INFLUENCE OF LACTOBACILLUS ACIDOPHILUS ON SERUM CHOLESTEROL LEVELS

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Story in Brief

The uptake of cholesterol by Lactobacillus acidophilus occurred only when the culture(s) was growing in the presence of bile under anaerobic conditions. Strains of Lactobacillus acidophilus isolated from the fecal flora of pigs varied with regard to the ability to assimilate cholesterol from a laboratory growth medium. Dietary supplementation with L. acidophilus RP32, which was selected for its ability to grow well in the presence of bile and to assimilate cholesterol from the laboratory medium, significantly inhibited ($P<0.05$) increases in serum cholesterol levels of pigs fed a high cholesterol diet. Supplementation with L. acidophilus P47 which was selected for its ability to grow in the presence of bile and lack of ability to remove cholesterol from the growth medium, failed to have a similar effect. This indicates that certain strains of L. acidophilus can act directly on cholesterol in the gastrointestinal tract, and thus may be beneficial in reducing serum cholesterol levels.

(Key Words: Lactobacillus acidophilus, cholesterol, cultured dairy products)

Introduction

Results from a seven year study conducted by the Lipid Research Clinics Program (1984) indicates that reduction of total plasma cholesterol can lower the incidence of coronary heart disease in that relatively small portion of the population suffering from primary hypercholesterolemia. Because of the associated risk of heart disease there has been a thrust toward finding ways to lower plasma cholesterol levels in such people.

Consumption of certain cultured dairy products can result in reduction of serum cholesterol (Grunewald, 1982; Hepner et al, 1979; Mann, 1977; Mann and Spoerry, 1974). Conclusions from some of these studies have indicated that the starter culture bacteria produce metabolites, during their growth in milk, which inhibit cholesterol synthesis in the body. None have suggested that the decrease in serum cholesterol levels was related to a direct action of the bacteria in the cultured milk products on cholesterol in the intestinal tract.

The objectives of this study were to determine whether L. acidophilus would assimilate cholesterol and to confirm whether or not consumption of selected strains would significantly prevent an increase of serum cholesterol in pigs fed a high cholesterol diet.

Materials and Methods

A broth culture of L. acidophilus was inoculated (1 percent) into 10

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ml of sterile lactobacilli MRS broth (Difco Laboratories) containing 1 percent pleuropneumonia-like organism (PPLO) serum fraction (Difco Laboratories) as the cholesterol source and 0.3 percent dried bile (oxgall). The broth was incubated anaerobically 24 h at 37 C. Cells were removed from the broth by centrifugation and resuspended in a volume of distilled water equal to that of the original broth culture. A colorimetric method (Rudel and Morris, 1973) was used to determine the amount of cholesterol in the resuspended cells and spent broth.

Pigs were selected as an animal model to determine if selected cultures varied in their ability to influence serum cholesterol since their digestive system, distribution of coronary arteries, and atherosclerotic tendencies resemble that of the human (Ratcliffe and Luginbuhl, 1971). Since L. acidophilus exhibits host specificity in the intestinal tract, strains of L. acidophilus of pig origin were tested. Rectal swabs were obtained from 4 to 6 month old pigs in the Oklahoma State University swine herd and from a commercial producer. The fabric portion of the swab was broken off into a sterile plastic bag and placed in ice water for transport to the laboratory. The swab was aseptically transferred to a tube containing sterile diluent, appropriate dilutions were prepared and the material was plated on a medium selective for lactobacilli. Plates were incubated at 37 C in a partial CO_2 atmosphere. Cultures were isolated from individual colonies and identified using established procedures (Gilliland, et al, 1980).

Cultures of selected strains of L. acidophilus were grown in 2.4 l of sterile MRS broth for 18 hour at 37 C. Cells were harvested by centrifugation and resuspended in two times their weight of cold sterile 10 percent nonfat milk solids (NFMS). The resulting concentrated culture was dispensed in 2 g portions into sterile cryogenic vials, frozen and stored in liquid nitrogen until used.

Eighteen five-week old Yorkshire gilts from seven litters were randomly assigned to individual metal pens and subsequently to three treatment groups to provide six pigs per treatment. The location of each pig was random with respect to treatments.

All pigs were fed a corn base diet without added crystaline cholesterol (Table 1) twice daily (0.28 kg each feeding) for a one-week adjustment period. During the experimental period which began the second week, all pigs were fed the corn diet supplemented with crystaline cholesterol to supply approximately 1,500 mg of cholesterol per day initially. On days 1 through 7 of the feeding trial, all pigs were fed 0.41 kg of feed at each feeding. For days 8-10 the amount of feed for each was increased to 0.69 kg per feeding. If any feed was left after a 2 hour period it was removed from the pens. Water was available at all times.

In addition to the corn base diet Group 1 (control group) was fed 50 ml of sterile 10 percent NFMS, Group 2 was fed 50 ml of 10 percent NFMS containing 5×10^{10} cells of L. acidophilus P47, and Group 3 was fed 50 ml of 10 percent NFMS containing 5×10^{10} cells of L. acidophilus RP32 once daily (at the evening feeding). The desired numbers of cells of L. acidophilus were provided by adding the appropriate amount of frozen concentrated cultures after thawing. The milk with and without lactobacilli was fed to the pigs in individual bowls just prior to feeding the corn base diet each evening.

Blood samples were taken from each pig after a 12 hour fast by anterior vena cava puncture during the experimental period on days 0, 5, and 10 to be analyzed for total serum cholesterol. Serum from each sample was analyzed for total serum cholesterol using an enzymatic assay kit (Sigma Chemical Co.).

Table 1. Composition of corn base diet used in pig feeding trial.

Component	Amount (kg)
Ground shelled corn	153.5
Butter	27.2
Dried Sweet Whey	72.6
Soybean meal	98.0
Salt	0.9
Dicalcium phosphate	5.4
Calcium Carbonate	3.3
Vitamin and trace mineral premix[a]	1.5
Total	362.4[b]

[a]Vitamin trace mineral premix supplied 1760 mg riboflavin; 8,800 mg pantothenic acid; 8,800 mg niacin; 8.8 mg vitamin B_{12}; 176,000 mg choline chloride; 1,760,000 I.U. vitamin A; 176,000 I.U. vitamin D_3; 4,400 I.U. vitamin E; 44 mg menadiane dimethyl-primidionol bisulfite; 39.6 mg selenium; 299.2 mg iodine; 19.8 g iron; 11 g manganese; 2.2 g copper; and 39.6 g zinc per kilogram of premix.

[b]After mixing with a Marion Mixer (Rapids Machinery Co., Marion, Iowa) 78.7 kg was removed for adjustment period feeding and 450 g cholesterol (purity at least equivalent to USP; Sigma Chemical Co., St. Louis, MO) was added and mixed into the remaining 283.7 kg. Including that from the butter, the diet contained 1775 mg cholesterol/kg.

Results and Discussion

Cholesterol was taken up by L. acidophilus in the broth medium containing PPLO serum and oxgall only during anaerobic growth of L. acidophilus.

Individual strains of L. acidophilus exhibited considerable variation with regard to the ability to assimilate cholesterol. For example L. acidophilus RP32 removed more cholesterol from the growth medium and accumulated more in the cells than did strain P47 (Table 2).

Table 2. Assimilation of cholesterol by Lactobacillus acidophilus during anaerobic growth in MRS broth containing PPLO serum and oxgall.

Culture	Cholesterol (μg/ml)[a]		
	Control Broth	Spent Broth	Cells
RP32	67.2	40.6	28.6
P47	67.2	61.4	8.2

[a]Each value represents the average from 3 trials.

In the feeding trial all pigs appeared to remain healthy and there were no significant differences in weight gains among the treatment groups. Consumption of the diet fed was not a problem and intake did not differ among treatment groups. The pigs were fed a diet high in cholesterol in order to cause an increase in serum cholesterol. All groups exhibited increases in serum cholesterol during the feeding trial as expected (Table 3). The mean serum cholesterol concentration for all treatament groups on day 0 was between 52 and 56 g/dl; there were no significant differences among groups ($P>0.05$). There were no significant interactions among treatments and days ($P>0.05$). The mean serum cholesterol values on Day 5 for the control group and P47 group had increased significantly ($P<0.05$) to 69.10 and 72.01 g/dl respectively. The group receiving L. acidophilus RP32 did not exhibit a significant ($P>0.05$) increase from day 0 to day 5. The mean concentration for the latter group on day 5 was significantly lower ($P<0.05$) than for the P47 group. The mean value of the RP32 group on day 10 was also significantly lower ($P<0.05$) than both the control and P47 groups. None of the groups exhibited significant increases from day 5 to day 10 ($P>0.05$).

Table 3. Influence of feeding cells of Lactobacillus acidophilus on serum cholesterol levels in pigs on a high cholesterol diet.

	Cholesterol (mg/dl)[a]		
Group	Day 0	Day 5	Day 10
Control	52.23 (1.88)^{C1}	69.10 (3.91)^{C2D2}	74.44 (4.64)^{C2}
L. acidophilus P47	55.58 (4.70)^{C1}	72.01 (3.02)^{C2}	73.48 (4.68)^{C2}
L. acidophilus RP32	52.84 (3.00)^{C1}	61.48 (3.30)^{D1}	62.29 (4.91)^{D1}

[a]Each value is the mean from six pigs; numbers in parentheses = standard deviation; values in same column followed by different superscript letters are significantly different ($P<.05$); values in the same row followed by different superscript numbers are significantly different ($P>.05$). There were no significant interactions among days and treatments ($P>.05$).

Some strains of L. acidophilus have the ability to assimilate cholesterol as indicated by the appearance of cholesterol in the cells during growth which was associated with decreases in the concentrations of cholesterol in the growth medium during anaerobic growth in a medium containing bile. The amount of bile required to enable the cultures to remove cholestrol from the growth medium was not in excess of the levels normally encountered in the intestines. Thus, the conditions required in the in vitro system for cholesterol uptake by L. acidophilus would also be expected to occur in the intestinal tract. This should enable the organism to assimilate at least part of the cholesterol ingested in the diet, thus making it unavailable for absorption into the blood. A similar action could be exerted on endogenous cholesterol in the intestines.

Both strains of L. acidophilus selected for use in the pig feeding trial exhibited resistance to bile; however, strain RP32 exhibited maximal ability and strain P47 exhibited minimal ability to assimilate cholesterol in vitro. These two strains were selected for the feeding trial to determine if the difference in ability to assimilate

cholesterol would influence any effect that consuming cells of L. acidophilus might have on serum cholesterol levels in the pigs. The significantly lower levels of serum cholesterol in the pigs that received the "cholesterol-assimilating" strain (RP32) further supports the idea that the ability of L. acidophilus to cause reductions in serum cholesterol is a result of the direct action of the culture on cholesterol.

Further research is needed to determine the mechanism of cholesterol uptake and to determine whether or not ingestion of cells of a selected strain of L. acidophilus could decrease serum cholesterol levels in adult humans with primary hypercholesterolemia.

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