had noticeable health-promoting impacts on the levels of plasma and liver lipids. Yogurt samples with *Bifidobacterium* plus *L. acidophilus* and supplemented with BBG were very effective in lowering the levels of TC in plasma and liver lipids, while the excretion of bile acids in the feces was enhanced. The treatments

containing yogurt fermented with *Bifidobacterium* plus *L. acidophilus* and supplemented with BBG have also antioxidant properties. These hypocholesterolemic effects of yogurt preparations containing BBG and probiotic bacteria could create an effective and economic contribution in treating hypercholesterolaemia, if these effects could be confirmed in human volunteers.

Conflict of interest statement

The authors declare no conflict of interests.

References

[1] Cui W, Wood PJ. Relationship between structural features, molecular weight and rheological properties of cereal β-glucan. In: Nishinari K, editor. Hydrocolloids. Amsterdam: Elsevier, 2000.

[2] Shen R, Shuangqun L, Dong J. Application of oat dextrin for fat substitute in mayonnaise. Food Chemistry. 2010;7.

[3] Barsanti L, Passarelli V, Evangelista V, Frassanito AM, Gualtieri P. Chemistry, physico-chemistry and applications linked to biological activities of β-glucans. Natural Product Reports. 2011;28:457-66.

[4] Gangopadhyay N, Hossain BM, Rai KD, Nigel P, Brunton PN. A review of extraction and analysis of bioactives in oat and barley and scope for use of novel food processing technologies. Molecules. 2015;20:10884-909.

[5] World Health Organization (WHO). Cardiovascular disease. Fact sheet no. 317, WHO, Geneva, Switzerland, 2009.

[6] Abd El-Gawad IA, El-Sayed EM, Hafez SA, El-Zeini HM, Saleh FA. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing *Bifidobacteria* in rats fed on a cholesterol-enriched diet. Int Dairy J. 2005;15:37-44.

[7] Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev. 2003;16:658-672.

[8] Gill HS, Rutherfurd KJ, Cross ML, Gopal PK. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. Am J Clin Nutr. 2001;74:833-9.

[9] Lee DK, Jang S, Baek EH, Kim MJ, Lee KS, Shin HS, Chung MJ, Kim JE, Lee KO, Ha NJ. Lactic acid bacteria affect serum cholesterol levels, harmful fecal enzyme activity, and fecal water content. Lipids Health Dis. 2009;8:21-9.

[10] Begley M, Hill C, Gahan CG. Bile salt hydrolase activity in probiotics. Appl Environ Microbiol. 2006;72:1729-38.

[11] Hatakka K, Mutanen M, Holma R, Saxelin M, Korpela R. *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp shermanii JS administered in capsules is ineffective in lowering serum lipids. J Am Coll Nutr. 2008;27:441-7.

[12] Simons LA, Amansec SG, Conway P. Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. Nutr Metab Cardiovasc Dis. 2006;16(8):531-5.

[13] Singroha G, Pandey N, Kumar Malik R, Kumar MS. Hypocholesterolemic effect of a *Lactobacillus gasseri* strain Lg70 isolated from breast fed human infant feces. Journal of Innovative Biology. 2014;1(2):105-11

[14] Aman P, Rimsten L, Andersson R. Molecular weight distribution of β-glucan in oat-based foods. Cereal Chem. 2004;81:356-60.

[15] Tosh SM, Brummer Y, Miller SS, Regand A, Defelice C, Duss R, Woelver MS, Wood PJ. Processing affects the physicochemical properties of β-glucan in oat bran cereal. J Agric Food Chem. 2010;58:7723-30.

[16] Moriarety S, Temelli F, Vasanthan T. Effect of formulation and processing treatments on viscosity and solubility of extractable barley β-glucan in bread dough evaluated under in vitro conditions. Cereal Chem. 2010;87:65-72.

[17] Wood PJ. Oat and rye β-glucan: Properties and function. Cereal Chem. 2010;87:315-30.

[18] Corbo RM, Bevilacqua A, Petruzzi L, Casanova PF, Sinigaglia M. Functional beverages: The emerging side of functional foods. Commercial Trends, Research, and Health Implications. 2014;13:1192-206.

[19] Elsanhoty R, Zaghlol A, Hassanein A. The manufacture of low fat labneh containing barely beta-Glucan 1-Chememical composition, microbiological evaluation and sensory properties. Current Research in Dairy Science. 2009;1:1-12.

[20] Sah BNP, Vasiljevic TS, McKechnie S, Donkor ON. Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. LWT-Food Science and Technology. 2016;65:978-86.

[21] Worrasinchai S, Suphantharika M, Pinjai S, Jamnong P. β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. Food Hydrocolloids. 2006;20(1):68-78.

[22] Satrapai S, Suphantharika M. Influence of spent brewer's yeast β-glucan on gelatinization and ret rogradation of rice starch. Carbohydrate Polymers. 2007;67:500-10.

[23] Santipanichwong R, Suphantharika M. Influence of different β-glucans on the physical and rheo logical properties of egg yolk stabilized oil-in-water emul sions. Food Hydrocolloids. 2009;23:1279-87.

[24] Ferreira IMPLVO, Pinho O, Vieira E, Tavarela JG. Brewer's Saccharomyces yeast biomass: Characteristics and potential applications. Trends in Food Science & Technology. 2010;21:77-84.

[25] Mahrous H, Kholy WME, Elsanhoty RM. Production of new synbiotic yoghurt with local probiotic isolate and oat and study its effect on mice. J Adv Dairy Res. 2014;2:121.

[26] Hassan KL, Haggag HF, ElKalyoubi MH, Abd EL-Aziz M, El-Sayed MM, Sayed AF. Physico-chemical properties of yoghurt containing cress seed mucilage or guar gum. Annals of Agricultural Science. 2015;60(1):21-8.

[27] Ibrugger S, Kristensen M, Poulsen MW, Mikkelsen SM, Ejsing J, Jespersen BM, Dragsted LO, Engelsen SB, Bugel S. Extracted Oat and Barley β-Glucans so not affect cholesterol metabolism in young healthy adults. J Nutr. 2013;143:1579-85.

[28] Yang JL, Kim YH, Lee HS, Lee MS, Moon YK. Barley beta-glucan lowers serum cholesterol based on the up-regulation of cholesterol 7alpha-hydroxylase activity and mRNA abundance in cholesterol-fed rats. J Nutr Sci Vitaminol (Tokyo). 2003;49:381-7.

[29] Singh M, Kim S, Liu XS. Effect of purified Oat β-glucan on fermentation of set-style yogurt mix. Journal of Food Science. 2012;77(8):10514-23.

[30] Sahan N, Yasar K, Hayaloglu AA. Physical, chemical and flavor quality of non-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage. Food Hydrocolloids. 2008;22:1291-7.

[31] Brennnan CS, Tudorica CM. Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: Comparative study of the utilisation of barley beta-glucan, guar gum and inulin. Inter J Food Sci Technol. 2008;43:824-33.

[32] Benito-Román O, Alonso E, Lucas S. Optimization of the β-glucan extraction conditions from different waxy barley cultivars. J Cereal Sci. 2011;53:271-76.

[33] Ravula RR, Shah NP. Selective enumeration of *Lactobacillus casei* from yogurt and fermented milk drinks. Biotechnol Tech. 1998;12:819-22.

[34] Reeves PG, Roossow KL, Landlauf J. Development and testing of the AIN-93 purified diets for rodents: Results on growth, kidney calcification and bone mineralization in rats and mice. J Nutrition. 1993;123:1923-31.

[35] AOAC International. Official methods of analysis of AOAC International. 17th edition. Gaithersburg, MD, USA, Association of Analytical Communities, 2000.

[36] Fernandez-Garcia E, McGregor JU. Fortification of sweetened plain yogurt with insoluble dietary fiber. Z Lebensm Unters Forsch A. 1997;205:433-7.

[37] Erdincler DS, Seven A, Inci F, Beğer, T, Candan G. Lipid peroxidation and antioxidant status in experimental animals: Effects of aging and hypercholesterolemic diet. Clinica Chimica Acta. 1997;265:77-84.

[38] Onning G, Akesson B, Oste R, Lundquist I. Effects of consumption of oat milk, soyamilk or cow's milk on plasma lipids and antioxidative capacity in healthy subjects. Annals of Nutrition and Metabolism. 1998;42:211-20.

[39] Rudel LL, Morris MD. Determination of cholesterol using o-phthalaldehyde. J Lipid Res. 1973;14:364-6.

[40] Al-Sheraji HS, Ismail A, Manap YM, Shuhaimi Mustafa S, Yusof RM, Hassan AF. Hypocholesterolaemic effect of yoghurt containing *Bifidobacterium pseudocatenulatum* G4 or *Bifidobacterium longum* BB536. Food Chemistry. 2012;135:356-61.

[41] Ogunremi RO, Sanni IA, Renu Agrawa R. Hypolipidaemic and antioxidant effects of functional cereal-mix produced with probiotic yeast in rats fed high cholesterol diet. Journal of Functional Foods. 2015;17:742-8.

[42] Sharifuzzaman SM, Al-Harbi AH, Austin B. Characteristics of growth, digestive system functionality, and stress factors of rainbow trout fed probiotics Kocuria SM1 and Rhodococcus SM2. Aquaculture Amsterdam, Netherlands. 2014;418-419:55-61.

[43] Geraylou Z, Souffreau C, Rurangwa E, De Meester L, Courtin CM, Delcour JA, Buyse J, Ollevier F. Effects of dietary arabinoxylan oligosaccharides (AXOS) and endogenous probiotics on the growth performance, nonspecific immunity and gut microbiota of juvenile Siberian sturgeon (*Acipenser baerii*). Fish and Shellfish Immunology. 2013;35:766-75.

[44] Kheadr EE, Abd El-Rahman AM, Abd El-Soukkary FAH, Abd El-Rahman AM. Impact of yoghurt and probiotic strains on serum cholesterol and lipoprotein profile in rats. Alexandria Journal of Agricultural Research. 2000;45(3):81-100.

[45] Xie N, Cui Y, Yin NY, Zhao X, Yang J, Wang Z, Fu N, Tang Y, Wang X, Liu X, Wang C, Lu F. Effects of two *Lactobacillus* strains on lipid metabolism and intestinal microflora in rats fed a high cholesterol diet. BMC Complementary & Alternative Medicine. 2011;11:47-53.

[46] Tahri K, Crociani J, Ballongue J, Schneider F. Effect of three strains of bifidobacteria on cholesterol. Letters in Applied Microbiology. 1995;21:151-449.

[47] Ivey KL, Hodgson JM, Kerr DA, Thompson PL, Stojceski B, Prince RL. The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a randomised controlled trial. Nutrition, Metabolism & Cardiovascular Diseases. 2015;25:46-51.

[48] Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, et al. Ingestion of a tea rich in catechins lead to a reduction in body fat and malondialdehyde-modified LDL in men. American Journal of Clinical Nutrition. 2005;81:122-9.

[49] Ou C, Ko J, Lin M. Antioxidative effects of intracellular extracts of yogurt bacteria on lipid peroxidation and intestine 407 cells. Journal of Food and Drug Analysis. 2006;14:304-10.

[50] Uskova MA, Kravchenko LV. Antioxidant properties of lactic acid bacteria–probiotic and yogurt strains. Vopr Pitan. 2009;78:18-23.

[51] Harisa GI, Taha EI, Khalil AF, Salem MM. Oral administration of Lactobacillus acidophilus restores nitric oxide level in diabetic rats. Australian Journal of Basic and Applied Sciences. 2009;3:2963-9.

[52] Lin MY, Chang FJ. Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. Digestive Diseases and Sciences. 2000;45:1617-22.

[53] Abubakr ASM, Hassan Z, Muftah M, Imdakim A, Sharifah NRSA. Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA). African Journal of Microbiology Research. 2012;6(34):6358-64.

[54] FDA: Food and Drug Administration. Food labeling: health claims; oat and coronary heart disease; final rule federal register doc. 97-1598, filed (2005), 1-22-1997.

[55] Vasiljevic T, Kealy T, Mishra VK. Effects of β-glucan addition to a probiotic containing yogurt. J Food Sci. 2007;72(7):405-11.

[56] Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann Clin Biochem. 1969;6:24-27.

[57] Singh PT, Malik KR, Katkamwar GS, Kaur G. Hypocholesterolemic effects of *Lactobacillus reuteri* LR6 in rats fed on high-cholesterol diet. Int J Food Sci Nutr 2015;66:71-75.