Performance and plasma metabolites of dairy calves fed starter containing sodium butyrate, calcium propionate or sodium monensin

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This study was conducted to examine the influence of supplementation of sodium butyrate, sodium monensin or calcium propionate in a starter diet on the performance and selected plasma metabolites (plasma glucose, non-esterified fatty acids and β-hydroxybutyrate) of Holstein calves during pre- and post-weaning periods. Twenty-four newborn Holstein calves were housed in individual hutches until 10 weeks of life, receiving water free choice, and fed four liters of milk daily. Calves were blocked according to weight and date of birth, and allocated to one of the following treatments, according to the additive in the starter: (i) sodium butyrate (150 g/kg); (ii) sodium monensin (30 mg/kg); and (iii) calcium propionate (150 g/kg). During 10 weeks, calves received starter ad libitum, while coast cross hay (Cynodon dactylon (L.) pers.) was offered after weaning, which occurred at the 8th week of age. Weekly, calves were weighted and evaluated for body measurements. Blood samples were taken weekly after the fourth week of age, 2 hours after the morning feeding, for determination of plasma metabolites. No differences were observed among treatments for starter or hay intake, BW and daily gain of the animals. Mean concentrations of selected plasma metabolites were similar in calves fed a starter supplemented with sodium butyrate, sodium monensin and calcium propionate. There was significant reduction in the concentrations of plasma glucose as calves aged. The inclusion of sodium butyrate, calcium propionate or sodium monensin as additives in starter feeds resulted in equal animal performance, before and after weaning, suggesting that sodium monensin may be replaced by organic acid salts.

Keywords: additives, early weaning, ionophores, ruminal development

Implications

This study was conducted to evaluate organic acid salts inclusion in calf starters as a replacement to the traditional use of ionophore, since these antibiotics were banned as growth promoters in the European Union. Since all commercial starters include ionophores, an alternative additive that could result in the same pattern of animal performance should be studied. The organic acid salts were chosen because they lack the antibiotic effect but could have beneficial effects on the rumen development processes. In addition, as seen in the non-ruminant industry, acidified feeds may have a beneficial effect on the health of the lower gastrointestinal tract.

Introduction

Calf rumen development, crucial for the early weaning program, is strongly related to the intake of fermentable organic matter and the resulting short-chain fatty acids (SCFA; Greenwood et al., 1997). Ruminal metabolism studies have pointed out butyric and propionic acids as the main stimuli for rumen development and the maturation process (Quigley et al., 1992), which suggests that additives that furnish higher SCFA concentrations or alter the rumen fermentation pattern may be used as a tool for reducing the milk feeding period.

The inclusion of ionophores in ruminant diets has shown changes in the SCFA concentrations, mainly with increases in propionic acid and reduction in acetic and butyric acid, as a result of its effect in selecting against Gram-positive bacteria in the rumen (Russell and Strobel, 1989). Moreover, positive effects are observed in animal growth in early weaning program systems with the use of ionophores (Fitzgerald and Mansfield, 1984; Watkins et al., 1987; Anderson et al., 1988). However, other authors did not observe differences in performance when ionophores were fed via milk replacer (Quigley et al., 1992) or starter (Nussio et al., 2002; Nussio et al., 2003a; Klotz and Heitmann, 2006), probably because...
of variation in the rumen development among evaluated animals. However, even though in several countries sodium monensin is included in most commercial starters, the use of ionophores as growth promoters has been already banned in the European Union, which may be a tendency in other countries.

Salts of SCFA may be considered as an alternative to replace sodium monensin as additives for starters for calves with under-developed rumen. In vivo investigations with non-ruminant tissues have shown that sodium butyrate is effective in stimulating cellular growth (Janssens and Nollet, 2002). Calcium propionate has been used as a readily available energy source to prevent metabolic disorders in cows. Even though in vitro and in vivo trials with infusion of acids suggest positive effects of the use of butyric or propionic acids on rumen development, no literature was found regarding its inclusion in starters and effects on forestomach maturation and animal growth.

The objective of this study was to evaluate the effects of inclusion of sodium butyrate, sodium monensin and calcium propionate in starters on heifer calves’ performance and plasma metabolites, before and after weaning.

Material and methods

Twenty-four Holstein heifer calves were utilized in a completely randomized block experimental design. Trial was conducted at the Embrapa Southeast Cattle, SP, Brazil, between March and November 2006. After birth, calves were separated from the dams and housed in individual hutches, receiving 2 l of colostrum as soon as possible, and every 12 h during 2 days. After that, calves received daily 4 l of whole milk, split into two meals (7 and 18 h), starter ad libitum and water free choice.

Calves were blocked considering age and weight of birth, and distributed in one of the treatments according to the additive included in the commercial starter: (i) sodium butyrate (150 g/kg of dry matter (DM) basis; Adimix Hay (Cynodon dactylon (L.) pers.) was offered ad libitum, in regardless of concentrate intake. After weaning, coast cross buckets, after orts removal, which was weighted daily.

The calves were weighed at birth and once every week, before morning feeding, until 10 weeks of age. Using a ruler with a centimeter scale, wither heights and hip width were measured weekly; heart girth was measured by a flexible tape, also graded in centimeters.

Blood samples were taken weekly, 2 h after morning feeding, from the 4th week of age until the end of the trial, via jugular venipuncture by vacuum tubes containing sodium fluoride and potassium ethylenediamine tetraacetic acid. Samples were centrifuged at 2000 × g (20 min at 4°C) and plasma was stored until analyses. As indicators of rumen development, plasma glucose, β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFAs) concentrations were evaluated. Plasma glucose was determined by the auto analyzer YSI 2700 (Biochemistry Analyser, Yellow Spring, OH, USA). NEFAs were measured using the commercial kit NEFA-c (Wako Chemicals GmbH, Richmond, VA, USA), with modifications for a microplate reading (BIO-RAD, Hercules, CA, USA), using an absorbance filter of 540 nm. Determinations of plasma BHBA were conducted using a commercial kit by CATAGEM C444-10, with adaptations for a microplate reading (BIO-RAD, Hercules, CA, EUA), using an absorbance filter of 340 nm.

Data of DM intake, BW, average daily gain (ADG) and body measurements were analyzed as repeated measures by the PROC MIXED of SAS according to the model (1). Plasma concentrations of glucose, NEFA and BHBA were analyzed by the PROC MIXED of SAS, including week of age in the model (2). Significance was adopted for values of P < 0.05 for all parameters.

\[ Y_{ijk} = \mu + T_i + B_j + E_{ijk} \]  

\[ Y_{ijk} = \mu + T_i + B_j + W_k + E_{ijk} \]  

Table 1 Chemical composition of starters feed and coast-cross hay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B</th>
<th>P</th>
<th>M</th>
<th>Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (g/kg)</td>
<td>900</td>
<td>905</td>
<td>898</td>
<td>916</td>
</tr>
<tr>
<td>CP (g/kg DM)</td>
<td>213</td>
<td>223</td>
<td>224</td>
<td>81</td>
</tr>
<tr>
<td>Ether extract (g/kg DM)</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Starch (g/kg DM)</td>
<td>377</td>
<td>359</td>
<td>354</td>
<td>–</td>
</tr>
<tr>
<td>Neutral detergent fiber (g/kg DM)</td>
<td>239</td>
<td>213</td>
<td>256</td>
<td>784</td>
</tr>
<tr>
<td>Total digestible nutrients (g/kg DM)</td>
<td>721</td>
<td>718</td>
<td>714</td>
<td>519</td>
</tr>
</tbody>
</table>

DM = dry matter.  
*Starter containing B = sodium butyrate; P = calcium propionate; M = sodium monensin.

Table 1 Chemical composition of starters feed and coast-cross hay
Additives inclusion in starter for dairy calves

Table 2 Least-square means of starter (g DM/day) and hay (g DM/week) intake by heifer calves receiving starter containing sodium butyrate, sodium monensin or calcium propionate

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B</th>
<th>P</th>
<th>M</th>
<th>s.e.m.</th>
<th>T</th>
<th>A</th>
<th>T × A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-weaning</td>
<td>343.8</td>
<td>483.8</td>
<td>429.0</td>
<td>65.0</td>
<td>0.31</td>
<td>&lt;0.0001</td>
<td>0.12</td>
</tr>
<tr>
<td>At weaning</td>
<td>962.1</td>
<td>1231.4</td>
<td>1091.1</td>
<td>124.6</td>
<td>0.32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Post-weaning</td>
<td>1664.3</td>
<td>1643.1</td>
<td>1578.7</td>
<td>97.2</td>
<td>0.82</td>
<td>0.005</td>
<td>0.77</td>
</tr>
<tr>
<td>Hay intake</td>
<td>316.5</td>
<td>267.7</td>
<td>349.7</td>
<td>66.42</td>
<td>0.70</td>
<td>0.50</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 DM = dry matter.
2 Starter containing B = sodium butyrate; P = calcium propionate; M = sodium monensin.

where, $Y_{ijk}$ is the response variable, $\mu$ is the overall mean, $T_i$ is the treatment effect, $B_j$ is the block effect, $W_k$ is the age effect and $E_{ijk}$ is the residual effect.

Results and discussion

Table 2 presents DM intake of starter and hay. Starter intake was not affected by treatments; however, as expected, there was a significant age effect, with increasing starter intake as calves aged. Other authors have also observed increases in starter intake with age of calves, mainly as a result of rumen development and increased intake capacity during the first weeks of life (Klein et al., 1987; Luchini et al., 1991; Greenwood et al., 1997; Klotz and Heitmann, 2006).

The interaction effect of treatment and age was significant, with lower starter intake for the sodium butyrate treatment as compared to the starter containing sodium monensin or calcium propionate during the 6th and 7th weeks and as compared only to calcium propionate during the 8th week (Figure 1). Even though the sodium butyrate-containing starter has resulted in lower intake during those weeks, there were no differences among treatments at weaning, when animals consumed about 1 kg DM/day.

Starter intake values were higher than expected according to the review of Davis and Drackley (1998), although observed starter intakes before weaning were similar to those presented by Quigley (1996b). However, Nussio et al. (2003a) observed low intake by calves receiving starter with or without monensin inclusion during the milk feeding period, which may have decreased animal performance during the subsequent period.

Several authors have found lower starter intake by calves fed a starter containing monensin when compared to control. According to Nussio et al. (2002), inclusion of monensin in the starters for calves may have a negative influence on solid feed intake. These authors observed lower intakes of starter with monensin during the period after weaning, suggesting that the negative effect may occur when the animal presents the rumen partly developed. In addition, literature frequently shows the inhibitory effect of monensin on DM intake by adult animals (McGuffey et al., 2001). However, no control treatment was tested during this trial, since most commercial starters have monensin in their composition.

Our results show adequate starter intake by heifer calves fed monensin, which may be fed to enhance performance in programs that use solid intake as a criterion for weaning, as recommended by Quigley (1996a). In a review by Goodrich et al. (1984), monensin has no effect on feed intake.

In the only published work evaluating calcium propionate, the additive was fed via milk replacer and the starter intake was not monitored (Bunting et al., 2000). The inclusion of calcium propionate was supposed to reduce starter intake since propionate is associated with the feed intake-controlling process, probably by reducing the time to indicate satiety during a meal (Bradford and Allen, 2007). Theoretically, feeding readily available energy for growing calves could result in reduced starter intake or increased daily gain without changes in intake, since these animals are not on negative energy balance. However, this effect was not seen at the present trial.

Data of hay intake refer only to the post-weaning period and are presented in Table 2. Hay intake (g DM/day) was not different among treatments, even though there were significant effects of the interaction of treatment and age, with lower intake for sodium butyrate treatment during the week after weaning (week 9). After weaning, calves took about 3 to 4 days to initiate hay intake; however, after that, the intake was always increasing. The adequate starter intake

Figure 1 Starter feed intake (g DM/day), according to week of age, by heifer calves receiving starter containing sodium butyrate (B), sodium monensin (M) or calcium propionate (P).
and consequent ruminal development may have contributed to the satisfactory hay intake after weaning. Forage intake is important to promote rumen wall muscular growth and maintain ruminal epithelium health (Quigley, 1996a). Lizieri et al. (2002) observed a lower starter intake when star-grass pasture or alfalfa hay was available during the milk feeding phase. According to those authors, hay feeding may reduce starter intake as a result of the low intake capacity, being recommended only after weaning. Nussio et al. (2003a) observed low hay intake by calves weaned with 6 weeks of age, probably because of a minor rumen development as a result of the low starter intake. Similar results were found by Bernardes et al. (2007) and Suárez et al. (2007), who observed low hay intake during the first weeks of age of dairy calves.

Table 3 shows ADG and live weight (LW) observed during periods before and after weaning. ADG and the average of LW were not affected by treatments during all evaluated periods. These animal growth rates are in agreement with those observed in the literature for milk-fed calves (Hoffman, 1997; Heinrichs and Losinger, 1998; Kertz and Chester-Jones, 2004), as a result of the adequate starter intake, which allowed adequate daily gains even after weaning.

Figure 2 reveals the progression on daily gain during the experimental period as a result of the simultaneous increase in feed intake. The daily gain reduction after week 8 for all treatments resulted from the weaning process, a period in which large digestive and metabolic changes take place, with the cessation of milk feeding. Nevertheless, it can be seen that daily gain increased rapidly during the following week because of the increased starter intake. It is important to emphasize that starter intake was still increasing during the weaning week and after that, but probably not at an adequate rate to maintain animal growth rate.

Even though calves on sodium butyrate treatment consumed a smaller amount of starter during weeks 6, 7 and 8 (Figure 1), it did not significantly affect weight gain ($P = 0.85$) or LW ($P = 0.23$). Figure 2 shows clearly the lower weight gain of this treatment exactly during the weeks when smaller intakes were seen, even though significant differences among treatments were not found.

The supplementation of monensin or other ionophore for dairy calves has resulted in inconsistent outcome with regard to animal performance. Although some authors have shown benefits from ionophore supplementation on animal performance (Fitzgerald and Mansfield, 1984; Watkinson et al., 1987; Sinks et al., 1992; Quigley, 1996c), others have not (Stockdale et al., 1982; Foreyt et al., 1986).

In the study of Bunting et al. (2000), the supplementation of calcium propionate via milk replacer had no effect on weight gain, as it was observed in the current trial. Sumner et al. (2007) have also not observed better weight gains or LW of heifers with about 11 months of age-fed chromium propionate in several doses. Even though several commercial milk replacers have included sodium butyrate as an acidifier, data regarding the performance of calves fed this additive via a starter are scarce.

The final measures of wither heights and hearth girth were not affected by treatments and are presented in Table 4. Although initial measures of hip width were also not affected by treatments, there was a tendency ($P = 0.09$) for calves fed the starter containing calcium propionate to have smaller values at the end of the experimental period. Likewise, there were significant differences among treatments, with calves on calcium propionate starter presenting smaller average value of hip width as compared to the other treatments. According to Heinrichs et al. (2007), the main body

![Figure 2](https://doi.org/10.1017/S1751731110001965)
development measures have high correlation with LW and animal performance. Data of wither heights are in agreement with data from Heinrichs and Hargrove (1987) and Hoffman (1997) with commercial herds in United States.

Weekly gains of all body measurements were not affected by treatments and are in agreement with those observed for calves of similar age by other authors (Cowles et al., 2006; Kehoe et al., 2007). Wither heights are in conformity with values recommended in the literature for milk-fed calves, which suggest optimal weekly gains of around 1 to 1.4 cm for animals up to 2 months of age (Hoffman, 1997).

Figure 3 presents the average values for plasma glucose, NEFA and BHBA concentrations 2 h after morning milk feeding. There were no significant treatment effects for plasma glucose concentration in any of the evaluated periods. Observed values are in agreement with those found in the literature for calves of similar age (Quigley and Bernard, 1992; Klotz and Heitmann, 2006) with calves of similar age.

In the current trial, NEFA concentrations were not increased during the period from weeks 6 to 9 and were not affected by treatments. The values observed are in agreement with those presented by others with calves of similar age (Quigley et al., 1991b; Quigley and Bernard, 1992; Nussio et al., 2003b; Klotz and Heitmann, 2006) with calves of similar age. The plasma BHBA concentrations were determined only in the literature for calves of similar age (Quigley et al., 1991b; Nussio et al., 2003b). However, Quigley and Bernard (1992) have not seen reductions in glucose levels according to week of age during the milk-feeding phase, probably because of the low DM intake observed and consequent delayed rumen development. According to Fraser (1991), adult cattle plasma glucose concentrations fluctuate from 2.3 to 4.1 mmol/l, suggesting that calves already had the rumen developed by weeks 8 and 9 (Figure 3).

There were no significant differences among treatments for NEFA concentrations. The assessment of plasma NEFA concentration was carried out only at the weaning week and the weeks thereafter to evaluate possible body reserves mobilization as a consequence of the weaning. As emphasized by Quigley (1996b), circulating NEFA is negatively correlated with solid diet intake, with reductions in the concentrations being expected with advancing age of calves. In the current trial, NEFA concentrations were not increased after weaning, suggesting no body reserve mobilization as a result of adequate starter intake. Plasma NEFA concentrations are comparable to data found in the literature (Quigley et al., 1991b; Quigley and Bernard, 1992; Nussio et al., 2003b; Klotz and Heitmann, 2006) with calves of similar age.

The plasma BHBA concentrations were determined only during the period from weeks 6 to 9 and were not affected by treatments. The values observed are in agreement with those presented by others with calves of similar age (Quigley et al., 1991b; Quigley and Bernard, 1992; Klotz and Heitmann, 2006). The high BHBA values observed after the 6th week of life are related to the high starter intake (Figure 1), indicating rumen development and the beginning of metabolism of final fermentation products by the rumen epithelium (Bergman, 1990).

### Table 4 Least-square means of wither height, heart girth and hip width of heifer calves receiving starter containing sodium butyrate, sodium monensin or calcium propionate

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B</th>
<th>P</th>
<th>M</th>
<th>s.e.m.</th>
<th>T</th>
<th>A</th>
<th>T×A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Withers height (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>75.5</td>
<td>72.9</td>
<td>74.5</td>
<td>1.07</td>
<td>0.24</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Final</td>
<td>86.0</td>
<td>84.3</td>
<td>87.2</td>
<td>1.29</td>
<td>0.31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gain (cm/week)</td>
<td>1.36</td>
<td>1.34</td>
<td>1.45</td>
<td>0.15</td>
<td>0.83</td>
<td>0.25</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Heart girth (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>77.6</td>
<td>76.0</td>
<td>77.7</td>
<td>1.57</td>
<td>0.68</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Final</td>
<td>95.7</td>
<td>93.1</td>
<td>97.7</td>
<td>1.84</td>
<td>0.26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gain (cm/week)</td>
<td>2.05</td>
<td>2.04</td>
<td>2.14</td>
<td>0.16</td>
<td>0.91</td>
<td>&lt;.0001</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Hip width (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>20.4</td>
<td>19.6</td>
<td>20.6</td>
<td>0.53</td>
<td>0.39</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Final</td>
<td>25.1</td>
<td>24.2</td>
<td>25.7</td>
<td>0.45</td>
<td>0.09</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gain (cm/week)</td>
<td>0.57</td>
<td>0.51</td>
<td>0.55</td>
<td>0.05</td>
<td>0.73</td>
<td>0.34</td>
<td>0.49</td>
</tr>
</tbody>
</table>

1Starter containing B = sodium butyrate; P = calcium propionate; M = sodium monensin.

2*T = treatment effect; A = age (week) effect; T×A = treatment and age interaction effect.

Additives inclusion in starter for dairy calves
Figure 3 Plasma glucose (a), non-esterified fatty acids (b) and \( \beta \)-hydroxybutyrate (c) concentration (mmol/L), according to week of age.

Even though there was a tendency \( P = 0.08 \) for age effect, significant increases on BHBA concentrations were not observed. It is possible that the adequate starter intake already observed. It is possible that the adequate starter intake already at the 6th week (about 800 g DM/day; Figure 1) resulted in early rumen development, suppressing the observation of age effect on plasma BHBA. On the other hand, evaluation of BHBA reveals an increase in plasma concentrations with advancing age of calves (Quigley et al., 1992; Klotz and Heitmann, 2006). According to those authors, the BHBA concentration increase reflects the increased concentration of butyrate in the rumen, mainly because of the increased solid feed intake as animals mature.

Conclusion

The inclusion of sodium butyrate, calcium propionate or sodium monensin as additives in starter feeds resulted in equal animal performance, before and after weaning, suggesting that sodium monensin may be replaced by SCFA salts. Performance and changes in plasma metabolites suggest that rumen development is more dependent on starter intake than on feed additive.

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