

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.705.009>

Effect of Supplementation of Encapsulated *Bifidobacterium bifidum* 235 in Milk Fat Rich Diet on the Feed Intake, Body Weight Gain, and FCR of Experimental Rats

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ABSTRACT

Keywords

Feed intake, Body weight gain, FCR, *Bifidobacterium bifidum* 235, Encapsulation

Article Info

Accepted:
04 April 2018
Available Online:
10 May 2018

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. Commercially available prebiotic FOS (3%) was used for co-encapsulation and 2% alginate was used as a coating material by extrusion method. The feed intake, body weight gain and FCR were recorded weekly. The body weight gain, feed intake and FCR were better in rats which were fed with encapsulated synbiotic and probiotic compared to control rats. The rats feed with encapsulated *Bifidobacterium bifidum* 235 have shown increased body weight gain, feed intake and better FCR when compared with rats of control group. The rats fed on non-encapsulated *Bifidobacterium bifidum* 235 had better body weight gain, feed intake and FCR in comparison with the rats which fed on non-encapsulated *Bifidobacterium bifidum* 235.

Introduction

Ghee, also known as clarified butter or anhydrous milk fat, is prepared by heating butter or cream to just over 100°C to remove water content by boiling and evaporation, then filtering out the precipitated milk solids. Ghee is known as *ghrta* (Acharayya *et al.*, 1997) (commonly spelled *ghrita*) in Sanskrit. *Ayurveda* has traditionally considered ghee to be the healthiest source of edible fat, with

many beneficial properties. According to *Ayurveda*, ghee promotes longevity and protects the body from various diseases (Tirtha, 1998).

It increases the digestive fire (*agni*) and improves absorption and assimilation. It nourishes *ojas*, the subtle essence of all the body's tissues (*dhatu*s). It improves memory and strengthens the brain and nervous system. It lubricates the connective tissues, thereby

rendering the body more flexible. With regard to the three *doshas* (organizing principles that govern the physiology), ghee pacifies *Vata* and *Pitta* and is acceptable for *Kapha* in moderation (Lad, 1998).

Ghee is heavily utilized in *Ayurveda* for numerous medical applications, including the treatment of allergy, skin, and respiratory diseases. Many Ayurvedic preparations are made by cooking herbs into ghee. Ghee carries the therapeutic properties of herbs to all the body's tissues.

It is an excellent *anupana* (vehicle) for transporting herbs to the deeper tissue layers of the body (Lad, 1998). Proper digestion, absorption and delivery to a target organ system are crucial in obtaining the maximum benefit from any therapeutic formulation; the lipophilic action of ghee facilitates transportation to a target organ and final delivery inside the cell since the cell membrane also contains lipid (Sharma, 1990).

Ghee is considered sacred and used in religious rituals as well as in the diet in India (Rajorhia *et al.*, 1993). In ancient India, ghee was the preferred cooking oil. It was considered pure and was felt to confer purity to foods cooked with it (Acharyya *et al.*, 1997). Ghee and other similar products such as *samna* (variant of the Arabic term *samn*) are used in many parts of the world (Sserunjoi *et al.*, 1998).

Fatty acid analysis of ghee indicated it contains 47.8% saturated fat (Dwivedi *et al.*, 2002). There has been concern about the possibility of ghee contributing to an increased risk of cardiovascular disease since it contains a high percentage of saturated fatty acids, leading to increased synthesis of cholesterol.

The American Heart Association recommends limiting the consumption of saturated fats to

less than 7% of energy to reduce the risk of cardiovascular disease (Lichtenstein *et al.*, 2006).

Materials and Methods

Place of work

Department of Livestock Products Technology, College of Veterinary Science, Rajendranagar, Hyderabad-30.

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

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.

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.

Methods: 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.

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 10⁶cfu/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.

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%.

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.

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 @ 10^6 CFU/kg feed, Group: Negative control supplemented with non-encapsulated *Bifidobacterium bifidum* 235 @ 10^6 CFU/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 @ 10^6 CFU/kg feed and prebiotic @ 0.1% by weight.

Analytical procedures

The following analysis was carried in the treatments and control samples

Body weights

Rats were allowed to consume their respective diets and water *ad libitum* for 6 weeks. Body weight and feed intake were recorded weekly.

Feed Conversion Ratio (FCR)

Rats were allowed to consume their respective diets and feed intake is recorded weekly and FCR is calculated using formula. $FCR = \text{g feed intake} / \text{g body weight gain}$.

Results and Discussion

The mean feed intakes of the rats were recorded weekly and presented in the table 1. There was a significant difference observed in the 1st week with the highest feed intake in group II (93.00g) rats and the lowest mean feed intake (87.83g) was in the group I rats. There was no significant difference in the mean feed intakes of groups III (90.77g) and V (90.77g) rats. The mean feed intake of group V (91.15) rats was significantly higher when compared with the group I rats. The

mean feed intake of group VI (90.33g) rats was greater than the group I (87.83g) rats. At the end of 6th weeks of experimental period, the mean feed intakes were significantly different among the groups. The highest mean feed intake was seen in the group II (91.72g) rats which were fed on only normal diet. There was no significant difference seen in the feed intakes of group III (91.66g), V (91.66g) and VI (91.27g) rats when compared with group II. The lowest mean feed intake was seen in the group I (85.00g) rats that were fed on high cholesterol diet. The mean feed intake of group IV (89.14g) rats was higher than the group I rats. The mean body weight gains in rats with the experimental diet are presented in table 2. The results on the mean body weight gain revealed that there was no significant difference seen in all the groups during the 1st week. The highest mean body weight gain was observed in group IV (24.33g) rats and the lowest in group V (23.66g) and VI (23.66g) rats.

At the end of 6 weeks of experimental study, it was observed that there was a significant increase ($P < 0.05$) in the mean body weights in all the groups. The highest mean body weight was seen in the group V (29.33g) and the least body weight gain was seen in the group II (25.33g) rats. The mean body weight gain of group I (29.00g) rats was not significantly different from group V. Mean body weight gains of group III (28.33g) rats were significantly lower than the group I. In group IV (27.00g) rats, the mean body weight gain obtained was lower than the group I. The mean body weights of group VI (27.33g) rats were less than the group I. The table 3 presents the feed conversion ratio (FCR). There was no significant difference seen in the mean FCR values obtained in the first week. In the last week of the experimental period, there was significant difference seen in all the groups. The highest FCR was seen in the group II (3.62) rats and lowest FCR was seen

in the group I (2.93) rats. The mean FCR of group VI (3.34) rats was higher than the group I. Mean FCR of group V (3.13) rats was higher than the group I. There was no significant difference seen in the FCR of group III (3.24) and group IV (3.30) rats, but FCR was higher than the group I. The mean feed intakes in all the groups have shown increasing trend except in group I which showed slight reduction in feed intake. At the end of 6th week of feeding period the highest feed intake was seen in the group II (91.72±0.5) which was fed with normal diet, and lowest feed intake was seen in the group I (85.00±1.0) which was fed on only high cholesterol diet. These results may be attributed to the higher caloric content of high fat diet as compared to basal diet. Similar trend of results was seen with Ning *et al.*, (2011) where there was a reduction in the feed intake of groups which were fed on high cholesterol diet compared with that of normal diet. Mahrous *et al.*, (2011) reported that there was reduction in feed intake of group fed with high cholesterol diet when compared with that of normal diet. Increase in the feed intake was observed in the group which was fed with combination of yoghurt starter and *L. acidophilus* P106 with 1.0% (w/w) cholesterol and 0.2% (w/w) oxgal.

Similar results were also reported by Amr *et al.*, (2011), Cavallini *et al.*, (2009) and Chovancikova and Simek (2001). There was an increasing trend of body weight gains in all the groups. By the end of feeding trail period of 45 days, the highest body weight gain was observed in group V (29.33± 1.1) which was fed on high cholesterol diet *al.*,ong with encapsulated prebiotic and *Bifidobacterium bifidum* 235. Increased body weight gain in this group can be attributed to the addition of prebiotic to the diet which may have contributed to increased survivability of *Bifidobacterium bifidum* 235, and also increased feed intake and body weight gain.

Similar results were seen by Sang-oh *et al.*, (2011) who observed that feeding of microencapsulated inulin as prebiotic resulted in higher body weight gain than that of the control group. Group I fed on only high cholesterol diet did not show significant difference compared to group V (29.33±1.1). The mean body weight gains of group VI rats (27.33± 1.0) were significantly lower than that of the group V probably encapsulation offered protection to bacteria to reach colon effectively, which may not occur in group VI. Similarly better weight gain was seen in the group III rats (28.33±1.1) when compared with that of group IV (27.00±1.0) which was fed with encapsulated *Bifidobacterium bifidum* 235. Similar trend was seen by Hu *et al.*, (2004) who reported that feeding only high cholesterol diet has shown increasing trend of body weight gain and high cholesterol diet *al.*,ong with *Lactobacillus plantarum* NS5 supplemented in water has shown slight decrease in body weight gain. Ning *et al.*, (2011), also reported reduction in body weight gain of group that were fed with high cholesterol diet along *L. plantarum*9-41-A, when compared to the group fed on only high cholesterol diet. By the end of 6th week feeding trail, the FCR of group I (2.93±0.09) was better when compared with that of group II (3.62±0.09) which were fed on the normal diet. The FCR of group V (3.13±0.1) was significantly higher than group I. This can be attributed to the higher caloric value of high cholesterol diet which has shown better FCR in group II and group V. similar results observed by Sang-oh *et al.*, (2011), who observed better FCR in groups fed with microencapsulated inulin along with high fat diet than the control group and Xie *et al.*,(2011) also reported better FCR in groups that were fed with high cholesterol diet with *L. plantaraum* 9-41-A and in high cholesterol diet with *L. fermentum* M1-16 compared with normal diet.

Table.1 Effect of feeding normal and high cholesterol diet supplemented with encapsulated and non-encapsulated *Bifidobacterium bifidum* 235 and prebiotics on body weight gain

| GROUP | TREATMENTS | Weight gain (g) | | | | | |
|-----------|--|----------------------|----------------------------|--------------------------|-------------------------|--------------------------|---------------------------|
| | | 1 st Week | 2 nd Week | 3 rd Week | 4 th Week | 5 th Week | 6 th Week |
| GROUP I | High Cholesterol Diet(NC) | 24.00±1.0 | 25.33 ^{bc} ±1.0 | 26.66 ^{ab} ±0.5 | 28.33 ^a ±0.5 | 28.33 ^{ab} ±0.5 | 29.00 ^{ab} ±1.0 |
| GROUP II | Normal diet | 24.00±1.0 | 24.66 ^c ±1.0 | 23.66 ^c ±0.5 | 24.33 ^c ±0.5 | 25.33 ^d ±0.5 | 25.33 ^d ±0.5 |
| GROUP III | NC+encapsulated <i>B.bifidum</i> 235 | 23.00±0.5 | 25.66 ^{abc} ±0.9 | 26.33 ^{ab} ±0.5 | 28.66 ^a ±1.1 | 27.33 ^{bc} ±0.5 | 28.33 ^{abc} ±1.1 |
| GROUP IV | NC+Non encapsulated <i>B.bifidum</i> 235 | 24.33 ±1.1 | 25.66 ^{abc} ± 0.5 | 27.00 ^a ±1.0 | 25.00 ^c ±1.0 | 26.66 ^c ±0.5 | 27.00 ^{cd} ±1.0 |
| GROUP V | NC+encapsulated prebiotic + <i>B.bifidum</i> 235 | 23.66±1.5 | 26.00 ^{ab} ±0.8 | 27.33 ^a ±0.5 | 28.33 ^a ±0.5 | 28.66 ^a ±0.5 | 29.33 ^a ±1.1 |
| GROUP VI | NC+Non encapsulated prebiotic + <i>B.bifidum</i> 235 | 23.66 ±0.5 | 26.66 ^a ±0.5 | 25.66 ^b ±0.5 | 26.66 ^b ±0.5 | 26.66 ^c ±0.5 | 27.33 ^{bc} ±0.5 |

**abcd Means with different superscripts in the same column differ significantly, (p<0.05); means are obtained at weekly interval

Table.2 Effect of feeding normal and high cholesterol diet supplemented with encapsulated and non-encapsulated *Bifidobacterium bifidum* 235 and prebiotics on feed intake of S.D. rats

| GROUP | TREATMENTS | Feed intake (g) | | | | | |
|-----------|--|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|-------------------------|
| | | 1 st Week | 2 nd Week | 3 rd Week | 4 th Week | 5 th Week | 6 th Week |
| GROUP I | High Cholesterol Diet(NC) | 87.83 ^d ±0.1 | 87.33 ^d ±0.5 | 84.51 ^c ±0.5 | 86.66 ^d ±0.5 | 86.33 ^d ±0.5 | 85.00 ^c ±1.0 |
| GROUP II | Normal diet | 93.00 ^a ±1.0 | 92.00 ^a ±0.8 | 91.85 ^a ±0.8 | 92.77 ^a ±0.5 | 91.66 ^a ±0.5 | 91.72 ^a ±0.5 |
| GROUP III | NC+encapsulated <i>B.bifidum</i> 235 | 90.77 ^{bc} ±0.6 | 90.00 ^{bc} ±1.0 | 90.33 ^b ±0.5 | 90.33 ^c ±0.5 | 90.33 ^{bc} ±0.5 | 91.66 ^a ±0.5 |
| GROUP IV | NC+Non encapsulated <i>B.bifidum</i> 235 | 92.27 ^{ab} ±0.8 | 89.33 ^c ±0.5 | 90.00 ^b ±1.0 | 89.33 ^c ±0.5 | 89.33 ^c ±0.5 | 89.14 ^b ±0.1 |
| GROUP V | NC+encapsulated prebiotic + <i>B.bifidum</i> 235 | 91.15 ^{bc} ±0.9 | 90.96 ^{ab} ±0.6 | 91.78 ^a ±0.6 | 91.66 ^b ±0.5 | 91.25 ^{ab} ±0.6 | 91.66 ^a ±0.5 |
| GROUP VI | NC+Non encapsulated prebiotic + <i>B.bifidum</i> 235 | 90.33 ^c ±0.5 | 90.37 ^{bc} ±0.3 | 90.33 ^b ±0.5 | 90.33 ^c ±0.5 | 89.33 ^c ±0.5 | 91.27 ^a ±0.3 |

**abcd Means with different superscripts in the same column differ significantly, (p<0.05); means are obtained at weekly interval

Table.3 Effect of feeding normal and high cholesterol diet supplemented with encapsulated and non-encapsulated *Bifidobacterium bifidum* 235 and prebiotics on feed conversion ratio (FCR) of S.D. rats

| GROUP | TREATMENTS | Feed conversion ratio | | | | | |
|-----------|--|-----------------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| | | 1 st week | 2 nd week | 3 rd week | 4 th week | 5 th week | 6 th week |
| GROUP I | High Cholesterol Diet(NC) | 3.66±0.1 | 3.45 ^b ±0.08 | 3.17 ^d ±0.08 | 3.06 ^d ±0.05 | 3.05 ^d ±0.04 | 2.93 ^d ±0.09 |
| GROUP II | Normal diet | 3.88±0.1 | 3.73 ^a ±0.1 | 3.88 ^a ±0.06 | 3.81 ^a ±0.04 | 3.62 ^a ±0.04 | 3.62 ^a ±0.09 |
| GROUP III | NC+encapsulated <i>B.bifidum</i> 235 | 3.95±0.2 | 3.50 ^b ±0.05 | 3.43 ^{bc} ±0.08 | 3.15 ^d ±0.06 | 3.30 ^{bc} ±0.04 | 3.24 ^{bc} ±0.1 |
| GROUP IV | NC+Non encapsulated <i>B.bifidum</i> 235 | 3.79±0.1 | 3.48 ^b ±0.1 | 3.33 ^{cd} ±0.1 | 3.57 ^b ±0.09 | 3.35 ^b ±0.03 | 3.30 ^{bc} ±0.1 |
| GROUP V | NC+encapsulated prebiotic + <i>B.bifidum</i> 235 | 3.86±0.2 | 3.50 ^b ±0.1 | 3.36 ^{bc} ±0.09 | 3.23 ^{cd} ±0.03 | 3.18 ^{cd} ±0.05 | 3.13 ^{cd} ±0.1 |
| GROUP VI | NC+Non encapsulated prebiotic + <i>B.bifidum</i> 235 | 3.81±0.04 | 3.39 ^b ±0.06 | 3.52 ^b ±0.1 | 3.38 ^c ±0.03 | 3.35 ^b ±0.05 | 3.34 ^b ±0.07 |

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

The body weight gain, feed intake and FCR were better in rats which were fed with encapsulated synbiotic and probiotic compared to control rats. The rats fed with encapsulated *Bifidobacterium bifidum* 235 have shown increased body weight gain, feed intake and better FCR when compared with rats of control group. The rats fed on non-encapsulated *Bifidobacterium bifidum* 235 had better body weight gain, feed intake and FCR in comparison with the rats which fed on non-encapsulated *Bifidobacterium bifidum* 235.

Acknowledgments

Authors are grateful to the Department of Livestock product Technology, College of Veterinary Science, P.V. Narasimharao Veterinary University, Hyderabad, India for giving laboratory support, financial support and encouragement to the present research project.

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How to cite this article:

Karthik Kotha, Kotinagu Korrapati and Kondal Reddy, K. 2018. Effect of Supplementation of Encapsulated *Bifidobacterium bifidum* 235 in Milk Fat Rich Diet on the Feed Intake, Body Weight Gain, and FCR of Experimental Rats. *Int.J.Curr.Microbiol.App.Sci*. 7(05): 62-70. doi: <https://doi.org/10.20546/ijcmas.2018.705.009>