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Effect of feeding synbiotic products on the faecal flora of albino rats and healthy volunteers

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Abstract
Considering the excellent bio-therapeutic benefits of *Lactobacillus* and *Bifidobacterium*, this study exploited the probiotic characteristics of *Lactobacillus acidophilus-015*, *Lactobacillus casei-297* and *Bifidobacterium bifidum-229* strains with natural prebiotic substances available in India *viz*. banana powder, malto-dextrin and honey to produce synbiotic food formulations and to study the beneficial/implantation effect of synbiotic food formulations on the faecal flora of albino rats and healthy volunteers. The growth pattern of *Bifidobacterium bifidum* and *Lactobacillus* strain showed an increasing trend in all synbiotic products fed group and also exhibited an excellent adoption in the intestinal tract. The proportional increase in the population of these probiotics was progressively enhanced with the passage of time. Due to increase in the bifidobacterial and lactobacilli population and their competitive ability in the gastrointestinal tract, a decrease in the coliform count was observed. It was concluded that all the combination of synbiotic food formulations showed an excellent adoption in the intestinal ecology and creat favourable environment for the proliferation of intestinal friendly selected probiotics and discourage the growth of harmful organisms.

Keywords: Synbiotics, prebiotics

Introduction
A number of ancient scripts of different religions had recognized the role of diet in health and nutrition and the present generation of human beings should be indebted to our ancestors for identifying and developing varieties of food materials. In the last few decades consumer demands in the field of food production has changes dramatically. The present need and market demand is driving the scientific community to rethink about these inherited foods and various combinations need to be identified, developed and improved upon based on factors like availability, abundance, adaptability, ease of preparation, present market demand coupled with nutritional and therapeutic values. So many new terms have appeared in the world food market, e.g., Functional food, Foodiceuticals, Nutraceuticals, Juiceceuticals, Bioceuticals, Therapeutic foods, Pharma foods, Designer foods, Medi-foods, Super foods, Bio-foods, Bio-active food, Performance foods, Dietetic foods, Probiotic foods, Prebiotic foods, Power foods, Energy foods, Ethnic foods, Convenience foods, Natural foods, Organic foods, Sportsman foods or in general Health foods. The latest in this chain and the most exciting and revolutionary power food in the world today is "Synbiotic food". These health foods occupy the borderline between food and medicine that can have a preventive effect on human health by maintaining the ecological balance of colon. Hundreds of microbial species live in association with humans-on skin and in oral, intestinal and vaginal tracts. Bacterial populations have been estimated to reach 100,000,000,000,000 (10^{14}) cells at all sites of the human body (Tannock, 1994), a number that is more amazing when considered in the context of exceeding by 10-fold the number of human cells associated with the human body. Colon or lower intestine plays crucial role in our health and happiness and there are 500 different species of bacteria present in colon known as microbial factory. Probiotics are non-pathogenic organisms (yeast or bacteria, especially lactic acid bacteria) in foods that can exert a positive influence on the host's health (Marteau *et al*., 2001) [2]. Probiotics are microorganisms that have demonstrated beneficial effects on human health, working in the gastrointestinal (GI) tract to enhance nutrient uptake, promote digestive function and bolster immunity. They may play a beneficial role in several medical conditions, including diarrhoea,

gastroenteritis, irritable bowel syndrone, inflammatory bowel disease etc. Prebiotics help the growth of a healthy micro-flora in the gut by providing the energy source. The ability of gut microflora to utilize different prebiotic ingredients depends on the chemical structure and relative chain length of the prebiotic ingredient.

Synbiotics refers to a food ingredients or dietary supplements combining probiotics and prebiotics in a form of synergism (Panday, 2015) [3]. Synbiotic foods are a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplement in the gastrointestinal tract. This study was planned considering the excellent bio-therapeutic benefits of *Lactobacillus* and *Bifidobacterium* strains with natural prebiotic substances available in India *viz*. banana powder, malto-dextrin and honey to produce synbiotic food formulations. It was believed that supplementation with synbiotic therapy may be a safe and effective strategy to maintain remission in gastro-intestinal tract. Thus, this study was planned to assess the effect of feeding synbiotic product on the faecal flora copositon of healthy volunteers and experimental animal.

Material and Methods

Procurement of standard probiotic cultures: Three probiotic cultures of lactic acid bacteria *viz*, *Lactobacillus acidophilus* NCDC-015, *Lactobacillus casei* NCDC-297 and *Bifidobacterium bifidum* NCDC-229, respectively, were procured from the National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, (India). These cultures were propagated and maintained in litmus milk (Davis,1955) [5] and *Bifidobacterium bifidum*-229 culture was propagated and maintained in supplemented sterile skim milk (Anand *et al*., 1984) [4] having the following composition: yeast extract, 0.1g; dextrose, 1g and skim milk 100 ml. This milk were filled up to the neck in standard corning screw capped tube (15×125 mm) and sterilized by steaming for 30 min on three consecutive days. All the cultures were propagated by incubating at 37±1^{0}C for 48 h. After incubation these culture tubes were preserved at 5-7^{0}C in refrigerator and transferred once in a week in the respective medium for their reactivation.

Procurement of prebiotic

Three natural prebiotic substances banana powder, malto dextrin and honey were selected after preliminary trials. Spray dried banana powder obtained from Dry tech processes Pvt Ltd., Andheri (E), Mumbai, Malto dextrin from Gujrat Ambuja Exports, Ltd., Navrangpura, Ahmedabad and Honey from Dabur India Ltd, New Delhi.

Production of synbiotic fermented dairy products

An attempt was made to develop the methodology for the preparation of synbiotic fermented products. The preliminary trial for selection of the best proportion of selected culture combination with individual prebiotics for preparing three different synbiotic products and a combination of all three prebiotics for preparing different synbiotic products was conducted. The selection of best combination of probiotic culture and natural prebiotic substances for synbiotic products were based on the activity (acid production) during incubation at different interval of time, 0, 2, 4, 6, 8, 10 and 12 h, changes in probiotic log count (*Lactobacillus* and *Bifidobacterium*) of selected culture combination with different proportion of prebiotics at different interval of time, 0, 4, 8, 12 h and the sensory evaluation of all the combination after achieving 0.65% of titrable acidity and cooling the product at refrigeration temperature (7±1^{0}C) at the same interval of time. Finally four synbiotic products were selected for further investigation *viz*.:

a. Synbiotic fermented dairy product with 2% banana powder (SFDP)
b. SFDP with 2% malto-dextrin
c. SFDP with 3% honey and
d. SFDP with 2% of banana powder, 2% malto-dextrin and 1% honey

The synbiotic products sample of different combination using all three cultures in all combination of natural probiotic substances were further used in present investigation.

Albino rat's trial

Albino rats: Thirty male albino rats (Wistar albino) of five to six weeks Age with a body weight ranging from 135 to 140 gm were purchased. All rats were initially given a standard laboratory stock feed (Hindustan Lever Ltd.) on *ad libitum* for one week before the treatment.

Rat feeding trials: A minimum of 3 albino rats were used to test each product in each trial. The albino rats were housed individually in stainless steel metalic cages in a temperature and humidity controlled room (25±1^{0}C, 50% RH). Light was controlled to give 12 h of light. The products were available *ad libitum* and clean feeding containers containing fresh product were exchanged daily for the prior day's residue, so that the feeding consumption could be determined. The products were prepared weekly and stored in refrigerator at 7±1^{0}C. Before feeding, the products were kept in water bath at 37±1^{0}C for about 1 h to obtain normal temperature of products and then provided to the animal to consume. Except for the laboratory diet, the synbiotic products were the only food available to the albino rats. Distilled water was made available to them round the clock. The control albino rats were given the normal laboratory stock feed (Hindustan Lever Ltd.). The feed without protein was formulated containing 40 g starch, 10 g sucrose, 10 g maltose, 20 g dextrose, 10 g ghee, 2 g sodium chloride and 800 mg vitamin B-complex. The feeding trial was carried up to 45 days and its influence on faecal flora was studied.

Human volunteers feeding trials

Selection of human volunteers: Five groups of four students having good health were selected from the S. N. Bose Hostel, Mohanpur campus, Nadia (West Bengal), India for studying the beneficial/implantation effect of synbiotic food formulation. The age of students varied from 18 to 24 years.

Feeding protocol: The students were fed with 100g of synbiotic food product containing minimum of 10^8 cells/ g every day after meals for a period of 12 days.

Collection of stool samples: In a sterile petridish approximately 1.0 g of stool sample was collected using a sterile spatula. First stool of the day passed by the test subjects on 0, 4th and 8th and 12th day were analysed for the enumeration of total viable counts, coliform count, lactobacilli and bifidobacterial count. Wet faecal material was collected separately from each rat of different treatments and control group in a sterile 'clinicol' (HiMedia) container and brought to the laboratory for analysis.

Analysis of stool samples

Preparation of faecal sample for microbiological analysis: 1.0 g portion of wet faeces were taken from the interior of the stool samples of albino rat and human volunteers were added to a 99 ml dilution blank containing 1% peptone as suggested by Gilliland *et al.* (1978) [6].

Enumeration of total viable count: The petridishes containing required dilutions were poured with Nutrient agar and after solidification plates were incubated at 37±1^{0}C for 48 h and results were expressed in terms of cfu/g of sample.

Enumeration of coliforms: The petridishes containing required dilution were poured with violet red bile agar medium as suggested by Gorbach *et al.,* (1967) [7]. After solidification of media plates were incubated at 37±1^{0}C for 48 h and results were expressed in terms of cfu/g of sample.

Enumeration of *Lactobacillus*: Lyophilised cultures of *L. acidophilus*-015, *L. casei*-297 and *B. bifidum*-229 were grown in sterilized litmus milk medium separately at 37±1^{0}C for 48 h with the successive transfer at 24 h interval. The organisms were propagated by transferring in MRS broth for its activation.

Enumeration of *Bifidobacterium*: *Bifidobacterium bifidum*-229 culture was propagated and maintained in supplemented sterile skim milk (Anand *et al*., 1984) [4]. To ensure the best survival and growth, the individual cultures were streaked on *Bifidobacterium* growth medium yoshioka agar (Yoshioka, 1971) [71] slant.

Statistical Analysis

All result's collected during the present investigation for the individual cultures and culture combination effect on antibacterial activity were analysed using one way Analysis of Variance (ANOVA) with the general linear model and univariate data from the Statitical Analysis System software package version SPSS 10.0 with the significance level set at $P<0.05$. The experimental data are presented as the means and standard error of the means. Multiple comparisons have been done by Duncan multiple range tests (Montgomery, 1991) [9] which was used to detect differences between treatment means.

Effect of feeding synbiotic products on the faecal flora (total bacterial and coliform log count) of albino rats

The effect of feeding synbiotic products on the faecal count (total bacterial and coliform log count) evaluated on 0, 15th, 30th and 45th day after feeding are depicted in table-1. The total bacterial count of all groups taking synbiotic formulations showed a significant decrease ($P<0.01$) of total bacterial count at all the intervals of stool analysis, whereas in control there was non-significant change observed between 15th and 30th day as well as in between 30th and 45th day results. All combinations showed a significant ($P<0.01$) decrease in total bacterial log count. The effect of treatment and days of feeding both showed a significant decrease ($P<0.01$) in the total bacterial count and the interaction effect of the treatment and days of analysis also showed a significant ($P<0.01$) effect when results were analyzed statistically.

The effect of feeding all the synbiotic products, control as well as the laboratory stock feed on the coliform log count is depicted in table 1. When the results of stool analysis of albino rats for coliform count was statistically analysed, the result of days interval showed no-significant effect on the group fed with laboratory stock feed whereas all other combination showed a significant decrease ($P<0.01$) in coliform at all level of intervals selected for the analysis. The maximum decrease in count was observed in case of S_{16} product which showed 3.60±0.084 coliform count on 45th day from 5.58±0.085 which was observed on 0 day of analysis (table-1). Akalin *et al.* (1997) [10] found that the number of coliform bacteria was decreased more in faeces of mice fed acido yogurt as compare to mice fed plain yoghurt. Christopher *et al.* (2006) [11] indicated that the coliform count in faecal material was decreased in experimental animal after feeding of plain yoghurt or probiotic yoghurt containing *Bifidobacterium bifidum.* The effect of days and the interaction effect of treatment and days both were found significant ($P<0.01$) for the change in coliform count of stool after feeding of synbiotic products.

Effect of feeding synbiotic products on *Lactobacillus* and *Bifidobacteria* log count of albino rats

The results of faecal flora log count (*Lactobacillus* and *Bifidobacterium* log count) of albino rats were statistically analysed and expressed as the mean value ± standard error with superscripts which shows the effect of feeding different synbiotic formulations. There was a significant increase ($P<0.01$) in *Lactobacillus* count in all the synbiotic products at different interval for stool analysis i.e. 0, 15th, 30th and 45th day of analysis. Group-I fed with the laboratory feed showed significant result on 15th day analysis and thereafter no-significant increase in count of *lactobacillus* was observed. The highest *Lactobacillus* count was observed in S_{16} which was 6.91±0.026, 7.43±0.045, 7.98±0.130 and 8.42±0.040 on the same interval of days. This significant increase ($P<0.01$) in *lactobacillus* count might be because of the fructo-oligosaccharides and other complex carbohydrates present in the prebiotic used in the formulation. The analysis of variable for the *Lactobacillus* count showed that there was a significant ($P<0.01$) effect of treatments (the different proportion of prebiotic) and the days interval of stool analysis on the increase of *Lactobacillus* count of synbiotic product. The *Bifidobacterium* count of all groups taking simple fermented product as control and other selected synbiotic preparations on the different interval of stool analysis showed a significant increasing ($P<0.01$) trend throughout the observation period. S_9 product fed group showed slightly lower count at all levels in comparison to other synbiotic products (S_2, S_5 and S_{16}). Yang *et al.,* (2005) [12] observed that the *Lactobacillus* and *Bifidobacterium* counts were significantly increased, but there was no change in the anaerobic bacteria counts in rats fed with low and high dose synbiotics, while the count of coliform organisms, the harmful bacteria, were significantly reduced in rats fed with low and high dose synbiotics in this study.

Effect of feeding synbiotic products on the faecal count of healthy volunteers

Human volunteers feeding protocol

Human feeding trials were conducted by feeding the synbiotic products to four healthy volunteers for each product separately. Five groups of four students having good health were selected from S. N. Bose Hostel, W. B. U. A. F. S. Mohanpur Campus, Nadia (West Bengal), India for studying the implantation effect of synbiotic products. Results pertaining to the effect of daily feeding of 100 g of synbiotic

products (S_2, S_5, S_9 and S_{16}) on the excretory pattern of total viable log count, coliform log count, l*actobacillus* by count and *Bifidobacterium* log count by sampling their stool during feeding up to 12 days, are shown in table-3.

Microbiological analysis of stool

Total viable log count: The result revealed an average log count for total viable organism in the stool of human volunteers receiving the synbiotic products were 9.02±0.015, 9.03±0.014, 9.01±0.018 and 9.03±0.018 log count/g for S_2, S_5, S_9 and S_{16} products, respectively, (table-3). However, insignificant increase of total viable log count (TVC) were registered (9.06±0.009, 9.07±0.023, 9.04±0.012 and 9.08±0.025 in S_2 S_5, S_9 and S_{16} respectively on 4th days). There was an increase in total viable count in faeces in S_2 (9.09±0.012), S_5 (9.11±0.015) and decrease in total viable count in S_9 (9.00±0.019) and S_{16} (9.05±0.035) on 8th day. Further analysis of faecal sample on 12th day revealed a significant (P<0.05) decrease in S_5 (9.05±0.017) and S_{16} (8.98±0.020) while insignificant decrease observed in case of S_2 (9.08±0.015) and S_9 (8.97±0.014). The average viable count in the stool from different infants were reported to vary from 9.3 × 10^8 to 10.8 × 10^8 cfu/g (Misra and Kuila, 1994b) on feeding of bifidus milk, while Sarkar and Misra (2002) [14] have reported a decline in the faecal sample of infant on feeding of Propiono-Acido-Bifido (PAB) milk and dietetic yoghurt, respectively. Thus the antagonistic effect exerted by S_5 and S_{16} against intestinal microflora seemed to be more efficacious than S_2 and S_9 product in restoring the normal intestinal flora of human volunteers.

Coliform count: The coliform count in the faecal sample of human volunteers prior to the feeding trial (i.e. on 0 day) 7.98±0.018, 7.92±0.012, 7.90±0.021 and 7.98±0.035 respectively. Effect of four synbiotic products on coliform log count showed a highly significant decrease (P<0.01) at each interval of analysis. The coliform log count of the faecal sample on 12th day were recorded as 7.69±0.014 (S_2), 7.66±0.032 (S_5), 7.68±0.018 (S_9) and 7.53±0.015 (S_{16}) while an insignificant decrease was recorded in control where only fermented product was fed without prebiotic. Coliform count of control decreased from 7.95±0.015 (0 day) to 7.86 ± 0.014 (12th day). The maximum decrease in count was observed in case of S_{16} product fed group followed by S_2, S_5 and S_9 groups. Reduction in coliform count may be ascribed as the combined antagonistic behaviour of the culture combination with natural prebiotic substances *viz*. banana powder, maltodextrin and honey. It was elucidated by John (2000) [15] that *B. bifidum* with *L. acidophilus* @ 1% inoculum level in prebiotic dahi could reduce the coliform count to the extent of 1000 times. Dolezalek (1979); Pahwa (1982); Khedkar *et al*. (1990b) [18] and Patel *et al*. (1992) [19] reported a significant reduction in faecal coliform count by conjugated cultures of *L. acidophilus* with *B. bifidum*. It may be concluded that S_{16} product is more efficacious than other synbiotic products to inhibit faecal coliform (table-3). Misra and Kuila (1994a) also showed higher antagonism of *B. bifidum* culture against coliform.

***Lactobacillus* count:** An average count of lactobacilli in the stool sample of volunteers fed with control and four different snbiotic products prior to feeding (0 day) were recorded as 6.29±0.020, 6.25±0.015, 6.38±0.039, 6.28±0.012 and 6.31±0.015 cfu/g, respectively. Such variations in the mean count of *lactobacillus* in the faeces of human subjects with different individuals have been reported (Khedkar *et al*. 1990a; Patel *et al*., 1992) [21, 19]. On the prolongation of feeding of synbiotic products, the lactobacilli counts were registered as 6.32±0.021, 6.35±0.015 and 6.38±0.018, cfu/g of faeces with control product fed to human volunteers which was found statistically insignificant between days, whereas in case of S_2 fed group, *lactobacillus* log count recorded was 6.35±0.012 (4th day), 6.57±0.017 (8th day) and 6.65±0.015 (12th day). The results of S_2 fed group were found significant (P<0.05) in the *Lactobacillus* log count. The same trend was observed in case of groups fed with S_5 and S_9 product. The maximum increase in lactobacilli population was observed in the case of the group fed with S_{16} product which showed 6.45±0.012, 6.64±0.032 and 6.69±0.015 of *Lactobacillus* log count for 4th, 8th and 12th days' respectively in faecal sample of healthy volunteers.

Results indicate that S_{16} product is more efficacious followed by S_2, S_5 and S_9 for implantation of lactobacilli in the intestine of human volunteers. Increase in the *Lactobacillus* count in the faeces of human subjects fed with acidophilus milk has also been reported (Khedkar *et al*., 1990a; Patel *et al*., 1992) [21, 19]. Myers (1931) [22] reported the implantation of *L. acidophilus* in the human intestinal tract within two days of acidophilus milk consumption. The finding of the present study indicate higher adherence and multiplication of lactobacilli in the intestinal tract of human volunteers fed with synbiotic products compare to plain fermented product used as control. It indicates the higher implantation ability of prebiotic added products than the control. An increase in the lactobacilli count on the faeces of humans with administration of bifidus yoghurt (Ogato *et al*., 1999) [23] and infant fed with Propiona-Acido-Bifido Milk (Sarkar and Misra, 1998b) [24] and dietetic yoghurt (Sarkar and Misra, 2002) [14] were reported.

***Bifidobacterium* count:** An average count of *B. bifidum* in the faeces of human volunteers fed with synbiotic products prior to feeding (0 day) trial was not recorded because no growth was observed on the yoshika agar medium. The absence of organism in the faeces may be attributed to the nature of *B. bifidum* as it was not present in higher number in the gastrointestinal tract of human being and it was not reported in control as well as synbiotic products used in the present investigation. The count were 6.05±0.015, 7.04±0.018, 7.29±0.012 for *Bifidobacterium* log count of human volunteers fed with S_2 product, S_5 showed 6.26±0.015, 6.69±0.012 and 6.87±0.015 log count, S_9 counts were 6.15±0.020, 6.44±0.014 and 6.68±0.015 *Bifidobacterium* log count S_{16} results was 6.22±0.015, 7.12±0.012 and 7.35±0.015 *Bifidobacterium* log count on 4th, 8th and 12th day of feeding, respectively.

The growth pattern of *B. bifidum* showed an increasing trend in all synbiotic products fed group and it exhibited an excellent adoption in the intestinal ecology. The proportional increase in the population of bifidobacteria was progressively enhanced with the passage of time. Due to increase in the bifidobacterial and lactobacilli population, a decrease in the coliform count was observed (table-3). It may thus be inferred that the bactericidal activity of *B. bifidum*, enhance the competitive ability of this strain to acquire resistance in the gastrointestinal tract and also to exert an antagonistic effect on coliform. Bezkorovainy **(**2001) [25] also observed the effect of fructo-oligosaccharides incorporated into the human diet which altered both the microbial flora and the metabolic activity of the colon.

Table 1: Effect of Feeding Synbiotic Products* on the Faecal Flora (Total Bacterial and Coliform Count) of Albino Rats

Type of feed	Total bacterial log count					Coliform log count				
	Interval for stool analysis of rat (days)				P value	Interval for stool analysis of rat (days)				P value
	0 Day	15 Day	30 Day	45 Day		0 Day	15 Day	30 Day	45 Day	
Laboratory stock feed	9.32±.032^{c}	9.37±.025^{bc}	9.43±.01^{ab}	9.49±.020^{a}	.006 ^{NS}	5.28±.049^{a}	5.31±.052^{a}	5.33±.055^{a}	5.37±.049^{a}	.66 ^{NS}
Control	9.18±.090^{a}	8.60±.075^{b}	8.23±.157^{bc}	7.90±.157^{c}	0.00**	5.34±.055^{a}	5.29±.263^{b}	4.57±.067^{c}	4.12±.061^{d}	0.00**
Product with 2% banana powder (S_2)	9.29±.040^{a}	8.59±.049^{b}	7.98±.049^{c}	7.56±.076^{d}	0.00**	5.54±.062^{a}	4.91±.047^{b}	4.27±.030^{c}	3.82±.082^{d}	0.00**
Product with 2% malto-dextrin (S_5)	9.26±.043^{a}	8.66±.072^{b}	8.06±.091^{c}	7.70±.094^{d}	0.00**	5.50±.087^{a}	4.99±.092^{b}	4.50±.069^{c}	3.99±.070^{d}	0.00**
Product with 3% Honey (S_9)	9.40±.026^{a}	8.84±.052^{b}	8.37±.049^{c}	7.87±.070^{d}	0.00**	5.44±.049^{a}	4.98±.066^{b}	4.51±.052^{c}	4.18±.044^{d}	0.00**
Product with 2% each of banana powder and malto-dextrin and 1% honey (S_{16})	9.35±.054^{a}	8.52±.019^{b}	8.08±.047^{c}	7.55±.038^{d}	0.00**	5.58±.085^{a}	4.93±.093^{b}	4.25±.135^{c}	3.60±.084^{d}	0.00**

* Products with selected culture combination (C_1+C_2+C_3, 1:1:1) @ 2% and selected proportion of prebiotics were analysed after achieving 0.65% LA; C_1=*Lactobacillus acidophilus*-015; C_2= *Lactobacillus casei*-297 and C_3= *Bifidobacterium bifidum*-229
Cultures were incubated at 37±1^{0}C in skim milk (95^{0}C/30 min)
** Highly significant (P<0.01); NS- Non-significant
Similar superscript marked groups are non-significant and dissimilar superscripts are significant at the level of P< 0.01.

Table 2: Effect of feeding synbiotic products* on the faecal flora (lactobacillus and Bifidobacterium) count of albino rats

Type of feed	*Lactobacillus* count (log count/ml)					*Bifidobacterium* count (log count/ml)				
	Interval for stool analysis of rat (days)				P value	Interval for stool analysis of rat (days)				P value
	0 Day	15 Day	30 Day	45 Day		0 Day	15 Day	30 Day	45 Day	
Laboratory stock feed	7.07±.035^{b}	7.11±.026^{ab}	7.14±.024^{ab}	7.18±.026^{a}	0.119 NS	–	–	–	–	–
Control	6.88±.017^{d}	7.30±.023^{c}	7.65±.040^{b}	7.99±.058^{a}	0.00**	5.60±.051^{c}	5.98±.055^{b}	6.38±.059^{a}	6.72±.062^{a}	0.001**
Product with 2% banana powder (S_2)	6.82±.070^{d}	7.27±.077^{c}	7.78±.067^{b}	8.15±.075^{a}	0.00**	5.60±.074^{d}	6.15±.029^{c}	6.70±.046^{b}	7.14±.067^{a}	0.00**
Product with 2% malto-dextrin (S_5)	6.75±.052^{d}	7.17±.059^{c}	7.65±.047^{b}	8.00±.055^{a}	0.00**	5.74±.050^{d}	6.22±.052^{c}	6.68±.069^{b}	7.12±.050^{a}	0.00**
Product with 3% honey (S_9)	6.84±.046^{d}	7.19±.023^{c}	7.56±.019^{b}	7.81±.038^{a}	0.00**	5.58±.063^{d}	6.02±.066^{c}	6.50±.087^{b}	6.83±.067^{a}	0.00**
Product with 2% each of banana powder and malto -dextrin and 1% honey (S_{16})	6.91±.026^{d}	7.43±.045^{c}	7.98±.130^{b}	8.42±.040^{a}	0.00**	5.62±.100^{d}	6.11±.097^{c}	6.63±.050^{b}	7.13±.076^{a}	0.00**

* Products with selected culture combination (C_1+C_2+C_3, 1:1:1) @ 2% and selected proportion of prebiotics were used for feeding rats after achieving 0.65% LA; C_1=*Lactobacillus acidophilus*-015; C_2= *Lactobacillus casei*-297 and C_3= *Bifidobacterium bifidum*-229
Cultures were incubated at 37±1^{0}C in skim milk (95^{0}C/30 min)
** Highly significant (P<0.01); NS- Non-significant
Similar superscript marked groups are non-significant and dissimilar superscripts are significant at the level of P< 0.01.

Table 3: effect of feeding synbiotic products on the faecal flora count of healthy volunteers

Synbiotic product	Interval of stool analysis (days)	Total Viable log count	Coliform log count	*Lactobacillus* log count	*Bifidobacterium* log count
Control	0	9.03±0.015	7.95±0.015	6.29±0.020	not detected
	4	9.04±0.012	7.93±0.041	6.32±0.021	6.02±0.012
	8	9.05±0.015	7.89±0.012	6.35±0.015	6.12±0.020
	12	9.06±0.020	7.86±0.014	6.38±0.018	6.21±0.012
Product with 2% Banana powder (S_2)	0	9.02±0.015	7.98±0.018	6.25±0.015	not detected
	4	9.06±0.009	7.93±0.009	6.35±0.012	6.05±0.015
	8	9.09±0.012	7.87±0.012	6.57±0.017	7.04±0.018
	12	9.08±0.015	7.69±0.014	6.65±0.015	7.29±0.012
Product with 2% malto-dextrin (S_5)	0	9.03±0.014	7.92±0.012	6.38±0.039	not detected
	4	9.07±0.023	7.81±0.014	6.45±0.018	6.26±0.015
	8	9.11±0.015	7.74±0.023	6.54±0.015	6.69±0.012
	12	9.05±0.017	7.66±0.032	6.63±0.012	6.87±0.015
Product with 3% honey (S_9)	0	9.01±0.018	7.90±0.021	6.28±0.012	not detected
	4	9.04±0.012	7.82±0.020	6.35±0.015	6.15±0.020
	8	9.00±0.019	7.74±0.015	6.42±0.012	6.44±0.014
	12	8.97±0.014	7.68±0.018	6.49±0.018	6.68±0.015
Product with 2% each of Banana powder and Malto -dextrin and 1% Honey (S_{16})	0	9.03±0.018	7.98±0.035	6.31±0.015	not detected
	4	9.08±0.025	7.90±0.029	6.45±0.012	6.22±0.015
	8	9.05±0.035	7.77±0.035	6.64±0.032	7.12±0.012
	12	8.98±0.020	7.53±0.015	6.69±0.015	7.35±0.015

The results are the representative of an average of 5 samples for each product, separately. Analysis was carried out on 0th, 4th, 8th and 12th day of feeding of the synbiotic product to four healthy volunteers for each product, separately.

Conclusion

The result of the present study revealed many fold increase in the bifidobacterial population after 12 days due to regular consumption of synbiotic food formulations. Thus a dosage of 100 g synbiotic product with 2% of banana powder, 2% malto-dextrin and 1% honey containing 10^8cells/g daily after meal for a period of 12 days can be recommended to provide lactic acid, antibiotic factor and live bacteria in addition to all other nutrient which are available in prebiotic substances. The combination of all these factors will create favourable environment for the proliferation of intestinal bifidobacteria and discourage the growth of harmful organisms. Therefore an improvement of the intestinal environment is necessary in order to make bifidobacteria grow.

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