



Hypocholesterolemic effect of a *Lactobacillus gasseri* strain Lg70 isolated from breast fed human infant feces

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Abstract: In this study we evaluated the hypocholesterolemic effect of a *L. gasseri* strain Lg70 isolated from human infant feces. Twenty four rats were randomly assigned to three groups comprising eight rats in each group. The three groups were negative control group (fed on normal basal diet), positive control group (fed on cholesterol enriched diet only) and test group (fed on cholesterol enriched diet supplemented with milk fermented with Lg70) for 90 days. Blood samples and fecal samples were collected after every 30 days and total cholesterol level, HDL-C, LDL-C, serum triglycerides, atherogenic index and coliform & LAB counts were analyzed on monthly basis. After 90 days feeding on their respective diets it was found that in test group receiving milk fermented with Lg70 total cholesterol level were decreased significantly by 29.71% ($p < 0.05$), LDL-C concentration decreased by 49.54% ($p < 0.05$), HDL-C level increased by 36.70% and the atherogenic index was reduced by 62.52%. The fecal coliforms were reduced from 6.4 ± 0.6 to 3.9 ± 0.5 log cfu in feces of rats receiving milk fermented by Lg70 and lactobacilli counts were increased from 6.8 ± 0.3 log cfu to 10.8 ± 0.7 log cfu. Rats that received milk fermented with Lg70 displayed least weight gain among all groups suggesting that Lg70 might have inhibitory effect on excess weight gain due to cholesterol enriched diet and so besides having hypocholesterolemic effect Lg70 can also be used to control obesity.

Keywords: *L. gasseri*, Total Cholesterol, Triglycerides, HDL-C, LDL-C, Fecal coliforms

Received: 14 May 2014 / Accepted: 05 June 2014 / Published Online: 14 June 2014

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The applications of probiotics have been well established throughout generations. The interest in the microorganisms in the recent years emanated from the discovery of their salubrious effect in lowering plasma cholesterol. The reported health promoting ability of LAB in humans and livestock include the inhibition of pathogenic microorganisms (Reid et al. 2003; Basu et al. 2007) immune-modulation (Fernandes and Shahani 1990; Gill et al. 2001), cholesterol lowering effect (Park et al. 2008) and neutralization of food mutagens produced in the colon and halting of intestinal dysfunction (Lee et al. 2009).

Elevated serum cholesterol level is widely considered as a contributory risk factor for the development of cardiovascular diseases (CVD) such as atherosclerosis, coronary heart disease 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 (WHO, 2009). It has been reported that even a 1% reduction in serum cholesterol could reduce the risk of coronary heart disease by 2-3% (Manson et al. 1992). Since Mann and Spoerry (1974) discovered the Hypocholesterolemic effects of fermented milk ingested by the Massai tribes people, the relationship between lactic acid bacteria (LAB) and the serum cholesterol has become a focus of great interest. Studies evaluating this relationship have found that lactobacilli or bifidobacteria can exhibit hypocholesterolemic properties in animal cholesterols (Fukushima and Nakano 1996; Gilliland et al. 1985; Nguyen et al. 2007) and in humans (Keim et al. 1981;

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Agerbaek et al. 1995; Anderson and Gilliland 1999; Xiao et al. 2002). Several hypotheses have been proposed to explain these findings: (1) consumption of cholesterol by intestinal bacteria, thus reducing the amount of cholesterol available for absorption (Pigeon et al. 2002; Pereira et al. 2002) (2) cholesterol may be bound to the bacterial cellular surface (Liong and Shah 2005) or incorporated into the bacterial cellular membranes (Lye et al. 2010a) or converted into coprostanol by cholesterol reductase, which is produced by strains of lactobacilli (Lye et al. 2010b); (3) inhibition of micelle formation by certain probiotic strains (Cheeke et al. 2000); (4) short-chain fatty acids produced upon selective fermentation of food by intestinal bacterial microflora may lower plasma cholesterol levels (Trautwein et al. 1998); and (5) some bacterial species excrete bile salt hydrolase, leading to increased bile excretion in feces (Begley et al. 2006). However, other reports are contradictory and fail to show hypocholesterolemic effects of probiotics (Hatakka et al. 2008; Simons et al. 2006). Consequently, this area remains controversial. Therefore, more information is required to strengthen the proposed hypotheses and improve our understanding of how bacteria affect cholesterol metabolism, which might lead to more appropriate use of probiotics. This study was conducted to evaluate cholesterol lowering effect of *L. gasserii* strain Lg70 using rats as animal cholesterol.

MATERIALS AND METHODS

Grouping and diet of animals

Twenty four male albino rats of Wistar strain weighing from 140 g to 150 g were procured from the Small Animal House of the Institute. All rats were housed individually in metal cages under a normal controlled room temperatures ($20\pm 2^{\circ}\text{C}$) and humidity ($50\pm 5\%$), and maintained on a constant 12-hour/12-hour light/dark cycle. The animal study was approved by Institute Animal Ethical Committee (IAEC) of NDRI, Karnal. The animals were maintained in accordance with the guidelines of the Institute Animal Ethics Committee. The rats were randomly divided into three groups (eight rats in each group). The initial average body weight was similar among the four groups. The three groups were assigned diets according to the following regimen: (1) negative control group: normal basal diet (2) positive control group: high-cholesterol diet (3) test group: high-cholesterol diet + milk fermented with *L. gasserii* Lg70. Rats had free access to water and their group-specific diet for three months. The rats were maintained at this diet for 90 days. The day on which the feeding of the diets started was referred to as 0 d. The test groups received 5 mL (10^9cfu/mL) of milk fermented with Lg70 daily for 90 days.

Collection of blood and fecal samples

Before sacrificing, the animals were kept for overnight fasting. Blood samples and fecal samples were collected on monthly basis starting from 0 d to 90 d. The animals were subjected to fasting for 12 h prior to blood

collection. Then, the animals were anesthetized, by exposing them to diethyl ether in a glass jar for a brief period of time. Blood sample (4 mL) was obtained from the celiac vein and transferred to non-heparinized vacuum collection tubes. Tubes were initially held stationary at 0°C for 30 minutes, and then centrifuged at 3500 rpm for 10 min at 4°C in a refrigerated centrifuge. The plasma was harvested and analyzed for plasma lipid profile.

Assay for Serum Lipids

Plasma total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured with commercial kits (Biovision HDL/LDL quantification kit). Atherogenic Index (AI) was calculated as LDL / HDL

Fecal Sample Collection and Analysis

For the microbiological analysis of fecal samples, fresh samples of feces were collected from each group at the end of every month by gently squeezing the rectal part of rats. Fecal materials were put into sterile test tubes and analyzed within 30 min as per the method of Xie et al. (2011). Each fecal sample (1 g) was homogenized in a homogenizer with 9 mL of 0.9% NaCl solution and subsequent serial dilutions were plated in triplicate on Violet Red Bile Agar for coliforms and MRS agar for lactic acid bacteria. The plates were incubated at 37°C for 24-48 h and the no of colonies counted were expressed as log cfu/ml.

Statistical Analysis

All data was analysed with (SAS, V. 9.2, SAS Institute Inc). One-way analysis of variance (ANOVA) with Duncan's multiple range test was performed to compare any significant differences ($p < 0.05$) in the variables between groups. Experimental data were presented as the mean \pm standard deviations (SD) of the mean.

RESULTS AND DISCUSSION

Effect of feeding different diets on total cholesterol (TC) levels

The effect of feeding different diets on plasma total cholesterol level during the 90 days experiment is presented in Table 1. The total plasma cholesterol concentrations between three groups ranged between 59.58 ± 6.09 - $60.82\pm 5.51\text{mg/dL}$ at 0 day. On 30th day after feeding them on their respective diet the total cholesterol (TC) level increased in both the positive control group and test groups though the increase in TC level was significantly ($p < 0.05$) higher in positive control group. The trend for total cholesterol reduction in test group remained same and on 60th day the Total Cholesterol values of positive control group increased to 101.08 ± 6.54 as against $72.06\pm 4.92\text{ mg/dL}$ in test group. Thus on 60th day 19.57% reduction in TC was observed for test group. On 90th day, there was significant ($p < 0.05$) reduction (29.71%) in total cholesterol content of test group ($81.29\pm 5.54\text{ mg/dL}$) in

contrast to positive control group (115.66 ± 7.02 mg/dL). It might be concluded that our strain Lg70 has very good potential to control the TC levels in rats fed on cholesterol enriched diet for three months. In a clinical study conducted by Mohan et al. (1990) it was observed that when *Lactobacillus sporogenes* was given to hyperlipidemic patients for 90 days, the total cholesterol level decreased by 32%. In another study Nguyen et al. (2007) administered *L. plantarum* PH04 (4×10^8 cfu/mL dose per mouse daily) to twelve male hypercholesterolemic mice for 14 days and observed a significant ($p < 0.05$) reduction of total serum cholesterol (7%) and triglycerides (10%) as compared to normal control. In 2011, Xie et al. (2011) reported that as compared to rats fed on a high cholesterol diet without LAB supplementation, serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels were significantly decreased in LAB fed rats.

Effect of feeding different diets on HDL-cholesterol (HDL-C) levels

HDL (High density Lipoprotein), known to be good for health, adds to the health benefits of the consumer. It has very little triglycerides and cholesterol and has a particular protein covering. High-density lipoprotein collects the surplus cholesterol that cholesterol metabolizing cells cannot utilize. That's why HDL is considered as good cholesterol. On 0 day, there was no difference in HDL-cholesterol concentrations among the different groups and the HDL values varied between 38.32 ± 4.63 - 41.34 ± 5.28 mg/dL (Table 2). On 30th day, after feeding different groups on their respective diets variable changes in HDL concentrations were observed.

A decrease was observed in HDL-Cholesterol level of positive control group and test group though the decrease was significantly low in test group (38.66 ± 4.44 mg/dL) as compared to positive control group (32.97 ± 6.48 mg/dL). During whole experimentation period, the level of HDL-C had increased to notable amount in test group. On 90th day, the test culture had shown remarkable HDL-C increase (27 ± 2.38 mg/dL) against positive control group (17.09 ± 3.26 mg/dL) with test group showing significant ($p < 0.05$) increase of 36.76% (27 ± 2.38 mg/dL). Thus an increase of 36.70% was observed in HDL-C of rats receiving milk fermented with *L. gasserii* strain Lg70. Our results are in agreement with Hashimoto et al. (1999) who reported that a diet containing *Lactobacillus casei* TMC 0409 could increase the concentration of HDL-C in rats. Our results are in agreement to the study conducted by Taranto et al. (1998), who reported that administration of *Lactobacillus reuteri* was effective in preventing hypercholesterolemia in mice, in addition to this, he observed 17% increase in HDL cholesterol. In another study Xiao et al. (2002) evaluated the effects of a low-fat yogurt containing 10^8 cfu/g of *B. longum* BL1 on lipid profiles and observed a 14.5% increase in HDL-cholesterol when

comparing to the negative control (yoghurt without *B. longum* BL1; $p < 0.05$). Shin et al. (2010) recently reported that the serum HDL-C levels in rats were increased by sonication-killed *Bifidobacterium longum*.

Effect of feeding different diets on LDL-Cholesterol (LDL-C)

LDL-C is the main component of serum cholesterol therefore; lowering of the LDL-C level may be an important factor for reducing serum total cholesterol. The LDL concentrations between the different groups ranged between 9.81 - 11.57 mg/dL (Table 3). On 30th day, a significant increase in LDL cholesterol was observed for positive control group (35.52 mg/dL) whereas the increase in LDL-C was significantly ($p < 0.05$) less (16.37 ± 9.13 mg/dL) in test group receiving milk fermented with *L. gasserii* Lg70. On 60th day the LDL-C further increased to 63.28 ± 5.48 mg/dL in positive control group whereas it only increased to 29.06 ± 7.19 mg/dL in test group which was significantly ($p < 0.05$) lower than the positive control group.

At the end of the experiment the LDL-C level further increased to 82.77 ± 8.39 mg/dL in positive control group whereas in test group which was fed on milk fermented with *L. gasserii* Lg70, a 49.54% reduction in LDL-C was observed and the LDL-C level was significantly ($p < 0.05$) lower than the positive control group. Mortensen et al. (2002) found that mice fed with a purified diet with 10% of long-chained fructan for 16 weeks showed that the fructan significantly reduced blood cholesterol by 29.7% ($p < 0.01$), LDL-cholesterol concentration by 25.9% ($p < 0.01$), in comparison to normal control. Fernandez et al. (2000) administered 10 g/100 g of resistant starch (obtained from the Meer Corporation) to male Hartley guinea pigs (body weight of 300-400 g) for four weeks. This study showed that the resistant starch significantly reduced ($p < 0.01$) plasma cholesterol by 27.4% and LDL-cholesterol concentration by 28.0% as compared to the normal positive control group. In a study Andrade and Borges (2009) studied the effect of fermented milk containing *L. acidophilus* and *B. longum* on plasma lipids of women with normal or moderately elevated cholesterol and found that fermented milk may help to reduce LDL levels in hypercholesterolemic adult women.

Effect of feeding different diets on Triglycerides (TG) Levels

The total plasma triglycerides concentrations among the different groups on 0 day ranged between 52.44 ± 4.56 - 55.47 ± 5.28 mg/dL and no difference was observed (Table 4). On 30th day, triglycerides level increased in both groups through the increase was significantly less in test group (56.46 ± 4.44 mg/dL) as against positive control group (64.48 ± 5.61 mg/dL). The triglyceride level further increased up to 90th day in both the groups. The triglycerides level increased to 78.96 ± 1.29 mg/dL in positive control group (45.63% increase) whereas in comparison to positive control group a comparison to positive control group

Table 1 Effect of feeding different diets on serum Total Cholesterol

Group	Total Cholesterol (mg/dL)			
	0 day	30 day	60 day	90 day
Negative control	60.4±5.88 ^a	60.41±5.05 ^a	60.06±4.81 ^a	60.2±4.24 ^a
Positive control	60.82±5.51 ^a	81.39±5.03 ^b	101.08±6.54 ^b	115.66±7.02 ^b
Test Group	60.09±5.99 ^a	66.13±6.42 ^a	72.06±4.92 ^c	81.29±5.54 ^c

^{a,b,c} Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

Table 2 Effect of feeding different diets on serum HDL-Cholesterol

Groups	HDL-Cholesterol (mg/dL)			
	0 day	30 day	60 day	90 day
Negative control	40.09±5.3 ^a	40.44±5.11 ^a	41.15±5.36 ^a	40.18±2.53 ^a
Positive control	38.32±4.63 ^a	32.97±6.48 ^b	23.29±5.11 ^b	17.09±3.26 ^b
Test Group	41.34±5.28 ^a	38.66±4.44 ^c	31.74±5.58 ^c	27±2.38 ^c

^{a,b,c} Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

Table 3 Effect of feeding different diets on serum LDL-Cholesterol

Groups	LDL- Cholesterol (mg/dL)			
	0 day	30 day	60 day	90 day
Negative control	9.81±5.34 ^a	9.16±6.93 ^a	8.38±8.3 ^a	9.52±3.41 ^a
Positive control	11.57±7.33 ^a	35.52±3.62 ^b	63.28±5.48 ^b	82.77±8.39 ^b
Test Group	7.85±6.37 ^a	16.37±9.13 ^a	29.06±7.19 ^c	41.76±6.54 ^c

^{a,b,c} Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

Table 4 Effect of feeding different diets on serum Triglycerides level

Group	Triglycerides (mg/dL)			
	0 day	30 day	60 day	90 day
Negative control	52.44±4.56 ^a	54.01±4.53 ^a	52.64±4.11 ^a	52.52±1.07 ^a
Positive control	54.61±6.12 ^a	64.48±5.61 ^b	72.53±3.65 ^b	78.96±1.29 ^b
Test Group	54.45±5.63 ^a	56.46±4.44 ^a	59.31±4.77 ^c	62.62±2.42 ^c

^{a,b,c} Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

a 20.69% (p<0.05) reduction of serum triglyceride was observed in test group. In a similar study Ngugen et al. (2007) isolated the *Lactobacillus plantarum* from infant feces and evaluated its hypocholesterolemic effects by feeding it to hypercholesterolemic mice for 14 days and observed that the triglycerides level decreased by 10% in experimental group. In another study, Abd El-Gawad et al.(2005) conducted a randomized, placebo normal controlled and parallel designed study to assess the efficiency of buffalo milk-yogurts (fortified with *Bifidobacterium longum* Bb-46) in exerting a cholesterol-lowering effect. In this study, the researcher found that the administration of *B. longum* Bb-46-fermented buffalo milk-yogurt significantly reduced triglycerides by 51.2% as compared to the negative control (p<0.05).

Effect of feeding different diets on Atherogenic Index (AI)

AI values were calculated from the LDL and HDL value. The Atherogenic Index values among all the three groups ranged between 0.2-0.3 on 0 day. On 30th day, the Atherogenic Index of positive control group increased to 1.13 whereas it was found to be 0.44 in case of test group. On studying the change in Atherogenic Index of test group a significantly (p<0.05) low value of 1.57 was observed in contrast to positive control group at the end of experiment (Table 5). Thus Atherogenic Index of rats receiving milk fermented with Lg70 was reduced by 62.52% (p<0.05) which is needful to treat high lipid pertaining diseases. Abd El-Gawad et al. (2005) also reported that rats fed on cholesterol enriched diet and then giving them buffalo

milk yoghurt (YBMBb-12, YBMBb-46) and soy-yoghurt (YSMBb-12) supplemented with *B. lactis* or *B. longum* help in reducing the atherosclerotic index values than the YBM (buffalo milk yoghurt) and NFSM (non-fermented soymilk), which did not contain bifidobacteria. These findings are also in agreement with those reported by (Hayakawa 1998, 2000)

Analysis of Faecal Sample for Probiotic Colonization

The concept of "gut health" has emerged as a major target for development of functional food with enhanced functional claims. In addition to its important physiological and immunological functions, the gut is also involved in miscellaneous diseases from acute infections and diarrhea or constipation to chronic diseases such as inflammatory bowel diseases, irritable bowel syndrome, or cancer (Lye et al. 2009). The fecal LAB counts of the different treatment groups are summarized in Table 6. On 0 day, the lactic acid bacteria count of all the groups ranged from $6.7 \pm 0.6 - 7 \pm 0.3$ log cfu. The LAB count tend to decrease in positive control group as the high cholesterol diet is thought to disturb GIT (gastrointestinal) microflora. But there was a gradual increase of LAB counts in test group. After 90 days of treatment, rats fed with milk fermented with Lg70 had shown significantly higher ($p < 0.05$) colonization of LAB in their gut. The lactic acid bacteria count significantly increased from 6.8 ± 0.3 to 10.8 ± 0.7 log cfu in faeces of rats fed with Lg70. The coliform counts of different groups are presented in Table 7. The coliform count showed a decrease in probiotic fed group and increased in rats fed

cholesterol enriched diet. The count of coliforms increased from 6.3 ± 0.4 to 9.9 ± 0.4 log cfu in feces of positive control group whereas it decreased from 6.4 ± 0.6 to 3.9 ± 0.5 log cfu in rats fed with milk fermented by Lg70.

When the number of probiotic organism (lactobacilli) increases, the number of harmful bacteria decreases. As reflected in the results that when rats were in the period of fermented product consumption, their intestinal ecosystem tend to improve by an increase in the populations of the so-called "good bacteria". Among all groups test group had shown noteworthy increase in LAB counts, which favors regular consumption of milk fermented with Lg70 for increasing the population of LAB in gut. Analyses of the rat intestinal microflora thus demonstrated that coliforms increased while lactobacilli were decreased in the positive control group, implying that the high-cholesterol diet might have interfered with the intestinal microbiota. Stepankova et al. (2010) found that absence of gut microbiota (germ-free conditions) accelerates the atherosclerosis in apoE-deficient mice, which also indicates the existence of the relationship between intestinal microbiota and serum cholesterol levels. Here we presume that LAB may have successfully ameliorated the intestinal microbiota disorder induced by a high-cholesterol diet and exerting a beneficial effect that influenced lipid profiles. In our study, *L. gasserii* inhibited the growth of *E. coli*. In addition, the numbers of lactobacilli were significantly higher in the LAB-fed rats, suggesting that the *L. gasserii* strain Lg70 used in this study can

Table 5 Effect of feeding different diets on Atherogenic index

Groups	AI index			
	0 day	30 day	60 day	90 day
Negative control	0.25 ± 0.15^a	0.24 ± 0.2^a	0.22 ± 0.22^a	0.23 ± 0.08^a
Positive control	0.31 ± 0.24^a	1.13 ± 0.35^b	2.86 ± 0.85^b	4.19 ± 0.29^b
Test Group	0.2 ± 0.16^a	0.44 ± 0.29^a	0.98 ± 0.45^c	1.57 ± 0.38^c

^{a,b,c} Values (means \pm SEM; n = 8) with different superscript in a column are significantly different at the level of $P < 0.05$

Table 6 Effect of feeding different diets on Lactobacilli count

Groups	Lactobacilli count (log cfu/g feces)			
	0 day	30 day	60 day	90 day
Negative control	7 ± 0.3^a	7.1 ± 1^a	7 ± 0.7^a	7.1 ± 1^a
Positive control	6.7 ± 0.6^a	6.4 ± 0.6^b	5.5 ± 0.4^b	4.9 ± 0.6^b
Test Group	6.8 ± 0.3^a	8.4 ± 0.6^c	9.7 ± 0.6^c	10.8 ± 0.7^c

^{a,b,c} Values (means \pm SEM; n = 8) with different superscript in a column are significantly different at the level of $P < 0.05$

Table 7 Effect of feeding different diets on Coliform count

Groups	Coliform count (log cfu/g feces)			
	0 day	30 day	60 day	90 day
Negative control	6.3 ± 0.6^a	6.5 ± 0.5^a	6.3 ± 0.5^a	6.3 ± 0.6^a
Positive control	6.3 ± 0.4^a	7.2 ± 0.4^b	8.2 ± 0.7^b	9.9 ± 0.4^b
Test Group	6.4 ± 0.6^a	5.6 ± 0.5^c	4.8 ± 0.4^c	3.9 ± 0.5^c

^{a,b,c} Values (means \pm SEM; n = 8) with different superscript in a column are significantly different at the level of $P < 0.05$

successfully tolerate gastric acid and bile salts, and establish itself in the gut. Imbalances in the gut microflora could be considered to be environmental factors involved in the development of obesity and its associated metabolic disorders (Cani, 2009). Chiu et al. (2006) also studied the effects of *Lactobacillus* fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet and reported an increase in lactobacilli counts after treatment.

Growth of Rats

All the rats appeared healthy throughout the feeding period. Body weight of rats in all the groups was monitored on monthly basis, as mentioned in Table 8. On 0 day, weight of all the groups were similar and had ranged between 149.66±2.88-150.91±2.03 g. During whole of the experiment duration, weight of rats in all the four groups increased, irrespective of the diet given to them. Though weight was gained by all rats the group fed on cholesterol enriched diet gained maximum weight among all groups whereas the group receiving milk fermented with Lg70 gained least weight among all the three groups. On 90th day, maximum weight was gained by positive control group 200.31±2.38g followed by negative control group (171.46±3.82) and test group (171.45±4.4). We observed significantly ($p < 0.05$) less weight gain in the rats fed on milk fermented with Lg70 as compared to positive control groups. Generally, a high-cholesterol diet could increase the body weight. To the best of our knowledge, only a few studies have reported a weight-lowering effect of LAB besides a hypocholesterolemic ability. Some bacterial strains are considered to have preventive effects on excessive body weight gain (Hamad et al, 2009; Kang et al. 2010). Dairy foods have been proposed to play a role in various physiological processes associated with weight management and the metabolic syndrome (Barba & Russo 2006; Pfeuffer & Schrezenmeir 2007). Weight-regulatory effects of dairy foods are likely to acts in concert with other bioactive components found in milk-derived products (Zemel et al. 2004) and this could be the reason for low weight of rats present in test group. Fat digestion and absorption in the small intestine may be affected by the gut microbiota. Hamad et al. (2009) reported a reduction in the adipocyte size of rats treated with fermented skimmed milk containing *Lactobacillus gasserii* SBT2055. The researchers, therefore, considered that the skimmed milk fermented by *L. gasserii* SBT2055 would reduce fat storage through the inhibition of dietary fat absorption.

Conclusion

Feeding rats on cholesterol enriched diet supplemented with milk fermented by *L. gasserii* strain Lg70 significantly reduced serum total cholesterol, triglycerides level, LDL cholesterol level and atherogenic index in comparison to rats that received cholesterol enriched diet only. Whereas the HDL cholesterol level, that is considered as good cholesterol was increased significantly in rats receiving milk fermented with *L. gasserii* Lg70 in comparison to positive control group. In addition the fecal coliforms were significantly reduced and LAB were increased in feces of rats receiving milk fermented with Lg70. From these results it can be inferred that *L. gasserii* strain Lg70 has cholesterol lowering potential so it can be used in management of serum cholesterol levels.

Acknowledgements

We greatly acknowledge the ICAR institutional fellowship received during this research work.

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Table 8 Effect of feeding different diets on body weight gain

Groups	Body weight (g)			
	0 day	30 day	60 day	90 day
Negative control	150.13±2.88 ^a	156.51±3.1 ^a	169.24±3.17 ^a	171.46±3.82 ^a
Positive control	150.91±2.03 ^a	163.35±3.43 ^b	181.24±2.92 ^b	200.31±2.38 ^b
Test Group	149.66±2.84 ^a	154.43±1.68 ^a	161.54±2.42 ^c	171.45±4.4 ^a

^{a,b,c} Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of $P < 0.05$

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