A Continuous Dietary Supply of Free Calcium Formate Negatively Affects the Parietal Cell Population and Gastric RNA Expression for H⁺/K⁺-ATPase in Weaning Pigs¹–³

Paolo Bosi,* Maurizio Mazzoni,* Sara De Filippi,* Paolo Trevisi,* Luisa Casini,* Gregorio Petrosino,† and Giovanna Lalatta-Costerbosa**

ABSTRACT Baby formula acidification can be used to reduce diarrhea. Calcium formate is a dietary acidifier frequently used in animal weaning diets; it is also a source of available calcium. Gastric acidification reduces gastrin release and hydrochloric acid (HCl) secretion. To study the medium-term effects on fundic gastric mucosa, we fed weaning pigs control diets or diets supplemented with free or fat-protected calcium formate. We evaluated the following: 1) the number of HCl-secreting parietal cells, by immunohistochemistry using an antibody against H⁺/K⁺-ATPase; 2) the number of enteroendocrine cells immunohistochemically stained with chromogranin A (CGA), somatostatin, and histamine (HIS); and 3) the expression of the H⁺/K⁺-ATPase gene, by real-time RT-PCR in the oxyntic mucosa. Cells co-staining for CGA and HIS were defined as enterochromaffin-like (ECL) cells. Pigs fed calcium formate had fewer parietal cells and a lower expression of the H⁺/K⁺-ATPase gene than the controls (P < 0.05). This reduction did not occur in pigs fed fat-protected calcium formate. Somatostatin immune-reactive cells were also more numerous in pigs fed free calcium formate than in controls (P < 0.05). The number of ECL cells was not affected. Using covariance analysis, the number of parietal cells explained part of the differences in the expression of H⁺/K⁺-ATPase gene (positive correlation, r = 0.385, P < 0.01), and excluded the statistical significance of the diet. In the future, the effects on the oxyntic mucosa should be checked when the diet supplemented with calcium formate is discontinued. Furthermore, a reduction in the number of parietal cells could impair the absorption of vitamin B-12 due to a reduced secretion of the intrinsic factor by these cells. J. Nutr. 136: 1229–1235, 2006.

KEY WORDS: • pigs • weaning • acidifiers • parietal cells • H⁺/K⁺-ATPase

Gastric acid secretion contributes to the gut barrier against pathogens. In breast-fed young mammals, this secretion is reduced in relation to the fermentative activity of milk lactose in the stomach (1). At weaning, the passage of maternal milk into the stomach ceases, but the secretion of hydrochloric acid (HCl) in the stomach remains limited (2,3). The successive intake of solid food/feed will progressively stimulate this HCl secretion.

Carboxylic acids are not only a metabolic fuel originating from gut fermentation, but also a bioregulator of gut microflora (4). These organic acids are frequently added to the diet of weaning pigs to compensate for the insufficient acidification of the stomach due to the reduced production of HCl by gastric parietal cells. Formate is one of the most interesting of these acids in the control of porcine postweaning diarrhea (5). The acidification of human infant formula including fermented products is a similar practice used to reduce the risk of diarrhea (6). The acidification of formula reduced bacterial translocation and gut colonization in a neonatal rabbit model (7), and citric acid supplementation was proposed to reduce the risks of necrotizing enterocolitis in neonates. Compared with controls, feedings acidified by HCl addition reduced gastric colonization of critically ill adults in a multicenter randomized trial (8). Among organic acids and their salts, calcium formate may also offer additional advantages because it is also a dietary calcium supplement; it has higher calcium availability in humans than either calcium carbonate or calcium citrate (9). Pharmacokinetic analysis in women showed that calcium formate has a short plasma half-life, with no risk of progressive accumulation in case of repeated use during the day (10). In the stomach, HCl is secreted by an ATP-dependent H⁺/K⁺ exchanger, an integral membrane protein of parietal cells.

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³ Supplemental Table 1 is available with the online posting of this paper at www.nutrition.org.
⁴ To whom correspondence should be addressed: E-mail: paolo.bosi@unibo.it.
present in both membranes of the microvilli of luminal membrane invaginations (secretory canaliculi) and the extensive cytoplasmatic system of membranes (11), often referred to as the tubulovesicular system. In cell cultures, the gene expression of H⁺/K⁺-ATPase is strongly related to the activity of parietal cells (12). Acid secretion is related to energy generation through oxidative phosphorylation; parietal cells are, in fact, rich in mitochondria, and enzyme histochemistry of mitochondria has been used in their detection (13).

Gastric acid secretion from parietal cells is under the regulatory control of both the central and the enteric nervous systems, and a complex network of neuroendocrine cells acting in an auto- or paracrine manner (14). Many different specialized endocrine cells staining with antisera to chromogranin A (CGA)⁵ are present in the oxyntic mucosa (15,16). The enterochromaffin-like (ECL) cells, such as histamine (HIS) endocrine cells, are included in this group; they are intermingled with parietal and chief cells (17) and exert their paracrine effect on parietal cells by secreting HIS. Histamine is a powerful acid secretagogue in the oxyntic mucosa; it is also present in mast cells, which are numerous in most animals, including pigs; they are found scattered throughout the mucosa (18). Mast cells, unlike HIS endocrine cells, are not stained with the antiserum to CGA, but they can be identified by metachromasia upon toluidine staining; however, no acid-related regulation of HIS secretion from mast cells has been described, probably due to the topographic distribution of the gastric mast cells and their low content of the enzyme histidine decarboxylase required for HIS synthesis (19).

Intragastric acidification causes a sudden reduction in the gastric secretion of gastrin (20–22). This peptide hormone is released by G cells and contributes to the stimulation of the secretion of HCl primarily through the activation of cholecystokinin (CCK)₂ receptors on ECL cells through the release of HIS. On the contrary, somatostatin (SST) inhibits HCl secretion through ECL or G cells (23). However, little is known about the effect of a time-extended supply of organic acids on gastric acid secretion and on the presence of HCl-secreting cells.

The aim of the present study was to assess the effect of calcium formate on the number of both parietal and endocrine cells that act on the acid secretion in the oxyntic mucosa of weaning pigs, and to correlate the number of parietal cells to the RNA expression for H⁺/K⁺-ATPase. A dietary treatment with fat-protected calcium formate was used to test whether the eventual effect of this additive on the stomach is related to actions in other parts of the digestive tract or whether it can be prevented by gastric protection.

**MATERIALS AND METHODS**

**Animals, diet and oral challenge with enterotoxigenic Escherichia coli (ETEC).** Large White pigs (n = 60), purchased from Suidea (Reggio Emilia, Italy) and weaned at 21 d (d 0), were used in 4 experimental cycles. For each cycle, the pigs were divided into 3 groups, n = 5/group, balanced for litter and live weight. For the entire experimental period, a control group (Group C) was fed a standard diet (Group) P diet was microencapsulated by fat protection consisting of hydrogenated vegetal triglycerides. To compensate for the presence of calcium from calcium formate, the control diet was supplemented with dicalcium phosphate and calcium sulfate, whereas the experimental diets contained monosodium phosphate in addition to calcium formate. The pigs were housed individually in pens with a mesh floor in a temperature-controlled room; tap water was freely available. The pigs were orally challenged with 1.5 mL of a 10¹⁵ cfu Escherichia coli (ETEC) K88 O148 (F4) suspension on d 2, and killed on d 7 or 8, equally distributed by treatment and on a random basis within each treatment. ETEC is currently an important agent of infection and diarrhea in weaning pigs (24), and the presence of intestinal receptors for its fimbriae is required for its pathogenicity (25).

The procedures followed were conducted according to the Italian law pertaining to experimental animals and were approved by the Ethic-Scientific Committee for Experiments on Animals of the University of Bologna.

**Kill and samplings.** On the day of killing, the pigs had access to their meal for 1 h. Then, for the last hour before killing, their access to the trough was excluded. They were then deeply anaesthetized with sodium thiopental (10 mg/kg body weight, Zoletil 100, Virbac) and killed by an intracardiac injection of Tanax® (0.5 mL/kg BW; Intervet Italia).

For each pig, the stomach was removed, opened along the greater curvature and rinsed with bidistilled water. Tissue specimens of ~1 cm² were removed throughout the whole thickness of the oxyntic gland area near the greater curvature and pinned tautly on balsa wood; they were then immersed in 10% buffered formalin for 24 h. At the end of 24 h, the samples were 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, an additional sample of the entire oxyntic wall was collected, flash frozen in liquid nitrogen, and stored at −80°C for quantification of the expression of the H⁺/K⁺-ATPase gene and for histochemical staining.

**Immunohistochemistry.** Formalin-fixed, paraffin-embedded specimens were serially sectioned (5 μm), mounted on gelatin-coated slides, and then deparaffinized in xylene. To unmask the antigenic sites, the slides were heated in 10 mmol/L sodium citrate buffer (pH 6.0) for 2 periods of 5 min each in a microwave oven at 750 W. After

**Table 1**

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¹ Provided per kilogram diet: retinyl acetate, 3.3 mg; cholecalciferol, 27.5 μg; menadione, 2 mg; α-tocopherol, 45 mg; thiamin, 4 mg; riboflavin, 8 mg; pyridoxine, 4 mg; vitamin B₁₂, 0.04 mg; niacin, 55 mg; biotin, 0.15 mg; d-pantothenic acid, 30 mg; folic acid, 1 mg; iron (as FeSO₄), 200 mg; zinc (as ZnSO₄), 175 mg; copper (as CuSO₄), 150 mg; manganese (as MnSO₄), 80 mg; iodine (as KI), 1 mg; selenium (as Na₂SeO₃), 0.3 mg.

² Diet C: lard, 1.00%; dicalcium phosphate, 1.20%; calcium sulfate, 0.80%; sepiolite, 0.05%; diet F: lard, 1.00%; calcium formate, 1.20%; monosodium phosphate, 0.85%; Diet P: fat-protected calcium formate (microencapsulated by fat protection consisting of hydrogenated vegetal triglycerides), 2.20%; monosodium phosphate, 0.85%.
microwave irradiation, the sections were allowed to cool to room temperature for 20 min. After cooling, the sections were washed in bidistilled water and adjacent sections underwent immunohistochemical staining to detect parietal and endocrine cells as described in detail previously (26). Supplemental Table 1 summarizes the antibodies used in this study and their dilutions in 1 mmol/L PBS, pH 7.4.

**Immunostaining of parietal cells.** Immunostaining was performed to detect parietal cells using a mouse monoclonal antibody against an α-subunit of H⁺/K⁺-ATPase. All samples were immunostained with the avidin-biotin-peroxidase complex (ABC) method and examined under a conventional microscope for morphometric analyses; 2 randomly selected samples per diet were also stained using the indirect immunofluorescent method (IF) and examined under the confocal laser scanning microscope (CLSM).

For the ABC method, the sections were treated with 90 mmol/L H₂O₂ and then incubated at 4°C overnight in the primary antibody. Sections were then coated with biotin-conjugated goat anti-mouse IgG and treated with the ABC complex (Vector elite kit, Vector Laboratories) according to the directions provided by the manufacturer. The sections were then counterstained with Mayer’s hematoxylin.

For the FITC method, sections were incubated at 4°C overnight in the primary antibody and then incubated in the dark for 1 h in the donkey anti-mouse FITC-labeled secondary antibody. The sections were examined and the images were acquired on a laser confocal scanning microscope, Olympus Fluo View 500 equipped with an appropriate Argon (488 nm) filter. Single- and multiplane scanning were used as 0.1-μm “optical sections” of stained cells.

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

**Immunostaining of endocrine cells.** The sections were processed for double staining with CGA and HIS to identify HIS endocrine cells and with CGA and SST to identify SST endocrine cells, using the IF method as described above. Adjacent sections were incubated at 4°C overnight in a solution containing a mixture of the primary antibodies: mouse anti CGA/rabbit anti-HIS and mouse anti CGA/rabbit anti-SST. The sections were then incubated in a mixture of the secondary antibodies: donkey anti-mouse-fluorescein isothiocyanate (FITC)-labeled and donkey anti-rabbit-tetramethylrhodamine isothiocyanate (TRITC)-labeled. The specimens were examined under a Zeiss Axiosplan microscope equipped with the appropriate filter cubes to discriminate between FITC and TRITC.

**Histochemical staining.** Histochemical staining of reduced NADH-tetrazolium reductase (NADH-TR) according to Novikoff et al. (27) was used to detect oxidative enzymes, rich in parietal cells. Histochemical staining was performed on frozen sections cut at 6 μm on a cryostat microtome at −20°C.

**Metachromasia.** This staining was used on paraffin-embedded tissue as 0.1-μm sections, expression as the mean number of positive cells in the total area of 0.24 mm² in this study and their dilutions in 1 mmol/L PBS, pH 7.4.

**Immunostaining of parietal cells.** Immunostaining was performed to detect parietal cells using a mouse monoclonal antibody against an α-subunit of H⁺/K⁺-ATPase. All samples were immunostained with the avidin-biotin-peroxidase complex (ABC) method and examined under a conventional microscope for morphometric analyses; 2 randomly selected samples per diet were also stained using the indirect immunofluorescent method (IF) and examined under the confocal laser scanning microscope (CLSM).

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Individual susceptibility to E. coli adhesion. The individual susceptibility to E. coli K88 adhesion was assessed in vitro on vili collected from the small intestines of the pigs using the procedure of Van den Broeck et al. (25).

**Statistics.** Data were analyzed by ANOVA using the General Linear Model (GLM) procedure (SAS version 8.1, SAS Institute) with a 3-factor design, including diet, cycle, sensitivity of intestinal villus to ETEC adhesion, and 1st level interactions. Sensitivity of intestinal villus to ETEC adhesion has relevance for growth, and for intestinal health and microbial and immune variables, in E. coli K88- and K99-challenged pigs (30). We decided to include this variable in the model to exclude the side effects of this challenge on gastric variables. The interactions, never statistically significant, were removed from the model and only the main effects were tested. The values presented are least square means ± SEM. Pair-wise comparisons between diets were done using Tukey’s test (Adjust procedure in SAS). Differences were considered significant when P < 0.05. The expression values of the H⁺/K⁺-ATPase gene did not fulfill the assumptions of normality of residue distribution and a logarithmic transformation of the data was used. These last values were also processed by intraclass covariance analysis (GLM procedure), considering the 3 class factors of the general design, the number of cells (obtained with different stainings), and the number of cells within each diet. This analysis also revealed the correlations between H⁺/K⁺-ATPase and cell counts.

**RESULTS**

Preliminary considerations on challenge and on sensitivity of intestinal villus to ETEC adhesion. The factors "sensitivity of intestinal villus to ETEC adhesion" and cycle of the experiment in general did not affect the variables assessed and did not interact with the diet. However, in pigs that were susceptible to ETEC adhesion, there tended to be more parietal cells in NADH-TR counting (P = 0.078), but not in H/KATPase counting, and increased gene expression for H⁺/K⁺-ATPase in the oxyntic wall (P = 0.13) compared with nonsusceptible pigs (data not shown). Four pigs (2 consuming diet C and 1 from each experimental diet) died after the challenge with colibacillosis (data concerning the sensitivity of intestinal villi to ETEC adhesion not shown).
General description of the oxyntic mucosa of piglets. The oxyntic mucosa was packed with relatively straight, unbranched glands opened in the foveolae (Fig. 1A). Because a strict division of the gland regions in isthmus, neck, and base linked to the distribution of cell types is somewhat difficult and arbitrary, we divided the glands into deep, middle, and superficial thirds. Parietal cells were scattered throughout the oxyntic glands, but were more numerous in the middle and superficial thirds. A different pattern of immunoreactivity was observed with the H⁺/K⁺-ATPase antibody in the majority of the parietal cells. Ring-like structures formed a network throughout the cytoplasm or surrounded the nucleus (reticular pattern) in the deep and middle thirds, and a few cells showed small granules filling the entire cytoplasm (diffuse pattern) (Fig. 1c). When parietal cells were examined in CLSM, the ring-like structures appeared as a network of hollow tubular formations throughout the cytoplasm (Fig. 1D). In all experimental groups, the cells showing the reticular pattern were prominent throughout the gland, whereas few cells with a diffuse pattern were scattered in the deep and middle thirds. Only in a few pigs did the diffuse pattern predominate throughout the gland. The cell areas varied greatly; small cells were intermingled with large round or typical polyhedral parietal cells. Round or oval endocrine cells stained by CGA were detected; they were intermingled with secretory exocrine cells throughout the oxyntic mucosa (Fig. 1E) and were most numerous in the deep and middle thirds of the gland. In the selected area of 0.24 mm², from 35 to 37 ECL cells (CGA+/HIS+) were counted. Very few HIS+/CGA− cells were found in the lamina propria (Figs. 1F, and 2); they were identified as mast cells when adjacent sections were stained with toluidine blue. In the selected area of 0.24 mm², from 10 to 12 SST cells (CGA+/SST+) were counted.

Dietary effect on the number and area of parietal cells. Glands from pigs fed the F diet had fewer parietal cells than the controls (Figs. 1A, B). Indeed, the number of parietal cells was significantly reduced in pigs fed the F diet for both H⁺/K⁺-ATPase and NADH-TR counting (P < 0.05, Fig. 2). In the P group, the number of parietal cells did not differ from that of the C group. The area of parietal cells and the depth of the lamina propria (data not shown) were not affected by the diet.

Effect on the number of enteroendocrine cells. The numbers of endocrine cells secreting SST in an area of 0.24 mm² were 9.15 ± 0.55 for C, 11.20 ± 0.51 for F, and 10.71 ± 0.52 for P. Compared with the controls, the number of endocrine cells secreting SST was higher in the stomachs of pigs fed the F diet (P < 0.05). On the contrary, the number of ECL cells (CGA+/HIS+), the number of remaining enteroendocrine cells (only CGA+), and the number of mast cells (CGA-/HIS+) were not affected by the diets. However, in pigs fed the F diet,

FIGURE 1 Effect of the diet on parietal cells in weaning pigs fed C (A, D, E, F) or F (B, C) diets. The oxyntic mucosa was immunostained with antibody to the α-subunit of H⁺/K⁺-ATPase (A–D) and ECL cells double immunostained with antibody to HIS and CGA (E, F). Antibody binding was detected by the ABC method (A–C) and by the IF method (D–F). In the C group (A), parietal cells were more numerous than in the F group (B). The majority of the cells showed a reticular pattern; a few cells (arrows) showed a diffuse pattern (C). Multiple plane scanning showed hollow tubular structures (D); corresponding to the reticular pattern of the parietal cell shown at higher magnification in the small insert, ECL-like cells, CGA (E) and HIS (F) immune-reactive cells were spread throughout the gland (short arrows); few mast cells (arrows) were detectable.
the number of ECL cells in the superficial part of the gland was increased (P < 0.05, Fig. 3).

Expression of the gastric H⁺/K⁺-ATPase gene. This measure varied greatly among the groups as follows: Log10 (gene copies/μg RNA) 3.60 ± 0.27 for C; 2.83 ± 0.23 for F; and 3.08 ± 0.28, for P. The oxyntic wall of pigs fed the P diet had a reduced expression for H⁺/K⁺-ATPase compared with tissue from those fed the C diet (P < 0.05). With the addition of fat-protected formate in the diet, the expression for H⁺/K⁺-ATPase did not differ from that of either the C or F group.

The gene expression of H⁺/K⁺-ATPase in the oxyntic wall was positively correlated with the number of parietal cells, as identified by H⁺/K⁺-ATPase staining (r = 0.385; P < 0.01, Table 2). The correlation between the gene expression of H⁺/K⁺-ATPase and the number of parietal cells was not affected by the diet as shown by the absence of interaction between the covariate and the diet. The effect of the diet, which was significant in the original ANOVA, was not significant when the number of parietal cells was included as a covariate. These data indicate that the difference between the F and the C groups in H⁺/K⁺-ATPase gene expression was due in part to a different number of cells capable of secreting HCl. However, the percentage of total variability explained by the number of cells was not high, indicating that other factors affect the short-term activation of parietal cells.

**DISCUSSION**

The immunostaining of pig parietal cells was remarkably similar to the labeling pattern observed in both mice (31) and rats (32). When these species are stimulated by HIS or gastrin, a reticular pattern predominates in a majority of cells that are in an active secreting stage, whereas a diffuse pattern predominates in the resting stage. Thus, it was suggested that the activity in acid secretion of individual parietal cells can be evaluated morphologically by the immunostaining of H⁺/K⁺-ATPase and that the reticular pattern corresponds to the active stage characterized by the developed tubulovesicular network, whereas the diffuse pattern corresponds to the resting state (31,32). Consequently, we hypothesize that, in the pigs in the present study, the majority of the cells were stimulated by acid secretion, probably due to the meal that the pigs consumed 1 h before they were killed.

Using the weaning pig as a model, we demonstrated that the constant supply of an organic acid for 8 d strongly affects the structure of the oxyntic mucosa. The trend does not change substantially if the number of parietal cells is evaluated by immunostaining with H⁺/K⁺-ATPase antibody or by immunohistochemical stain with NADH-TR. However, with NADH-TR, the cell number was higher; this is not surprising because this stain is not a specific marker of parietal cells but is the expression of oxidative enzymes.

What led to the observed reduction in parietal cell number? It is thought that the endocrine cells, which constitute only a small percentage of the total number of cells in the mammalian oxyntic mucosa (33), are important factors for the maturation and development of the oxyntic mucosa. The reduction of parietal cell number in calcium formate–fed pigs could be consistent with the hypothesis of a negative effect of the acidifier on gastrin secretion. It is interesting to observe that, in mice, the blockade of gastrin-inducible CCK2 receptors of parietal cells and ECL cells determines long-term hypotrophy and hypoplasia of this mucosa (34). Moreover, in gastrin gene knock-out mice, a reduction in the number of parietal cells was observed, whereas the ECL cell number is not affected by gene deletion (35–37). In the present experiment, the antral mucosa was not sampled; thus, we cannot say whether the number of gastrin-secreting G cells was also affected, although that is likely. In our trial, the free formate–induced reduction in parietal cells is concomitant with the increase in SST positive cells, but not with variations in total ECL numbers. Somatostatin acts as a potent inhibitor of gastric acid secretion. In mice, type 2 receptors for SST are found on both parietal and ECL cells (38). This suggests that SST may act to reduce acid secretion by directly affecting parietal cells or by attenuating HIS release from ECL cells. A third mode of action for SST is its inhibition of the release of the acid secretagogue hormone gastrin (39,40). Gastric SST-producing cells (D cells) are regulated by CCK through the activation of CCK1 receptors (23); more acidic meals strongly stimulate the release of CCK (41); this could explain the increased number of SST positive cells in the oxyntic mucosa after the addition of free calcium formate. ECL cells can also be involved in gastric morphology regulation; in fact, mice lacking histidine decarboxylase (the enzyme required for the synthesis of HIS) had an increased number of parietal cells, as demonstrated by Nakamura et al. (42). In our trial, the entire number of ECL cells was not changed by the diet; thus, it is not likely that HIS could have affected the numerical reduction of the parietal cells that occurred. It is not clear whether gastrin has some relevance for the growth of ECL cells in pigs. In gastrin gene knock-out mice, no effect of gene deletion was reported on the number of...
E. coli (0.007 0.07 0.58). Covariates are the number of parietal cells and of ECL cells. A phenotypic test on E. coli strain). A phenotypic test on E. coli.

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(a K88 strain). A phenotypic test on E. coli with functional capacity.

membrane that is observed in parietal cells is more correlated with the gene expression for H+/K+-ATPase but not with functional capacity.

To our knowledge, this is the first time that the gene expression for H+/K+-ATPase has been assessed in the oxyntic gastric mucosa of swine. We showed that the number of parietal cells is correlated with gene expression for H+/K+-ATPase but independent of the diet, as revealed by the analysis of covariance; this agrees with the observations done on cell culture (12). However, there was a large individual variation for the gene expression for H+/K+-ATPase and other undetermined factors presumably affected by the quantity of specific mRNA. We standardized the time for the final meal (1 h) and the period of food deprivation before killing (1 h), but we could not take into account individual variations in the distribution of feed intake within the allotted time. Furthermore, the pigs had free access to water, and this could have diluted the gastric content to varying degrees. Finally, we cannot exclude that H+/K+-ATPase protein abundance within the luminal facing membrane that is observed in parietal cells is more correlated with functional capacity.

The oxyntic mucosa from rats fed the P diet showed an intermediate pattern for the number of the different types of cells considered. Concerning the parietal cells, the data show that the protection of the acidifier could alleviate the depressive effect compared with the formate salt. On the other hand, the outcome for ECL cells and SST-positive cells is less clear. The protection of calcium formate was obtained by means of a triglyceride external layer. The release of formate from the globules was not tested, and we cannot exclude the fact that the fats were partially digested by salivary and gastric lipases. The use of protected acidifiers as supplements in infant diets could also be a tool for reducing the risk of acute lung injury, which is often observed in the case of the instillation of acidified diets into the lungs (44). Another reason for reducing the gastric availability of calcium formate is that the reduction of parietal cells can impair the production of the intrinsic factor required for vitamin B-12 absorption from the vitamin in the gastrointestinal tract (45). Indeed, a link between long-term gastric acid suppressive therapy and significant decreases in serum vitamin B-12 was demonstrated (46).

In our experiment, all of the pigs were challenged with an enterotoxigenic E. coli (a K88 strain). A phenotypic test on jejunal villi showed that half of the pigs were susceptible to ETEC adhesion to the jejunum, whereas the others were not. The stomach is not a typical site of action for ETEC; however, our pigs that were susceptible to ETEC intestinal adhesion had a reduced daily live weight gain, a worse fecal score, and more days of diarrhea (Bosi et al., unpublished data), and we speculate that a general more inflammatory condition could have affected the stomach morphology of these pigs. Indeed, local inflammation related to a greater bacterial load in the stomach leads to an increase in the number of parietal cells and of gastrin output (47). Because the number of parietal cells in NADH-TR counting tended to increase, as did the gene expression for H+/K+-ATPase in K88 susceptible pigs, it might be possible to extend previous observations for stomach infection to intestinal infection. However, in H+/K+-ATPase counting, the number of parietal cells was not affected; thus, we cannot definitively conclude that pigs that are more affected by ETEC infection respond with an increased stimulus for acid secretion.

In conclusion, supplementation with the salt of an organic acid and any change from a diet containing a relevant portion of these additives, such as calcium formate, to a diet without them should be carefully considered.

ACKNOWLEDGMENT

We are grateful to Prof. Laura Calzà for her valuable assistance with the laser confocal scanning microscope.

LITERATURE CITED


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<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Sensitivity of intestinal villus to ETEC adhesion</td>
<td>1</td>
<td>4.6</td>
<td>0.12</td>
<td>6.6</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>14.8&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.007</td>
<td>0.07</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Covariate within diet</td>
<td>2</td>
<td>5.5</td>
<td>0.24</td>
<td>3.1</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Covariates are the number of parietal cells and of ECL cells.
<sup>2</sup> The common regression coefficient is +0.0147 ± 0.0056 SE.


