Effect of protected sodium butyrate on performance and intestinal morphology

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The inclusion of organic acids in piglet diets helps prevent intestinal problems in the post-weaning period and improves animal growth performance.

Butyrate has a specific role in improving the growth performance of weaned piglets. Butyric acid is the main energy source for epithelial cells in the intestine and the ileum. In trials with growing pigs, butyrate not only improves animal growth but also increases the length of ileal microrilli and the depth of the colon, ileum and jejunum epithelium.

Butyrate increases intestine development in piglets and improves the digestive and absorptive capacity of the small intestine in pigs.

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The crypt of Lieberkühn represents proliferative compartment of intestinal epithelium (Ki67 positive cells). The differentiated compartments are specialised cells, adapted for food processing, and are found on the villi in the small intestine. Crypts harbour slowly dividing multipotent stem cells. The descendants of these stem cells in the crypt undergo more rapid proliferation, and are referred to as transit amplifying (TA) cells.

During migration of these TA-derived daughter cells to the villi surface, they differentiate into the four mature epithelial lineages:

1. The absorptive enterocytes who produce enzymes to digest sugars and proteins. The other three lineages are secretory types:
   - goblet cells produce mucus, thereby providing protection to the epithelium against stress and chemical damage.
   - Enteroendocrine cells secrete a great variety of hormones such as secretin, serotonin, substance P and somatostatin.
   - Paneth cells differentiate while moving to their functional location, (bottom of crypt). They specialise in the secretion of antimicrobial peptides and enzymes such as lysozyme and phospholipase.

Ki67 antigen is expressed in proliferating cells but not in quiescent cells, and it is a new nuclear proliferation marker. Ki-67 cells can be detected by using a monoclonal antibody, which reacts with a proliferation marker Ki-67.

Mitosis rate is a morphological sign of a very short process (<1h) to be detected, which is why the mitotic rate is less than the Ki67 rate. A correlation between the level of Ki67 cells (%) and pH variation can also be shown. Reduction of pH linearity of the intestinal content increases the level of Ki67 cells, level of replication cells.

The inclusion of butyrate in animal nutrition has a beneficial effect on the villi enlargement of the absorptive surface and in the rate of number of villi per plica. It may be possible to develop and use redox potential measurements in conjunction with pH as an early indicator of whether fermentation is proceeding in a normal or abnormal course.

Bacterial cell growth is strongly dependent on environmental conditions such as pH, redox potential and inhibitory compounds. Lactobacillus changes the oxidation-reduction potential through its production of metabolites such as lactic acid.

During growth of lactobacillus, decreasing of pH and dropping of oxidation-reduction potential changed the membrane permeability, and the result showed that oxidation reduction potential level of -170 mV was preferable for the formation of lactic acid.

Protection against infection of salmonella and shigella also appears to be related to high level of volatile acids, low pH, and low oxidation reduction potential of the intestinal contents.

The relationship of the normal flora to the intestinal epithelium is a prime example of a complex microbrial community associated with a higher eukaryote. The intestinal luminal flora produces a massive and diverse metabolic output that varies according to anatomical location, nutrient composition and host health.

It may be more illuminating to view the normal flora as a holistic community rather than as a collection of semi-independent organisms mediating 'beneficial' or 'detrimental' effects. Some results suggest a novel mechanism by which this complex community can collectively influence key epithelial signalling pathways. Metabolic products/small mole-

<table>
<thead>
<tr>
<th>Initial body weight (kg)</th>
<th>Final body weight (kg)</th>
<th>DWG (g)</th>
<th>DFI (kg)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.5</td>
<td>61.5</td>
<td>786</td>
<td>2.339</td>
</tr>
<tr>
<td>Butrex C4</td>
<td>40.5</td>
<td>63.8</td>
<td>830</td>
<td>2.321</td>
</tr>
<tr>
<td>Difference</td>
<td>+2.5% ±3.7%</td>
<td>+5.6% -0.7%</td>
<td>-5.9%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Performance results of experimental animals fed with different sources of butyrate at 1.5kg/t of feed during four weeks.

Fig. 1. A correlation can be seen between the level of Ki67 cells (%) and pH variation.
In the present study, the effects of protected butyrate on performance and intestinal health were evaluated. The aim of the experiment was to test the effect of protected butyrate (Butirex C4) in the intestinal tract parameters and the response on growing and feed conversion rate.

Special consideration for the intestinal health and parameters to indicate the effect of Butirex C4, were the intestinal value of pH, redox potential, lactobacillus population, lactate and VFA amount and Ki67 cells.

Material and methods

The trial was carried out in Budapest. A total of eight pigs of 40kg body weight were used during four weeks. There were two treatments, Control group without butyrate, and Butirex C4 (Na butyrate protected 54%) 1.5kg/m2. For the experimental period, the initial and final body weight and feed intake was measured, to evaluate daily weight gain (DWG), daily feed intake (DFI) and feed conversion rate (FCR).

At the end of the trial, all the animals were euthanised and different samples of the intestinal tract content were taken, to measure the intestinal pH, redox potential, VFA, Lactobacillus count and Ki67 cells.

Results

No significant differences were observed for performance parameters, by the low number or replicates (Table 2). Variation of final body weight showed that the best results were obtained with Butirex C4, 63.8 kg (Fig. 2).

Similar results were observed for daily weight gain (DWG) and feed conversion rate (FCR).

Intestinal parameters of pigs showed differences with the inclusion of butyrate. Intestinal pH at jejune level was reduced from 5.9 in the control group to 5.5 with Butirex C4, and could decrease redox potential of the gastrointestinal tract (Table 3). The reason for this change in the intestinal pH values could be due to a variation in the intestinal microflora.

Lactobacillus count (UFC/g) in the ileum was significantly increased (P<0.05) with the inclusion from 2.5 x 10^7 in the control to 2.65 x 10^8 in the Butirex C4 protected group. This increase in lactic acid microflora could be the reason for the increase of L-lactate (mM/kg) in the ileum (P<0.05) of Butirex C4 (Table 3).

The metabolic flux, influenced by the productivity of lactic acid was obviously affected by redox potential reduction in Butirex C4 samples. Electrochemical measurement of redox potential is a good control parameter of fermentation processes by the sensitivity of its measurement.

The selective changes of microflora in the small intestine and pH reduction promote the VFA significantly increase (P<0.05) and changes of microflora in the caecum.

The VFA concentration in the caecum increases from 10^9 mM/kg in the control group to 142 mM/kg for Na-butyrate buffer.

The increase of VFA is mainly due to an increase of acetic and propionic acid, significantly (P<0.05) higher for Butirex C4 in the caecum (Table 3).

According to the experience of Wang et al. (2004), changes in the intestinal parameters of pigs fed with Butirex C4 could induce VFA production increasing the flow of ileal substrate into the hindgut.

These VFA are physiologically active in the large intestine and absorbed through the gut wall, providing an additional source of energy for the host. Short-chain fatty acids are rapidly absorbed from the large intestine.

The main effect of butyrate is developed in the villi area of the ileum by the length and the number of villi. An increase (P<0.1) in Ki67 positive cells/crypt (cellular membrane for proliferation) was observed in the ileum, with the inclusion of Butirex C4.

Conclusions

The results of performance parameters showed more efficiency with Butirex C4. Butirex C4 protection allows the release of butyrate in the small intestine to promote intestinal development and villi growth. The different parameters evaluated at intestinal level showed better intestinal health with the inclusion of Butirex C4 in the diet compared with the control diet.

It could be concluded that, on the basis of the performance and intestinal results of the study, the action buffer Na-butyrate had a good effect on the global results of the pigs.

References are available from the author on request.

Table 3. Intestinal parameters of experimental animals fed with butyrate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Butirex C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach pH</td>
<td>3.4</td>
<td>3.25</td>
</tr>
<tr>
<td>Jejune pH</td>
<td>5.95</td>
<td>5.7</td>
</tr>
<tr>
<td>Ileum pH</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>-345</td>
<td>-285</td>
</tr>
<tr>
<td>L-lactate in ileum (mM/kg)**</td>
<td>22 b</td>
<td>38 a</td>
</tr>
<tr>
<td>Acetic acid in caecum (mM/kg)**</td>
<td>65 b</td>
<td>84 a</td>
</tr>
<tr>
<td>Propionic acid in caecum (mM/kg)**</td>
<td>31 b</td>
<td>41 a</td>
</tr>
<tr>
<td>Butyric acid in caecum (mM/kg)</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>VFA cecum (mM/kg)**</td>
<td>108 a</td>
<td>142 b</td>
</tr>
<tr>
<td>Lactobacillus ileum (UFC/g)*</td>
<td>2.5 x 10^7</td>
<td>2.65 x 10^7 x</td>
</tr>
<tr>
<td>Ki67 positive cells/crypt in ileum*</td>
<td>17 y</td>
<td>26 x</td>
</tr>
</tbody>
</table>

* P<0.1; ** P<0.05