

## Effect of Bioyogurt Consumption on Fatty Metabolites of Serum and Colonic Microflora in Healthy Subjects

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### ABSTRACT

Probiotic products highly affect the health of consumers by reducing the risk of heart attacks and improving the desirable microflora in the intestinal tract. In this study the effect of yogurt (500g/d for 3 wk) enriched with either *Bifidobacterium bifidum* or *Lactobacillus acidophilus* on the level of cholesterol, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) of serum and as well on the intestinal microflora were investigated in 24 healthy volunteers. Statistical analysis indicated that lower levels of cholesterol and HDL were found after consumption of either one of bio-yogurts as compared to the control states ( $P<0.05$ ), whereas changes of LDL were not significant. Also, in comparison with control periods the results of microbial counts indicated that the number of fecal coliforms excreted during the consumption of either one of the yogurt types were not noticeable, while the number of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* increased significantly ( $P<0.05$ ).

**Keywords:** Cholesterol, High Density Lipoprotein (HDL), Intestinal micro flora, Low Density Lipoprotein (LDL), Probiotic yogurt,

### INTRODUCTION

Nowadays it is in general recognized that an optimum balance in microbial population in one's digestive tract is associated with sound nutrition and good health (Lourens-Hattingh *et al.*, 2001). Bacterial population in the large intestine is very high reaching maximum counts of  $10^{12}$  cfu/g and consisting of both beneficial and harmful species. Beneficial species play an efficacy role in production of vitamins, organic acids and bacteriocins and as well inhibit some of some the diseases, while harmful ones produce toxins and carcinogen substances (Kailasapathy *et.al* 2008). Increasing evidence indicates that consumption of probiotic microorganisms can help maintain such favorable microbial profile and result in several therapeutic benefits (Fernades *et al.*, 1987; Lourens-Hattingh *et al.*, 2001).

Adequate number of viable cells, namely the "therapeutic minimum" needs to be consumed regularly for transferring probiotic effects to consumers (Lourens-Hattingh *et al.*, 2001).

One of the most popular dairy products used as carrier for viable *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cells is bio-yogurt.

*Bifidobacteria* are the predominant flora in breast-fed infants representing 99% of the fecal microbial flora. *Lactococci*, *Enterococci* and Coliforms represent less than 1% of the intestinal population, with *Bacteriodes*, *Clostridia* and other organisms being normally absent. With weaning and ageing of the infant, gradual changes in the intestinal flora profile occur. The proportion of bifidobacteria, lactobacilli and other beneficial bacteria declines significantly, while harmful bacterial counts increase.

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However, such good nutrition and dietary regimes as the consumption of bio-yogurt can maintain desirable microbial balance in the large intestine (Shakeri, 2003; Lourens-Hattingh *et al.*, 2001).

There are claims that a consumption of probiotic products significantly reduces serum cholesterol (Fernades *et al.*, 1987; Lourens-Hattingh *et al.*, 2001; Shakeri, 2003; Bartram *et al.*, 1994). Several interrelationships between increase in serum cholesterol levels and heart attacks are demonstrated (Dosti, 1998; Siunat, 1999).

Fat metabolism results in the production of some four substances namely: total cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and triglycerides. An excess of in the above substances (more than normal limit) causes development of heart diseases as a primary event. Cholesterol accumulated in blood vascular walls causes their constriction. LDL intensifies it but HDL plays an important role in cholesterol removal and transfer (Amini *et al.*, 2004; Dosti, 1998; Siunat, 1999; Zarate *et al.*, 2002). Differences between HDL and LDL are in their goal cell. In fact, LDL carries cholesterol from liver to peripheral cells of the body, while, HDL carries excessive cholesterol from peripheral cells to liver changing it into bile. Since changing cholesterol to bile is the main means of its removal, HDL reduces serum cholesterol levels (Dosti, 1998). Claims are strong that certain *L.acidophilus* strains and some *bifidobacteria* species are able to lower cholesterol levels within the intestine. Cholesterol co-precipitates with deconjugated bile salts with the decline in pH as a consequence of lactic acid formation by the lactic acid bacteria. Another theory is that *L.acidophilus* deconjugates bile acids into free ones, which are excreted more rapidly from the intestinal tract than the conjugated bile acids (Shakeri, 2003; Fernades *et al.*, 1987; Lourens-Hattingh *et al.*, 2001). Some scientists suggest that propionic acids produced by probiotic species reduce cholesterol synthesis in liver

(Zarate *et al.*, 2002). In addition, it is reported that an active factor i.e. Hydroxyl Methyl Glutarate (HMG) synthesized by lactic acid bacteria, inhibits cholesterol synthesis in the body (Fernades *et al.*, 1987; Lourens-Hattingh *et al.*, 2001; Shakeri, 2003).

Thus, in this study, considering the importance of *L.acidophilus* and *B.bifidum* as probiotic species endowed with potential therapeutic properties, the effects of these probiotic cultures on human LDL, HDL and cholesterol levels and as well on colonic microbial flora are investigated.

## MATERIALS AND METHODS

### Yogurt Preparation

All products were weekly produced under sanitary food preparation conditions at the Padratooos Factory of Ghouchan (Khorasan, Iran). The products were produced using pasteurized milk. The yogurt was added with the cultures of *B.bifidum* (yogurt B, BB-12, chr. Hansen Co., Denmark) and *L.acidophilus* (yogurt L, LA-5, chr. Hansen Co., Denmark). All products were

fermented at 37°C to a final pH of 4.6. The fresh product batches contained viable bacteria counts exceeding 10<sup>9</sup> colony forming units (cfu)/l. Freshly prepared products were dispensed into 500 g-cups, packaged and stored at 7°C prior to daily delivery to the experimental subjects.

### Subjects

Twenty four volunteers in good health (fifteen female, nine male; aged 20-30 y, average 23.5 y) participated in the study. Selection was carried out through completing forms as well as through a pre-study of colonic microbial counts along with and routine blood test, to ensure health of volunteers. During three week periods the subjects received 500 ml of yogurt drink per day (yogurt B and L) in a randomized order.

In addition to the yogurts taken the volunteers kept their usual dietary habits. Additionally, each volunteer kept a record of the occurrence of any abdominal discomfort during the study periods.

### Measurements

At the end of each week test period (yogurt B and L), feces was collected and bacteriological examination done within 8h after sampling. Fecal samples were diluted in physiological serum in  $1:10^4$  ratios. Out of each of these dilutions 0.1 ml was plated onto the following media: Eosin methylen blue agar (Merck, Darmstact, Germany) for *Escherichia coli*, Salmonella Shigella Agar (Merck, Darmstact, Germany) for fecal coliforms and de Man Rogoso & Sharp Agar (Merck, Darmstact, Germany) for *Bifidobacteria* and *Lactobacilli*. Bacterial colonies were enumerated and identified according to their morphology, growth characteristics and the usual biochemical criteria.

Also before consumption of yogurt (B and L) and at the end of each week of test period, blood tests including HDL, LDL and cholesterol determination were carried out on the volunteers. These experiments were done by courtesy of the Medical Diagnosis Laboratory (Doctor Bigdeli, Mashhad, Iran).

### Statistical Analysis

The results obtained during test periods were compared with data gathered before yogurt (B and L) consumption (control

status) individually. Kruskal- Wallis one way ANOVA and Dunnet's method were used to compare cholesterol, LDL and HDL as well as microbial counts in EMB, SSA and MRS media. Values are expressed as mean + SEM (Standard Error of Mean). Also culture kinds, period of consumption and in between interaction effects were analyzed through CRP two way ANOVA.

## RESULTS AND DISCUSSION

### Cholesterol, LDL and HDL

The results of blood tests obtained during the test period and the corresponding control data are summarized in Table 1. As observed, serum cholesterol levels gradually declined during the test period so that at the end of the third week, there were significant differences ( $P<0.05$ ) in cholesterol levels in consumers consuming the L-yogurt vs. control. No significant difference was found for the serum cholesterol levels of consumers consuming the B-yogurt.

As evident from Table 1, both probiotic species were able to reduce cholesterol levels in volunteers, but *L.acidophilus* was more effective than *B.bifidum* as also confirmed by results obtained by other scientists (Lourens-Hattingh *et al.*, 2001). Mean comparisons showed *L.acidophilus* lowered cholesterol levels at 13.5 percent, while *B.bifidum* reduced it at 4.47%. Since viable cells consist of possible therapeutic effects, then they must tolerate digestive tract to benefit the colon. James W. Anderson *et.al* (1999) reported that serum

**Table 1.** Fat metabolite levels in yogurt L and B consumers ( $\text{mg ml}^{-1}$ )<sup>a</sup>

Source	Blood test	Control	First week	Second week	Third week
yogurt L	Cholesterol	189.000 $\pm$ 7.69	187.000 $\pm$ 8.44	174.700 $\pm$ 5.36	163.500 $\pm$ 4.31
	LDL	122.200 $\pm$ 6.43	124.800 $\pm$ 6.70	112.500 $\pm$ 4.25	109.100 $\pm$ 3.02
	HDL	38.800 $\pm$ 1.13	36.100 $\pm$ 1.43	37.800 $\pm$ 1.70	33.800 $\pm$ 1.08
yogurt B	Cholesterol	172.200 $\pm$ 11.39	175.200 $\pm$ 9.18	168.700 $\pm$ 6.32	164.500 $\pm$ 4.58
	LDL	108.500 $\pm$ 6.20	115.500 $\pm$ 5.21	109.500 $\pm$ 3.85	105.800 $\pm$ 3.71
	HDL	37.100 $\pm$ 1.91	34.200 $\pm$ 1.04	34.400 $\pm$ 1.13	35.700 $\pm$ 1.22

<sup>a</sup> Mean $\pm$  SEM

**Table 2.** Results of analysis of variance of cholesterol.

Source	DF	SS	MS	F	P
Yogurt kind	1	1411.200	1411.200	2.4938	0.1187
Consumption period	3	3975.400	1325.133	2.3417	0.0803
Interaction effect between them	3	881.200	293.733	0.5191	-

cholesterol concentrations in the groups consumed fermented milk enriched by *L.acidophilus* ATCC FM decreased significantly by 2.4% following treatment.

*Bifidobacteria* in particular usually exhibits weak growth and requires an anaerobic environment, a low redox potential and the addition of bifidogenic factors to achieve the desired levels of growth, furthermore, it is reported that *L.acidophilus* is also more tolerant to acidic conditions than *B.bifidum* (Lourens-Hattingh *et al.*, 2001; Martin-Diana *et al.*, 2003). For these reasons a number of viable cells of *L.acidophilus* are more and more effective than *B.bifidum* in colon. Results of analysis of variance (Table 2) shows that bio-yogurt consumption period significantly affected the reduction of cholesterol levels ( $P<0.08$ ).

There was no interaction effect observed between bio-yogurt kind (B and L) and consumption period on reduction of cholesterol levels.

The result of HDL determination (Table 1) showed that high density lipoprotein levels, in comparison with control, were lowered during yogurt consumption (B & L). But at the end of the third week, it was not significant in yogurt B while serum HDL levels were significantly lowered at that time ( $P<0.05$ ). These results are confirmed by other scientists (James W. Anderson *et al.* 1999). They described that serum HDL-cholesterol values decreased significantly in hypercholesterolemic groups (3.9% in the *L.acidophilus* L1 FM group and 5.9% in *L.acidophilus* ATCC FM group).

Also, statistical analysis of LDL showed

that there was no noticeable difference in the average LDL during yogurt B and L consumption, while yogurt L affected the LDL reduction a little more than yogurt B. The results obtained by James W. Anderson *et al.* (1999) also showed the overall serum LDL-cholesterol values after treatments to be 2.6% and 1.1% lower than those of the baseline in *L.acidophilus* L1 and *L.acidophilus* ATCC 43211 hypercholesterolemic group, respectively, but they were not significant. However, LDL-cholesterol concentrations in group L1 FM decreased significantly after receiving yogurt for a period of 2 weeks. Bartram *et al.* (1994) showed that fecal concentration of Short Chain Fatty Acids (SCFAs) did not differ during the period of consumption of bio-yogurt enriched by *B.longum* containing lactulose as a bifidogenic factor. They demonstrated that the unchanged spectrum of SCFA excretion may be the result of differences in SCFA absorption from the colonic lumen. As mentioned, LDL is undesirable because of constipation on blood vascular walls and accelerating state of being affected to atherosclerosis. In contrast, HDL carries LDL and fouling of vessel wall to liver in order to degrade and remove cholesterol synthesis. The LDL and HDL levels must be kept low and high respectively (Amini *et al.*, 2004; Dosti, 1998; Siunat, 1999). Thus, serum lowering of HDL levels through probiotic culture is not desirable.

The results of analysis of variance of LDL and HDL (Tables 3& 4) also revealed that consumption of yogurt B and L did not noticeably affect LDL and HDL levels.

**Table 3.** Results of analysis of variance of HDL

Source	DF	SS	MS	F	P
Yogurt kind	1	32.512	32.512	1.7486	0.1902
Consumption period	3	121.938	40.646	2.1861	0.0971
Interaction effect between them	3	75.838	25.279	1.3596	-

**Table 4.** Results of analysis of variance of LDL

Source	DF	SS	MS	F	P
Yogurt kind	1	1073.113	1073.113	4.1358	0.0457
Consumption period	3	1809.938	603.313	2.3252	0.0820
Interaction effect between them	3	397.238	132.413	0.5103	-

It is necessary to note that yogurt containing viable *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* can lower cholesterol too, but they, because of being very sensitive, could not tolerate the digestive tract (Fernades *et al.*, 1987; Vinderola *et al.*, 2002). Synergistic cholesterol reducing effects between probiotic and traditional culture are known to be occurring. Along with both means of yogurt preparation other bacterial species (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) that might have acted as bifidogenic factors on the resident colonic flora were chronically transferred to the subjects (Bartram *et al.*, 1994).

Abdominal discomforts and drowsiness were rarely (and only by a few volunteers) reported throughout the study, while on the contrary some volunteers reported even an improved function of their digestive system.

### Microbial counts

The results of microbial counts of fecal samples of volunteers during study period and the control are summarized in Table 5. As shown there was no difference in the average EMB medium colony counts during yogurt B and L consumption and control as well as during SSA medium colony counts. It means bio-yogurt consumption did not significantly affect fecal coliform and particularly *E.coli*.

Microbial cultures in SSA medium were

carried out to enumerate other fecal coliforms (red colonies), since yellow colonies in SSA medium indicate *Salmonella* and *Shigella* presence, which show microbial infection of intestine (Saeidi Asl, 1999). No yellow colonies were observed in fecal samples of the volunteers. Bartram *et.al* (1994) showed that the bacterial counts for *Escherchia coli* and *Clostridia* were also not different during consumption of yogurt enriched by *B.longum* in duration of 21 days. Their results revealed a great stability of the human fecal microflora (Bartram *et al.*, 1994). There are some reports which demonstrate infant diarrhea, caused by *E.coli* activity, which was cured through acidophilus milk consumption (Fernades *et al.*, 1987). Since, some researches have demonstrated that beneficial bacteria can influence the number of harmful bacteria of colon (Lourens-Hattingh *et al.*, 2001), so, microbial flora of colon are mainly affected by dietary, age and antagonism as well as synergism effects among colonic bacteria (Shakeri, 2003; Lourens-Hattingh *et al.*, 2001).

The results of MRS medium enumeration showed that in comparison with the corresponding control statuses, a more significant number of *Bifidobacteria* and *Lactobacilli* were excreted following the consumption of either of the yogurt preparation, ( $P<0.05$ ). Uyeno *et.al* (2008) have suggested that the human fecal bacterial community could be altered by ingesting yogurt, even though probiotic lactobacilli are present or absent in the yogurt, meaning that this does not seem to be

**Table 5.** Cultural counts of fecal bacteria (log cfu/g fecal) <sup>a</sup>.

Media	Control B	Yogurt B	Control L	Yogurt L
EMB	8.909±0.13	8.949±0.14	8.910±0.10	8.636±0.12
SSA	8.310±0.18	7.820±0.37	8.400±0.28	7.958±0.29
MRS	1.960±0.92	5.467±0.81	2.397±0.93	5.547±0.82

<sup>a</sup> ± SEM; n=24



a factor affecting the change. Mattila-Sandholm (1999) has emphasized that probiotic cultures play a main role in a healthy and large intestine balance. If beneficial species tolerate digestive tract and maintain their viability, they can act therapeutically and improve metabolic activity of intestine (Shakeri, 2003; Zarate et al., 2002). Vinderola et al. (2002) showed that produced substances by such dairy starters as diacetyl, acetoin, bacteriocins can inhibit growth of some dairy microbial species. It has been reported that *L.acidophilus* survives far better than the traditional yogurt culture organisms, *Lactobacillus delbrueckii subsp. bulgaricus* and *S.thermophilus* in yogurt under acidic conditions (Mortazavi et al., 1997; Vinderola et al., 2002). It was also found that *L.acidophilus* is also more tolerant to acidic conditions than *B.bifidum*. The pH of fermented milk can influence the resident cells. It was also found that *L.acidophilus* and *B.bifidum* showed satisfactory populations at pH values above 4. But this is not true regarding yogurt traditional cultures (Mortazavi et al., 1996; Zarate et al., 2002).

In conclusion, the result of this study demonstrated that regular probiotic yogurt consumption generally exerts positive effects on the lowering of fat metabolites in particular cholesterol. Favorable effects of yogurt enriched by *L.acidophilus* were more noticeable than those of *B.bifidum*. Also revealed was a great stability of the human fecal microflora to this kind of dietary change. In all, regular probiotic yogurt consumption can increase the number of beneficial species present in colon and prosper their positive effects. In order to enhance the results, obtained in this project, further extended studies in hypercholesterolemic subjects or patients with intestinal disturbances are needed.

#### ACKNOWLEDGEMENT

We thank Zohorian (Padratoos Factory) for providing the yogurt preparations, Bigdeli (Diagnosis Laboratory), and

Tavasoli (Microbiology Laboratory, College of Agriculture, Ferdowsi University) for providing the needed materials and equipment.

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### اثر مصرف ماست پروبیوتیک بر متابولیت های چربی سرم و فلور میکروبی روده در افراد سالم

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#### چکیده

فرآورده های پروبیوتیک اثر زیادی بر سلامت مصرف کنندگان از طریق کاهش خطر حمله قلبی و بهبود فلور میکروبی مطلوب در مسیر روده ای دارد. در این مطالعه ماست ۵۰۰ گرم در روز به مدت ۳ هفته (غنی شده با بیفیدوباکتریوم بیفیدوم یا لاکتوباسیلوس اسیدوفیلوس بر مقدار کلسترول، لیپوپروتئین با دانسیته پایین (LDL) و لیپوپروتئین با دانسیته بالا (HDL) سرم و فلور میکروبی روده ای در ۲۴ داوطلب سالم بررسی شد. تجزیه آماری نشان داد که بعد مصرف هر دو نوع ماست پروبیوتیک مقدار کلسترول و HDL در مقایسه با شاهد کمتر بود. ( $P > 0.05$ ) در حالی که تغییر در LDL معنی دار نبود. همچنین در مقایسه با شاهد نتایج شمارش میکروبی نشان داد که تعداد کلیفرم های مدفوعی در مدت مصرف ماست قابل توجه نبود. این در حالی است که تعداد بیفیدوباکتریوم بیفیدوم و لاکتوباسیلوس اسیدوفیلوس به طور معنی داری افزایش یافت. ( $P > 0.05$ )