OPEN ACCESS Freely available online

Advances in Dairy Research

Research Article

Nutrigenomic Effects of Fermented Milk Containing *Bifidobacterium longum* Bb536 on Hepatic Cholesterologenic Genes in Hypercholesterolemic Rats

Amin Ismail[1,2], Azrina Azlan[1,2], Mohd Manap[3] and Sadeq Hasan Al-Sheraji[1,4*]

[1]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; [2]Halal Products Research Institutes, Laboratory of Halal Science Research, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; [3]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; [4]Department of Food Science, Faculty of Agriculture, Ibb University, Ibb, Yemen

ABSTRACT

The aim of this study was to investigate the effects of fermented milk contains *bifidobacteria* on plasma lipid levels and selected genes in hypercholesterolemic rats. Effects of *Bifidobacterium longum* Bb536 with prebiotics on body weight of rats, level of bile acids and selected genes were studied. Sprague-Dawley rats were randomly divided into groups. Negative control group received standard diet, positive control group received high-cholesterol diet, and intervention groups received high-cholesterol diet added with fermented milk contains *Bifidobacterium longum* Bb536 in presences of prebiotics. After 8 weeks, body weight, level of bile acids were measured and low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A reductase were investigated in rats' liver using quantitative real-time polymerase chain reaction. Fermented milk contains *Bifidobacterium longum* Bb536 increased weight of rats and level of bile acids over 8 weeks of intervention. Low density lipoprotein receptor gene level was increased significantly in fermented milk treated rats compared to positive control. Whereas, 3-hydroxy-3-methylglutaryl-coenzyme. A reductase gene level was decreased significantly in fermented milk treated compared to positive control. The present study showed that fermented milk contains *Bifidobacterium longum* Bb536 effectively regulated the expression of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes influencing the cholesterol metabolism in liver cells.

Keywords: Nutrigenomic; *Bifidobacteria;* Hypercholesterolemic; Fermented; Prebiotic

INTRODUCTION

Alhaj [1] have reported that the major cause of morbidity and mortality in the world is cardiovascular disease. Cholesterol is required for certain hormones and vitamin formation, being an essential component in the nerve cells and cell membranes. It is one of the risk factors in cardiovascular disease as well as other chronic health conditions including arthrosclerosis. However, Manson [2] has shown that a percent decrease in serum cholesterol level may decrease cardiovascular disease risk to about 3%.

One of the intervention measures aimed at maintaining a disease free condition to manage and/or control triglycerides and blood cholesterol levels in our diet is with drug treatment, for example the use of statins [3]. Other intervention to reduce blood cholesterol consumption of food with low fat and cholesterol [4], probiotic bacteria [5] and dietary fibre [6].

Attention has focused on the use of probiotic bacteria in commercial and scientific fields as a result of the different beneficial effects of these bacteria on humans. Probiotics are living microorganisms, which after consumption to a certain amount is capable of promoting the host health beyond normal nutrition [7]. They form part of the food components in food products as supplements. However, probiotics bacteria have been used for years in food fermentation, examples include some strains of *lactobacillus* and *bifidobacteria.*

***Corresponding author:** Sadeq Hasan Al-Sheraji, Department of Food Science, Faculty of Agriculture, Ibb University, Ibb, Yemen, Tel: +967774726464; E-mail: sadek_975@yahoo.com

Received date: February 19, 2019; **Accepted date:** March 05, 2019; **Published date:** March 13, 2019

Citation: Ismail A, Azlan A, Manap M, Al-Sheraji HS (2019) Characterization of Dairy Production Constraints, Existing Feeding Practices and Mineral Supplementation in Dairy Feeds in Two Districts of East Shoa Zone, Ethiopia. J Adv Dairy Res 7: 220.

Bifidobacteria is one of human intestinal microflora which assists in maintenance of good health [8]. Research has assessed the probiotic bacteria effects on lipid profile. Although the mechanism about cholesterol reduction by probiotic bacteria is yet unclear; some hypotheses are suggested to determine the hypocholesterolemic activity of probiotic bacteria, and bioactive peptides produced by their proteolytic activities. Probiotic bacteria could ferment non-digestible carbohydrates in intestinal to produce Short Chain Fatty Acids (SCFAs) such as propionic acid which is capable of reducing hepatic cholesterol synthesis. Pereira et al. [3,9-11] stated that probiotics are capable of inhibiting intestinal cholesterol absorption by binding bile acids and cholesterol to probiotic bacterial cells, absorption cholesterol by probiotic bacteria.

Different attempts have been adopted including supplementation with prebiotics ingredients, which are non-digestible by human gastrointestinal tract, to maintain required *bifidobacteria* levels. Promotion of growth in the colon and overall well-being of human health by *lactobacilli* and *bifidobacteria* have been reported [9]. Recently, the interest in prebiotics was to combine them with probiotic bacteria in food to improve the functional properties for special health importance [12].

Mangifera pajang Kosterm (*M. pajang*) belongs to the Anacardiaceae family and is ovoid in shape and is an underutilised fruit that is known in Malaysia (Sabah and Sarawak), Brunei, and Indonesia [13]. The size of the fruit is three times larger than commercial mango (*Mangifera indica*) [14], and the fruit pulp which represents 50%-67% of the total weight, is fibrous. In a previous study, [15] investigated the Soluble Dietary Fibre (SDF) and Insoluble Dietary Fibre (IDF) compositions and physicochemical properties for *M. pajang fibrous* (MPF) and reported that MPF contains appreciable amounts of beneficial chemical and physiochemical properties. The fibrous pulp of *M. pajang* fruit consisted of heteropolysaccharide and belonged to α and β-type of the pyran group [16]. In another study [17] demonstrated that MPF and its polysaccharides (MPFP) showed strong fermentation and non-digestibility properties, and thus it might be a prospective prebiotic that could be incorporated into food products. MPFP significantly improve physical and sensory properties of the yoghurt [18], also increased the viability and activity of *B. longum* BB 536in yoghurt, thus the production of short chain fatty acids as well as the proteolytic activity of these organisms significantly increased in the presences of MPFP [19]. *B. longum* Bb536 had significantly lower plasma total cholesterol, low-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol. In addition, faecal excretion of bile acids was markedly increased in the rats fed the yoghurt containing *B. longum* Bb536 [20]. Although, *Bifidobacterium longum* Bb536 administration significantly reduced plasma total cholesterol, liver lipid deposition and adipocyte size and these effects was significantly increased in the presence of *Mangifera pajang fibrous* polysaccharides [21]. However, till date no report has been carried on the effects of *Bifidobacterium longum* Bb536 on the regulation of genes encoding the LDLR and HMGCR genes. Thus, this study designed to investigate ability of *Bifidobacterium longum* Bb536 in the presence of *Mangifera pajang fibrous* polysaccharides on the regulation of LDLR and HMGCOR genes using real-time polymerase chain reaction (PCR) with a TaqMan assay.

MATERIALS AND METHODS

Materials

The fibrous of *M. pajang* fruit was prepared by removing its skin, peel and separating the seed from the pulp. The pulp was wet-milled and the juice was separated. The pomace was dried and ground. Crude polysaccharide was extracted using hot water, deproteinated, dialysed, concentrated, precipitated and lyophilized.

Chemicals

Bovine milk casein, alpha-cellulose, corn starch, lithocholic acid, and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Butylated hydroxytoluene (BHT) was purchased from Sigma-Aldrich Fine Chemicals. Vitamin mixture, mineral mixture, choline chloride, DL-methionine, cholesterol, cholic acid, chenodeoxycholic acid and deoxycholic acid were purchased from MP Biomedicals, California, USA.

Preparation of synbiotic yoghurt

Yoghurt was prepared according to method described by [20] with some modifications. Skim milk powder instant (NZMP, Fonterra, Auckland, New Zealand) was weighed, dissolved in water to constitute 8.25% skim milk (w/v) and divided into three experimental lots; one lot was fortified with 0.75 gL^{-1} non-fat dried milk (YCBB), the second lot with 0.75 gL^{-1} inulin (YCBBI) and the third lot with 0.75 gL^{-1} MPFP (YCBBM). After mixing the mixtures were separately homogenised with an APV homogeniser (Albertslund, Denmark) until all ingredients were dissolved in the milk. The homogenates were then pasteurised at 85°C for 30 min and cooled by immersion of the flasks in an ice water bath until they reached temperatures of 40°C-43°C. Exactly 3% (v/v) yoghurt starter culture; *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* and *B. longum* BB 536 were added to each lot. The mixtures were distributed into 100 mL plastic cups and were then incubated at 40°C, until they reached a pH of 4.55, followed by cooling to 4°C and storage at that temperature no longer than 7 days before feeding. The number of all bacteria in all yoghurt products was enumerated to be approximately as below:

YCBB contains 7.5 Log CFU/mL *Bifidobacterium longum* Bb536, 8.17 Log CFU/mL *Lactobacillus delbrueckii ssp. Bulgaricus* and 8.45 Log CFU/mL *Streptococcus thermophilus*. YCBBI contains 8.48 Log CFU/mL *Bifidobacterium longum* Bb536, 8.44 Log CFU/mL *Lactobacillus delbrueckii ssp. Bulgaricus* and 8.44 Log CFU/mL *Streptococcus thermophilus*. YCBBM contains 8.39 Log CFU/mL *Bifidobacterium longum* Bb536, 8.18 Log CFU/mL *Lactobacillus delbrueckii ssp. Bulgaricus* and 8.44 Log CFU/mL *Streptococcus thermophiles*.

Animal handling, feeding and induction of hypercholesterolemia

Forty male Sprague-Dawley rats at 4 weeks of age were housed in stainless steel cages in a room with relative humidity 60% and temperature 20°C-25°C, with 12 h light exposure in a daily cycle from 6 am to 6 pm. Experiment was carried out according to the guidelines for the use of animals approved by the Animal Care and Use Committee (ACUC) of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia with approval no. UPM/FPSK/PADS/BR-UUH/00362. All animals were fed on a basal diet for one week. After this acclimatization period, the rats were divided randomly into 5 experimental groups of 8 rats. First group was given a basal diet (cholesterol-free diet) and water during the experimental time of 8 weeks and considered as a negative control group (NC). The other four groups were nourished on the diet with cholesterol added at a level of 2.0% (w/w) (cholesterol-enriched diet), one of the 4 groups, which considered as a positive control group (PC), was nourished only on a cholesterol-enriched diet and tap water for an 8-week period. The other 3 groups were provided the cholesterol-enriched diet along with a variety of liquid yoghurt instead of normal water: group YCBB, milk fermented with 2% (v/v) *Lactobacillus delbrueckii subsp. Bulgaricus, Streptococcus thermophilus* (Chr. Hansen Laboratories, Copenhagen, Denmark) and 1% *B. longum* Bb536; group YCBBI, milk fermented with 2% (v/v) *L. delbrueckii subsp. Bulgaricus*, *S. thermophilus* and 1% *B. longum* Bb536; and group YCBBM, milk fermented with 2% (v/v) *L. delbrueckii subsp. Bulgaricus*, *S. thermophilus* and 1% *B. longum* Bb536. Throughout the feeding period, the environmental conditions were well controlled, and both diet and drink were available ad libitum.

Food intake and body weight

The animals were fed for an 8-week period, during which time body weight was recorded every 2 weeks (AND, HR-200, Singapore), and food intake was recorded daily.

Faeces collection

Faeces were collected on the last 2 days of the feeding period and kept frozen at -20°C for the analysis of bile acids.

Determination of bile acids in faeces

Extraction of bile acids from faeces was carried out according to the method of Kikuchi-Hayakawa [22], in brief lyophilized ground feces were accurately weighed (0.25 g) into a 16 × 150 mm screw-capped test tube and extracted with 5 mL of ethanol at 80°C after that the ethanol was evaporated by pure nitrogengases, and then the residue was dissolved in methanol and passed through a 0.45 µm membrane filter.

The level of extracted bile acids was determined according to the method of Roda et al. using an Agilent series 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto sampling injector, solvent degasser and Diode Array Detector (DAD). Thirty five microlitres of the extracted sample was injected on the Sphrosorb BDS C18 column (250 mm × 4.6 mm, ID 5 µm particle size). The operating parameters were set as follows: column temperature, 35°C; flow rate 1.0 mL/ min; UV-detector, 210 nm. The isocratic elution was with methanol/ acetonitrile/ 0.01% M phosphate buffer pH 5.3 (50/25/25, v/v/v) as a mobile phase. The standard bile acids used were cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid (Sigma Chemical Co., St Louis, MO, USA). Each sample was injected twice. The peak area of bile acids standard solution was used to prepare a standard curve. From these standard curves (Appendices D.7, D.8, D.9 and D.10), the concentration of bile acids in the test samples was calculated.

RNA isolation

Rats were sacrificed after 8 weeks of intervention and their livers preserved in RCL2® Solution (ALPHELYS, Toulouse, France) within 5-10 min of death. RNA was isolated from frozen liver samples using the RNeasy® Lipid Tissue Isolation Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The purity of the extracted RNA was determined by measuring the ratio of the optical density at 260 and 280 nm using a spectrophotometer (BioRad, USA). The total RNA concentration was determined by measuring the absorbance at 260 nm. The integrity and size distribution of the total RNA was determined by using a 1.5 agarose gel. The 18 s and 28 s RNA bands on gel electrophoresis were visualized under UV light using gel image instrumentation.

cDNA synthesis

RNA samples were reverse transcribed into first-strand cDNA using QuantiTect® Reverse Transcription Kit (QIAGEN GmbH, Hilden, Germany). According to the manufacturer's instructions, 1 µg of total extracted RNA was mixed with 2 µl of gDNA and completed to 14 µl with RNAse free water. The mixture was mixed gently and incubated at 70°C for 5 min. Then, the microcentrifuge tubes were placed on ice and the reverse-transaction rxn components were added: 1 µl of quantiscript reverse transcriptase, 4 µl of quantiscript RT Buffer, 1 µl of RT primer mix and the previous mixture (14 µl). The mixture was mixed gently and incubated for 15 min at 42°C Finally, the reaction was stopped by heating at 95°C for 3 min. cDNA samples were labeled and stored at –20°C for real time PCR work.

Primers and probes design

TaqMan Primers and probes specific for LDLR, HMG-COAR and β-actin genes were designed and synthesized by (Proligo France SAS) and supplied by First BASE Laboratories (Selangor, Malysia) from the gene sequence of rat (*Rattus norvegicus*) adopted from the NCBI (National Center for Biotechnology Information) GenBank Database (www.ncbi.nlm.nih.gov). GenBank accession number code for LDLR is NM- 175762, for HNG-COAR is NM_013134 and β-actin gene is NM_031144.

Quantitative real-time PCR

Real-time PCR was performed using the QuantiFast™ SYBR® Green PCR Kit (QIAGEN GmbH, Hilden, Germany). According to the manufacturer's instructions, a reaction (25µl)

containing 12.5µl of QuantiFast SYBR Green PCR master mix with 2µl of 400 nM of each forward and reverse primer, 1 µl of the probe (200 nM) and 1 µl of the template cDNA (10 ng/µl). Real-time PCR amplification of cDNA was performed for 40 cycles. After an initial incubation for 15 min at 95°C the PCR cycle consisted of a denaturation period for 10 s at 95°C and an annealing period for 30s at 60°C. Analysis of the gene expression data was performed using a Δ Δ CT method of relative quantification, according to a previous report by Kenneth et al. (2001). RotorGene analysis software (version 6.0) was used to analyze all the results from the PCR assays.

Statistical analysis

All the data are presented as the mean ± standard deviation (SD). Group differences were analysed using a one-way ANOVA (SPSS version 19.0., SPSS, Inc., Chicago, IL, USA) followed by a least significant difference (LSD) test. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Body weight gain, total food intake and food efficiency of rats

Table 1 shows the changes of body weight gain, total food intake, and food efficiency of rats between the baseline and in the week 8 of the treatment. At the beginning of experiment the body weight of all groups was between 133, 136 g and there is no significant ($p>0.05$) difference between all groups, after that the body weights of all treatment groups showed increases from the beginning until the end of the experiment period it reached 428 g (NC), 406 g (PC), 392 g (YCBB), 415 g (YCBBI), and 472 g (YCBBM).

Table 1: Body weight gain, total food intake, and food efficiency of rats fed a high- cholesterol diet for 8 weeks.

Group	Weight at baseline (g)	Weight at 8 weeks (g)	Body weight gain (g) a	Food intake (g)	Food efficiency (%)b
NC	135.88 ± 21.98^{a}	428.13 ± 27.59^{c}	287.42 ± 26.29^{c}	1029.84± 13.27^{d}	27.91 ± 2.4^{b}
PC	133.75 ± 12.08^{a}	406.50 ± 36.80^{ab}	282.00 ± 33.29b^{c}	1040.48 ± 16.16^{d}	27.10 ± 2.1^{a}
YCBB	135.09 ± 14.85^{a}	392.88 ± 19.29^{a}	257.63 ± 16.80^{a}	861.28 ± 12.24^{a}	29.91 ± 1.4^{c}
YCBBI	134.27 ± 11.67^{a}	415.79 ± 25.23^{bc}	287.51 ± 14.72^{c}	957.59 ± 14.15^{c}	30.02 ± 1.6^{c}
YCBBM	134.09 ± 13.15^{a}	472.64 ± 23.13^{d}	296.84 ± 19.97^{d}	932.54 ± 11.37^{b}	31.85± 2.3^{d}

[a]% Body weight gain=[(final weight - initial weight)/initial weight] × 100.

[b]Food efficiency (%)=(body weight gain/food intake) × 100.

NC=Negative Control, PC=Positive Control, YCBB=group treated with yoghurt culture+*B. longum* Bb536" YCBBI=group treated with yoghurt culture+*B. longum* Bb536+inulin and YCBBM=group treated with yoghurt culture+*B. longum* Bb536+MPFP. Each value represents means of 8 rats ± SD. Means with different small letter superscripts in the same column are significantly different ($p<0.05$).

The food was taken by rats throughout the experiment. The highest amount of food intake was by PC (1040 g) followed by NC (1029 g). However, the lowest amount was in YCBB (861 g). Also the average of food intake by YCBBI and YCBBM is 957 g and 932 g respectively. Results showed food intake of treated rats with different yoghurt significantly lower compared to control groups. Although food intake of treated rats with different yoghurt was significantly lower than other groups, however, the percentage of body weight gain of these groups was significantly higher than other groups at the end of the study maybe due to consumption of yoghurt that contains nutrient/ calorie more than the positive and negative control. The findings are in agreement with those found by [22] Kikuchi-Hayakawa, [23] Suzuki et al., mentioned that no significant ($p>0.05$) difference in body weight gain in rats treated with *L. acidophilus* or bifidobacteria.

Faecal bile acid excretion of experimental rats at the end of study

As tabulated in Table 2, there was no significant ($p>0.05$) difference in the levels of total bile acids between rats in NC and PC, however rats fed on YCBB, YCBBI and YCBBM diets excreted significantly higher level of bile acids than those PC and NC. It is noteworthy that the excretion of bile acids in rats fed on the YCG4I, YCG4M and YCBBM diets was significantly higher than those fed on YCG4 diet. This result demonstrates an opposite relationship between the level of bile acids excreted in the faeces and the total levels of TC, LDL-C or VLDL-C in rats fed with YCG4I, YCG4M and YCBBM diets.

The above results are in agreement with those reported by Kikuchi- Hayakawa et al. The present study proposed that hepatic cholesterol metabolism could be altered in order to supply more cholesterol for synthesis of bile acids. Imaizumi et

al. [24] and Suzuki reported that *Bifidobacterium* species 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. Moreover, *B. pseudocatenulatum* G4 or *B. longum* Bb536 cells can eliminate cholesterol through both assimilation and coprecipitation with deconjugated bile salts [25]. Consequently, *B. pseudocatenulatum* G4 or *B. longum* Bb536 caused an increment in the excretion of free bile salts in the faeces [26-30]. The excretion of bile acids was increased when MPFP and inulin were added due to increase the survival of *B. pseudocatenulatum* G4 or *B. longum* Bb536.

Table 2: Faecal bile acid excretion.

Treatment	Bile acids (mg/g)			
	Cholic acid	Chenodeoxycholic acid	Deoxycholic acid	Lithocholic acid
NC	28.67 ± 0.25^{a}	21.78 ± 0.26^{a}	05.82 ± 0.13^{a}	05.72 ± 0.41^{a}
PC	28.67 ± 0.25^{a}	21.78 ± 0.26^{a}	05.82 ± 0.13^{a}	05.72 ± 0.41^{a}
YCBB	31.62 ± 0.59^{c}	27.90 ± 0.37^{c}	10.57 ± 0.37^{c}	07.29 ± 0.17^{b}
YCBBI	31.62 ± 0.59^{c}	27.90 ± 0.37^{c}	10.57 ± 0.37^{c}	07.29 ± 0.17^{b}
YCBBM	35.04 ± 0.40^{d}	51.81 ± 0.76^{d}	12.37 ± 0.27^{d}	08.85 ± 0.56^{d}

NC: Negative Control; PC: Positive Control; YCBB: Group Treated with Yoghurt Culture+*B. longum* Bb536"; YCBBI: Group Treated with Yoghurt Culture+*B. longum* Bb536+inulin and YCBBM: Group Treated with Yoghurt Culture *B. longum* Bb536 + MPFP. Each value represents means of 8 rats ± SD. Means with different small letter superscripts in the same column are significantly different ($p<0.05$).

Regulation of LDLR mRNA level by *Bifidobacterium longum* Bb536 in presence of prebiotic

From the amplification plot for LDLR gene, the β -actin gene had CT values ranging from 18 - 19 cycles and LDLR gene had CT values between 19-23 cycles. The effects of *Bifidobacterium longum* Bb536 in presence of prebiotic on the regulation of LDLR gene are shown in Table 3. When cells were incubated with HLPDS and treated with *Bifidobacterium longum* BB536 in presence of prebiotics, LDLR mRNA level was increased significantly by 4.11, 7.46 and 8.45 fold in group treated with yoghurt culture+*B. longum* Bb536, group treated with yoghurt culture+*B. longum* Bb536+inulin and group treated with yoghurt culture+*B. longum* Bb536 + MPFP respectively, compared to untreated cells. The effect of *B. longum* Bb536 was significantly increased in presence of either inulin or MPFP as. LDLR activity was significantly upregulated by *Bifidobacterium longum* Bb536 in presence of prebiotic. Although inhibition of LDL oxidation by a range of different plant sources has been widely reported [25]. The LDLR modulation effects have not been described previously for the *Bifidobacterium longum* Bb536 in presence of prebiotics which used in this study.

Table 3: Regulation of LDLR mRNA level by *Bifidobacterium longum* Bb536 in presence of prebiotic.

Sample	Average CT of LDLR	Average CT of β -actin	Δ CT	Δ Δ CT	2 – Δ Δ CT (Fold)
NC	29.24 ± 1.05	26.91 ± 0.91	2.33	-1.65	3.14*b*
PC	29.34 ± 0.93	25.36 ± 0.57	3.98	0	1.00*a*
YCBB	29.63 ± 0.57	27.69 ± 0.87	1.94	-2.04	4.11*c*
YCBBI	30.29 ± 1.12	29.22 ± 0.79	1.07	-2.9	7.46*d*
YCBBM	28.31 ± 0.92	27.41 ± 0.86	0.9	-3.08	8.45*e*

Δ CT: CT of target gene (LDLR) - CT of reference gene (beta actin), Δ Δ CT: Δ CT of experimental cells- Δ CT of control. NC: Negative Control; PC: Positive Control; YCBB: Group treated with yoghurt culture+*B. longum* Bb536; YCBBI: Group Treated with Yoghurt Culture+*B. longum* Bb536+inulin and YCBBM: Group Treated with Yoghurt Culture+*B. longum* Bb536 + MPFP. Each value represents the mean ± SD of 4 rats.

Regulation of HMG-COAR mRNA level by *Bifidobacterium longum* Bb536 In presences of prebiotics

The effects of *Bifidobacterium longum* Bb536 in presence of prebiotic on the regulation of LDLR gene are shown in Table 4. When cells were incubated with HLPDS and treated with *Bifidobacterium longum* Bb536 in presence of prebiotics, HMGCR mRNA level was decreased significantly by 0.92, 0.69 and 0.48 fold in group treated with yoghurt culture+*B. longum* Bb536, group treated with yoghurt culture+*B. longum* Bb536+inulin and group treated with yoghurt culture+*B. longum* Bb536 + MPFP respectively, compared to untreated cells. Presence of prebiotics either inulin or MPFP showed significant decrease in HMGCR mRNA.

Table 4: Regulation of HMG-COAR mRNA level by *Bifidobacterium longum* Bb536 in presences of prebiotics.

Sample	Average CT of HMG-COAR	Average CT of β-actin	Δ CT	Δ Δ CT	2 – Δ Δ CT (Fold)
NC	27.05 ± 1.05	25.77 ± 0.41	1.28	0.51	1.49*d*
PC	26.70 ± 0.49	25.93 ± 0.95	0.77	0	1.00*c*
YCBB	26.76 ± 0.50	24.91 ± 0.21	1.85	1.08	0.92*b*
YCBBI	26.30 ± 2.60	24.22 ± 0.12	2.08	1.31	0.69*a*
YCBBM	27.01 ± 0.87	24.72 ± 0.45	2.29	1.52	0.48*a*

Δ CT: CT of target gene (HMG-COAR) -CT of reference gene (beta actin), Δ Δ CT: Δ CT of experimental cells- Δ CT of control.

NC: Negative Control; PC: Positive Control; YCBB: Group Treated with Yoghurt Culture+ *B. longum* Bb536; YCBBI: Group Treated with Yoghurt Culture+*B. longum* Bb536+inulin, and YCBBM: Group Treated with Yoghurt Culture+*B. longum* Bb536 +MPFP. Each value represents the mean ± SD of 4 rats.

CONCLUSION

Synbiotic yoghurt contains *Bifidobacterium longum* Bb536 in presence of prebiotics significantly increased the body weight of rats which excreted significantly higher level of bile acids than those PC and NC and effectively regulated the expression of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes influencing the cholesterol metabolism in liver cells.

ACKNOWLEDGEMENT

The authors are grateful for financial support by the Research University Grant Scheme (RUGS) (Project No: 02-01-09-0703RU) from the Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

REFERENCES

1. Alhaj OA, Kanekanian AD, Peters AC, Tatham AS. Hypocholesterolaemic effect of Bifidobacterium animalis subsp. lactis (Bb12) and trypsin casein hydrolysate. Food Chemistry. 2010;123: 430-435.
2. Manson JE, Tosteson H, Ridker PM, Satterfield S, Herbert P. The primary prevention of myocardial infarction. N Engl J Med. 1992;326: 406-16.
3. Pereira DIA, Gibson GR. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Appl Environ Microbiol. 2002;68:4689-93.
4. Lora KR, Morse KL, Gonzalez-Kruger GE, Driskell JA. High saturated fat and cholesterol intakes and abnormal plasma lipid concentrations observed in a group of 4- to 8-year-old children of Latino immigrants in rural Nebraska. Nutrition Research. 2007;27:483-91.
5. Schaafsma G, Meuling WJA, Bouley C. Effects of milk product, fermented by Lactobacillus acidophilus and with fructo-oligosaccharides added, on blood lipids in male volunteers. Eur J Clin Nutr. 1998;52:436-40.
6. Theuwissen E, Mensink RP. Water-soluble dietary fibers and cardiovascular disease. Physiol Behav. 2008'94:285-92.
7. Begley M, Hill C, Cormac GMG. Bile salt hydrolase activity in probiotics. Appl Env Microbiol.2006;72: 1729 -38.
8. Tannock GW. Analysis of the intestinal microflora: a renaissance. Antonie Van Leeuwenhoek.1999;76:265-78.
9. Nagaoka S, Futamura Y, Miwa K, Awano T, Yamauchi K. Identification of novel hypocholesterolemic peptides derived from bovine milk b-lactoglobulin. Biochem Biophys Res Commun. 2001;281:11-17.
10. Gibson GR. Fibre and effects on probiotics (the prebiotic concept). Clinical Nutrition Supplements. 2004;1:25-31.
11. Liong MT, Shah NP. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J Dairy Sci. 2005;88:55-66.
12. O'Neill J. the lifelong benefits of inulin and oligofructose. Cereal Foods World. 2008;53:65-68.
13. Hassan FA, Ismail A, Abdulhamid A, Azlan A. Identification and quantification of phenolic compounds in bambangan (Mangifera pajang Kort.) peels and their free radical scavenging activity. J Agri Food Chem. 2011;59:9102-11.
14. Hassan FA, Ismail A, Abdul Hamid A, Azlan A, Al-sheraji SH. Characterisation of fibre-rich powder and antioxidant capacity of Mangifera pajang K. fruit peels. Food Chemistry 2011;126:283-88.
15. Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM. Functional properties and characterization of dietary fiber from Mangifera pajang Kort Fruit Pulp. J Agric Food Chem. 2011;59:3980-985.

16. Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusuf RM. Survival and activity of bifidobacteria during refrigerated storage of yoghurt containing Mangifera pajang fibres polysaccharides. J Food Sci.2012;77:624-30.
17. Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusuf RM. Fermentation and non-digestibility of Mangifera pajang fibrous and its polysaccharide. J Func Food.2012;4:933-40.
18. Al-Sheraji SH, Alburihi S, Nasser YH, Nagi YM. Effects of Polysaccharides from Mango' Peel on Physicochemical and Sensory Properties of Non-Fat Yoghurts. Adv Dairy Res. 2017;5:181.
19. Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM. Purification, characterization and antioxidant activity of polysaccharides extracted from the fibrous pulp of Mangifera pajang fruits. LWT-Food Scie Tech. 2012;48:291-96.
20. Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusuf RM. Hypocholesterolaemic effect of yoghurt containing Bifidobacterium pseudocatenulatum G4 or Bifidobacterium longum Bb536. Food Chem.2012;135:356-61.
21. Al-Sheraji SH, Ismail A, Azlan MY, Hassan FA. Effects of Bifidobacterium longum Bb536 synbiotic on lipid profile, toxicity and histopathologic changes in hypercholesterolaemic rats. Benef Microbes. 2015;6:661-68.
22. Kikuchi HH, Onodera N, Matsubara S, Yasuda E, Shimakawa Y.Effects of soya milk and bifidobacterium-fermented soya milk on plasma and liver lipids, and faecal steroids in hamsters fed on a cholesterol-free or cholesterol-enriched diet. Br J Nutr. 1998;79:97-105.
23. Suzuki Y, Kaizu H, Yamauchi Y. Effect of cultured milk on serum cholesterol concentrations in rats fed high-cholesterol diets. Nihon Chikusan Gakkaiho. 1991;62:565-71.
24. Imaizumi K, Hirata K, Zommara M, Sugano M, Suzuki Y. Effects of cultured milk products by Lactobacillus and Bifidobactrium species on the secretion of bile acids in hepatocytes and in rats. J Nutr Sci Vitaminol. 1992;38:343-51.
25. Tahri K, Crociani J, Ballongue J, Schneider F. Effects of three strains of bifidobacteria on cholesterol. Lett Appl Microbiol. 1995;21:149-151.
26. Salleh MN, Runnie I, Roach PD, Mohamed S, Abeywardena MY. Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in hepg2 cells by tropical plant extracts. J Agric Food Chem. 2002;50:3693-697
27. Dambekodi PC, Gilliland SE. Incorporation of cholesterol into the cellular membrane of Bifidobacterium longum. J Dairy Sci. 1998;81:1818-24.
28. El-Gawad IA, El-Sayed EM, Hafez SA, El-Zeini HM, Saleh FA.The hypocholesterolemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. Int Dairy J. 2005;15:37-44.
29. Roda A, Piazza F, Baraldini M. Separation techniques for bile salts analysis J Chromatogr B Biomed Sci Appl. 1998;717:263-78.
30. Suzuki Y. Effects of fermented milk on lipid metabolism. Snow Brand Reports. 1995;105:67-108.