Fecal Trimethylamine, Lactate and Volatile Fatty Acid Concentrations and Their Relationships with Diarrhea in Newborn Dairy Calves

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ABSTRACT. To clarify the significance of fecal trimethylamine, lactate and volatile fatty acids (VFAs) in fermentative development of the hindgut in neonatal calves and the occurrence of diarrhea, 143 fecal samples (47 diarrheic, 93 normal and 3 meconium) were collected from 58 dairy calves fed milk. An additional 20 fecal samples were obtained from 10 weaned calves aged 13 to 18 weeks. Fecal pH, trimethylamine, ammonia, lactate and VFAs were analyzed. Compared with weaned calves, the trimethylamine level was higher in milk-fed calves and was associated with a large number of cases of diarrhea, but it had little relationship with the fecal ammonia level. Feces collected from the youngest (<2 weeks age) calves were more acidic and were associated with a higher lactate concentration. Lactic fermentation and fecal acidity were tremendously accelerated, particularly in diarrhea. Despite large fluctuations, the VFA concentrations were lower in diarrheic than in normal feces. In diarrheic feces at the youngest stage, the lower proportion of n-butyrate in the VFAs was accompanied by the elevated proportion of acetate. However, the fermentative differences between the diarrheic and normal feces were less apparent with advancing age. Thus, the fecal lactate and VFA profiles revealed marked changes with advancing age and suffering from diarrhea. Upon comparison with weaned calves, the trimethylamine level was clearly higher in the milk-fed calves and showed huge elevations in diarrheic cases.

KEY WORDS: calf feces, colonic lactate, diarrhea, fecal trimethylamine, fecal VFA.

Unlike adult ruminants, newborn calves depend on the colon rather than the rumen (foregut) for digestive fermentation because they gain most of their nutrients from milk feeding as opposed to solid food [1]. For this reason, they are therefore called hindgut animals or preruminants during the neonatal period involving milk feeding [1, 2]. Thus, the colon is the primary site of early microbial colonization in these calves, although their gut flora remains immature at this life stage [2]. Nutrients that escape digestion and absorption in the small intestine undergo large-bowel fermentation, which is accompanied by the production of lactate, volatile fatty acids (VFAs), gas and nitrogenous intermediates such as trimethylamine. The physiological significance of VFAs and lactate in gut health has received considerable attention in humans [7, 15, 16] and animal nutrition [16, 18] during the last few decades. In addition, trimethylamine, which is a degenerative product of intestinal bacteria, is often associated with foul odors in the atmosphere and also has significance in nutritional, clinical and malodor characterization in healthy humans [13, 21]. Aside from the air pollution resulting from excess trimethylamine production in the livestock industry [20], little attention has been focused on trimethylamine formation in the gut and its clinical significance in dairy and beef cattle. The aims of this comparative study, therefore, were to examine the production of trimethylamine and fermentative organic anions, including lactate and VFAs, in the colon in relation to normal hindgut development and occurrence of sporadic diarrhea in young calves.

MATERIALS AND METHODS

Animals: A total of 58 (32 male, 26 female) milk-fed calves, consisting of 55 Holstein and 3 Jersey calves, were used. The calves were less than 60 days old, were fed 3 to 5 kg of a milk diet (milk replacer) daily in two meals and had free access to starter feed, hay and fresh water. The protein of the milk replacer consisted entirely of skim milk and whey and did not include proteins of vegetable or fish origin.

To allow comparisons to be made with calves having higher solid feed intake and functional rumen, an additional 10 weaned calves (13–18 weeks old) were used.

Fecal collection and analyses: From the 58 milk-fed calves, a total of 143 fecal samples, including 47 diarrheic and 3 meconium samples, were collected by rectum grabbing. Multiple samples were collected from 37 individuals during the course of the study, which consisted of duplicate samples in 19 calves, 3 to 5 samples in 13 calves and 6 to 7 samples in 5 calves. In several diarrheic feces, the shedding of cryptosporidial or coccidial oocysts was confirmed; however, the pathogenesis of all the diarrheic cases could not be determined. Additionally, 20 fecal samples were collected from 10 weaned calves that did not suffer from diarrhea.

Immediately after collection, the fresh feces were subjected to extraction with four parts water, and the pH of each extract was then measured using a glass electrode. The extract was centrifuged at 2,500 × g for 10 min, and the
RESULTS

As multiple sampling was conducted in 37 calves, both normal and diarrheic samples were collected from 14 individuals. Accordingly, all of the obtained data were divided into two categories of normal or diarrhea at sampling, irrespective of the individuals from which they were collected.

Postnatal comparisons in normal feces: For normal feces, comparisons among the four age stages, including the weaned stage, are summarized in Table 1. In the youngest calves (<2 wk), the fecal pH (mean 6.26) was lower compared with the other stages. There were no significant differences in the trimethylamine levels among the three stages during milk feeding; however, the level from the milk-fed calves was markedly higher than in those from the weaned calves ($P<0.05$). The fecal ammonia and lactate levels at <2 wk and 2 to <4 wk were higher than in samples from calves older than 4 weeks ($P<0.05$). Fecal VFA concentrations showed no significant difference among the four stages, although there was a higher tendency in the weaned calves. Higher proportions of n-butyrate along with lower proportion of acetate were observed at the younger stages, particularly at <2 wk, than those thereafter.

Comparisons between normal and diarrheic feces: Table 1 also shows comparisons between normal and diarrheic fecal profiles. The fecal acidity and lactate levels were markedly higher in diarrheic samples particularly at <2 wk, whereas the VFA level in diarrheic feces was significantly lower than that in normal feces throughout the milk-fed stages ($P<0.001$ or $P<0.01$). Despite an increased tendency for a higher trimethylamine level in diarrhea, the differences between normal and diarrheic feces were not significant for either trimethylamine or ammonia. The proportion of n-butyrate was significantly lower in diarrheic feces than in normal feces at <2 wk and 2 to <4 wk and was inversely

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Table 1. Fecal trimethylamine, ammonia, lactate and volatile fatty acids (VFAs) concentrations in newborn calves

<table>
<thead>
<tr>
<th>Feces</th>
<th>Age (week)</th>
<th>pH</th>
<th>Trimethylamine</th>
<th>Ammonia</th>
<th>Lactate</th>
<th>VFAs</th>
<th>Acetate</th>
<th>Propionate</th>
<th>n-Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>to &lt;2†</td>
<td>Mean</td>
<td>6.26(a)</td>
<td>0.55(a)</td>
<td>39.9(a)</td>
<td>4.4(a)</td>
<td>126</td>
<td>51.0(a)</td>
<td>18.4(b)</td>
</tr>
<tr>
<td></td>
<td>(n=23)</td>
<td>SD</td>
<td>0.37</td>
<td>0.37</td>
<td>19.4</td>
<td>9.6</td>
<td>54</td>
<td>13.9</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>to &lt;4</td>
<td>Mean</td>
<td>6.83(b)</td>
<td>0.44(b)</td>
<td>26.0(b)</td>
<td>3.4(b)</td>
<td>109</td>
<td>58.5(b)</td>
<td>22.2(b)</td>
</tr>
<tr>
<td></td>
<td>(n=36)</td>
<td>SD</td>
<td>0.51</td>
<td>0.35</td>
<td>16.9</td>
<td>8.2</td>
<td>44</td>
<td>8.5</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>4 to 8</td>
<td>Mean</td>
<td>7.38(c)</td>
<td>0.32(c)</td>
<td>16.5(c)</td>
<td>0.3(c)</td>
<td>116</td>
<td>62.4(c)</td>
<td>22.7(c)</td>
</tr>
<tr>
<td></td>
<td>(n=32)</td>
<td>SD</td>
<td>0.34</td>
<td>0.25</td>
<td>11.2</td>
<td>0.2</td>
<td>50</td>
<td>7.3</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>13 to 18</td>
<td>Mean</td>
<td>7.31(d)</td>
<td>0.08(d)</td>
<td>12.9(d)</td>
<td>0.3(d)</td>
<td>149</td>
<td>70.6(d)</td>
<td>17.4(d)</td>
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<tr>
<td></td>
<td>(n=20)</td>
<td>SD</td>
<td>0.23</td>
<td>0.06</td>
<td>7.9</td>
<td>0.1</td>
<td>42</td>
<td>7.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>to &lt;2†</td>
<td>Mean</td>
<td>5.51</td>
<td>0.69</td>
<td>13.2</td>
<td>32.3</td>
<td>44***</td>
<td>71.3</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>(n=18)</td>
<td>SD</td>
<td>0.93</td>
<td>0.66</td>
<td>13.4</td>
<td>22.9</td>
<td>36</td>
<td>14.6</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>to &lt;4</td>
<td>Mean</td>
<td>6.53</td>
<td>1.23</td>
<td>18.6</td>
<td>10.4</td>
<td>68**</td>
<td>59.9</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>SD</td>
<td>0.63</td>
<td>1.64</td>
<td>12.9</td>
<td>19.4</td>
<td>44</td>
<td>9.8</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>4 to 8</td>
<td>Mean</td>
<td>7.28</td>
<td>0.42</td>
<td>16.7</td>
<td>0.3</td>
<td>71**</td>
<td>64.2</td>
<td>21.1</td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>SD</td>
<td>0.44</td>
<td>0.27</td>
<td>12.7</td>
<td>0.2</td>
<td>36</td>
<td>5.8</td>
<td>4.6</td>
</tr>
</tbody>
</table>

†: Samples less than 5 days old were not included.
*, **, ***: Significant difference compared with normal feces at the same age (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).
a), b), c): In normal feces, means with different superscripts within a column are significantly different ($P<0.05$).
related to that of acetate at the youngest stage. The deviated fecal profiles observed in the diarrheic samples were resolved with advancing age, with the exception of that of the VFAs (Table 1).

**Distribution and relationships among fermentative products:** The distribution of fecal pH and concentrations of trimethylamine, lactate and VFAs during the milk-fed period and their regression lines are shown in Fig. 1. The meconium samples contained trace amounts of trimethylamine and organic acids; however, fecal acidity and lactate increased promptly after birth, particularly in the diarrheic samples ($R^2=0.58$). Fecal trimethylamine showed obscure transitions with advancing age in either the normal or diarrhea during the milk-fed period, although several diarrheic samples showed extremely high trimethylamine levels. Furthermore, fecal trimethylamine showed no definite relationships with any other fecal profiles, including ammonia. In spite of the wide fluctuation of VFA concentrations in both normal and diarrheic feces, a trend of decreasing VFAs in diarrhea was clearly evident.

**DISCUSSION**

**Postnatal changes of trimethylamine and diarrhea:** The appearance of a higher trimethylamine level in the colon of milk-fed calves than weaned calves has attracted much interest. Compared with adults in humans, the fluctuations in colonic and fecal microbial populations are generally larger in young infants and are characterized by wide day-to-day and dietary-induced variations [7]. The degradation of dietary proteinaceous substrates by the gut microflora frequently produces many nitrogenous metabolites, including bioactive amines. Although seafood generally contains large amounts of trimethylamine or its precursors, the milk replacer fed to the present calves did not contain any fish-based products. Based on the present finding of a higher fecal trimethylamine level in milk-fed calves, but not weaned calves, it is therefore concluded that trimethylamine was produced from milk substrates and not from the starter feed (concentrate diet). Indeed, trimethylamine is also produced by bacterial degeneration of lecithin, choline and betaine, which are often included as nutrients in milk replacer [5].

In several of the diarrheic feces, extremely huge levels of trimethylamine were found (Fig. 1b); however, no significant difference was identified on an average between the normal and diarrheic feces (Table 1). The diarrheic colon might promote a degeneration of proteinous nutrients that escaped absorption in the small intestine, particularly in the youngest neonates, leading a higher level of fecal trimethylamine. Similar to the high trimethylamine level observed in several feces (Fig. 1b) in the present study, a substantial amount (3.5 mM on the average; highest 9.2 mM) was found in the feces of healthy human adults who consumed western-style diets [13].

**Fate of trimethylamine:** Trimethylamine has been specified as an illegal material by the Malodor Prevention Law in Japan. Limiting the production of trimethylamine from livestock yards has been declared an urgent task [20], even though the trimethylamine absorbed by animals is mainly oxidized by the liver to odorless and tasteless metabolites
that are subsequently excreted in urine [13]. However, it has been reported that certain humans [21] and dairy cows [3, 5] excrete substantial amounts of trimethylamine in their breath, urine, sweat and milk, resulting in a characteristic fishy odor and an off-flavor of bovine milk, although the detailed mechanisms remain a matter of debate. In the present study, a definite relationship was not observed between the trimethylamine level and fecal pH or VFA concentrations, although a potential decrease of toxic amine excretions was suggested by increasing the supply of fermentable carbohydrates or dietary fiber in human food [13]. The lack of association between the fecal ammonia and trimethylamine levels may be a reflection of the independent route by which each precursory compound is metabolized. Higher ammonia level and increased acidity were observed in normal feces at <2 wk. This result may be a reflection of a functional excretion of protons via ammonia into the colonic lumen, but this process may be hindered under diarrheic conditions.

**Fecal acidity and organic anions:** Postnatal transitions of fecal pH and the lactate level were typically observed (Fig. 1a and 1c) in accordance with previous results [12]. Nearly all alimentary lactate, succinate and ethanol represent intermediates in the global fermentation cascade of VFA production [6]. Lactate accumulation is not only a result of enhanced lactate production; it can also be caused by the collapse of lactate-utilizing bacterial populations, at the expense of VFA production in the acidic luminal milieu [6]. This was evident upon comparison of the lactic acid and VFA levels between the normal and diarrheic feces at <2 wk (Table 1). Lactate is a stronger acid (pK=3.7) than VFAs (pK=4.8 – 4.9) and acts as a proton sink in the fermentation process [6, 8]. The role of lactic fermentation in the colon is well recognized for promoting gut health, particularly in the neonatal period [4, 11]; however, with advancing age, lactate production is replaced by production of VFAs. Despite its beneficial effects at moderate levels, excessive accumulation of lactate and increased acidity have a deleterious impact on the colonic mucosa [14, 17], which suggests that excessive accumulation of lactate must be alleviated to maintain gut integrity, as reported in humans [14, 17, 19].

After birth, the sterile colon is rapidly exposed to the surrounding microbes from the dam, bedding, barn and other numerous sources. Immediately after birth, microorganisms such as *Escherichia coli* and *Streptococci* spp. dominate initially in the colon, followed by the appearance of obligate anaerobes some days later in most mammals [6, 7]. Although the first colonizing microflora also produces lactate and VFAs, the colonic flora and VFA profiles are not yet stable during the neonatal period [6].

The lower proportion of n-butyrate in diarrhea is worth noting. This result is consistent with a finding that increased n-butyrate proportions were associated with recovery from diarrhea in young calves [9]. This implies a physiological significance of n-butyrate for intestinal health. In fact, the integrity of colonocytes is controlled by n-butyrate, which acts as an oxidative fuel of intestinal cells, as a driving force for water and sodium absorption, on normal proliferation and differentiation of colonocytes, as an anticancer substance and on other process [15, 18, 19].

In conclusion, the youngest calves had more acidic feces with higher lactate production. Lactate and n-butyrate have a strong significance for colonic health and also diarrhea in the neonatal period. Despite in diarrhea; however, the deviated fecal profiles with exceptions of VFAs returned to those in normal ones with advancing age, even as early as in the preruminant period prior to weaning. On the other hand, fecal trimethylamine in neonatal calves was controlled mainly by colonic degeneration of milk substrate, not solid feed.

**REFERENCES**

15. Topping, D. L. and Clifton, P. M. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and non-


