Changes of Serum Lipids and Proteins during Probiotics Feeding and Its Exposure

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Abstract- The aim of this study was to investigate the effect of L. bulgaricus and S. thermophilus genera on serum cholesterol and triglyceride lowering potential of probiotic in guinea pigs and the changing pattern of above serum parameters after termination of the treatment. The effects of oral supplementation of probiotics to animals have been carried out in many occasions but their outcome after the termination of treatment is lacking. Twenty four eight weeks old guinea pigs were selected randomly divided into two groups (n=12)) the test and the control, were reared in six cages (four per cage). Both groups received control mash diet (based on corn and soybean) during the experiment, to meet the nutrient requirements. A 5 ml of aqueous medium culture of L. bulgaricus and S. thermophilus with 1.76 x 109 and 1.5 x 108 colony forming units/ml, respectively were given to test animals for fourteen days and the control animals were given the same volume of distilled water. Blood samples were withdrawn by heart puncture at 14 and 21 days after the initiation of probiotic supplementation and serum total cholesterol, triglycerides, HDL cholesterol and total protein levels were assayed in both groups. The total cholesterol level of the test group was significantly lower ($p<0.01$) than the control group after 2 weeks and it increased slightly after the termination of probiotic supplementation. HDL cholesterol level was significantly higher in test animals after 3 weeks ($p<0.05$). There were no significant differences ($p>0.05$) in serum triglycerides, total protein, weight gain, body temperature, food and water intakes and body temperature between the control and treated groups. It was concluded that live probiotics significantly reduced the serum total cholesterol during the treatment period and increased the HDL cholesterol significantly following termination of the treatment.

It is suggested that the feeding of probiotic does not affect food intake and bring up the metabolic changes during the treatment period and the changes reversibly return normal following termination of treatment but there is significant persisting effect for serum HDL cholesterol. However further studies are required to gain insights into the understanding the treatment of probiotics with time interval in order to economize the beneficial effects.

Index Terms- Probiotics, Serum cholesterol, Serum HDL, Serum triglycerides L. bulgaricus and S. thermophilus

I. INTRODUCTION

Probiotics are live microbial supplements when administered in adequate amounts, confer a beneficial effect on the health of the host by improving its intestinal microbial balance (FAO/WHO, 2001; Fuller, 1989). The control of serum cholesterol and triglyceride levels by using probiotics has been well recognized (Lin et al. 1989; Taranto et al. 1998 ; Kalavathy *et al*., 2006). The use of probiotics has only acquired scientific recognition in recent years although their applications as functional foods have been well-established throughout generations. Among the numerous intestinal microbes, selected as probiotics, include species of the genera Lactobacillus, Bifidobacterium, and *S. thermophilus* with many health beneficial effects (Harish and Varghese, 2006). In the interest of their promising effects on health and well being, probiotics has become increasingly recognized as supplements for human consumption.

It is known that elevated levels of total blood cholesterol or other blood lipids such as triglycerides are the main component could be considered as risk factors for developing many health problems of human including coronary heart diseases (Lim et al., 2004). People affected with hypercholesterolemia may avert the use of cholesterol-lowering drugs, because of the expense of drug therapy and the unwanted side effects.

Studies examining the efficacy of hypocholosteremic effect of probiotics often do not sufficiently address the optimum dose, frequency, duration of treatment and the effect of post exposure. Many studies have used hamsters (Lin *et al*, 2004), guinea pigs (Madsen *et al* , 2007) and pigs (Patterson *et al*, 2008) as models due to their similarities with humans in terms of cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of hepatic cholesterol enzymes (Fernandez *et al*, 2000). Although probiotics have been delivered in the range of 10^7 to 10^{11} CFU/day in humans (Naruszewicz *et al*., 2002) and 10^7 to 10^9 CFU/day in animals (Lubbadeh *et al* , 1999), some probiotics have been shown to be efficacious at lower levels, while some require a substantially higher amount to exert a hypocholesterolemic effect.

Higher dosage may not necessarily translate to better effects on cholesterol, as compared to lower dosage. Strains of probiotics have also been found to exhibit antibiotic resistance and have raised concerns about horizontal resistant gene transfer to the host and the pool of gastrointestinal pathogenic microflora (Huys et al., 2006). Considering these facts, the safety

verification of probiotics with respect to the effect of post exposure has to be studied extensively.

Although the hypocholesterolemic potential of probiotics has been widely studied, an accurate dosage of administration and efficacy after termination of the treatment yet to be established. The objectives of this study were to determine the effect of probiotic containing *L. bulgaricus* and *S. thermophilus* bacteria on serum lipid profiles, total protein concentration, weight gain of guinea pigs and their changing pattern of above parameters after termination the treatment.

II. Materials and Methods

The experiment was conducted at the Faculty of Medicine, University of Peradeniya, during February to June 2011.

2.1 Subjects

A total of twenty four, eight weeks old female guinea pigs (*Cavia porcellus*) (350 g±10), were obtained from Medical Research Institute, Colombo with a good reputation of producing disease free laboratory animals. They were randomly divided into two groups (Test and control n=12). Each group had six replicate pens were randomized with respect to the treatment. They were reared under sanitary conditions and fed for two weeks on rations based on corn and soybean formulated to meet the nutrient requirement of guinea pigs (National Research council,1994). Feed and water were provided *ad libitum* . Ethical approval for the study was obtained from the local authority and the animal experiment was carried out according to the International Guiding Principles for Biomedical Research Involving Animals (Council for the International Organizations of Medical Sciences 1985).

2.2 Probiotic culture for colony counting

L. .bulgaricus and *S. thermophilus*, the two probiotic bacteria used in this study were obtained from MILCO Private Ltd (YO-MIXTM621 bulk freeze dried yoghurt culture manufactured in Germany. "Pour plate method" was used to measure the number of colony forming units in the initial highly concentrated culture, Pour plates were prepared using MRS (de Man, Rogosa, Sharpe) and NRC (Neutral Red Chalk) agar and serial dilutions of organisms from 10^{-1} to 10^{-9} were used. Plates were incubated at 42˚C for 24 hours. *Lactobacillus* colonies appeared as white patches in MRS agar and *Streptococcus* colonies appeared as reddish pink patches in NRC agar. The number of colonies in each plate was counted and the number of colony forming units in the initial probiotic culture was calculated.

2.3 Treatment

Following an adaptation period of one month, 5 ml of probiotic solution contained *L.bulgaricus* and *S.thermophilus* with 1.76 x 10^9 and 1.5 x 10^8 colony forming units / ml respectively was given to each test animal orally for two weeks . Animals in the control group were given the same volume of distilled water. The body weights and body temperatures were recorded daily throughout the experimental period.

2.4 Biochemical parameters

After termination of probiotic supplementation at 14 days, three milliliters of blood was collected from each animal through heart puncture following anesthetizing animals with chloroform. They were kept fasting for 12 hours before the collection of blood. Samples were centrifuged for 10 min at 2000 rpm, sera was separated and were stored at -20°C until assayed. Second bleeding was carried out one week after termination of the treatment as described above and serum was stored.

2.5 Chemical analysis

Serum total cholesterol, triglyceride and HDL cholesterol levels were measured using commercial diagnostic kits (Randox Laboratories Ltd, Ardmore, Diamond Road, Crumin, co. Anrtim, United Kingdom). The total protein in serum was estimated with commercial kits from Biolabo Reagents, Maizy, France.

2.6 Data Analysis

Data were analyzed using the statistical package GenStat Discovery Edition 5 (VSN International Ltd., Hemel Hempstead, U.K.). Statistically significant differences between group means were determined by analysis of variance (ANOVA). Mean values were considered significantly different at $P < 0.05$.

III. Results

3.1 Total cholesterol concentration

Table 1 gives the mean total cholesterol concentration in serum samples at 2 weeks and 3 weeks after probiotic feeding were T1,T2 and C1 and C2 for control respectively. T_1 group had a significantly lower serum total cholesterol level (about 30%) than the control group (C_1) ($p<0.05$). However, in T2 there wasn't a significant difference between the control and the test. However, one week after termination of probiotic supplementation, the level of total cholesterol in control (C_2) guinea pigs was quite close to that of treatment group (T_2).

3.2 Serum triglyceride concentration

The mean triglyceride concentration in both T1 and T2 were lower than the control groups although the changes were not statistically significant ($p>0.05$) (Table 1). Eventhough, there is a tendency to increase the mean triglyceride levels with aging, there was a non- significant decline ($p>0.05$) with probiotic feeding. At 2 weeks (T1) it was a significantly lower than the control group (C1), whereas there was no significant difference ($p>0.05$) among C2 and T2.

3.3 DL Cholesterol concentration in serum

The mean HDL cholesterol levels in test group (T_1) and control group (C_1) were quite closer to each other two weeks after initial probiotic introduction. However, HDL cholesterol level of T2 test group animals one week after termination of probiotic supplementation (T_2) was significantly higher than the control group C2 ($p<0.05$) .

3.4 Total protein concentration in serum

The total protein concentrations in serum after two weeks of probiotic feeding and one week after termination of probiotic feeding are shown in Table 1. Probiotics feeding had not altered

the serum concentration of protein to a significant level at two weeks after probiotic treatment. However after termination of live micro organisms feeding, total protein level of test group (T_2) was appreciably higher than control group (C_2) even though the changes were not statistically significant.

3.5 Feed and water intake, body temperature and body weight gain Probiotics did not have a significant influence on either the weight gain or the body temperatures of guinea pigs. Food intake, water intake were also not significantly affected by probiotic supplementation.

IV. Discussion

The test group (T_1) had a significantly low level of serum total cholesterol compared to the control group (C_1) at 2 weeks of probiotic supplementation. However, one week after termination of probiotic supplementation, there wasn't a significant difference between the control (C2) and the test (T2). Cholesterol lowering effect of probiotic bacteria is in agreement with the studies conducted on bottle fed babies (Harrison and Peat, 1975), (Grunewald, 1982), in pigs (Gilliland, *et al*, 1985), rats (Pulusani and Rao,1983) and hens (Tortuero and Brenes, 1975). However reason for the cholesterol lowering action of these bacteria still remains to be clarified. Mechanisms for the cholesterol lowering effect of *L.acidophilus* group (Gilliland, *et al*, 1985, Ra˘si´c *et al* 1992, Noh et al,1997) and *Bifidobacteria* (Tahri *et al* ,1992) had been discussed. The co-precipitation with deconjugated bile salts in an acidic environment by interfering absorption from the intestinal lumen (Klaver and Van-der-Meer, 1993, De-Rodas *et al*, 1996) is also considered. Thus, increased excretion of bile acids should result in lowered serum concentrations, which in turn would decrease the enterohepatic circulation.

According to the results of this study, decrease in serum cholesterol was limited to the time of probiotic bacteria feeding. The increase level of total cholesterol after termination of the treatment could be explained as increased *de novo* synthesis of cholesterol in liver in order to replace the excreted bile acids. This could be due to inability of the particular strain of probiotic bacteria to attach permanently to the gut wall and hence continuous supply might be necessary to exert the effects.

There was no significant difference in serum triglyceride concentrations between test and control groups. But the probiotic feeding has an influence on lipid metabolism of animals to decrease serum triglyceride. The feeding period of two weeks may not be sufficient to give a significant change of serum triglyceride concentration. Sometimes *L. bulgaricus* and *S. thermophilus* may not have an direct influence on serum triglyceride, but effect may be indirectly through cholesterol metabolism.

HDL cholesterol concentration of treatment (T1) and control groups (C1) were not significantly different. But, 3 weeks after initial probiotic feeding (T2) HDL cholesterol concentration was significantly higher than the control group (C2). This is one of the key findings of this study. This result is in agreement with certain studies conducted on rats and humans (Usman and Hosono, 2000; Keim, et al., 1981). HDL is also known as friendly cholesterol, which has been increased considerably and hence, will be an important contributor for reducing the risk of heart disease.These animals also share an almost similar digestive anatomy and physiology, nutrient requirements, bioavailability and absorption, and metabolic processes with humans, making them useful experimental models for research applications (Patterson *et al.*, 2008). Hence, the positive hypocholesterolemic effects shown in animal studies suggest a similar potential in humans.

Since the probiotic feeding did not influence the feed intake of the subjects, there had no effect on serum protein and this finding is in agreement with work reported earlier (Fernandez, 2001).

Probiotic supplementation had no influence on feed and water intake, body temperature and body weight gain of the guinea pigs. This indicate that for changes of serum cholesterol levels are not related to their feed in take and it is exclusively for the probiotic supplementation. This fact can be further confirmed by normal weight gain of the animals in the treated group.

V. Conclusion

Live probiotics significantly reduced the serum total cholesterol and increased the HDL cholesterol of guinea pigs during the treatment period. But there was no influence on serum protein, body weight gain, feed and water intake with two weeks treatment period. To the best our knowledge, this is the first study which the effects of probiotic feeding is compared after termination of treatment. The results of this study suggests that the effect of probiotic is independent from the food intake and the live organisms bring up the metabolic changes only during the treatment period and all the changes reversibly return normal following termination of treatment. However further studies are required to gain insights into the understanding the treatment of probiotics with time interval in order to achieve the maximum beneficial effect with minimum cumbersome.

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Table 1- Effects of feeding probiotics on serum cholesterol ,triglyceride, HDL cholesterol, protein (mg/dl) in guinea pigs

	Cholesterol		Triglyceride		HDL cholesterol		Protein	
	2wk	3 wk	2 wk	3 wk	2 wk	3 wk	2 wk	3 wk
Control	52.4	51.2	57.6	77.1	5.05	5.09	5.38	5.52
Test	36.8	50.8	34.2	49.9	7.96	8.85	5.43	6.01
Standard error	4.12	8.85	14.65	14.06	1.44	1.31	0.32	0.27
F value	0.004	0.486	0.146	0.256	0.547	0.002	0.874	0.12

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