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Research Article

Prebiotic Functional Food Supplements Enhance Gut Health

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Abstract

Purpose: Prebiotics, typically non-digestible carbohydrates may give a competitive advantage to the live-fed probiotic bacteria in the gastrointestinal tract through modification of both composition and metabolism of intestinal microbiota. Fructooligosaccharides (FOS) are renowned to have various health promoting effects in animals and humans. Present study was aimed to assess the prebiotic effects of three formulations (F1, F2 and F3) using adult Wistar rats.

Methods: Adult Wistar rats of weight 262 ± 20 g were grouped into 5 of 6 rats each namely; control, FOS, F1, F2, and F3. Rats were fed on control AIN93, FOS, F1, F2 and F3 diet respectively for 6 weeks. Fecal samples were collected at 0, 2, 4 and 6 weeks, whereas cecal and colonic content was obtained at the end of the experiment. These samples were analyzed for the changes in the microbial counts in the *lactobacilli* and *E. coli.* In addition to this, plasma and serum samples were analyzed for the biomarkers of lipid profile and antioxidant status.

Results: In comparison with FOS and control, *lactobacilli* were significantly increased in feces after 6 weeks with the order F2 > F3 > F1 ($p < 0.001$); cecum ($p < 0.05$) and colon content ($p < 0.01$) (F3 > F1 > F2). Additionally, F1, F2 and F3 showed a significant decrease in the *E. coli* count as compared to control. Significant decrease was also observed in total cholesterol for F1, F3 ($p < 0.001$) followed by F2, FOS ($p < 0.01$) and decrease in serum glucose for F1 ($p < 0.05$), F2 ($p < 0.001$) and decrease in MDA for F2 ($p < 0.01$). HDL and TEAC of plasma for F3 ($p < 0.01$) and F1 ($p < 0.05$) were significantly increased. Maintenance of normal architecture and increased adherence of *lactobacilli* to colon was observed in formulation groups.

Conclusion: Present formulations exhibit prebiotic effects with improvement in gut microbiota, lipid and antioxidant profile of the rats. This study may be useful to develop novel multifunctional supplements.

Keywords: *Fructooligosaccharides; Functional Foods; Prebiotic; Probiotics; Wistar Rats*

Abbreviations

AIN: American Institute of Nutrition; ANOVA: Analysis of Variance; BIF: *Bifidobacteria*; CD: Critical Difference; CFU: Colony Forming Unit; FISH: Fluorescent In Situ Hybridization; FOS: Fructooligosaccharides; GIT: Gastro-Intestinal Tract; GOS: Galactooligosaccharides; LAB: *Lactobacilli*; MDA: Malondialdehyde; MRS: DeMann's Rogosa Agar; SCFA: Short Chain Fatty Acids; TEAC: Trolox Equivalent Antioxidant Capacity; TVC: Total Viable Count.

Introduction

The human gut is colonised with very diverse population of the microorganisms around 1014 cells g-1 of the content [1]. The gut microbiota improves the absorption of the nutrients and energy in addition to this, influences the physiology, biochemistry and immunology of the host [2,3]. Altering composition or functioning of bacterial communities in the bowel might be achieved through the use of probiotics or prebiotics which will promote health or be used in the prophylaxis or treatment of specific diseases [4]. Prebiotics are dietary com-

ponents or supplements that pass undigested through the small bowel and become sources of carbon and energy for bacterial residents (autochthonous strains) and thus boost bacterial population and/or metabolism in colon [5].

The diet rich in fruits and vegetables showed inverse proportion to the risk of the cardiovascular and intestinal diseases through various epidemiological studies [3]. Fruits and vegetables are rich in dietary fibers including oligosaccharides and polysaccharides with prebiotic nature which are selectively fermented by the gut microbiota improving health status of the health [6]. Vegetables like leeks, asparagus, chicory, Jerusalem artichokes, garlic, onions, and cereals like wheat, oats, and soybeans are found as the natural sources for prebiotics [6].

Now-a-days, many of the studies are suggesting the prebiotic nature of the oligosaccharides and synthesized products [7]. Yadav., *et al.* [7] reviewed various ways of the synthetic production of the prebiotics including enzymatic and microbial synthesis, hydrolysis of the polysaccharides which involves many synthetic chemicals processes and enzymes. This causes to increase the cost of the prebiotic products and hence they are not accessible to the common man. However the green chemistry approach of using natural sources of prebiotics as fruits and vegetables is recommended by the authors over the synthetic one. The natural sources such as fruits and vegetables and their products are safe as foods as well as they are cost effective and eco-friendly. Hence the need of finding the novel prebiotic natural sources is demanding the more research in screening of the various natural sources including fruits and vegetables.

The present study therefore was aimed to screen the three formulations developed from various fruits and vegetables for their prebiotic nature using *in vivo* model. The study also reports the multifunctional benefits of these formulations associated with the dietary fibers for their antioxidant and preventive approach against the cardiovascular and intestinal diseases.

Materials and Methods

Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of Vitamin E and ABTS (2, 2' azinobis 3-ethylbenzothiazoline 6-sulphonic acid diammonium salt) and lysozyme were purchased from Sigma. All analytical grade reagents were used. Biochemical kits for estimation of glucose, total and HDL cholesterol, triglycerides were purchased from Accurex Biomedical Pvt. Ltd., India. Hemoglobin kit was purchased from Ranbaxy fine chemical limited. The probes for FISH assay were purchased from Integrated DNA Technologies, USA.

Experimental prebiotic formulations

Three most promising formulations were designated as F1, F2 and F3 and used as experimental formulations for animal study. These three formulations were made up of dried and finely powdered edible portions of the materials viz., ber, fig, guava, grapes, okra, papaya, pomegranate, red tamarind, spinach, gum and apple. The materials were mixed in various proportions for development of three formulations. These formulations were tested *in vitro* for prebiotic potential using consortium A (Probiotic acidophilus, USA), *S. thermophilus* and *L. casei* [8].

Animal model and experimental design

AIN 93 diet was prepared as per American Institute of Nutrition guidelines with the composition reported by Reeves., *et al.* [9] as shown in Table 1. Based on pilot experiment for dose of FOS and formulations, 3% and 6% were used respectively in the diet by replacing respective amount of sucrose.

AIN-93G diet formulated for the growth, pregnancy and lactation phases of rodents	
Ingredients	g kg^{-1} diet
Cornstarch	397.486
Casein (>85 % protein) Skimmed Milk Powder	200.000
Dextrinized cornstarch (90-94 % tetrasaccharides)	132.000
Sucrose	100.000
Soyabean oil (no additives)	70.000
Fiber	50.000
Mineral mix (AIN-93G-MX)	35.000
Vitamin mix (AIN-93 G-VX)	10.000
L-Cystine	3.000
Choline bitartrate (41.1 % choline)	2.500
Tert-butyl hydroquinone	0.014
Vitamin mix (AIN-93 –VX) that supplies the recommended concentrations of vitamins for AIN-93G diet	
Ingredients	g kg^{-1} mix
Nicotinic acid	3.000
Ca Pantothenate	1.600
Pyridoxine-HCl	0.700
Thiamin-HCl	0.600
Riboflavin	0.600
Folic acid	0.200
D-Biotin	0.020
Vitamin B-12 (cyanocobalamin) (0.1% in mannitol)	2.500
Vitamin E (all-rac-a-tocopheryl acetate) (500 lU/g)	15.00
Vitamin A (all-trans-retinyl palmitate) (500,000 IU/g)	0.800
Vitamin D3 (cholecalciferol)(400,000 lU/g)	0.250
Vitamin K (phylloquinone)	0.075
Powdered sucrose	974.655
Mineral mix (AIN-93 –MX) that supplies the recommended concentrations of elements for AIN-93G diet	
Ingredients	g kg^{-1} mix
Essential mineral elements	
Calcium carbonate, anhydrous, 40.04% Ca	357.00
Potassium phosphate, monobasic, 22.76 % P; 28.73 K1	196.00
Sodium citrate, tri-potassium, monohydrate, 36.16 % K	70.78
Sodium chloride, 39.34 % Na, 60.66 % Cl	74.00

Potassium sulfate, 44.87 % K; 18.39 % S	46.60
Magnesium oxide, 60.32 % Mg	24.00
Ferric citrate, 16.5 % Fe	6.06
Zinc carbonate, 52.14 % Zn	1.65
Manganous carbonate, 47.79 % Mn	0.63
Cupric carbonate, 57.47 %Cu	0.30
Potasssium iodate, 59.3 % I	0.01
Sodium selenate, anhydrous, 41.79 % Se	0.01025
Ammonium paramolybdate, 4 hydrate, 54.34 % Mo	0.00795
Potentially beneficial mineral element	
Sodium meta-silicate, $9H_2O$, 9.88 % Si	1.45
Chromium potassium sulfate, $12H_2O$, 10.42 % Cr	0.275
Lithium chloride, 13.38 % Li	0.0174
Boric acid, 17.5 B	0.0815
Sodium fluoride, 45% F	0.0635
Nickel carbonate, 45 % Ni	0.0318
Ammonium vanadate, 43.55 % V	0.0066
Powdered sucrose	221.026

Table 1: *AIN-93G diet.*

Adult (week old) male albino Wistar rats (n = 30, weight 262 ± 20g) were obtained from the animal house of Agharkar Research Institute, Pune and housed separately in stainless steel cages in a room with controlled temperature (20 - 22°C) and humidity (50 - 55%) and maintained in a cycle of light and dark for 12h each to acclimatize for seven days to laboratory conditions before the commencement of experiments. Feed intake and weights of rat were recorded weekly and weight gain was calculated.

Dietary treatments

During the acclimatization, animals were fed with lactose free AIN 93 diet and water *ad libitum* for a week. After acclimatization, rats were divided into five groups (n = 6 each) as control (lactose free AIN 93 diet), standard (Standard FOS, Nutraflora USA); F1; F2 and F3 groups and fed for six weeks with respective diet and water *ad libitum*. The respective diets were prepared replacing sucrose from AIN93 diet with respective formulations and hence this helped to reduce excess sugar from the diet. At the end of first week all groups were given a single dose of probiotic suspension of standard marketed consortium with nine probiotic organisms (Probiotic Acidophilus Nutrition Now, Inc. USA) (9 mg l^{-1}/rat= $8x10^9$ cells).

Sample collection

Faecal samples were collected at 0, 2, 4 and 6 weeks for microbial load immediately after defecation in separate sterile tubes at the end of every week and processed within an hour of collection.

At the end of the sixth week feeding trial, rats were fasted overnight and anaesthetized using anesthetic ether saturated chamber and blood samples were collected by heart puncture method. The rats were sacrificed by cervical dislocation. The cecum was tied off immediately after dissection to avoid leakage of cecal contents into the colon due to relaxation of gut muscle after death.

Serum and plasma samples were collected. Colon and cecum content was collected in PBS (pH 7.2) and used for microbiological analysis. Colon segments were collected in 50mM Tris-HCl buffer and 10% formalin for histological examination for adherence of probiotics.

The entire protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Agharkar Research Institute (Registration number 101/1999/CPCSEA), Pune.

Microbiological analysis of faeces, cecal and colon content

The samples were homogenized in saline (1:10). Ten-fold serial dilutions were prepared and 0·1ml of each dilution was plated on an appropriate agar for total viable count. Samples were analysed for total LAB on De Man–Rogosa–Sharpes (MRS) agar, for *E. coli* on MacConkey's agar. All plates were incubated at 37°C for 24h, under partial anaerobic condition using glass desiccators. Results were recorded as log10 values of probiotics and *E. coli* as CFU g^{-1} dry weight of faeces.

Enumeration of lactobacilli by FISH assay

The enumeration of *lactobacilli* was done using FISH assay [10,11] with slight modification. In brief, after 10th step of final centrifugation, the cells were suspended in 150 µl of PBS, and 100 µl aliquots were used for quantification of fluorescence at excitation wavelength of 530/525 and emission at 590/535 using fluorescence spectrophotometer. Standard culture of *Lactobacillus* spp., *E. coli* and *Bifidobacterium* spp. hybridized with respective standard probes Eub 338, NON 338, Lab 158, EC 1531 and Bif 164 [11]. The 50 µl aliquot was fixed on slide and fluorescence was captured using Nikon-Eclipse Camera under fluorescent microscope at 565 nm for green fluorescence of Cy3 labelled bacterial probes [12]. Results were expressed as $\log_{10}$ No. of cells as CFU g^{-1} dry weight.

Biochemical parameters of serum and plasma

Serum and plasma analysis was done for glucose, hemoglobin, total cholesterol, triglycerides, HDL cholesterol. TEAC was studied for analyzing antioxidant status of plasma while plasma MDA was estimated as a marker of lipid peroxidation in different experimental groups.

Plasma antioxidant capacity (TEAC)

The estimation was done as per the method of Miller., *et al* [13]. The method gives direct measure of radical scavenging capacity of the samples. Results were expressed as mg of trolox equivalent ABTS+ radical scavenging capacity dl^{-1} plasma.

Lipid peroxidation (LPO)

The direct oxidative stress marker malondialdehyde (MDA), the secondary product of LPO, was estimated in the plasma samples utilizing the colorimetric reaction of thiobarbituric acid (TBA) using modified method of Placer., *et al* [14]. The results were expressed as nM of MDA mg^{-1} protein.

Histology of colon

Distal colon segment fixed in fresh 10% formalin were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax. The thin sections (5 – 7 µm) were double stained with hematoxylin and eosin. The microscopy and result interpretation was performed with the help of Dr. Suryawanshi, expert histopathologist from Omega Laboratories, Pune, India.

Statistical Analysis

All the observations were done in triplicates and data were summarized as mean values and standard deviations. Statistical tests like ANOVA, student paired t test were used for the analysis. The faecal and colonic bacterial population usually shows high inter-rat variation. Therefore, bacterial counts were expressed in $\log_{10}$ CFU g^{-1} dry weight.

Results

All animals were in good health condition and behaved normal throughout the experimental period and no side effects such as diarrhoea were recorded. The diets were made isocaloric for all groups replacing sucrose with 6% formulations and 3% FOS with similar energy values around 3740 ± 10 Kcal kg^{-1} of diet. Formulation groups F3 and F1 showed significant ($P < 0.05$) lowered weight gain as compared to control. Similarly % moisture content of the faeces in F2 and F3 groups was found increased as compared to zero weeks. However, there was no significant increase in the total faecal weights of formulation and FOS groups as compared to control group.

Microbiota analysis of faeces

Analysis of *lactobacilli*: Two way ANOVA showed significant increase in the *lactobacilli* count between all animal groups ($P < 0.0001$) from zero to sixth week ($P < 0.0001$) (Table 2). The result indicated significant ($P < 0.0001$) interaction between feeding of formulations and improved intestinal flora towards healthy profile during the experimental time period of six weeks. Further, inter group analysis for the *lactobacilli* count at sixth week by one way ANOVA indicated significant differences ($P < 0.0001$) in the count. The CD when compared with control group showed highly significant increase in the count for F2 ($P < 0.001$) and F3 ($P < 0.001$) followed by FOS ($P < 0.02$) and F1 ($P < 0.02$) group.

	0 week	2 week	4 week	6 week
Lactobacilli count as Log_{10} CFU g^{-1} dry weight of faeces				
C	8.52 ± 0.72	8.20 ± 0.54	8.60 ± 0.44	8.66 ± 0.37 ^{NS}
S	8.50 ± 0.41	7.63 ± 0.43	8.76 ± 0.24	9.09 ± 0.45 ^{a}
F1	8.92 ± 0.57	8.16 ± 0.58	8.21 ± 0.78	9.08 ± 0.23 ^{a}
F2	8.95 ± 0.26	8.94 ± 0.61	10.08 ± 0.22	10.62 ± 0.14 ^{ab}
F3	8.79 ± 0.70	8.09 ± 0.49	9.79 ± 0.20	10.18 ± 0.14 ^{ab}

Table 2: *Faecal microbiota analysis for lactobacilli.*

Data represented Log_{10} values as mean ± S.D.

a: indicates significance level when compared for inter group difference as compared to control group at sixth week. ($^{a}P < 0.001$)

b: indicates significance level when compared for inter group difference as compared to FOS group at sixth week. ($^{b}P < 0.001$)

NS: not significant

E. coli: Inter group analysis at sixth week indicated all the formulation groups (F2, $P < 0.001$; F3, $P < 0.001$ and F1, $P < 0.01$) showed significant decrease as compared to control and standard FOS (Figure 1). Two way ANOVA showed significant differences in the *E. coli* count for weekly analysis ($P < 0.0001$), inter group differences ($P < 0.0001$) and the interaction of groups and weeks ($P < 0.02$).

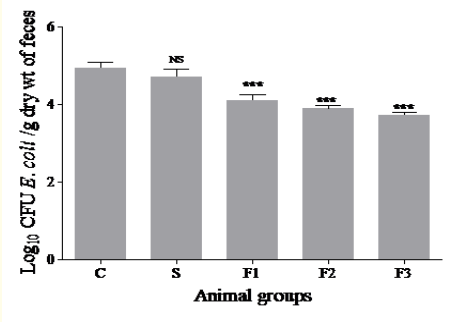

Figure 1: *Effect of different formulations on E. coli count at 6th Week.*

These results indicated effectiveness of formulations in terms of increasing the *lactobacilli* count with simultaneous decrease in the *E. coli* count when the animals were fed for six weeks.

Microbiota analysis of cecum and colon content: Analysis for colon content showed increase in the *lactobacilli* in experimental groups as compared to control. F3 showed increased count of *lactobacilli* followed by F2 and F1 as compared to control (Figure 2). Similarly, cecum content showed increase in the count of *lactobacilli* for F3 followed by F1 and F2 as compared to control (Figure 3).

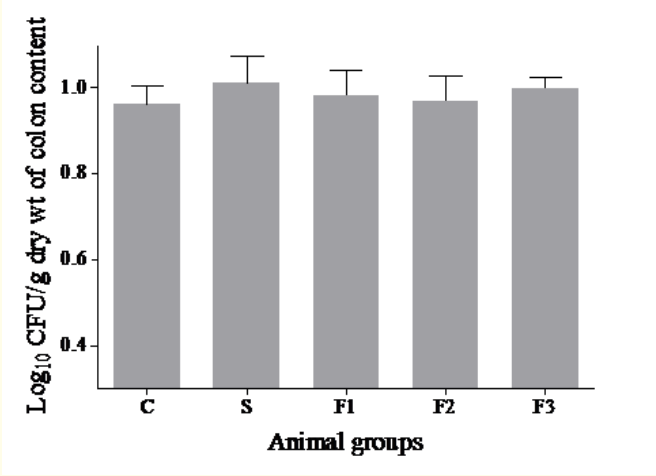

Figure 2: *Colon content analysis for lactobacilli.*

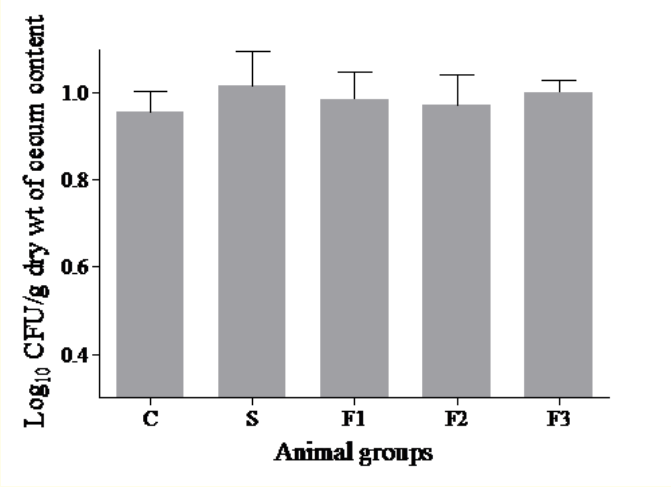

Figure 3: *Cecum content analysis for lactobacilli.*

Enumeration of *lactobacilli* and *E. coli* by FISH assay

***Lactobacilli, bifidobacteria* and *E. coli* in Faeces**

Lactobacilli: Two way ANOVA showed significant difference in the animal groups and for weekly intervals ($P < 0.0001$) for *lactobacilli* count. Further one way ANOVA for sixth week and CD showed significant increase in the F2 ($P < 0.001$), F3 ($P < 0.001$), F1 ($P < 0.001$) and standard group ($P < 0.01$) (Figure 4) as compared to control.

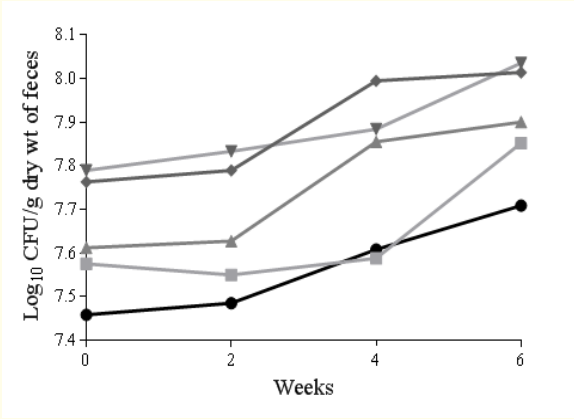

Figure 4: *Analysis of lactobacilli in faeces by FISH technique (● C, ■ S, ▲ F1, ▼ F2, ♦ F3).*

E. coli: One way ANOVA and CD analysis for 6th week showed significant decrease in the count ($P < 0.005$) for formulations F2 ($P < 0.001$) and FOS ($P < 0.01$) as compared to control (Figure 5).

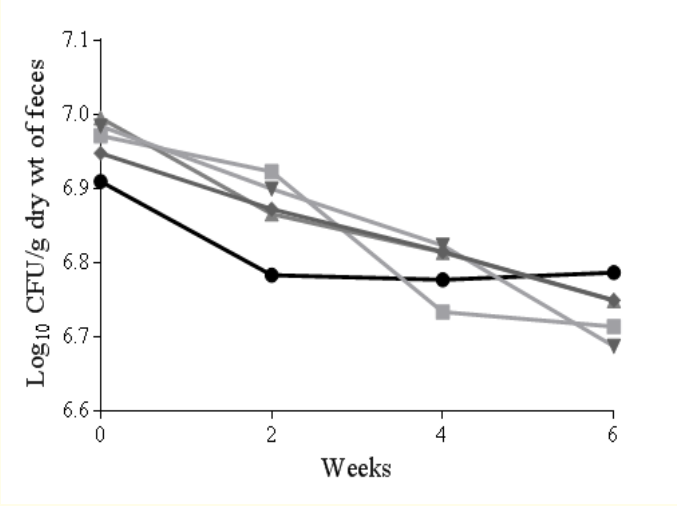

Figure 5: *Analysis of E. coli in faeces by FISH technique (● C, ■ S, ▲ F1, ▼ F2, ♦ F3).*

Bifidobacteria: Two way ANOVA for *bifidobacteria* count revealed significant differences between animal groups ($P < 0.0001$) and weeks ($P < 0.0001$). Further one way ANOVA for count at sixth week showed significant differences ($P < 0.01$) in the groups wherein based on CD significant increase was observed for formulations F2 ($P < 0.001$) followed by F3 ($P < 0.02$) and standard FOS ($P < 0.05$) as compared to control group (Figure 6).

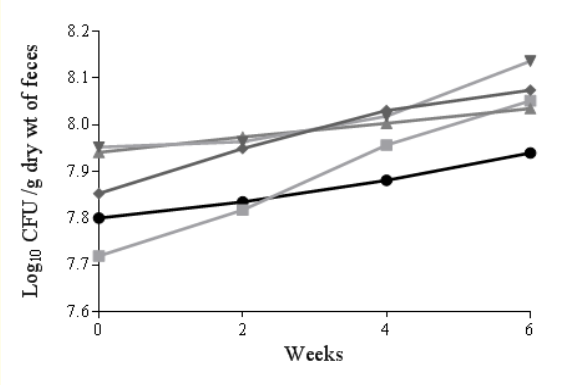

Figure 6: *Analysis of bifidobacteria in faeces by FISH technique (● C, ■ S, ▲ F1, ▼ F2, ♦ F3).*

Lactobacilli, bifidobacteria and *E. coli* in cecum content

One way ANOVA showed a significant increase in the count of *lactobacilli* for only formulation group F2 ($P < 0.05$) and *bifidobacteria* ($p = 0.06$) for formulation F3 ($P < 0.05$) and F1 ($P < 0.05$) as compared to control in cecum content. Additionally, non-significant decrease in *E. coli* count was observed for all formulation groups (Figure 7).

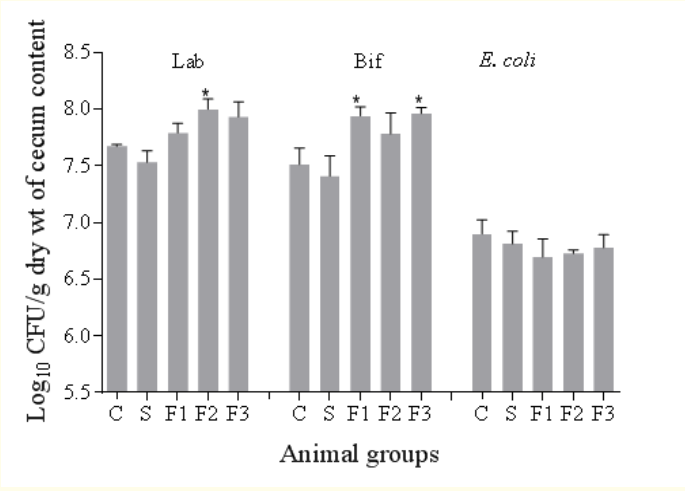

Figure 7: *FISH analysis of cecal lactobacilli, bifidobacteria and E. coli.*

Lactobacilli, bifidobacteria and *E. coli* in colon content

Analysis of colonic content for *lactobacilli* detected non-significant difference in the count for formulation groups as compared to FOS group, whereas *bifidobacteria* were not detected in the colonic content in present experiment. *E. coli* count in colonic content for standard FOS ($P < 0.05$), F1 ($P < 0.02$) and F3 ($P < 0.01$) was significantly decreased as compared to control group (Figure 8).

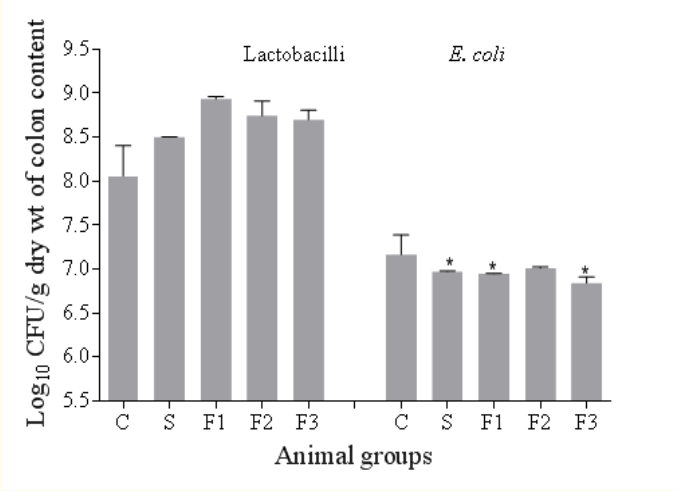

Figure 8: *FISH analysis of lactobacilli and E. coli in colon content.*

Biochemical parameters of serum and plasma

Biochemical parameters (Table 3) showed significant decrease in total cholesterol for F1, F3 ($P < 0.001$) followed by F2 and FOS ($P < 0.01$) as compared to control. Further, F1, F2 and FOS showed decreasing trend in the TG levels. All experimental groups showed increase in HDL but only F3 ($P < 0.01$) and F1 ($P < 0.05$) showed significant increase as indicated by one way ANOVA and CD. FOS and formulations showed increase in hemoglobin levels.

Parameters	Control	FOS	F1	F2	F3
Glucose$	135.8 ± 16.3	122.2 ± 14.5^{NS}	115.5 ± 23.2^{a3}	92.1 ± 14.0^{a3b3}	142.2 ± 6.7^{NS}
Hemoglobin$	13.6 ± 0.6	14.5 ± 1.0^{NS}	14.4 ± 1.2^{NS}	14.7 ± 0.9^{NS}	15.3 ± 2.1^{NS}
HDL$	41.3 ± 5.0	44.3 ± 2.1^{NS}	45.7 ± 4.5^{a1}	41.8 ± 3.1^{NS}	48.1 ± 2.9^{a2}
Total Cholesterol$	97.1 ± 9.7	81.4 ± 3.8^{a2}	71.6 ± 13.4^{a3}	79.7 ± 5.6^{a2}	76.4 ± 7.7^{a3}
Triglyceride$	53.2 ± 15.4	50.6 ± 19.7^{NS}	51.7 ± 14.1^{NS}	42.7 ± 6.5^{NS}	71.0 ± 15.6^{NS}
PlasmaMDA#	5.7 ± 2.7	4.3 ± 3.0^{NS}	3.7 ± 1.8^{NS}	2.0 ± 0.3^{a2}	4.5 ± 0.4^{NS}
Plasma TEAC$	31.5 ± 3.5	29.1 ± 2.3^{NS}	33.8 ± 4.0^{NS}	31.2 ± 3.8^{NS}	32.5 ± 5.0^{NS}

Table 3: *Biochemical parameters of blood sample of rats.*

Data represented as mean ± S.D. $: mg dl^{-1}, #: nm ml^{-1}

a: indicates significance level when compared to control ($^{a1}P < 0.05$, $^{a2}P < 0.01$, $^{a3}P < 0.001$)

b: indicates significance level when compared for inter group difference as compared to FOS group at sixth week. ($^{b1}P < 0.05$, $^{b2}P < 0.01$, $^{b3}P < 0.001$)

NS: non-significant

One way ANOVA and CD showed significant decrease in the serum glucose levels of formulation groups F1 ($P < 0.05$), F2 ($P < 0.001$) as compared to control. Increase in TEAC of plasma in formulations F1 and F3 was observed as compared to FOS group. MDA levels for all formulations were found significantly different but CD analysis showed significant decrease in F2 ($P < 0.01$) as compared to control. Increased TEAC and decreased MDA indicate improved antioxidant status of the animals under study.

Histology of colon

The effect of different dietary treatments on the adherence of the bacterial colonies and cellular composition of the gut tissues was studied. Colony adherence frequency was counted and scored for respective groups as number of colonies adhered per unit field. Adherence of bacteria was found increased significantly ($P < 0.05$) in all experimental groups as compared to control. Formulation F3 showed more increase in the colony numbers than FOS while F1 and F2 were comparable to FOS (Figure 9).

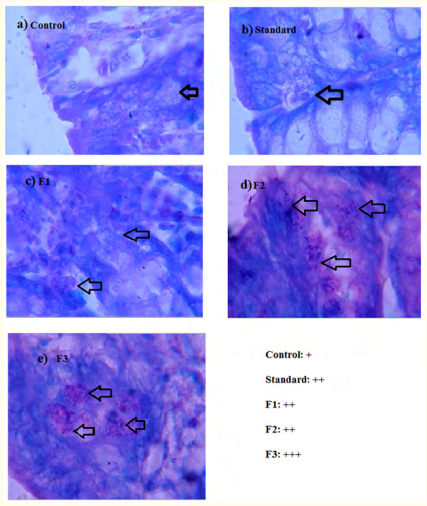

Figure 9: *Bacterial colonies adhered to colon segment (Magnification 100X): a) Control, b) Standard, c) F1, d) F2 and e) F3.*

Length of villi and thickness of lamina propria was measured using stage micrometer. Similarly parameters like villus atrophy, active lymphoid follicles; goblet cell hyperplasia infiltration of MNC were scored for each observation considering their active functions. Increased villi length was observed in formulation F3 followed by F2 and F1 as compared to FOS and control group. Whereas, thinner lamina propria was observed for formulations F3 followed by F2 and F1 as compared to FOS and control group (Figure 10). This can be explained as more active functions of villi and lamina propria indicating increased and active absorption of nutrients. Dietary treatment of prebiotic material might have actively increased the functions of intestinal cells and thus will be considered as important parameters for healthy intestine.

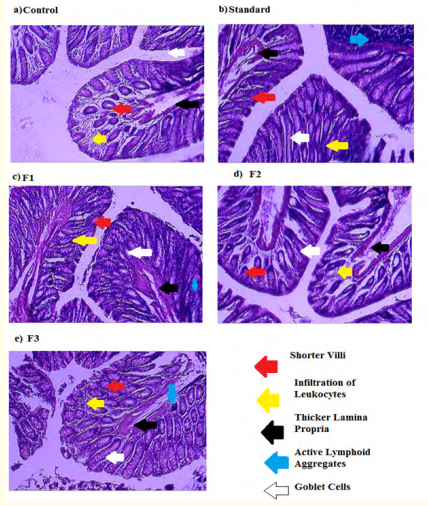

Figure 10: *Changes in intestinal architecture after treatment with different formulations (Magnification 100X): a) Control, b) Standard, c) F1, d) F2 and e) F3.*

Overall formulation F3 was found more effective than other groups in terms of maintaining the normal architecture of intestine and increasing the adherence of *lactobacilli* to colon.

Discussion

Prebiotics are an exciting and challenging new concept in nutrition and digestive function. While the mechanism of their effect in gut microbiota is slowly being discovered, their effects on health are much more difficult to demonstrate. Nevertheless, this is an important new area for food and nutrition science [15].

In the present study, as the animals were adult, decreased weight gain in standard FOS and F1 might be related to the decrease in the body fat. These results are indirectly supported by Nakamura., *et al*. [16] suggesting effect of FOS on lowering down the body fat absorption. Higher faecal water content in the formulation group might be related to the water holding capacity of the dietary fibers from formulations by intestinal bacteria [17]. Formulation F2, F3 and F1 showed significant increase in the faecal *lactobacilli* with simultaneous decrease in *E. coli* count. Cecal and colon content also showed improvement in the *lactobacilli* count.

Few animal studies concluded that supplementing the diet with inulin type fructans decreased the cecal pH and increased the size of the cecal pool of short chain carboxylic acids [18-22]. Short chain FOS administration dose-dependently increases faecal *bifidobacteria* in healthy human volunteers, with an optimal and well-tolerated dose ranging from 2.5 to 10 g/d [23,24]. During present study, faecal and cecal analysis showed increased numbers of *bifidobacteria*; however colon content was devoid of *bifidobacteria*. This might be because colon content was not collected by scraping the mucosa, thus *bifidobacteria* remained adhered to the colon mucosa. Tiihonen., et al. [25] supports present results, suggesting that in the rat study the baseline cell numbers of *bifidobacteria* were below the detection limit but GOSPRO treatment increased the cell numbers of faecal BIF in rats significantly. Sembries., *et al*. [26] in Wistar rats and Wang., *et al*. [27] in Balb/c mice showed similar results. Attachment of BIF to intestinal mucosa may result into different baseline cell numbers of BIF in humans and rats [28] and the bifidogenic factors in the diet [27]. Similarly, Rowland and Tanaka [29] showed corresponding increase in BIF and LAB with solely 5% GOS (transgalactosylated oligosaccharide) supplementation in germ-free rats inoculated with a human microbiota.

In vitro and *in vivo* studies indicated that oligofructose (OF) and inulins (IN) selectively stimulate the growth of *lactobacilli* and *bifidobacteria* in the intestine and faeces of rats or human subjects [21,30-34]. Present results confirmed increased *lactobacilli* and *bifidobacteria* numbers by feeding experimental diets to healthy rats with all the three formulations. Further, histology of colon has showed improved intestinal architecture as well as improved adherence of probiotics to intestinal epithelial cells. These results suggest that, mucin production might have increased due to presence of prebiotic formulations which supports organisms to adhere in to mucosal membrane. These results are in accordance with the study of Kleessen., *et al*. [35] which states that mucins and/or fructans may be important growth substrates for these mucosa-associated species and bacteria differ in their capacity to colonize the mucus layer or the mucosal epithelium. Kleessen., *et al.* [10,35] summarized the effect of fructans in diet on mucosal morphometry, histochemical composition of intestinal mucosubstances, cecal, colonic and faecal short chain fatty acid (SCFA) concentration and gut microbiota. Moreover, adhesion to the intestinal mucus supports persistence in the human gut ecosystem. He F., *et al.* [36] suggested that the mucosal adhesion of *bifidobacteria* might be strain-specific and dependent on substrate availability such as mucins or fructans. Thus, it is probable that such dietary manipulations could be of benefit in both the protection of the intact intestinal epithelium and the therapy of a disturbed mucosal barrier.

Further, present study revealed that consumption of three formulations at 6% in the diet of a healthy rat model; lowered weight gain, lowered the total serum cholesterol and triglycerides levels and lead to an increased HDL and antioxidant status in the plasma. Reduction of the total cholesterol levels can be explained on the basis of the cholesterol binding and bile acid binding activity of the plant materials used in the formulations as seen through in vitro study. These results are supported by Liong and Shah [37] where reduction in serum cholesterol in male Wistar rats was observed with the use of synbiotic diets of *Lactobacillus casei* ASCC 292 with FOS, maltodextrin and

mixture of FOS and maltodextrin. Dietary fibers adsorb bile salts and cholesterol which get excreted through faeces. This excretion results into utilization of cholesterol for bile salt synthesis and thus directly and indirectly lowers down the serum cholesterol. The reduction of cholesterol and triglycerides indicated the lowering down body fat absorption by FOS and formulation groups and thus maintaining the lipid profile of Wistar rats. The materials also showed inhibition of α-amylase by dietary fibers in our previous study (data not shown, Yadav., *et al*. unpublished) which might have resulted into controlling the serum glucose levels by lowering down the rate of release of free glucose in blood. The plant materials used in this study are rich in polyphenols which may result into increase in the antioxidant status of the rats. In addition, prebiotic are claimed to have effect [15,38-41] on gut microbiota which in turn inhibit colonisation of pathogens, improve bowel function, calcium and micronutrients bioavailability, immune stimulation, exhibit anti-colon cancer properties and lipid lowering action which justifies the present research interest into prebiotics.

Conclusion

Hence, the formulations in the present study indicated multifunctional properties based on dietary fiber and oligosaccharides which might help in regulations in total cholesterol and triglycerides levels, stool bulking and hence reducing intestinal transit time. This work represents one of the first efforts to develop cheap indigenous nutraceutical supplement based on indigenous fruits and vegetables. However, further experiments on humans are necessary.

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