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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 total cholesterol levels of S.D.rats

Karthik Kotha, Kotinagu Korrapati and Kondal Reddy

Abstract
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 total cholesterol were analysed at 15 days interval. The total cholesterol (9.40%) of rats feed on high cholesterol diet supplemented with encapsulated synbiotic were significantly lower than all the other groups. There was further reduction in total cholesterol (9.73%) 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 total cholesterol (4.80%) 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 total cholesterol (2.89%) when compared to the group fed only on control diet.

Keywords: *Bifidobacterium bifidum* 235, total cholesterol, encapsulation, synbiotic.

1. Introduction
Lipid is the scientific term for the word "fat" in blood. At proper levels, lipids perform important functions in our body, but can cause health problems if they are present in excess [1]. The WHO has predicted that by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the World [2]. It was reported that hypercholesterolemia contributed to 45% of heart attacks in Western Europe and 35% of heart attacks in Central and Eastern Europe from 1999 to 2003 [3]. The risk of heart attack is three times higher in those diets with hypercholesterolemia, compared to those who have normal blood lipid profiles. The WHO delineated that unhealthy diets with high fat, salt and free sugar and low in complex carbohydrates, fruits and vegetables lead to increased risk of cardiovascular diseases [4]. People affected with hypercholesterolemia may avert the use of cholesterol-lowering drugs by practicing dietary control or supplementation of probiotics and/or prebiotics. Probiotics are defined as '*living microbial supplements that beneficially affect the host animals by improving its intestinal microbial balances*' [5]. Prebiotics are '*indigestible fermented food substrates that selectively stimulate the growth, composition and activity of micro flora in gastrointestinal tract and thus improve hosts' health and well-being*'. When probiotics and prebiotics are used in combination, they are known as 'synbiotics'. The use of probiotics and prebiotics has only acquired scientific recognition in recent years although their applications as functional foods have been well-established throughout generations. In the interest of their promising effects on health and well-being, probiotics and prebiotics have become increasingly recognized as supplements for human consumption. Prebiotics are utilized by the intestinal microbial population to produce short-chain fatty acids which may lead to the reduced incidence of gastrointestinal disease, cancers and cardiovascular diseases; and improvement of lipid profiles. Different approaches have been attempted to increase the resistance of the probiotic bacteria against adverse conditions like inclusion of appropriate selection of acid and bile-resistant strains, stress adaptation, incorporation of micronutrients such as peptides and amino acids, and micro-encapsulation [6]. Providing probiotic living cells with a physical barrier against adverse conditions is an approach currently receiving major interest. Encapsulation is a physico chemical or mechanical process to entrap a substance in a material in order to produce particles with

diameters of a few nanometers to a few millimeters [7]. Probiotic encapsulation is used to protect the cells against an adverse environment more than controlled release [8, 9]. Little work has been carried out to investigate the role encapsulated of synbiotics in maintaining healthy serum lipid profile in diets containing milk fat rich products like ghee.

2. Materials and methods

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

2.2 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, Di potassium phosphate, Calcium chloride, 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 Methods: **Culture activation and maintenance** *B. bifidum* 235 strain was rehydrated in MRS broth and incubated for 24 h at 37^0C. Cells were then cultured in the same conditions for three successive transfers in MRS broth at 37^0C 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 37^0C and then the cells were harvested by centrifugation at 5000 rpm for 15 minutes at 4^0C and washed with 0.9% normal saline and lyophilised to get bacterial powder and stored at 4^0C.

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 10^6 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 40^0C for 48 h and alginate beads were stored at 4^0C.

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 @ 10^6CFU /kg feed, Group: Negative control supplemented with non-encapsulated *Bifidobacterium bifidum*235 @ 10^6CFU /kg feed. Group 5-Negative control diet supplemented with *Bifidobacterium bifidum* 235 @10^6 CFU/kg feed and prebiotic @ 0.1% by weight, Group 6-Negative control supplemented with non encapsulated*Bifidobacterium bifidum*235 @ 10^6CFU /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 anaesthesia into serum vacutainers and centrifuged at 3000 RPM for 15 min and serum was separated and stored at -20^0C till analysis. The sera samples were analyzed for the total cholesterol, HDL cholesterol, LDL cholesterol and 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: Total cholesterol

2.11 Analytical Procedures The following analysis was carried in the treatments and control samples. **Estimation of**

cholesterol: The estimation of total cholesterol in serum is done by CHOD-PAP method [10]. (Table No.1). Mix well incubate at 37°C for 10 min. Aspirate Blank followed by Standard and Tests. Read the absorbance of standard and each test tube against Blank at 505/670 nm on bichromatic analyzer

Table 1: Assay Procedure

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

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

	Treatments	Total Cholesterol Levels (g/dl)			
		1st Day	15th Day	30th Day	45th Day
GROUP I	High Cholesterol Diet(NC)	74.45^{ab}±1.6	95.75^{a}±0.8	112.65^{a}±0.7	124.87^{a}±0.8
GROUP II	Normal diet	74.0^{ab}±1.6	85.87^{e}±0.9	101.37^{d}±1.0	113.12^{d}±1.3
GROUP III	NC+encapsulated *B.bifidum 235*	75.50^{a}±1.4	91.98^{c}±0.9	102.87^{c}±0.6	113.16^{d}±0.8
GROUP IV	NC+Non encapsulated *B.bifidum 235*	74.87^{a}±1.7	95.25^{a}±0.7	106.87^{b}±0.6	121.25^{b}±0.8
GROUP V	NC+encapsulated prebiotic +*B.bifidum 235*	74.50^{ab}±0.9	90.62^{d}±0.7	100.25^{e}±1.0	109.62^{e}±0.7
GROUP VI	NC+Non encapsulated prebiotic + *B.bifidum 235*	73.37^{b}±0.5	93.62^{b}±1.7	102.37^{c}±0.9	118.87^{c}±0.9

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

Calculation

$$\text{cholesterol} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \text{X concentration of standard (mg/dl)}$$

3. Results and Discussion

Table 2 shows the results regarding the mean total cholesterol levels. On initial day, there was not much significance difference was seen in the groups. The highest mean total cholesterol was seen in the group III (75.50 mg/dl) rats and least was seen in group VI (73.37 mg/dl). Among the groups I (74.45 mg/dl), II (74.00 mg/dl), IV (74.50 mg/dl), and V (74.87 mg/dl), there was no significant difference seen but, when compared with group III the mean total cholesterol was less. By the end of 45 days of feeding trail, there was a significant difference seen in all the treatments the highest level of mean total cholesterol was observed in the group I (124.87 mg/dl) rats fed on only high cholesterol diet and the lowest mean total cholesterol was seen in the group II (109.62 mg/dl) rats which were fed on normal diet. There was a significant increase seen in the mean total cholesterol of group IV (121.25 mg/dl) rats which were fed on the high cholesterol diet along with non encapsulated *Bifidobacterium bifidum 235*, when compared with group III (113.16 mg/dl) rats which were fed on high cholesterol diet with encapsulated *Bifidobacterium bifidum 235.* The mean total cholesterol of group V (113.12 mg/dl) rats which were fed on high cholesterol diet along with encapsulated synbiotic was reduced when compared with group VI (118.87 mg/dl) rats which were fed on high cholesterol diet with non encapsulated synbiotic. A reduction in serum total cholesterol, 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 [11, 12, 13] and (2) bile salt deconjugation by bile salt hydrolase to produce free bile acids [14, 15, 16]. 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 [17, 18]. The serum samples of rats at the end of at 6th week showed significant difference in the Total Cholesterol between the of normal and high cholesterol diets. The total cholesterol (9.40%) 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 total cholesterol [19]. Consumption of 375 ml synbiotic milk in humans (containing of 10^7-10^8 CFU/g of *Lactobacillus acidophilus* and 2.5% (g/100 g) of fructooligosaccharides) resulted in a significant decline in total cholesterol by 4.4% [20].

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 Total Cholesterol 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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