

Original Research Article

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Effect of Administration of Fatless Probiotic Food Containing Whey Protein Concentrate on the Faecal Count, Blood Cholesterol Level and Growth Performance of Albino Rats

P.K. Bisoi¹, Rakesh Kumar², Binita Rani³ and B.K. Singh^{4*}

¹Dairy Microbiology, OUAT, Bhubneshwar, India

²Department of Dairy Microbiology, ³Dairy Nutrition, ⁴Dairy Technology, Faculty of Dairy Technology, SGIDT, Jagdeo Path, Patna, Bihar, India

*Corresponding author

ABSTRACT

Increased industrialization, urbanization and mechanization are being associated with the dramatic changes in perception of health beneficial food which in turn has led to a significant decrease in the occurrence of chronic non-communicable diseases viz. obesity, diabetes-mellitus, cardiovascular diseases, stroke and hypertension and some types of cancer. Fatless fermented milk added with whey protein concentrate at 3% level was prepared by inoculating 1% inoculum of *Lactobacillus acidophilus*-015 and *Lactobacillus casei*-297 at 1:1 ratio and fed to albino rats for 45 days. Lactic count in faecal material and body weight of experimental animal were more increased in Group III rats which were fed whey protein concentrate added fatless fermented milk as compare to Group II rats which were fed plain fatless fermented milk than control group-I rats at the end of experiment. Coliform count in faecal material and serum cholesterol level were declined more in Group III rats comparatively to Group II rats than control Group-I rats whereas cholesterol level was increased in control group rats at the end of the experiment. So, it can be recommended to use of WPC as prebiotic in the preparation of fat less fermented milk products for maintaining the bacterial environment of intestine and controlling blood cholesterol level.

Keywords

Nonfat fermented milk, Whey protein concentrate, *Lactobacillus acidophilus*, *Lactobacillus casei*, Albino rats

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Introduction

Fermented dairy products have been playing a major role in the part of the diet of people throughout the world for their taste, nutritive value and therapeutic properties. During recent years, an increasing interest has developed in foods that contribute to a positive effect on health beyond their nutritional value. Among these functional

foods, much attention has been focused on probiotic products. Probiotic foods contain microorganisms or components of microbial cells that have a beneficial effect on the health and well-being of the consumer host (Salminen *et al.*, 1999). Sometimes the probiotic organisms fail to grow properly and to fight against pathogenic organisms due to lack of their foods. This has led to the coining of the term synbiotic food, which includes

probiotic microorganisms and their food materials known as prebiotic substances in the system. Klaver and Van der Meer (1993) suggested that probiotic organisms assimilate cholesterol within bacterial cell, increased excretion of bile salts due to deconjugation by bile salt hydrolase, antioxidative effect. Kawase *et al.*, (2000) found that Lactic acid bacteria inhibit the cholesterol absorption by binding of cholesterol to lactic acid bacterial cells, the promotion of excretion through faeces by the binding of bile acid to lactic acid bacterial cells and the assimilation of cholesterol by lactic acid bacteria. Oyetayo *et al.*, (2003) investigated that *Lactobacillus casei* had a better probiotic effect than *Lactobacillus acidophilus* in terms of live function improvement, anticholesterolaemic property and protection of the gastrointestinal tract from infection. Yadav *et al.*, (2008) reported that *Lactobacillus acidophilus* and *Lactobacillus casei* significantly increased the counts of Lactobacilli adherent to epithelial walls and free in the lumen of the small and large intestine, while decreasing attached as well as free coliform counts. Nagaoka (1996) described that β -lactoglobulin inhibited cholesterol absorption through changes of micellar cholesterol solubility in the intestine, accompanied by an increase of faecal steroid excretion.

Newburg *et al.*, (1998) investigated that whey protein concentrate might have enhanced the immune response to rotavirus challenge bacterial action because of immunoglobulin and reduced pathogenically clearance time by increasing neutrophil or natural killer cell activity. Naidu *et al.*, (1999) found that stalic acids (types of oligosaccharides) are typically attached to whey proteins and possess the prebiotic effects. Naidu *et al.*, (1999) suggested that whey contains several unique components including Lactose, Immunoglobulin, Lactoferrin, Lactoperoxidase, Glycomacro peptides and

Sphingolipids and significant levels of these survive passage through the stomach and small intestine and survive in the large intestine and intact proteins, where they exert their biological effects. Walzem *et al.*, (2002) found that whey protein content about 26% of branched chain amino acids which stimulated protein synthesis and muscle building. The present investigation was conducted to exploit the prebiotic character of whey protein concentrate with selected probiotics for maintaining the gastro-intestinal environment as well as controlling blood cholesterol level.

Materials and Methods

Two strains of lactic acid bacteria in freeze dried ampules namely *Lactobacillus acidophilus*-015 and *Lactobacillus casei*-297 were obtained from national collection of dairy culture centre, NDRI, Karnal, India. Both the cultures were maintained in sterile litmus milk media having reconstituted skim milk (10%) 100 ml, 1 percent litmus solution 1–2 ml and final pH 1.0, and then activated by three successive transfers at 24 h interval before use. Purity of the organisms was confirmed before use by microscopic examination using Gram staining technique and Catalase test according to Manual in Dairy Bacteriology, ICAR Sub-committee on Dairy Education (1972).

Skim milk (10% TS) was prepared by dissolving 10.5 gm of skimmed milk powder in 100 ml of distilled water. 3.17 gm of Whey protein concentrate was added into 100 ml Skim milk (10% TS) to maintain 13% TS. Fatless milk was heated up to 115⁰C for 15 min and cooled down to 38-40⁰C. 1% Mixed culture of *Lactobacillus acidophilus*-015 and *Lactobacillus casei*-297 having 1:1 ratio was inoculated into the fatless milk and incubated for 7 h at 37⁰C after inoculation. The acidity was measured at 2 h interval during the incubation period of milk as per provision of

ISI handbook of food analysis, part XI, Dairy products (1981).

Fifteen male albino rats (Wistar albino) of five to six weeks age with a body weight ranging from 135 to 140 gm were purchased. All rats were initially given a standard diet of thirteen gm rat feed for one week before the treatment, purchased from Shankar Feed Pvt. Ltd, Biprannapara, Jalan Complex, Howrah, West Bengal.

Fifteen albino rats were randomly divided into three treatment groups with five rats in each group. All rats were caged individually and allowed to drink water from water bottle separately. All the rats were fed corresponding diet for forty-five days according to their groups. Which are given as follows:

Group I (Control): Basal diet

Group II (Treatment): Basal diet supplemented with fatless fermented milk.

Group III (Treatment): Basal diet supplemented with whey protein concentrated fortified fatless fermented milk

About 13 gm of vet care rat feed was given to each rat individually of the group I. 4 gm of fatless fermented milk, which was prepared by inoculating probiotic cultures i.e. *Lactobacillus acidophilus-015* and *Lactobacillus casei-297*, having 13% TS from skim milk powder source, was given with 9 gm rat feed (vet care) per day to the group treatment-II rats separately. 9 gm vet care rat feed, with 4 gm whey protein concentrate added fatless fermented milk, prepared by inoculating probiotic cultures of *Lactobacillus acidophilus-015* and *Lactobacillus casei-297* having 10% TS from skim milk powder source and 3% TS from whey protein concentrate source, was given per day to the group treatment – III rats individually. On 0th

day the start of experiment, 15th day & 30th day during the experimental period and 45th day the close of experiment, body weights of all albino rats were recorded by using an electronic balance. Faecal samples of albino rats were collected at 0, 15, 30, and 45 days of feeding experiment. Wet faecal material was collected separately from each rat of different treatments and control group in a sterile 'clinical' (HiMedia) container and brought to the laboratory for analysis.

One gram faeces were homogenized in 9 ml of sterile normal saline solution and serially diluted according to desired dilution by using sterile one ml pipettes. Coliform count was done in accordance with the procedures laid down in Manual in Dairy Bacteriology by ICAR sub-committee on Dairy Education (1972). Blood samples were collected directly from the heart of control and treatment groups of rats on 0, 15, 30 and 45 days of feeding experiment under the sedation of Ketamine hydrochloride (35 mg/Kg body weight; I.M). Blood samples were collected in dry test tubes and kept in slanting position for 2-3 h. Serum was separated and stored at -20^oC till estimation of Cholesterol. The total cholesterol level was estimated according to the method described by Wybenga and Pileggi method by using the diagnostic kits (span diagnostics, Surat).

Reagent1: Cholesterol Reagent.

Reagent 2: Working cholesterol standard, 200 mg%

Reagent 3: Precipitating Reagent

3 ml of Cholesterol reagent-1 was taken into blank (B), standard (S) and test (T) tubes each. Reagent-2 working cholesterol standard (200 mg %) about 0.015 ml was added into standard tube (S). 0.015 ml serum was taken into Test (T) tube by the help of micropipette.

All the tubes were mixed well and kept immediately in the boiling water bath exactly for 90 seconds, immediately cooled to room temperature under running tap water. Required sample was taken in a cuvette, and cuvette was kept on a spectrophotometer to measure the optical density at 560 nm.

$$\text{Total Cholesterol (mg/dl)} = \frac{\text{O.D Test}}{\text{O.D Std.}} \times 200$$

Where,

O. D Test = Optical Density of required sample

O. D Std = Optical Density of standard solution which was given by company span

Results and Discussion

Effects of feeding of whey protein concentrate fatless fermented milk to the experimental animal were recorded and analysed as follows:

Effect on the lactic count in faecal materials

Plain fatless fermented milk having 13% total solid was prepared by using C₁+C₂ (1:1) culture @ 1% inoculum. Then WPC added fatless fermented milk (10% TS from SMP and 3% T.S. from WPC) having 13% TS was prepared by inoculating @ 1% culture of C₁+C₂ (1:1). Rats were divided into three groups having five in each. Group – I was considered as control, Group-II and Group-III were taken as treatment group. Rat feed was fed to control group, PFFM was fed to Group-II and WFFM was fed to Group-III as treatment. Feeding experiment was continued up to forty-five days. At 0, 15, 30 & 45 days of feeding experiment, lactic count in faecal material was observed which is presented in

Table 1 and Figure 1. It can be observed that the increasing of lactic count in faecal materials of Group-II and Group-III rats were different from control group as well as from each other significantly ($P < 0.05$) at each day of experiment, where as the lactic count in control group was almost remain same or slight changed during experimentation.

Lactic count in faecal material of Group II rats was increased as compare to control group. Yadav *et al.*, (2008) reported that probiotic dahi containing *L. acidophilus* and *L. casei* significantly increased counts of Lactobacilli adherent to epithelial walls and free in the lumen of the small and large intestine. So the increment of lactic count in Group-II rats was observed. Naidu *et al.*, (1999) suggested that whey contains Lactose, Immunoglobulin, Lactoferrin, Lactoperoxidase, Glycomacropetides and Sphingolipids which survive the passage through the stomach and act as prebiotic agent. Hence by addition of WPC into fatless fermented milk, the lactose composition be increased and also the above ingredient level. By increasing of the above mentioned ingredients, which provide good source of nutrients to Lactic acid bacteria, the population and viability of lactic acid bacteria may be increased. Thus by taking of such WPC added fatless fermented milk regularly more lactic acid bacteria in vivo may be seen and thereby more number of Lactic count might be obtained in faecal materials of Group-III rats among the groups.

Effect on the coliform count in faecal materials

At 0, 15, 30, 45 days of experiments, coliform count in faecal material to corresponding group of rats was observed which is presented in Table 2 and Figure 2.

Table.1 Lactic acid bacteria count (c.f.u $\times 10^5$ / gm) in faecal materials of different groups of rat

Group	Day			
	0	15	30	45
I	78.80 \pm 5.45	84.20 \pm 3.44	82.60 \pm 2.42	89.80 \pm 2.85
II	75.80 \pm 4.29	358.00 \pm 20.83	724.00 \pm 35.30	982.00 \pm 17.15
III	79.40 \pm 5.20	500.00 \pm 28.81	888.00 \pm 24.58	1154.00 \pm 46.65

(Average of five replications)

Table.2 Coliform count (c.f.u $\times 10^2$ / gm) in faecal materials of different groups of rat

Group	Day			
	0	15	30	45
I	3860.00 \pm 169.12	4100.00 \pm 343.51	4100.00 \pm 277.49	3980.00 \pm 455.41
II	4340.00 \pm 273.13	802.00 \pm 40.67	410.00 \pm 28.98	73.80 \pm 3.89
III	4820.00 \pm 515.17	576.00 \pm 45.01	266.00 \pm 18.60	41.40 \pm 2.86

(Average of five replications)

Table.3 Serum cholesterol level (mg/dl) of different groups of Albino rat

Group	Day			
	0	15	30	45
I	72.85 \pm 0.47	76.00 \pm 0.41	79.93 \pm 0.89	86.93 \pm 2.38
II	73.88 \pm 3.50	66.71 \pm 2.46	60.49 \pm 2.28	54.08 \pm 1.90
III	82.41 \pm 3.26	67.44 \pm 2.96	56.23 \pm 1.98	44.32 \pm 1.50

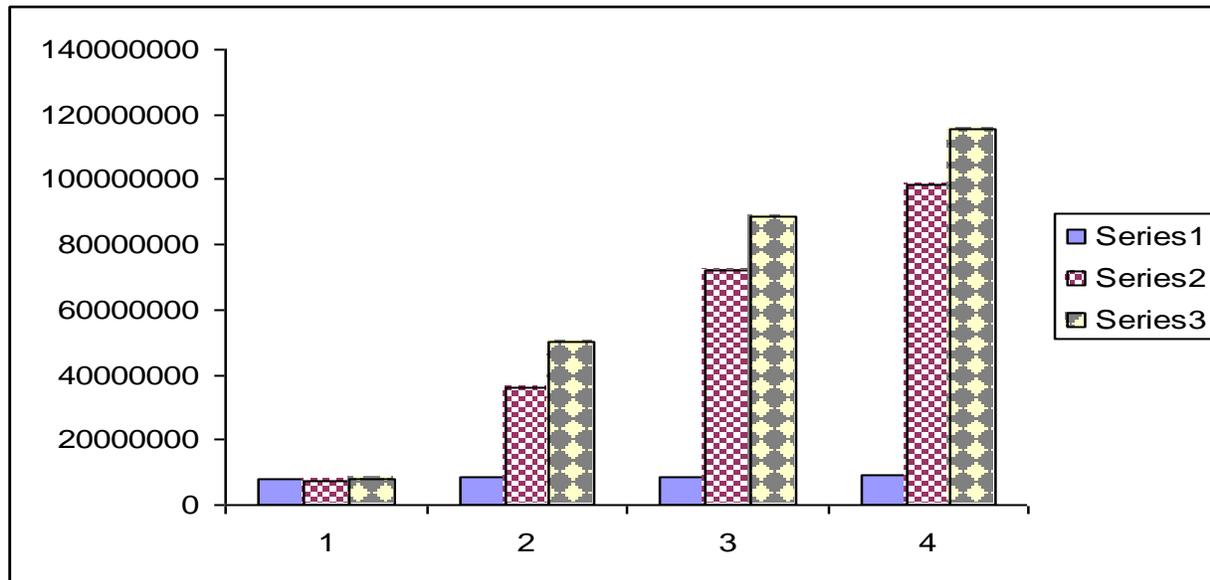
(Average of five replications)

Table.4 Body weight (gm) of different groups of Albino rat

Group	Day			
	0	15	30	45
I	137.20 \pm 0.86	157.20 \pm 1.07	178.40 \pm 1.21	199.60 \pm 1.44
II	137.60 \pm 0.87	170.80 \pm 0.86	199.40 \pm 1.50	221.80 \pm 1.16
III	137.80 \pm 0.86	180.20 \pm 1.16	211.60 \pm 1.08	240.40 \pm 1.03

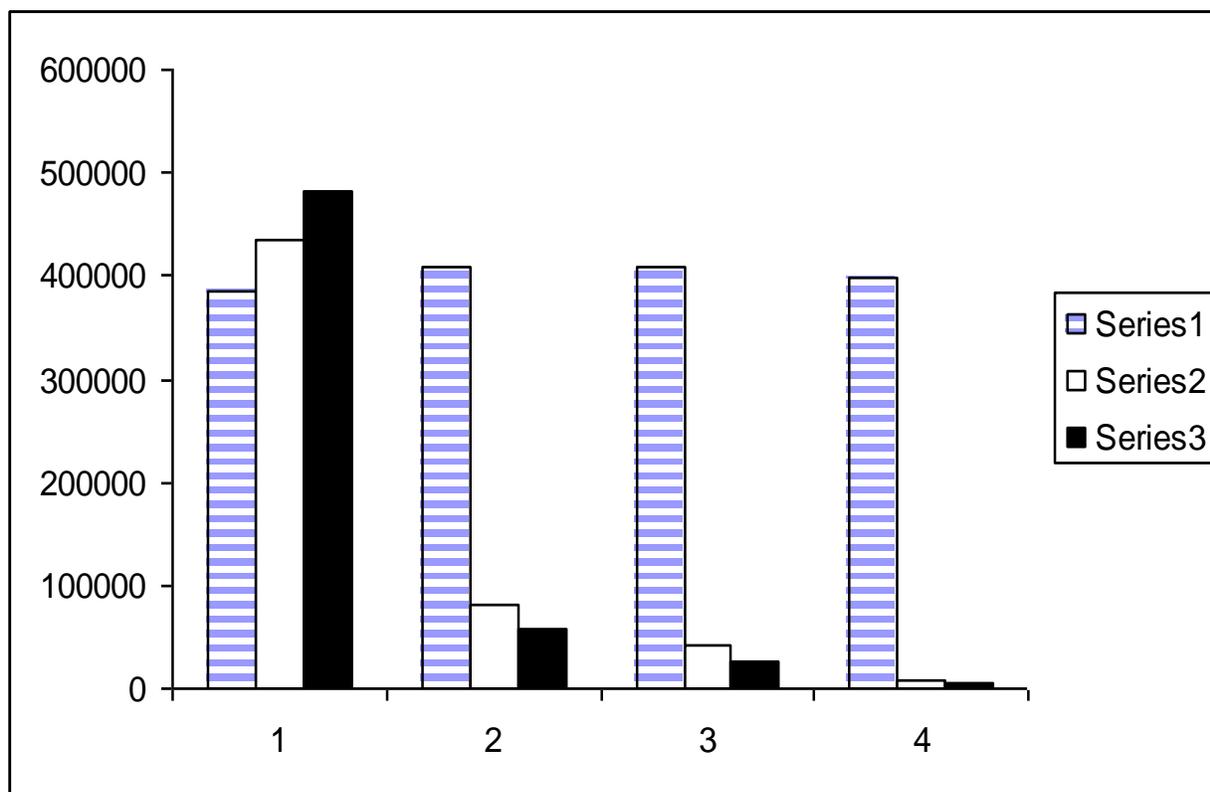
(Average of five replications)

Fig.1 Change in lactic acid bacteria count in faecal materials



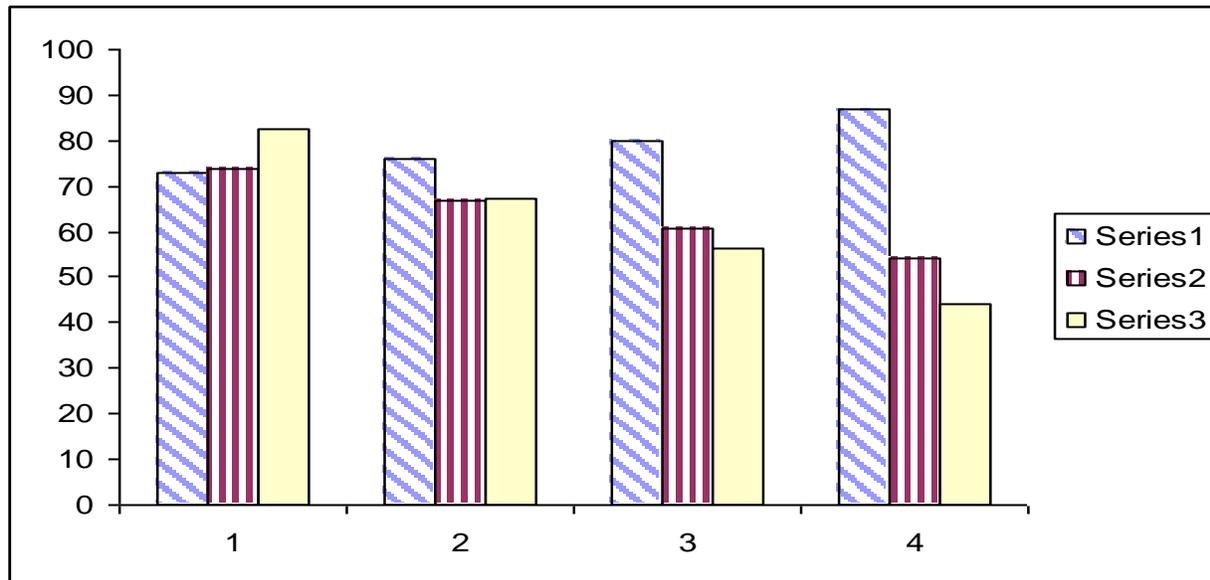
Series -1 is Group I, Series - 2 is Group II, and Series 3 is Group III
1 stands for 0 day, 2 for 15 day, 3 for 30 day and 4 for 45 day.

Fig.2 Change in coliform count in faecal materials



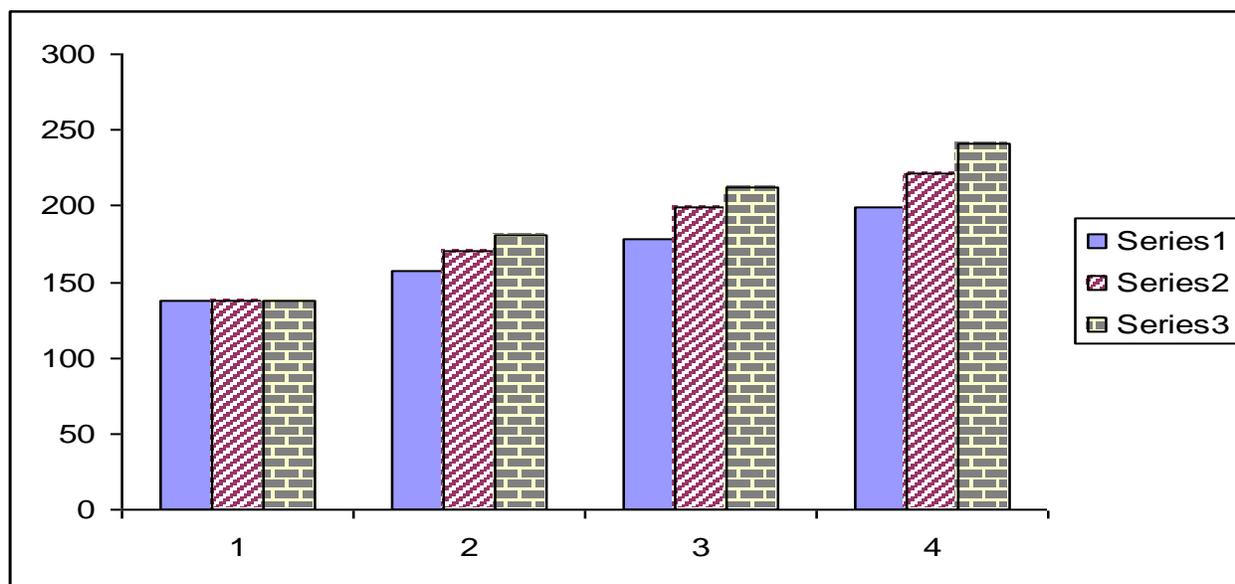
Series -1 is Group I, Series - 2 is Group II, and Series 3 is Group III
1 stands for 0 day, 2 for 15 day, 3 for 30 day and 4 for 45 day.

Fig.3 Change in serum cholesterol level



Series -1 is Group I, Series - 2 is Group II, and Series 3 is Group III
1 stands for 0 day, 2 for 15 day, 3 for 30 day and 4 for 45 day.

Fig.4 Change in body weight



Series -1 is Group I, Series - 2 is Group II, and Series 3 is Group III
1 stands for 0 day, 2 for 15 day, 3 for 30 day and 4 for 45 day.

The coliform count was declining significantly ($p < 0.05$) in faecal materials in Group-II and Group-III rats where as in control group constant or increase trend was observed. Alojz Bonma Radomie *et al.*,

(2002) suggested that production of bacteriocin like substances or competitive exclusion that involved the ability of bifidobacteria to occupy binding sites on intestinal wall inhibited the coliform or other

intestinal pathogens, thereby prevented attachment and growth of enteric pathogens competing for nutrients and stimulation of immunity. Yadav *et al.*, (2008) suggested that the number of coliforms in faeces of experimental diabetic animals was decreased after feeding of probiotic dahi. Klaenhammer (1998) found that *Lactobacillus acidophilus* produces variety of antimicrobial compounds like lactic acid (>85%), hydrogen peroxide and bacteriocins viz. Lactacin B, Acidophilucin A, Acidocin A, Acidocin 8912 and Acidocin B etc. which have the ability against pathogenic microorganisms.

So we agree with the above reasons that the coliform count in faecal material was decreased due to the antibacterial activity of such taken probiotic microorganisms. Hence the coliform count was significantly decreased in case of Group II albino rats as compare to control Group-I. Coliform count in faecal materials of Groups-III rats was decreased significantly as compare with Group II and control Group-I rats. Because according to Newburg *et al.*, (1998) whey protein concentrate has natural killer cell activity against pathogens. Thus incase of Group-III rats, coliform count in faecal materials was decreased significantly as compare to Group-II and Group-I albino rats.

Effect on the total cholesterol level of rats

At 0, 15, 30, 45 days of experiments, total cholesterol level to the corresponding group of rats were observed, which is presented in Table 3 and shown in Figure 3. It may be observed that the decreasing trend of total cholesterol level of Group II and Group III rats was significant as compare with control Group and the rate of decreasing is more in Groups-III significantly as compare to Group II at each day of experiment, whereas

cholesterol level was increased gradually incase of control group rats.

Total cholesterol level in Group II and Group III rats were decreased and significantly from each other. Kawase *et al.*, (2000) suggested that serum cholesterol might be lowered in experimental animal due to the effect of the fermentation product on cholesterol metabolizing enzyme system in liver, the promotion of excretion of cholesterol through faeces, the inhibition of cholesterol absorption by the binding of cholesterol to lactic acid bacteria cells, the promotion of excretion by the binding of bile acid to lactic acid bacterial cells and the assimilation of cholesterol by lactic acid bacteria. Cholesterol level was decreased in both treatment Groups might be because of the above said reason. But the total cholesterol level was decreased significantly more in Group III than Group II because Group III rats were fed WPC added fatless fermented milk whereas Group II rats were fed plain fatless fermented milk. As β -lactoglobulin (a component of WPC) inhibited cholesterol absorption through changes of micellar cholesterol solubility in the intestine, accompanied by an increase of faecal steroid excretion (Nagaoka, 1996). So WPC added fatless fermented milk had better effect against cholesterol level on blood serum as compare to plain fatless fermented milk fed animals.

Effect on the body weight

The data related to body weight of rats of corresponding group at 0, 15, 30, 45 days of experiments were observed and presented in Table 4 and in Figure 4. It may be observed that the body weight of all groups of rat were increased but body weight was increased more significantly in case of Groups-III rats than that of Group-II and control Group.

Body weight was more increased in Group-II and Groups- III rats than control Group because of more availability of nutrition through fatless fermented milk. Rasic (1987) found that the body weight of rats, fed with diet containing cultured dairy product was increased because of nutrient utilization enhancement associated with availability of more digestible protein as well as enhanced bio-availability of minerals, especially iron and synthesis of B-complex Vitamins, particularly folic acid. But the gaining of body weight was significantly more in Group III than Group II rats because Group III rats were fed WPC added fatless fermented milk where as Groups II rats were fed plain fatless fermented milk. According to Walzem *et al.*, (2002) whey protein content about 26% of branched chain amino acids which stimulated protein synthesis and muscle building in experimented animal. The mineral content of fat less fermented milk was increased by adding of WPC. Hence due to these reasons body weight of Group III rats was increased more than Groups II rats.

An investigation was planned and conducted for preparation of fatless fermented milk and arbitrarily add the quantity of WPC into fat less fermented milk as well as to study the effect of administration of fat less fermented milk containing whey protein concentrate to Albino rats for determining the prebiotic effect of whey protein concentrate. Two cultures named *Lactobacillus acidophilus-015*(C₁) and *Lactobacillus casei-297* (C₂) were inoculated @ 1% for preparation of fatless fermented milk having 13% TS. C₁ + C₂ with 1:1 ratio by adding whey protein concentrate 3% in such a way that the total solid remained 13% in fat less fermented milk. Lactic count was more increased in Group III rats as compare to Group II followed by control group-I rats at the end of experiment. Coliform count in faecal

material was declined more in Group III rats than Group II rats and in Group II rats the coliform count was decreased than control Group-I rats. Serum cholesterol was more decreased in Groups III rats than Group II rats and in case of Group II rat's serum cholesterol was decreased as compare to initial level of experiment whereas cholesterol level was increased in control group rats at the end of the experiment. On the basis of this experiment it can be conclude that the addition of prebiotic as WPC (Level = 3%) to probiotic for the preparation of fatless fermented milk has remarkable increase in the lactic count, decrease in coliform count in faeces, decrease cholesterol in blood serum and dramatically increase in the body weight of rats. Therefore it can be recommended to use of WPC as prebiotic in the preparation of fat less fermented milk products for maintaining the bacterial environment of intestine and controlling blood cholesterol level.

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