Journal of Probiotics & Health
ISSN: 2329-8901

Hajare, J Prob Health 2017, 5:3
DOI: 10.4172/2329-8901.1000183

Research Article OMICS International

Oral Administration of LBKV-3 as Probiotic Enhances Immunoglobulin Level and Faecal Microflora in Malnutrate Children

Sunil T Hajare*

College of Natural and Computational Sciences, Dilla University, Dilla 419, Ethiopia

***Corresponding author:** Sunil T Hajare, Assistant Professor of Biotechnology, College of Natural and Computational Sciences, Dilla University, Dilla- 419, Ethiopia, Tel: +251-945910983; E-mail: sunilhajare@gmail.com

Received date: September 06, 2017; **Accepted date:** September 13, 2017; **Published date:** September 20, 2017

Abstract

Role of probiotics in regulation of immune system and development in microflora has begun to implicate in humans and animals. Inconsistent data exist regarding the effect of a single probiotic strain in malnutrate children. The aim of this study clinically proven *Lactobacillus* acidophilus strain LBKV-3 intended as probiotc for human was used to test its effect on modulation of immunoglobulin and composition of faecal microflora. To accomplish this task, 135 malnutrate children were orally administrated with buffalo milk as control, fresh curd as a special module for assessment of stimulatory effects and probiotic acidophilus milk used as a probiotic. During feeding, we evaluate the level of saliva IgA, serum IgG and IgE and faecal microflora. The result of our study demonstrated that the frequency of immunoglobulin and microflora was not affected when children consumed buffalo milk. In presence of fresh curd, the level of IgA, IgG and composition of friendly bacteria slightly increases and proportion of pathogenic bacteria declines. However, probiotic acidophilus milk significantly triggers the level of IgA and IgG as well as population of helpful bacteria rises and concentration of pathogenic bacteria declines in comparison to fresh curd. Irrespective of IgA and IgG, the ratio of IgE reduces in presence of fresh curd and probiotic acidophilus milk. Thus, our study showed that probiotic has positive effects on modulation of immunoglobulin and regulation of gut microflora. To confirm these findings, large-scale experiments involving the use of a large number of humans of different age groups and under different health and nutritional condition are required to be performed. Moreover, a different dose or strain of probiotic also warrants further confirmation.

Keywords: *Lactobacillus acidophilus*; Probiotic; Probiotic acidophilus milk; LBKV-3; Immunoglobulin; Microflora; ELISA

Introduction

In natural environment, the gastrointestinal tract of human is colonized by complex microflora that constitutes many different species of microorganism. Balancing the composition of these microbes in gastrointestinal tract is handy not only in promoting effective digestion and maximum gain of nutrients, but also important in increasing the capacity of host in excluding danger microorganism and hence contributes in preventing diseases [1]. *Lactobacilli* are known to be common inhabitant of animal intestinal microflora and many reports have suggested them to provoke various positive health attributes. This category of "good" bacteria is generally termed as probiotics. Therefore, probiotics can be defined as living microbes which upon on ingestion in certain number exhibit health effects beyond excluding inherent of basic nutrients [2]. Other described probiotics as microbial diet that effectively control host physiology by regulating their mucosal and systemic immunity as well as promoting the nutritive and microbial balance in their intestinal tract [3]. Probiotics are potentially known to manage and treat various gastrointestinal diseases such as diarrhea, inflammatory bowel disease and colon cancer [4,5].

Human immune system can mount both innate and adaptive immune response under the eventually condition of infections by pathogenic microbes. Many types of immune cells such as epithelial cells, natural killer cells, macrophages, neutrophiles, dendritic cells and lymphocytes are involved in eluciding immune response and subsequently neutralize deadly pathogens. These cells are known to be quickly activated in the event of infection which leads to the production of array of defensive molecules. Some of these cells may change their physiology and become phagocytic while, others are involved in secretion and synthesis of antibodies. Thus, activation of these cells may provide reflecting protection against pathogenic microbes by promoting specific immune responses. Therefore, these cells may have potential in the probiotic enhancement of immunological barrier in human gastrointestinal tract.

Probiotics have found to exert potential effect on both arms of immune response. For example, oral administration of probiotic containing *Bifidobacterium breve* promotes humoral immunity in mice by enhancing the secretion of immunoglobulin A (IgA) that are previously challenged by cholera toxin [6]. It is well known that immune function tends to decline with increasing age, supplement of probiotic twice a daily with *B. lactis* reported significant increase and improve various role of immune function in a group of healthy elderly people [7]. In other study, probiotics have found to augent humoral arm of adaptive immunity and subsequently enhance the immunological barrier of the intestine [8]. Our previous result also suggests that probiotic has potential to enhance the phagocytic activity in the children [9]. Thus, it is clear from above reports that probiotic can be human friendly in attributing and regulating the immunity in human.

Today in the era of continue use of antibiotics in controlling diseases has emerge out the problem of antibiotic resistant bacteria, which is becoming very difficult to control and have adverse effects on human. With the above positive attributes of probiotic bacteria on the

prevention and disease managements in animals thus, they hold a great potential as a better alternative to the use of antibiotics.

In this present study previously isolated Lactobacillus strain LBKV-3 with the best probiotic attribute based upon the results [10] was selected as potential probiotic for malnutrate children and its ability to modulate the level of IgA, IgG, and IgM was investigated. Secondly, the faecal microflora of malnutrate children was also demonstrated in the presence of LBKV-3. The results of the proposed is briefly highlighted and discussed below.

Materials and Methods

The children were selected using the slandered procedure given by Medical Council India as follow: the weight and height of 135 six-to nine-year-olds of the public school system in the poorest community of Yeotmal district (Maharashtra) were measured. The height/age (H/A), weight/height (W/H) and BMI (Body Mass Index) were computed using LMS software and compared with the WHO (2006) standards. The children's parents signed informed consent forms agreeing with their children's participation. One hundred and fifty children, comprising fifty each in the three age groups viz. 6-7 years, 7-8 years and 8-9 years, were randomly selected from various parts of a tribal village named Adgaon, Dist Yeotmal (India) which is 20 km from the Institute. The village is well attached with road facility. More than 75% of the population of this village belongs to tribal community including Adivashi, Banjara, Hatkar, Dhangar etc. Before recruitment and enrolment into the study, Ethical approval was obtained from the College of Dairy Technology, MAFS University Research Ethics Committee

Study design and size of sample

Previously [10] isolated and tested *Lactobacillus* strains (LBKV-3) with the best probiotic attributes was selected as potential probiotics for malnourished children below 10 year. The good probiotic character of this strain is its immunomodulatory ability to increase phagocytic cells, immunoglobulin and fecal microflora level in malnourished children below 10 years [10]. As this strain has previous history of human consumption, it can be regarded as "safe" sharing the same "GRAS" status as traditional Lactobacillus strains. Thus in present study *Lactobacillus* strain, LBKV-3 used to test its effect on lactase activity in undernourished children below 10 years.

A total number of 135 children were utilized for the study. All the children were grouped into three categories on the basis of age. The first group consist of kids with 6-7 years of age. Second group include the kids with age group of 7-8 years and third group constitute the kids of age from 8-9 years. 45 malnutrate children were included in each group. Among each group of children, 15 were feeded with 100 ml/ children/day of Buffalo Milk (BM), 15 were given 100 g/children/day Fresh Curd (FC) and 15 were inoculated with 100 g/children/day Probiotic Acidophilus Milk (PAM). The group of children that consumed BM were served as control. The feeding trial was continued for a period of 12th week. During and after feeding trial, the level of immunoglobulin and composition of microflora was measure at a period of 2nd, 4th, 8th and 12th week respectively.

Sampling criteria

The inclusion and exclusion criteria for children to enter the study were as follows:

Inclusion criteria

Malnutrition was the principle criteria for the kids.

Capable of giving consent information.

Volunteers in the age group of 6-7, 7-8 and 8-9 years, male or female.

No objection from respective kids parents to be included in the study.

Before recruitment and enrolment into the study, parents/ guardians of each subject was provided with a full explanation of the study and a formal informed consent sought and recorded.

Exclusion criteria

Food allergy problem.

Habituate to smoking, drinking alcohol and eating tobacco or tobacco products.

Kids with stress or stress induced symptoms.

Kids with any kind of disease symptoms or syndrome.

Collection of Buffalo Milk (BM)

Fresh BM was procured from Vasant Dairy Ltd. situated at Adgaon village, Taluka: Pusad, District: Hingoli from Maharashtra state of India. Fresh BM was used throughout the entire tenure of investigation.

Preparation of Fresh Curd (FC)

Pure culture of *Lactococcus* (lactis NCDC 60) for curd formulation was collected from National Collection of Dairy Cultures, Division of Dairy Microbiology, National Dairy Research Institute, Karnal, Haryana, India. For preparation of FC, fresh BM was collected from the above mentioned place. The collected milk was filtered and heated at 85°C for 30 minutes. After heating, the milk was allowed to cool down to 37°C followed by inoculation with 10^7 cfu/g of *Lactococcus* to the cooled milk. The milk was incubated at room temperature overnight for fermentation of milk by *Lactococcus* to FC. After incubation, FC was utilized to feed thechildren.

Preparation of Probiotic Acidophilus Milk (PAM)

For preparation of PAM, pure culture of *L. acidophilus* LBKV3 strain was collected from National Collection of Dairy Cultures, Division of Dairy Microbiology, National Dairy Research Institute, Karnal, Haryana, India. 10^7 cfu/g of *L. acidophilus* LBKV3 was utilized for the preparation of PAM to feed children by following methodology of Nahaisi [11] with slight modification. Care was taken to avoid the rise in acidity beyond 0.6% as thechildren do not preferred the product with higher acidity content. The protocol for preparation of PAM is highlighted in Figure 1.

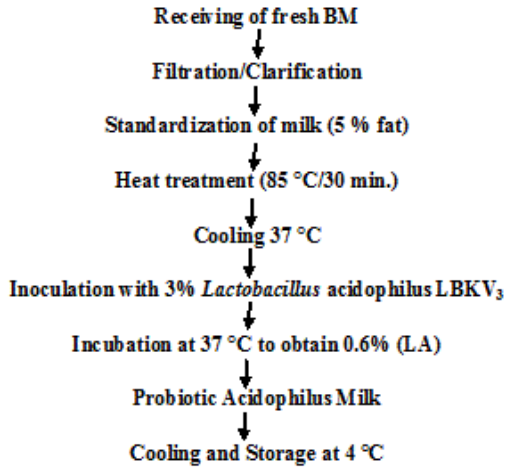

Figure 1: Flow diagram for manufacture of Probiotic Acidophilus Milk.

Measurement of total immunoglobulin

1-2 ml of whole saliva (for quantification of IgA) was taken with a sterile, soft plastic dropper on two separate occasions i.e. 2 to 3 days apart and pooled before and after feeding of BM, FC and PAM up to 12th week. The saliva was centrifuged at 1500 × g for 10 min and the supernatant was stored at 4°C with 0.1% thymol to retard the growth of contaminating microorganism. For quantification of serum IgG and IgE, blood samples were collected using two 5-mL vacutainer tubes before feeding BM, FC and PAM as well as during 2nd, 4th, 8th and 12th week of interval after feeding BM, FC and PAM. The blood was centrifuged at 1500 × g for 15 minutes and supernatant was stored at -20°C. IgG and IgE concentrations in serum and IgA concentration in saliva was measured by using ELISA Starter Accessory Kit (E101, Bethyl Laboratories, Montgomery, TX, USA) along with horseradish peroxidise conjugated goat anti-human IgA, IgG and IgE (A80-102P, A80-104P and A80-108P, Bethyl Laboratories, Montgomery, TX, USA). All assays were performed in duplicate of each sample.

Microflora count

20 g of faecal sample was mixed with 80 ml of sterilized physiological solution and homogenized. Microflora count was carried out as described by Francavilla et al. [12]. The following selective media were used: MRS agar (*lactobacilli*); Bifidobacterium agar modified (*bifidobacteria*); M17 agar (*lactococci*); Mannitol salt agar (*staphylococci*); Reinforced Clostridial Medium supplemented with 8 mg/L novobiocin, 8 mg/L colistin (clostridia); MacConkey agar (*coliforms*); Slanetz and Bartley (*enterococci*); Salicin agar (*propionibacteria*). All the media were purchased from Hi-Media Ltd. Mumbai, India.

Statistical analysis

Mean and standard deviation from the mean was calculated in MS-Excel 2007. Statistical analysis of data was performed using Student's t test to compare antibody responses between probiotic-treated and control groups using SAS 9.1.3 software. Statistical significance was assessed at a P value of ≤ 0.05.

Results

Level of immunoglobulin in malnutrate children with age group of 6-7 years that consumed BM, FC and PAM

Data on the effect of BM for stimulatory production of immunoglobulin revealed that BM recorded no regulatory effect on IgA, Ig and IgE production. When the children were feeded with FC as stimulatory of immunoglobulin, FC showed increase in IgA concentration from 3.9 to 4.3 mg/dl. Similarly, the concentration of serum IgG also reported observable increment from 850 to 921 mg/dl at the end of 12th week of feeding trial. On the other hand, slight decrease in IgE concentration recorded from 101 to 94 mg/dl over the period of 10th week in comparison to standard value of 103 mg/dl. Data on PAM as a test probiotic recorded significant (P ≤ 0.05) stimulatory effect over that of FC. Linear increase in IgA over the period of 4th to 12th week i.e. from 4.2 to 4.6 mg/dl notified its functional range over lag phase of 2nd where stimulation remained unaltered. Similar pattern of increment was observed for serum IgG which produced 870 mg/dl to 940 mg/dl form 2nd to 12th week. However, level of IgE did not altered in presence of PAM over the period of 12th week also (Table 1).

Tested Immunoglobin	Normal range (mg/dl)	Before Feeding buffalo milk (mg/dl)	Before Feeding fresh curd (mg/dl)	Before Feeding PAM (mg/dl)	Concentration of immunoglobins (mg/ml)											
					After feeding buffalo milk				After feeding fresh curd				After feeding PAM			
					2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week
IgA (saliva)	4.7 -5.2	3.6 ±0.9	3.7 ± 0.9	3.9 ± 0.4	3.7 ± 0.9	3.6 ± 0.5	3.6 ± 0.9	3.6 ± 0.4	3.9 ± 0.9	4.1 ± 0.2	4.1 ± 0.9	4.3 ± 0.1	3.9 ± 0.9	4.2 ± 0.5	4.3 ± 0.7	4.6 ± 0.3
IgG (Serum)	633 -1280	850 ± 21.20	850 ± 21.20	856 ± 21.20	850 ± 2.51	849 ± 2.10	844 ± 16.11	850 ± 7.11	860 ± 12.51	875 ± 21.10	910 ± 16.11	921 ± 17.11	870 ± 16.51	905 ± 19.10	915 ± 20.11	940 ± 17.20
IgE (Serum)	48 -207	103 ± 6.61	103 ± 7.12	104 ± 10.11	103 ± 6.62	102 ± 6.62	102 ± 6.11	101 ± 7.71	98 ± 4.12	95 ± 2.10	96 ± 6.12	94 ± 7.12	102 ± 4.00	101 ± 2.10	99 ± 10.21	98 ± 6.12

Table 1: Effect of feeding buffalo milk, fresh curd and PAM on concentration of immunoglobins in the children of age group from 6-7 years. *Values are expressed as mean. ± indicates standard deviation.

Level of immunoglobulin in malnutrate children with age group of 7-8 years that consumed BM, FC and PAM

Children with the age group of 7-8 did not show any significant effect in the regulatory role of immunoglobulin when they consumed fresh BM. When this group of volunteers received FC, concentration level of saliva IgA increased from 3.9, 4.2, 4.5 and 4.7 mg/dl during 2nd, 4th, 8th and 12th week respectively in comparison to that of pre-treatment level (3.8 mg/dl). Serum IgG level also found inclined over 8th and 12th week as 810 and 821 g/dl was noted when compared to pre-treated IgG level in children of 790 mg/dl. During 2nd and 4th week no rise in the level of IgG was recorded in presence of FC. On the other hand opposite pattern were observed in the level of serum IgE which is reduced from 101, 98, 97 and 96 mg/dl during 2nd, 4th, 8th and 12th week when compared to pre-treatment level of 103 mg/dl. The test group which received PAM as tested probiotic, significant ($P \leq 0.05$) rise in the level of saliva IgA was noted by 4.1, 4.4, 4.6 and 4.9 mg/dl during 2nd, 4th, 8th and 12th week as these children showed 3.9 mg/dl IgA level before consuming PAM. When observing serum IgG proportion, it was noted efficient rise from 850 mg/dl pre-treated level to 910, 926, 1011 and 1125 mg/dl at all the week tested. In contrast, the level of serum IgE of volunteers in this group was declined from 102-99 mg/dl than that of level of serum IgE of 104 mg/dl before consuming the PAM (Table 2).

Tested Immunoglobin	Normal range (mg/dl)	Before Feeding buffalo milk (mg/dl)	Before Feeding fresh curd (mg/dl)	Before Feeding PAM (mg/dl)	Concentration of immunoglobins (mg/ml)											
					After feeding buffalo milk				After feeding fresh curd				After feeding PAM			
					2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week
IgA (saliva)	4.7 - 5.2	3.7 ± 0.5	3.8 ± 0.4	3.9 ± 0.8	3.8 ± 0.7	3.7 ± 0.4	3.6 ± 0.8	3.8 ± 0.9	3.9 ± 0.4	4.2 ± 0.8	4.5 ± 0.9	4.7 ± 0.9	4.1 ± 0.4	4.4 ± 0.6	4.6 ± 0.2	4.9 ± 0.9
IgG (Serum)	633 -1280	840 ± 15.20	790 ± 21.20	850 ± 21.20	822 ± 3.51	820 ± 3.10	820 ± 6.11	840 ± 7.11	782 ± 12.51	789 ± 21.10	810 ± 16.11	821 ± 17.11	910 ± 16.51	926 ± 19.10	1011± 20.11	1125± 17.20
IgE (Serum)	48 - 207	103 ± 5.12	103 ± 6.18	104 ± 9.87	103 ± 4.18	103 ± 6.11	103 ± 6.81	102 ± 5.12	101 ± 2.11	98 ± 5.10	97 ± 1.12	96 ± 3.10	102 ± 3.92	101 ± 3.12	98 ± 9.16	99 ± 8.16

Table 2: Effect of feeding buffalo milk, fresh curd and PAM on concentration of immunoglobins in the children of age group from 7-8 years. *Values are expressed as mean. ± indicates standard deviation.

Level of immunoglobulin in the children with age group of 8-9 years that consumed BM, FC and PAM

Concentration of saliva IgA before and after feeding BM for volunteers of 8-9 years reported no difference. Serum IgG proportion was also found to be decreased throughout the period on comparing to pre-treatment measurement. Similarly, serum IgE level remain unchanged up to 2nd and 4th week compare to before feeding concentration of 121 mg/dl and slightly decreased at the end of period (117 and 118 mg/dl) during 8th and 12th week. In the group where volunteers received FC, the concentration of IgA and IgG was higher compare to pre-treated value. Level of IgA were found to be increased during 8th and 12th week i.e. 4.7 and 4.9 mg/dl was noted. While the level of IgG found to rise proportionally from 2nd week to 12th week as 888 to 910 mg/dl of IgG was recorded. The concentration of IgE in presence of FC was remarkably lower at all the period that those of before feeding level of 125 mg/dl. When the level of immunoglobulin tested in presence of PAM, IgA concentration remains unchanged during 2nd week (4.1 mg/dl) but increased significantly at 4th, 8th and 12th week from 4.7, 4.8 and 5.1 mg/dl respectively. Similarly, significant rise ($P \leq 0.05$) in the proportion of serum IgG was observed. A pre-treated quantity of 852 mg/dl was raised to 925, 936. 1106 and 1210 mg/dl over 2nd, 4th, 8th and 12th week. In contrast to level of IgA and IgG, IgE quantity did not alter tremendous to that of pre-treated quantity of 126 mg/dl where 121, 119. 124 and 130 mg/dl were recorded at all the period (Table 3).

Tested Immunoglobin	Normal range (mg/dl)	Before Feeding buffalo milk (mg/dl)	Before Feeding fresh curd (mg/dl)	Before Feeding PAM (mg/dl)	Concentration of immunoglobins (mg/ml)											
					After feeding buffalo milk				After feeding fresh curd				After feeding PAM			
					2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week
IgA (saliva)	4.7 - 5.2	3.9 ± 0.9	3.9 ± 0.5	4.1 ± 0.2	3.9 ± 0.3	3.9 ± 0.6	4.0 ± 0.2	4.0 ± 0.2	4.1 ± 0.4	4.0 ± 0.5	4.7 ± 0.8	4.9 ± 0.4	4.1 ± 0.2	4.7 ± 0.2	4.8 ± 0.2	5.1 ± 0.9
IgG (Serum)	633 -1280	836 ± 15.20	848 ± 21.20	852 ± 21.20	829 ± 3.51	821 ± 3.10	824 ± 6.11	821 ± 7.11	888 ± 12.51	876 ± 21.10	881 ± 10.11	910 ± 27.10	925 ± 16.01	936 ± 10.10	1106± 25.11	1210± 7.20
IgE (Serum)	48 -	121 ± 0.23	125 ± 2.03	126 ± 12.23	124 ± 5.11	121 ± 2.10	117 ± 16.11	118 ± 7.71	134 ± 5.11	141 ± 2.00	164 ± 6.11	191 ± 7.01	121 ± 5.10	119 ± 2.00	124 ± 2.10	130 ± 7.01

	207														

Table 3: Effect of feeding buffalo milk, fresh curd and PAM on concentration of immunoglobins in the children of age group from 8-9 years. *Values are expressed as mean. ± indicates standard deviation.

Effect of feeding BM, FC and PAM on faecal microflora in malnutrate children of age 6-7 years

Faecal microflora of children in presence of BM increased throughout the period from 2nd to 12th week for both friendly and harmful bacteria as shown in Table 4. Results of thechildren feeded with FC showed slight increased in friendly bacteria. For example, *Lactobacilli* concentration increases from0.5 cfu/g before feeding to 1.6 cfu/g at 12th week. Similar pattern was observed for *bifidobacteria* where 1.0 cfu/g before treatment was increased to 1.4 cfu/g at 12th week. *Lactococci* and *propionibacteria* also showed slight rise in concentration in before treatment (0.7 and 0.4 cfu/g) and after treatment (2.4 and 2.5 cfu/g) up to 12th week. The microflora of harmful bacteria was more in before feeding the FC than that of after feeding the FC. The composition of coliforms decline from 4.5 cfu/g to 3.0 cfu/g at 12th week. Clostridia decreased from 5.2 cfu/g to 3.4 cfu/g for 12th week of period. *Staphylococci* and *enterococci* also reduced from 3.3 and 4.2 cfu/g to 1.3 and 2.1 cfu/g till 12th week of period. Consuming of PAM for 2nd week resulted into striking increased in the microbial count of human friendly bacteria i.e. *lactobacilli*, *bifidobacteria*, *lactococci* and propionibacteria and very sharp decline in the composition of harmful microflora like *coliforms*, *clostridia*, *staphylococci* and *enterococci* count and the pattern was continue till 12th week of period. *Lactobacilli* count was increased by five fold, *bifidobacteria* by threefold, *lactococci* by three fold and *propionibacteria* by fivefold at 2nd week of feeding in comparison to before feeding composition of PAM. The drift of increment was further continue during 4th, 8th and 12th week of feeding trial with eleven fold rise in the log viable count of *lactobacilli*, five fold in case of *bifidobacteria*, seven fold for *lactococci* and sixteen fold rise for *propionibacteria* count at the end of feeding trial was recorded. The faecal harmful microflora was sharply declined and the extent of decline for *coliforms* was 0.3 cfu/g from 4.4 cfu/g, *clostridia* measure 0.5 cfu/g which is almost negligible compare to before feeding level i.e. 4.8 cfu/g. similarly, *staphylococci* reduced to 0.4 cfu/g and *enterococci* declined to 0.2 cfu/g which were found to be negligible in compare to their respective pre-treatment level counterpart (Table 4).

Microorganism tested	Before Feeding buffalo milk (cfu/g)	Before Feeding fresh curd (cfu/g)	Before Feeding PAM (cfu/g)	Composition of faecal microflora (cfu/g)											
				After feeding buffalo milk				After feeding fresh curd				After feeding PAM			
				2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week
Lactobacilli	0.6	0.5	0.4	0.7	0.9	1.4	1.5	0.6	0.9	1.4	1.6	2.6	3	3.3	4.6
Bifidobacteria	1.6	1	1.1	1.8	1.9	2.3	2.7	1.3	0.9	1.3	1.4	4.3	4.9	5	5.7
Lactococci	1	0.7	0.6	1.3	1.5	1.7	2.7	1.1	1.4	1.7	2.4	1.8	1.9	2.4	4.3
Propioniobacteria	0.3	0.4	0.2	0.6	0.7	0.9	1.7	1	1.3	1.8	2.5	1	1.6	1.8	3.3
Coliforms	4.7	4.5	4.4	4.8	5.4	6.3	6.7	4.4	4.1	3.5	3	3.7	3.3	1.5	0.3
Clostridia	5.2	5.2	5.8	5.6	6.7	7.3	8.6	5	4.9	4.3	3.4	4.4	4.1	2	0.5
Staphylococci	3.4	3.3	3.1	3.2	3.6	4.2	4.6	2.8	2.7	1.9	1.3	2.6	2.5	1.7	0.4
Enterococci	3.4	4.2	3.8	4	4.2	4.5	5.2	2.8	2.7	2.9	2.1	3.6	3.4	1.4	0.2

Table 4: Effect of feeding buffalo milk, fresh curd and PAM on composition of faecal microflora in the children of age group from 6-7 years. *Values are expressed as mean.

Effect of feeding BM, FC and PAM on faecal microflora in the children of age 7-8 years

Slight to moderate increase in the composition of both friendly and pathogenic microbes was recorded with the volunteers in the age of 7-8 those consumed BM. However, when the children were feeded with FC, showed rise in the count of *lactobacilli* (0.4 to 2.4 cfu/g), *bifidobacteria* (0.7 to 2.2 cfu/g), *lactococci* (0.8 to 3.3 cfu/g) and *propionibacteria* (0.5 to 3.4 cfu/g). In contrast to friendly microbes, sharp decline in the composition of coliforms (5.5 to 3.1 cfu/g), *clostridia* (5.2 to 2.1 cfu/g), *staphylococci* (6.5 to 1.5 cfu/g) and *enterococci* (7.9 to 3.3 cfu/g) at the end of 12th week. In the test group in which the volunteers received PAM proved best compare to the BM and FC. The initial count for *lactobacilli* measured was 0.4 which reached to 7.3 cfu/g at the end of 12th week. Similarly, *bifidobacteria* ranged from 1.1 to 8.3 cfu/g, *lactococci* ranged from 0.6 to 9.1 cfu/g and the range for *propionibacteria* observed during 12th week was 0.2 to 7.4 cfu/g. On the other side, significant decline was seen in coliforms count from 4.4 to 0.4 cfu/g, clostridia count from 5.8 to 0.3, *staphylococci* count from 3.1 to 0.3 and *enterococci* count from 3.9 to 0.4 cfu/g at the last period of feeding trial i.e. 12th week (Table 5).

Microorganism tested	Before Feeding buffalo milk (cfu/g)	Before Feeding fresh curd (cfu/g)	Before Feeding PAM (cfu/g)	Composition of faecal microflora (cfu/g)											
				After feeding buffalo milk				After feeding fresh curd				After feeding PAM			
				2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week
Lactobacilli	0.6	0.4	0.4	0.7	1.5	1.7	2.1	0.1	1.1	1.5	2.4	2.1	2.8	4.6	7.3
Bifidobacteria	1.5	0.7	1.1	1.9	2.3	2.5	2.8	1	1.2	1.5	2.2	3.2	4.2	6.9	8.3
Lactococci	1.4	0.8	0.6	2.5	2.8	3.2	4.7	1.6	2	2.5	3.3	3	4.7	7.2	9.1
Propioniobacteria	0.5	0.5	0.2	1.2	1.5	2.4	3.6	1.1	1.9	2.8	3.4	2.2	3.7	5.9	7.4
Coliforms	5.3	5.5	4.4	5.5	5.9	6.3	6.8	5.1	4.8	3.8	3.1	2.4	1.5	0.5	0.4
Clostridia	4.9	5.2	5.8	4.4	4.5	5	5.5	4.4	3.9	3.2	2.1	2	0.3	0.2	0.3
Staphylococci	4.4	6.5	3.1	4.6	5.2	5.3	5.4	4.7	4.3	2.8	1.5	2.5	2.4	0.7	0.3
Enterococci	4.5	7.9	3.9	4.9	5.2	5.7	6.2	7	6.5	5.2	3.3	2.8	2	1.4	0.4

Table 5: Effect of feeding buffalo milk, fresh curd and PAM on composition of faecal microflora in the children of age group from 7-8 years. *Values are expressed as mean.

Effect of feeding BM, FC and PAM on faecal microflora in the children of age 8-9 years

While comparing the microflora of children with the age of 8-9 years to that of children with age of 6-7 and 7-8 years, similar pattern of result was noted when they consumed BM. For instant, slight to moderate increased in the composition of friendly and pathogenic bacteria were observed. Microflora of the children who consumed FC depicts the significant rise in the composition of friendly bacteria i.e. *lactobacilli*, *bifidobacteria*, *lactococci* and *propionibacteria*. In present of FC no tremendous decline in the population of pathogenic bacteria was recorded as coliforms declined from 5.3 to 3.8 cfu/g, clostridia from 6.8 to 5.0 cfu/g, *staphylococci* from 5.7 to 3.6 cfu/g and slight increased in the composition of *enterococci* i.e. from 5.5 to 7.0 cfu/g at the end of 12th week was seen. The effect of feeding PAM on the intestinal microflora in test group of kids 8-9 years revealed that the average number of *lactobacilli* increased from 0.7 to 7.5 cfu/g, *bifidobacteria* raised from 1.4 to 6.8 cfu/g, *lactococci* from 1.4 to 6.0 cfu/g and *propionibacteria* rises from 0.5 to 6.3 cfu/g at the end of 12th week of feeding trial. During the trial period, significant decreases in the count of four pathogenic bacterial flora was recorded. Coliforms reduced from 6.2 to 1.7 cfu/g, *clostridia* reduced from 6.4 to 0.0 cfu/g, staphylococci reduced from 4.5 to 0.0 cfu/g and *enterococci* reduced from 4.7 to 0.7 cfu/g which is almost negligible at the end of 12th week (Table 6).

Microorganism tested	Before Feeding buffalo milk (cfu/g)	Before Feeding fresh curd (cfu/g)	Before Feeding PAM (cfu/g)	Composition of faecal microflora (cfu/g)											
				After feeding buffalo milk				After feeding fresh curd				After feeding PAM			
				2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week	2 week	4 week	8 week	12 week
Lactobacilli	0.7	0.8	0.7	1.1	1.4	1.9	2.7	1.4	1.5	2	2.8	2.7	3.8	5.6	7.5
Bifidobacteria	1.3	1.3	1.4	1.7	2.2	2.7	2.8	1.7	2.3	2.8	3.2	1.9	2.6	4.6	6.8
Lactococci	1.9	1.6	1.4	2.3	2.7	3.2	3.9	2.4	2.9	3.4	4.3	2.8	3.6	5.3	6
Propioniobacteria	0.5	0.8	0.5	1	1.3	1.8	2.5	1.2	1.7	2.6	3	1.8	3.4	4.7	6.3
Coliforms	5	5.3	6.2	5.2	5.4	6.4	7.1	5.9	5.4	4.6	3.8	5.2	5	2.8	1.7
Clostridia	4.6	6.8	6.4	4.8	4.2	4.5	5.1	6.4	6.3	5.6	5	3.3	1.5	1.1	0
Staphylococci	4.8	5.7	4.5	5.3	5.7	6	6.4	5.8	5.5	4.3	3.6	3.1	2.5	0.7	0
Enterococci	5.1	5.5	4.7	5.7	6.1	6.8	7.4	5.9	6.3	6.7	7	3.2	3	1.6	0.7

Table 6: Effect of feeding buffalo milk, fresh curd and PAM on composition of faecal microflora in the children of age group from 8-9 years. *Values are expressed as mean.

Discussion

It has been suggested that gut microflora is an important component of human in the intestine mucosal barrier and give rise to concept of bacteriotheraphy i.e. therapeutic application of potential beneficial bacteria, which function as probiotic. Therefore, probiotic are nothing but feed supplement containing live microbes that beneficially support the host by improving its intestinal microflora balance [13]. These beneficial bacteria also shown to regulate humoral immune response and thereby promotes intestine immunological barrier [14].

Thus it is clear from the previous evidence that probiotic provide health benefit to human by modulation of immune functions. However, in order to understand how these bacteria improve human health especially in children is not well studied except one report which showed that food in the form of probiotic triggers phagocytic activity in the malnutrate children [9]. By considering this view, the present investigation focused on the role of *L. acidophilus* LBKV3 strain on the regulation of immunoglobulin production and whether, PAM supports the composition of microflora in malnutrate children or not.

The results of the present study demonstrate that the concentration of saliva IgA, serum IgG and serum IgE in presence of BM s control is not affected in all the age group studied. When the children consumed FC as special module for assessment of stimulatory effects, the level of IgA and IgG altered over the period of time of feeding. The concentration of IgA appeared in the saliva resulted in increased proportion in all the age group studied up to 12^{th} week when comparing the level of IgA before consuming the FC. Similar pattern was observed in the level of IgG also. However, slight decreased in the level of was recorded as kids preferred FC. PAM, which is used as test probiotic reported significant ($P \leq 0.05$) effect on the level of IgA and IgG over that of FC and BM in all the age groups tested. In contrast to the proportion of IgA and IgG in presence of PAM, IgE level was not altered during the course of feeding trial (Tables 1-3). Many probiotic strains are reported to trigger the production of immunoglobulin. Karamese et al., [15] observed 10% increment in the level of IgA concentration after consumption of probiotic strains *L. johnsonii* in mouse model. The same study also determines the increased in the concentration of IgG in rat serum in presence of probiotic strain compared to control group. Parallel with the current information, our results supported in the rise of IgG and IgA level in presence of PAM in all age groups of children. Our study also explored the level of IgE declined with consumption of probiotic which is in line by another report who proposed similar pattern of results [16, 17, and 14]. Furthermore, another probiotic strain *L. plantarum* 299v has also reduced the proportion of serum IgE in critically ill patients [18] and also in heavy smokers [19]. IgE is involved in the allergy processes, so a decrease in the levels of this immunoglobulin could be beneficial for allergic subjects. In the present study, single strain of *L. acidophilus* proved best for production of IgA and IgG. A significant increase in the proportion of IgA and IgG was detected, but only in the group which received the PAM. IgA is the main immunoglobulin involved in mucosal defense thus, an increase in concentration of IgA may enhances its protection against pathogens. The findings of present investigation are also in close conformity of McFarland et al., [19-21] who concluded that concentration of IgA is the serum of Colombian children was significantly elevated after feeding probiotic.

Infancy stage, as one of the intermediate physiological development phase recorded its proven susceptibility to environmental microflora responsible for wide range of diseases. Host pathogen relationship depends upon defence mechanism of host and adaptability of pathogen associated with nurturing mode during the growth. Monitoring of defence mechanism depends upon quantitative regularization of *in vivo* immunoglobulin and there relative stability. Proportionate combination of IgA, IgG and IgE decides the efficacy of overall defence mechanism. Monitoring the level of IgA, IgG and IgE by probiotic stimulatory mechanism in form of hypothesis emerged out from the present investigation could be the model for commercial exploitation of probiotic in regulating malnutrition [22]. The present investigation could be handy for the developing countries of Africa, where food insecurity and hunger is challenging problem.

Another objective of our investigation is to demonstrate the effect of probiotic on microflora of study sample. It has been observed that when children consumed BM there microflora is not disturbed. However, in the presence of FC, the composition of useful bacterial increases and concentration of pathogenic bacteria declines over the course of period up to 12^{th} week in all age groups of children. In the presence of test probiotic i.e. PAM, a significant rise in the population of friendly bacteria was observed. In contrast, reduced number of pathogenic bacteria was noted in all age groups till 12^{th} week (Tables 4-6). Human intestinal flora contains as many as 1014 bacteria classified into 400-500 species, which are ten times higher than all the cells in the human body. Some bacteria of the intestinal flora such as coliforms, *clostridia*, *staphylococci* and *enterococci* can be harmful, while others like *lactobacilli*, *bifidobacteria*, *lactococci* and *propionibacteria* belonging to the so-called probiotic strain are favourable for the organism. The microflora in the large intestine plays an important part in the life of the host organism. Its composition may change several times during our life. However, it can still be regarded as nearly constant. Non-pathogenic, pathogenic and potentially pathogenic microorganisms living in a state of equilibrium determined by their own ecosystem within the large intestine take part in the local immunological and metabolic processes as well as in those affecting the organism as a whole. Probiotic food containing *Bifidobacterium* and/or *Lactobacillus*, both is reported to be extremely important for proper function of intestine [23]. Researcher also showed that consumption of food as probiotic containing the above strain can give notable rise in their abundance and with a reduction of more pathogenic organism in the gut [24]. A recent in vitro study showed promising effect of probiotic on the elderly gut microflora. Species of *Bifidobacterium* and *Lactobacillus* as two probiotic when added as batch culture significantly increase the composition of *Bifidobacterium* and at the same time lower down the Bacteroides count after fermentation [25]. Thus it is clear from previous report and from our study that ingestion of probiotic may possesses beneficial approach to maintain the balance of intestinal microflora. Use of specific probiotic may aims to modulate host immune response to potential harmful antigens. Oral administration of *B. bifidum* was reported to stimulate IgA response to ovaalbumin [26] and B. breve was shown to regulae IgA response to cholera toxin in mice [27]. In similar manner, rise in humoral response in comparison to control study including an increment in rota virus specific antibody secreting cell in the IgA class was observed in the children with acute rotavirus diarrhea who received *L. rhamnosus* during the acute phase of diarrhea [8]. In research done on humans, it has been reported that intake of yoghurt increased activity of phagocytes and natural killer cell and production of cytokine and antibody. In vitro studies on cells showed similar results, after exposure to lactic acid bacteria [28-31]. In a study by Perdigon et al. [32] on mice, it was observed that feeding yoghurt and

L. acidophilus and *L. casei* increased IgA and IgA producing cells in the animal small intestine. The greater the dose of probiotic, the greater the effect. Puri et al. [33] showed in their study that plasma IgA levels in mice that consumed yoghurt were considerably higher than those in mice who feeded milk. They concluded that the IgA produced by B cells in the intestine gets into the blood and elevates plasma IgA levels. Thus, there is adequate information available in the form of literature to support the hypothesis that administration of probiotic culture to human will impact on intestinal microflora and regulation of immune systems specially over malnutrition. Results of our study also provide useful information and baseline to support this hypothesis.

Conclusion

Results of our study conclude that probiotic has positive effects on modulation of immunoglobulin and regulation of gut microflora especially in malnutrate children. However, our study only emphasize on children up to age of 9 years because high influence of malnutrition during this age. Moreover, the efficacy of probiotic under different condition may be associated with method of production of probiotic itself or some other factors likewise: low survival rate and stability of strain, low dosage and frequency of administration, interactions with some medicines, health and nutritional status of the kids, effect of age, stress and genetics to an array of other factors. Therefore, it demands large-scale experiments involving the use of a more number of humans of different age groups and under different health and nutritional condition are required to confirm this conclusion. Moreover, a different dose or strain of probiotic also warrants future investigation.

Acknowledgements

The authors are thankful to parents of all the children who permitted them to be the part of this study, without them this work could not be possible.

Conflict of Interest

The Authors agree that they don't have any conflict of interest.

References

1. Collado MC, Bauerl C, Perez Martinez G (2012) Defining microbiota for developing new Probiotics Microbial Ecology in Health and Disease 23: 18579.
2. Fuller R (1989) Probiotics in man and animals. Journal of Applied Bacteriology 66: 365-378.
3. Leahy SC, Higgins DG, Fitzgerald GF, Van Sinderen D (2005) Getting better with bifidobacteria. Journal of Applied Microbiology 98: 1303-1315.
4. Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF (2003) Probiotics for treating infectious diarrhoea. Cochrane Database of Systematic Reviews 2: CD003048.
5. Capurso G, Marignani M, Delle Fave G (2006) Probiotics and the incidence of colorectal cancer: when evidence is not evident. Digestive and Liver Disease 38: S277-S282.
6. Yasui H, Kiyoshima J, Hori T, Shida K (1999) Protection against influenza virus infection of mice fed Bifidobacterium breve YIT4064. Clinical Diagnostic and Immunology 6: 186-192.
7. Arunachalam K, Gill HS, Chandro RK (2000) Enhancement of natural immune function by dietary consumption of Bifidobacterium lactis (HN019). European Journal of Clinical Nutrition 54: 263-267.
8. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, et al. (1992) Enhancement of the circulating antibody secreting cell response on human diarrhea by a human lactobacillus strain. Pediatric Research 32: 141-144.
9. Hajare ST, Pawar VN (2014) Oral administration of Probiotic strain, Lactobacillus acidophilus LBKV-3 enhances the phagocytic function of Tribal Children. Research in Biotechnology 5: 05-12.
10. Khedkar CD (2004) Standardization of a method for preparation of probiotic acidophilus milk and studies on implantation abilities of the culture in the GI tract of tribal children below five years. A PhD thesis submitted to SRTMU. Nanded.
11. Nahaisi MH (1986) Lactobacillus acidophilus: therapeutic properties, products and enumeration. In: Robinson RK (ed.). Developments in Food Microbiology, pp: 153-178.
12. Francavilla R, Calasso M, Calace L, Siragusa M, et al. (2012) Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. Paediatric, Allergy and Immunology 23: 420-427.
13. Toole PWO, Cooney JC (2008) Probiotic Bacteria Influence the Composition and Function of the Intestinal Microbiota. Interdisciplinary Perspectives on Infectious Diseases.
14. Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S (2001) Probiotics: effects on immunity. American Journal of Clinical Nutrition 73: 444S-450S.
15. Karamese M, Aydin H, Sengul E, Gelen V, Sevim C, et al. (2007) The Immunostimulatory Effect of Lactic Acid Bacteria in a Rat Model. Iranian Journal of Immunology 13: 1174-1180.
16. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, et al. (2007) Probiotics in prevention of IgEassociated eczema: a doubleblind, randomized, placebocontrolled trial. Journal of Allergy and Clinical Immunology 119: 220-228.
17. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, et al. (2004) Lactobacillus GG in inducing and maintaining remission of Crohn's disease. BMC Gastroenterology 4: 5.
18. McNaught CE, Woodcock NP, MacFie J, Mitchell CJ (2002) A prospective randomised study of the probiotic Lactobacillus plantarum 299V on indices of gut barrier function in elective surgical patients. Gut 51: 827-831.
19. Naruszewicz M, Johansson M, Zapolska Downar D, Bukowska H (2002) Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers. American Journal of Clinical Nutrition 76: 1249-1255.
20. McFarland LV (2006) Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. American Journal of Gastroenterology 101: 812-822.
21. McFarland LV (2009) Evidence based review of probiotics for antibiotic-associated diarrhea and Clostridium difficile infection. Anaerobe 15: 274-280.
22. McFarland LV (2015) Probiotics for the Primary and Secondary Prevention of C. difficile Infections: A Meta-analysis and Systematic Review. Antibiotics 4: 160-178.
23. Satokari RM, Vaughan EE, Smidt H, Saarela M, Matto J, et al. (2003) Molecular approaches for the detection and identification of bifidobacteria and lactobacilli in the human gastrointestinal tract. Systematic and Applied Microbiology 26: 572-584.
24. Toward R, Montandon S, Walton G, Gibson GR (2012) Effect of prebiotics on the human gut microbiota of elderly persons. Gut Microbes 3: 57-60.
25. Likotrafiti E, Tuohy K, Gibson KGR, Rastall RA (2014) An in vitro study of the effect of probiotics, prebiotics and synbiotics on the elderly faecal microbiota. Anaerobe 27: 50-55.
26. Moreau MC, Hudaul S, Bridonneau C (1990) Systemic antibody response to ovalbumin in gnotobiotic C3H/HeJ mice with Bifidobacterium bifidum or Escherichia coli. Microecological Theory 20: 309-312.
27. Yasui H, Nagaoka N, Mike A, Hayakawa K, Ohwaki M (2009) Detection of Bifidobacterium strains that induce large quantities of IgA. Microbial Ecology and Health Disease 5: 155-162.
28. Pereyra BS, Lemonnier D (1993) Induction of human cytokines by bacteria used in dairy foods. Nutrition Research 13: 127-1140.

29. Aattouri N, Lemonnier D (1997) Production of interferon induced by Streptococcus thermophilus: role of CD4+and CD8+lymphocytes. Journal of Nutritional Biochemistry 8: 25-31.

30. Galdeano C, De Moreno A, Vinderola G, Bibas Bonet ME, Perdigon G (2007) A proposal model: mechanisms of immunomodulation induced by probiotic bacteria Review. Clinical Vaccine and Immunology 14: 485-492.

31. Peng S, Tasara T, Hummerjohann J, Stephan R (2011) An overview of molecular stress response mechanisms in Escherichia coli contributing to survival of Shiga toxin-producing Escherichia coli during raw milk cheese production. Journal of Food Protection 74: 849-864.

32. Perdigon G, Fuller R, Ray R (2001) Lactic Acid Bacteria and their Effect on the Immune System. Current Issues in Intestine Microbiology 2: 27-42.

33. Puri P, Rattan A, Bijlani RL, Mahapatra SC, Nath I (1996) Splenic and intestinal lymphocyte proliferation response in mice fed milk or yogurt and challenged with Salmonella tythimurium. International Journal of Food Science and Nutrition 47: 391-398.