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Effect of feeding normal and high cholesterol diet incorporated with encapsulated and non-encapsulated *Bifidobacterium bifidum* 235 and prebiotics on serum triglycerides of S.D. Rats

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Abstract

In the present experiment, effect of encapsulated and non encapsulated synbiotic (prebiotic and probiotic) on the feed intake, body weight gain, and feed conversion ratio and serum lipid profile is studied. The probiotic bacteria used in the study is *Bifidobacterium bifidum* 235. The feeding experiment was conducted in S.D. rats which were fed with high cholesterol diet (group I), normal diet (group II), high cholesterol diet with encapsulated *Bifidobacterium bifidum* 235 (group III), high cholesterol diet with non encapsulated *Bifidobacterium bifidum* 235 (group IV), high cholesterol diet with encapsulated synbiotic (group V), and high cholesterol diet with non encapsulated synbiotic (group VI). The effect of supplementation of encapsulated *Bifidobacterium bifidum* 235 with prebiotics in milk fat rich high cholesterol diet on serum profile of experimental rats was studied for 45 days. The serum samples for estimation of triglycerides were analyzed at 15 days interval. The triglycerides (10.49%) of rats feed on high cholesterol diet supplemented with encapsulated synbiotic were significantly lower than all the other groups. There was further reduction in triglycerides (10.14%) has observed in rats fed with high cholesterol diet supplemented with encapsulated *Bifidobacterium bifidum* 235 which may be due to encapsulation has protected organisms from adverse conditions of gastro intestinal tract, relatively lesser in triglycerides (7.89%) was seen rats fed with high cholesterol diet with non encapsulated synbiotic may be due to feeding of non encapsulated synbiotic. In rats fed with high cholesterol diet with non encapsulated *Bifidobacterium bifidum* 235 there was least reduction in triglycerides (7.97%) when compared to the group fed only on control diet.

Keywords: *Bifidobacterium bifidum* 235, triglycerides, encapsulation, synbiotic

1. Introduction

Probiotics have been defined as “live microbial food supplements which beneficially affect the host improving the intestinal microflora balance”^[1], or more broadly as “living microorganisms, which upon ingestion exert health benefits beyond inherent general nutrition”. Prebiotics have been defined as a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, and thus improves host health^[2]. Probiotic benefits include: Increased resistance to infectious diseases, particularly of the intestine, decreased duration of diarrhea, reduction in blood pressure, reduction in serum cholesterol concentration, reduction in allergy, stimulation of phagocytosis by peripheral blood leucocytes, modulation of cytokine gene expression, regression of tumors and reduction in carcinogen or co-carcinogen production^[3]. Cholesterol plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. Excess cholesterol in the bloodstream can form plaque (a thick, hard deposit) in artery walls. The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart. When blood flow is restricted, angina (chest pain) can result. A heart attack will result when blood flow to the heart is severely impaired and a clot stops blood flow completely. When there is too much LDL cholesterol in the blood, it is deposited inside the blood vessels, where it can build up to hard deposits and cause atherosclerosis, the disease process that underlies heart attacks^[4]. Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and triglycerides. They're transported in the blood as part of lipoproteins.

These are the five major families of blood (plasma) lipoproteins: (1) chylomicrons, (2) very low-density lipoproteins (VLDL), (3) intermediate-density lipoproteins (IDL), (4) low-density lipoproteins (LDL), (5) high-density lipoproteins (HDL). When hyperlipidemia is defined in terms of class or classes of elevated plasma lipoproteins, the term hyperlipoproteinemia is used [5]. Intestinal bacteria convert cholesterol into a less absorbable form coprostanol thus hampering its absorption from the intestinal tract [6]. Lactic acid bacteria in intestine have the cholesterol lowering effect [7]. Some oral bacteria such as *Lactobacillus acidophilus* have been commercially available for the cholesterol lowering. Feeding friendly bacteria can do: (1) reduce the growth of unfriendly bacteria, (2) maintain regular bowel movements, (3) maintain cholesterol and triglyceride levels, (4) maintain healthy blood sugar levels [8] (Fazeli *et al.*, 2010). Homopolysaccharides are a group of polysaccharides composed of one monosaccharide type.

The aim of this study was to focus on the Effect of feeding high cholesterol diet with encapsulated synbiotic *Bifidobacterium bifidum* 235 on serum triglycerides of S.D. Rats.

2. Materials and Methods

2.1 Place of work: Department of Livestock Products Technology, College of Veterinary Science, Rajendranagar, Hyderabad-30.

2.2 Materials: Probiotic Bacterial culture the probiotic bacterial strain used in this study was pure freeze dried culture of *Bifidobacterium bifidum* 235 which was already characterized as probiotic in the laboratory of Department of Livestock Products Technology, College of Veterinary Science, Rajendranagar, Hyderabad

2.3 Chemicals: Agar agar Type I, Tri ammonium citrate extra pure, Di potassium phosphate, D (+) Dextrose anhydrous, FOS (carbohydrate composition on % dry basis: 96.2% FOS and 3.8% of glucose, fructose, sucrose), *Lactobacillus* MRS agar, Magnesium sulphate, Manganous sulphate, Polysorbate. MRS Agar was used for the enumeration of *Bifidobacterium bifidum* 235.

2.4 Equipments and Instruments Air Compressor, Refrigerated Centrifuge, Lyophiliser, pH meter, Electronic balance, Bacteriological Incubator, Laminar Flow, Peristaltic Pump, Magnetic stirrer with hot plate, Orbital Shaker Incubator, Vortex mixer Touch type, Kits for total cholesterol, from Transasia Bio-Medicals Ltd, Solan, India, Erba Mannheim semi automatic serum analyser.

2.5 Culture activation and maintenance *B. bifidum* 235 strain was rehydrated in MRS broth and incubated for 24 h at 37 °C. Cells were then cultured in the same conditions for three successive transfers in MRS broth at 37 °C for 20-24 h. It was then properly activated and served as the inoculum. Then, it was cultured in MRS broth for production of freeze dried *B. bifidum* 235 using 5% inoculum respectively and incubated for 48 h at 37 °C and then the cells were harvested by centrifugation at 5000 rpm for 15 minutes at 4 °C and washed with 0.9% normal saline and lyophilised to get bacterial powder and stored at 4 °C.

2.6 Micro-encapsulation procedure: The micro-encapsulation of *B. bifidum* 235 using sodium alginate as coating material was carried out according to the method of Chen *et al.* (2005), with some modification using micro-encapsulator. Solutions of sodium alginate (2%) containing approximately 10⁶ cfu/g of *B. bifidum* 235 with 0.1% by weight of commercial prebiotic FOS were atomized in 0.1 M calcium chloride, respectively. The atomization was achieved by forcing the sodium alginate solution through the micro-encapsulator device with the help of a peristaltic pump for 20 rpm and compressed air with 1MPa pressure. The solution of calcium chloride remained under constant magnetic stirring until the end of encapsulation. Alginate beads remained at rest for 30 minutes and were separated from the calcium chloride solution with sieves and washed with distilled water and dried at 40 °C for 48 h and alginate beads were stored at 4 °C.

2.7 Feed: Rat feed in the form of pellet (NIN standard feed) was procured by National Institute of Nutrition, Hyderabad, with the following formulation and specification: Composition of normal diet: Wheat flour-22.5%, Roasted Bengal gram flour-60.0% Skim milk powder -5.0%, Casein -4.0%, Refined sun flower oil -4.0%, Salt mixture -4.0%, Vitamin mixture-0.5%.

2.8 High Fat Diet Composition: (NIN, Hyderabad): Normal mice diet-750.0g, Dextrose monohydrate-75.0g, Sucrose-16.25g, Dextrin-16.25g, Ghee-75.00g, Cholesterol: 12.50g, Sodium cholate :5.0g, Cellulose:12.50g, Mineral mix (AIN 93G)-8.75g, Vitamin mix (AIN 93UX)-2.5g, Choline chloride-1.25g, Note: The total cholesterol content is 12.6 g/Kg of High fat diet.

2.8.1 Methods: Forty eight male *Sprague dawley* (S.D.) rats of uniform age and weight were procured from NIN, Hyderabad for the study. Feed and water was provided *ad libitum* throughout the experiment. Animals were housed in polypropylene cages in a well ventilated animal house with 12h - 12h light - dark cycles. Acclimatization period of 2 weeks was observed before the start of experiment. After an acclimatization period of 2 weeks, rats were randomly divided into 6 groups of 8 rats in each and serum samples were collected for total cholesterol estimation. Subsequently, group 1 was kept as normal control throughout the experimental period. Remaining 5 groups were kept on high cholesterol diet incorporated with encapsulated prebiotics and probiotics and non-encapsulated prebiotics and probiotics. The rats were provided with water for 24 h. Blood samples were collected and serum was separated for total cholesterol estimation. Experimental animal design: Six experimental diets were prepared as follow: Group 1: Negative control (high cholesterol diet) incorporated with ghee, Group 2: Positive control (normal diet), Group 3: Negative control supplemented with encapsulated *Bifidobacterium bifidum* 235 @ 10⁶CFU /kg feed, Group: Negative control supplemented with non-encapsulated *Bifidobacterium bifidum* 235 @ 10⁶CFU /kg feed. Group 5-Negative control diet supplemented with *Bifidobacterium bifidum* 235 @ 10⁶ CFU/kg feed and prebiotic @ 0.1% by weight, Group 6-Negative control supplemented with non encapsulated *Bifidobacterium bifidum* 235 @ 10⁶CFU /kg feed and prebiotic @ 0.1% by weight.

2.9 Blood collection: Blood collection was carried out at every 15 days interval for sero-biochemical analysis till the end of experiment (8 wks). Feed was withdrawn 12 h before the blood collection and blood was collected through retro-orbital plexus after ether anesthesia into serum vacutainers and centrifuged at 3000 RPM for 15 min and serum was separated and stored at -20 °C till analysis. The sera samples were analyzed for the triglycerides and on 1st, 15th, 30th, and 45th day.

2.10 Biochemical Profile: Plasma was separated from the blood and used for Total cholesterol analysis by using diagnostic kits: Triglycerides

Analytical Procedures The following analysis was carried in the treatments and control samples. Estimation of Triglycerides: The estimation of total cholesterol in serum is done by CHOD-PAP method [9]. (Table No.1). Mix and incubate for 10 mins at 37 °C read the absorbance of standard and each sample at 546/670 nm on biochromatic analyzers against reagent blank.

Table 1: Assay Procedure

Pipette into tubes marked	Blank	Standard	Test
Working Reagent	1000µl	1000µl	1000µl
Distilled Water	10µl	--	--
Standard	--	10µl	--
Test	--	--	10µl

2.11 Calculation

$$\text{Triglycerides (mg/dl)} = \frac{\text{absorbance of test} \times \text{Concentration of Standard (mg/dl)}}{\text{absorbance of standard}}$$

3 Results and Discussion

Mean serum triglycerides values are shown in Table 2. There was no significant difference seen in the mean serum triglyceride values on the initial day, the highest mean serum triglyceride value was seen in the group V (83.50 mg/dl) rats when compared with group I (81.87 mg/dl) rats. Between group III (83.50 mg/dl) and group V (83.50 mg/dl) rats there was no significant difference. The group IV (83.00 mg/dl) rats have shown significant increase when compared with group I. By the end of 45 days of feeding period there was a significant difference seen in all the groups in comparison with positive (102.5 mg/dl) and negative controls (115.3 mg/dl) diets. The reduction of mean serum triglycerides was seen in group V (103.2 mg/dl) rats which were fed with high cholesterol diet along with encapsulated synbiotic, when compared with group VI (106.2 mg/dl) rats which were fed on high cholesterol diet with non-encapsulated synbiotic. There was a greater reduction in mean serum triglyceride values of group III (103.6 mg/dl) rats which were fed on high cholesterol diet along with encapsulated *Bifidobacterium bifidum* 235, in comparison with group IV (106.1 mg/dl) rats fed on high cholesterol diet with non-encapsulated

Bifidobacterium bifidum 235. A reduction in serum triglycerides was observed, in group fed on the high cholesterol diet along with encapsulated synbiotic. The probable reason may be due to enhanced survivability of encapsulated probiotic and *Bifidobacterium bifidum* 235 with prebiotic, withstanding exposure to the adverse conditions of gastro intestinal tract such as gastric acidity and bile reaction. The cholesterol-lowering activity of lactic acid bacteria has not yet been worked out completely, probiotics may alter serum cholesterol by two possible mechanisms: (1) directly binding dietary cholesterol into the small intestine before cholesterol can be absorbed into the body [10, 11, 12] and (2) bile salt deconjugation by bile salt hydrolase to produce free bile acids [13, 14, 15]. Free bile acids thus formed by the deconjugation of conjugated bile salts are less soluble and are less likely to be reabsorbed by the intestinal lumen compared to bile salts, and are lost from the human body through faeces [16, 17]. The serum samples of rats at the end of at 6th week showed significant difference in the lipid profiles between the of normal and high cholesterol diets. The triglycerides (10.49%) of group V rats were significantly lower than all other groups. This may be due to feeding of high cholesterol diet with encapsulated synbiotic. Feeding of synbiotic containing *L. acidophilus* ATCC 4962, fructooligosaccharides, inulin, and mannitol decreased plasma triglycerides [18]. A reduction in triglycerides (10.14%) was seen in the group III rats. This may be attributed due to feeding of high cholesterol diet with encapsulated *Bifidobacterium bifidum* 235. The plasma triglycerides values obtained from high cholesterol diet supplemented with microencapsulated *L. plantarum* Lp91 were significantly lower than the hypercholesterolemic control group [19]. The reduction of triglycerides (7.89%) in group VI rats was better when compared to group IV, may be due to feeding on high cholesterol diet with non encapsulated synbiotic. Similar results were observed by feeding of soybean oligosaccharides to 50 wistar rats @ 450mg/kg BW /day for 45 days showed a reduction in triglycerides by 40.8% [20]. The reduction was higher probably due to addition of high concentration of prebiotic sugars. Feeding of Xylo-oligosaccharides in 40 male S.D. rats @ 60g/kg diet for 35 days showed reduction in triglycerides by 33.9% [21]. In group IV rats which were on high cholesterol diet with non encapsulated *Bifidobacterium bifidum* 235, there was a reduction in the triglycerides (7.97%) when compared with group II fed with only high cholesterol diet. Administration of 10⁹ CFU/ml of *L. plantarum* KCTC 3928 for 4 weeks in hypercholestermia induced rats resulted in reduction in triglycerides by 32% [22]. Similar results were attributed with the feeding of *Bifidobacterium longum* Bb-46 in 48 male albino hypercholesterolemic rats @ of 0.07% (w/v) for 35 days showed a reduction of triglycerides (51.2%) [23]. A report studied in 32 human subjects fed with *B. longum* BL1 @ 10⁸ CFU/g for 4 weeks showed reduction in serum triglycerides²⁴.

Table 2: Effect of feeding normal and high cholesterol diet incorporated with encapsulated and non encapsulated *Bifidobacterium bifidum* 235 and prebiotics on serum Triglycerides of S.D. Rats.

Group	Treatments	Serum triglycerides (g/dl)			
		1 st Day	15 th Day	30 th Day	45 th Day
Group I	High Cholesterol Diet(NC)	81.87 ^a ±0.6	98.3 ^a ±0.7	109.2 ^a ±1.0	115.3 ^a ±0.9
GROUP II	Normal diet	84.00 ^a ±1.3	94.00 ^a ±0.7	98.12 ^a ±0.8	102.5 ^a ±1.5
GROUP III	NC+encapsulated <i>B. bifidum</i> 235	83.5 ^a ±1.7	96.12 ^b ±1.1	103.5 ^b ±1.0	103.6 ^b ±1.0
GROUP IV	NC+Non encapsulated <i>B. bifidum</i> 235	83.00 ^{ab} ±1.5	95.00 ^b ±0.7	105.0 ^b ±1.0	106.1 ^b ±0.6
GROUP V	NC+encapsulated prebiotic + <i>B. bifidum</i> 235	83.5 ^a ±1.2	95.12 ^c ±0.9	103.6 ^c ±1.0	103.2 ^{cd} ±0.7
GROUP VI	NC+Non encapsulated prebiotic + <i>B. bifidum</i> 235	83.17 ^{ab} ±1.2	96.48 ^b ±0.6	105.5 ^b ±0.7	106.2 ^b ±0.8

^{abcd} Means with different superscripts in the same column differ significantly, ($p < 0.05$); means are obtained at every 15days interval

4. Conclusion: From this study it may be concluded that feeding of encapsulated *Bifidobacterium bifidum* 235 and prebiotic has shown better reduction in the serum Triglycerides probably encapsulation with prebiotic has enhanced survivability of *Bifidobacterium bifidum* 235 with prebiotic which helped in enhancing survival during exposure to the adverse conditions of gastro intestinal tract such as gastric acidity and bile reaction.

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