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Effect of method of delivery of sodium butyrate on maturation of the small intestine in newborn calves

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ABSTRACT

The effect of sodium butyrate (SB) supplementation in milk replacer (MR), starter mixture (SM), or both on small intestine maturation in newborn calves was investigated. Twenty-eight male calves with a mean age of 5 (±1) d were randomly allocated into 1 of 4 groups (7 animals per group) and fed (1) MR and SM, without SB (MR^- and SM^-, respectively; MR^-/SM^-); (2) MR^- and SM supplemented with SB encapsulated within triglyceride matrix (SM^+, 0.6% as fed; MR^-/SM^+); (3) MR supplemented with crystalline SB (MR^+, 0.3% as fed) and SM^- (MR^+/SM^-); or (4) MR^+ and SM^+ (MR^+/SM^+). The MR was offered in amounts equal to 10% of initial body weight of the calf. The SM was blended with whole corn grain (50/50; wt/wt) and offered ad libitum as a starter diet. Calves were slaughtered at 26 d (±1) of age and small intestine development was investigated. Treatment with MR^+ decreased villus height in the proximal jejunum and decreased villus height, crypt depth, and tunica mucosa thickness in the middle jejunum, whereas treatment with SM^+ tended to increase small intestine weight and crypt depth in the proximal jejunum, and increased villus height in the distal jejunum. In the duodenum, crypt depth and tunica mucosa thickness were greater for the MR^-/SM^+ group compared with MR^-/SM^-, MR^+/SM^-, and MR^+/SM^+ groups. In the ileum, crypt depth was less for MR^-/SM^+ compared with MR^-/SM^-. Supplementation with SB in both MR and SM enhanced cell proliferation and decreased apoptosis in the middle jejunum mucosa. Regarding brush border enzyme activities, addition of SB to MR increased lactase activity in the middle jejunum and maltase activity in the distal jejunum, and tended to increase lactase activity in the distal jejunum, aminopeptidase A activity in the middle jejunum and ileum, and aminopeptidase N activity in the ileum. In contrast, SM^+ increased dipeptidylpeptidase IV activity in the distal jejunum and tended to increase aminopeptidase N in the distal jejunum. In conclusion, both MR^+ and SM^+ affected small intestine development in newborn calves. This effect depended on the method of SB delivery but MR^+ generally had a more pronounced effect. No synergistic effect of SB supplementation into MR and SM was found.

Key words: milk replacer, starter mixture, gastrointestinal tract, development

INTRODUCTION

The stimulatory effect of dietary sodium butyrate (**SB**) on gastrointestinal tract development in the calf is well established (Guilloteau et al., 2009; Górka et al., 2011a,b). Because early rumen development is desired in dairy calves (Greenwood et al., 1997; Baldwin et al., 2004), SB supplementation in the starter mixture (**SM**) seems to be especially justified. When supplemented in SM, butyric acid is released mainly in the rumen and thus rumen epithelium development is directly stimulated. This results in higher solid feed intake in first weeks of life (Górka et al., 2011a), and thus, potentially improves performance, especially after weaning. However, until regular intake of solid feed starts, liquid feed (milk or milk replacer) is the main source of nutrients for the calf.

In newborn calves, the lower gut (abomasum and small intestine) is the main site of liquid feed digestion. The development of those compartments of the gastrointestinal tract can be stimulated predominantly by SB supplementation in liquid feed (Guilloteau et al., 2009; Górka et al., 2011b). In preruminant calves, liquid feed bypasses the reticulorumen via the reticular groove and thus, when supplemented in liquid feed, SB can be delivered directly to the abomasum and then the small intestine. When added into milk replacer (**MR**), SB accelerates small intestine epithelium maturation, exerts a trophic effect on the small intestine mucosa, and stimulates some functions of the small intestine (Guilloteau et al., 2009; Górka et al., 2011b). Sodium

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butyrate in MR also stimulates pancreas development and increases pancreatic secretion and, consequently, nutrient digestibility (Guilloteau et al., 2009, 2010b). Together, these factors result in better performance of calves fed MR supplemented with SB (Guilloteau et al., 2009; Górka et al., 2011a).

Although SB addition into SM affects mainly rumen development and its supplementation in MR mainly affects small intestine development, we cannot exclude the possibility that SB in SM may affect small intestine development; vice versa, SB in MR may affect rumen development. It has been shown that SB supplementation in MR may affect development of not only the small intestine but also the rumen (Górka et al., 2011a,b). Less is known about the effect of SB addition into SM on maturation of the calf small intestine, although stimulation of intestinal stem cell proliferation and reduced apoptosis have been demonstrated in several animal species when SB was delivered in solid feed (Guilloteau et al., 2010a). Although butyrate supplemented in SM is expected to be released and absorbed in the rumen, at least part of the butyric acid may pass to the lower gut, stimulating small intestine functions. Increasing the butyric acid concentration in the rumen may also indirectly affect small intestine development. Infusion of short-chain fatty acids into rat colon affects not only growth of the colon but also that of the jejunum (Reilly et al., 1995). When supplemented in SM, sodium butyrate increases plasma glucagon-like peptide-2 in calves (Górka et al., 2011a), which is known to play an important role in small intestine development (Burrin et al., 2005). Therefore, supplementation of SB not only in MR but also in SM may stimulate small intestine maturation in newborn calves.

Based on this information, we hypothesized that simultaneous addition of SB into both MR and SM would synergistically affect small intestine development in the calf. The aim of this study was to determine the effect of SB inclusion in MR, SM, or both, on small intestine maturation in neonatal calves.

MATERIALS AND METHODS

Animals and Diets

The animal study protocol was approved by the local ethics committee before the onset of the trial. A detailed description of the study is presented elsewhere (Górka et al., 2011a,b). Briefly, 28 healthy male calves (Holstein or Holstein × Limousin) with mean age of 5 (±1) d were randomly allocated into 1 of 4 experimental groups (7 calves per group) and fed (1) MR and SM, both without SB ($\mathbf{MR^-/SM^-}$); (2) MR^- and SM supplemented with SB encapsulated within a triglyceride matrix ($\mathbf{SM^+}$, 0.6% as fed; $\mathbf{MR^-/SM^+}$); (3) MR supplemented with crystalline SB ($\mathbf{MR^+}$, 0.3% as fed) and SM^- ($\mathbf{MR^+/SM^-}$); or (4) MR^+ and SM^+ ($\mathbf{MR^+/SM^+}$). Animals were kept and fed individually. Milk replacer (22% CP and 18% fat in DM) was offered in amounts equal to 10% of calf BW at the initiation of the experiment. The MR, with or without SB, was mixed (1:9) with warm water (about 40°C) and fed from a bucket with a teat 2 times a day (0800 and 1500 h). The SM (38% CP in DM), with or without SB, was blended with whole corn grain (50/50; wt/wt) and offered as a starter diet (about 24% CP and 15% NDF in DM) daily for ad libitum intake, after the morning MR feeding. Sodium butyrate encapsulated within a triglyceride matrix (30:70 butyrate:triglyceride matrix) was used in SM^+ to slow the release of butyric acid in the rumen. Because SM was blended with whole corn grain (50/50; wt/wt) and encapsulated SB contained 30% of SB and 70% of triglyceride matrix, the starter diet offered to calves contained 0.3% encapsulated SB product and 0.09% SB. For MR^+, crystalline SB was used in an amount equal to 0.3% in milk replacer powder (as fed).

Tissue Sampling and Analysis

Calves were fed experimental diets for a period of 3 wk. At the end of the trial, all calves (26 ± 1 d of age) were euthanized by captive bolt stunning and exsanguinated. Within 20 min after slaughter, the abdominal cavity was opened and the gastrointestinal tract was removed. The duodenum, jejunum, and ileum were separated, emptied, rinsed repeatedly with water, drained, and measured and weighed individually.

One square centimeter of whole-thickness tissue samples from the duodenum, proximal, middle and distal jejunum, and ileum were taken for analyses and placed in 4% buffered formaldehyde (Sigma-Aldrich Corp., St. Louis, MO) for 5 d and then stored in ethanol. Then, the samples were embedded in paraffin, and serial histological sections (5 μm thick) were stained with hematoxylin and eosin for histometric analysis under the light microscope. Villus length, crypt depth, and tunica mucosa and tunica muscularis thickness were measured in 5 to 8 slides for each tissue sample with an optical binocular microscope (Olympus BX 61, Olympus, Warsaw, Poland) coupled via a digital camera to a PC equipped with Cell P (Olympus) software. Thirty measurements for each analyzed parameter were collected.

Whole-thickness middle jejunum samples were fixed in embedding medium (OCT, Cell Path, Newtown, UK), frozen in a liquid nitrogen, and stored at – 80°C. Samples were cut into 15-μm slides, rinsed with PBS (Gibco, Paisley, UK), incubated in 1% BSA/10% goat