Effects of Na-butyrate supplementation in milk formula on plasma concentrations of GH and insulin, and on rumen papilla development in calves

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Abstract

Although the growth-promoting action of sodium-butyrate (Na-butyrate) used as a feed additive has been observed in calves and pigs, the precise mechanisms involved remain to be clarified. In this study, pre-weaning calves were given milk formula (MF) supplemented with butyrate for 6 weeks to investigate its effects on postprandial changes in the plasma concentrations of metabolic hormones, and, simultaneously, on growth performance, the weight of the digestive organs and rumen papilla development. Ingestion of MF increased ($P < 0.05$) the plasma concentrations of GH and insulin as well as the glucose level, but decreased the non-esterified fatty acid concentration. Na-butyrate supplementation in MF or in lactose solution (with the same quantity of lactose contained in the MF, 5%) suppressed the increase in plasma insulin and

GH concentrations, and the plasma IGF1 level was not changed. The length of the rumen papilla and the weight of the perirenal fat tended to increase in the calves fed with Na-butyrate-supplemented MF, but the weight of the liver, spleen, and stomach were not changed. In addition, there was no difference in the expression of mRNA for sodium-dependent glucose transporter-1 in the small intestinal epithelial tissues. We conclude that the accelerated growth performance related to the intake of Na-butyrate used as a feed additive reported previously in several species is partly due to improved insulin sensitivity and a better digestive functional development. These data could be applicable to animal and human nutrition.

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Introduction

Butyric acid, one of the short-chain fatty acids (SCFAs), is a natural substance present in the gastrointestinal (GI) tract (GIT; colon in monogastric species, forestomachs in ruminants), in milk with the exception of sow milk and only traces in human milk (0.16 g/l in cow milk; Alais 1984), as well as in the sweat and feces of most mammals. Butyric acid is also available as the Na, K, Mg, or Ca salts. The advantage of salts over free acids is that they are generally odorless and easier to handle in the feed manufacturing process owing to their solid and less volatile forms. A recent review (Guilloteau *et al.* 2010a) highlighted the biological role of these substances (acid and/or salts) when they are naturally produced by the GI microbial ecosystem or orally ingested as a feed additive. Many studies have been conducted on the use of the salts of butyric acid as a feed additive at low doses (0.1–0.4% of dry matter). These experiments were realized mainly in milk-fed calf (Guilloteau *et al.* 2004, 2009,

2010b) or in piglet before weaning (Kotunia *et al.* 2004, Mazzoni *et al.* 2008, Le Gall *et al.* 2009), and sometimes during (or after) weaning in calf (Gorka *et al.* 2009, 2011, Flaga *et al.* 2009) or in piglet (Gálfi & Boroki 1990, Wang *et al.* 2005, Biagi *et al.* 2007, Le Gall *et al.* 2009). These substances were found to have widespread positive effects on digestive secretions (pancreas and small intestine), digestibility and feed efficiency, growth rate, adiposity through the modulation of cell proliferation, differentiation, and function in the GIT, especially mucosal epithelial cells, and on defence systems (barrier function, antimicrobial potency, and immune system) in healthy and sick animals (Pouillart 1998, Partanen & Mroz 1999, Gauthier 2002, Scheppach & Weiler 2004, Franco *et al.* 2005, Mroz 2005, Manzanilla *et al.* 2006, Mazzoni *et al.* 2008, Le Gall *et al.* 2009, Guilloteau *et al.* 2009, 2010b). Moreover, sodium-butyrate (Na-butyrate) supplementation in milk formula (MF) and/or starter diet intake by neonatal calves had a beneficial effect on calf growth and nutrient utilization, modified the relative weight of the different parts of the

forestomach, and produced larger rumen papillae length and width, suggesting an enhancement of the rumen (Gorka *et al.* 2009, 2011, Slusarczyk *et al.* 2010). The stimulated papillae growth in newborn calves was confirmed in other experiments (Tamate *et al.* 1962, Mentschel *et al.* 2001). In their review, Guilloteau *et al.* (2010a) concluded that since, in most circumstances, these molecules seem to act in a similar way when in the acid or salt form, it appears that only the radical ($\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-COO-}$) is of major importance. Thus, in the present paper, the term 'butyrate' is sometimes used interchangeably for the acid, the salt and the anion forms.

The dietary level of butyrate did not cause changes in the serum β -hydroxybutyrate concentration in calves (Slusarczyk *et al.* 2010) and butyrate disappears from the upper GIT (mainly in the stomach). It is thought to be metabolized in the GIT wall (and/or in the liver) because it is not found in the peripheral blood (Manzanilla *et al.* 2006, Guilloteau *et al.* 2009, 2010a). Thus, for GIT, the effects on the stomach and hindgut seem to be direct (Mentschel *et al.* 2001, Gorka *et al.* 2009, 2011), whereas the effects on the pancreas and small intestine are probably indirect. In these last conditions, the actions of butyrate are believed to involve the hormono-neuro-immunosystem (Guilloteau *et al.* 2010a). As examples, it was suggested that Na-butyrate may act through growth factors (Tsubaki *et al.* 2001, Flaga *et al.* 2009) or endocrine systems (Simon *et al.* 1997, Bartholome *et al.* 2004, Katoh *et al.* 2007, Flaga *et al.* 2009). Na-butyrate may also stimulate defence systems through heat shock proteins and modify immune and inflammatory responses (Ren *et al.* 2001, Millard *et al.* 2002, Böcker *et al.* 2003, Guilloteau *et al.* 2009). However, the intervention of butyrate by way of the hormono-neuro-immunosystem remains poorly demonstrated and unclear.

This study was conducted in neonatal calves receiving MF (supplemented or not with Na-butyrate) and calf starter, and was carried out on the basis of the finding that Na-butyrate used as a feed additive ingested at low doses is a good growth promoter. We hypothesized that the promoting effect of Na-butyrate may be caused by improvement of GIT development which could be linked to the effects of bioactive peptides and/or hormones secreted in the blood and produced mainly by the insulino/somatotropic axis (GH, insulin-like growth factor 1 (IGF1), and insulin). Simultaneously, GIT, liver and perirenal fat were weighed. Moreover, the digestion of MF in calves results in a rapid gastric emptying of lactose just after meal ingestion, which is followed by the arrival of high levels of lactose in the small intestine (Guilloteau & Zabielski 2005). This phenomenon could modify nutrient absorption and the metabolite profile. Thus, the effects of butyrate supplementation on GIT were studied by measuring eventual modifications of the expression of sodium-dependent glucose transporter-1 (SGLT-1) in small intestinal epithelial tissues.

Materials and Methods

Animals and diets

The treatments and experiments were conducted according to Tohoku University regulations concerning the protection and care of experimental animals (GSAS20-49 and GSAS20-50). The animal studies were performed at the Tohoku University Farm (Kawatabi, Osaki, Japan).

Twenty-four Holstein milk-fed male calves, aged 3 days (day 1), were held in individual calf hutches for a further 42 days (6 weeks). They were divided into two groups of 12 animals each (control group, C; and Na-butyrate-supplemented group, B), and were subjected to the two experiments described below. The mean body weight (BW) at day 12 was similar in groups C and B (Table 1). A standard feeding schedule was applied for both groups. Each animal in both groups received the same amounts of MF (Meiji Feed Co., Tokyo, Japan) and was fed twice daily, at 0830 and 1630 h (2 l each time) with a milk bottle throughout the experiment. From day 7, the calves were fed a starter diet, made available *ad libitum*. The MF and calf starter consisted of 245 and 200 g crude protein and 219 and 20 g crude fat per kg respectively (Kitade *et al.* 2002, Yonekura *et al.* 2002). MF given to the animals in the B group was supplemented with increasing amounts of Na-butyrate (3, 5, and 7 g/day from day 1 to 3, from day 4 to 7, and from day 8 to 42 respectively; Norel, Barcelona, Spain) so the animals could adapt to the chemical. There were no refused quantities of MF.

Experimental design

In Experiment 1, 16 animals (eight for each group, C and B) were fed MF and the starter diet as described above. Among them, eight calves (four from each group) were fitted with a blood catheter at day 41, which was used for plasma sample collections at day 42. All the animals were finally killed by stunning at a slaughterhouse on days 43/44, 18–22 h after their last meal and then subjected to tissue sample collections.

In Experiment 2, since the digestion of MF in calves resulted in the rapid arrival of lactose in the small intestine, the other eight animals (four from each group, C and B) were fed as described above and fitted with a catheter on day 41, which was used for plasma sample collections on day 42. On day 42, MF was replaced by lactose solution (100 g/2 l tap water). The amount of lactose was chosen to be equal to the quantity of lactose present in milk replacer. Lactose solution was ingested without butyrate (group C) or with 3.5 g of butyrate (group B). For both experiments, MF or lactose solution was given to each animal at 0900 h using a milk bottle. During the blood sampling, the starter was removed.

Sample collection and analyses

Plasma concentrations of hormones and metabolites (Experiments 1 and 2) On day 42, blood samples were collected via a catheter inserted into the left jugular vein

Table 1 The effect of sodium-butyrate supplementation in milk formula and starter on growth parameters, organs and/or tissue weight. Data are mean $\pm$ S.E.M.

	Control group ^a	Butyrate group ^a	P values
Calves (n)	Eight	Eight	
Growth parameters			
BW at day 12 (kg)	47.2 $\pm$ 1.1	50.5 $\pm$ 4.5	0.56
BW (kg) at the end of the experiment (day 42)	72.7 $\pm$ 0.4	78.2 $\pm$ 5.8	0.15
BW gain (kg; from days 4 to 42 of age)	25.5 $\pm$ 1.2	27.7 $\pm$ 1.0	0.19
Starter feed consumption (kg; from days 4 to 43 of age)	16.2 $\pm$ 1.1	15.7 $\pm$ 1.4	0.81
Feed/BW gain ratio (kg/kg)	1.8 $\pm$ 0.1	1.7 $\pm$ 0.1	0.75
Organs and/or tissues			
Liver (g)	1330 $\pm$ 45	1362 $\pm$ 76	0.37
Spleen (g)	208 $\pm$ 13	206 $\pm$ 11	0.90
Peri-renal fat (g)	112 $\pm$ 9	161 $\pm$ 23	0.07
Fore stomach (weight)			
Whole stomach (g)	1496 $\pm$ 46	1475 $\pm$ 79	0.82
Reticulorumen (g)	890 $\pm$ 30	884 $\pm$ 41	0.91
Omasum (g)	212 $\pm$ 18	194 $\pm$ 16	0.48
Abomasum (g)	389 $\pm$ 19	386 $\pm$ 23	0.93
Fore stomach (% of the whole stomach weight)			
Reticulorumen (g/g)	59.7 $\pm$ 2.0	60.4 $\pm$ 2.8	0.85
Omasum (g/g)	14.2 $\pm$ 1.2	13.3 $\pm$ 1.1	0.58
Abomasum (g/g)	26.1 $\pm$ 1.3	26.5 $\pm$ 1.3	0.80
Rumen papilla length (mm)	2.24 $\pm$ 0.23	2.54 $\pm$ 0.35	0.08

P values are based on (unpaired) Student's *t*-test (*n*=8 for each group).

^aThe calves were fed 'milk formula + starter' without butyrate (control group) or with butyrate supplementation (butyrate group).

in tubes containing heparin (500 IU/ml, Wako Pure Chem., Osaka, Japan). Blood samples were withdrawn at every 15 min for each calf (5 ml for each sampling time), from 30 min before to 2 h after the morning meal (MF or lactose solution) for hormone and metabolite analysis (Katoh *et al.* 2004, Kobayashi *et al.* 2006). The plasma concentrations of GH, insulin, and IGF1 were measured by RIA as described previously (Katoh *et al.* 2004). Glucose and non-esterified fatty acid (NEFA) concentrations were determined using a commercially available kit (Wako Pure Chem.).

Morphological and gene expression analyses

(Experiment 1) Immediately after slaughter in Experiment 1, the liver, forestomachs, spleen, and kidneys were removed as well as the perirenal fat and then weighed. Five rumen epithelial pieces (5 $\times$ 5 cm) were sampled for each animal at the cranial ventral blind sac. The tissue was immediately fixed in Bouin's solution for histological analyses. Digital pictures of thin slices from each mucosal pieces were taken by the CCD camera with the dissection microscopy and the papilla length (*n*=30–50) was measured on the digital pictures by the NIH image program.

Ten centimeter-long mucosal samples of the middle jejunum and the ileum (50% of their length) were also removed and soaked in liquid nitrogen to analyze the mRNA expression of *Sglt-1*. Total RNA extraction was performed from mucosal samples using TRIzol reagent (Gibco BRL) according to the manufacturer's instructions and was resuspended in RNase-free water, treated with diethyl pyrocarbonate (Sigma–Aldrich Japan). The purity of RNA was acceptable if the ratio of optical density (OD)

measurements at 260 and 280 nm (OD 260/280 nm) was >1.9 . Electrophoresis using ethidium bromide staining was used to check for possible RNA breakdown. RT-PCR for the expression of *Sglt-1* mRNA was performed by random hexamer primers. The RT-PCR quantification was made with the Light Cycler System (Roche Molecular Biochemicals; Hayashi *et al.* 2006). Glyceraldehyde-3-phosphate dehydrogenase was included in the assays as a housekeeping gene.

Statistical analysis

Data are expressed as the mean $\pm$ S.E.M. All data were analyzed using SAS (version 9.1; SAS Institute Inc., Cary, NC, USA). One-way ANOVA was employed, followed by Duncan's multiple range tests and Student's *t*-test. In statistical analyses, *P* < 0.05 was the level of significance, and *P* < 0.10 indicated a tendency. The area under the curves for 150 min (AUCs) was calculated by a trapezoidal integration method. The incremental area (ICA) for each hormone was calculated for 120 min after feeding. For calculating ICA, the mean concentration of the three preprandial values from –30 to 0 min was used as the base value.

Results

BW, organ weight, and papillae length

The calves of both groups remained clinically healthy during the entire study. At the end of the experiment (day 42), the

mean BW and the BW gain of group B were 107.6 and 108.6% of those observed in group C respectively (Table 1), but these differences were not significant. During the entire experiment, total dry matter (DM) feed consumption was similar in both groups. This means that the amount of starter feed refused was very small for both groups.

The weights (absolute and relative to BW values) of various organs and stomachs did not show a difference due to butyrate supplementation except for perirenal fat, which tended to be greater for group B compared with group C (+43.8%, $P=0.07$; Table 1).

In the rumen, the mean papilla length (Fig. 1, Table 1) tended to be greater ($P=0.08$) in the animals of group B than group C.

Hormonal and metabolite responses to milk replacer feeding

The basal GH concentrations (mean of the three values obtained at -30, -15, and 0 min before the morning meal) were not different in the calves of group B compared with that of group C (5.8 ± 1.3 and 4.3 ± 1.2 ng/ml, respectively, $P=0.37$; Fig. 2). However, during the first postprandial hour (at 30–60 min), the plasma GH concentrations were smaller ($P<0.05$) in group B compared with those in group C. In addition, AUC measured with group B tended to be lower ($P=0.07$) than that obtained with group C. For ICA, no difference between the groups was observed ($P=0.14$). Despite the suppression in GH level differences, however, there was no difference in the plasma IGF1 concentrations on day 42 between groups C and B (50.2 ± 3.0 vs 57.4 ± 4.0 ng/ml, respectively, $P=0.64$, not shown).

The time course of postprandial increase in the plasma insulin concentrations for group B compared with group C was smaller ($P<0.05$) at 75 min and showed a tendency to be

Figure 1 Length of rumen papilla for the calves in the C (white bar) and B (black bar) groups (left), and microphotographs of the tissues (right). All values for papilla length represent mean $\pm$ S.E.M. for eight animals in each group, and were analyzed by Student's *t*-test ($P=0.08$). Scale units in both photographs are 0.5 mm.

Figure 2 The time course of postprandial changes in levels of plasma GH, areas under the curve (AUC), and incremental area (ICA) for the calves after ingestion of MF in the C (open circle) and B (filled circle) groups. All values represent mean $\pm$ S.E.M. for four animals in each group, and were analyzed by Student's *t*-test. * $P<0.05$ ($n=4$ for each group).

smaller at 60 min (Fig. 3 left). In addition, the AUC and ICA showed a tendency to be smaller for group B than for group C (Fig. 3 right; $P=0.06$ and 0.05 respectively).

There was no difference in the basal glucose concentrations between the two groups ($P=0.66$). For the postprandial time course of plasma glucose concentrations, the value for group B showed a tendency to be smaller only at 60 min than that for group C (Fig. 4 left). There was, however, no difference in AUC and ICA for both groups ($P=0.17$ and 0.11 , respectively, data not shown).

There was also no difference in the basal NEFA concentrations between the two groups ($P=0.36$). For the postprandial time course of the plasma NEFA concentrations, the values for group B showed a tendency to be greater only at 105 min than those for group C (Fig. 4 right).

Hormonal and metabolite responses to lactose feeding

Before ingestion of lactose solution, there was no difference in the basal GH concentrations between groups C and B. Ingestion of lactose solution, however, did not cause either an increase in the plasma GH concentration or a difference for both groups in AUC and ICA ($P=0.14$ and 0.57 , respectively, data not shown).

The basal insulin concentration was greater ($P<0.05$) for group C than for group B at -15 and 0 min. After ingestion of lactose solution, the increase in group B was still smaller, or tended to be smaller ($P<0.10$), than that in group C (Fig. 5 left). The AUC of insulin for group B was smaller ($P=0.02$), and ICA showed a tendency to be smaller ($P=0.07$), than that for group C.

There was no difference ($P=0.31$) in the basal glucose concentrations between both groups. On the time course curves, the glucose concentrations for group B at 30 and

Figure 3 The time course of postprandial changes in levels of plasma insulin, areas under the curve (AUC), and incremental areas (ICA) for the calves after ingestion of MF in the C (open circle) and B (filled circle) groups. All values represent mean $\pm$ S.E.M. for four animals in each group, and were analyzed by Student's *t*-test. * $P < 0.05$ and † $P < 0.10$ ($n = 4$ for each group).

45 min showed a tendency to be smaller than those for group C (Fig. 6, left). However, there was no difference between the two groups for AUC and ICA ($P = 0.17$ and 0.11 , respectively, data not shown). In addition, there was no difference between the two groups for the basal ($P = 0.23$) and time course curve for NEFA (Fig. 6 right).

Gene expression of *Sglt*

Gene expression of *Sglt-1* in the epithelial tissues of the ileum showed a tendency to be greater than that of the jejunum (0.470 ± 0.163 and 0.875 ± 0.126 , respectively, $P = 0.09$, data not shown) in the control animals. However, no difference was observed between the two groups either in the jejunum or in the ileum ($P = 0.88$ and 0.16 , respectively, data not shown).

Discussion

In the present experiment, the dose of Na-butyrate used as a feed additive in MF (but not in the starter) was similar to that employed in most of the experiments already cited (see Introduction). Positive effects of butyrate on animal performance and GIT functions and development have been reported, although no effects on performance were noticed in the present work. However, we have shown enhanced rumen papilla growth and perirenal fat accumulation in the calves fed MF supplemented with butyrate compared with the calves fed MF without butyrate. Moreover, we have demonstrated that the postprandial increase in the plasma concentrations of insulin and GH as well as glucose, induced by MF or lactose solution intake, was reduced or moderately suppressed by the presence of butyrate in MF. To date and to the best of our knowledge, this is the first time that it has been shown that

butyrate supplementation at a low dose simultaneously modifies the concentrations of several plasma hormones produced by the insulino/somatotropic axes as well as the blood glucose concentration.

The effects of butyrate supplementation resulted in increased nutrient digestibility, stimulation of digestive enzyme secretion, enhancement of proliferation, differentiation, and maturation, reduction apoptosis in the enterocytes, and modification of the intestinal luminal microflora and/or an improvement of the epithelial integrity and defence systems (Cummings *et al.* 1995, Guilloteau *et al.* 2010a). These effects could explain the increase in BW gain of the animals. In this study, however, there was no difference in BW gain between the two groups (with and without butyrate supplementation), as it was observed by Gorka *et al.* (2009, 2011) and Flaga *et al.* (2009). These observations could be due to the feeding experimental schedule, which is commonly less intensive, or to the different quality of MF used in Japan from that in EU countries; Japanese MF contains casein but that of the EU does not. The main reason, however, is probably due to the status of the calves. The reported experiments were conducted in very young calves (≤ 1.5 months) which ingested MF and starter diet from 3 days after birth, whereas the measurements for other studies were obtained in milk-fed (Guilloteau *et al.* 2004, 2009, 2010b) or in weaned (Slusarczyk *et al.* 2010) calves aged at least 2 months. In fact, it was demonstrated, in different species, that butyrate must be ingested immediately after birth, but the growth performances were observed later in life (Guilloteau *et al.* 2009, 2010b, Le Gall *et al.* 2009).

In previous studies, we have found that bolus injection of SCFAs (acetate, propionate, and butyrate) mixture in the rumen of milk-fed calves every day from 7 days to 13 weeks of age changed the expression of leptin and cholecystokinin (CCK) receptors in the stomachs and small intestine (Yonekura *et al.* 2002). In addition, butyrate injection in the rumen of calves and sheep stimulated epithelial growth and the mitotic index (Tamate *et al.* 1962, Sakata & Tamate 1976).

Figure 4 The time course of postprandial changes in levels of plasma glucose and NEFA for the calves after ingestion of MF in the C (open circle) and B (filled circle) groups. All values represent mean $\pm$ S.E.M. for four animals in each group, and were analyzed by Student's *t*-test. † $P < 0.10$ ($n = 4$ for each group).

Figure 5 The time course of changes in levels of plasma insulin, areas under the curve (AUC), and incremental areas (ICA) for the calves after ingestion of lactose solution in the C (open circle) and B (filled circle) groups. All values represent mean $\pm$ S.E.M. for four animals in each group, and were analyzed by Student's *t*-test. * $P < 0.05$ and † $P < 0.10$ ($n = 4$ for each group).

These findings suggest that SCFAs, which are actively produced in the rumen after weaning, affect the GIT functions. Because butyrate (or β -hydroxybutyrate) was not found in peripheral blood circulation (Manzanilla *et al.* 2006, Guilloteau *et al.* 2010a) and because the effects of butyrate were observed at various GIT epithelia (Sakata & Yajima 1984) and butyrate can act directly or indirectly on tissue development and repair (Guilloteau *et al.* 2010a), several humoral factors can be postulated. The pre- and postprandial concentrations of plasma gastrin and CCK, regulatory peptides known for their trophic effects in the upper gut, were not affected by butyrate supplementation, suggesting that the effects of Na-butyrate were not mediated by these substances (Guilloteau *et al.* 2009). In contrast, in young calves (26 days of age), butyrate supplementation of MF and/or starter diet exerted a pronounced effect on GIT development, particularly on the abomasum and small intestine, which may be controlled by IGF2 and ghrelin (Flaga *et al.* 2009). Moreover, Glucagon-like peptide-2 could be implicated (Gorka *et al.* 2011).

Milk replacer feeding in young calves and goat kids or drinking milk directly from their dam's teats in goat kids caused an increase in the plasma concentrations of insulin and GH (Katoh *et al.* 2004, Kobayashi *et al.* 2006). A simultaneous increase in both hormones seems to be a typical endocrine feature in pre-weaning ruminant animals, but this observation remains to be clarified. Butyrate (or SCFAs) injection in either the jugular blood circulation or the rumen reduced plasma GH concentrations *in vivo* (Matsunaga *et al.* 1993, 1999). These responses may be due to the suppression in basal and GH-releasing-hormone-induced cellular calcium ion concentrations in pituitary somatotrophs (Katoh 1995, Katoh *et al.* 1996, Katoh & Ishiwata 1998, Ishiwata *et al.* 2000, 2005). However, the suppressive effects of butyrate on insulin level were more controversial than those on GH level, because the

bolus injection of butyrate as well as glucose or L-arginine in the jugular vein was demonstrated to increase the plasma insulin and glucagon concentrations (Sasaki *et al.* 1982). Thus, there is probably a difference between the acute and chronic effects of butyrate administration. However, the reason for the abolished postprandial insulin levels was not due to suppressed glucose absorption capacity in the intestinal epithelium. This assumption is supported by several findings. First, in both groups (C and B) in the present experiment, the postprandial increases in plasma glucose concentration were similar; moreover, *Sgt-1* mRNA was expressed at a similar level in the intestinal epithelium. Secondly, previous studies with calves (Guilloteau *et al.* 2009) as well as with neonatal (Kotunia *et al.* 2004) and weaned (Mazzoni *et al.* 2008, Le Gall *et al.* 2009) piglets demonstrated that the ingestion of Na-butyrate resulted in improved performances linked to a better GIT development.

The alternative explanation is that Na-butyrate supplementation improved the insulin sensitivity. In veal calves, milk feeding for a long period was demonstrated to cause insulin resistance. The responses of plasma glucose and insulin to a MF ingestion in calves became greater by aging and remained elevated for more than 6 h after feeding (Vicari *et al.* 2008). In addition, a significant urinary excretion of glucose was also observed, showing type 2 diabetes symptoms in human subjects (Ferrannini & Camastra 1998). In this study, however, the postprandial increase in plasma insulin and glucose concentrations was suppressed by butyrate supplementation. Improvement of insulin resistance by Na-butyrate ingestion might result in the increased perirenal fat accumulation, as shown in Table 1. This assumption may be supported by a finding that was recently reported in mice (Gao *et al.* 2009). In dietary obese C57BL/6J mice, Na-butyrate supplementation (at 5% wt/wt) in the high-fat diet prevented development of insulin resistance and obesity. The mechanism of butyrate action is related to the promotion of energy expenditure through enhanced mitochondrial expression of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) and uncoupling protein-1. Butyrate may thus be a useful

Figure 6 The time course of postprandial changes in levels of plasma glucose and NEFA for the calves after ingestion of lactose solution in the C (open circle) and B (filled circle) groups. All values represent mean $\pm$ S.E.M. for four animals in each group, and were analyzed by Student's *t*-test. † $P < 0.10$ ($n = 4$ for each group).

nutrient for preventing the progressive diabetes commonly observed in milk-fed calf.

In conclusion, the application of Na-butyrate as a feed additive seems to be promising in animal and human nutrition. Owing to the effects of butyrate on improving GIT development and consequently growth performances, this feed additive could be useful in animal nutrition, as was demonstrated in calf and piglet. Moreover, when added to MF or lactose solution, butyrate suppressed the postprandial elevation in plasma insulin and glucose levels, although it reduced GH levels. In this way, butyrate could be a good candidate for preventing metabolic diseases in animals and for human nutrition.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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