INFLUENCE OF DEGREE OF BILE TOLERANCE ON ABILITY OF LACTOBACILLUS ACIDOPHILUS TO DEVELOP IN THE INTESTINES

S.E. Gilliland[1], T.E. Staley[2] and L.J. Bush[1]

Story in Brief

Cultures of lactobacilli identified as Lactobacillus acidophilus from the intestinal contents of young calves varied in their ability to grow in broth containing 0.3 percent ox bile as compared to control broth. Frozen concentrated cultures were prepared from a strain exhibiting low bile tolerance and from a strain exhibiting high bile tolerance. Results from a feeding trial involving new born dairy calves showed that supplementing the diet with the more bile resistant strain of L. acidophilus caused greater increases in the numbers of facultative lactobacilli in the upper small intestines than did the strain exhibiting lower bile resistance.

Introduction

The use of Lactobacillus acidophilus as a dietary adjunct can provide several benefits to the digestive system such as helping to control undesirable microorganisms in the intestinal tract. It also in beneficial to those individuals who cannot adequately digest lactose. Recent studies have suggested that the organism may be useful in helping control serum cholesterol levels.

The ability to grow in the presence of bile is a characteristic of L. acidophilus which has been identified as being important in permitting it to survive and grow in the intestinal tract. However, information is lacking as to the degree of bile resistance necessary to enable L. acidophilus to most effectively grow in the intestinal tract.

The objective of this study was to compare the ability of two strains of L. acidophilus exhibiting different degrees of bile resistance to increase the number of facultative lactobacilli in the intestinal tract.

Materials and Methods

Broth media prepared with and without 0.3 percent dried ox bile (bile salts) was inoculated with a freshly prepared broth culture of L. acidophilus. Growth at 37 C was monitored by measuring increases in turbidity. Growth curves were plotted and times required for the turbidity to reach an optical density of 0.3 determined.

Newborn Holstein and Ayrshire bull calves were randomly assigned to the respective treatment groups. The treatments included, Control, L. acidophilus C28, and L. acidophilus 27SC. Each calf was fed twice daily an amount of raw mixed herd milk equal to 11.25 percent of its metabolic size. The control group received raw milk without any addition. Sufficient concentrated culture of the appropriate strain of L. acidophilus was added to the milk of the remaining groups to yield a

[1]Professor, Animal Science [2]Professor, Physiological Sciences

total population of 1×10^{11} viable cells just prior to each feeding. After the seventh and eighth feeding, each calf was sacrificed and segments of the mid-jejunum, terminal ileum, and mid-large intestine were removed surgically, for microbiological analysis. The numbers of facultative lactobacilli were enumerated.

Results and Discussion

Comparison of the ability of 7 strains of L. acidophilus from the intestines of calves to grow in the broth with and without 0.3 percent bile salts revealed considerable variation among strains (Table 1). The bile salts exerted some inhibitory effect on all strains tested. Strains 27SC, 25SB, and 36SB were the most resistant strains. Strains C28, FR1 and FR2 has not reached an optical density of 0.3 at 1 hr. Because of identity characteristics and degree of bile tolerance, strain C28 was selected as one with low bile tolerance and strain 27SC as one with high bile tolerance for comparison in the feeding trial.

Table 1. Comparison of bile tolerance of strains of Lactobacillus acidophilus isolated from intestinal contents of calves.

	Hr to Reach Optical Density of 0.3	
Strain	MRS Broth	MRS Broth +0.3% Ox Bile
C28	2.62[a]	>6
30SC	3.67	5.60
27SC	2.37	2.82
25SB	2.17	2.62
36SB	2.38	2.72
FR1	3.02	>6
FR2	3.50	>6

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

Comparison of the influence of supplementing milk with the two strains of L. acidophilus upon the numbers of facultative lactobacilli in the intestinal tract are shown in Table 2. The data are presented as the mean $\log_{10}$/g dry wt of intestinal material for each segment sampled. In the jejunum, the numbers of facultative lactobacilli appeared to be greater in both groups of calves which received L. acidophilus, however, the difference was significant for only the group that received L. acidophilus 27SC ($P<.05$). In the ileum, the numbers were significantly higher ($P<.05$) for groups of calves receiving both strains of L. acidophilus than in the control group. The difference between the groups receiving the two strains of L. acidophilus was not significant ($P<.05$). In the large intestine, while the numbers of lactobacilli appeared to be greater in both groups which received L. acidophilus, neither was significantly greater than in the control group. It is interesting to note that in all groups the numbers of lactobacilli were higher in the large intestine than in either the jejunum or ileum.

Table 2. Influence of feeding cells of low and high bile tolerant Lactobacillus acidophilus on numbers of facultative lactobacilli in the intestines of dairy calves.

Treatment	Bile Resistance[b]	Log_{10} count/g dry wt[a]		
		Jejunum	Ileum	Large Intestine
Control	--	5.77[c]	5.57[c]	6.91
L. acidophilus C28	low	6.57[d]	7.00[d]	7.87
L. acidophilus 27SC	high	7.07[d]	6.79[d]	8.24

[a] Each value is a mean from 7 calves.
[b] Bile resistance based on comparison of culture growth in broth with and without 0.3% ox bile.
[c,d] Means in same column followed by different superscripts are significantly different ($P<.05$).

Of the two strains tested in the feeding trial, the one which was more bile tolerant resulted in greater numbers of facultative lactobacilli in the upper part of the small intestine (jejunum) than did the lesser bile resistant strains. The concentration of bile salts might be expected to be higher in this segment of the intestine than in the ileum since it is closer to the point where bile enters the intestinal tract. No significant difference in the numbers of lactobacilli was detected in the ileum in the groups receiving the two strains of L. acidophilus, although both strains caused significantly higher numbers than in the control groups. This was perhaps due to the bile concentration not being as great in the ileum as in the jejunum. The ability of L. acidophilus to cause significant increases in the numbers of lactobacilli in the upper portion of the small intestine may be very critical with regard to controlling the growth of intestinal pathogens entering the digestive system.