Food Chemistry 119 (2010) 1121–1126

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cholesterol-lowering effect of phytosterol-containing lactic-fermented milk powder in hamsters

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ARTICLE INFO

Article history:
Received 17 June 2009
Received in revised form 21 July 2009
Accepted 17 August 2009

Keywords:
Blood lipid
Fermented milk
Hypercholesterolemia
LDL-C/HDL-C
Phytosterol

ABSTRACT

Male hamsters were fed six different diets for 4 weeks. Group 1, fed with control diet of regular rodent chow. Group 2, fed with high-fat-high-cholesterol diet containing cholesterol and corn/coconut oil mixture. Group 3, the positive control fed with high-fat-high-cholesterol diet containing 0.74% (w/w) phytosterols. Groups 4–6 were the high-fat-high-cholesterol diet mixed with phytosterol-containing lactic-fermented milk powder (PSFMP) which contains 0.37%, 0.74% and 1.85% (w/w) of phytosterols, respectively. Results demonstrated that PSFMP could significantly decrease ($P < 0.05$–$P < 0.001$) the levels of total cholesterol (serum cholesterol), serum triacylglycerol, liver lipids and atherogenic index (LDL-C/HDL-C), while it could also significantly ($P < 0.001$) increase the level of fecal cholesterol. The pronounced hypolipidemic effects of PSFMP might be attributed to its ability to enhance cholesterol excretion. These results suggest that PSFMP could be used as a potential cholesterol-lowering ingredient in the management of hypercholesterolemia.

1. Introduction

Atherosclerosis and its related complications are the leading causes of death in western world and many developed countries. A consistent and strong association between total cholesterol level and atherosclerosis has been well documented. Dyslipidemia, including elevation of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triacylglycerol (TG) concentrations and a decrease in high density lipoprotein cholesterol (HDL-C) concentration in the blood, is the major risk factor of atherosclerosis (Glass & Witztum, 2001). The prevalence of atherosclerosis is increasing all over the world due to the adaptation of Western life-style and is likely to reach epidemic proportions in the coming decades (Bonow, Smaha, Smith, Jr-Mensah, & Lenfant, 2002).

Phytosterols occur widely in small quantities in plant foods, such as vegetable oils, seeds, and nuts (Ling & Jones, 1995; Ramadan, Zayed, & El-Shamy, 2007). They include sitosterol, campesterol, stigmasterol and other phytosterols. Phytostanols are a fully-saturated subgroup of phytosterols (contain no double bonds). (Moreau, Whitaker, & Hicks, 2002). Phytosterols and phytostanols may interfere with the intestinal cholesterol absorption by inhibiting cholesterol incorporation into micelles in the lumen of the intestine (Moghadasian & Frohlich, 1999; Ntanios & Jones, 1999). Recent studies showed that hamster administered with plant sterol esters in the high cholesterol high fat diet could significantly lower the plasma total cholesterol and LDL-C (Meijer, Bressers, de Groot, & Rudrum, 2003). Piironen, Lindsay, Miettinen, Toivo, and Lampi (2000) concluded from a literature survey that 1.8–2 g/d of plant stanols or sterols in the adult human diet could be an ideal dose for lowering blood cholesterol.

Latic-fermented milk (yogurt) is a dairy product containing organic acids produced by lactic acid bacteria during the fermentation. Many studies indicated that yogurt could reduce serum cholesterol level in rat and human (Akalin, Gonc, & Duzel, 1997; Xiao et al., 2003). It was demonstrated that intestinal lactic acid bacteria, such as *Lactobacillus acidophilus*, caused bile salts to deconjugate and coprecipitate with cholesterol under anaerobic conditions (Hepner, Fried, Stjeor, Fusetti, & Morin, 1979). Chiu, Lu, Tseng, and Pan (2006) found that Lactobacillus-fermented milk fed to hamsters was very effective in reducing cholesterol in blood and in liver. It was proposed that mechanisms of the hypocholesterolemic activity of lactic acid bacteria may involve the inhibition of exogenous cholesterol absorption from the small intestine by the binding of cholesterol and bile acids with the bacterial cells, as well as suppressing bile acid resorption by deconjugation as a function of the bacterial bile salt hydrolase activity (De Smet, De Boever, & Verstraete, 1998; Gilliland, Nelson, & Maxwell, 1985).

Recent report indicated that the phytosterol-enriched milk and fermented skim milk significantly reduced LDL-C (Ho, Chein, &

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doi:10.1016/j.foodchem.2009.08.023

Hwang, 2007; Plana et al., 2008). The introduction of phytosterol-enriched fermented milk in powder form may be advantageous over liquid form in controlling high cholesterol, because the fermented milk in liquid form does not have long shelf-life and prone to bacteriological changes. However, efficacy of powder form in controlling cholesterol level must be verified. Therefore, the aim of this study was to investigate the effect of a lactic-fermented milk powder, supplemented with plant sterols/stanols mixture, on lipid metabolism (including TG, TC, and lipoprotein cholesterol concentrations in serum, liver cholesterol, liver TG and fecal neutral sterols) of hyperlipidemic hamster. Syrian golden hamster was chosen as the animal model because the cholesterol and bile acid metabolism in hamster are similar to human and this model has been widely used in atherosclerosis research (Nistor, Bulla, Filip, & Radu, 1987; Spady & Dietschy, 1988).

2. Materials and methods

2.1. Phytosterol-containing lactic-fermented milk powder preparation

Whole milk containing phytosterol/phytostanol mixture (3.7 mg/mL) was heated using a plate heat exchanger at a temperature of 120 °C for 3 s and then cooled to 40 °C. The milk was inoculated with $1 \times 10^{5-6}$ CFU/mL probiotic mixture (*L. acidophilus, Bifidobacterium lactis, Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and incubated at 40 °C for 7 h, then cooled and stored at 4 °C. Phytosterol-containing lactic-fermented milk powder was obtained by lyophilising the fermented milk along with phytosterol/phytostanol mixture in various proportions. The phytosterol/phytostanol mixture was composed of 70–75% β-sitosterol, 12–16% β-sitostanol, 7–9% campesterol, and 4–6% campestanol. The test product powder (100 g) contained 17.0 g protein, 3.6 g fat, 69.47 g nitrogen-free extract (NFE), 5.25 g crude fibre, 1.6 g ash and 3.08 g phytosterol/phytostanol.

2.2. Experimental animals and diets

Eighty male 6-week-old Golden Syrian hamsters were obtained from National Laboratory Animal Center (Taipei, Taiwan). Hamsters were housed in polycarbonate cages (4 per cage) and maintained in a controlled environment (22 ± 2 °C) with a 12-h light/dark cycle (lights on 07:00–19:00 h). These animals were maintained according to the guidelines established in Taiwan Government Guide for the Care and Use of Laboratory Animals. The animals were given free access to regular rodent chow (LabDiet®, 5001 Rodent diet, Purina, St. Louis, MO, USA) and water for 2 weeks to acclimatise. Eight animals which were extremely (10%) overweight or underweight were eliminated. The remaining of 72 hamsters was weighed and randomly divided into 6 experimental groups of 12 animals each and were fed with 6 different diets for 4 weeks. Group 1 was the control (C) group, hamsters were fed with rodent chow diet. The other five groups of hamsters were fed with high-fat-high-cholesterol diet, which was prepared from regular rodent chow supplemented with 0.5% (w/w) cholesterol and oil mixture (corn oil/coconut oil = 1:1, w/w) to adjust the fat content to 12% (w/w). Group 2 was the high fat control (HFC) group, hamsters were fed with the high-fat-high-cholesterol diet without any additional supplements. Group 3 was the positive control (PC) group, 0.74% (w/w) phytosterols (containing 75% β-sitosterol, 10% campesterol and matrix, Merck, Darmstadt, FRG) were added to the high-fat-high-cholesterol diet. Groups 4–6 were the experimental groups, animals were fed with various dosages of lyophilised powder of phytosterol-containing lactic-fermented milk powder (PSFMP) along with high-fat-high-cholesterol diet. Group 4 were the basic dosage (1×) group, the diet contained 12% (w/w) PSFMP supplemented with 370 mg of phytosterol/phytostanol mixture per 100 g of diet. Group 5 and 6 were the 2 and 5 folds (2× and 5×) basic dosage groups, the diets consisted of 24% and 60% PSFMP supplemented with 740 mg and 1850 mg of phytosterol/phytostanol mixture per 100 g of diet, respectively. The detailed compositions of the experimental diets are shown in Table 1. All diets were prepared every week and stored at −20°C. A chow-based, rather than a semi-purified, diet was used because it can result in the hamster's lipoprotein profile (predominately LDL-C) more similar to human's profile (Ausman, Rong, & Nicolosi, 2005; Terpstra, Holmes, & Nicolosi, 1991).

2.3. Animal experiment and sample collection

During the 4-week experimental period, hamsters were given free access to experimental diets and tap water. Tap water was refreshed every 3 days and experimental diets were refreshed every day. Body weights were recorded twice a week. Fecal samples from each cage were collected and combined for 2 successive days before animals were sacrificed and stored at −70 °C for fecal neutral sterols determination. At termination of the study, hamsters were fasting overnight (~16 h), then weighed and anesthetised prior to killing by carbon dioxide inhalation. Blood samples were drawn from the abdominal vein and collected in tubes. Tubes were placed at 37 °C in water bath for 1.5 h to coagulated blood and then kept at 4 °C in a refrigerator for 1 h. Serum was separated from the blood by centrifugation (365g) for 10 min at 4 °C then stored at −20 °C for further measurement of serum total cholesterol (TC), serum triacylglycerol (TG) and lipoprotein cholesterol concentrations. After exsanguinations, liver was excised, washed in ice-cold saline, weighed, and frozen in liquid nitrogen then stored at −70 °C for liver lipids analysis.

2.4. Serum total cholesterol (TC) determination

Serum total cholesterol (TC) concentration was determined by the enzymatic CHOD–PAP (cholesterol oxidase–peroxidase aminophenazone) method using a total cholesterol test kit (Merck,

Table 1
Composition and nutrients of the experimental diets.

	Feed composition (%)					
	C[a]	HFC[b]	PC[b]	1×[b]	2×[b]	5×[b]
Ingredients						
Chow diet	100	91.6	90.86	78.1	64.5	23.9
Oil		7.9	7.9	8.4	9	10.6
Test product[c]				12	24	60
Casein				1	2	5
Phytosterols			0.74			
Cholesterol		0.5	0.5	0.5	0.5	0.5
Nutrients						
Protein	23	21.1	20.9	21.0	20.9	20.7
Fat	4.5	12.0	12.0	12.0	12.0	12.0
NFE[d]	56	51.3	50.9	52.1	52.8	55.1
Fibre	6	5.5	5.5	5.3	5.1	4.6
Phytosterol			0.74	0.37	0.74	1.85
Cholesterol		0.5	0.5	0.5	0.5	0.5
Calorie (kcal/100 g)	356.5	397.7	395.1	400.2	403.1	411.1

[a] The guaranteed analysis of chow:crude protein not less than 23.05%, crude fat not less than 4.5%, crude fibre not more than 6.0%, ash not more than 8.0%.

[b] HFC, high-fat-high-cholesterol control; PC, positive control (group HFC plus phytosterol standards); 1×, group HFC with basic dosage of phytosterols in test product; 2×, group HFC plus twice basic dosage; 5×, group HFC plus five times basic dosage.

[c] The test product powder (100 g) contained 17.0 g protein, 3.6 g fat, 69.47 g NFE, 5.25 g crude fibre, ash 1.6 g and 3.08 g phytosterol/phytostanol.

[d] NFE, nitrogen-free extract (NFE = 100 − (protein + fat + fibre + ash)).

Darmstadt, Germany) (Allain, Poon, Chan, Richmond, & Fu, 1974). Cholesterol esters were hydrolysed to free cholesterol by cholesterol esterase. The free cholesterol produced was oxidised by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide (H_2O_2), which was oxidatively coupled with 4-aminophenazone and phenol via the presence of peroxidase to yield quinoneimine with maximum absorption at 500 nm. An aliquot of 5 μL serum was incubated with 200 μL enzyme reagent in a 37 °C water bath for 10 min and the absorbance at 500 nm was measured. Concentrations of TC in the samples were determined from a standard curve constructed by using cholesterol standard (Merck).

2.5. Serum triacylglycerols (TG) determination

Serum triacylglycerols (TG) was analysed according to the fully-enzymatic GPO (glycerol phosphate oxidase)–PAP method using a total triacylglycerol test kit (Merck, Darmstadt, Germany) (Bucolo & David, 1973). Triacylglycerols were hydrolysed to glycerol and fatty acids by lipase. Under a successive enzymatic reactions catalysed by glycerol kinase and glycerol-3-phosphate oxidase, H_2O_2 was produced and oxidatively coupled with 4-aminophenazone and phenol via the presence of peroxidase to yield quinoneimine with maximum absorption at 500 nm. An aliquot of 5 μL serum was incubated with 200 μL enzyme reagent in a 37 °C water bath for 15 min and the absorbance at 500 nm was measured. Concentrations of TG in the samples were determined from a standard curve constructed by using triacylglycerol standard (Merck, Darmstadt, Germany).

2.6. Lipoprotein isolation and determination

Serum samples were isolated by continuous density gradient ultracentrifugation. The lipoprotein fractions of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) were isolated from serum by preparative ultracentrifugation at $d < 1.019$ g/mL, $d = 1.019–1.063$ g/mL and $d = 1.063–1.21$ g/mL, respectively, using a Beckman Optima™ LE-80K ultracentrifuge (Beckman, Palo Alto, CA) and a Ti 50.4 rotor. Cholesterol concentrations of each isolated lipoprotein were determined according to the enzymatic CHOD–PAP method as described previously.

2.7. Hepatic lipids analysis

Liver lipid was extracted by the method of Folch and Solane (1957). Liver tissue (0.2 g) was homogenised with chloroform/methanol (2/1, v/v) to a final volume of 20 times the tissue sample (0.2 g in 4 mL of solvent mixture) in an ice bath. The homogenate was filtered (with the Whatman No. 1 filter paper) to obtain the liquid phase and replenished the volume to 5 mL. An aliquot of 10 μL filtrate with 5 μL Triton X-100 were evaporated under a nitrogen stream in an eppendroff. TC and TG concentrations in the filtrates were determined according to the enzymatic CHOD–PAP and GPO–PAP methods. Liver TC and TG were express as mg/g liver.

2.8. Fecal neutral sterols determination

Lyophilised fecal samples were ground to a fine powder and extracted with 20-fold weight to volume ratio of Folch solution (chloroform:methanol = 2:1; v/v) at room temperature for 12 h in a screw-capped glass tube. The extract was filtered through Whatman No. 1 filter paper and the filtrate was replenished the volume to 5 mL. Fecal neutral sterols (cholesterol, campesterol and β-sitosterol) were analysed by reverse phase high performance liquid chromatography (HPLC) (Hitachi, Tokyo, Japan) with UV–VIS detector and Hypersil HS C18 column (4.6 mm ID × 250 mm, 5 μm particle size, ThermoQuest Hypersil Division, Runcorn, UK) using a method modified from Holen (1985). The mobile phase was methanol/acetonitrile (56/44, v/v), flow rate was 1.0 mL/min and detected with short-wave UV detection (205 nm). Sterol concentrations of samples were determined from a standard curve constructed with cholesterol and phytosterols (containing 10% campesterol and 75% β-sitosterol, Merck) standards.

2.9. Statistical analysis

All data were shown as mean ± standard deviation. Significant differences were determined using Student's t test where differences were considered significant if $p < 0.05$. All of the samples were measured in triplicate. Serum and liver lipid compositions data in each group of animals were compared with HFC group.

3. Results and discussion

3.1. Dosage in the diets

In this study, we intend to test the efficacy of the phytosterol-containing lactic-fermented milk powder (PSFMP) on lowering blood lipid of hamsters. From literature reports (Piironen et al., 2000), we estimated that the adequate content of phytosterols in a healthy adult's diet is 1.85 g in 500 g (dry weight of diet)/day, that is equivalent to 0.37%. Therefore, we used 0.37% phytosterol mixture (which consists of sterols/stanols at about 4/1 ratio) in the hamster's diet as the basic dose (1×). Considering the difference between human and hamster, diets containing 0.74% (2×) and 1.85% (5×) phytosterol mixtures were also examined for the blood lipid lowering effect. Since PSFMP contained 3.08 g/100 g phytosterols, the amounts of PSFMP used in the 1×, 2×, and 5× doses groups were 12, 24 and 60 g/100 g diet, respectively. The detailed compositions of the experimental diets are listed in Table 1.

3.2. Hamsters condition and body weight gain

There were no significant differences in the mean final body weights among the different groups except for groups 2× and 5× at termination of the study (Table 2). Both groups 2× and 5× had more body weight gain than the hyperlipidemic control (group HFC) ($p < 0.05$). This result was similar to the study of Akalin et al. (1997), they showed that mice fed yogurt or acidophilus yogurt for 28 days significantly increased their body weights. This might be contributed by milk protein.

3.3. Serum lipid and lipoprotein profiles

Table 3 shows the changes of hamster serum lipids and lipoprotein profiles after 4 weeks of feeding period. Hamster serum total cholesterol (TC) and triacylglycerol (TG) of the control group (C) were 116 and 108 mg/dL, respectively. Serum TC and TG in high-fat-high-cholesterol diet group (HFC) were significantly raised to 559 and 232 mg/dL ($p < 0.001$). This indicated that 12% fat and 0.5% cholesterol diet has successfully induced hyperlipidemia in the test animals. As compared with the high-fat-high-cholesterol diet group, serum TG levels of all experimental groups (PC, 1×, 2× and 5×) were not significantly different. When comparing serum TC, two groups (2× and 5×) fed PSFMP exhibited significant lower TC value than HFC group and showed dose dependent cholesterol-lowering effect. Serum TC were reduced (compared with the HFC group) by 37.6% and 65.1% in 2× and 5× groups, respectively.

Table 2
The body weights and liver weights of the different groups of test animals[a].

	C	HFC	PC	1×	2×	5×
Body weight (g)						
Initial	108.0 ± 4.1	109.5 ± 3.5	108.8 ± 4.0	108.8 ± 4.5	107.8 ± 2.8	107.1 ± 3.5
Final	114.5 ± 3.7	117.3 ± 4.4	120.2 ± 4.6	119.2 ± 4.2	122.7 ± 4.7**	127.9 ± 4.8***
Liver weight (g)	3.32 ± 0.31***	5.30 ± 0.22	4.80 ± 0.32***	5.06 ± 0.43	5.00 ± 0.44*	4.44 ± 0.35***
Liver wt/body wt (g/100 g)	2.90 ± 0.27***	4.52 ± 0.14	3.99 ± 0.25***	4.24 ± 0.26*	4.07 ± 0.34***	3.47 ± 0.23***

[a] Values are shown as the mean ± SD (n = 12).
* $p < 0.05$ significantly different when compared with HFC group by Student's t test.
** $p < 0.01$ significantly different when compared with HFC group by Student's t test.
*** $p < 0.001$ significantly different when compared with HFC group by Student's t test.

Table 3
Total cholesterols (mg/dL) and triacylglycerol (mg/dL) concentrations in different groups of test animals[a].

	C	HFC	PC	1×	2×	5×
Triacylglycerol	107.5 ± 17.0***	231.8 ± 71.5	227.1 ± 87.6	281.3 ± 112.7	251.7 ± 74.9	245.8 ± 93.6
Total cholesterol	116.5 ± 16.0***	559.3 ± 54.2	371.8 ± 46.4***	486.0 ± 111.1	349.4 ± 62.3***	195.4 ± 13.7***
(VLDL + IDL)-C	14.1 ± 6.8***	111.8 ± 31.5	61.5 ± 17.3***	102.5 ± 39.0	69.5 ± 16.0***	37.4 ± 13.0***
LDL-C	29.9 ± 5.4***	301.5 ± 40.5	191.6 ± 45.5***	218.8 ± 49.9***	147.9 ± 38.2***,#	31.4 ± 8.4***
HDL-C	58.8 ± 11.1***	118.7 ± 23.0	107.5 ± 12.5	120.7 ± 17.8	110.6 ± 14.7	111.6 ± 13.0
LDL-C/HDL-C	0.52 ± 0.11***	2.61 ± 0.54	1.82 ± 0.54**	1.85 ± 0.54**	1.34 ± 0.31***,#	0.29 ± 0.10***

[a] Values are shown as the mean ± SD (n = 12).
** $p < 0.01$ significantly different when compared with HFC group by Student's t test.
*** $p < 0.001$ significantly different when compared with HFC group by Student's t test.
# $p < 0.05$ significantly different while 2× group compared with PC group by Student's t test.

In lipoprotein profile, VLDL-C + IDL-C ($p < 0.001$) and LDL-C ($p < 0.001$) were significantly lowered by 2× and 5× PSFMP diets. Compared with the HFC group, LDL-C of 2× and 5× groups were reduced by 50.9% ($p < 0.001$) and 89.7% ($p < 0.001$). As for HDL-C levels, dietary 0.74% phytosterols or 1×, 2× and 5× PSFMP supplementation showed no effect. We noticed a significant increase of the ratio of LDL-C/HDL-C, which is an index of atherogenicity, in high-fat-high-cholesterol diet (HFC group) when compared with the control group. Comparing with the HFC group, there was a significant decrease in the LDL-C/HDL-C of the groups with dietary supplementation of 0.74% phytosterols ($p < 0.01$) or 1× ($p < 0.01$), 2× ($p < 0.001$) and 5× ($p < 0.001$). Our data also showed that the 2× group (diet containing 0.74% phytosterols in fermented milk) could significantly lower LDL-C and LDL-C/HDL-C of hamster by 22.9% ($p < 0.05$) and 27.8% ($p < 0.05$) comparing with PC group (diet containing 0.74% phytosterols without fermented milk).

Xiao et al. (2003) reported that feeding 20% fermented milk powder could reduce 22% total cholesterol and 40% LDL-C of rat fed 0.5% cholesterol-containing diet. Previous study of our lab showed that feeding 25.9% phytosterol-containing milk powder (containing 0.72% phytosterol) reduced 31% total cholesterol and 52.0% LDL-C of hamster on 0.5% cholesterol-containing diet (Ho et al., 2007). In this study, feeding 24% PSFMP (2× group, containing 0.74% phytosterol) reduced 37.6% total cholesterol and 50.9% LDL-C of hamster on 0.5% cholesterol-containing diet. Our results clearly showed that the hypocholesterolemic effect of phytosterol-containing lactic-fermented milk powder is better than either fermented milk powder alone or phytosterol-containing milk powder.

The chemical structures of phytosterols are similar to cholesterol, they can displace cholesterol from bile salt micelles and compete the site for absorption in the brush border, thus suppress the absorption of exogenous (dietary) cholesterol (Ikeda, Tanaka, Sugano, Vahuny, & Gallo, 1988). Most human and animal studies demonstrated that dietary phytosterols significantly reduce total cholesterols, especially LDL-C concentration, without affecting HDL-C or serum TG concentrations. Our results showed the significant reductions in hamster's serum TC, non HDL-C concentrations and LDL-C/HDL-C ratio, while serum TG level was not affected. These results were in consistent with the previous research findings (Meijer et al., 2003; Ntanios & Jones, 1999). Chiu et al. (2006) found that the Lactobacillus-fermented milk fed to hamsters was very effective in reducing LDL-C in the blood. Some studies also indicated that the fermented milk could reduce cholesterol due to the inhibition of lactic acid bacteria on exogenous cholesterol absorption from the small intestine by the binding of cholesterol and bile acids with the bacterial cells (De Smet et al., 1998; Gilliland et al., 1985). Our result showed that with the same dose of phytosterols 2× group is more effective than PC group in reducing LDL-C and LDL-C/HDL-C ratio, indicating diet containing phytosterols in fermented milk might have better cholesterol-lowering effect than phytosterols alone without fermented milk in hamster model.

3.4. Liver weight and liver lipid composition

All hamsters had developed fatty liver after feeding high fat and high cholesterol diets for 4 weeks by visual observation after sacrifice. The PC group and PSFMP diet groups (2× and 5×) showed significant reduction in liver weight as compared with HFC (Table 2). Our data showed that hamster fed with 0.74% phytosterol (PC group) and PSFMP were significant lower in relative liver weight comparing with HFC group (the relative liver weight decreased by 11% in PC group and 4%, 8.9%, 22% in 1×, 2× and 5× PSFMP groups, respectively). The result was similar to Ntanios and Jones' (1998) finding, they reported that hamsters fed with high cholesterol diet and 1% sitostanol could reduce 20% relative liver weight. Table 4 indicated that hepatic cholesterol and TG concentrations were significantly increased in HFC group as compared with control group (C). Our result showed that hamsters in the PC group and PSFMP group had significantly lower hepatic cholesterol (decreased by 30.7% in PC group and 15.1%, 41.3%, 79.6% in 1×, 2× and 5× PSFMP groups, respectively) and TG (decreased by 25%, 27% in 2× and 5× PSFMP groups, respectively) concentrations

Table 4
Hepatic lipid profiles in the different groups of test animals[a].

	C	HFC	PC	1×	2×	5×
Cholesterol (mg/g liver)	6.1 ± 1.5***	66.8 ± 11.2	46.3 ± 8.0***	56.7 ± 6.7*	39.2 ± 3.9***,###	13.6 ± 2.6***
triacylglycerol (mg/g liver)	11.1 ± 3.6*	15.2 ± 5.1	15.9 ± 3.3	15.1 ± 2.7	11.4 ± 3.0*,###	11.1 ± 1.7*

[a] Values are shown as the mean ± SD (n = 12).
* $p < 0.05$ significantly different when compared with HFC group by Student's t test.
*** $p < 0.001$ significantly different when compared with HFC group by Student's t test.
$p < 0.001$ was significantly different while 2× group compared with PC group by Student's t test.

Table 5
The fecal cholesterol and phytosterol contents in the different groups of test animals[a].

	C	HFC	PC	1×	2×	5×
Cholesterol (mg/g feces)	nd	1.1 ± 0.3	7.5 ± 0.6***	3.9 ± 0.3***	8.0 ± 1.5***	18.8 ± 1.3***
Phytosterol (mg/g feces)	nd	nd	20.8 ± 2.9***	8.0 ± 0.9***	19.2 ± 1.4***	70.6 ± 3.7***

[a] Values are shown as the mean ± SD (n = 12).
*** $p < 0.001$ significantly different when compared with HFC group by Student's t test.
nd = not detected.

when compared with HFC group. Lin, Meijer, Vermeer, and Trautwein (2004) reported that feeding 0.24% phytosterol esters could decrease hepatic cholesterol concentration by 44.3% in hamsters which were fed with high fat diet for 5 weeks. Earlier study also indicated that the increase in hamster's relative liver weight by high-fat-high-cholesterol diet might be due to the rise in accumulation of cholesterol and TG in liver (Lin et al., 2004). Compared with PC group, liver cholesterol and TG concentrations of PSFMP 2× group were significantly decreased 15.3% and 28.3%, respectively. Chiu et al. (2006) found Lactobacillus-fermented milk fed to hamsters could reduce both cholesterol and TG levels in the liver. The addition of phytosterols in lactic-fermented milk can thus show additional lowering effect of liver lipid than phytosterol alone.

3.5. Fecal cholesterol and phytosterols analysis

Table 5 showed the fecal cholesterol and phytosterol contents in the different groups. In the control group (group C), fecal cholesterol and phytosterol contents were not detected. In the HFC group, fecal cholesterol content was 1.1 mg/g feces while phytosterols (campesterol and β-sitosterol) were still absent in the HFC group. Compared with group C, feces of HFC group showed slight increase in cholesterol content possibly due to adding 0.5% cholesterol in the diet. Our data showed that diets supplemented with phytosterols (PC group) or PSFMP (1×, 2×, 5× groups) significantly increased fecal cholesterol and phytosterol concentrations ($p < 0.001$), and the increase was proportional to PSFMP contents of the diets.

It has been reported that the net effect of dietary cholesterol absorption, endogenous cholesterol synthesis and biliary cholesterol excretion regulates body cholesterol balance (Jolley, Woollett, Turley, & Dietschy, 1998; Lu, Lee, & Patel, 2001). Our results confirmed that the cholesterol-lowering effect of phytosterol-containing lactic-fermented milk powder is not only due to the inhibition of intestinal dietary cholesterol absorption but also due to the interference of biliary cholesterol re-absorption.

4. Conclusions

Feeding with high fat (12%) and high cholesterol (0.5%) diet for 4 weeks successfully induce hypercholesterolemia in hamster model. Phytosterol-containing lactic-fermented milk powder containing 0.74% and 1.85% phytosterol/phytostanol showed significant effects on lowering serum TC, non HDL-C, LDL-C/HDL-C (atherogenic index), hepatic lipid levels and relative liver weight, while it could also increase the levels of fecal cholesterol and fecal phytosterols. There were no adverse effects of PSFMP on the growth and health condition of hamsters. We conclude that the phytosterol-enriched fermented milk powder will be a useful dietary supplement in lowering cholesterol level. However the efficacy of this formulation in human subjects needs to be verified.

Acknowledgements

We deeply appreciated the financial support and supply of phytosterol-containing lactic-fermented milk powder by Uni-President Enterprises Corp.

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