IN VIVO HYPOCHOLESTEROLEMIC ACTION OF *LACTOBACILLUS ACIDOPHILUS* AND CALCIUM: INTERFERENCE WITH ENTEROHEPATIC CIRCULATION OF BILE ACIDS

B.Z. de Rodas[1], S.E. Gilliland[2], C.V. Maxwell[3] and M.L. Rose[1]

Story in Brief

The objective of this study was to determine if the hypocholesterolemic effect of *Lactobacillus acidophilus* and dietary calcium was related to reduced absorption of bile acids from the intestines in swine. Thirty three Yorkshire barrows (4 replicates; 92 kg) with indwelling jugular catheters were fed a diet supplemented with .5% crystalline cholesterol for 14 days. On day 15, cholesterol was removed from the diet and pigs within each replicate were assigned to one of four treatments (2 x 2 factorial) including 2 levels of calcium (.7% and 1.4%) with and without *L. acidophilus*. These diets were fed for 15 consecutive days. Blood samples were collected daily and sera was analyzed for total, high density lipoprotein, and low density lipoprotein cholesterol and for total bile acids. Concentrations of total serum cholesterol were reduced by 11.8% in pigs fed *L. acidophilus* compared to pigs fed a diet without *L. acidophilus*. Similarly, serum total cholesterol was reduced by 11.3% in pigs fed 1.4% calcium compared to pigs fed .7% calcium. Neither, *L. acidophilus* nor calcium has an effect on HDL cholesterol levels. Concentrations of LDL cholesterol tended to follow those of total cholesterol. Serum bile acid levels were reduced by 23.9% by feeding *L. acidophilus* and by 21.4% by feeding 1.4% calcium when compared to their controls. Moreover, total bile acid concentration was positively correlated with total cholesterol concentration in pigs fed *L. acidophilus* or 1.4% calcium. These data suggest that hypocholesterolemic actions of *L. acidophilus* and calcium are probably mediated through interference of the enterohepatic circulation of bile acids.

(Key Words: *Lactobacillus acidophilus*, Calcium, Hypocholesterolemic.)

Introduction

High levels of serum total cholesterol and low density lipoprotein (LDL) cholesterol are strongly associated with an increased risk of coronary heart disease, the number-one cause of death in the United States (Lipid Research Clinics Program, 1984a,b). Thus, ways to reduce serum cholesterol levels are being sought.

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[1]Graduate Assistant [2]Regents Professor [3]Professor

Several studies have indicated that dietary supplementation with *Lactobacillus acidophilus* or calcium resulted in reduction of serum cholesterol, but the mechanism of this effect is not universally agreed upon. In vitro studies have indicated that *L. acidophilus* can take up cholesterol during growth and deconjugate bile acids (Walker and Gilliland, 1993). Therefore, *L. acidophilus* offers two potential mechanisms for reducing the amount of cholesterol absorbed from the small intestine which in turn may decrease serum cholesterol levels. In addition, it has been reported that calcium can bind bile acids and may interfere with the enterohepatic circulation of bile acids.

The purpose of this study was to evaluate the hypocholesterolemic effect of *L. acidophilus* and dietary calcium in pigs previously fed a high cholesterol diet and to determine if the effect was related to reduced absorption of bile acids from the small intestine.

Materials and Methods

Thirty three Yorkshire barrows (4 replicates) weighing approximately 92 kg were surgically fitted with an indwelling jugular catheter. Following recovery, all pigs were fed a diet supplemented with .5% crystalline cholesterol for 14 days (cholesterol feeding period) at 12 hour intervals (1.4% BW) to cause an increase in serum cholesterol (Table 1). On day 15, crystalline cholesterol was removed from the diet and pigs within each replicate were assigned in a 2 x 2 factorial arrangement to one of four treatments including two levels of calcium (.7% and 1.4%) with and without 2.5×10^{11} cells of *L. acidophilus* ATCC 43121 (Table 1). These diets were fed for 15 consecutive days (experimental period) as in the cholesterol feeding period. *L. acidophilus* ATCC 43121 was selected for use in this study because of its ability to assimilate cholesterol and to deconjugate bile acids (Walker and Gilliland, 1993). All pigs received 250 ml of sterile 10% reconstituted nonfat dried milk just prior to feeding the dried ration. Cells of *L. acidophilus* were added to the milk for those treatments that included *L. acidophilus*.

Blood samples were collected daily from the jugular catheter prior to the morning feeding. The sampling period started following the convalescence period and continued for 29 days after initiation of the trial. Sera was analyzed for total and HDL cholesterol and for total bile acids. Concentrations of LDL cholesterol were calculated by difference between total and HDL cholesterol.

Data obtained during the experimental period (day 15 to 29) were evaluated by least squares analysis of variance for repeated measures in a split plot design. Pigs were defined as the experimental unit with treatment as the main plot and sampling day as a repeated measure (subplot). The effects of *L. acidophilus*, calcium and their interaction were tested using pig within replicate x *L. acidophilus* x calcium mean square as the error term. The effects of sampling day, sampling day x *L. acidophilus*, sampling day x calcium and the three-way interaction were tested by the residual error.

Table 1. Composition of experimental diets.

	Diets[a]		
	Days 1 to 14	Days 15 to 29	
Ingredient		.7 % calcium	1.4 % calcium
Yellow corn	67.425	68.095	66.245
Nonfat dried milk	20.00	20.00	20.00
Butter	10.00	10.00	10.00
Lysine, HCl	.03	.03	.03
Calcium carbonate	.32	.15	2.00
Dicalcium phosphate	1.15	--	--
Deflourinate phosphate	--	1.15	1.15
Vitamin, TM premix[b]	.25	.25	.25
Salt	.30	.30	.30
Ethoxyquin	.025	.025	.025
Cholesterol[c]	.50	--	--
	100.00	100.00	100.00
Calculated analysis (%)			
Crude protein	12.36	12.41	12.26
Lysine	.70	.70	.70
Calcium	.70	.70	1.40
Phosphorus	.60	.60	.60

[a] As fed basis; each ingredient presented on percentage basis.

[b] Supplied 4950 IU vitamin A, 550 IU vitamin D, 27.5 IU vitamin E, 24.75 mg pantothenic acid, 38.5 mg niacin, 6.6 mg riboflavin, 3.63 mg menadione, 27.5 ug vitamin B12, 110 mg choline, .30 mg selenium, 19.96 mg manganese, 99.79 mg zinc, 99.79 mg iron, 9.97 mg cooper and .20 mg iodine per kg of feed.

[c] Purity at least equivalent to USP/NF (Sigma Chemical Co., St. Louis, MO).

Results and Discussion

All pigs appeared to remain healthy, and there were no significant differences in weight gains, feed intake or cholesterol intake during the length of the experiment. Hypercholesterolemia was induced, as expected, by feeding .5% crystalline cholesterol and 10% butter in the diet. Total serum cholesterol concentrations averaged 85.4 mg/dl on day 1 (just prior to the initiation of the cholesterol feeding period) and increased to 294.6 mg/dl on day 15, a rise of 209.2 mg/dl (2.4 fold increase) for the entire cholesterol feeding period.

The overall effect of *L. acidophilus* and calcium on serum total cholesterol, HDL cholesterol, LDL cholesterol and total bile acids during day 15 to 29 is shown in Table 2. Main effect means are presented since no

Table 2. Effect of *Lactobacillus acidophilus* and calcium on serum total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein cholesterol (LDL), and total bile acids of pigs previously fed a high cholesterol diet (day 15 to 29)[a].

	L. acidophilus		Calcium	
Item	Without	With	.7%	1.4%
Cholesterol, mg/dl[bc]	167.9±5.2	148.0±5.3	167.5±5.2	148.5±5.3
HDL, mg/dl[d]	57.9±4.7	55.1±4.1	57.7±4.2	55.3±4.6
LDL, mg/dl[be]	114.6±7.7	95.9±7.7	113.5±7.3	97.0±7.6
Bile acids, µmol/l[f]	9.2± .7	7.0± .9	9.1± .7	7.1± .8

[a] Values are least squares means ± SE. LA=*L. acidophilus.*

[b] Means were adjusted for initial (day 15) sera values using covariate analysis.

[c] LA effect (P<.01); Ca effect (P<.01) and LAxCa interaction (P>.6).

[d] LA effect (P>.5); Ca effect (P>.7) and LAxCa interaction (P>.3).

[e] LA effect (P<.09); Ca effect (P<.1) and LAxCa interaction (P>.6).

[f] LA effect (P<.06); Ca effect (P<.08) and LAxCa interaction (P>.8).

significant *L. acidophilus* x calcium interaction was observed for any of the variables tested. When averaged over days, pigs fed *L. acidophilus* had an 11.8% lower (P<.01) total cholesterol concentration than pigs fed a diet without *L. acidophilus* (167.9 ± 5.2 vs 148.0 ± 5.3). Similarly, pigs fed 1.4% calcium had an 11.3% lower (P<.01) total cholesterol concentration than those fed .7% calcium (167.5 ± 5.2 vs 148.5 ± 5.3). No differences were observed between the two levels of calcium (P>.5) and the two levels of *L. acidophilus* (P>.7) for serum HDL cholesterol concentration. However, serum LDL cholesterol level tended to decrease when pigs were fed *L. acidophilus* (P<.09) or 1.4% calcium (P<.1). The concentration of LDL cholesterol was reduced by 16.4% in pigs fed milk supplemented with *L. acidophilus* when compared to pigs fed milk without *L. acidophilus*, and by 14.6% in pigs fed the diet containing 1.4% calcium when compared to those fed the diet containing .7% calcium. Pigs fed milk with *L. acidophilus* also tended to have lower (P<.06) total serum bile acid than those fed milk without *L. acidophilus* and pigs fed the diet containing 1.4% calcium tended to have lower (P<.08) bile acid levels than those fed the diet containing .7% calcium. Serum bile acid concentrations were reduced by 23.9% by feeding *L. acidophilus* and by 21.4% by feeding 1.4% calcium.

Daily concentrations of serum total cholesterol in response to dietary calcium level and *L. acidophilus* are shown in Figure 1. Regardless of dietary calcium or *L. acidophilus* level, total cholesterol concentrations decreased after cholesterol was removed from the diet (day on treatment effect P<.01); however, the decrease was greater for pigs fed the highest level of calcium or for those fed *L. acidophilus*. A significant day on treatment x calcium

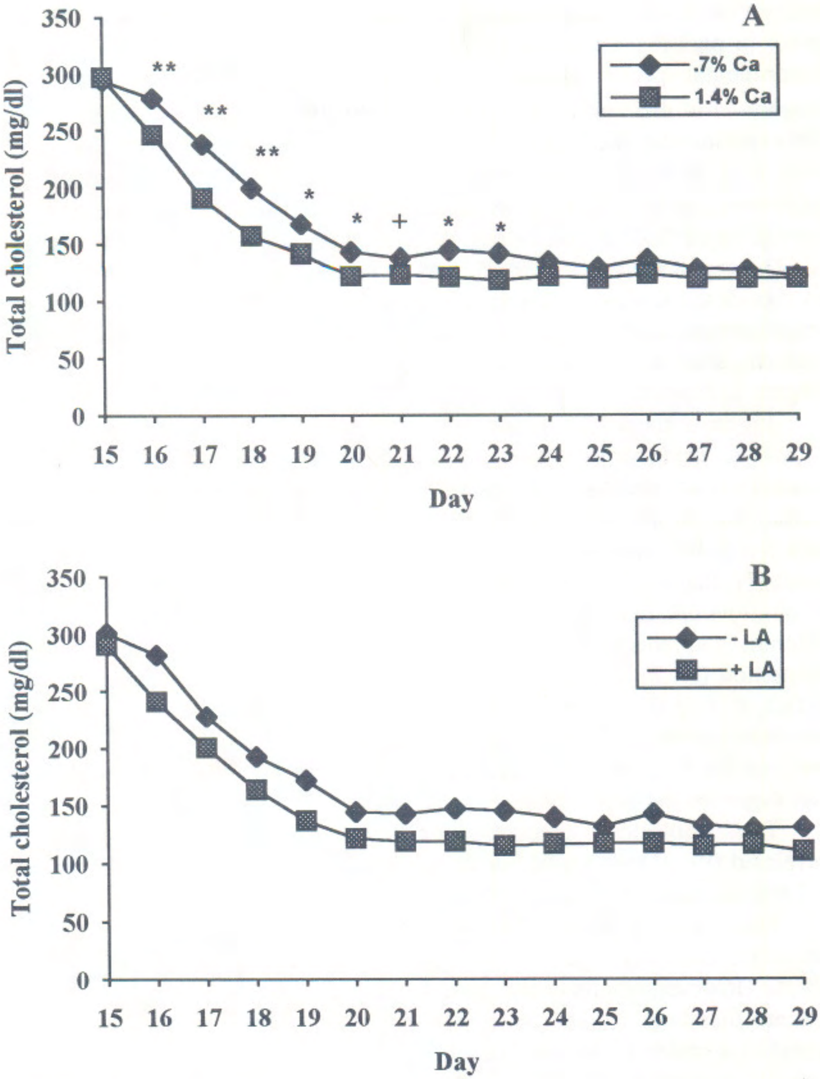

Figure 1. Effect of dietary calcium (Panel A) and *Lactobacillus acidophilus* (Panel B) on total serum cholesterol level of pigs previously fed a high cholesterol diet. Values are least squares means. Pooled SE=7.7 mg/dl. Calcium effect (P<.01), *L. acidophilus* effect (P<.01), day on treatment effect (P<.01), and day on treatment x calcium interaction effect (P<.06). **Means differ (P<.01) within day. *Means differ (P<.05) within day. +Means differ (P<.1) within day.

interaction (P<.06) was observed for total cholesterol. Cholesterol level was lower in pigs fed 1.4% calcium than in pigs fed .7% calcium throughout the experimental period (Figure 1, Panel A), but as expected the greatest magnitude of difference occurred during the first three days after initiation of the experimental diets (days 16, 17, and 18; serum cholesterol reduction of 32.7, 47.1, and 42.3 mg/dl, respectively; P<.01). From day 19 through 23 the difference due to calcium level continued but the magnitudes of the differences were reduced (25.7 mg/dl for day 19, 21.5 mg/dl for day 20, 23.5 mg/dl for day 22, 23.4 mg/dl for day 23; P<.05, and 14.8 mg/dl for day 21; P<.1). There was no significant day on treatment x *L. acidophilus* interaction; pigs fed the diet supplemented with *L. acidophilus* had a lower total cholesterol concentration each day after day 15 while on test than pigs fed a diet without *L. acidophilus* (Figure 1, Panel B).

Figure 2 shows the response of total bile acids to dietary calcium (Panel A) and *L. acidophilus* (Panel B) by each day on treatment. Total bile acid concentrations decreased in all treatment groups during the experimental period (day on treatment effect; P<.01). However, the decrease was greater in pigs fed 1.4% calcium than in pigs fed .7% calcium (Figure 2, Panel A). Similarly, the reduction in total bile acid concentrations was greater in pigs fed *L. acidophilus* than in pigs fed the diet without *L. acidophilus*. These differences in reduction resulted in a tendency for a day on treatment x calcium interaction (P<.2) and a day on treatment x *L. acidophilus* interaction (P<.2). In fact, during the overall 15-day experimental period pigs fed 1.4% calcium had 21.4% lower (P<.08) bile acid concentrations than pigs fed .7% calcium, and pigs fed *L. acidophilus* had 23.9% lower (P<.06) bile acid concentrations than those fed the diet without *L. acidophilus*.

Total cholesterol concentrations during day 15 to 29 were positively correlated (P<.01) with total bile acid concentrations in pigs fed *L. acidophilus* or 1.4% calcium (r=.55 and r=.48, respectively).

There is no apparent relationship between the ability of *L. acidophilus* to assimilate cholesterol and to deconjugate bile acids (Walker and Gilliland, 1993). However, results of the present study and that of Gilliland et al. (1985) indicate that both actions are important in enabling *L. acidophilus* to exert hypocholesterolemic actions and that these actions are mediated, at least partially, through interference of the enterohepatic circulation of bile acids.

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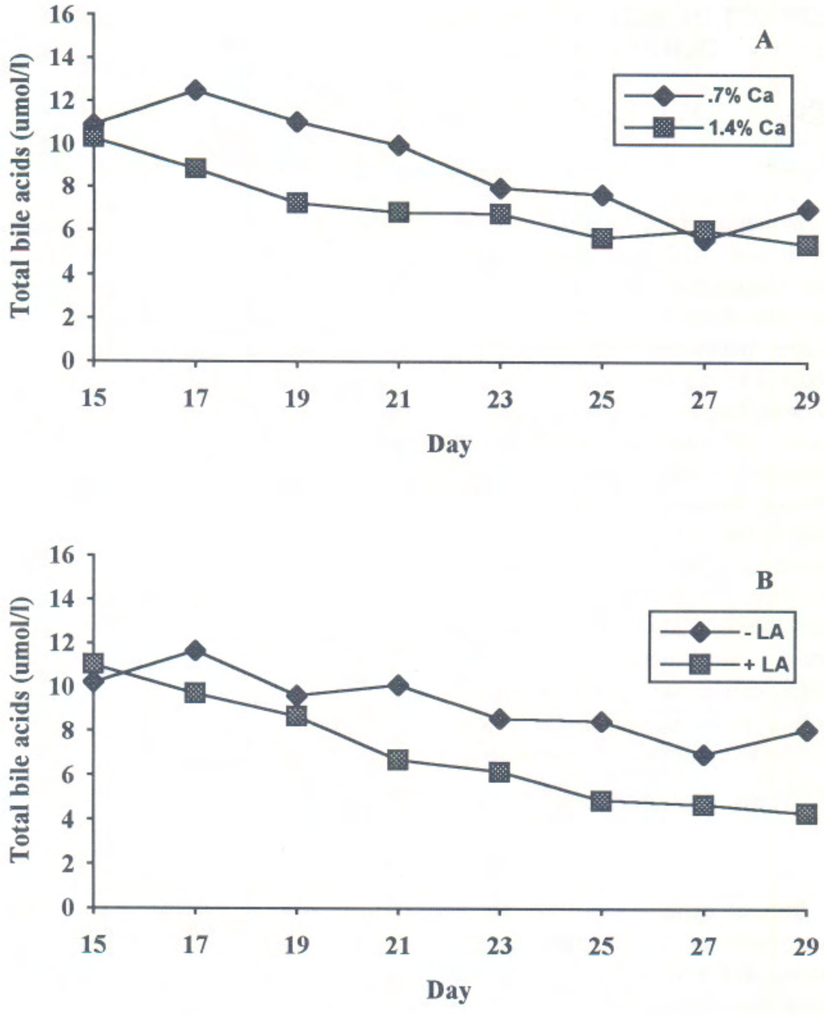

Figure 2. Effect of dietary Calcium (Panel A) and *Lactobacillus acidophilus* (Panel B) on total serum bile acids of pigs previously fed a high cholesterol diet. Values are least squares means. Pooled SE=1 µmol/l. Calcium effect (P<.08), *L. acidophilus* effect (P<.06), day on treatment effect (P<.01), day on treatment x calcium interaction (P<.2), and day on treatment x *L. acidophilus* interaction (P<.2).