

## Effect of feeding traditionally prepared fermented milk *dahi* (curd) as a probiotics on nutritional status, hindgut health and haematology in dogs

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According to Indian system of traditional medicine (Ayurveda), *dahi* is beneficial in promotion of health and vitality due to its antibacterial action against pathogenic microbes and improvement in nutrient digestibility. Hence, in the present study traditionally prepared Indian fermented milk *dahi*/curd was evaluated as a probiotics for its health benefits in canine model. Eight Labrador dogs were divided in completely randomized design (CRD) in two groups, one control (CON, without supplement) and other treatment group (*dahi*, supplemented with measured amount (100 ml) of *dahi*/curd having  $\sim 10^{6-7}$  cfu of *Lactobacillus* sp /ml). Nutrient digestibility and hindgut health was assessed after 6 weeks and haematology was done after 7 weeks of experimental feeding. There was a slight ( $P>0.05$ ) increase in dry matter (DM,  $P=0.055$ ), organic matter (OM,  $P=0.073$ ), crude fibre (CF,  $P=0.104$ ) digestibility while that of calcium improved significantly ( $P<0.05$ ) due to feeding of *dahi*. Significant reduction in fecal pH ( $P<0.001$ ) and ammonia ( $P<0.01$ ), whereas lactate ( $P<0.001$ ) and total short chain fatty acids (SCFAs,  $P<0.05$ ) were increased in *dahi* fed group. The health positive microbial count (lactobacilli and bifidobacteria) were significantly ( $P<0.05$ ) increased with decrease in health negative coliforms ( $P<0.01$ ) in *dahi* fed animals. Total erythrocyte count (TEC) was increased ( $P<0.01$ ) and mean corpuscular volume (MCV) decreased ( $P<0.01$ ) in *dahi* compared to CON. In conclusion, traditionally prepared *dahi*/curd can be used as a probiotics with beneficial effect on digestibility of some nutrients, hindgut health characteristics, intestinal microbial balance and haematology.

**Keywords:** Fermented milk, *Dahi*/curd, Digestibility, Probiotics, Health, Haematology

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Fermentation an oldest traditional method for producing and preserving foods in a wide variety of flavors, aromas and textures, which enrich the human diet; additionally fermented foods can also have the added benefits of enhancing digestibility, improving nutritional as well as therapeutic value<sup>1</sup>. The role of fermented milk in human diet was known from Vedic times. *Dahi* (Curd), buttermilk (*chass*), sweetened yoghurt (*lassi*) are some of the traditionally fermented dairy products being used in every household of the Indian subcontinent. 'Ayurveda' a traditional Indian medicinal system being practiced for thousands of years, which includes more than thousand species of plants, nearly 100 minerals and over hundreds of animal products as a remedy for various ailments and according to WHO the primary health needs of countries in Africa, Asia and Latin America are met

by traditional medicines<sup>2,3</sup>. In the Ayurveda *dahi* has been recommended for treatment of diarrhoea and other acute/chronic gastrointestinal disorders from time immemorial.

Traditionally prepared fermented milk *dahi*/curd contains promising lactic acid bacteria known for their probiotic potential with beneficial health effects on consumers<sup>4</sup>. Probiotics are viable microorganisms that are beneficial to the host when consumed in appropriate quantities; these are inherently present in fermented food products. Probiotic bacteria have long history of association with dairy products which provide a desirable probiotic delivery vehicle and among those yoghurt and fermented milk have received most attention as carriers of live probiotic cultures<sup>5</sup>. Evidence suggests that many diseases caused by pathogenic bacteria invading the digestive tract can be prevented, if proper intestinal flora were maintained<sup>7</sup>, this can be accomplished by

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consumption of live microbes in the form of probiotics/fermented milk products including *dahi*. Thus fermented milks offer tremendous potential for improving nutrition, soothe intestinal disorders, improve immune function, optimize gut ecology and promoting overall health.

*Dahi/curd* an Indian fermented milk product known for its palatability, nutritious and therapeutic role<sup>6</sup>, it has a viable bacterial count of  $10^6 - 10^7$  per ml which includes lactobacilli, yeast and mold. Typical bacterial strains are variable and include *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactococcus lactis*, *L. helveticus*, *L. cremoris*, *L. casei* and *L. acidophilus*, these microbes are recognized as a potential probiotics. Although, probiotics are present in different forms in the market either in freeze dried capsules or spray dried foods but it is stated that when provided in live form such as yoghurt or fermented milk, these may give more beneficial effects. *Dahi/curd* is an integral constituent of the Indian diet and has been recognized for its positive probiotics impacts on health other than high nutritive value<sup>8</sup>. In this backdrop the present study was conducted to investigate the use of traditionally prepared fermented milk *dahi* (curd) as a probiotics on nutrient utilization, hindgut health and haematology in canine model.

### Methodology

Experimental protocol and animal management was approved by Institutional Animal Ethical Committee (IAEC) of Indian Veterinary Research Institute, Izatnagar (India). An experiment was conducted for the period of 8 weeks to investigate probiotic potential of traditional fermented milk *Dahi/curd* on nutrient utilization, hindgut health and haematology. Eight Labrador dogs were divided into two groups: one control (CON, without supplement) and other treatment group (*dahi*, supplemented with 100 ml of *dahi/curd* as a probiotics (providing  $\sim 10^{8-9}$  cfu/head/d of *Lactobacillus* sp.). All the animals were healthy with normal appetite and fed to meet the maintenance energy requirement.

Basal diet containing different cereal-protein (rice, jowar, soybean meal, red gram, lentil) and mineral-vitamin premix was formulated to meet the nutrient requirements as per NRC<sup>9</sup>. Raw cow milk was obtained from the dairy of Division of Livestock Production and Management of the Institute. *Dahi/curd* was prepared by inoculating the milk with

household *dahi* containing  $\sim 10^{6-7}$  colony forming units (cfu)/mL *Lactobacillus* spp (Fig. 1).

A four-day digestion trial was conducted after 6 weeks of experimental feeding. After digestion trial hindgut health characteristics and haematology was assessed. Samples of foods and faeces were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), total ash (TA), phosphorus as per the standards<sup>10</sup> and calcium by titrametric method. Organic matter (OM) and nitrogen free extract (NFE) was calculated by difference.

Hindgut health characteristics were analyzed by estimation of physical, chemical and microbiological changes in faeces which represents the happening in hindgut. Faecal pH (using digital pH meter Eutech Instruments, Malaysia), lactate<sup>11</sup>, ammonia<sup>12</sup> and short chain fatty acids (SCFAs)<sup>13</sup>, were determined by adopting standard methods. Microbial population were enumerated in faeces by three sets of serial ten fold dilutions ( $10^{-1}$  to  $10^{-9}$ ) and plated onto selective media: Rogosa agar (BBL-Difco Laboratories, USA) for lactobacilli, MacConkey agar (BBL-Difco Laboratories, USA) for coliforms. Reinforced clostridial agar (Himedia, Mumbai, India) for clostridia and bifidobacteria agar (Himedia, Mumbai, India) for bifidobacteria. For lactobacilli and coliforms count relevant agar plates were incubated aerobically at 37°C for 24 and 48 hrs, respectively; whereas, bifidobacteria and clostridial agar plates were incubated anaerobically at 37°C for 24-48 hrs. The bacterial colonies were counted as colony

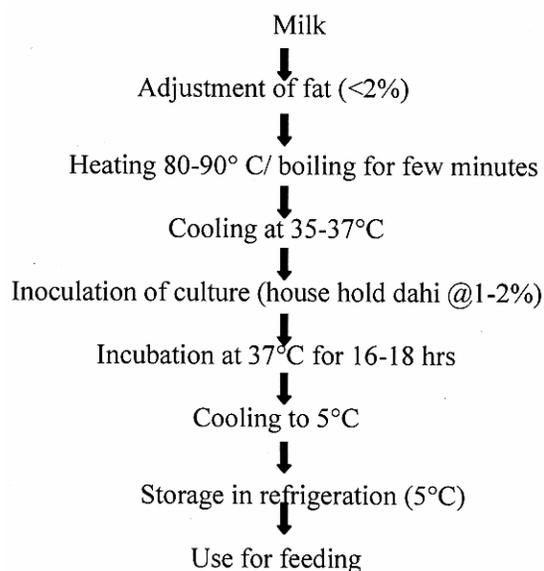


Fig.1—Procedure for preparation of *dahi/curd* (Flow diagram)

forming units (cfu)/per gm faeces. Blood haematology was done just after bleeding as per standard procedures<sup>14</sup>. The experimental data generated were analyzed adopting standard statistical procedures<sup>15</sup>.

### Results and discussion

Fermented milk products are milk substrates that are prepared by using edible microorganisms which hydrolyze the carbohydrates, proteins and fats to nontoxic products with flavours, aromas and textures pleasant to the consumers<sup>16</sup>, it can be Yoghurt, Kefir, Kumiss, Acidophilus milk, Bulgarian milk, Leben, *Dahi* and *Lassi*. *Dahi/curd* is considered the oldest Indian fermented milk product and is equivalent to western yogurt which is palatable, refreshing, and has characteristic taste liked by the people of all age group<sup>17</sup>. The scale of production ranges from household level to industrial scale including preparation by *halwai*'s milk shops in urban area. Now a days use of fermented foods including probiotics in various forms containing variety of microorganisms is increasing in human and veterinary medicine. Hence, this study tried to validate efficacy of household *dahi/curd* as a probiotics in canine model.

### Nutrient utilization

The composite diets fed to the experimental animals contained 91.56% OM, 22.06% CP, 4.62% EE, 3.96% CF, 60.91% NFE, 64.87% total carbohydrate, 8.44% total ash, 1.19% calcium and 1.05% phosphorus on DM basis. All the dogs consumed food (on fresh basis) at almost similar level with mean values being 2408.34±10.93 and 2440.00±20.05 gm/d, respectively for CON and supplemented group of dogs (*dahi*). The intake of DM and various nutrients (viz. CP, EE, CF, NFE, Ca and P) was almost similar (P>0.05) in all the animals, which was also observed in earlier studies when probiotics was fed<sup>18</sup>. *Dahi/curd* is believed to improve appetite, vigor and has been recommended for indigestion, dysentery or other intestinal disorders<sup>19</sup>. During preparation of *dahi* microbial fermentation produces antibacterial compounds, lowers intestinal pH thus preventing growth of undesirable organisms and helps to improve digestibility either because of enhanced retention of nutrients or partial breakdown of undigestible compounds<sup>19</sup>. Hence, fermented foods including probiotics are hypothesized to positively affect the absorption of various nutrients, and may also defend against infectious diseases. There was significant (P<0.05) increase in DM digestibility but

the digestibility of OM (P=0.073) and CF (P=0.104) were tended to increase due to supplementation of *dahi* compared to CON. Similar to our findings dogs fed probiotics (*L. acidophilus*) tended to have greater total tract DM and OM digestibility than those fed control diet containing cellulose<sup>20</sup>, further, Kumagai *et al*<sup>21</sup> observed subtle improvement (P=0.11) in crude fibre digestibility due to feeding of probiotics (Table1).

The action of microorganisms during the preparation of cultured foods or in the digestive tract has been shown to improve the quantity, availability and digestibility of some dietary nutrients<sup>22</sup>. Lactic acid bacteria as probiotics are known to create a favorable environment in the intestinal tract by lowering the pH and help in absorption of minerals which was justified by significantly (P<0.05) improved calcium digestibility in animals those consumed *dahi*. An increase of calcium uptake was demonstrated in some probiotic bacteria such as *L. salivarius*<sup>23</sup>.

### Hindgut health characteristics

The data pertaining to hindgut health characteristics is presented in Table 2. The physical, biochemical and microbiological characteristics of faeces although not absolute give an idea about what's happening in the hindgut. There was no influence of feeding *dahi* on physical characteristics of faeces (frequency of defecation, stickiness, quantity of faeces voided) between two groups. Corroborating the present findings, Pasupathy *et al*<sup>24</sup> did not found impact of *Lactobacillus acidophilus* (probiotics) supplementation on faecal score, defecation frequency, amount of faeces voided in mongrel pups. However, in another study probiotic

Table 1—Total tract nutrient digestibility (%) of dogs supplemented with or without *Dahi/curd*

Attribute	CON	DAHI
Dry matter	80.2±0.26 <sup>a</sup>	81.03±0.25 <sup>b</sup>
Crude protein	73.82±1.15	73.59±0.64
Ether extract	86.65±1.81	90.49±0.73
Crude fibre	27.91±1.77	32.06±1.26
Organic matter	81.93±0.21	82.81±0.35
Nitrogen free extract	88.03±0.61	88.87±0.49
Total carbohydrates	84.35±0.67	85.40±0.38
Calcium	56.65±1.17 <sup>a</sup>	62.02±1.20 <sup>b</sup>
Phosphorus	60.59±2.29	66.04±1.42

<sup>ab</sup>Means bearing different superscripts in a row differ significantly (\*P<0.05);

NS, non significant (P>0.05)

supplementation led to reduction in incidence of liquid faeces and post-weaning diarrhea in piglets<sup>25</sup>. This indicates the more pronounced effects of fermented milk products or probiotics in diarrhea or unhealthy conditions. There was significant reduction in faecal pH ( $P<0.001$ ) and ammonia ( $P<0.01$ ) due to supplementation of *Dahi*, on the other hand faecal lactate was higher ( $P<0.001$ ) in *dahi* compared to CON. Ammonia is formed during colonic fermentation of protein and it may be speculated that smaller amounts are formed in the presence of probiotic bacteria<sup>26</sup>. Lactobacilli in the form of fermented dairy products or probiotics known to increase production of SCFAs by accelerating the breakdown of carbohydrates that are resistant to indigenous bacteria<sup>27</sup>; similarly in present study there was significant improvement in concentration of total SCFAs ( $P<0.05$ ) in *dahi* supplemented animals compared control. The SCFAs serve as an energy source for the host, providing 10-30% of basal metabolic requirements including energy for liver cells, colonocytes and peripheral tissues<sup>28</sup>.

The gastrointestinal tract microbiota plays an important role in host health due to its involvement in nutritional and physiological functions. Consumption of fermented foods with added probiotic microbes favorably alters hindgut microbes and known to maintain intestinal microbial balance. The data on microbial population is presented in Fig. 2. The faecal count of lactobacilli ( $P<0.001$ ) and bifidobacteria ( $P<0.05$ ) were significantly improved due to feeding of *dahi*. The mean value ( $\log_{10}$  cfu/g) were significantly higher in *dahi* group ( $8.79\pm 0.21$  and  $9.87\pm 0.06$ ) compared to CON ( $7.96\pm 0.15$  and  $9.39\pm 0.12$ , respectively). Both *Lactobacillus* spp. and *Bifidobacterium* spp. are the major lactate producing bacteria in the hind gut, and the present increase in their population in *dahi* supplemented group is in congruence with the observed higher faecal lactate concentrations in the said groups. In contrast to health positive microbes, the faecal coliform count ( $\log_{10}$  cfu/g) was significantly ( $P<0.01$ ) decreased in *dahi* group ( $5.87\pm 0.20$ ) compared to control ( $6.87\pm 0.12$ ), however, faecal clostridia count did not vary significantly ( $P>0.05$ ) when *dahi* was supplement to control diet ( $9.67\pm 0.12$  vs.  $9.29\pm 0.12$   $\log_{10}$  cfu/g in DAHI vs. CON group). Similar to our results feeding of probiotics was associated with increased count of faecal lactobacilli but in contrast the authors also observed decreased colistridial count due to feeding of

Table 2—Hindgut health characteristics as influenced by feeding of *Dahi*/curd

Attributes	CON	DAHI
Frequency of defecation	4.58±0.44	5.42±0.31
Stickiness <sup>†</sup>	2.54±0.08	2.79±0.08
Faeces voided (g/d)	640.1±20.37	649.7±12.42
Faecal DM (g/d)	118.3±1.64	114.6±1.70
pH***	5.02±0.01 <sup>b</sup>	4.43±0.05 <sup>a</sup>
Ammonia <sup>‡*</sup>	36.03±1.39 <sup>b</sup>	30.03±0.96 <sup>a</sup>
Lactate <sup>‡**</sup>	25.87±2.24 <sup>a</sup>	44.63±1.71 <sup>b</sup>
Total SCFAs <sup>‡*</sup>	393.6±29.60 <sup>a</sup>	500.6±32.89 <sup>b</sup>

<sup>†</sup>based on 1-4 point scale; <sup>‡</sup> all the values in  $\mu\text{mol/g}$  dry faeces

<sup>ab</sup>Means bearing different superscripts in a row differ significantly (\* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ );

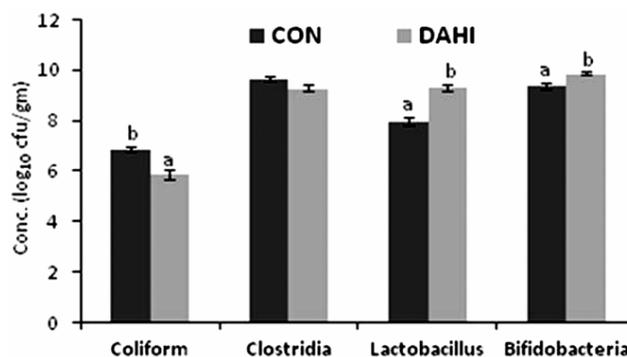


Fig. 2—Health positive (*Lactobacillus* and *Bifidobacteria* spp.) and health negative (*Coliform* and *Clostridium* spp.) faecal microbes as influenced by feeding of *Dahi*/curd, a,bMeans within the same series followed by different lowercase letters are significantly different ( $P<0.05$ )

probiotics<sup>29</sup>. Probiotic *dahi* was known to maintain intestinal microbial balance by reducing potential pathogens including coliforms in diseased conditions like diabetes in rats<sup>30</sup>.

#### Haematology

The data pertaining to various haematological indices in presented in Table 3. The total erythrocyte count ( $\times 10^{12}/\text{L}$ ) was significantly ( $P<0.001$ ) higher and MCV was significantly ( $P<0.01$ ) reduced in *dahi* compared to CON. However, TLC and blood indices (Hb, PCV, MCH, MCHC) did not vary attributable to dietary intervention. The increased TEC due to feeding of probiotics was also reported earlier in dogs<sup>29</sup>. Likewise, Cetin *et al*<sup>31</sup> had reported significant increase in TEC while TLC was uninfluenced due to feeding of probiotics.

In conclusion, the results indicated that traditionally prepared Indian fermented milk *dahi*/curd (with viable count of  $10^6$ - $10^7$  cfu/ml *Lactobacilli* sp.) can be used as a probiotics to

Table 3—Hematological indices of experimental animals as influenced by feeding of *Dahi*/curd

Parameters	CON	DAHI
Haemoglobin (g/dl)	13.16±0.17	14.59±0.64
PCV (%)	44.25±0.25	45.00±0.41
TEC (x 10 <sup>12</sup> /L) *	7.17±0.24 <sup>a</sup>	9.06±0.25 <sup>b</sup>
TLC (x 10 <sup>9</sup> /L)	14.95±0.47	16.15±1.14
MCV (fL) *	61.93±3.04 <sup>b</sup>	49.81±1.61 <sup>a</sup>
MCH (pg)	18.44±1.00	16.18±1.06
MCHC (mg/dl)	29.80±0.34	31.92±1.02

<sup>ab</sup>Means bearing different superscripts in a row differ significantly (\*P<0.05)

improve utilization of some of the nutrients, hindgut health and haematological indices.

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