Influence of Feeding Fermented Colostrum and *Lactobacillus acidophilus* on Fecal Flora of Dairy Calves

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ABSTRACT

Twenty Holstein calves were assigned alternately at birth to diets of 1) fermented colostrum, 2) colostrum treated with 1% propionic acid, 3) whole milk, or 4) whole milk treated with *Lactobacillus acidophilus* (frozen concentrate culture) at 5 × 10⁸ organisms per litter. Diets were fed once daily for 3 wk at 10% of birth weight as the sole source of nutrients. Fecal samples were collected at 0, 7, 14, and 21 days of age and analyzed for coliform and lactobacilli numbers. Fermented colostrum diets did not alter coliform counts in feces of healthy calves. Fecal coliform counts of calves fed *L. acidophilus* decreased with time. Average fecal lactobacilli counts were lower for the colostrum diets than milk diets. The apparent lowered incidence of scours frequently reported in calves fed fermented colostrum diets was not reflected in major changes in fecal microflora under the conditions of this study.

INTRODUCTION

Fermented colostrum for young calves is an excellent source of nutrients (6). In addition to the nutritional and economic advantages, fermented colostrum has decreased the incidence of diarrhea in young calves from whole milk (10, 21, 27) and milk replacer (4, 26). Reasons for decreased diarrhea are not clear. One hypothesis is that lactobacilli in fermented colostrum adhere to and colonize the intestinal tract and, thereby, inhibit coliform growth either through bactericidal properties, the production of acids, or some other mechanism. The primary cause of diarrhea in calves is infection by *E. coli* (7).

The value of lactobacillus, specifically *L. acidophilus*, for humans was proposed originally by Metchnikoff (12). Recent reviews (20, 22, 24) have emphasized some of the potential benefits of lactobacillus, including a suppression of coliform growth, implantation in the intestinal tract, and decreased gastrointestinal problems and diarrhea. Administration of a nonfermented milk product containing *L. acidophilus* increased numbers of lactobacilli in the feces of healthy men (8). The value and use of acidophilus food products is based on some of these potential benefits (23, 24).

The use of *L. acidophilus* for livestock has not been studied extensively or with conclusive results. Feeding a dried commercial preparation (25) or a viable culture (1) of *L. acidophilus* reduced the incidence of diarrhea in dairy calves. Findings have been similar with pigs (15, 16). Feeding cultured milk prepared by inoculating pasteurized milk with *L. acidophilus* and *L. lactis* did not alter growth or health of calves compared to a control diet (13). Bruce et al. (3) recently reported that feeding *L. acidophilus* cultures of human or calf intestinal origin increased the numbers of lactobacilli in feces of calves and reduced the numbers of coliform in feces.

Since it is difficult to obtain intestinal samples for microbial analyses, enumeration of fecal microbial flora has been used as an indirect method of determining bacteria inhabiting the intestinal tract (3, 8, 15, 17, 19). The fecal flora is assumed to represent only the luminal flora and not that associated with mucosal epithelial surfaces and is assumed to vary with different types of diets (2).

The objective of this study was to observe
changes in fecal flora of calves fed excess colostrum, naturally fermented and acid preserved, and milk treated with a *L. acidophilus* culture of human origin.

**MATERIALS AND METHODS**

**Experimental Diets and Animal Handling**

Twenty Holstein calves (11 males and 9 females) were separated from their dam immediately after birth and hand-fed approximately 2.5 kg of their dam’s colostrum. The calves were fed an additional 2.5 kg of dam’s colostrum within the next 12 to 24 h and then moved to individual solid-walled pens in a heated and mechanically ventilated calf barn. Calves were assigned alternately at birth to one of four diets with five calves per diet. Diet 1 was naturally fermented colostrum (FC) composed of the first six milkings postpartum from Ayrshire, Holstein, and Brown Swiss cows. Diet 2 was the same colostrum treated with 1% by weight daily of propionic acid (PA). Calves on diets 1 and 2 were fed 3 kg of the respective diets diluted with 1.5 kg warm water daily. Diet 3 was whole milk (WM) drawn daily from the bulk cooler. Diet 4 was whole milk treated with *L. acidophilus* (LA). A commercial, viable human *L. acidophilus* product (Fargo 606 frozen concentrate by Microlife Technics) was added to warm whole milk immediately prior to feeding at a concentration of 1.06 x 10^6 organisms per liter. This dosage was based on a 10^8 dosage for humans (8). Calves on diets 3 and 4 were fed 4 kg of their respective diets daily. All diets were fed once daily by nipple pail for 21 days as the sole source of nutrients. A few calves were fed twice daily for 2 to 3 days until adjusted to diets, then once daily. Water was available free choice.

Colostrum for diets 1 and 2 were stored in 80-liter plastic containers for at least 7 days and not longer than 21 days prior to feeding. Colostrum was added daily to the containers until they were full; then they were covered tightly. Colostrum was stirred only after additions, before feeding and before samples were collected. Each calf was fed from one specific container of colostrum. The trial was from January to May during which the temperature of the calf barn was maintained at approximately 10 C.

**Sampling Procedures**

Sampling (200 to 300 ml) of each liquid diet was weekly for laboratory analysis. Also, 5 ml of each diet were collected into sterile tubes and mixed with 5 ml of a sterile buffered glycerol-saline solution adjusted to pH 7.4 for subsequent microbial analyses. Fecal samples were obtained at birth and 7, 14, and 21 days of age. Samples obtained from the rectum with sterile gloves were placed into sterile, plastic graduated centrifuge tubes. The fecal samples were diluted with five parts of the sterile buffered glycerol-saline solution to one part feces and mixed. The buffered glycerol-saline solution has been a widely used medium from preservation of fecal samples (5).

Since immediate analysis of liquid and fecal samples was not possible, samples were frozen for later analyses. Freezing of fecal samples in the buffered glycerol solution has yielded coliform counts nearly 80% of those from a fresh, unfrozen sample, while samples frozen without the buffered glycerol yielded counts of only 10% of the fresh samples (9). Fecal lactobacilli counts were similar to those reported by Bruce et al. (3) for fresh fecal samples, thus suggesting that freezing in buffered glycerol solution did not alter fecal lactobacilli counts.

Fecal samples were thawed and centrifuged at 2000 x g to obtain a wet packed volume of feces, and results were recorded as log per milliliter wet packed feces. This fecal precipitate was diluted 1:10 with .85% sterile saline solution. Serial dilutions then were with .85% sterile saline solution. Serial dilutions then were with .85% sterile saline in test tubes. Violet Red Bile agar (VRB) BBL and Lactobacillus Selective agar (LBS) BBL were used to enumerate coliforms and lactobacilli, respectively. Pour plates were made with the VRB agar and spread plates, with a glass spreading bar, were made on the LBS agar. Dilutions were 10^-3 to 10^-7. The VRB plates were incubated at 37 C for 24 h. The LBS plates were incubated at the same temperature for 48 h in a GasPak BBL anaerobic system with two disposable hydrogen and carbon dioxide generator envelopes. Colony counts were done manually with numbers over 300 designated as too numerous to count (TNTC). Plates that contained the 10^-7 dilution and were TNTC were assigned arbitrarily the number of 1 x 10^10 for analytical purposes.
Milk samples also were analyzed for coliform and lactobacilli numbers. Serial dilutions were made directly from the 1:1 dilution of milk and buffered glycerol. The dilutions that were used were $2 \times 10^{-1}$ to $2 \times 10^{-5}$. The agars and methods described for the fecal analyses were identical to those for milk samples except for the difference noted above.

Statistical Analyses

The test for determining significant differences among treatment effects was the F-ratio from the analyses of variance analyzing for log of bacterial counts. An orthogonal comparison technique was used to determine differences between means.

RESULTS AND DISCUSSION

Results of the chemical and microbial analyses for the four liquid diets are in Table 1. The chemical composition generally agrees with published components for colostrum and whole milk (6). Numbers of coliforms in both FC and PA diets were similar. Numbers in fermented colostrum agree with other reports but in PA were higher than reported (6). Numbers of lactobacilli were comparable to those in (11, 14).

Results of fecal microbial analyses are in Table 2, and Figure 1 and 2. All fecal samples at birth contained less than $10^3$ lactobacilli and less than $10^3$ coliforms per milliliter of wet packed feces and are not reported. No differences were significant in the numbers of coliforms in feces for all sampling times (Table 2), although counts tended to be lower from calves fed the fermented diets and the *L. acidophilus* supplemented diet. Calves fed the FC diet tended toward less coliforms for the three sampling times (Figure 1). Calves fed the LA diet had a linear decrease in coliforms (Figure 1), suggesting an antagonistic action toward coliforms or possible implantation of lactobacilli in the intestinal tract. This response is similar to studies with pigs (17). Bruce et al. (3) reported a greater decrease in coliforms from feces of calves fed *L. acidophilus* for the first 2 wk of age compared to a control diet. Differences were small between *L. acidophilus* cultures of human or calf origin (3). The largest decrease in fecal flora was at 3 wk for calves fed the LA diet ($P<.05$). No other study is available for comparison since Bruce et al. (3) did not measure fecal counts after 2 wk.

Calves fed the milk diets had significantly more lactobacilli per milliliter feces than calves fed colostrum diets. The lactobacilli counts from feces of calves fed the WM diet was unexpected and not readily explainable but persisted for all 3 wk (Figure 2). Bruce et al. (3) reported a greater increase in lactobacilli from feces of calves fed the *L. acidophilus* culture. A possible explanation for the lower lactobacilli from feces of calves fed the colostrum diets (FC and PA) is that lactobacilli in the diets were colonizing the small intestine. Only those organisms in excess were removed via the feces, resulting in little change in lactobacilli. Perhaps

<table>
<thead>
<tr>
<th>Item</th>
<th>FC</th>
<th>PA</th>
<th>WM</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (%)</td>
<td>15.50</td>
<td>15.80</td>
<td>12.18</td>
<td>12.18</td>
</tr>
<tr>
<td>SE</td>
<td>.28</td>
<td>.65</td>
<td>.18</td>
<td>.18</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.92</td>
<td>5.65</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>SE</td>
<td>.25</td>
<td>.25</td>
<td>.21</td>
<td>.21</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>4.90</td>
<td>5.01</td>
<td>2.81</td>
<td>2.81</td>
</tr>
<tr>
<td>SE</td>
<td>.16</td>
<td>.16</td>
<td>.10</td>
<td>.10</td>
</tr>
<tr>
<td>Coliforms b</td>
<td>3.81</td>
<td>4.20</td>
<td>&lt;1.30</td>
<td>&lt;1.30</td>
</tr>
<tr>
<td>SE</td>
<td>.07</td>
<td>.32</td>
<td>.10</td>
<td>.04</td>
</tr>
<tr>
<td>Lactobacilli b</td>
<td>5.56</td>
<td>5.82</td>
<td>2.66</td>
<td>3.18</td>
</tr>
<tr>
<td>SE</td>
<td>.27</td>
<td>.22</td>
<td>.10</td>
<td>.04</td>
</tr>
</tbody>
</table>

a14 samples for each of the FC and PA diets; 9 samples for each of the WM and LA diets.

b $\log_{10}$ of organisms/ml liquid diet.
TABLE 2. Mean fecal microbial counts averaged across the 7, 14, and 21 day sampling period for all calves on diets fermented colostrum (FC), FC treated with propionic acid (PA), whole milk (WM), and WM treated with \textit{L. acidophilus} (LA).

<table>
<thead>
<tr>
<th>Diet</th>
<th>FC</th>
<th>PA</th>
<th>WM</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms [^a]</td>
<td>7.03</td>
<td>7.28</td>
<td>7.33</td>
<td>6.86</td>
</tr>
<tr>
<td>Lactobacilli [^a]</td>
<td>6.93[^c]</td>
<td>7.34[^c]</td>
<td>8.60[^d]</td>
<td>8.15[^d]</td>
</tr>
<tr>
<td>L:C ratio [^b]</td>
<td>.99:1[^e]</td>
<td>1.01:1[^e]</td>
<td>1.17:1[^f]</td>
<td>1.19:1[^f]</td>
</tr>
</tbody>
</table>

\(^a\)Log\(_{10}\) of organisms/ml wet packed feces.
\(^b\)Lactobacilli to coliform ratio.
\(^c,d\)WM and LA>FC and PA (P<.01).
\(^e,f\)WM and LA>FC and PA (P<.05).

A factor is in fermented colostrum and not in whole milk which facilitates colonization in the intestinal tract.

Several researchers (8, 18) have suggested that feeding lactobacilli may not be effective unless stress is applied. Gilliland et al. (8) fed \textit{L. acidophilus} to healthy human males and found an increase in the number of lactobacilli in their feces but found no effect on coliform numbers. They suggested that this response may have been related to the test subjects being healthy and not experiencing intestinal disorders. In our study, diarrhea and health disorders were not

![Figure 1](image1.png)

Figure 1. Average number of coliforms in the feces of calves on all diets for individual sampling periods. (Vertical lines above the bars are standard errors.) FC, fermented colostrum; PA, propionic acid treated FC; WM, whole milk; LA, \textit{L. acidophilus} treated WM.

![Figure 2](image2.png)

Figure 2. Average number of lactobacilli in the feces of calves on all diets for individual sampling periods. (Vertical lines above the bars are standard error. \(\text{C}_1\) indicates WM and LA>FC and PA, \(P<.05\).) FC, fermented colostrum; PA, propionic and treated FC; WM, whole milk; LA, \textit{L. acidophilus} treated WM.
present and may account for the absence of significant changes in coliform numbers.

The results of this study suggest a possible relationship between the numbers of lactobacilli and coliforms in feces, particularly with calves fed a \textit{L. acidophilus} culture. The L:C ratio (Table 2) is consistent with that in healthy, nonscouring pigs (17). The L:C ratio increased most with age for the \textit{L. acidophilus} supplement diet, a similar increase with age as reported (3). Additional studies are needed to determine if \textit{L. acidophilus} administration can control enteric pathogens and to determine microbial survival in the intestinal tract and attachment to the intestinal mucosa. The apparent lowered incidence of diarrhea frequently reported in calves fed fermented colostrum diets (4, 6, 10, 21, 26, 27) was not reflected in major changes in fecal microflora under the conditions of this study, although a trend existed for lower fecal coliforms from calves fed fermented colostrum compared to the control.

REFERENCES


