Effect of *Lactobacillus acidophilus* NCDC 13 supplementation on the progression of obesity in diet-induced obese mice

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Abstract

There is an increased interest in investigating the relationship between the gut microbiota and energy homeostasis. Probiotics are health beneficial microbes mainly categorised under the genus *Lactobacillus* and *Bifidobacterium*, which when administered in adequate amounts confer health benefits to the host, and have been implicated in various physiological functions. The potential role of probiotics in energy homeostasis is a current and an emerging area of research. In the present study, *Lactobacillus acidophilus* NCDC 13 was used to evaluate its anti-obesity potential in diet-induced obese (C57BL/6) mice. The probiotic bacterial culture was administered in Indian yogurt preparation called 'dahi', prepared using native starter cultures, and compared with control dahi containing only dahi starter cultures. The dietary intervention was followed for 8 weeks, and whole-body fat composition, and liver and muscle adiposity were measured using MRI. Changes in gut microbiota were assessed by fluorescent *in situ* hybridisation in faeces and caecal contents. The feeding of the probiotic brought no changes in body-weight gain, food and dahi intake when compared with the control dahi-fed animals. No significant changes in body fat composition, liver and muscle adiposity were also observed. At the end of the dietary intervention, a significant increase ($P<0.05$) in the number of total *Bifidobacterium* was observed in both faeces and caecal contents of mice as a result of probiotic dahi administration. Thus, *L. acidophilus* NCDC 13 supplementation could be beneficial in shifting the gut microbiota balance positively. However, its anti-obesity potential could not be established in the present study and warrants further exploration.

Key words: Probiotics: *Lactobacillus acidophilus* NCDC 13: Dahi: Obesity: Microbiota

Obesity has now attained pandemic proportions in many parts of the world. There is a recent interest in studying the gut microbiota of obese and lean individuals, with differences in composition being suggested. As this is the case, the microbiota is susceptible to change through the diet. Probiotics are live microbes which when administered in adequate amounts confer health benefits to the host[1]. This occurs through targeting the gut microbiota. Lactobacilli and bifidobacteria are the most widely used probiotic species. For probiotics to have an effect on metabolism, it is necessary that they should reach distal parts of the intestine in sufficient numbers to exert effects therein. For this purpose, they must be able to bypass gastric acidity and possess bile-tolerant properties to

ensure survival in the lower gut. The potential role of probiotics in metabolic diseases has recently gained wide attention owing to rising evidence of the importance of the gut microbiota balance in energy homeostasis[2]. Firmicutes and Bacteroidetes represent dominant microbial phylum members of gut ecology and their ratio has been linked to obesity in mice[3] as well as in human subjects[4]. Recent studies have indicated the anti-obesity roles of different probiotics in terms of lowering of food intake and body weights, though the possible mechanism of action is not yet clear.

In the Indian population, there is an increased prevalence (10 %) of overweight individuals, of which the majority are from urban areas consuming energy-dense food products[5].

'Dahi' is a fermented milk product of the Indian subcontinent having low glycaemic index and produced by multiple starter strains of *Lactococcus*. It is perceived as a 'healthy' food product among the Indian population. It differs from European yogurt in having a higher diacetyl content produced by multiple strains of *Lactococcus*, which also gives a characteristic flavour to the product[6]. Therefore, addition of probiotics to dahi may act as an important vehicle for probiotic administration and may produce a physiological response in the host. Some earlier reports have indicated the anti-diabetic potential of probiotic dahi containing *Lactobacillus acidophilus* and *L. casei*[6–9].

L. acidophilus NCDC 13 is an indigenous probiotic strain isolated from fermented milk sources in India. The strain was selected from twenty different lactobacilli strains and has been shown to exhibit the best probiotic properties such as acid tolerance, bile tolerance and surface hydrophobicity as assessed under *in vitro* conditions[10]. The probiotic has also been used in animal experiments and been shown to survive in the gastrointestinal tract of mice[11,12]. The present study was designed to evaluate the effects of *L. acidophilus* NCDC 13 supplementation on intestinal microbiota modulation and on the progression of obesity under high-fat dietary conditions in diet-induced obese C57BL/6 mice. We investigated the anti-obesity effects of the probiotic by supplementing it in a fermented milk product, dahi, compared with the control dahi alone.

Materials and methods

Cultures

L. acidophilus NCDC 13 and control dahi culture NCDC 167 (*Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis* biovar. *diacetylactis*) were procured from the National Collection of Dairy Cultures, Dairy Microbiology Division, National Dairy Research Institute, Karnal, India.

Preparation of control and probiotic dahi

The methodology has been reported previously[6] and demonstrated to give good viable counts. Semi-skimmed milk (1·5 %, w/v fat) was purchased for the preparation of dahi. The control dahi (mixed *L. lactis* sp.) and probiotic (*L. acidophilus* NCDC 13) cultures were propagated and maintained in M17 and de Man–Rogosa–Sharpe broth, respectively. The cultures were activated in sterile skimmed milk before their usage for the preparation of dahi. For the present study, two types of dahi (control and probiotic) were prepared. Control dahi was prepared by inoculating milk with starter dahi culture at the 1 % (v/v) level. Probiotic dahi was prepared by inoculating milk with both starter dahi culture and probiotic (*L. acidophilus* NCDC 13) cultures, each at the 1 % (v/v) level. Dahi is a fermented milk product having specific textural properties (set-type, semi-solid). Adding the probiotic after fermentation would result in the alteration of the texture of the product, resulting in the control dahi and probiotic dahi with different textures. Therefore, it is recommended to add the probiotic

before dahi fermentation. There were no other nutritional differences between the two preparations other than the presence of the probiotic in the latter. Incubations were done at 30°C in both cases for 12 h and the final products so formed were stored at 4°C. The dahi products were prepared every week and bacterial enumeration was performed repeatedly. Viable counts of the control and probiotic cultures in dahi preparations were determined by serially diluting the aliquots and plating on M17 and de Man–Rogosa–Sharpe agar plates, respectively, and are expressed as colony-forming units (cfu)/ml. The viable counts of total *Lactococcus* in control dahi as determined on M17 agar were in the range of $3 \times 10^7 – 4 \times 10^7$ cfu/ml. The viable counts of *L. acidophilus* in probiotic dahi as determined on de Man–Rogosa–Sharpe agar were in the range of $5 \times 10^7 – 9 \times 10^7$ cfu/ml.

Animals and treatments

All animal procedures were performed in accordance with the UK Animals Scientific Procedures Act (1986). A total of twenty-four male C57BL/6 mice (6–8 weeks old; Charles River) were singly housed under controlled temperature (21–23°C) and light conditions (12 h light–12 h dark cycle; lights on at 07.00 hours). After acclimatisation for 1 week on a normal chow diet, they were then randomised according to their body weights and divided into two groups (control dahi and probiotic dahi), each having twelve mice. Both groups were fed on a 21 % high-fat diet (Table 1) and their respective dahi products.

Both the control and probiotic dahi were provided in special hoppers to prevent spillage, for a brief period (about 6 h) during the daytime. During this period, the high-fat diet was removed from the cages to ensure that the animals in both groups consumed their respective dahi products. Animals were resumed on the high-fat diet after dahi supplementation. Water was given *ad libitum*. The feeding schedule was followed for 8 weeks.

Table 1. Composition of the high-fat diet

Ingredients	g/kg
Energy	
kcal/g	4·6
kJ/g	19·0
Casein	195·0
DL-Met	3·0
Sucrose	342·9
Maize starch	75·0
Maltodextrin	75·0
Anhydrous milk fat	210·0
Cellulose	110·0
Mineral mix (AIN-76)	35·0
Calcium carbonate	4·0
Vitamin mix*	10·0
Ethoxyquin, antioxidant	0·04

AIN, American Institute of Nutrition.
* Vitamin mixture, g/kg (#Teklad 40 060): vitamin A palmitate, 3·9; vitamin D$_3$ (cholecalciferol), 0·4; vitamin E (α-tocopheryl acetate), 24·2; vitamin K$_3$, 4·9; thiamin hydrochloride, 2·2; riboflavin, 2·2; niacin, 9·9; folic acid, 0·19; pyridoxine hydrochloride, 2·2; vitamin B$_{12}$, 2·9; inositol, 11·0; *p*-aminobenzoic acid, 11·0; biotin, 0·04; choline dihydrogen citrate, 349·6; calcium pantothenate, 6·6; vitamin C, 101·6; mixed in maize starch.

Whole-body MRI and localised ^{1}H magnetic resonance spectroscopy for intrahepatocellular (IHCL) and intramyocellular (IMCL) lipid levels were performed after 8 weeks, at the end of the dietary intervention. Faecal pellets were collected at week 0 before the start of the dietary intervention and at week 8 before the start of the scanning experiments and kept frozen at $-20°C$ until further analysis. Following MRI procedures, mice were culled, epididymal fat collected and stored on ice. Caeca were precisely excised and the contents were frozen.

Fluorescent *in situ* hybridisation to assess microbial changes

The protocol was followed as described previously[13] with some modifications. Briefly, frozen faecal and caecal samples were diluted in ten volumes of PBS (pH 7·2; Oxoid). The samples were homogenised and the supernatant fraction (375 μl) containing bacterial cells was fixed with 4 % (w/v) paraformaldehyde in a ratio of 1:3 at 4°C. The bacterial cells were subjected to centrifugation (1000 g; 3 min), washed twice with PBS and finally suspended in an equal volume of PBS and ethanol (150 μl each). The cells were appropriately diluted and 20 μl of the suspension were applied to Teflon poly-L-lysine-coated six-well glass slides (Tekdon, Inc.). Permeabilisation of the cells was carried out in an ethanol series (50, 80 and 96 %) for 3 min each and hybridised with group-specific Cy-3-labelled (at the 5′-end) probes for 4 h at 50°C. The probes used were LAB158[14], BIF164[15], EREC482[16] and MIB663[17] to enumerate total *Lactobacillus–Enterococcus*, bifidobacteria, *Eubacterium rectale– Clostridium coccoides* and mouse intestinal bacteria belonging to the Bacteroides subgroup in the phylum Cytophaga– Flavobacter–Bacteroides, respectively. The slides were subsequently washed, mounted in ProLong® Gold antifade reagent and stored at 4°C under dark conditions. The fluorescent cells were manually counted in fifteen random fields of view per well. Total cell counts were obtained with 4′,6-diamidino-2-phenylindole dihydrochloride staining.

Magnetic resonance studies

Whole-body ^{1}H magnetic resonance spectroscopy. Mice were fasted for 16 h and anaesthetised with a 1–2 % isoflurane–oxygen mix which was maintained throughout the scan. The animals were scanned on a 4.7 Tesla Varian INOVA imaging system (Varian, Inc.) using pulse sequence with the following parameters: repetition time, 10 s; pulse angle, 45°; averages, 4. The spectra obtained were analysed and body adiposity calculated as described earlier[18].

Whole-body MRI. Whole-body MRI was carried out to determine the amount of visceral and subcutaneous adipose tissue. Consecutive 2 mm-thick slices were acquired using a spin-echo sequence with the following parameters: repetition time, 2·2 s; echo time), 20 ms; matrix size, 256 × 192; field of view), 45 × 45 mm; averages, 2. The slices/images were then subjected to segmentation analysis (SliceOmatic™, Tomovision®) by an observer blinded to the experimental

groups and the masses of different adipose tissue depots calculated.

Localised ^{1}H magnetic resonance spectroscopy. The IHCL and IMCL lipid content was assessed by placing a voxel of $2 × 2 × 2\,mm^3$ on liver and muscle images. A point resolved spectroscopy sequence with the following parameters, repetition time = 10 s, echo time = 9 ms and averages = 64, was applied on the voxel to obtain the spectra and the relative percentage of lipid determined, by integration of the lipid peak[18].

Adipocyte cell size and number

White adipose tissue (epididymal depot) was collected and finely minced in Dulbecco's modified Eagle's medium solution supplemented with 4 % (w/v) glucose and collagenase (1 mg/ ml). The tissue suspension was incubated in a vibrating water-bath set at 140 cycles/min for 45 min at 37°C. The suspension was filtered through a polypropylene mesh (400 μm) after complete digestion of the tissue. This was followed by washing twice using Dulbecco's modified Eagle's medium solution containing 4 % (w/v) bovine serum albumin and 1 mg/40 ml trypsin inhibitor. An aliquot of the adipocytes was collected, diluted with trypan blue and the cells were counted in a haemocytometer. Cell number and size were calculated from the images acquired using software Cellprofiler after setting an appropriate threshold.

Statistical analysis

All data are presented as means with their standard errors. The data were statistically analysed using GraphPad Software (GraphPad Software). Two-way ANOVA with Bonferroni *post hoc* tests were used for the analysis of body weights, food intake and fluorescent *in situ* hybridisation data using time (d/weeks) and treatment as two independent variables.
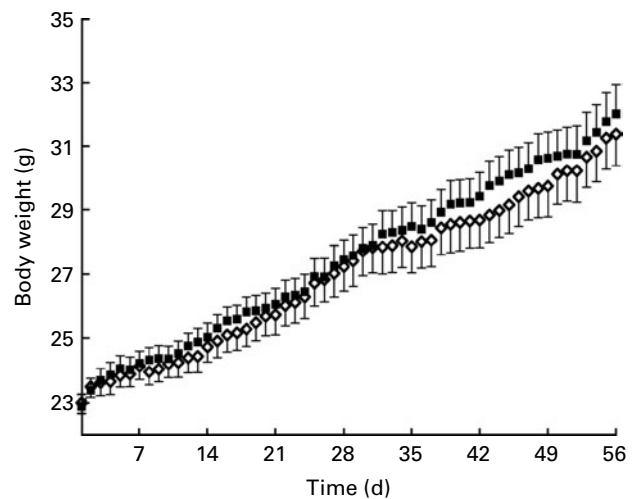

Fig. 1. Effect of control and probiotic dahi on body weight in mice fed a high-fat diet. Mice were fed either control (◇) or probiotic (■) dahi along with a high-fat diet and their body weight was measured daily over the 8-week intervention period. Values are means corresponding to twelve mice, with standard errors represented by vertical bars.
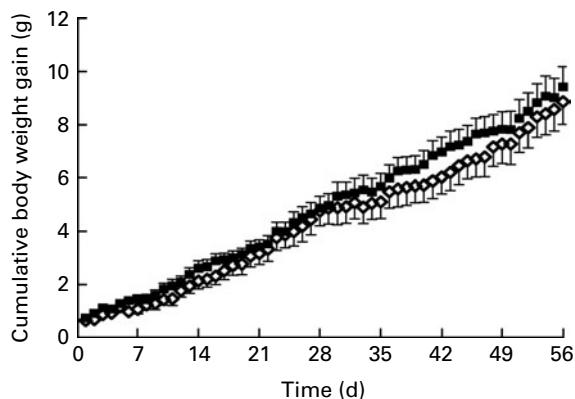
Fig. 2. Effect of control and probiotic dahi on cumulative body-weight gain in mice fed a high-fat diet. Mice were fed either control (◊) or probiotic (■) dahi along with a high-fat diet and their body weight was measured daily. Cumulative body-weight gain was calculated by subsequently adding the daily weight gains of mice over the 8-week intervention period. Values are means corresponding to twelve mice, with standard errors represented by vertical bars.

Other parameters such as whole body, liver and muscle lipid levels, adipocyte size and number were analysed by an unpaired t test to compare the two groups. P values <0.05 were considered significant.

Results

Body weight, cumulative food and dahi intake

The feeding of the probiotic brought no significant changes in body weight and cumulative body-weight gains of mice over control dahi supplementation. Similarly, no differences in cumulative food and dahi intake were observed between the two groups (Figs. 1–4).

Whole-body fat composition

Measurements of whole-body adiposity and fat deposition in visceral and subcutaneous fat depots by MRI revealed no differences between the control and probiotic dahi-fed mice. Lipid levels in the IHCL and IMCL fluids also differed non-significantly between the two groups (Table 2).

Adipocyte size and number

The average adipocyte size was measured to be 78·65 (SEM 12·31) and 69·96 (SEM 12·41) μm for the control and probiotic dahi-fed groups, respectively (Table 2). Adipocyte size, though apparently smaller in the case of the probiotic dahi-fed group, was statistically non-significant. Adipocyte number also differed non-significantly between the two groups (Table 2).

Microbial modulation

Data on different bacterial groups in the faecal and caecal samples, expressed as $\log_{10}$ cells/g, are shown in Figs. 5 and 6. The baseline number of total bacteria as measured by
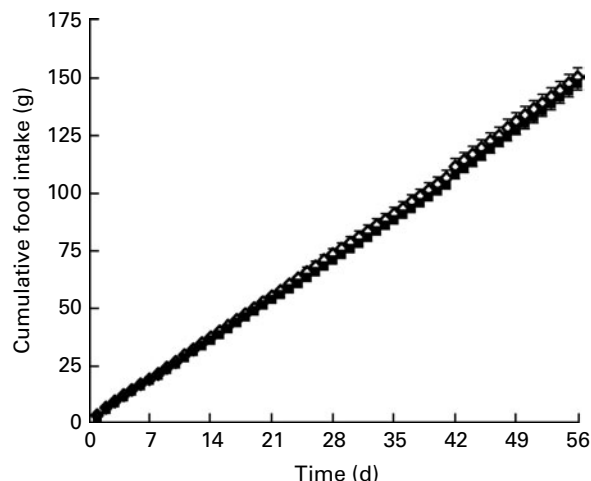

Fig. 3. Effect of control and probiotic dahi on cumulative food intake in mice fed a high-fat diet. Mice were fed either control (◊) or probiotic (■) dahi along with a high-fat diet and their food intake was measured daily. Cumulative food intake was calculated by subsequently adding the daily food intake of mice over the 8-week intervention period. Values are means corresponding to twelve mice, with standard errors represented by vertical bars.

4′,6-diamidino-2-phenylindole dihydrochloride and other bacterial groups targeted by different probes in the faecal samples were similar between the control and probiotic dahi-fed groups before the start of the dietary intervention. At week 8, a significant difference was observed in bifidobacterial numbers ($P<0.05$) between the faecal samples of the control and probiotic dahi-fed groups (7·59 (SEM 0·12) $v.$ 8·04 (SEM 0·10) $\log_{10}$ cells/g). However, no difference was observed in the other bacterial groups such as total bacteria, E. rectale– C. coccoides and mouse intestinal bacteria. A similar trend was observed in the caecal contents collected terminally, with a significant difference in bifidobacterial numbers. The feeding of a high-fat diet along with control yogurt resulted in
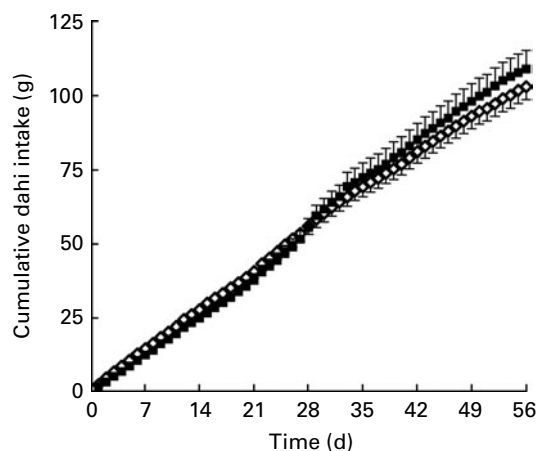

Fig. 4. Effect of control and probiotic dahi on cumulative dahi intake in mice fed a high-fat diet. Mice were fed either control (◊) or probiotic (■) dahi along with a high-fat diet and their dahi intake was measured daily. Cumulative dahi intake was calculated by subsequently adding the daily dahi intake of mice over the 8-week intervention period. Values are means corresponding to twelve mice, with standard errors represented by vertical bars.

Table 2. Effect of control and probiotic dahi on tissue weights, body fat composition, and adipocyte size and number in mice fed a high-fat diet

(Mean values with their standard errors, *n* 12)

Parameter	Control dahi		Probiotic dahi		P
	Mean	SEM	Mean	SEM	
Liver weight (g)	1·29	0·11	1·38	0·11	0·6189
Epididymal WAT mass (g)	1·09	0·11	1·26	0·16	0·4719
Caecal weight (g)	0·21	0·01	0·20	0·01	0·6872
Whole-body adiposity (%)	19·01	2·10	21·47	2·33	0·4457
Liver adiposity (%)	10·71	1·20	13·01	1·77	0·3009
Muscle adiposity (%)	0·84	0·11	1·00	0·28	0·5814
Visceral fat (g)	2·26	0·31	2·39	0·35	0·7921
Subcutaneous fat (g)	3·34	0·31	3·53	0·25	0·7482
Adipocyte size (μm)	78·65	12·31	69·96	12·41	0·6299
Adipocyte number	$1·7 \times 10^8$	$5·3 \times 10^7$	$2·1 \times 10^8$	$5·3 \times 10^7$	0·6246

WAT, white adipose tissue.

a significant decrease in the faecal *Lactobacillus–Enterococcus* counts at week 8 when compared with baseline. However, probiotic (*L. acidophilus*) feeding was found to maintain a similar count of *Lactobacillus–Enterococcus* after 8 weeks under high-fat dietary conditions.

Discussion

The study aimed to investigate the effects of feeding a probiotic, *L. acidophilus* NCDC 13, on intestinal microbiota modulation and the progression of obesity in diet-induced obese mice under a high-fat dietary environment. The probiotic was fed in the form of a dahi preparation and the results were compared with the control dahi-fed animals. The feeding of the probiotic dahi resulted in no significant differences in body weight, cumulative body-weight gain, cumulative food and dahi intake in comparison with the control dahi-fed group of mice. The present results are consistent with the observations of Esposito *et al.*[19] who reported no change in the body weight and food intake of rats fed a high-fat diet supplemented with a commercial probiotic preparation, VSL#3, containing multiple strains of *Lactobacillus, Bifidobacterium* and *Streptococcus thermophilus*. Similarly, no change in body weight as well as food intake has been reported in rats fed high-cholesterol diets supplemented with *L. plantarum*[20]. However, body-weight gain was rather high with no change in food consumption in rats fed non-fermented milk produced with *L. gasseri*[21]. On the contrary, Lee *et al.*[22] observed that administration of *L. rhamnosus*, a conjugated linoleic acid-producing bacterium, resulted in the lowering of body-weight gain in mice with no change in average energy intake. In another study, the probiotic strain, *L. paracasei*, reduced the body-weight gain in rats fed a high-fat diet with no change in food consumption[23]. Similarly, no effect on food intake was observed, despite an increase in the plasma levels of peptide YY and neuropeptide Y, the hormones implicated in central appetite regulation, when rats were fed a mixture of probiotics (*L. delbrueckii* and *B. lactis*) and a prebiotic (inulin)[24]. The efficacy of a probiotic is highly strain-specific, which could account for variations in the outcome of studies employing different probiotic strains.

We measured whole-body adiposity and lipid levels in the IHCL and IMCL fluids, and lipid deposition in visceral and subcutaneous fat depots in both groups of mice using MRI/S imaging techniques. However, we found no significant differences in the aforementioned parameters, which are in agreement with the observed non-significant difference in the adipocyte size of mice. There are recent reports which demonstrated the effects of different probiotic strains in the lowering of adipose tissue mass[19,22,25] and adipocyte size[26–28]. This discrepancy may be explained by the difference in the mode of the probiotic administration. In the present study, the probiotic was supplemented in the form of a fermented milk preparation along with dahi starter cultures, whereas in other studies, the probiotic was administered alone either by oral administration or was incorporated in the skimmed milk. Recent elegant studies in axenic mice harbouring simplified gut bacterial members have shown that the presence of two different bacterial species alters the metabolic activity of
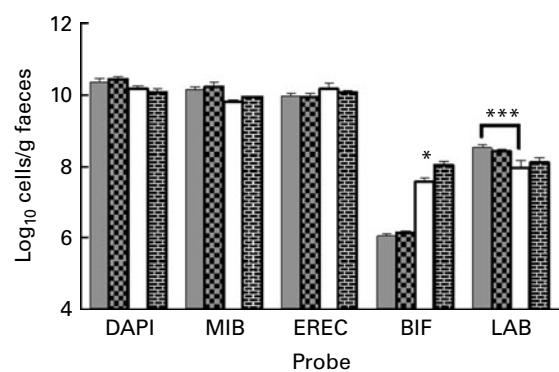

Fig. 5. Effect of control and probiotic dahi on faecal bacterial groups in mice fed a high-fat diet. Mice were fed either control or probiotic dahi along with a high-fat diet. Faecal samples were collected either at baseline (■, control; ▨, probiotic) or after 8 weeks at the end of dietary intervention (□, control; ▤, probiotic). The different bacterial groups belonging to mouse intestinal bacteria (MIB), *Eubacterium rectale–Clostridium coccoides* (EREC), and total bifidobacteria (BIF) and *Lactobacillus–Enterococcus* (LAB) were measured using fluorescent *in situ* hybridisation. Total bacteria were determined by 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining. Values are means corresponding to six mice, with standard errors represented by vertical bars. *$P<0·05$, ***$P<0·001$.
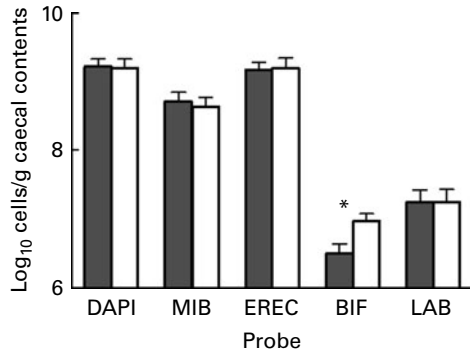
Fig. 6. Effect of control and probiotic dahi on caecal bacterial groups in mice fed a high-fat diet. Mice were fed either control or probiotic dahi along with a high-fat diet. Caecal contents were collected after 8 weeks at the end of dietary intervention from both control (■) or probiotic (□) dahi-fed mice. The different bacterial groups belonging to mouse intestinal bacteria (MIB), *Eubacterium rectale–Clostridium coccoides* (EREC), and total bifidobacteria (BIF) and *Lactobacillus–Enterococcus* (LAB) were measured using fluorescent *in situ* hybridisation. Total bacteria were determined by 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) staining. Values are means corresponding to six mice, with standard errors represented by vertical bars. * $P < 0.05$.

each other[29,30]. Therefore, it is also possible that though the multiple starter strains of *Lactococcus* sp. used in the preparation of dahi did not affect the viability of the probiotic in the product, they might have hindered the efficacy of the probiotic culture in the gut of the animals. Also, it is demonstrated that permanent colonisation of species from common probiotic drinks has proven difficult[31]. There could be interactions between two cultures during fermentation *in vitro* but the interaction in the gut *in vivo* is far more complex. The desired property is the proliferation of the probiotic in the presence of the starter culture in the product so that the probiotic reaches the gut in good amounts.

In the present study, the number of total *Lactobacillus–Enterococcus* recovered in the faeces and caecal contents of mice after 8 weeks of probiotic supplementation did not differ significantly when compared with the control dahi group, which lacked the probiotic bacteria. Nevertheless, the probiotic supplementation maintained the total *Lactobacillus–Enterococcus* count (at week 8) similar to the initial baseline count (at week 0), while in the control dahi group, a significant reduction in the total lactobacilli counts was observed at week 8 under a high-fat diet. This may be indicative of the inability of the probiotic strain used in the present study to colonise the intestinal walls and proliferate in the intestinal environment under high-fat dietary conditions. *L. acidophilus* NCDC 13 has been shown to exhibit probiotic properties in terms of acid, and bile tolerance under *in vitro* conditions by other workers[10]. However, it is generally stated that *in vitro* studies are not often predictive of the *in vivo* situation, since conditions are so vastly different[32]. On the contrary, Kushal *et al.*[11] reported an increase in total *Lactobacillus* counts in the faeces of mice (maintained on a normal chow diet) after 8 d of *L. acidophilus* NCDC 13 supplementation. However, a conventional plating technique in selective media was used for the enumeration of total faecal *Lactobacillus* when

compared with fluorescent *in situ* hybridization hybridisation used in the present study. The fluorescent *in situ* hybridisation hybridisation technique relies upon specific hybridisation of the 16s rRNA-based probes in the target organism and provides a better measure of the bacterial number accurately. It also alleviates the laborious cultivation and purification tasks[33]. The dose given may also make a difference in the potential to colonise the intestinal walls. Kushal *et al.*[12] used sterile skimmed milk to provide 1×10^{10} cfu/ml of *L. acidophilus* NCDC 13 per mouse per d, while in the present study, mice consumed about 2 g of the probiotic dahi, which provided $1–2 \times 10^8$ cfu of *L. acidophilus* NCDC 13 per mouse per d. The difficulty in achieving colonisation has been highlighted by Lee *et al.*[31] who demonstrated decreased replication rates of probiotic organisms in the mouse gut. The addition of prebiotic carbohydrates has been shown to enhance the survival and proliferation of probiotics both under *in vitro*[34] and *in vivo* conditions[35]. Therefore, it would be interesting to supplement *L. acidophilus* NCDC 13 with suitable prebiotics to enhance the survivability of the probiotic in the gut and to study its potential anti-obesity role.

We have reported here a significant increase in the number of *Bifidobacterium* in both the caecal contents and faeces of mice fed the probiotic dahi preparation. This is in contrast to the expected rise in *Lactobacillus* numbers. Dinoto *et al.*[36] also reported similar results where supplementation of a probiotic, *B. breve*, into the diet of rats produced no change in total bifidobacteria counts as well as no recovery of the probiotic strain in the caecal contents, whereas the number of total lactobacilli increased. Some other reports have also revealed an increase in bifidobacterial numbers upon administration of probiotic lactobacilli strains in different animal models[37,38]. Thus, certain unexplained metabolic interactions are undergoing in the colonic environment upon supplementation of dahi and probiotic cultures. With regard to anti-obesogenesis, an increase in bifidobacterial number is desirable and has been positively correlated with weight loss[39] and the lean phenotype in rodents[40].

Thus, it is concluded that supplementation of *L. acidophilus* NCDC 13 did not result in the lowering of body weight and food intake. However, supplementation of *L. acidophilus* NCDC 13 in the diet of high-fat fed mice increased bifidobacterial numbers in the caecal contents as well as in the faeces. Further investigation with the probiotic could provide further insights into the mechanism of probiotic functioning and its potential anti-obesity role.

developed the study protocol. T. A., J. A. and K. T. carried out the studies. T. A., J. A., G. G., K. T., R. S., J. B. and G. F. analysed the data and wrote the manuscript. The authors state that there is no conflict of interest.

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