Fecal D- and L-Lactate, Succinate, and Volatile Fatty Acid Levels in Young Dairy Calves

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(Received 14 February 2006/Accepted 22 May 2006)

ABSTRACT. For evaluation of physiologically significant organic anions in the colonic environment, 87 samples of normal feces were collected from the rectum of 15 calves less than 60 days old. The calves were fed milk replacer with free access to starter diet and hay. After fecal extraction with water, pH, D- and L-lactate, succinate, and volatile fatty acid (VFA) concentrations were determined. There was wide variation in fecal pH (4.3 to 7.7). Higher lactate concentrations were observed in feces samples with lower pH, and most of these samples were collected during the first 4 weeks of life. Elevated lactate concentrations included both the D- and L-isomers, and the D-isomer comprised approximately 30–50% of total lactate. Elevated succinate concentrations were observed in only 8 fecal samples, while other samples had lower or trace amounts of succinate. Elevated fecal succinate showed no relationship with fecal pH or VFA concentrations. Fecal VFA concentrations were lower in samples collected in the early postnatal stage, but fecal VFA concentrations were not necessarily related to age. We confirmed that fecal D- and L-lactate concentrations increased with a concomitant decrease of VFA in the acidic lumen of the colon, although acidic feces were not necessarily accompanied by elevated concentrations of lactate. In contrast, succinate production was not related to fecal pH or VFA concentrations.

KEY WORDS: calf feces, colonic fermentation, fecal lactate, fecal succinate, fecal VFA.

Newborn calves depend on the colon rather than the rumen for digestive fermentation because they obtain most nutrients from milk, not from solid diets, and this classifies them as hindgut-animals in the neonatal period [4]. As fermentation products, not only volatile fatty acid (VFA), but also organic acids such as lactate and succinate are present as intermediary metabolic products under the acidic conditions of the luminal digesta [8]. Since lactate and succinate are stronger acids compared with VFA [2, 3], it is believed that excess production of these intermediates affects the nutrition and pathophysiology of calves. Lactate is comprised of the D- and L-isomers; however, D-lactate originates from microorganisms in the gut, not from endogenous metabolism within the tissues of mammals [3, 9]. Absorption of a large quantity of D-lactate from the gut causes severe metabolic acidosis, including acidemia in neonatal calves, due to slower metabolic clearance of D-lactate in host mammals [3]. Thus, calves easily suffer serious clinical disturbances, such as impaired palpebral reflex and nervous signs [7]; however, their pathophysiological conditions depend not only on acid-base status or dehydration, but also on the specific effect of D-lactate [3, 6, 7]. Succinate production was elevated in some cases of non-pathogenic diarrhea in piglets [14] and in human patients with severe ulcerative colitis [13], although the importance of colonic and cecal succinate is unknown in calves. On the other hand, VFAs are easily absorbed, not only from the rumen, but also from the large intestine, and their absorption is most significant as a promoter of sodium and water absorption from the gut [4, 10]. Clarification of fermentation properties including colonic or fecal organic anions is required for clinical work by veterinarians. Therefore, the aim of the present study was to serve as a preliminary survey to evaluate normal fecal conditions in healthy young calves with regard to organic anions, such as lactate, that are related to isomers and succinate together with VFA concentrations.

MATERIALS AND METHODS

Animals: A total of 15 unweaned Holstein calves (male, 10; female, 5) were used. They were less than 60 days old and were given 4 or 5 kg of milk replacer daily in two meals with free access to starter diet, hay, and water.

Fecal samples and analyses: A total of 87 fecal samples were collected from the rectum of the 15 calves. Maximum sample numbers were 18 from one calf, followed by 12 from 2 calves, 5 to 7 samples from 3 calves, and 2 to 4 samples from 9 calves. The ages of the calves at sampling differed among individuals. Abnormal feces, such as diarrhea, were not included. Fresh feces (5 g) were extracted with 20 ml of water, and pH was measured by glass electrode. A portion of the extract was centrifuged (2,000 × g, 10 min), and the supernatant was frozen at –30°C until analysis. After thawing, VFA was analyzed by gas chromatography as reported previously [12]. The thawed supernatant was deproteinized by zinc sulfate and potassium hydroxide (Somogyi method). The D-lactate and L-lactate in the deproteinized supernatant were analyzed by UV method using D-lactate dehydrogenase (Oriental Yeast, Tokyo) in combination with a test kit (L-Lactic acid, 139084, Boehringer, Germany), and succinate was analyzed using a UV test kit (Succinic acid, 176281, Boehringer, Germany).

Data analyses: The effects of fecal pH and age of the calves at time of sampling on fecal organic acid concentra-
tions were evaluated by scatter and regression analyses. Since fecal lactate concentration was strongly related to fecal pH rather than age at time of sampling, all fecal samples were divided into three groups by pH as follows: 1) acidic group, pH less than 5; 2) sub-acidic group, pH 5 to less than 6; and 3) neutral group, pH higher than 6. Fecal data were statistically examined among the three groups by non-parametric test using Friedman’s and Scheffe’s tests. Statistical significance was defined as P<0.05.

RESULTS

The relationships between age and fecal pH, total lactate, and VFA concentrations are shown in Fig. 1. During the first 4 weeks of life, fecal pH fluctuated extensively within a wide range (4.3–7.7), and elevated concentrations of fecal lactate were observed. However, fecal samples collected during early life did not necessarily show elevated concentrations of lactate. Fecal VFA concentrations also fluctuated widely in the early postnatal period.

Comparisons of fecal properties among the 3 groups by fecal pH level are given in Table 1. The concentrations of lactate were the highest in the acidic group followed by the sub-acidic group. Significant differences were confirmed for D- and L-lactate between the neutral and other two groups. The proportion of D-isomer in total lactate was approximate 50% in the neutral and sub-acidic groups, but was 31% in the acidic group. On the other hand, VFA concentrations in the acidic group were significantly lower than those in the sub-acidic group, which had the highest concentrations, and the neutral group. Fecal samples with elevated concentrations of succinate were fewer than those with elevated concentrations of lactate. In 8 samples with elevated concentrations of succinate (>4 mM), 5 were collected from the same calf and the other 3 were from 2 other calves. There were no significant differences in succinate concentration among the 3 groups.

The relationships between fecal pH and organic acid concentrations are shown in Fig. 2. Most of the elevated lactate concentrations were observed in feces with a pH of less than 6. Elevation of lactate concentrations was accompanied by increases in both the D- and L-isomers. No clear relationship was observed between pH and succinate.

The relationships between fecal VFA and lactate and suc-

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Table 1. Comparison of lactate, succinate, and volatile fatty acid (VFA) concentrations among the acidic, sub-acidic, and neutral feces of the neonatal calves

<table>
<thead>
<tr>
<th>Group*</th>
<th>Lactate (mM)</th>
<th>Succinate (mM)</th>
<th>VFA (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>D</td>
<td>Total</td>
</tr>
<tr>
<td>Acidic</td>
<td>53.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sub-acidic</td>
<td>16.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neutral</td>
<td>1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Acidic, pH <5.0; Sub-acidic, 5.0 ≤ pH <6.0; Neutral, 6.0 ≤ pH.

a-c) Means with different superscripts within a column are significantly different (P<0.05).
Samples showing remarkable increases in lactate had a VFA concentration of less than 150 mM. However, samples with lower VFA concentrations were not necessarily accompanied by higher lactate concentrations. There was no clear relationship between fecal VFA and succinate concentrations.

**DISCUSSION**

Despite the importance of microbes in hind-gut digestion, colonic fermentation was investigated chemically by analyzing lactate, succinate and VFA concentrations in fresh feces from the present calves. Thus, discussion of the microbial significance is limited in the present study.

The fecal pH of the present calves showed a wide distribution (pH 4.3–7.7) compared with the narrower and more neutral range (pH 7.13 ± 0.13) of lactating dairy cows [12]. Most of acidic feces were observed in the first 4 weeks of life. According to the dissociation constants (pK) of lactate (pK=3.7) and VFA (pK=4.9) [3], lactate is a much stronger acid than VFA. Many cases of higher fecal lactate were confirmed in the early postnatal period. Considering the
neutrality of meconium (pH 6.8) in newborn calves [11], acidification of feces in early life is thought to be due to lactate production in the colon. Similar to the present data, higher lactic fermentation activity was well observed in the stomachs of newborn monogastric animals and in the rumen and abomasum of neonatal calves [3].

Critical lowering of colonic pH, which shifts the metabolism from VFA to lactate fermentation, is responsible for generating electron sink as colonic lactate [8], pathogenesis of epithelial damage of the colon [1], colitis in human patients [15], and osmotic diarrhea in domestic animals [1, 13, 14]. Pathogenesis of higher lactate was not obvious in our calves, although its significance cannot be disregarded. However, lactate is considered to function as the main anion in the immature gut flora of the colonic lumen in early life. Thereafter colonic lactate is replaced by VFA with advancing age.

Digestion of excess amounts of fermentable carbohydrate should lead to production of lactate, which should include both isomers. The increased lactate concentrations of the present calves included both the D- and L-lactate isomers. Besides the digesta or feces, elevation of plasma D-lactate in normal calves has also been observed previously [6]. The alimentary digesta and some diets include both D- and L-isomers, with somewhat lower proportions of the D-isomer than the L-isomer, although the ratio varies case-by-case [3]. The present result, which show approximate 30–50% D-isomer in total lactate, are in agreement with the above-mentioned findings [3]. From the standpoint of metabolic peculiarity of D-lactate in mammals [3, 6, 7], most neonatal calves seem to be confronted frequently with the challenge of D-lactate burden, despite having no native production of D-lactate.

Succinate is also an intermediary metabolite in the cascade of propionate production, and more succinate is accumulated under some acidic luminal conditions [5, 13]. As with lactate, succinate acts as a strong acid (pK=4.2) [2]. However, there were a few fecal samples with higher concentrations of succinate, and elevated concentrations of succinate were not necessarily found in acidic feces. Consequently, it seems likely that succinate production is not necessarily influenced by acidification of the digesta. As in our study, notable individual differences in cecal succinate concentrations were also observed in rats despite the same dietary conditions [5]. It seems to be difficult to evaluate colonic fermentation by fecal succinate concentration. Succinate and lactate, which are intermediates in global fermentation, are finally metabolized to VFA to varying extents in the gut [8, 14].

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Fecal VFA is a general reflection of colonic fermentation of nutrients that have escaped gastric or small intestinal digestion. Many of the feces samples of the present calves had higher VFA concentrations than those of adult cows, which are generally less than 100 mM [12]. However, lower VFA was observed in many feces samples during the postnatal period and in some samples thereafter (Fig. 1). Lower fecal VFA in early life appears to reflect accelerated lactic fermentation in the acidic lumen at the expense of VFA production. However, lower VFA concentrations with advancing age might be due to inadequate feed intake, and this is likely given that adult cases with lower fecal VFA have been observed in dry cows, which have a lower feed intake than lactating cows [12]. Luminal VFA is rapidly absorbed from the cecum and colon, as well as from the rumen. This process provides energy for sodium and water absorption from the large intestine [4]. Consequently, neonatal calves with less VFA production are also likely to suffer diarrhea.

Conclusion: Decreased fecal pH due to accelerated production of lactate was accompanied by lower VFA production in newborn calves. Elevated fecal lactate concentrations were comprised of both the D- and L-isomers, but not by any one isomer. Elevated lactate production accompanying lower VFA might be related to pathogenesis in the colon. The number of samples with higher concentrations of succinate was less than those with higher concentrations of lactate, and the succinate concentration had no relationship with fecal pH and the VFA concentration.

Fig. 3. Relationships among fecal volatile fatty acid (VFA), succinate, and lactate concentrations (n=87).
This work was supported by a grant to the High Technological Research Center, Rakuno Gakuen University, from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES