Supplemental Sodium Butyrate Stimulates Different Gastric Cells in Weaned Pigs1–3

Maurizio Mazzoni,4 Maud Le Gall,4 Sara De Filippi,4 Laura Minieri,4 Paolo Trevisi,4 Jaroslaw Wolinski,7 Giovanna Lalatta-Costerbosa,8 Jean-Paul Lallès,6 Paul Guilloteau,6 and Paolo Bosi4*  

4Department of Agri-food Protection and Improvement, University of Bologna, 42100 Reggio Emilia, Italy; 5Department of Agro-Environmental Science and Technology, University of Bologna, 42100 Bologna, Italy; 6Institut National de la Recherche Agronomique (INRA), Joint Research Unit for Livestock Production Systems, Animal and Human Nutrition, 35590 Saint-Gilles, France; 7Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Science, Jabłonna, Poland; and 8Department of Veterinary Morphophysiology and Animal Production, University of Bologna, 40064 Ozzano dell’Emilia, Italy

Abstract

Sodium butyrate (SB) is used as an acidifier in animal feed. We hypothesized that supplemental SB impacts gastric morphology and function, depending on the period of SB provision. The effect of SB on the oxyntic and pyloric mucosa was studied in 4 groups of 8 pigs, each supplemented with SB either during the suckling period (d 4–28 of age), after weaning (d 29 to 39–40 of age) or both, or never. We assessed the number of parietal cells immunostained for H+/K+-ATPase, gastric endocrine cells immunostained for chromogranin A and somatostatin (SST) in the oxyntic mucosa, and gastrin-secreting cells in the pyloric mucosa. Gastric muscularis and mucosa thickness were measured. Expressions of the H+/K+-ATPase and SST type 2 receptor (SSTR2) genes in the oxyntic mucosa and of the gastrin gene in the pyloric mucosa were evaluated by real-time RT-PCR. SB increased the number of parietal cells per gland regardless of the period of administration (P < 0.05). SB addition after, but not before, weaning increased the number of enteroendocrine and SST-positive cells (P < 0.01) and tended to increase gastrin mRNA (P = 0.09). There was an interaction between the 2 periods of SB treatment for the expression of H/K-ATPase and SSTR2 genes (P < 0.05). Butyrate intake after weaning increased gastric mucosa thickness (P < 0.05) but not muscularis. SB used orally at a low dose affected gastric morphology and function, presumably in relationship with its action on mucosal maturation and differentiation. J. Nutr. 138: 1426–1431, 2008.

Introduction

Sodium butyrate (SB)9 is a very interesting molecule, because it is an energy source made available from bacterial fermentations, particularly for colonocytes. It also has important regulatory functions regarding cell proliferation, differentiation, and apoptosis, which differ between normal and cancer colorectal cells (1). Finally, SB modulates the gut microflora (2,3), depending on the adaptation of the bacteria to variations in chyme acidity (4).

The role of organic acids used as additives in the diet has received great attention. Acidification of infant formula with fermented products was proposed as a practical tool to prevent diarrhea (5). The protective action of organic acids can also be powerful when the passage from the maternal (milk) diet to the weaning diet is abrupt, as occurs in some farm animals. Butyrate is a digestion product normally released from milk triacylglycerol in the stomachs of suckled veal calves as a result of the sustained action of preintestinal lipases (6). In suckling pigs, the production of lactic acid from dietary lactose fermentation also contributes to acidifying the gastrointestinal contents (7), but it creates the conditions for delaying the full maturation of hydrochloric acid (HCl) secretion by the oxyntic mucosa (7). It can be hypothesized that dietary lactic acid or other organic acids can mimic the effects of the endogenous production of this acid. Immediately postweaning, supplementation of a diet with organic acids also reduced growth depression in piglets during this transition period (8). This effect could be related to the control of the development of Escherichia coli, as demonstrated with another organic acid salt, calcium formate (9). Among organic acids, the use of butyrate in the diet of weaning pigs has been less frequently studied. In piglets fed standard weaning diets, the amount of SB in the gastric content is very low (10) or not detected (11). Butyrate supplementation improved the gain:feed ratio in the first 2 wk postweaning (10). Conversely, SB administered for 6 wk postweaning did not affect growth performance (12).
The parietal cells in the oxyntic mucosa are responsible for HCl secretion through H\(^+\)/K\(^+\)-ATPase-dependent proton pump and Cl\(^-\) secretion via an apical channel. The activation of acid secretion can act directly on the parietal cell (by the calcium-sensing receptor) (13) or indirectly via the gastric cell pathway. In this case, the release of gastrin from G cells in the pyloric mucosa acts on the enteroendocrine gastrin cells of the stomach. Following stimulation, ECL cells secrete histamine, causing the parietal cell to insert proton pumps (H\(^+\)/K\(^+\)-ATPase) into its apical membrane. Luminal acidification depresses gastrin secretion. Calcium formate added to the weaning diet for piglets reduces the number of HCl-secreting cells and H\(^+\) gastrin secretion. Calcium formate did not inhibit gastric acid secretion.

In addition, it is not known if SB can stimulate a physiological pattern of gastric mucosa growth such as that observed for the colon (16) and cell proliferation from the jejunum to the distal colon (17). Furthermore, it has recently been shown that SB supplementation to formula-fed piglets from 3 to 10 d of age affected the development of the jejunum and ileal mucosae (18). This raises the interesting point that butyrate supplementation in suckling pigs can hasten the maturation of the gut.

In this study, we hypothesized that supplemental SB alters the morphology and function of the gastric mucosa and that its action can be modulated by the period of administration. We tested this hypothesis by providing SB orally during the suckling period and/or the postweaning period in piglets and by assessing the effects of SB on various aspects of stomach morphology and physiology after the postweaning period.

Materials and Methods

Piglets, experimental design, and feeding. For the ethical treatments of the piglets, the experimental procedures were carried out according to the guidelines of the French Ministry for Animal Research. The experiment involved 2 periods: 1) the suckling period from d 4 after birth until weaning at 28 d of age; and 2) the postweaning period from d 29 to 39–40 (day of slaughter). Two experimental factors were applied: butyrate before weaning (BE) and butyrate after weaning (AF). The combinations of these factors were defined as: CC, pigs never supplemented with SB; BC, pigs supplemented only before weaning; CB, pigs supplemented only after weaning; BB, pigs supplemented both before and after weaning.

For a total of 32 subjects, quadruplicate of Piétrain × Large White × Landrace piglets of the same birth weights and growth rates over the first 4 d of life were selected within litters and were assigned to the 4 dietary combinations. The experiment was conducted in 2 consecutive batches. The experiment involved 2 periods: 1) the suckling period from d 4 after birth until weaning at 28 d of age; and 2) the postweaning period from d 29 to 39–40 (day of slaughter). Two experimental factors were applied: butyrate before weaning (BE) and butyrate after weaning (AF). The combinations of these factors were defined as: CC, pigs never supplemented with SB; BC, pigs supplemented only before weaning; CB, pigs supplemented only after weaning; BB, pigs supplemented both before and after weaning.

For a total of 32 subjects, quadruplicate of Piétrain × Large White × Landrace piglets of the same birth weights and growth rates over the first 4 d of life were selected within litters and were assigned to the 4 dietary combinations. The experiment was conducted in 2 consecutive batches with 4 litters each. During the suckling period, an SB solution or saline solution was prepared for oral administration during the suckling period: 1) saline solution (9 g NaCl/L) for the control groups (CC and CB); and 2) SB solution (60 g/L, Sigma, 303410) dissolved in saline solution for the SB groups (BC and BB). The pH was adjusted to 7.0 by adding NaOH. Two weaning diets offered only from the day of weaning were formulated (Tables 1 and 2). In combination with the BB groups, SB was introduced (3 g/kg) in 1 of these starter diets by replacing corn starch. Thus, the daily consumption of SB ranged from 0.4 to 0.7 g/d during the suckling period and 0.7 to 1.5 g/d in the postweaning period. No antimicrobial agent was added to the diets. These diets were offered to piglets as a mash with a feed:water ratio of 1:1 by weight during the first 3 d after weaning and 2:1 thereafter.

Pig slaughter and tissue sampling. The piglets from 2 litters were randomly killed 4 h after the last meal on d 11 and those from the remaining 2 litters on d 12 after weaning (d 39–40 of age). The piglets were stunned by electric shock and then exsanguinated. For each pig, a midline abdominal incision was made and the whole gastrointestinal tract was gently removed. The stomach was separated, opened along the greater curvature, emplanted of its contents, and rinsed with twice-distilled water. Whole thickness tissue specimens of approximately 1 cm\(^2\) were removed from the oxyntic and pyloric gland areas near the greater curvature and from the antral region. Tissue samples were pinned tightly to balsa wood and were fixed in 10% buffered formaldehyde (immunohistochemistry) or 10% buffered formalin (morphology).

### TABLE 1 Control diet composition on an as-fed basis

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley meal</td>
<td>300.0</td>
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<tr>
<td>Wheat meal</td>
<td>297.0</td>
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<tr>
<td>Peas (44% stalk)</td>
<td>50.0</td>
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<tr>
<td>Whey powder</td>
<td>80.0</td>
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<tr>
<td>Wheat bran</td>
<td>25.0</td>
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<tr>
<td>Soy protein concentrate(^a)</td>
<td>40.0</td>
</tr>
<tr>
<td>Corn starch(^b)</td>
<td>40.0</td>
</tr>
<tr>
<td>Potato protein, purified (Protastar)</td>
<td>50.0</td>
</tr>
<tr>
<td>Maize gluten meal</td>
<td>22.0</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>25.0</td>
</tr>
<tr>
<td>Palm oil (50%) + soybean oil (50%)</td>
<td>31.0</td>
</tr>
<tr>
<td>Melasses</td>
<td>10.08</td>
</tr>
<tr>
<td>Lime stone</td>
<td>10.2</td>
</tr>
<tr>
<td>CaHPO(_4), H(_2)O</td>
<td>7.80</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.8</td>
</tr>
<tr>
<td>Trace mineral and vitamin premix(^c)</td>
<td>4.0</td>
</tr>
<tr>
<td>i-Methionine (99%)</td>
<td>1.1</td>
</tr>
<tr>
<td>L-Lysine - HCl (79%)</td>
<td>3.4</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.31</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\(^a\) Calculated nutrient composition, as-fed basis (g/kg): DM, 888; CP, 191; crude fiber, 34; fat, 50; Ca, 7.2; Na, 2.5; P, digestible, 3.65; lysine, 12.5; methionine, 4.4; tryptophan, 2.5; threonine, 8.0; net energy, MJ/kg, 10.0.

\(^b\) Soycomil.

\(^c\) Corn starch (4%) is addressed as an ingredient to be partially substituted by 0.3% SB in the experimental treatment.

\(^d\) This trace mineral-vitamin premix supplied per kilogram diet as follows: retinol, 525 μg; cholecalciferol, 5 μg; α-tocopherol, 7.4 mg; phylloquinone, 0.5 μg; thiamin, 1 mg; riboflavin, 4 mg; pantothenic acid, 9 mg; niacin, 12.5 mg (available); biotin, 50 μg; cyanocobalamin, 15 μg; folic acid, 0.3 mg; pyridoxine, 1.5 mg; choline, 400 mg; Fe, 80 mg; Mn, 54 mg; Zn, 10 mg; Cu, 0.15 mg; I, 0.14 mg; Se, 0.25 mg; antioxidants (E310,320,321), 50 mg; and corn starch as a carrier.
**TABLE 2** Effect of butyrate supplementation before or after weaning, at both times, or never on gastric morphological parameters of pigs

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th></th>
<th></th>
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<th></th>
<th>Statistical significance of the effects, ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>BC</td>
<td>CB</td>
<td>BB</td>
<td>SEM</td>
<td>BE</td>
<td>AF</td>
<td>BE × AF</td>
</tr>
<tr>
<td>Oxyntic mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gland depth, ( \mu )m</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Parietal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n/\text{gland} )</td>
<td>606</td>
<td>666</td>
<td>615</td>
<td>647</td>
<td>27.3</td>
<td>0.108</td>
<td>0.855</td>
<td>0.625</td>
</tr>
<tr>
<td>( n/100-\mu \text{m gland} )</td>
<td>40.6</td>
<td>47.7</td>
<td>47.8</td>
<td>48.0</td>
<td>2.01</td>
<td>0.081</td>
<td>0.087</td>
<td>0.093</td>
</tr>
<tr>
<td>Enteroendocrine cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n/\text{gland} )</td>
<td>21.2</td>
<td>26.0</td>
<td>32.2</td>
<td>32.7</td>
<td>2.66</td>
<td>0.337</td>
<td>0.004</td>
<td>0.423</td>
</tr>
<tr>
<td>( n/100-\mu \text{m gland} )</td>
<td>3.60</td>
<td>3.95</td>
<td>5.57</td>
<td>5.08</td>
<td>0.517</td>
<td>0.961</td>
<td>0.012</td>
<td>0.551</td>
</tr>
<tr>
<td>SST (^*) cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n/\text{gland} )</td>
<td>3.34</td>
<td>3.66</td>
<td>3.90</td>
<td>6.08</td>
<td>0.677</td>
<td>0.082</td>
<td>0.043</td>
<td>0.190</td>
</tr>
<tr>
<td>( n/100-\mu \text{m gland} )</td>
<td>0.56</td>
<td>0.55</td>
<td>0.66</td>
<td>0.95</td>
<td>0.106</td>
<td>0.208</td>
<td>0.033</td>
<td>0.186</td>
</tr>
<tr>
<td>( n/\text{total enteroendocrine cells} )</td>
<td>0.156</td>
<td>0.140</td>
<td>0.117</td>
<td>0.179</td>
<td>0.0178</td>
<td>0.205</td>
<td>0.985</td>
<td>0.044</td>
</tr>
<tr>
<td>Pyloric mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrin cells, ( n/\text{gland} )</td>
<td>17.7</td>
<td>17.0</td>
<td>16.1</td>
<td>17.5</td>
<td>2.51</td>
<td>0.846</td>
<td>0.902</td>
<td>0.703</td>
</tr>
</tbody>
</table>

1 Values are means and pooled SEM, \( n = 7–8 \) per treatment. AF, after weaning; BB, pigs supplemented with sodium butyrate before and after weaning; BC, pigs supplemented with sodium butyrate only before weaning; BE, before weaning; CB, pigs supplemented with sodium butyrate only after weaning; CC, pigs never supplemented with sodium butyrate.

2 Contrast “Butyrate whichever period vs. never butyrate,” \( P < 0.01 \).

3 Contrast “Butyrate, 1 period vs. 2 periods,” \( P < 0.01 \).

Bouin’s solution (morfometry) for 24 h. The tissue samples were then removed from the fixative and washed in 5.14 mol/L ethanol. The specimens were then dehydrated in a graded series of ethanol and embedded in paraffin. For each pig, additional samples of the entire oxyntic and pyloric walls were collected for molecular biology, snap-frozen in liquid nitrogen, and stored at \(-80^\circ \)C until analysis.

The pH of the fresh gastric digesta was determined immediately upon collection using a pHmeter (704 model, Metrohm) and the content was removed from the fixative and washed in 5.14 mol/L ethanol. The tissue samples were then dehydrated in a graded series of ethanol and embedded in paraffin. For each pig, additional samples of the entire oxyntic and pyloric walls were collected for molecular biology, snap-frozen in liquid nitrogen, and stored at \(-80^\circ \)C until analysis.

The pH of the fresh gastric digesta was determined immediately upon collection using a pHmeter (704 model, Metrohm) and the content was then frozen, freeze-dried, milled, and stored for analysis.

**Muscularis and mucosa morphometry.** Tissue sections of 5 \( \mu \)m thickness were cut from paraffin blocks to estimate the muscularis and mucosa thickness. The tissue sections were then stained with hematoxylin and eosin according to a standard protocol. Thirty whole sections of muscularis and mucosa per slide were measured using Axiostar Vision 4.3 software (Zeiss) under a light microscope (Zeiss).

**Immunohistochemistry.** Adjacent formalin-fixed sections (5 \( \mu \)m) underwent immunohistochemical staining for detecting parietal and endocrine cells. We used microwave treatment for unmasking relevant antigenic sites before immunodetection. All the antibodies used in this study are listed in Supplemental Table 1.

Immunostaining of parietal cells was performed as previously reported (14). Briefly, the sections were treated with 90 mmol/L \( \text{H}_2\text{O}_2 \) in methanol for 30 min to block endogenous peroxidase activity, then with normal goat serum for 1 h, followed by a primary antibody against the \( \alpha \)-subunit \( \text{H}^+/\text{K}^-\text{ATPase} \) incubated at 4\(^{\circ} \)C overnight by a biotin-conjugated goat anti mouse IgG and then by ABC complex (Vector Laboratories). The immune reactions were visualized applying a 3–3\(^{\prime}\)-diaminobenzidine chromogen solution (Vector Laboratories).

For each pig, we counted all the parietal cells in 20 randomly selected glands located perpendicularly to the surface of the mucosa using an optical microscope. The depth of the lamina propria, from the pits to the muscularis mucosae, was measured in the same areas using a Zeiss Axioplan microscope (10\( \times \) objective) connected to KS 300 image analysis software (Kontron Elektronik).

Immunostaining of the endocrine cells was performed using the indirect double-labeling immunofluorescent technique. Anti-chromogranin A antibody, which labels endocrine cells (21,22), was used in association with anti-somatostatin (SST) antibody for the oxyntic mucosa and anti-gastrin antibody was used for the pyloric mucosa. The sections were incubated at 4\(^{\circ} \)C overnight in a solution containing chromogranin A/SST or SST/gastrin mixtures of the primary antibodies. After washing in PBS, the sections were incubated in the dark for 1 h with a mixture of goat anti-rabbit antibody labeled with fluorescein isothiocyanate and goat anti-mouse antibody labeled with Alexa 594 (Supplemental Table 1).

Negative controls to prove the specificity of the secondary antibodies were obtained by incubating the sections without the primary antibody or with appropriate nonimmune g globulins.

For each pig, all the endocrine cells in 20 randomly selected glands located perpendicularly to the mucosal surface were counted using a Zeiss Axioplan microscope equipped with the appropriate filter cubes for discriminating between fluorescein isothiocyanate and Alexa 594 stainings.

**Gene quantification by real-time RT-PCR.** To quantify mRNA abundance for ATPase, SST type 2 receptor (SSTR)-2 and gastrin genes in the fundic and pyloric mucosa, total RNA was isolated and its integrity controlled as previously reported (9). For each gene investigated, 2 pairs of primers were designed on the specific pig nucleic acid sequence (GenBank) by Primer 3 (23) (Supplemental Table 2). An absolute quantitative analysis, using an external standard curve for each gene, was performed in a LightCycler instrument (Roche), as described previously (14). Data were expressed as gene copies per microgram RNA.

**Statistics.** ANOVA using the GLM procedure of SAS (version 8.1, SAS Institute) with a 2-level full factorial design was carried out: BE, AF, and interaction. Batch and litter within batch were also included in the model. When the \( P \)-value for the BE by AF interaction was \(< 0.10 \), the following preplanned orthogonal contrasts were performed: “Butyrate whichever period vs. never butyrate,” \((\text{CB} + \text{BC} + \text{BB}) \) vs. \((\text{CC} + \text{BE}) \); “Butyrate, 1 period vs. 2 periods,” \((\text{CB} + \text{BC}) \) vs. \((\text{BB}) \); “Butyrate, only before vs. only after,” \((\text{BB} + \text{BC}) \) vs. \((\text{BB}) \). The values presented are least square means ± SEM and effects were considered significant at \( P < 0.05 \). The values for gene expressions did not display a normal residue distribution. Therefore, a log base 10 transformation of the data were used. The correlation between \( \text{H}^+/\text{K}^-\text{ATPase} \) and SST type 2 receptor (SSTR2) genes expressions was also calculated.
Results

Except for 2 piglets, the piglets from the experimental groups remained clinically healthy during the study. One pig from the BB group of the first batch and 1 pig from the BC group of the 2nd batch had to be removed from the experiment, because they did not consume the weaning feed. Excluding those subjects, the final body weights were 10.5, 11.4, 11.0, and 12.1 ± 0.4 kg, respectively, for CC, BC, CB, and BB groups (effect of SB supplementation before weaning, \(P < 0.05\)). SB did not change growth before weaning and the pooled daily gain from d 4 to 28 was 284 ± 17 g. In the postweaning period, the interaction between SB supplementation before and after weaning tended to be significant (\(P = 0.10\)). The daily gains of the BC (195 g), CB (174 g) and BB (192 g) groups were higher than that of the CC group (133 g, pooled SE = 12 g) (\(P < 0.05\)).

The weight of the empty stomach relative to body weight was 9.68 ± 0.35 g/kg and the pH of the gastric contents was 3.40 ± 0.09 and did not differ among the groups. The amount of residual DM in the stomach, calculated as a percentage of the DM intake of the last meal and normalized for body weight, was greater (\(P < 0.05\)) in the groups that received SB during the suckling period (BC, 45.0; BB, 51.4) than in those that did not (CC, 36.9; CB, 42.1 ± 3.8; pooled SE = 3.8 gastric residual DM amount/DM intake, %). SB addition postweaning and the interaction between the 2 SB supplementation periods did not affect this variable.

The depth of the oxyntic gland tended to be increased by butyrate BE (\(P = 0.108\)) (Table 2). The interaction between SB addition before and after weaning approached significance for the number of parietal cells per gland (\(P = 0.097\)), which was increased by the administration of SB whatever the period of treatment (see also Fig. 1A, CC group; Fig. 1B, BB group). The number of parietal cells per 100-μm depth of the gland tended (\(P = 0.099\)) to be increased with the addition of SB after weaning. The number of enteroendocrine cells, per gland and per 100-μm depth of the stomach, was strongly increased by SB after weaning (Fig. 1C, CC diet; Fig. 1D, BB diet). SB after weaning also increased the number of SST cells (Fig. 1E, CC diet; Fig. 1F, BB diet). However, when these cell numbers were expressed per gland, SB addition during the suckling phase tended to have an effect (\(P = 0.082\)). All the SST-positive cells were also chromogranin A-positive. The 2 SB treatments interacted to affect the ratio of SST-positive:total enteroendocrine cells. The pigs receiving SB in both periods had relatively more SST-positive cells than the pigs receiving SB only before or after weaning. Finally, the counts of gastrin-positive cells in the pyloric mucosa was not affected by the treatments.

Butyrate supplementation after weaning increased gastric mucosa thickness, with no effect on the muscularis thickness. Mucosal thickness in the CB (467 μm) and BB (482 μm) groups were greater than in the CC (403 μm) and BC (431 μm; pooled SE = 31 μm) groups. SB treatment in the suckling period did not affect this variable.

There was an interaction between the pre- and postweaning periods of SB supplementation for H/K-ATPase and SSTR2 mRNA abundance (Table 3) but no significant contrast for H/K-ATPase. Supplying SB in either period only tended to increase SSTR2 (\(P = 0.098\)) mRNA abundance compared with SB addition in both periods. In the pyloric mucosa, feeding SB postweaning tended to increase gastrin mRNA (\(P = 0.087\)).

Discussion

The major findings of the present study are that SB supplementation postweaning increased the densities and numbers of enteroendocrine and SST cells in the fundic mucosa of pigs.

ENTEROENDOCRINE CELLS IN THE OXYNTIC MUCOSA OF PIGS

Enteroendocrine cells in the oxyntic mucosa include various endocrine/paracrine cells (ECL cells, A-like cells secreting ghrelin and obestatin, and D cells secreting SST). For the intestine, the enteroendocrine L cells, which produce the intestinotrophic glucagon-like peptide-2, have been proposed to be target cells for butyrate (16). The selective induction of enteroendocrine cells in the stomach by SB has, to our knowledge, never been reported before. It is generally assumed that oral SB, at a low concentration, increases the amount of residual DM in the stomach, calculated as a percentage of the DM intake of the last meal and normalized for body weight, was greater (\(P < 0.05\)) in the groups that received SB during the suckling period (BC, 45.0; BB, 51.4) than in those that did not (CC, 36.9; CB, 42.1 ± 3.8; pooled SE = 3.8 gastric residual DM amount/DM intake, %). SB addition postweaning and the interaction between the 2 SB supplementation periods did not affect this variable.

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Butyrate supplementation after weaning increased gastric mucosa thickness, with no effect on the muscularis thickness. Mucosal thickness in the CB (467 μm) and BB (482 μm) groups were greater than in the CC (403 μm) and BC (431 μm; pooled SE = 31 μm) groups. SB treatment in the suckling period did not affect this variable.

There was an interaction between the pre- and postweaning periods of SB supplementation for H/K-ATPase and SSTR2 mRNA abundance (Table 3) but no significant contrast for H/K-ATPase. Supplying SB in either period only tended to increase SSTR2 (\(P = 0.098\)) mRNA abundance compared with SB addition in both periods. In the pyloric mucosa, feeding SB postweaning tended to increase gastrin mRNA (\(P = 0.087\)).

TABLE 3 Effect of butyrate supplementation before or after weaning, at both times, or never on gene expression in the stomach mucosa of pigs

<table>
<thead>
<tr>
<th>Groups</th>
<th>CC</th>
<th>BC</th>
<th>CB</th>
<th>BB</th>
<th>SEM</th>
<th>BE</th>
<th>AF</th>
<th>BE × AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyntic mucosa</td>
<td>Log10 (gene copies/μg RNA)</td>
<td>6.24</td>
<td>6.61</td>
<td>6.47</td>
<td>6.33</td>
<td>0.125</td>
<td>0.739</td>
<td>0.029</td>
</tr>
<tr>
<td>H/K-ATPase</td>
<td>6.55</td>
<td>6.86</td>
<td>6.76</td>
<td>6.52</td>
<td>0.114</td>
<td>0.851</td>
<td>0.680</td>
<td>0.029</td>
</tr>
<tr>
<td>SSTR2</td>
<td>4.27</td>
<td>4.06</td>
<td>4.92</td>
<td>4.77</td>
<td>0.374</td>
<td>0.730</td>
<td>0.087</td>
<td>0.946</td>
</tr>
</tbody>
</table>

1 Values are means and pooled SEM, \(n = 7–8\) per treatment. AF, after weaning; BE, before weaning; BC, pigs supplemented with sodium butyrate before and after weaning; BB, pigs supplemented with sodium butyrate only before weaning; BE, before weaning; BB, pigs supplemented with sodium butyrate only after weaning; CC, pigs never supplemented with sodium butyrate.

2 Contrast “Butyrate, 1 period vs. 2 periods,” \(P = 0.098\).
gastric pH, is rapidly protonated and absorbed by nonionic diffusion in the stomach (24), but the moderate presence of the monocarboxylate transporter 1 in the basolateral surface of gastric epithelial cells could facilitate butyrate exchanges (25). The movements of butyrate can also be favored by the presence of the monocarboxylate transporter 2 in the area of parietal cells (25). However, there is no evidence yet that enteroendocrine cells selectively utilize butyrate.

The increased numbers of SST-positive enteroendocrine D cells after SB addition postweaning can be explained by the action of this peptide as an inhibitory feedback messenger on acid secretion, which has already been observed in piglets fed free calcium formate (14). This apparently contrasts with the trend of increased gastrin mRNA abundance. Indeed, SST has an inhibitory effect on gastrin gene transcription (26). But, if the number of SST-positive cells is related to the total number of enteroendocrine cells, the effect of SB is more complex and variable according to the period of supplementation. Pigs that received SB for both periods had a relatively greater number of SST cells than pigs that were supplemented with SB only before or after weaning. Conversely, in the BB group, a negative trend was seen for mRNA abundance of SSTR2, which mediates the action of SST in parietal, ECL, and G cells. Both observations could indicate a sort of long-term adaptation to SB supplementation. The pattern of expression of SSTR2 mRNA abundance probably reflects the various cell populations on which the receptors are found. Direct feedback after gastrin secretion should be excluded; in fact, SSTR2 gene expression was not reduced in gastrin-knockout mice or gastrin/CCK-knockout mice (27). Finally, we should also consider a direct effect of SB on the growth or activity of D cells, because SB induces SST production on cultivated cells (28).

The control of luminal pH in the gut is critical to the digestive function and the integrity of the different parts of the gastrointestinal tract. This is achieved using various mechanisms mainly governed by acid-sensing primary afferent neurons (29), resulting in feedback on gastric acid secretion, mucosal function, and motility. Because SB was used here at a low dose and is a moderate acid, the lack of reduction in gastric pH with SB treatment is not surprising and agrees with other observations that were reached using the same dose of SB (10). Calcium formate (12 g/kg feed) decreased parietal cell numbers and H+/K+-ATPase gene expression in the porcine oxyntic mucosa (14). These signs of short- or long-term adjustments were not observed with SB at the present dose.

One mechanism used by the digestive system to control luminal pH is the delay of gastric emptying. Manzanilla et al. (10) have shown that SB after weaning did not affect feed intake but increased the DM percentage of the gastric contents, which is an indicator of slower gastric emptying or of faster liquid phase outflow from the stomach. In the present study, residual DM in the stomach at slaughter increased in pigs fed SB before weaning, whatever the treatment after weaning (30). However, this was accompanied by increased feed intake. The persistence of an effect after the suspension of the supplementation with SB suggests that control of the pH in the digesta contents flowing to the duodenum is not sufficient to explain our observation. This is additionally supported by the fact that there was no effect on residual DM with the SB treatment after weaning (30). Mechanisms involving the long-term effects of butyrate could be advocated. We herein present data showing that some morphological variations (parietal cells, SST+ cells) in the fundic mucosa tend to remain for at least 1 wk after the suspension of SB treatment. More data, also including information from physiological events in the intestine, are required to better understand gastric physiology mechanisms.

The number of parietal cells per gland in the oxyntic mucosa increased in the pool of SB treatments compared with the controls. Acid secretion is a high energy-demanding process and SB can rapidly penetrate through cell membranes. Butyrate supported H+ secretion, although less effectively than glucose, acetate, and pyruvate in the isolated gastric mucosa of neonatal pigs (31). However, there is no indication that SB can stimulate the proliferation of parietal cells, notwithstanding the demonstrated effect on colonocytes (16). Here, gastrin gene expression also tended to increase after SB supply. This observation may be related to the delay of gastric emptying, because stomach distension is known to stimulate gastric acid production. Recent research demonstrates that gastrin, besides its effect on gastric secretion, regulates the organization of the gastric mucosa (32) and also acts as a proliferation agent on parietal cells and ECL cell progenitors (33,34). In our trial, SB provided postweaning also tended to increase the number of parietal cells per unit depth of the gland but did not affect the depth of the gland. This result is consistent with the observations of Kotunia et al. (18) who did not find any effect on the plasma gastrin level in neonatal pigs fed SB in milk formula, but contrasts with those of Bakke et al. (35) who reported that SB increased the number of parietal cells in parallel with the total number of mucosal cells when hypergastrinaemia was induced in rats. However, a paracrine effect of gastrin without any change in its blood concentration, via activation of other growth factors (36), can be hypothesized.

SB did not affect the number of gastrin-secreting cells in our trial. In the piglets provided with oral SB, the effect should have persisted for 11 – 12 d after the end of the treatment. The lifetime of parietal cells in mice is estimated to be ~80 d (37). Therefore, it can be hypothesized that the persistent effect of SB on parietal cell numbers is the residual result of the same effect seen on parietal cells with SB supplementation postweaning. However, the number of parietal cells per unit depth of the gland was not changed by the preweaning provision of SB. Indeed, the depth of the gland tended to increase after preweaning SB administration; thus, the increase of the cell number reflected, at least partially, an overall effect of SB on oxyntic gland development.

Notwithstanding the average increase in the number of parietal cells with oral SB and the trend for increased gastrin mRNA abundance with SB after weaning, H+/K+-ATPase gene expression in the oxyntic mucosa was not changed by the supplementation. In a previous experiment supplementing piglets with calcium formate, individual variations in ATPase gene expression were partially explained by individual variations in parietal cell numbers (14). However, the time elapsed between the second meal and the killing differed between experiments (2 h vs. 4 h here). To the best of our knowledge, the synchrony between the expressions of genes related to the short-term control of gastric secretion has not been studied. More information about the effect of the time from the stimulus induced by the meal and/or the presence of feed in the stomach on gene expression would be useful for a better interpretation of such experiments.

The data on different gastric cells can be integrated with the results from mucosa morphometry obtained in the antrum region. SB treatment after weaning resulted in increased mucosal thickness. This observation suggests that SB action on stomach tissue is relevant to its functional development. Elevated growth of the gastric mucosa may result from high proliferation and/or low apoptosis rates. Kien et al. (17) showed thatecal butyrate
infusion at a rate equal to that produced in the colon did not affect the apoptosis index but caused a 78–119% increase in cell proliferation in the jejunum, ileum, distal colon, and cecum. Such information is still not available for the stomach.

In conclusion, our investigation provides evidence that SB has a complex impact on porcine gastric morphology and function that cannot be explained by its acid function. Supplementation with butyrate from weaning to death stimulated more cells to differentiate into enteroendocrine cells; furthermore, dietary butyrate increased the number of parietal cells per gland, whatever the period of supplementation (including a trend for pigs after 11–12 d of suspension of the treatment). This is the first study to our knowledge that demonstrates the ability of supplemental SB to affect cellular mechanisms of differentiation and to control tissue growth in the normal healthy stomach, such as is observed in the large intestine in vivo or in in vitro systems. Finally, oral SB proved to be of interest for stimulating growth performance and feed intake when provided to young pigs, especially before weaning.

**Literature Cited**

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