Influence of Feeding Cells of *Lactobacillus acidophilus* on the Fecal Flora of Young Dairy Calves

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Story in Brief

Newborn dairy calves were removed from their dams after nursing one time. All calves received one additional feeding of colostrum. They were then placed on a diet of pasteurized milk or non-fermented pasteurized milk containing a culture of *Lactobacillus acidophilus* of human intestinal origin or one of calf intestinal origin. During 14-day feeding periods, the numbers of lactobacilli in the feces of calves being fed *L. acidophilus* increased greater than in feces of control calves. The culture of calf origin caused the greatest increase. The increases in numbers of lactobacilli were accompanied by decreases in numbers of coliforms in the feces. The *L. acidophilus* of human origin caused the greatest decrease in numbers of coliforms.

Introduction

Some species of lactobacilli are involved in maintaining a proper balance among microorganisms in the intestinal tract (Sandine et al., 1972 and Speck 1976). Consumption of a product(s) containing viable cells of *L. acidophilus* has been useful in re-establishing the normal intestinal flora after oral antibiotic therapy (Sandine et al., 1972 and Speck 1976) as well in treating patients infected with intestinal pathogens (Tomic-Karovic and Fanjek, 1962). The administration of a non-fermented milk product containing *L. acidophilus* resulted in the appearance of significantly increased numbers of lactobacilli in the feces of healthy men (Gilliland et al., 1978). Some investigators have suggested that there exists host specificity among strains of *L. acidophilus*. Different biotypes of *L. acidophilus* were isolated from humans and chickens (Mitsuoka 1969). A strain isolated from a human could not be implanted in chickens (Morishita et al., 1971).

The objective of this study was to determine the effect(s) of feeding non-fermented milk containing *L. acidophilus* upon the numbers of lactobacilli and coliform bacteria in the intestinal contents of calves during the first one to two weeks of life. Two strains of *L. acidophilus* were compared, one isolated from human intestinal tract and one from the intestinal tract of a calf.

Experimental Procedure

Fifteen bull calves (Holstein or Ayrshire) were assigned to three treatment groups of five calves each. One group, designated as the control group, was fed cold pasteurized whole milk. A second group received cold pasteurized whole milk containing *L. acidophilus* NCFM (a human isolate) and a third group was fed the same milk containing *L. acidophilus* C-28 (a calf isolate).

After birth, the calves were allowed to nurse their dams once. The navel cords of the calves were then swabbed with iodine and covered to help prevent infection while transporting the calves to the experimental area. The calves were placed in individual stalls so that no two calves of the same treatment group were adjacent. A second colostrum feeding was given with a nursing bottle 14 hr after birth. This colostrum was from a supply of frozen pooled colostrum. The feeding of the experimental milks was

started with the third feeding. All animals were fed the indicated milk at 12.5 percent of metabolic size ($W^{.75}$) per day. The animals were fed twice daily.

To prepare the experimental milk, cells of *L. acidophilus* NCFM (or *L. acidophilus* C-28) were harvested by centrifugation from cultures grown in lactobacilli MRS broth (Difco). The cells were resuspended in a small amount of cold pasteurized whole milk then sufficient amounts were added to six gallon containers of cold pasteurized whole milk to achieve a population of about 1×10^7/ml. The milk was prepared fresh weekly and stored under refrigeration until fed.

Fecal samples were obtained by rectal stimulation from each animal on days 1, 7 and 14. The samples were collected in unused 8 oz cottage cheese cartons and immediately transported to the laboratory for analysis. Each sample was mixed and diluted with sterile 0.1 percent peptone; the appropriate dilutions were plated with the required media. Lactobacillus Selection Agar (LBS; BioQuest) was used to enumerate lactobacilli and violet red bile agar (VRBA; BioQuest) to enumerate coliforms. The LBS agar plates were incubated in a CO_2 atmosphere 48 hr at 37 C (Gilliland et al., 1978). The VRBA plates were incubated 24 hours at 37 C. The percent dry weight of each sample was determined using an oven drying method. The counts (lactobacilli and coliforms) were calculated on a dry weight basis.

Results and Discussion

Both lactobacillus cultures used to prepare the milk in this study were identified as being *L. acidophilus* using procedures described by Gilliland and Speck (1977). Cells from the same volumes of broth cultures were used to prepare each batch of milk for both strains of *L. acidophilus*. This resulted in the milk containing *L. acidophilus* C-28 having a population of approximately 1×10^7/ml and that containing *L. acidophilus* NCFM having a population of approximately 5×10^6/ml. The populations remained stable for each strain during the period (one week) the milk was held in refrigerated storage while being used in the feeding trial.

The numbers of lactobacilli detected in the feces of the calves increased during the 14-day feeding period for all three groups (Table 1). The increases observed for the control group probably represent the normal development of the intestinal flora in neonatal calves. The average numbers increased greater in the groups being fed milk containing cells of *L. acidophilus* than in the control group. The group receiving milk containing the strain (C-28) isolated from the intestinal contents of a calf exhibited the greatest increase. The difference in results observed using the two strains of *L. acidophilus* from different hosts may be due to host specificity of the strains. The strain (NCFM) from a human did not appear to establish as well as the other strain. Additional experiments will be needed to determine if host specificity occurs among strains of *L. acidophilus*. Greater increases in the numbers of lactobacilli appearing in the feces might occur if higher numbers were added to the milk. In experiments with

Table 1. Average numbers of facultative lactobacilli in feces from young calves being fed non-fermented milk containing *Lactobacillus acidophilus*.

Group	Lactobacilli[a]/g		
	Day 1	Day 7	Day 14
Control	7.50	8.24	8.20
L. acidophilus NCFM (Human Origin)	6.99	7.88	8.57
L. acidophilus C-28 (Calf Origin)	6.84	8.83	9.17

[a]Counts expressed as $\log_{10}$ of count per g dry wt. of feces.

Table 2. Average numbers of coliforms in feces from young calves being fed non-fermented milk containing *Lactobacillus acidophilus*.

Group	Coliforms[a]/g		
	Day 1	Day 2	Day 14
Control	9.52	9.44	9.21
L. acidophilus NCFM (Human Origin)	9.67	9.48	8.67
L. acidophilus C-28 L. (Calf Origin)	9.56	9.40	8.90

[a]Counts expressed as $\log_{10}$ of count per g dry wt. of feces.

humans, the numbers of *L. acidophilus* in milk being consumed influenced the number of lactobacilli that appeared in the feces (Gilliland et al.; 1978).

There was a decrease in the average numbers of coliforms in all groups during the 14-day feeding period (Table 2). The number decreased most in the two groups receiving milk that contained cells of *L. acidophilus*.

As the numbers of lactobacilli appearing in the feces increased (Table 1), the numbers of coliforms decreased (Table 2). This suggests that intestinal lactobacilli may exert an antagonistic action toward coliform bacteria in the intestine of calves. Such an antagonistic action could be very useful in controlling enteric pathogens such as enteropathogenic *Escherichia coli*.

While results in this study suggest a relationship between the numbers of lactobacilli and coliforms in the feces of young dairy calves, additional studies will be needed to determine if administration of *L. acidophilus* as a dietary adjunct can control enteric pathogens. These should include challenging the test animals with a pathogen such as enteropathogenic *E. coli*. Important factors to consider in selecting a *L. acidophilus* for such use are identity and viability of the organism, ability of the culture to survive in the host animal's intestinal tract, and its ability to inhibit enteric pathogens. Cultures of *L. acidophilus* could be evaluated for their ability to inhibit the pathogens in laboratory experiments prior to selecting one for an animal feeding study.

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