DAHI CONTAINING PROBIOTIC *LACTOBACILLUS ACIDOPHILUS* AND *LACTOBACILLUS CASEI* HAS A PROTECTIVE EFFECT AGAINST *SALMONELLA ENTERITIDIS* INFECTION IN MICE

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***Salmonella enteritidis* infection has received attention during recent years owing to its high prevalence worldwide. In the present study, the protective effect of probiotic dahi (curd) supplemented with *Lactobacillus acidophilus* and *L. casei* against *Salmonella enteritidis* infection in mice is investigated. Seven days pre-feeding with probiotic dahi significantly increased anti-*S. enteritidis* sIgA (secretary IgA) antibodies and lymphocyte proliferation in *S. enteritidis* infected mice. IL-2, IL-6 and IFNγ production were significantly increased in supernatant of cultured splenocytes collected from mice pre-fed with probiotic dahi, while IL-4 levels were not changed significantly. Moreover, activities of β-galactosidase and β-glucuronidase, and counts of *S. enteritidis* in intestine, liver and spleen were decreased, whereas total lactobacilli in faeces were increased in mice pre-fed with probiotic dahi. Pre-feeding of probiotic dahi for 7 days was more effective than 2 days pre-feeding. Thus, the results indicate that, pre-feeding with probiotic dahi ameliorated *S. enteritidis* infection by stimulating specific and non-specific immune response. Above all, it lowered colonization of gastrointestinal tract as well as translocation of *S. enteritidis*.**

Salmonella enteritidis is a food-born pathogen and its infection is a major public health threat, worldwide. It needs the serious attention of medical professionals and researchers to develop preventive strategies that can ameliorate the risk of *S. enteritidis* infection (1). Over the years, various strategies have been investigated to reduce *S. enteritidis* infection, of which the strengthening of immune system of host is considered to be most important (2). Regarding this, it has been reported that consumption of functional foods containing probiotic lactic acid bacteria (LAB) enhances host immune system by modulating gut microflora (3). Probiotic LAB, especially *Lactobacillus* and *Bifidobacterium,* are known to enhance the capacity of host to fight against intestinal infections by stimulating the mucosal immune system (4). Ingestion of LAB also exerts immunomodulatory effects through modulation of the systemic immune system by activating antigen specific B and T cells which produce antibodies and various types of cytokines, respectively, to exclude pathogen invading into the intestinal tract (9). LAB

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have been shown to exert protective effects against infections of a wide variety of enteropathogens such as *Salmonella typhimurium*, *Listeria monocytogenes*, *E. coli* etc. (5-7). De Waard et al (8) demonstrated that mice fed with *L. casei* (Shirota), prior to oral challenge with *L. monocytogenes,* exhibited reduced pathogen burden in gastrointestinal tract and restrained the pathogen translocation to the spleen and liver when compared to non-probiotic-fed control mice. This indicates that, the enhancement of anti-*Listeria* activity in mice might be due to feeding of *L. casei*.

Although stimulation of the immune system by LAB against enteropathogens has been demonstrated to be strain-dependent (10), search for new immunomodulatory LAB strains and the validation of those for human consumption is an important area of current research in food science. We have characterized two lactobacilli strains i.e. *L. acidophilus* and *L. casei* for best probiotic attributes such as acid and bile tolerance, surface hydrophobicity, antimicrobial, cholesterol removal and bile-salt deconjugation out of 50 strains (11). Further, employing these strains as probiotics we have developed a probiotic dahi (curd/yogurt) by some technical modifications (12). Preliminary studies showed that probiotic dahi have higher efficacy to modulate innate immune response in normal mice than control dahi (13), indicating that feeding of probiotic dahi may strengthen the innate and/or specific immune system to fight various infections. In the present study we have analysed whether prior feeding with probiotic dahi could prevent *S. enteritidis* infection in mice, and following this approach how the immune response is modulated.

MATERIALS AND METHODS

Bacterial strains and preparation of probiotic dahi

L. acidophilus NCDC14, *L. casei* NCDC19 and *L. lactis* biovar *diacetylactis* NCDC60 (normal dahi culture) were obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, India. *S. enteritidis* strain was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. Probiotic dahi was prepared according to the method described elsewhere (12). In brief, raw buffalo milk was procured from the experimental dairy of the NDRI, Karnal, India. Milk fat was adjusted to 2.5% by adding fresh skimmed milk and was boiled at 90°C for 15 min and then cooled aseptically to 37°C. Reconstituted milk was inoculated with *L. acidophilus*, *L. casei* and *Lactococcus lactis* biovar *diacetylactis* for the preparation of probiotic dahi, and was incubated at 37°C for 12-14 h. Control dahi was prepared by inoculating *L. lactis* alone and incubating at 30°C for 14 h.

Animals, feeding and challenge procedures

Six-week-old, male Swiss albino mice (23-28 g body weight) were housed individually in a small animal house of the Institute with 12-12 h light/dark cycle. Animals were divided into 4 groups (n = 15): i) control group (CON) fed with synthetic diet (Table I); ii) milk-fed group (MFG) fed with reconstituted non-fermented milk with 2.5% fat; iii) control dahi fed group (CDG) fed with control dahi; and iv) probiotic dahi fed group (PDG) fed with probiotic dahi. Additionally, the ii, iii and iv groups were fed with milk/dahi(s) along with a synthetic diet, and all the groups were provided with water *ad libitum.* In this study, two types of experiments were conducted to find the time-dependent effect of probiotic dahi, i.e. animals were fed with experimental diets in the respective groups for 2 and 7 consecutive days and then orally challenged with $1x10^8$ cfu/ live *S. enteritidis* using oral catheter. After challenge, the animals were continuously fed with the respective diets for a further 8 days. Body weight was measured on alternate days. No mortality was observed after *S. enteritidis* administration in mice. Five animals from each group were sacrificed on days 2, 5 and 8 of the post-challenge period. Intestine, liver and spleen were collected and weighed, aseptically. Study protocols were approved by the National Dairy Research Institute Animal Ethics Committee and the animals were maintained as per rules and regulations of the ethics committee.

Microbial analysis

Fresh faecal samples were collected by gentle squeezing of the rectal part of the mice. Faecal materials were put into sterile test tubes and transferred to aseptic conditions. The organs (intestine/ liver/ spleen) or fecal samples (1 g) were gently homogenized in 5 ml 0.1% peptone water. Suspensions were serially diluted in peptone water and plated (in duplicate) on Salmonella-Shigella agar and MRS agar (Hi-Media Pvt. Ltd. Mumbai, India) and incubated at 37°C for 72 and 48 h for enumeration of *S. enteritidis* and total lactobacilli, respectively.

Collection of intestinal fluid

Intestinal fluid was collected by the procedure described by Lim et. al (14), with slight modifications.

Intestine was carefully removed from gastro-duodenal to ileocaecal junctions and the contents were washed out with 5 ml phosphate buffer saline (PBS, pH 7.2). Content was centrifuged at 2000 x *g* for 30 min at 4°C using cooling centrifuge (Himac CR22, New Delhi, India), and supernatant was collected in another tube for further analysis.

β-Galactosidase and β -Glucuronidase assay

ONPG (o-nitro phenyl β-D-galactopyranoside) and PNPG (p-nitrophenyl β-D glucuronide), synthetic substrates for β-galactosidase and β-glucuronidase, were used for assaying enzyme activities according to Conchie et al (15) and Stossel (16), respectively. In brief, 0.25 ml of intestinal fluid was added to 0.25 ml of ONPG/ PNPG solution and a final volume was made to 1 ml with citrate phosphate buffer (pH 3.8). Mixtures were incubated for 5 h at 37°C and added with 0.8 ml of 0.5 M sodium carbonate and 1 ml of 0.1N NaOH to stop β-galactosidase and β-glucuronidase activities, respectively. Absorbance was measured at 430 and 410 nm using a UV-VIS spectrophotometer (Specord 200 Analytikjena AG, Germany). Activities of β-galactosidase and β-glucuronidase enzymes were determined by estimating released ortho-nitrophenol (ONP) and p-nitrophenol (PNP), respectively and considering respective standard curves for calculations.

Estimation of sIgA in intestinal fluid

sIgA was estimated by the method described in Engwall and Perlmann (17). In brief, wells of polystyrene microtitre plates were coated with autoclaved suspension of *S. enteritidis* (100 µl) at the rate of 10^8cfu/ ml in 0.06 M carbonate buffer (pH 9.6). Control wells were coated with 100 µl of carbonate buffer alone. Plates were incubated for 18-20 h at 37°C for drying. After dry, 200 µl of 70% methanol was added and left for 20 min to fix antigen on the plate surface and then dried again with dryer. Free binding sites were blocked by adding 350 µl of blocking solution (2% BSA in PBS-Tween-20 [PBS/T)) and incubated at room temperature for 2 h with occasional shaking. Blocking solution was eliminated and wells were washed three times with 0.05% PBS/T. Samples (100 µl) were added and the plates were incubated for 1 h at 37°C with occasional shaking. After antigen-antibody reaction, the plates were washed-out. Furthermore, 100 µl of goat anti-mouse peroxidase conjugate was added to each well and incubated at 37°C for 1 h, and again washed with PBS/T followed by 2 times with PBS, and 100 µl of α-phenylenediamine dihydrochloride (4g/ l) in 0.1 M citrate phosphate buffer (pH 5.0) was added. Plates were kept for 30 min in the dark at room temperature. Reaction was stopped with 100 µl of 4N H_2SO_4, and OD was measured at 493 nm using ELISA plate recorder. Antibody concentration in each sample was expressed as OD/ 100 µl.

Lymphocyte proliferation assay

Aseptically collected spleen tissues were gently teased with sterile needles and forceps to release splenocytes into the RPMI 1640 media. Tissue suspensions were allowed to stand for 2 min to sediment large tissue clumps. The upper portion containing splenocytes was collected and centrifuged at 1000 × *g* for 5 min at 4°C. Suspension was incubated with erythrocyte lysis buffer (0.17 M Tris HCl and 0.16 M NH_4Cl, pH 7.2) for 1 min and washed twice by centrifuging as above with RPMI 1640 medium. Cell viability was checked by trypan blue (0.4% solution). Viable splenocytes (10^6cells/ ml) were finally cultured in heat inactivated 10% fetal calf serum enriched RPMI 1640 medium supplemented with or without 50 µg/ ml lipopolysaccharide (LPS; Sigma Chemical Co, St. Louise, USA) and incubated at 37°C in a humidified atmosphere of 5% CO_2 incubator for 48 h. Then, 10 µl of MTT (5 mg/ml) was added in each well and incubated for 4 h at 37°C. Acidified isopropanol (100 µl of 0.1N HCl in anhydrous isopropanol) was added and mixed thoroughly to dissolve the dark blue crystals of formazan. Formazan quantification was performed using an ELISA plate reader with 540 nm test and 630 nm reference wavelengths.

Measurement of cytokine levels

Splenocytes were cultured as described above and supernatant was collected after 48 h to analyze cytokine levels. Mouse specific ELISA kits for measurement of IL-2 (Genetix Asia Pvt. Ltd., New Delhi India), IL-4, IL-6 (OSB Agencies Pvt. Ltd., Delhi India) and IFNγ (eBioscience Pvt. Ltd., New Delhi, India) were used. Assays were performed as per the instructions provided by the manufacturers.

Statistical analysis

Data was subjected to analysis of variance using Systat version 6.0. Significant differences among mean values of groups were analysed by Duncan's test. Values were expressed as means ± SEM. Differences with P values <0.05 were considered statistically significant.

RESULTS

Effects on body and organ weights

No significant changes were observed in the body weights of animals fed with milk, controls and probiotic dahi(s) during whole study period (data not shown here). At the end of the study, mean values

Table I. *Composition of diet fed to mice.*

Constituent(s)	Amount (g/100 g)
Starch	38.5
Casein	20.0
Sucrose	25.0
Refined oil	10.0
Vitamin mixture#	1.0
Mineral mixture#	5.0
Choline chloride	0.2
Methionine	0.3
# Vitamin and mineral mixtures were prepared and mixed according to AOAC (25)	

of body weight gains were slightly higher (3.8 g) in animals pre-fed for 7 days with probiotic dahi than other groups. Although, the mean weight of spleen collected from *S. enteritidis*-infected CON mice was slightly higher (~0.87 g) than others, but no significant differences were observed in the weights of liver (~1.89 g) collected from *S. enteritidis*-infected mice among all groups for both experiments (data not shown).

Effects on microbial counts

Counts of *S. enteritidis* in intestine, liver and spleen were significantly lower in animals pre-fed with probiotic dahi for both 2 and 7 days. This effect was more prominent in mice pre-fed for 7 days than for 2 days. As depicted in Table I, *S. enteritidis* counts were significantly lower in tissue samples of 7-day pre-fed animals than those 2- day pre-fed, whereas no significant differences were observed between CON and MFG animals. Furthermore, counts of *S. enteritidis* were moderately lower in the animals pre-fed for 7 days with control dahi, whereas no significant effect was observed in 2-day pre-feeding of control dahi.

Interestingly, the total faecal counts of lactobacilli were significantly increased (Fig. 1.) after feeding with probiotic dahi for both 2 and 7 days and consistently remained raised up to end point of the study. On the other hand, total lactobacilli counts in fecal samples from other groups consistently decreased after *S. enteritidis* challenge (Fig. 1).

Effects on β-galactosidase and β-glucuronidase activities

Enzymatic activities of β-galactosidase and β-glucuronidase in intestinal fluid collected from mice pre-fed for 2 days with milk, control and probiotic dahi significantly increased with the time of *S. enteritidis* infection, but the extent of increase was significantly lower in mice fed with probiotic dahi than in other groups (data not shown here). The extent of elevation of these values was highest in CON animals followed by MFG, CDG and PDG, respectively. Interestingly, the rate of increment in β-galactosidase and β-glucuronidase activities in intestinal fluid collected from mice pre-fed for 7 days with probiotic dahi was significantly even lower than that of milk and control fed mice. Such increasing effect in β-galactosidase activity was drastically suppressed ($p < 0.05$) in mice fed with probiotic dahi. Similarly the suppressing effect of probiotic dahi for β-glucuronidase activity was also observed (Fig. 2).

Effects on sIgA

The sIgA levels in intestinal fluid were significantly ($p < 0.05$) increased (61%) in the animals pre-fed for 7 days with probiotic dahi on day 5 after *S. enteritidis* infection, and remained higher (56%) till 8 days than those of other groups ($p < 0.05$). No significant differences were observed of sIgA levels in intestinal fluid collected from animals pre-fed for 7 days with milk (MFG), control dahi (CDG) and CON during experimental period (Fig. 3). Similarly, sIgA levels in intestinal fluid were not altered in the animals pre-fed for 2 days with milk, control and probiotic dahi after *S. enteritidis* infection (data not shown).

Effects on lymphocyte proliferation

Lymphocyte proliferation index was observed to be increased on day 5 after *S. enteritidis* infection in the cells collected from mice pre-fed with probiotic dahi for 2 days, and then it decreased till 8 days (Fig. 4A). On the other hand, proliferation index of cells collected from MFG and CDG mice did not change as compared to CON. However, pre-feeding of probiotic dahi for 7 days significantly increased

Table II. *The counts of S. enteritidis (log cfu/ g of tissues) in intestine, liver and spleen collected from mice pre-fed for 2 and 7 days with non-fermented milk, control dahi and probiotic dahi before challenge and during 8 days of the experiment*[*].

Group(s)	Intestine			Liver			Spleen		
	2 day	5 day	8 day	2 day	5 day	8 day	2 day	5 day	8 day
	← After 2 days pre-feeding →								
CON	6.28±0.13^{aA}	6.82±0.16^{bA}	4.98±0.09^{cA}	4.51±0.14^{aA}	5.98±0.11^{bA}	4.21±0.09^{cA}	3.34±0.10^{aA}	4.59±0.12^{bA}	3.68±0.08^{cA}
MFG	6.25±0.11^{aA}	6.78±0.17^{bA}	4.88±0.23^{cA}	4.40±0.19^{aA}	5.96±0.16^{bA}	4.12±0.25^{aA}	3.20±0.13^{aA}	4.52±0.11^{bA}	3.59±0.15^{cA}
CDG	5.92±0.13^{aC}	6.50±0.20^{bA}	4.62±0.27^{cA}	4.80±0.10^{aB}	5.90±0.13^{bA}	4.20±0.21^{aA}	3.30±0.06^{aA}	3.98±0.08^{bB}	2.59±0.11^{cB}
PDG	5.61±0.18^{aC}	6.20±0.11^{bB}	4.16±0.15^{bB}	3.86±0.09^{aC}	5.10±0.11^{bB}	3.50±0.13^{cB}	2.90±0.13^{aB}	3.27±0.14^{bC}	1.90±0.14^{cC}
	← After 7 days pre-feeding →								
CON	6.06±0.09^{aA}	6.61±0.12^{bA}	5.59±0.11^{cA}	4.88±0.11^{aA}	5.51±0.10^{bA}	4.45±0.08^{cA}	3.19±0.13^{aA}	4.51±0.11^{bA}	2.21±0.12^{cA}
MFG	6.00±0.25^{aA}	6.52±0.19^{bA}	4.56±0.14^{cA}	4.82±0.17^{aA}	5.43±0.15^{bA}	4.40±0.12^{cA}	3.10±0.17^{aA}	4.52±0.17^{bA}	2.10±0.17^{cA}
CDG	5.50±0.14^{aB}	6.23±0.15^{bA}	4.24±0.11^{cB}	4.20±0.13^{aB}	4.43±0.12^{aB}	3.51±0.17^{bB}	1.98±0.11^{aB}	3.25±0.12^{bB}	2.42±0.14^{cA}
PDG	4.30±0.18^{aC}	4.56±0.17^{aC}	2.20±0.12^{bD}	3.98±0.10^{aC}	3.80±0.11^{aC}	1.00±0.13^{bC}	1.10±0.09^{aC}	3.00±0.09^{bC}	1.10±0.11^{aC}

[*]*Values are means ± SEM of 5 animals in each group*
[a,b,c]*Values with different superscripts in a row in a particular parameter are significantly different at the level of* $p < 0.05$

Table III. *The levels of IL-2 and IL-4 in the supernatant of cultured splenocytes collected from animals pre-fed for 2 and 7 days with non-fermented milk, control dahi and probiotic dahi before S. enteritidis challenge and during 8 days of the experiment*[*].

Groups	IL-2 (pg/ ml)			IL-4 (pg/ml)		
	2 day	5 day	8 day	2 day	5 day	8 day
	← After 2 days pre-feeding →					
CON	41.0±4.88^{aA}	35.9±4.78^{aA}	40.1±4.01^{aA}	136.2±9.87^{aA}	128.4±5.89^{aA}	139.9±6.38^{aA}
MFG	40.2±5.30^{aA}	35.6±5.25^{aA}	38.0±3.78^{aA}	134.4±12.14^{aA}	127.2±6.33^{aA}	135.2±6.23^{aA}
CDG	44.2±5.22^{aA}	40.2±4.66^{aA}	39.8±4.65^{aA}	126.1±7.41^{aA}	130.1±5.72^{aA}	131.1±4.65^{aA}
PDG	39.9±5.14^{aA}	35.6±6.12^{aA}	38.6±5.45^{aA}	125.1±5.81^{aA}	128.5±4.89^{aA}	125.2±4.23^{aA}
	← After 7 days pre-feeding →					
CON	51.3±4.11^{aA}	59.2±5.77^{aA}	59.5±5.34^{aA}	93.4±5.83^{aA}	95.3±4.23^{aA}	121.2±3.76^{bA}
MFG	50.1±4.2^{aA}	55.0±6.4^{aA}	58.1±5.8^{aA}	91.5±5.33^{aA}	94.0±4.55^{bA}	121.1±3.23^{cA}
CDG	80.2±7.1^{aB}	155.3±7.8^{bB}	62.4±8.9^{cA}	47.5±3.87^{aB}	61.2±4.76^{bB}	50.1±3.55^{aB}
PDG	220.2±11.2^{aC}	327.3±16.3^{bC}	155.0±14.6^{cC}	35.1±4.76^{aC}	34.8±4.32^{aC}	30.7±5.11^{aC}

[*]*Values are means ± SEM of 5 animals in each group*
[a,b,c]*Values with different superscripts in a row for a particular parameter are significantly different at the level of* $p < 0.05$
[A,B,C]*Values with different superscripts in a column for a particular pre-feeding time are significantly different at the level of* $p < 0.05$

LPS-induced cell proliferation of splenocytes collected from *S. enteritidis* infected mice. Values were elevated on day 5 of the post-challenge period and then slightly decreased, but values still remained significantly higher at day 8 as compared to other groups. Furthermore, no significant changes were observed in cell proliferation index of cells collected from MFG and CDG compared to CON (Fig. 4B).

Effects on cytokine levels

Levels of IFN-γ were significantly increased after infection with *S. enteritidis* in all the groups, but levels were significantly increased in supernatant of cultured splenocytes collected from mice pre-fed

Table IV. *The levels of IL-6 and IFN-γ in the supernatant of cultured splenocytes collected from animals pre-fed for 2 and 7 days with non-fermented milk, control dahi and probiotic dahi before S. enteritidis challenge and during 8 days of the experiment*[*].

Group(s)	IL-6 (pg/ml)			IFN-γ (pg/ml)		
	2 day	5 day	8 day	2 day	5 day	8 day
	← After 2 days pre-feeding →					
CON	97.5 ± 6.33^{aA}	95.8 ± 5.87^{aA}	94.5 ± 6.34^{aA}	199.2 ± 8.54^{aA}	210.3 ± 16.56^{aA}	329.3 ± 15.2^{aA}
MFG	96.2 ± 7.82^{aA}	95.2 ± 6.6^{aA}	93.2 ± 9.5^{aA}	198.8 ± 10.4^{aA}	207.1 ± 21.4^{aA}	321.1 ± 22.1^{bA}
CDG	98.0 ± 6.91^{aA}	95.6 ± 5.1^{aA}	102.1 ± 4.6^{aA}	315.0 ± 21.3^{aB}	395.3 ± 26.3^{bB}	425.0 ± 25.3^{bB}
PDG	105.3 ± 12.5^{aA}	110.0 ± 7.8^{aA}	110.2 ± 9.3^{aA}	462.4 ± 26.2^{aC}	701.2 ± 36.2^{bC}	776.3 ± 29.7^{cC}
	← After 7 days pre-feeding →					
CON	106.2 ± 9.3^{aA}	104.3 ± 12.4^{Aa}	92.4 ± 8.4^{aA}	281.3 ± 12.4^{aA}	293.4 ± 11.3^{aA}	224.3 ± 14.1^{bA}
MFG	102.6 ± 11.6^{aA}	102.5 ± 10.6^{bA}	91.8 ± 8.7^{bA}	278.1 ± 12.5^{aA}	295.0 ± 21.1^{aA}	220.0 ± 21.5^{bA}
CDG	158.5 ± 13.2^{Ab}	84.6 ± 8.9^{bB}	110.2 ± 9.5^{cB}	400.2 ± 18.3^{aB}	828.2 ± 28.4^{bB}	302.1 ± 32.5^{cB}
PDG	250.0 ± 12.7^{aC}	162.3 ± 13.5^{bC}	185.0 ± 12.7^{bC}	633.3 ± 23.2^{Ac}	1769.0 ± 21.8^{bC}	1073.8 ± 32.3^{cC}

[*]*Values are means ± SEM of 5 animals in each group*
[a,b,c]*Values with different superscripts in a row for a particular parameter are significantly different at the level of p < 0.05*
[A,B,C]*Values with different superscripts in a row for a particular pre-feeding time are significantly different at the level of p < 0.05*

for 2 days with probiotic dahi compared to those of CON, MFG and CDG (p <0.05). The levels of IL-2, IL-4 and IL-6 did not change among various groups of animals pre-fed for 2 days with milk, control and probiotic dahi and infected with *S. enteritidis* during whole experimental period (Tables III and IV). In line with other findings, pre-feeding for 7 days was more effective for production of cytokines by spleen cells. As shown in Tables III and IV, the levels of IL-2, IL-6 and IFN-γ were dramatically increased after day 2 of *S. enteritidis* infection, and further increased values of cytokines were observed during whole post challenge period than other groups (p < 0.05). Levels of IL-4 were significantly lower in cells collected from PDG mice as compared to other groups (p < 0.05), while no significant differences were observed in these cytokine levels in supernatant of cultured splenocytes collected from 7 days pre-fed CON and MFG mice. Feeding of control dahi also exhibited a moderate activity to enhance cytokine production in cultured spleen cells.

DISCUSSION

In the present study, the effect of probiotic dahi was evaluated against *S. enteritidis* infection in mice pre-fed for 2 and 7 days. It has been observed that effects were more prominent in animals pre-fed with probiotic dahi for 7 days than that for 2 days. Feeding of probiotic dahi enhanced the innate, as well as specific immunity against *S. enteritidis* infection characterized by enhancing the production of sIgA, cytokines and lymphocyte proliferation, and decreasing *S. enteritidis*-induced inflammatory response. The infection with *S. enteritidis* frequently results in intestinal gut fluid secretion and is characterized by diarrhea, but the exact mechanism remains unknown (18). Generally, *S. enteritidis* invades to intestinal epithelium and stimulates intense acute inflammatory response (19). In accordance with this, results of the resent study indicate that inflammatory marker i.e. β-galactosidase and β-glucuronidase, activities were significantly increased in *S. enteritidis* infected mice. Interestingly, feeding of probiotic dahi significantly ameliorated the activities of these enzymes in the intestinal fluid, which indicates that probiotic dahi suppressed *S. enteritidis*-induced acute inflammatory reactions. The exact mechanism of this inhibitory effect of probiotic dahi against *S. enteritidis* is not clear, but our results suggest that, the feeding of probiotic dahi before infection may generate an inhibitory layer on

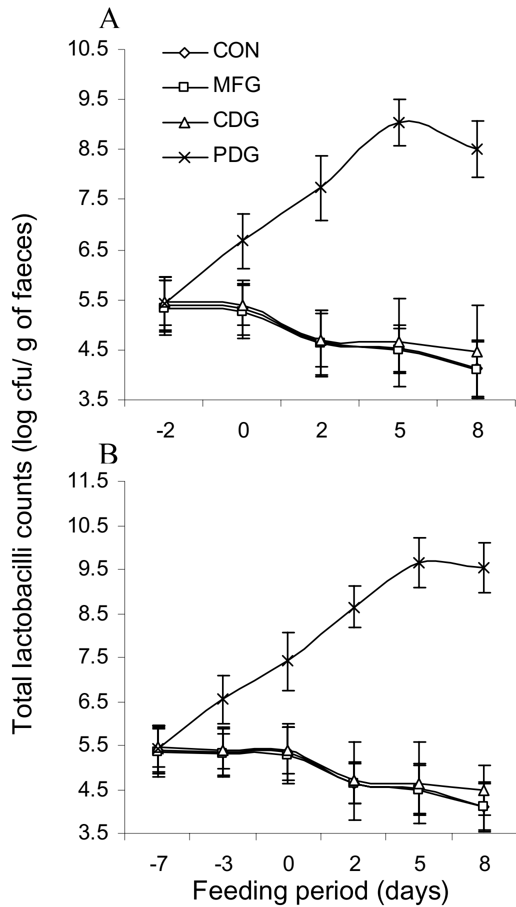

Fig. 1. *The total faecal lactobacilli counts in the samples collected from mice fed with synthetic diet (CON), milk (MFG), control dahi (CDG) and probiotic dahi (PDG) for 2 (**A**) and 7 (**B**) days before S. enteritidis.* [a,b,c]*Values (means ± SD of 5 animals in each group) within a group of different time intervals are significantly different at the level of P <0.05.*
[A,B,C]*Values (means ± SEM of 5 animals in each group) among various groups at particular time intervals are significantly different at the level of P < 0.05.*

the surface of intestinal epithelium, such as biolfilm. It has been clearly observed that feeding of probiotic dahi significantly increases intestinal and fecal total lactobacilli counts and decreases *S. enteritidis* counts in intestine. This indicates that lactobacilli are already established in the gut of mice before *S. enteritidis,* and inhibit the growth as well as invasion of *S. enteritidis*.

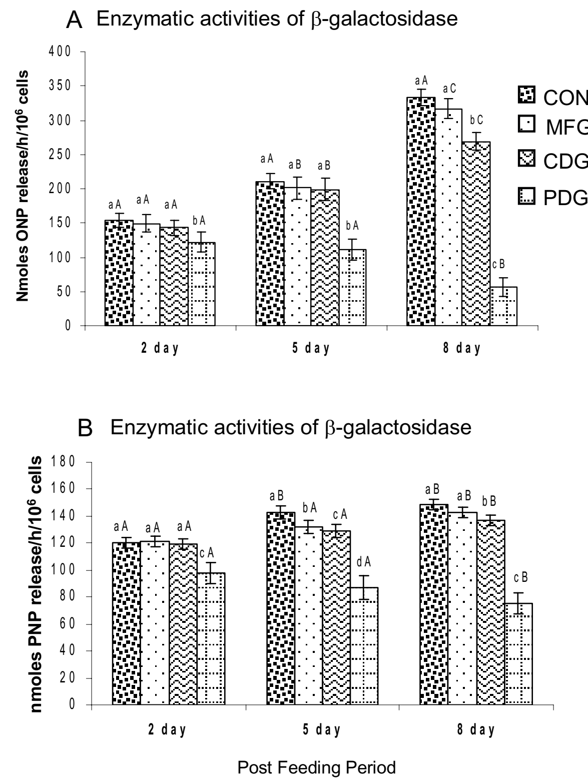

Fig. 2. *The enzymatic activities of β-galactosidase (A) and β-glucuronidase (B) in intestinal fluid collected from mice fed with synthetic diet (CON), non-fermented milk (MFG), control dahi (DFG) and probiotic dahi (PDG) for 7 days before S. enteritidis challenge and during 8 days of the experiment*. Description of symbols as in Fig. 1.*

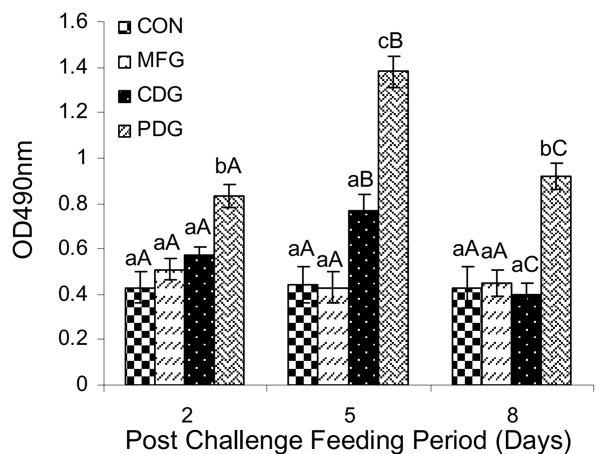

Fig. 3. *The levels of sIgA in intestinal fluid collected from mice fed with synthetic diet (CON), non-fermented milk (MFG), control dahi (CDG) and probiotic dahi (PDG) for 7 days before S. enteritidis challenge and during 8 days of the experiment*.*
Symbols as described as in Fig.1.

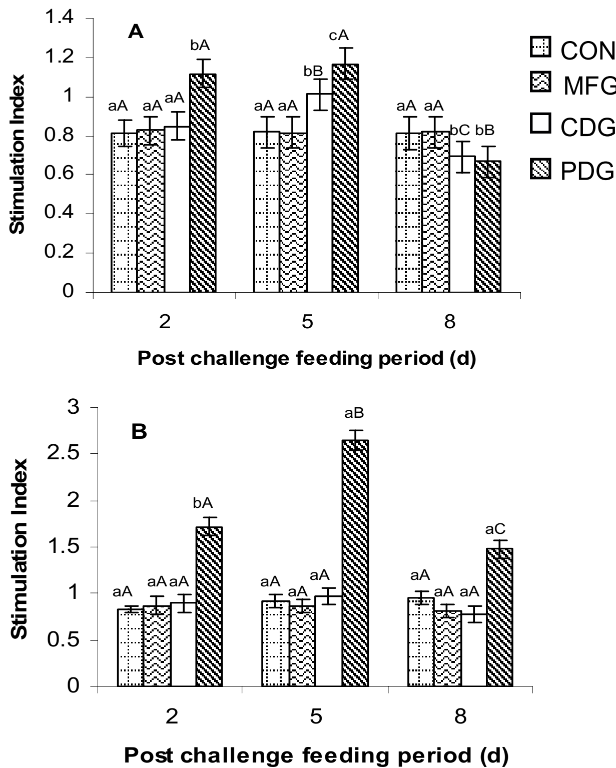

Fig. 4. *The splenocyte proliferation index of spleen cells collected from mice fed with synthetic diet (CON), non-fermented milk (MFG), control dahi (CDG) and probiotic dahi (PDG) for 2 (**A**) and 7 (**B**) days before S. enteritidis challenge and during 8 days of the experiment*. Description of symbols as in Fig. 1.*

S. enteritidis infection occurs when ingested organism by-pass gastric defenses, multiply within intestinal lumen, penetrate into intestinal mucosa and multiply within macrophages of the reticuloendothelial system to disseminate via systemic circulation, reaching different organs such as liver and spleen (20). In the present study, the feeding of probiotic dahi reduced *S. enteritidis* load in intestine as well as peripheral organs i.e. liver and spleen. This indicates that probiotic dahi has potential to inhibit *S. enteritidis* infection systemically. The first line of immune-mediated defense in gut against pathogens is the production of sIgA which acts against pathogen by forming complex with particulate antigen, thereby restricting the binding of antigen to epithelial surface or by neutralizing the biologically active antigenic sites (21). In this study, it has also been observed that feeding of probiotic dahi enhanced secretion of IgA into intestinal fluid. This indicates that feeding of probiotic dahi may inhibit *S. enteritidis* infection by enhancing production of anti-*Salmonella*-sIgA, and that might exclude *S. enteritidis* antigen either by precipitating and/or presenting for complement system and immune cells to be destroyed. In line with our results, several other workers have also reported that consumption of LAB and its fermented milk products augment production of sIgA against various enteropathogens such as *E. coli*, *Shigella* and *Salmonella* (7, 22-23).

LAB activates systemic immune response by increasing proliferation of various subtype lymphocytes [T (Th0, Th1, Th2 and CTLs) and B lymphocytes] and other immune cells i.e. NK cells, dendritic cells, which circulate continuously in the blood and lymphatic system, and also migrate into the tissues at the site of infection. Although Th1 type immune response helps in eradicating the infections by stimulating cell mediated immunity (24) and secretes specific cytokines such as IL-2, IFN-γ etc., Th2 immune cells appose the functions of Th1 cells and secrete IL-4. In the present study, feeding of probiotic dahi enhanced the ability of spleen lymphocytes to proliferate *in-vitro,* and enhanced production of IL-2, IL-6 and IFN-γ. These results indicate that probiotic dahi augmented lymphocyte proliferation and enhanced T-cell response towards Th1 by stimulating the production of IL-2, IL-6 and IFN-γ. This twist in immune response may help to eradicate *S. enteritidis* infection.

In conclusion, the results of the present study indicate that the feeding of probiotic dahi has beneficial immunomodulatory effects against *S. enteritidis* by increasing protective mechanisms such as enhanced production of sIgA, lymphocyte proliferation, and improved gastrointestinal barrier, and decreasing *S. enteritidis*-induced inflammatory responses. On the basis of these results, it may be recommended that feeding of this probiotic dahi can pave the way to strengthen the body immune system for protecting the host, probably, from various enteropathogens.

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