Assessment of health benefits of membrane extracted innate plant fructo-prebiotics and lacto-probiotics in rats.

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ABSTRACT

The effect of feeding prebiotic extract of onion (Allium cepa) and probiotic culture Lactobacillus fermentum 141 on selected health indicators such as body weight gain, fecal pH, fecal coliforms, serum cholesterol and serum triglycerides was studied in wistar rats for a period of six weeks. The prebiotics were extracted from onion by membrane technology (nanofiltration) and was subsequently used in the feeding experiments. Rats were grouped into four groups namely, Group-I was fed with basal diet alone i.e. control group, Group-II was fed with prebiotic extract of onion, Group-III was fed with Lactobacillus fermentum 141 and prebiotic extract of Onion and Group-IV was fed with Lactobacillus fermentum 141 alone. Each experimental group had 10 rats and was fed with basal diet. The rat feed was added with freeze-dried culture @ 0.1% by weight, and prebiotic extract was added @ 0.05% by weight. The treatment Groups-III and IV were added with probiotic species @ of 3.0 X 10^9 CFU/gm to the respective test diets. There was no significant (p<0.01) improvement in the live body mass throughout the experimental period for all the treatment groups. There was a significant effect on fecal coliforms in all the treatment groups as compared to the control group. Rats fed with Lactobacillus fermentum 141 showed consistently less fecal coliforms as compared to the control group. The rats fed with pre and probiotics separately and prebiotics alone showed lower fecal pH values than the control group. The serum cholesterol content was significantly (P<0.01) lower in all the treatment groups as compared to the control group. The serum triglycerides content showed a significant decrease in all the experimental rats compared to control group suggesting the influence of pre and probiotics in altering serum cholesterol and triglyceride levels.

Key words: Fructo-Prebiotics, Lacto-Probiotics, Wistar rats

INTRODUCTION

An ideal probiotic has number of beneficial functions in the host body, when it is ingested together with pre-biotic sugars, which act as substrate for growth and multiplication of probiotics in colon. The probiotics and prebiotics (primarily oligosaccharides) are the two increasingly popular ingredients that are incorporated in dairy based functional foods and other dietary supplements to improve overall health. In farm animals, probiotics and prebiotics are added to enhance growth rate and to prevent early mortality [1]. Various types of oligosaccharides have been found as natural components in many common foods including fruits, vegetables, seeds, onion, garlic, milk, honey and in Japanese traditional foods such as sake and sweet sake [2]. However, extraction of innate prebiotics was not attempted except by the Japanese researchers [3]. The present commercial production of prebiotics involves enzymatic synthesis using simple sugars or enzymatic hydrolysis of polymer such as inulin. Previous studies reported the feasibility of nano-filtration for separating monosaccharides from oligosaccharides [4]. The objective of the present study was to assess the utility and efficacy of separated prebiotics from onion (Allium cepa) in an rodent model, by feeding wistar rats in combination with probiotic culture (Lactobacillus fermentum 141) on selected...
health indicators such as body weight gain, fecal pH, fecal coliforms, serum cholesterol and serum triglycerides in all the experimental rats.

**MATERIALS AND METHODS**

**Membrane separation of oligosaccharide**
The source material onion (*Allium cepa*) was first ground into the fine paste and was subjected to hot water treatment at 98°C for about 2 h and then the crude extract was subjected to pre-filtration with muslin cloth so as to avoid the blocking of the membranes with the suspended particles. Later, it was subjected to membrane filtration. Earlier membrane processing was successively used to separate the protein and lipid contaminants from oligosaccharide solution [3]. After membrane separation, the oligosaccharide solution was subjected to drying by using either spray drying or freeze-drying.

Pre-filtered extract obtained from onion from the above process was subjected to Ultra filtration (UF) using a pilot scale membrane system of Nishotech Systems, Mumbai. Membranes used were PES 10 KD, PES 20 KD, RC 10 and PS 50 KD supplied by the same company. The PES 20 KD was found to be suitable and used for the extraction of prebiotic oligosaccharides from onion. Filtrate from UF was concentrated in a rotary evaporator to obtain thick syrup of sugars.

**High performance liquid chromatography analysis**
HPLC instrument of (Varian, USA) with solvent delivery module of binary pump (Prostar 210/215 and Prepstar 218), Column oven model (Prostar 510), Auto sampler (Prostar 410) and auto sampling for 100 vials with Refractive Index detector of Varian (Prostar 350/352) were used for analysis of oligosaccharides. Water’s Spherisorb (5 µm), NH₂-bound column of dimensions (4.6 mm x 250 mm) at a column temperature of 65°C with a mobile phase flow rate of 1.0 mL / min for a period of 30 min was used for the analysis of purified oligosaccharides. The mobile phase used for the analysis of sugar samples was comprised of acetonitrile and water [70:30 (v/v)]. Both solvents were of HPLC grade, procured from Qualigens fine chemicals, Mumbai. The data-processing software (Star work station version 7.2) used for the analysis was supplied along with the instrument by the manufacturer.

**Probiotic culture**
The probiotic organism namely *Lactobacillus fermentum* 141 was obtained from the National Dairy Research Institute, Karnal, Haryana. The above probiotic species was grown on a pilot scale using a seven-liter capacity lab fermenter (Lark Innovative Fine Teknowledge, Chennai) with deMann Rogosa and Sharpe (MRS) broth as medium at optimum conditions of temperature, pH and 5% CO₂ tension. The bacterial cells grown were harvested by centrifugation at 3500 rpm for 10 min at 4°C and then the bacterial cells were washed with normal saline solution and re-centrifuged to get the bacterial pellet. The culture thus obtained after centrifugation was lyophilized and used for animal experiments.

**Experimental design**
Forty male wistar rats were selected for the experimental study. The wistar rats and rat feed were procured from National Institute of Nutrition, Hyderabad. The rats were divided into four groups and each group consisting of 10 rats namely, Group-I was fed with basal diet alone i.e. control group, Group-II was fed with prebiotic extract of onion, Group-III was fed with *Lactobacillus fermentum* 141 and prebiotic extract of Onion and Group-IV was fed with *Lactobacillus fermentum* 141 alone. Each experimental group had 10 rats and was fed with basal diet. The rat feed was added with freeze-dried culture @ 0.1% by weight, and prebiotic extract was added @ 0.05% by weight. The treatment Groups-III and IV were added with probiotic species @ of 3.0 X 10⁹ CFU/gm to the respective test diets. The duration of the experiment was for a period of six weeks. The body weights of rats were recorded at the beginning and later weekly body weights and feed intake were recorded regularly.

**Feeding and management**
The rats were randomly distributed in to four groups and all animals were maintained in separate cages. Regular cleaning and aseptic measures were followed with regular feeding and watering. The rats were maintained on balanced ration through-out the experimental period (six weeks).

**Sampling, testing and observations**
The body weight gains (individual basis), feed intakes, and feed conversion ratios were determined for each week. This experiment was designed to determine the effect of prebiotics and probiotic strains on the performance of the rats with different parameters like fecal pH, fecal coliforms, serum cholesterol and serum triglycerides.
Collection of fecal samples
Freshly voided fecal samples were collected in the morning on last day of the experiment from each experimental group. The fecal samples were collected aseptically and taken within 30 min to the laboratory and analyzed for pH and also for assessing fecal coliforms by the most probable number [5].

Physico-chemical analyses
Fresh fecal samples of one gram collected from the litter were weighed accurately using an electronic weighing balance (Type BL-220H, Shimadzu Corporation, Japan) and aseptically transferred into a beaker and then added 50 mL of sterile distilled water to the fecal contents and uniformly mixed and the fecal pH was measured by a pH meter (Elico, Hyderabad).

Enumeration of fecal coliforms
In the present study, Most Probable Number (MPN) method was used to determine the coliform bacteria in the feces of poultry litter of the experimental groups. The Most Probable Number (MPN) method is a statistical, multi-step assay consisting of presumptive, confirmation and completed tests [5]. In the assay, serial dilutions of a sample were inoculated into lactose containing broth. Enumeration methods that are based on lactose fermentation are frequently used to detect E.coli and total coliforms in the fecal samples.

Blood sampling
Blood is collected from plexus and after collection, the capillary tube is gently removed and wiped with sterile cotton. Bleeding can be stopped by applying gentle finger pressure. The blood collected were transferred into Eppendorff tubes of 1.5 mL capacity and then centrifuged in the micro-centrifuge, (S V Instruments Analytical Pvt Ltd, New Delhi) at a speed of 3500 rpm for 15 minutes and the serum so separated was collected and used for the serum analysis. Auto-hematological analyzer, (ERBA Mannheim, Biomedical Limited, Germany) was used to analyze the serum cholesterol and serum triglycerides using ERBA Mannheim Instrument and ERBA chemical kits. Analysis of Variance test at 5% level of significance [6] was employed to evaluate the experimental data.

Estimation of serum cholesterol
The serum cholesterol was estimated by dynamic extended stability CHOD-PAP method or modified Roeschläu’s method [7].

Estimation of serum triglycerides
The serum triglycerides was estimated by dynamic extended stability with lipid clearing agent Glycerol Phosphate Oxidase (GPO) – Trinder method and this reagent is based on the method of Wako and the modification reported by McGowan [8].

RESULTS AND DISCUSSION
The results of feeding prebiotics and probiotics to the Wistar rats on body weight gain are presented in Table 1. There was no significant improvement (p<0.01) in the body mass throughout the experimental period (6 wks) for all the treatment groups. A few studies have reported a weight lowering effect of Lactobacillus species besides hypocholesterolemic ability. Researchers reported significant weight loss (p<0.05) in the rats supplemented with Lactobacillus plantarum 9-41-A compared with the model group rats [9]. Alteration of the intestinal microflora by supplementation with LAB strains may lead to body weight reduction. Mahrous et al. (2011) observed a mixed effect on weight gain in rats when two different Lactobacillus acidophilus strains were fed when compared to control group.

There was a significant lowering of fecal pH in rats fed with prebiotics (extracted from onion) and Lactobacillus fermentum 141 and prebiotics (extracted from onion) compared to control group (Table 2). Lower pH in the gut discourages the growth of undesirable bacteria and thus enhances the health of the host. The fecal pH values were observed to be lower in the fructo-oligosaccharides (FOS) and the galacto-oligosaccharides (GOS) groups than the control group [11]. In addition, the pH of caecum was significantly lowered in rats by ingestion of each oligosaccharide, while fecal pH value was only reduced by FOS and GOS [12]. The change of pH was probably caused by the higher levels of total short chain fatty acid (SCFA) production in probiotic fed group.

The fecal coliform count was significantly lower in all treatment groups compared to control group (Table 2). Strojny et al., (2011) reported that the administration of probiotic and prebiotic supplementation to a high fat diet decreases the total coliforms in treatment groups when compared to the control group in rats [13]. Similar reports were obtained by Hsu et al., (2004) in rats supplemented with prebiotics-xylooligosaccharides (XOS) and fructooligosaccharides (FOS) [14].
The serum cholesterol content was significantly (P<0.01) lower in prebioitc, pre- and probiotic combination and *Lactobacillus fermentum* 141 fed rats compared to control group (Table 3). The serum triglycerides content showed a significant decrease in all the treatment group rats compared to control group. The probiotics feeding alone did not influence the serum cholesterol and triglycerides levels, however feeding together with probiotics had a significant influence on serum cholesterol and triglycerides. The probiotic organisms are reported to deconjugate bile acids in small intestines and reduce the solubilisation and absorption of lipids and thus reducing the serum cholesterol and triglycerides [15]. The serum cholesterol levels decreased in the rats fed with probiotic supplemented diets in hypercholesterolemia-induced mice and rats [16]. Fazeli *et al.*, (2010) reported that the consumption of *L. plantarum* A7 (10^8 CFU/mL) for 14 day was effective in lowering serum lipid levels in rats [17]. Taranto *et al.*, (2000) reported that the administration of *Lactobacillus reuteri* was effective in preventing hypercholesterolemia in mice and observed a decrease in total cholesterol (22%) and triglycerides (33%) [18].

Table 1. Body weight (kg) changes in rats fed with pre and probiotics.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>1.71 NS</td>
</tr>
<tr>
<td>II. Prebiotics (Onion)</td>
<td>1.54 NS</td>
</tr>
<tr>
<td>III. LAB 141 + Prebiotics (Onion)</td>
<td>1.59 NS</td>
</tr>
<tr>
<td>IV. Lactobacillus fermentum 141</td>
<td>1.65 NS</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
</tr>
<tr>
<td>P Value</td>
<td>0.752</td>
</tr>
</tbody>
</table>

abc Means with different super scripts differ significantly, P<0.01.
The means are obtained at the end of 6 weeks feeding trial

Table 2. Fecal pH and coliform values of rats fed with pre and probiotics.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fecal pH values</th>
<th>Fecal coliforms (MPN Index/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>8.46^a</td>
<td>64.17^a</td>
</tr>
<tr>
<td>II. Prebiotics (Onion)</td>
<td>7.33^a</td>
<td>27.83^b</td>
</tr>
<tr>
<td>III. LAB 141 + Prebiotics (Onion)</td>
<td>7.31^a</td>
<td>23.17^b</td>
</tr>
<tr>
<td>IV. Lactobacillus fermentum 141</td>
<td>8.13^a</td>
<td>19.17^b</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>SEM</td>
<td>0.095</td>
<td>310.55</td>
</tr>
</tbody>
</table>

abc Means with different super scripts differ significantly, P<0.01.
The means are obtained at the end of 6 weeks feeding trial

Table 3. Effect on serum triglyceride and cholesterol levels (mg/dL) in rats fed with pre and probiotics.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Triglycerides (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>917.35^a</td>
<td>1512.00^a</td>
</tr>
<tr>
<td>II. Prebiotics (Onion)</td>
<td>533.28^bc</td>
<td>1104.30^a</td>
</tr>
<tr>
<td>III. LAB 141 + Prebiotics (Onion)</td>
<td>620.11^b</td>
<td>1146.69^bc</td>
</tr>
<tr>
<td>IV. Lactobacillus fermentum 141</td>
<td>429.59^c</td>
<td>1334.00^ab</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
<td>0.046</td>
</tr>
<tr>
<td>SEM</td>
<td>29.98</td>
<td>34.58</td>
</tr>
</tbody>
</table>

abc Means with different super scripts differ significantly, P<0.01.
The means are obtained at the end of 6 weeks feeding trial

CONCLUSION

Feeding of *Lactobacillus fermentum* 141, prebiotics extracted by membrane processing of onions and a combination of *Lactobacillus fermentum* 141 and prebiotics (from onion) to Wistar rats for six weeks showed favorable effect in reducing the serum cholesterol, serum triglycerides and also reduced fecal pH (may help to discourage growth of unwanted bacteria) and fecal coliform count. However the feeding of pro and prebiotics did not influence the body weight gain of rats after six weeks.

Acknowledgements

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REFERENCES

[8] MW McGowan; JD Artiss; DR Strandbergh; B Zak, Clinical Chemistry. 1983, 29, 538-42.
[9] Ning Xie; Yi Cui; Yu-Ni Yin; Xin Zhao; Jun-Wen Yang; Zheng-Gen Wang; Nian Fu; Yong Tang; Xue-Hong Wang; Xiao-Wei Liu; Chun-Lian Wang; Fang-Gen LU, BMC Complementary and Alternative Medicine, 2011, 11,53-63.
[13] L Strojný; A Bomba1; E Hjová1; A Chmelárová1; G Mojišová1; I Bertková1; J Koprovicová1; M Pomfy2; V Strompfová3; M Molokácová4,