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Probiotic LaVK2 Dahi Improves Lipid Profiles in Hypercholesterolemic Rats

Dheeraj Mohania[1]*, Vinod Kumar Kansal[1], Renu Sagwal[1], and Peter Kruzliak[2]

[1]*Division of Animal Biochemistry, National Dairy Research Institute (N.D.R.I.), Haryana, India*
[2]*Department of Cardiovascular Diseases, International Clinical Research Center, St. Anne´s University Hospital and Masaryk University, Pekarska, Czech Republic*

Abstract

Background: There is a growing interest concerning the role of probiotics as natural hypocholesterolemic agents in etiopathogenesis of cardiovascular and related heart diseases. Based on the growing evidence of the importance of probiotics with potential cardiovascular benefits, the efforts to improve the lipid profile using lactic acid bacteria in an optimal direction are gathering momentum.

Methods: The effects of probiotic Dahi containing *L. acidophilus* LaVK2 and Dahi culture on lipid profiles in rats with diet-induced hypercholesterolemia were studied. Three treatment groups of rats (n=7) were fed experimental diets: LaVK2 Dahi, Dahi or buffalo milk (BM) for 120 days. After the consumption of experimental diets, animals were fed a hypercholesterolemic diet *ad libitum.*

Results: Supplementation with LaVK2 Dahi decreased plasma total cholesterol by 22.6%; however, in BM and Dahi fed groups, the increase was over 70%. The decrease in triacylglycerol level in rats fed LaVK2 Dahi was 64.2%. Plasma high density lipoprotein (HDL) increased by 72.6% in LaVK2 Dahi fed group, whereas low density lipoprotein (LDL) + very low density lipoprotein (VLDL)-cholesterol decreased by 89%, respectively. As a result, the atherogenic index (AI), the ratio of HDL to LDL+VLDL was decreased by 91.2% on LaVK2 Dahi when compared to only 13.1% decline on BM. Futhermore, the accumulations of total cholesterol and triacylglycerols in liver and aortic tissues were significantly ($P<0.05$) reduced in rats fed with LaVK2 Dahi.

Conclusion: These observations suggest that oral administration of probiotic LaVK2 Dahi attenuated diet-induced hypercholesterolemia leading to cardiac protection by decreasing VLDL+LDL-cholesterol, LDL-cholesterol and TAGs and increasing HDL-cholesterol. Probiotic LaVK2 Dahi may have a therapeutic potential to improve the lipid profile and the risk of cardiovascular diseases.

Keywords: Cardiovascular disease; Hypercholesterolemia; *Lactobacillus acidophilus*; Probiotics; Rat

Abbreviations: AI: Atherogenic Index; BM: Buffalo Milk; ANOVA: Analysis of Variance; AOAC: Association of Official Analytical Chemist; CVD: Cardiovascular Disease; HDL: High Density Lipoprotein; LAB: Lactic acid Bacteria; LDL: Low Density Lipoprotein; NCDC: National Collection of Dairy Cultures; NDRI: National Dairy Research Institute; SCFA: Short Chain Fatty Acids; TAGs: Triacylglycerols; VLDL: Very Low Density Lipoprotein

Introduction

Cardiovascular disease (CVD) is a major cause of death in adults across the world and about 17.5 million people died from CVDs in 2005, representing 30% of all global deaths [1]. Increased levels of certain blood lipids have been reported to be the predominant risk factor for cardiovascular disease [2-4]. More recently it has been reported that probiotics have innumerable therapeutics benefits, such as lowering blood cholesterol [5,6], anticarcinogenesis [7-9], prevention of diarrhoea [10], improving lactose tolerance [11], immunomodulation [12], as an antidiabetic agent [13] and enhancement of resistance against various pathogens [14]. The possible mechanisms involved in the hypocholesterolemic effect include an assimilation of cholesterol by growing cells, the binding of cholesterol to the microbial cellular surface, the deconjugation of bile by bile salt hydrolase (Bsh), the co-precipitation of cholesterol with deconjugate bile, the binding action of bile by fiber, and the microbial production of short-chain fatty acids (SCFAs) in the colon [6-9,14,15-18]. Several recent studies from our laboratory have shown multifactorial health benefits of probiotics in animal models such as anti-aging [19,20], anticarcinogenic [21], detoxification [22] and hypocholesterolemic [23] effects. An indigenous isolate LaVK2 identified as *Lactobacillus acidophilus* by molecular-typing methods was studied extensively for its functional and probiotic attributes viz., acid and bile salt tolerance, cell surface hydrophobicity, autoaggregation and Caco-2 cell-binding as well as antibacterial and antioxidative activities. LaVK2 isolate could survive 2 h incubation at pH 1.5-2.0 and toxicity of 1.5-2.0% oxgall bile. LaVK2 could deconjugate major bile salts like taurocholic acid (TCA), glycocholic acid (GCA) and tauro-deoxycholic acid (TDCA), indicating its potential to cause hypocholesterolemia. The isolate exhibited cell-surface hydrophobicity of approximately 41.7% and possesses a functional bile salt hydrolase (BSH) enzyme activity that could help in the depletion of cholesterol in the host. Presence of these characteristics suggested the possibility of specific interaction and colonization potential of LaVK2 isolate in the gut. The isolate LaVK2 demonstrated higher free radical scavenging activity and also exhibited antibacterial activity against E. coli, L. *monocytogenes, S. typhi*, S. aureus and B. cereus (Unpublished data). Dahi is an Indian fermented milk product containing mixed mesophillic cultures of lactococci, which are not probiotic in nature.

***Corresponding author:** Dr. Dheeraj Mohania, Department of Research, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi-110060, India, Tel: +91-011-42251624; Fax: +91-011-25861002; E-mail: dmohania@gmail.com

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In the present study, we have developed the technology to incorporate the LaVK2 strain into Dahi that could be used as a good medium for delivery of probiotic strains for the protection against cardiovascular disease. In this context, the present study was undertaken to explore the therapeutic potential of probiotic LaVK2 Dahi, with a particular fous on the cholesterol-lowering potential for lowering the risks of CVD.

Materials and Methods

Bacterial strains

Lactococcus lactis ssp. cremoris NCDC-86 *and L. lactis ssp. lactis biovar diacetylactis* NCDC-60 were obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, India. *L. acidophilus* LaVK2 is our laboratory isolate with probiotic attributes tested through a battery of tests as per FAO/WHO guidelines. This isolate was identified by molecular-typing methods and studied extensively for their functional and probiotic attributes viz., acid and bile salt tolerance, cell surface hydrophobicity, autoaggregation and Caco-2 cell-binding as well as antibacterial and antioxidative activities (Unpublished data). Lactobacilli and lactococci were propagated and maintained in MRS-broth and M17 broth (Himedia Laboratories Pvt. Ltd., Mumbai, India) at 37 and 30°C, respectively, and were stored at 4-8°C between transfers.

Preparation of dahi and probiotic LaVK2 dahi

Bacterial cultures were revitalized three times in reconstituted and autoclaved skim milk prior to use for preparation of fermented milk. Buffalo milk obtained from the cattle yard of the Institute and standardized to 3.0% fat, was heated to 90°C for 15 min and was then cooled to 37°C. Dahi was prepared by culturing standardized buffalo milk with Dahi starter culture (*Lactococcus lactis ssp. cremoris and Lactococcus lactis ssp. lactis biovar diacetylactis*, 1% each) for 8 h at 30°C. Probiotic LaVK2 Dahi was prepared by inoculating buffalo milk with L. acidophilus LaVK2 (1%) and mesophillic Dahi culture (1%). The final product contained 2×109 cfu/g of lactococci, and 2×109 cfu/g of L. acidophilus.

Animals, diet and experimental design

Twenty-one male Wistar rats (each 110 day old) were allocated to one of three groups (n=7/group). The animals were maintained in small animal house of Institute at a controlled temperature of 25 ± 2°C and relative humidity of 55 ± 5% at a 12-h light/12-h dark cycle. After the supplements (LaVK2 Dahi, Dahi and BM) were consumed, the animals were fed a hypercholesterolemic diet *ad libitum* (Table 1). The animals were used and cared for in accordance with the principles and guidelines for human use, and the protocol was approved by the Institutional Ethics Committee.

Blood sampling and analytical procedures

Blood samples were collected from the orbital venous plexus of 12-hour fasted and anesthetized rats using heparinized tubes containing 10 µl of heparin solution (20 IU), and spun at 1000 x g for 10 minutes at 4°C for plasma separation. Plasma was analyzed for total cholesterol, HDL-cholesterol and triacylglycerols (TAGs) using Liquid Gold kits (Span Diagnostics Ltd, Surat, India) based on enzymatic oxidation of these molecules. Low density lipoprotein (LDL) concentration was calculated using the equations of Friedewald [24]. At the end of the experimental period, the animals were sacrificed. Fat was extracted from the aorta and liver with chloroform-methanol (2:1) mixture [25] evaporated to dryness under the stream of nitrogen, reconstituted in ethanol, and analyzed for plasma total cholesterol and TAGs.

Ingredients	Amount
Casein	20.0%
Hydrogenated vegetable oil	20.0%
Cellulose	5.0%
Choline chloride	0.2%
Starch	19.25%
Sucrose	30.0%
D, L-methionine	0.3%
Salt mixture	5.3%
Vitamin mixture	1.0%

AOAC: Association of Official Analytical Chemists, IU: International Units.
Salt mixture (AOAC, 2005) [26] required for 10 kg diet (500 g) contained $CaCO_3$- 190.7 g; $CoCl_2.6H_2O$- 0.0115 g; $CuSO_4.5H_2O$- 0.238 g; $FeSO_4.7H_2O$- 13.5 g; KH_2PO_4 - 194.5 g; KI- 0.4 g; $MgSO_4.7H_2O$-58.62 g; $MnSO_4.H_2O$, 2.005 g; NaCl-69.65g; and $ZnSO_4.7H_2O$-0.274g.
Vitamin mixture (100 g) comprised of biotin- 4 mg; folic acid- 20 mg; vitamin B_{12}- 0.3 mg; menadione- 50 mg; para aminobezoic acid- 1g; meso-inositol- 1g; thiamine- 50 mg; riboflavin- 80 mg; pyridoxine- 50 mg; calcium pentothenate- 0.4 g and starch-76.946 g.
Vitamin A ($2×10^5$ IU), vitamin E (103 IU) and vitamin D ($2×10^4$ IU) were administered to the diet through oil/fat (for 10 Kg diet)

Table 1: Composition of hypercholesterolemic basal diet

Statistical analysis

The results were expressed as the means ± standard deviation for each group (n=7) and analyzed by 1-way analysis of variance followed by the Tukey post hoc test (SYSTAT version 6.0.1, SPSS Inc, Chicago, Illinois). Differences were considered significant at $P<0.05$.

Results

Average feed intake and body weight gained among LaVK2 Dahi, Dahi and BM were not significantly different (data not shown). This observation suggests that BM, Dahi or LaVK2 Dahi administration did not affect the overall feed intake and body weight gain in the entire experiment. To investigate the protective effect of probiotic Dahi in lowering cholesterol in rats fed with hypercholesterolemic diet, plasma cholesterol was measured every 30 day interval upto 120 days. The effects of three dietary treatments on the plasma total cholesterol are presented in Table 2. Plasma total cholesterol was elevated throughout experimental period and increased above 73% when compared to baseline level in rats fed with BM. In rats fed with LaVK2 Dahi, plasma total cholesterol level was decreased by 22.6% when compared to rats fed with BM. The overall mean plasma total cholesterol was significantly lower in rat fed with LaVK2 Dahi and Dahi as compared to rats fed with BM. Plasma HDL-cholesterol levels were increased in all three dietary groups: BM, Dahi and LaVK2 Dahi (Table 3). However, increase in plasma HDL-cholesterol was non-significant among the 3 dietary groups. Plasma LDL- and very-low-density-lipoprotein (VLDL)-cholesterol in BM- and Dahi fed groups were elevated by 84.6% and 73%, respectively, on day 120, as compared to baseline level in the respective groups (Table 4). The decrease in level of LDL and VLDL-cholesterol was 89% on day 120 as compared to baseline level in LaVK2 Dahi group.

The statistical analysis showed that plasma LDL cholesterol levels differed significantly among the exprimental groups ($P<0.05$). As shown in Table 5, plasma levels of LDL were remarkably increased in Dahi and BM fed groups and remained elevated throughout the experimental period. In the LaVK2 Dahi group, level of LDL-cholesterol transiently increased on day 30 and then subsequently decreased below the baseline levels by 390.0% on days 120 as compared

	Buffalo milk	Dahi	LaVK2 Dahi	ANOVA (P value)
Baseline	57.03 ± 5.23	54.48 ± 15.88	58.76 ± 9.79	0.76
30 d	68.62^{ab} ± 8.17	62.17^{a} ± 6.66	75.93*,^{b} ± 13.34	0.07
Δ30 d	11.56^{a} ± 7.37 (20.1)	7.87^{b} ± 12.81 (14.1)	17.17^{a} ± 8.35 (29.1)	0.11
60 d	71.64*,^{a} ± 10.99	47.72^{b} ± 5.37	48.99^{b} ± 6.31	<0.001
Δ60 d	14.54^{a} ± 13.30 (25.6)	-7.64^{b} ± 12.02 (-12.3)	-9.77^{b} ± 8.55 (-16.7)	<0.001
90 d	81.02*,^{a} ± 14.90	72.06*,^{ab} ± 9.12	71.80*,^{ab} ± 13.37	0.24
Δ90 d	23.99 ± 16.44 (42.0)	17.50 ± 22.05 (32.1)	13.04 ± 18.22 (22.1)	0.70
120 d	98.59*,^{a} ± 15.31	95.68*,^{a} ± 5.92	45.54*,^{b} ± 6.38	<0.001
Δ120 d	41.53^{a} ± 17.23 (72.7)	41.16^{a} ± 20.09 (75.6)	-13.23^{b} ± 7.77 (-22.6)	<0.001
Overall mean	75.45^{a} ± 10.92	66.44^{b} ± 8.59	60.20^{b} ± 9.84	0.002

Values (mg/dl) are mean ± SD for n=7. ^{a,b,c}Values within row with different superscript letters are significantly different (P<0.05). *Values within column differ significantly from the corresponding value at baseline (P<0.05). Values in parentheses are percent change. Significance of difference was tested on actual change in total cholesterol.

Table 2: The effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on plasma total cholesterol concentration in rats

	Buffalo milk	Dahi	LaVK2 Dahi	ANOVA (P value)
Baseline	25.13^{a} ± 3.01	22.13^{a} ± 2.83	24.13^{a} ± 3.95	0.002
30 d	29.54*,^{a} ± 3.27	25.21^{b} ± 4.26	26.50^{b} ± 5.99	0.005
Δ30 d	4.41 ± 1.69 (17.5)	3.11 ± 2.00 (14.0)	2.37 ± 2.75 (10.0)	0.39
60 d	34.11*,^{a} ± 3.85	28.57^{b} ± 2.20	30.17^{b} ± 4.89	<0.001
Δ60 d	8.97^{a} ± 3.12 (35.3)	6.46^{a} ± 2.06 (29.4)	6.04^{b} ± 4.69 (25.3)	<0.001
90 d	36.93*,^{ab} ± 3.61	32.87*,^{b} ± 4.50	33.56*,^{ab} ± 5.66	<0.001
Δ90 d	11.80^{a} ± 3.05 (46.8)	13.14^{a} ± 4.50 (48.9)	9.43^{a} ± 5.81 (39.4)	<0.001
120 d	39.68^{a} ± 3.20	39.40*,^{a} ± 4.91	41.63*,^{a} ± 12.32	0.001
Δ120 d	14.54^{a} ± 2.90 (57.5)	17.43^{a} ± 4.25 (78.7)	17.50^{a} ± 10.06 (72.6)	0.001
Overall mean	33.08^{a} ± 3.39	29.64^{a} ± 3.74	31.18^{a} ± 6.56	0.71
Overall mean	33.08^{a} ± 3.39	29.64^{a} ± 3.74	31.18^{a} ± 6.56	0.71

Values (mg/dl) are mean ± SD for n=7. ^{a,b,c}Values within row with different superscript letters are significantly different (P<0.05). *Values within column differ significantly from the corresponding value at baseline (P<0.05). Values in parentheses are percent change. Significance of difference was tested on actual change in HDL-cholesterol.

Table 3: The effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi dahi on plasma HDL-cholesterol concentration in rats

	Buffalo milk	Dahi	LaVK2 Dahi	ANOVA (P value)
Baseline	31.85^{ab} ± 7.20	32.40^{ab} ± 15.29	34.63^{a} ± 10.10	0.89
30 d	40.81^{ab} ± 5.66	36.89^{ab} ± 5.84	45.89^{a} ± 15.22	0.181
Δ30 d	8.92^{a} ± 6.05 (27.9)	4.50^{a} ± 13.03 (13.9)	11.26^{a} ± 11.19 (32.7)	0.57
60 d	46.76*,^{a} ± 6.96	19.18*,^{b} ± 5.06	18.81*,^{b} ± 4.81	<0.001
Δ60 d	14.86^{a} ± 11.54 (46.7)	-7.47^{b} ± 18.12 (-40.7)	-15.81^{b} ± 12.33 (-45.7)	0.004
90 d	44.12*,^{a} ± 12.98	39.14^{a} ± 9.22	38.24^{a} ± 15.11	<0.001
Δ90 d	12.19^{a} ± 14.63 (38.2)	6.75^{a} ± 21.93 (21.0)	6.85^{a} ± 27.77 (10.4)	0.21
120 d	58.89*,^{a} ± 13.13	56.16*,^{a} ± 5.58	3.91*,^{b} ± 7.27	<0.001
Δ120 d	26.99^{a} ± 15.57 (84.6)	23.73^{a} ± 18.40 (73.1)	-30.71^{b} ± 14.77 (-88.7)	<0.001
Overall mean	44.51^{a} ± 9.19	36.71^{b} ± 8.20	28.31^{c} ± 10.48	<0.001

Values (mg/dl) are mean ± SD for n=7. ^{a,b,c,d,e}Values within row with different superscript letters are significantly different (P<0.05). *Values within column differ significantly from the corresponding value at baseline (P<0.05). Values in parentheses are percent change. Significance of difference was tested on actual change in VLDL+ LDL cholesterol.

Table 4: The effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on plasma LDL + VLDL cholesterol concentration in rats

to baseline levels. In addition, the decreased levels in plasma LDL-cholesterol was significantly lower (P<0.001) in LaVK2 Dahi group as compared to Dahi and BM fed groups. As shown in Table 6, plasma levels of TAGs remained increased in Dahi- and BM-fed groups and remained elevated throughout the experimental period. In the LaVK2 Dahi group, the levels of TAGs transiently increased on day 30 and then subsequently decreased and reached below baseline level by 64.2%. The levels of plasma TAGs were significantly (P<0.05) lower in LaVK2 Dahi-fed group when compared to Dahi- and BM-fed groups. The atherogenic index (AI), a ratio of VLDL + LDL cholesterol to HDL cholesterol, remained unaffected throughout the experimental period in rats fed with BM. In contrast, the AI decreased in rats fed with LaVK2 Dahi (Table 7). On day 120, the AI was significantly (P<0.05) lower in LaVK2 Dahi-fed rats when compared to BM- and Dahi-fed groups.

The contents of cholesterol and TAGs in aortic tissue were significantly lower in rats fed with Dahi and LaVK2 Dahi (Table 8). As anticipated, the LaVK2 Dahi was more efficacious than Dahi in reducing the levels of cholesterol and TAGs in aortic tissue. The contents of cholesterol in aortic tissue decreased by 7.2 and 26.4% on Dahi and LaVK2 Dahi groups, respectively, relatively to BM group and the corresponding declines in TAG contents in the aortic tissue were 14.4 and 29.1%, respectively. In addition, LaVK2 Dahi was also more

efficacious than dahi in reducing the deposition of cholesterol and TAGs in liver. Deposition of cholesterol in liver reduced by 14.0% and 29.8% in Dahi and LaVK2 Dahi-fed animals, respectively, compared to BM fed group (Table 9), the corresponding decline in TAG in the liver was 9.5% and 35.7%, respectively.

Discussion

It is well known that increased cholesterol levels constitute the predisposing factor associated with an increased risk of CVD. Hence, lowering plasma cholesterol levels in hypercholesterolemic patients lowers the incidence of CVD. A probiotic fermented dietary intervention could be a promising and cost-effective approach in improving the lipid profile and, hence, may play a crucial role in the management of CVD. Several studies showed that regular supplementation of functional foods such as probiotics may reduce plasma cholesterol levels [5,6,15,23]. Hence, the present study was designed specifically to explore the antihypercholesterolemic potential of probiotic LaVK2 Dahi in rat model. The present study clearly showed that rats fed with LaVK2 Dahi exhibit significantly ($P<0.05$) lower levels of plasma total cholesterol, TAGs, LDL-cholesterol, LDL+VLDL-cholesterol, AI and accumulations of tissue lipids in aorta and liver than Dahi- and BM-fed animals. The overall positive effects showed by LaVK2 Dahi group on the lipid profile in experimental animals were better than those rendered by Dahi and BM groups. These observations are consistent with preveiously published studies from author's laboratory on the hypocholesterolemic effect of probiotic fermented milk products [5]. These observations suggest that the major lipids in plasma and hepatic or aortic tissues of animals fed with buffalo milk along with hypercholesterolemic diets went beyond the level of the GI tract at each interval resulting in enhanced tissue uptake and/or *de novo* synthesis of these lipids in plasma during the 4 months. These observations of up-regulation of plasma and hepatic or aortic cholesterol and TAGs are in agreement with the results of other workers [26]. Though, the effects of consumption of LaVK2 Dahi on plasma lipids as well as on the major lipids of hepatic/aortic tissues have not previously been reported, the results of present study indicate that feeding rats with LaVK2 Dahi resulted in significant decrease ($P<0.05$) in plasma total cholesterol at day 60 and 120. The results were consistence with other studies [27,28]. Mechanistically, enhanced cholesterogenesis observed in the liver of animals may be attributed to activation of HMG CoA reductase [29]; while the accumulation of cholesterol by the aorta during this feeding period may have been mediated by an enhanced cholesterol efflux from the liver, since most extra-hepatic tissues depend on the liver for their cholesterol needs [30]. The immediate substrates for triacylglycerols and lipid synthesis are the free fatty acids (FFA). They are also the major source of energy in many tissues. In the present study, the excessive accumulation of TAGs in the tissues upon consumption of the BM and Dahi along with hypercholesterolemic diets indicates that most of the absorbed FFA may be directed towards the synthesis of TAGs. This may suggests an impairment of mitochondrial β-oxidation of FFA, hence, compromising energy production in these tissues.

In the present study, animals fed with LaVK2 Dahi reversed the hypercholesterolemia induced by the consumption of the hypercholesterolemic diet. Several interpretations of this observation could be considered. At the outset, the probiotic LaVK2 Dahi reduced plasma cholesterol and TAGs suggesting that the effects of the probiotic Dahi might have commenced at the level of the GI tract, inhibiting absorption of these lipids. Cholesterol reduction by lactic acid bacteria has already been attributed to the ability of these bacteria to bind cholesterol in the small intestine [31-35]. Our statistics indicate that in addition to cholesterol and TAGs could also be bound by these micro-

	Buffalo milk	Dahi	LaVK2 Dahi	ANOVA (P value)
Baseline	$7.54^{a} \pm 9.55$	$9.02^{a} \pm 17.80$	2.04a ± 13.16	0.60
30 d	$6.24^{a} \pm 9.08$	$5.82^{a} \pm 8.60$	$11.54^{*,a} \pm 12.06$	0.51
Δ30 d	$-1.27^{a} \pm 8.50$ (-17.0)	$-3.17^{a} \pm 12.22$ (-36.0)	$9.47^{b} \pm 6.96$ (466.0)	0.047
60 d	$1.41^{a} \pm 12.60$	$-13.51^{*,b} \pm 7.68$	$4.89^{a} \pm 7.08$	0.004
Δ60 d	$-4.82^{a} \pm 12.80$ (-82.0)	$-19.44^{b} \pm 4.34$ (-251.0)	$-6.64^{a} \pm 13.04$ (140.0)	0.043
90 d	$0.77^{a} \pm 16.35$	$4.82^{a} \pm 9.52$	$26.36^{*,b} \pm 14.87$	0.006
Δ90 d	$1.08^{a} \pm 6.53$ (-90.0)	$18.54^{b} \pm 11.13$ (-47.0)	$21.46^{b} \pm 19.27$ (1193.0)	0.022
120 d	$10.92^{a} \pm 17.02$	$19.01^{a} \pm 5.68$	$-5.91^{b} \pm 8.27$	0.002
Δ120 d	$10.15^{a} \pm 5.97$ (45.0)	$14.27^{a} \pm 4.75$ (111.0)	$-33.90^{b} \pm 17.56$ (-390.0)	<0.001
Overall mean	$5.38^{a} \pm 4.29$	$5.03^{a} \pm 11.87$	$7.78^{a} \pm 12.12$	0.90

Values (mg/dl) are mean ± SD for n=7. ^{a,b}Values within row with different superscript letters are significantly different ($P<0.05$). *Values within column differ significantly from the corresponding value at baseline ($P<0.05$). Values in parentheses are percent change. Significance of difference was tested on actual change in LDL cholesterol.

Table 5: The Effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on plasma LDL cholesterol concentration in rats

	Buffalo milk	Dahi	LaVK2 Dahi	ANOVA (P value)
30 d	$163.00^{*} \pm 25.08$	$155.24^{*} \pm 36.51$	$189.41^{*} \pm 25.93$	0.32
Δ30 d	$41.57^{ab} \pm 9.31$ (34.4)	$38.37^{ab} \pm 15.28$ (32.8)	$26.47^{a} \pm 4.67$ (16.3)	0.29
60 d	$180.45^{*,a} \pm 22.62$	$163.74^{*,a} \pm 23.09$	$69.56^{*,b} \pm 15.44$	<0.001
Δ60 d	$58.01^{a} \pm 10.05$ (48.0)	$46.84^{a} \pm 21.38$ (40.1)	$-93.39^{b} \pm 24.12$ (-57.3)	<0.001
90 d	$216.59^{*,a} \pm 30.09$	$171.69^{*,b} \pm 23.06$	$59.40^{*,c} \pm 15.35$	<0.001
Δ90 d	$94.17^{a} \pm 11.01$ (77.5)	$54.74^{b} \pm 20.92$ (46.9)	$-103.54^{c} \pm 30.36$ (-63.5)	<0.001
120 d	$239.71^{*,a} \pm 34.35$	$185.42^{*,b} \pm 18.92$	$58.29^{*,c} \pm 15.57$	<0.001
Δ120 d	$117.44^{a} \pm 12.36$ (96.6)	$68.54^{b} \pm 21.75$ (58.7)	$-104.66^{c} \pm 26.88$ (-64.2)	<0.001
Overall mean	$184.61^{a} \pm 28.32$	$158.60^{a} \pm 27.16$	$107.92^{b} \pm 19.54$	<0.001

Values (mg/dl) are mean ± SD for n=7. ^{a,b,c}Values within row with different superscript letters are significantly different ($P<0.05$). *Values within column differ significantly from the corresponding value at baseline ($P < 0.05$). Values in parentheses are percent change. Significance of difference was tested on actual change in plasma triacylglycerols.

Table 6: The effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on plasma triacylglycerols concentration in rats

	Buffalo milk	Dahi	LaVK2 Dahi	ANOVA (P value)
Baseline	1.30^{a} ± 0.44	1.46^{a} ± 0.66	1.48^{a} ± 0.51	0.70
30 d	1.39^{a} ± 0.27	1.50^{a} ± 0.37	1.76^{a} ± 0.60	0.02
Δ30 d	0.091 ± 0.104 (6.9)	0.033 ± 0.233 (2.0)	0.286 ± 0.158 (18.9)	0.67
60 d	1.36^{a} ± 0.27	0.66*,^{b} ± 0.19	0.64*,^{b} ± 0.21	<0.001
Δ60 d	0.077^{a} ± 0.185 (6.2)	-0.794^{b} ± 0.212 (-54.4)	-0.841^{b} ±0.232 (-56.8)	0.03
90 d	1.17^{a} ± 0.30	1.20^{a} ± 0.34	1.19*,^{a} ± 0.57	<0.001
Δ90 d	-0.117 ± 0.169 (-8.5)	-0.257 ± 0.326 (-17.7)	-0.293 ± 0.342 (-19.6)	0.42
120 d	1.46^{a} ± 0.26	1.43^{a} ± 0.26	0.13*,^{b} ± 0.17	<0.001
Δ120 d	0.171^{a} ± 0.176 (13.1)	-0.029^{ab} ± 0.256 (-2.0)	-1.346^{c} ± 0.210 (-91.2)	<0.001
Overall mean	1.34^{a} ± 0.31	1.24^{ab} ± 0.36	1.04^{b} ± 0.41	0.05

Values are mean ± SD for n=7. †Atherogenic Index is a ratio of LDL + VLDL- cholesterol to HDL- cholesterol. ^{a,b,c} Values within row with different superscript letters are significantly different (P<0.05). *Values within column differ significantly from the corresponding value at baseline (P<0.05). Values in parentheses are percent change. Significance of difference was tested on actual change in atherogenic index.

Table 7: The Effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on atherogenic index† in rats

	Cholesterol	Triacylglycerols		
	mg/g tissue (*P <0.001*)	mg/g fat (*P <0.001*)	mg/g tissue (*P <0.001*)	mg/g fat (*P = 0.005*)
Buffalo milk	2.32^{a} ± 0.10	7.04^{a} ± 1.13	26.90^{a} ± 1.98	81.28a ± 11.67
Dahi	2.16^{b} ± 0.13	6.67^{a} ± 1.20	23.01^{b} ± 1.81	71.02ab ± 13.72
LaVK2 Dahi	1.73^{c} ± 0.11	5.13^{b} ± 0.81	19.10^{c} ± 0.91	57.13c ± 10.78

Values are mean ± SD for n=7. ^{a,b,c}Values within column with different superscript letters are significantly different (*P < 0.05*)

Table 8: The effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on cholesterol and triacylglycerols contents in aortic tissue in rats

	Cholesterol		Triacylglycerols	
	mg/g tissue (*P <0.001*)	mg/g fat (*P <0.001*)	mg/g tissue (*P <0.001*)	mg/g fat (*P = 0.001*)
Buffalo milk	2.32^{a} ± 0.14	28.32^{a} ± 4.79	8.83^{a} ± 0.76	105.82^{a} ± 17.02
Dahi	2.01^{b} ± 0.23	23.01^{b} ± 4.49	7.90^{b} ± 1.34	93.04^{b} ± 11.33
LaVK2 Dahi	1.65^{c} ± 0.37	19.10^{bc} ± 3.74	5.68^{c} ± 0.70	66.09^{c} ± 6.46

Values are mean ± SD for n=7. a,b,c Values within column with different superscript letters are significantly different (P<0.05).

Table 9: The effect of feeding buffalo milk, Dahi or probiotic LaVK2 Dahi on contents of cholesterol and triacylglycerols in liver in rats

organisms resulting in their decreased absorption and homeostatic response, resulting in lowering of these plasma lipids. Secondly, with reference to lipid metabolism in the tissues, ingestion of probiotic dahi could have inhibited the activity of HMG CoA reductase, reducing the amount of cholesterol synthesized in the liver and aorta. This reduced cholesterol content of the hepatic tissues is consistent with the fact that their cholesterol is obtained from the liver. In addition to reducing hepatic cholesterol and hepatic TAGs, aortic tissue lipids were also decreased by ingestion of the probiotic Dahi. Furthermore, the indigenous *L. acidophilus* LaVK2 strain has also shown to possess bile salt hydrolase (BSH) activity *in vitro* and this activity remains in the product up to seven days (data not shown), thereby suggesting that this BSH activity could be responsible for lowering the plasma total cholesterol concentration.

Several workers have attributed cholesterol-lowering property of lactobacilli to their ability to deconjugate bile salts, resulting in excretion of free bile acids in faeces, which in turn enhances the requirement of cholesterol to produce more bile acids hepatic tissue [36,37].

Recently, a novel protein having a molecular weight of 12KDa has been characterized from *L. acidophilus* ATCC 43121 responsible for reduction of cholesterol *in vitro* [38]. It has also been suggested that lactic acid bacteria (LAB) may incorporate cholesterol from the surrounding environment into cellular membranes during growth, thus exerting beneficial effects on serum cholesterol levels [36,37]. Cholesterol incorporated into or attached to LAB cells in the intestine cannot be absorbed into the blood [34]. As observed in the present study, deconjugation of major bile salts by the probiotic LaVK2 strain could be responsible for lowering the cholesterol level in vivo. The deconjugation of bile salts such as cholate, chenodeoxycholate and deoxycholate may lead to decreased solubility, and hence, lowering the reabsorption in enterohepatic system, thereby, resulting in an increased demand for cholesterol as a precursor of bile salts [39]. The BSH activity present in LaVK2 strain could be responsible for depletion of cholesterol, hence may be involved in ameliorating the hypercholesterolemia.

In the present study, the levels of plasma LDL+VLDL cholesterol was significantly lower (P<0.05) on rats fed with LaVK2 Dahi when compared to Dahi and BM. These observations are in agreement with studies of Fukushima and Nakano [40] who have reported that the probiotic preparation reduced plasma lipids including the VLDL+LDL cholesterol and a decrease in hydroxymethylglutaryl coenzyme A reductase (NADPH) activity in liver and increased neutral and acidic sterol excretion in faeces of rats fed with probiotic preparation. The results recently obtained by Rajpal and Kansal [23] from the author's laboratory also support the significant decrease in blood and tissue lipids observed in present study. HDL-cholesterol is considered as good cholesterol and its level in plasma correlated inversely to CVD [5]. This is an important factor since HDL-cholesterol can prevent arteriosclerosis. In present study, levels of HDL-cholesterol in plasma statistically non-significant in groups fed with Dahi, BM and LaVK2 Dahi. This is inconsistent with the reports of other workers who reported augmentation in HDL-cholesterol levels in rats fed with probiotic Dahi containing L. *casei, L. acidophilus and Lactococcus lactis* [41].

Administration of LaVK2 Dahi significantly lowered the plasma LDL-cholesterol at the conclusion of the experiment. These results are

in agreement with the reports of several studies [42,43]. In the present study, the level of plasma TAGs was significantly ($P<0.05$) reduced in rats fed with LaVK2 Dahi when compared to Dahi or BM. These results are in agreement with the reports of Usman and Hosono [44,45] who studied the effect of *L. gasseri* on lipid profile in rats fed cholesterol-enriched diet, and observed reduction in serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels and diminution in AI. It has also been proposed that various milk components such as orotic acid, retentate, pyrimidine-like nucleotide, calcium or hydroxyl methyl glutaric acid reduces the *de-novo* synthesis of cholesterol through inhibition of NADPH formation (a reducing power required for biosynthesis of cholesterol) by HMG-CoA synthase (a rate limiting step in cholesterol biosynthesis) in liver and increases the cholesterol clearance from blood stream by enhancing the excretion of bile acids [46]. In addition, fermentation of milk with LAB increases the bioavailability of these components, and their absorption as well as function of such components in gastrointestinal tract [47]. Probably, this may be the reason why LaVK2 Dahi is more effective than BM.

Recently, the author's laboratory has shown that probiotics down-regulate carcinogen activating cytochrome P450 enzymes CYP1A1, CYP1A2 and CYP1B1 in liver, and up-regulate carcinogen detoxifying γ-glutamyltranspeptidase, UDP-glucuronosyl transferase and quinone reductase activities in liver as well as in colon [22]. The potential of this product to improve macrophage and lymphocyte functions [20], and antioxidative status [19] has also been established. Findings of this study indicate that consumption of probiotic Dahi may be more efficacious in reducing diet-induced hypercholesterolemia and decreasing the plasma, hepatic, and aortic lipids than dahi. However, future research may be needed to explore the molecular mechanism underlying the cholesterol-lowering effect of probiotic Dahi and production of bacterial metabolites that may modulate the hepatic cholesterol biosynthesis or degradation.

Conclusion

In conclusion, the present study has presented the anti-hypercholesterolemic effect of the probiotic LaVK2 Dahi in rats with diet-induced hypercholesterolemia, where plasma total cholesterol, LDL, VLDL, and TAG levels were found to be decreased significantly, and HDL cholesterol levels were observed to be increased but not significantly. Our study reveals that probiotic LaVK2 Dahi was more efficacious than Dahi in reducing plasma cholesterol and TAGs in diet-induced hypercholesterolemic rats. These findings suggest that probiotic LaVK2 Dahi could be exploited as potential alternate biotherapeutic agent to decrease cholesterol levels and lower the risk of CVD, although the area is open for further studies.

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References

1. World Health Organization (2009) Cardiovascular disease, Geneva, Switzerland.
2. World Health Organization (WHO) (2003) "Diet, Nutrition and Prevention of Chronic Diseases": Report of a Joint WHO/FAO Expert Consultation, Geneva, Switzerland.
3. Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, et al. (2012) Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res 2012: 902917.
4. Mohania D, Nagpal R, Kumar M, Bhardwaj A, Yadav M, et al. (2008) Molecular approaches for identification and characterization of lactic acid bacteria. J Dig Dis 9: 190-198.
5. Mohania D, Kansal VK, Shah D, Nagpal R, Kumar M, et al. (2013) Therapeutic effect of probiotic dahi on plasma, aortic, and hepatic lipid profile of hypercholesterolemic rats. J Cardiovasc Pharmacol Ther 18: 490-497.
6. Mohania D, Kansal VK, Nagpal R, Yamashiro Y, Marottta F (2013) Supression of diet-induced hypercholesterolemia by probiotic dahi containing *Lactobacillus acidophilus* and Lactobacillus plantarum. Inter J Pro and Probio 8: 75-84.
7. Mohania D, Kansal VK, Shah D (2013) Probiotic Dahi containing *Lactobacillus acidophilus* and Lactobacillus plantarum suppresses DMH induced preneoplastic lesions in early colorectal carcinogenesis in Wistar Rats. Am J Can Biol 1: 1-14.
8. Mohania D, Kansal VK, Sagwal R, Shah D (2013) Anticarcinogenic effect of probiotic Dahi and piroxicam on DMH-induced colorectal carcinogenesis in Wistar rats. Am J Can Ther Pharmacol 1: 8-24.
9. Mohania D, Kansal VK, Kumar M, Nagpal R, Yamashiro Y, et al. (2013) Modulation of expression of Programmed Death-1 by administration of probiotic Dahi in DMH-induced colorectal carcinogenesis in rats. Acta Biomed 84: 102-109.
10. Rosenfeldt V, Michaelsen KF, Jakobsen M, Larsen CN, Møller PL, et al. (2002) Effect of probiotic Lactobacillus strains on acute diarrhea in a cohort of nonhospitalized children attending day-care centers. Pediatr Infect Dis J 21: 417-419.
11. Ooi LG, Liong MT (2010) Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and *in vitro* findings. Int J Mol Sci 11: 2499-2522.
12. Klein A, Friedrich U, Vogelsang H, Jahreis G (2008) *Lactobacillus acidophilus* 74-2 and Bifidobacterium animalis subsp lactis DGCC 420 modulate unspecific cellular immune response in healthy adults. Eur J Clin Nutr 62: 584-593.
13. Yadav H, Jain S, Sinha PR (2007) Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and Lactobacillus casei in high fructose fed rats. Nutrition 23: 62-68.
14. Shu Q, Gill HS (2002) Immune protection mediated by the probiotic Lactobacillus rhamnosus HN001 (DR20) against Escherichia coli O157:H7 infection in mice. FEMS Immunol Med Microbiol 34: 59-64.
15. Liong MT, Shah NP (2005) Optimization of cholesterol removal by probiotics in the presence of prebiotics by using a response surface method. Appl Environ Microbiol 71: 1745-1753.
16. Lye HS, Rusul G, Liong MT (2010) Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. J Dairy Sci 93: 1383-1392.
17. Anal AK, Singh H (2005) Recent advances in microencapsulation of probiotic for industrial applications a targeted delivery. Trends Food Sci Tech 18: 240-251.
18. Reid G, Jass J, Sebulsky MT, McCormick JK (2003) Potential uses of probiotics in clinical practice. Clin Microbiol Rev 16: 658-672.
19. Kaushal D, Kansal VK (2012) Probiotic Dahi containing *Lactobacillus acidophilus* and Bifidobacterium bifidum alleviates age-inflicted oxidative stress and improves expression of biomarkers of ageing in mice. Mol Biol Rep 39: 1791-1799.
20. Kaushal D, Kansal VK (2011) Age-related decline in macrophage and lymphocyte functions in mice and its alleviation by treatment with probiotic Dahi containing *Lactobacillus acidophilus* and Bifidobacterium bifidum. J Dairy Res 78: 404-411.
21. Rajpal S, Kansal VK (2008) Buffalo milk probiotic Dahi containing *Lactobacillus acidophilus*, Bifidobacterium bifidum and Lactococcus lactis reduces gastrointestinal culture induced by dimethylhydrazine dihydrochloride in rats. Milchwissenschaft 63: 122-125.
22. Sharan S, Kansal VK (2011) Effect of feeding probiotic Dahi containing *Lactobacillus acidophilus* and Bifidobacterium bifidum on enzymes that catalyse carcinogen activation and detoxification in rats. Milchwissenschaft 66: 244-247.
23. Rajpal S, Kansal VK (2009) Probiotic Dahi containing *Lactobacillus acidophilus* and Bifidobacterium bifidum attenuates diet induced hypercholesterolemia in rats. Milchwissenshaft 64: 21-25.

24. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502.

25. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-499.

26. AOAC (2005) Official methods of analysis, In: Horowitz W. (edtr) (18thedn), Gaithersburg.

27. Chiu CH, Lu TY, Tseng YY, Pan TM (2006) The effects of Lactobacillus-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. Appl Microbiol Biotechnol 71: 238-245.

28. Kawase M, Hashimoto H, Hosoda M, Morita H, Hosono A (2000) Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. J Dairy Sci 83: 255-263.

29. Rao DR, Chawan CB, Pulusani SR (1981) Influence of milk and Thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. J Food Sci 46: 1339-1341.

30. Ademuyiwa O, Agarwal R, Chandra R, Behari JR (2009) Lead-induced phospholipidosis and cholesterogenesis in rat tissues. Chem Biol Interact 179: 314-320.

31. De Smet I, Hoorde, LV, Saeyer ND, Woestyne MV, Verstraete W (1994) *In vitro* study of bile salt hydrolase activity of BSH isogenic Lactobacillus plantarum 80 strains and estimation of cholesterol lowering through enhanced BSH activity. Micro Eco Healt Dis 7: 315-329.

32. Gilliland SE, Nelson CR, Maxwell C (1985) Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl Environ Microbiol 49: 377-381.

33. Buck LM, Gilliland SE (1994) Comparisons of freshly isolated strains of *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol during growth. J Dairy Sci 77: 2925-2933.

34. Kimoto H, Ohmomo S, Okamoto T (2002) Cholesterol removal from media by lactococci. J Dairy Sci 85: 3182-3188.

35. Dilmi-Bouras A (2006) Assimilation (*in vitro*) of cholesterol by yogurt bacteria. Ann Agric Environ Med 13: 49-53.

36. Grunewald KK (1982) Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J Food Sci 47: 2078-2079.

37. Corzo G1, Gilliland SE (1999) Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*. J Dairy Sci 82: 472-480.

38. Kim Y, Whang JY, Whang KY, Oh S, Kim SH (2008) Characterization of the cholesterol-reducing activity in a cell-free supernatant of *Lactobacillus acidophilus* ATCC 43121. Biosci Biotechnol Biochem 72: 1483-1490.

39. Fabian E, Elmadfa I (2006) Influence of daily consumption of probiotic and conventional yoghurt on the plasma lipid profile in young healthy women. Ann Nutr Metab 50: 387-393.

40. Fukushima M, Nakano M (1996) Effects of a mixture of organisms, *Lactobacillus acidophilus* or Streptococcus faecalis on cholesterol metabolism in rats fed on a fat- and cholesterol-enriched diet. Br J Nutr 76: 857-867.

41. Jain S, Yadav H, Sinha PR (2009) Antioxidant and cholesterol assimilation activities of selected lactobacilli and lactococci cultures. J Dairy Res 76: 385-391.

42. Zommara M, Tachibana N, Sakono M, Suzuki Y, Oda T, et al. (1996) Whey from cultured milk decreases serum cholesterol and increases antioxidant enzymes in liver and red blood cells in rats. Nut Res 16: 293-302.

43. Ali AA, Velasquez MT, Hansen CT, Mohamed AI, Bhathena SJ (2004) Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. J Nutr Biochem 15: 583-590.

44. Usman, Hosono A (2000) Effect of administration of Lactobacillus gasseri on serum lipids and fecal steroids in hypercholesterolemic rats. J Dairy Sci 83: 1705-1711.

45. Usman, Hosono A (2001) Hypocholesterolemic effect of Lactobacillus gasseri SBT0270 in rats fed a cholesterol-enriched diet. J Dairy Res 68: 617-624.

46. Agrawal RAK, Kansal VK (1991) Influence of skim milk on plasma lipids, lipoproteins and Lecithin: cholesterol acytransferase activity in cholesterol fed rabbits. Milchwissenschaft 46: 355-357.

47. Roberfroid MB (2000) Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 71: 1682S-7S.