Influence of Yogurt and Acidophilus Yogurt on Serum Cholesterol Levels in Mice

A. SIBEL AKALIN,* SIDDIK GÖNÇ,* and SELMIN DÜZEL[†]
*Department of Dairy Technology, Faculty of Agriculture,
University of Aegean, Bornova-Izmir, Turkey 35100
[†]Ministry of Agriculture and Rural Affairs,
Veterinary Control and Research Institute,
Bornova-Izmir, Turkey 35100

ABSTRACT

The effects of yogurt and acidophilus yogurt on the weight gain, serum cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, and the numbers of fecal lactobacilli and coliforms were investigated in mice assigned to three dietary treatments for 56 d: 1) commercial rodent chow and water (control), 2) commercial rodent chow and yogurt made from milk inoculated with a 3% (vol/vol) liquid culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (yogurt), and 3) commercial rodent chow plus yogurt made from milk inoculated with a 0.01% (wt/vol) freeze-dried culture of *Streptococcus thermophilus* plus *Lactobacillus acidophilus*. The weight gains of mice receiving yogurt or acidophilus yogurt were higher than those of the mice in the control group. The mean values for serum cholesterol concentrations and LDL cholesterol concentrations were significantly decreased when acidophilus yogurt was fed on d 28 and 56. High density lipoprotein cholesterol and triglycerides were not affected by yogurt or acidophilus yogurt. The highest number of fecal lactobacilli was found in mice receiving acidophilus yogurt, and the number of fecal coliforms of that group was also lower than in the other two groups. (**Key words**: mice, acidophilus yogurt, yogurt, serum cholesterol)

Abbreviation key: **HDL** = high density lipoprotein, **LDL** = low density lipoprotein.

INTRODUCTION

There is probably an increased incidence of atherosclerotic heart disease in patients with hypercholesterolemia (19), which indicates the importance of determining methods to reduce serum cholesterol. In 1974, Mann and Spoerry (26) discovered that the consumption of large quantities of fermented milk by the Masai tribesmen in Africa actually lowered their serum cholesterol level. Later, unfermented milk was also demonstrated to be hypocholesterolemic both in humans (13, 25) and animals (1, 3, 22, 23, 29). Mann (24) and Mann and Spoerry (25) claimed that more hypocholesterolemic activity was greater for fermented milk than for whole milk, and the general thought was that the hypocholesterolemic factor might be part of fermented milk products.

Recently, yogurt and other fermented milk products have been reported to contain some substances that lower serum cholesterol. Consumption of some cultured or culture-containing dairy products supplemented with *Lactobacillus acidophilus* has the potential to decrease serum cholesterol concentrations in rats, pigs, boars, and humans (4, 8, 10, 18, 37, 40). However, Grunewald and Mitchell (11) and Thompson et al. (41) found no hypocholesterolemic activity from acidophilus milk consumed by mice and humans, respectively. Examination of the literature shows that yogurt consumption has had various effects on serum cholesterol; some investigators demonstrated the hypocholesterolemic activity of yogurt in humans (2, 12, 17, 18, 24), but others did not (31, 41). Sources of variation may be different in levels of hypocholesterolemic compounds in yogurt and different bacterial strains used in yogurt fermentation. The superiority of acidophilus yogurt over yogurt has not been substantiated. The objective of this investigation was, therefore, to study the effects of dietary yogurt and acidophilus yogurt on concentrations of serum cholesterol, triglyceride, and lipoprotein levels in mice.

MATERIALS AND METHODS

Sixty white male mice (mean initial weight of 22 g) were fed a commercial rodent chow for ad libitum intake for 4 wk. Mice then were divided at random into three groups of 20 each. The diet was analyzed by the agricultural faculty in the Dairy Technology

Received October 28, 1996.
Accepted April 28, 1997.

Department of the Aegean University. The diet contained approximately 21.99% protein, 6.73% fat, 2.53% fiber, 4.73% ash, and 55.0% nitrogen-free extract (by difference). Group 1 received rodent chow plus water, group 2 received rodent chow plus yogurt, and group 3 received rodent chow plus acidophilus yogurt. No water was provided to groups 2 or 3.

For the production of yogurt and acidophilus yogurt, low fat milk (1% fat and 9% SNF) was heated at 85°C for 20 min prior to fermentation and divided into two parts. The portions were then cooled to 40 to 45°C, and milk was inoculated with a 3% (vol/vol) liquid culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* from the Department of Dairy Technology and 0.01% freeze-dried culture of *S. thermophilus* plus *L. acidophilus* from Chr. Hansen Laboratorium (Copenhagen, Denmark) for dietary treatments identified as yogurt and acidophilus yogurt, respectively. Each of the inoculated mixes was poured into containers and incubated at 42 and 37°C for yogurt and acidophilus yogurt, respectively, until pH 4.6. The products were cooled to 4°C and stored at that temperature no longer than 3 d before feeding. The number of lactobacilli in freshly fermented milk products was determined by established procedures (15, 16) to be approximately 10^7/ml.

The mice received their assigned diets for ad libitum intake for 56 d. Weight gains were calculated for respective groups at 28 and 56 d. Fecal samples were collected into sterile test tubes at 0, 14, 28, 42, and 56 d. Immediately following collection procedures, samples were homogenized in sterile phosphate buffer on a Vortex mixer (Griffin and George Ltd., Britain) for 4 min. To determine total lactobacilli, a sample of the feces was plated on acidified MRS agar. The plates were incubated for 48 h in a 99% CO_2 environment at 37°C. After the incubation period, colonies were selected for Gram stain. Regular, nonspore-forming, Gram-positive rods were the only bacteria that were retained for the enumeration. These were transferred to Lactobacilli MRS broth (Difco Laboratories, Detroit, MI), grown for 24 h, and tested for the enzyme catalase. All strains that were negative for catalase and Gram-positive rods were assumed to be lactobacilli. The number of fecal coliforms was determined on violet red bile agar (Oxoid Laboratories, Hampshire, England). The plates were incubated for 24 h at 37°C. The results were reported as $\log_{10}$ counts per gram wet weight of feces.

At the end of the 28- and 56-d feeding period, the mice were deprived of food for a minimum of 12 h and then anesthetized by ether. Blood samples were collected from abdominal aorta, placed in sterile tubes, and centrifuged at 4000 × *g*. The obtained serum samples were analyzed for cholesterol, high density lipoprotein (**HDL**) cholesterol, and triglycerides. Kits were used for the analysis (Boehringer Mannheim GmbH, Mannheim, Germany). Low density lipoprotein (**LDL**) cholesterol was calculated using the equations of Friedewald et al. (6).

Statistical Analysis

Two-way analysis of variance was used to determine variation within and between dietary periods for body weight, serum cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, lactobacilli, and coliforms. The least significant difference test for mean separation was used to determine the statistical significance within and between dietary periods of the same variables if analysis of variance was significant as outlined by SAS (36). All data are reported as means and standard deviations of the means.

RESULTS AND DISCUSSION

The effect of diet supplementation with yogurt or acidophilus yogurt on the weight gain of mice is illustrated in Figure 1. The mice that were fed yogurt and acidophilus yogurt had higher average weight gains than did the control group but were not different from each other. Grunewald (10) and Suzuki et al. (40) found no difference in weight gain among rats consuming skim milk and those consuming milk that contained *L. acidophilus*, but, in mice, weight gain was considerably greater for the group given fermented acidophilus milk, which is similar to other published findings (11).

The effect of yogurt and acidophilus yogurt on serum lipids is shown in Table 1. On d 28, the mean serum cholesterol concentrations for the group fed acidophilus yogurt was significantly lower ($P < 0.01$) than for the control group. The mean concentration for the group fed acidophilus yogurt on d 56 also was significantly lower than for the control group and the group fed yogurt. The hypocholesterolemic effect of yogurt occurred only at the end of 56 d and was lower than that of acidophilus yogurt.

The finding that acidophilus yogurt lowers serum cholesterol concentrations is in agreement with data from other studies involving various milk products containing *L. acidophilus*. Sinha (37) demonstrated that unfermented milk containing viable *L. acidophilus* cells significantly reduced the serum cholesterol concentrations in rats, and acidophilus yogurt had an anticholesterolemic effect in piglets (4, 18). Similar

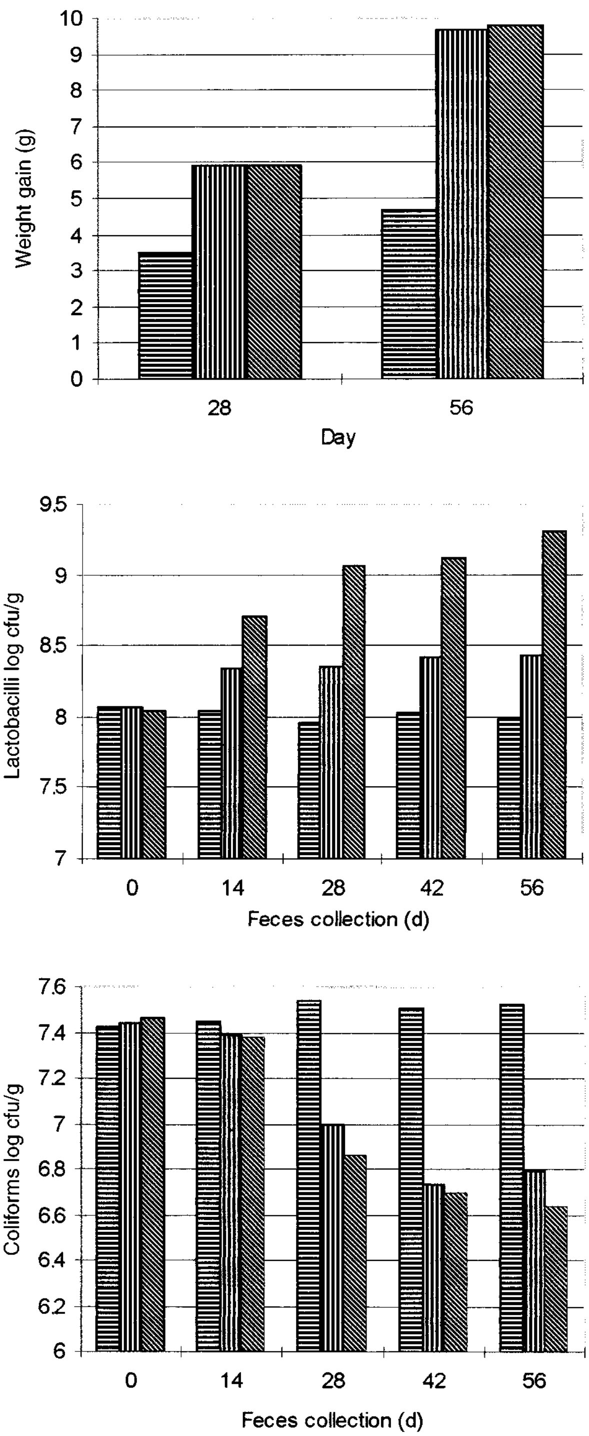

Figure 1. Effect of dietary yogurt and acidophilus yogurt on weight gain (top), fecal lactobacilli (middle), and fecal coliforms (bottom). Mice were assigned to one of three dietary treatments for 56 d: 1) commercial rodent chow and water (control; horizontally striped bar), 2) commercial rodent chow and yogurt made from milk inoculated with a 3% (vol/vol) liquid culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (yogurt; vertically striped bar), and 3) commercial rodent chow plus yogurt made from milk inoculated with a 0.01% (wt/vol) freeze-dried culture of *S. thermophilus* plus *Lactobacillus acidophilus* (acidophilus yogurt; diagonally striped bar).

results were obtained in studies of rats fed milk fermented by *L. acidophilus* or by *L. acidophilus*, *S. thermophilus*, *Streptococcus faecium*, and *Bifidobacterium* sp. (10, 14, 40). Khedkar et al. (20) found that the effect of acidophilus milk in lowering the serum cholesterol of human volunteers who were 50 to 60 yr of age varied depending on the strain of *L. acidophilus* that was used in fermentation. In addition, this effect was found to act directly on cholesterol in the gastrointestinal tract, making it unavailable for absorption into the blood (21). Conversely, Grunewald and Mitchell (11) reported that acidophilus milk had no effect on serum cholesterol in mice.

Both HDL cholesterol and triglycerides in mice were unaffected by dietary yogurt or acidophilus yogurt, but serum LDL cholesterol was reduced especially by the diet containing acidophilus yogurt. Because the LDL cholesterol was calculated from values for serum cholesterol, triglycerides, and HDL cholesterol, the reduction in serum LDL cholesterol concentrations would be expected (27). The concentration of HDL cholesterol decreased slightly in the group fed acidophilus yogurt during the experimental period, but the changes were not significant ($P > 0.01$) (Table 1). The results related to HDL cholesterol generally agree with the findings of Danielson et al. (4), Moussa et al. (27), Rao et al. (32), and Thompson et al. (41). However, Hruby et al. (14) reported that, for human volunteers, a fermented milk product containing *S. thermophilus*, *S. faecium*, *L. acidophilus*, and various *Bifidobacterium* spp. decreased total cholesterol and the LDL fraction with a simultaneous, moderate increase in the HDL fraction.

Supplementation of diets with either yogurt or acidophilus yogurt resulted in small but nonsignificant decreases in serum triglycerides over the 56-d period. In addition, none of the groups exhibited significant changes from d 28 to 56 ($P > 0.01$). A small but nonsignificant decline in triglyceride was also observed by Danielson et al. (4) in the mature boars fed acidophilus yogurt and by Hepner et al. (12) in humans fed yogurt. However, Jones et al. (18) found that yogurt had lowered triglyceride concentrations of piglets, and Moussa et al. (27) found that both yogurt and acidophilus yogurt had the same effect on triglyceride concentrations of rats.

The effect of dietary treatments on fecal lactobacilli and coliforms are shown in Figure 1. The groups of mice varied considerably in the numbers of lactobacilli and coliforms in the feces. The mice in the groups fed yogurt or acidophilus yogurt exhibited highly significant increases in the numbers of lactobacilli in their feces. The greatest increase in mean

TABLE 1. Effect of yogurt and acidophilus yogurt on serum lipids in mice.[1]

Dietary treatment group[3]	Cholesterol		HDL[2] Cholesterol		LDL Cholesterol		Triglycerides	
	d 28	d 56	d 28	d 56	d 28	d 56	d 28	d 56
	(mg/d)							
Control								
$\bar{X}$	171.2^{a,x}	168.1^{a,x}	54.6	54.2	97.8^{a,x}	96.6^{a,x}	94.3	90.9
SD	3.0	3.9	3.1	3.1	3.6	2.4	5.8	5.9
Yogurt								
$\bar{X}$	169.6^{a,y}	157.7^{b,x}	52.7	52.0	98.8^{a,y}	88.3^{b,x}	90.7	86.9
SD	5.0	6.8	3.0	3.8	5.4	4.3	6.7	5.3
Acidophilus yogurt								
$\bar{X}$	139.9^{b,x}	116.0^{c,y}	51.0	51.3	70.4^{b,x}	46.9^{c,y}	92.6	88.7
SD	3.1	4.2	3.7	4.2	3.8	5.0	5.2	4.3

[a,b,c]Means within a column with no common superscript letters differ ($P < 0.01$).

[x,y]Means within a row within a cholesterol group with no common superscript letters differ ($P < 0.01$).

[1]Each value is a mean (±SD) of nine observations.

[2]HDL = High density lipoprotein; LDL = low density lipoprotein. The LDL cholesterol was calculated by the equation of Friedewald et al. (6): LDL cholesterol = serum cholesterol – HDL cholesterol – serum triglycerides.

[3]Mice were assigned to one of three dietary treatments for 56 d: 1) commercial rodent chow and water (control); 2) commercial rodent chow and yogurt made from milk inoculated with a 3% (vol/vol) liquid culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (yogurt), and 3) commercial rodent chow plus yogurt made from milk inoculated with a 0.01% (wt/vol) freeze-dried culture of *S. thermophilus* plus *Lactobacillus acidophilus* (acidophilus yogurt).

number of lactobacilli was for the group receiving acidophilus yogurt. Numbers of coliform bacteria in feces decreased for mice in both yogurt and acidophilus yogurt groups during the 56-d feeding period. The decrease in the mean number of coliforms for the group of mice fed acidophilus yogurt appeared to be greater than that in the group fed yogurt. There was a trend toward more lactobacilli and fewer coliforms in mice that received acidophilus yogurt than in the other groups.

Mice that received acidophilus yogurt exhibited significantly more lactobacilli in their feces than did mice that received yogurt, indicating that *L. acidophilus* established itself more effectively in the murine intestinal tract than did the *L. bulgaricus* in the yogurt diet.

Lactobacillus bulgaricus, one of the two most important organisms used in yogurt manufacture, has been reported (5, 33, 38) to have poor survival in the gastrointestinal tract because of its low tolerance for bile salts, low resistance to acid pH, and rather selective requirements for sugars (5, 33, 38). Nevertheless, some researchers (34, 35) demonstrated that this organism survives passage through the intestinal tract of humans.

There is less controversy about the capability of *L. acidophilus* to survive passage through the gut. Increases in numbers of lactobacilli in the intestinal flora accompanied by concurrent reductions in numbers of coliform bacteria is apparently a normal development within the intestinal flora of mice. This result suggests that the lactobacilli in the developing intestinal flora exert a controlling effect on coliform bacteria. In our experiment, on average, numbers of coliform decreased more in feces of mice that received acidophilus yogurt.

Several investigators (4, 7, 9, 28, 30, 39) have observed increases in numbers of lactobacilli accompanying a decrease in coliforms after oral administration of *L. acidophilus*. Hruby et al. (14) found a positive effect on the composition of the intestinal flora of human volunteers fed by a fermented milk product containing *S. thermophilus*, *S. faecium*, *L. acidophilus*, and various *Bifidobacterium* strains.

In summary, our results strongly suggest that the acidophilus yogurt supplementation of the diet of mice significantly reduced serum cholesterol and LDL cholesterol, without effecting serum triglycerides or HDL cholesterol. Furthermore, the greatest number of lactobacilli occurred in the feces from mice that had received acidophilus yogurt.

ACKNOWLEDGMENT

This work was supported by Turkish National Council.

REFERENCES

1 Bagulawski, W., and J. Wrobel. 1974. An inhibitor of sterol-biosynthesis in cow's milk. Nature 247:210.

2 Bazzare, T. L., S. Liu Wu, and J. A. Yuhas. 1983. Total and HDL cholesterol concentrations following yogurt and calcium supplementation. Nutr. Rep. Int. 44:175.
3 Bernstein, B. A., T. Richardson, and C. H. Amundson. 1977. Inhibition of cholesterol biosynthesis and acetyl-coenzyme A synthetase by bovine milk and orotic acid. J. Dairy Sci. 60:1846.
4 Danielson, A. D., E. R. Peo, K. M. Shahani, A. J. Lewis, P. J. Whalen, and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966.
5 Davis, J. G. 1970. 'Dietary' or 'healthy' yogurt. Dairy Ind. 35: 827.
6 Friedewald, W. R., R. I. Levy, and D. S. Frederickson. 1972. Lipoproteins in serum. Clin. Chem. 18:499.
7 Gilliland, S. E., B. B. Bruce, L. J. Bush, and T. E. Staley. 1980. Comparison of two strains of *Lactobacillus acidophilus* as dietary adjuncts for young calves. J. Dairy Sci. 63:364.
8 Gilliland, S. E., C. R. Nelson, and C. Maxwell. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377.
9 Gilliland, S. E., and M. L. Speck. 1977. Use of the Minitek system for characterizing lactobacilli. Appl. Environ. Microbiol. 33:1289.
10 Grunewald, K. K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078.
11 Grunewald, K. K., and K. Mitchell. 1983. Serum cholesterol levels in mice fed fermented and unfermented acidophilus milk. J. Food Prot. 46:315.
12 Hepner, G., R. Fried, S. Leor, L. Fusetti, and R. Morin. 1979. Hypocholesterolemic effect of yogurt and milk. Am. J. Clin. Nutr. 32:19.
13 Howard, A. N., and J. Marks. 1977. Hypocholesterolemic effect of milk. Lancet 30:255.
14 Hruby, S., V. Spelina, L. Schlemmerova, and B. Turek. 1992. The significance of the consumption of specifically fermented dairy products for the composition of the intestinal microflora and for serum cholesterol values. Cesk. Hyg. 37:109.
15 International Dairy Federation. 1988. Yogurt: enumeration of characteristic microorganisms. Colony count technique at 37°C. IDF Stand. 117A. Int. Dairy Fed., Brussels, Belgium.
16 International Dairy Federation. 1991. Annex a. Lactic acid starters–guidance of sampling and analysis. IDF Stand. 149. Int. Dairy Fed., Brussels, Belgium.
17 Jaspers, D. A., L. K. Massey, and L. O. Luedecke. 1984. Effect of consuming yogurts prepared with three culture strains on human serum lipoproteins. J. Food Sci. 49:1178.
18 Jones, G., K. M. Shahani, and A. M. Amer. 1985. The effect of acidophilus yogurt on serum cholesterol, triglyceride and lipoprotein levels of weaning pigs. J. Dairy Sci. 68 (Suppl. 1): 84.(Abstr.)
19 Kannel, W. B., W. P. Castelli, and T. Gordon. 1971. Serum cholesterol, lipoproteins and the risk of coronary heart disease. The Framingham Study. Ann. Intern. Med. 74:1.
20 Khedkar, C. D., R. D. Garge, J. M. Mantri, S. A. Kulkarni, and G. D. Khedkar. 1993. Effect of feeding acidophilus milk on serum cholesterol in human volunteers of 50–60 years. J. Dairying Foods Home Sci. 12:33.
21 Khedkar, C. D., R. D. Garge, J. M. Mantri, A. M. Patil, B. R. Chavan, and G. D. Khedkar. 1993. Uptake of cholesterol by human strains of *Lactobacillus acidophilus*. J. Dairying Foods Home Sci. 12:57.
22 Kritchevsky, D., S. A. Tepper, R. D. Morissey, S. K. Garnecki, and D. M. Klurfeld. 1979. Influence of whole or skim milk on cholesterol metabolism in rats. Am. J. Clin. Nutr. 32:597.
23 Malinow, M. R., and P. McLaughlin. 1975. The effect of skim milk on plasma cholesterol in rats. Experimentia 31:1012.
24 Mann, G. V. 1977. A factor in yogurt which lowers cholesterol in man. Atherosclerosis 26:335.
25 Mann, G. V. 1977. Hypocholesterolemic effect of milk. Lancet 10:556.
26 Mann, G. V., and A. Spoerry. 1974. Studies of a surfactant and cholesterolemia in the Masai. Am. J. Clin. Nutr. 27:464.
27 Moussa, S. Z., F.M.M. Salama, and N. A. Taha. 1995. Effect of fresh cow's milk and some fermented milk products on rat serum cholesterol, triglycerides, total lipids and lipoprotein levels. Egypt. J. Dairy Sci. 23:69.
28 Muralidhara, K. S., G. G. Sheggeby, P. R. Elliker, D. C. England, and W. E. Sandine. 1977. Effect of feeding lactobacilli on the coliform and lactobacillus flora of intestinal tissue and feces from piglets. J. Food Prot. 40:288.
29 Nair, C. R., and G. V. Mann. 1977. A factor in milk which influences cholesteremia in rats. Atherosclerosis 26:363.
30 Patel, J. R., J. M. Dave, R. I. Dave, and S. S. Sannabhadti. 1992. Effect of feeding milk fermented with mixed culture of human strains of lactobacilli on fecal lactobacilli and coliform counts in human test subjects. Ind. J. Dairy Sci. 45:385.
31 Payens, W., E.J.M. Rethans, and H. Ward. 1979. Einfluß des konsums einer großen menge von joghurt oder milch auf den serumcholesterolgehalt. Milchwissenschaft 31:525.
32 Rao, D. R., C. B. Chawan, and S. R. Pulusani. 1981. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. J. Food Sci. 46:1339.
33 Rasic, J. L., and J. A. Kurmann. 1979. Yoghurt. Scientific Grounds, Technology, Manufacture and Preparations. 2nd ed. Tech. Dairy Publ. House, Copenhagen, Denmark.
34 Salvadori, P., and B. B. Salvadori. 1972. Studio sulle variazioni coproculturali Nell'u omo in rapporto alla somminis trazione di yogurt. Minerva Dietol. 13:1.
35 Salvadori, P., B. B. Salvadori, G. A. Ragazzi, and G. Ottogalli. 1973. Variations in the microflora of human feces with feeding yogurt. Minerva Dietol. 13:8.
36 SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.
37 Sinha, D. K. 1978. Development of a nonfermented acidophilus milk and testing its properties. M.S. Thesis, Univ. Nebraska, Lincoln.
38 Speck, M. L. 1975. Lactobacilli in the human diet. Dairy Ind. 40:129.
39 Speck, M. L. 1976. Interactions among lactobacilli and man. J. Dairy Sci. 59:338.
40 Suzuki, Y., H. Kaizu, and Y. Yamauchi. 1991. Effect of cultured milk on serum cholesterol concentrations in rats which fed high-cholesterol diets. Anim. Sci. Technol. 62:565.
41 Thompson, L. U., D.J.A. Jenkins, M. A. Amer, R. Reichert, A. Jenkins, and J. Kamulsky. 1982. The effect of fermented and unfermented milks on serum cholesterol. Am. J. Clin. Nutr. 36: 1106.