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Original articles

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EFFECT OF SODIUM BUTYRATE SUPPLEMENTATION IN MILK REPLACER AND STARTER DIET ON RUMEN DEVELOPMENT IN CALVES

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Rumen development is an important factor determining early solid feed intake and performance in cattle. A popular trend towards early weaning of newborn dairy calves necessitated looking for ways of accelerating the gastrointestinal tract (GIT) development. The present study aimed to determine the effect of sodium butyrate (NaB) supplementation in milk replacer and starter diet on rumen development in rearing calves. Fourteen bull calves (5-day-old) were randomly allocated to two groups: Control (C) and NaB. The later received 0.3 % NaB in milk replacer and starter diet. Animals were in experiment up to age of 26 days. Addition of NaB to milk replacer and starter diet had no effect on daily growth rate, but reduced the weight loss observed in C calves in first 11 days of age. Additionally, the NaB calves weighed more at the end of the study and tended to have higher growth rate in the whole trial period ($P<0.15$). The NaB calves showed a tendency toward higher reticulorumen weight ($P=0.13$) and higher reticulorumen weight expressed as a percent of whole stomach weight ($P=0.02$) as compared to control. Histometry analysis indicated larger rumen papillae length and width ($P<0.01$) in NaB group, and no change in muscle layer thickness, as compared to control. Plasma glucagon-like peptide-2 relative increase was higher in NaB group than in C group, and may be involved in rumen development. In conclusion, supplementation of the diet (milk replacer and starter diet) with NaB may enhance rumen development in neonatal calves.

Key words: *neonatal calves, volatile fatty acids, papillae growth, glucagon-like peptide-2*

INTRODUCTION

The development of forestomachs in calves, in particular rumen, is highly dependent on nutritional factors and it may be significantly accelerated by early introduction of solid feeds (1-4), which may be especially important in early weaning systems. Restricted amounts of liquid feed and ad libitum solid feed intake positively affected development of rumen weight, volume and function in calves (2, 3, 5). Additionally, solid feed intake starting from the first days of life increases solid feed intake and may positively affect development of rumen microflora, rumen fermentation as well as its epithelium (3, 5-7). For efficient solid feed utilization proper rumen epithelium development is especially desired. Fermentation products in the rumen such as volatile fatty acids (VFA) (mainly butyric acid and to a lesser extent propionic acid) are considered to be the main stimulators of rumen epithelium development (8, 9). Thus feeding calves with restricted amounts of liquid feed and starter mixtures containing carbohydrates which are rapidly fermented to butyric and propionate acids is practiced to accelerate rumen development (4, 5, 10, 11).

Liquid feed (colostrum, milk or milk replacer) is the most important nutrient source for preruminant calves until starter solid mixture intake reaches at least 1% of animal body weight (1). Liquid feed bypasses the forestomachs and enters the abomasum and small intestine, the main sites of their digestion (2). Development of abomasum and small intestine in neonatal calves is most pronounced in the first weeks of life (12, 13). It continues in the later age, although it is not as intensive as in simple stomached mammals (13-15). In contrast to milk, milk replacers provide less stimulation toward abomasum and small intestine development due to the presence of antinutritional factors and lack or bioactive substances normally present in colostrum and milk, e.g., hormones and growth factors (12, 13, 16). In contrast to calves fed with milk, atrophy of intestinal villi and decrease in intestinal brush border enzyme activity may be observed in calves fed with milk replacer (12, 16, 17, Kowalski *et al*., unpublished,). We also hypothesize that the negative effect of milk replacers on intestine development may reduce starter mixture intake thereby slowing rumen development.

Butyric acid was demonstrated to stimulate epithelial cell proliferation, and regulate cell differentiation and apoptosis in the gut (18, 19). It is also the preferable energy source for colon and rumen epithelial cells (19, 20), with anti-inflammatory, cytoprotective and antibacterial properties (21-23). Supplementation of sodium salt of butyrate (NaB) in milk replacer or starter diet positively affected stomach and small intestinal development as well as growth parameters in newborn

piglets, veal calves and broiler chicken (24-26). Additionally, infusion of butyric acid into the rumen stimulated rumen papillae growth in newborn calves (8, 9).

The aim of the present study was to determine the effect of NaB supplementation in milk replacer and starter mixture on rumen development in newborn calves. We hypothesised that concomitant addition of NaB to milk replacer and starter mixture would stimulate development of both rumen and intestine and thereby increase starter diet intake.

MATERIALS AND METHODS

Animals and diets

The animal study protocol has been approved by the Local Ethics Committee prior to the study. Fourteen clinically healthy male calves (Holstein or Holstein x Limousine), age 4-6 days, were randomly allocated to two experimental groups: Control (C) and Na-Butyrate (NaB) (7 calves per treatment). In the following the mean age of calves will be used. Before the onset of the trial the calves were routinely fed colostrum and than 2.5 L of whole milk given twice a day, and with no access to a starter diet. The calves were kept individually in pens (1.5 x 1.2 m) with rubber floor to avoid straw intake. Each calf was in the trial over a period of 3 weeks, until the age of approximately 26 days.

The animals were fed individually. Milk replacer (Primolac, Polmass S.A., Poland, 210 g of crude protein and 16.9 MJ/kg) was mixed with warm water (about 40°C) in the ratio of powder to water as 1 to 9 and fed twice a day from bucket with teat. The daily dose of milk replacer was stable during the whole experimental period and equaled 10% of initial body weight (BW) of the calf. The milk replacer offered to the NaB group contained 0.3% (as feed) crystalline Na-Butyrate (Vetagro, Italy) that was introduced to the milk replacer by the milk replacer producer. Refusals of milk replacer were recorded daily.

Commercial pelleted starter mixture (Koncentrat KCJ, Polmass S.A., Poland, 340 g of crude protein and 11.3 MJ/kg) was mixed with whole corn grain (50/50; wt/wt) and offered as a starter diet (215 g of crude protein and 11.5 MJ/kg) once daily ad libitum, beginning on the first day of the experiment. The starter mixture offered to the NaB group contained microencapsulated NaB (Vetagro, Italy) in amount equal to 0.6% (as feed), so the final starter diet contained 0.3% NaB. Microencapsulated form of NaB was used in order to slow down the butyric acid release in the reticulorumen. The starter diet intake was controlled daily.

Measurements and observations

The calves were individually weighed at the beginning of the trial, and on day 12, 19 and 26 of life, before morning feeding. The animal health condition was scored at the start of the trial and at every week using the five point health status scores: very good – 5, very bad – 1. Fecal score including fluidity, consistency and smell were controlled daily using the Larson *et al*. scale (27). Every abnormal condition as well as every veterinary treatment used, were recorded. Calves with diarrhea were treated with a commercial electrolyte solution. Electrolyte therapy was initiated when fecal fluidity equaled to 3 or 4. Medical treatment was used as recommended by veterinarian surgeon only when it was absolutely essential.

Rumen fluid and histometry analysis

At 26 day of life the calves were killed, and the entire forestomachs and abomasum were dissected. Rumen fluid was sampled for VFA concentration analysis (28), ammonium concentration analysis (29), rumen pH, and abomasum fluid was sampled for pH measurement. The reticulorumen, omasum and abomasum were separated, emptied, rinsed repeatedly with water, drained and weighed individually.

One cm^2 whole thickness samples from the right side of the cranio-dorsal sack of the rumen were immediately fixed in 4% buffered phormaldehyde for 5 days and stored in ethanol for preparation. They were then embedded in paraffin. Serial histological sections of 7 µm thickness were stained with hematoxylin and eosine for morphometry analysis under light microscope. Morphometry analysis involved measurements in the rumen wall (rumen papillae length and width, muscle layer thickness) in 5 to 8 slides for each tissue sample. In each tissue sample, thirty rumen papillae and rumen muscle layer measurements were done using optical binocular microscope (OLYMPUS BX 61, Poland) coupled *via* a digital camera to a PC computer equipped with a Cell^P (OLYMPUS) software.

GLP-2 radioimmunoassay

Blood (10 ml) was withdrawn from the left or right jugular vein of all calves at the beginning of the trial, and on day 12, 19 and 26 of life, before morning feeding. The blood was collected in tubes containing EDTA (VT-100 STK, 0.1 ml EDTA, 0.47 ml/l: 21 w/v %, CML, Nemours, France) and aprotinin (10,000 IU/ml, Trasylol, Bayer Pharma SAS, Puteaux, France). All tubes were centrifuged 2300 x g for 10 min at 8°C and plasma was frozen and stored at -20°C until further analysis. GLP-2 concentrations in plasma were measured by radioimmunoassay after extraction of plasma with 70 % ethanol (vol/vol, final concentration), employing antiserum code no. 92160 and standards of human GLP-2 (proglucagon 126-158, a gift from Novo Nordisk A/S) and monoiodinated Tyr-12 GLP-1, specific activity >70 MBq/nmol (30). The antiserum is directed against the N-terminus of GLP-2 and therefore measures only fully processed, intact GLP-2 of intestinal origin. Sensitivity for the assay is below 2 pmol/l, and intra-assay coefficient of variation below 6 %.

Statistical analysis

The homogeneity of variances between animal groups was checked using Levene's test. BW, BW gain, starter diet intake, whole corn grain intake from starter diet, GLP-2 concentration and relative increase (%) of GLP-2 concentration in blood plasma were analyzed as repeated measures using PROC MIXED (31). The statistical model included calf as a random effect, and group and its interaction with time (calf age) as a fixed effect (32). Additionally, the statistical model included animal breed as a main effect and initial age and initial BW of calves as a covariate. If the group effect or group x time effect was significant, differences between treatments in particular time were determined using ESTIMATE statements of SAS (31). Means for above mentioned data as well as for stomach development parameters were subjected to one-way analysis of variance using PROC GLM (31). The effect of breed, age and initial or end body weight of animals were incorporated into the statistical model. The significance was declared at $P<0.10$ and tendencies at $P<0.15$.

RESULTS

Calf performance and health

The results of BW, BW gain and starter diet intake are shown in *Table 1*. Animals from NaB group weighed slightly more at

the beginning of the trial (45.3 *vs* 47.7 kg for C and NaB, respectively), however, these differences were not significant. The BW of the animals changed significantly during the trial period (P<0.01). The NaB positively affected BW during the whole trial period (P=0.08). Those differences were especially apparent on days 12, 19 and 26 of age. At the end of the experiment the animals from the NaB group gained 1.7 kg more than control (P<0.10). It is also worth to notice that the initial and final body weights of animals from C group did not differ.

There was no significant effect of NaB on daily BW gain. However, the calves from NaB group seemed exhibit a reduced BW loss as compared to that of C group between 5-11 day of age. Additionally, mean BW gain of NaB group during the whole trial period was significantly higher (16 *vs* 95 g/day for control and NaB, respectively; P≤0.10).

The starter diet intake increased successively with age (P<0.01) and a significant group x time effect was observed (P=0.02). The starter diet intake was significantly higher for NaB group between 19-26 days of age (162 *vs* 270 g/day for C and NaB groups, respectively; P<0.10). Moreover, NaB animals tended to consume more starter diet in the whole trial period (95 *vs* 136 g/day for C and NaB groups, respectively; P<0.15). All calves selected more pellets of the starter mixture from the starter diet avoiding whole corn grains at the beginning of the trial, but the whole corn grain intake increased with age (P<0.01) (data not shown). However, there was no treatment effect on this sorting.

There were no significant effect of NaB supplementation on fecal score besides fecal consistency which was softer for NaB group (P=0.07; *Table 2*). The animals from NaB group had less scouring days (P=0.03) and electrolyte therapies (P=0.10) as compared to that of C group during the whole trial period. Additionally, the body condition score was improved by NaB supplementation (P=0.06).

Table 1. The effect of sodium butyrate supplementation in milk replacer and starter mixture on calves body weight, daily body weight gain and starter diet intake

Item	Group	
	Control	NaB
Body weight (kg)[1]		
5 day of life	45.3 ± 2.3[4]	47.7 ± 1.8
12 day of life	42.2 ± 2.2	47.9 ± 2.0**
19 day of life	42.8 ± 2.2	47.9 ± 2.1**
26 day of life	45.6 ± 2.1	49.7 ± 2.2***
Mean body weight gain (kg)	0.3 ± 0.4	2.0 ± 0.7**
Daily body weight gain (g/day)[2]		
5-11 days of life	-143 ± 71	10 ± 68
12-18 days of life	-12 ± 62	10 ± 36
19-26 days of life	214 ± 80	265 ± 48
Mean daily body weight gain (g/day)	16 ± 19	95 ± 35**
Starter diet intake (g/day)[3]		
5-11 days of life	25 ± 8	33 ± 10
12-18 days of life	97 ± 25	106 ± 15
19-26 days of life	162 ± 54	270 ± 30**
Mean starter diet intake (g/day)	95 ± 27	136 ± 17*

[1]Significant group effect and time effect (P=0.08 and P<0.01, respectively); [2]Significant group and time effect (P=0.13 and P<0.01, respectively); [3]Significant group x time effect and time effect (P=0.02 and P<0.01, respectively); [4]Mean ± Standard error; *P<0.15; **P<0.10; ***P<0.05

Rumen and abomasum fluid pH and rumen fermentation

NaB supplementation had no significant effect on the fluid pH and the fermentation process in the rumen, however, the sum of VFA, especially propionic, butyric and valeric acid concentrations, was higher and ammonium concentration was lower in the rumen fluid of NaB calves (*Table 3*). On the other hand, the abomasum fluid pH was reduced in NaB group (P=0.02).

Rumen development

The NaB supplemented animals tended to have higher empty reticulorumen weight (P=0.13), however, when it was expressed as percent of BW those differences were not significant (*Table 4*). On the other hand, expressed as a percent of whole stomach, reticulorumen weight was higher (42.6 *vs* 49.8% for C and NaB groups, respectively; P<0.01) and abomasum weight was lower (45.5 *vs* 39.5% for C and NaB groups, respectively; P=0.02) in

Table 2. The effect of sodium butyrate supplementation in milk replacer and starter mixture on health status of calves in whole trial period

Item	Group		P-value
	Control	NaB	
Fecal score			
Fluidity[1]	1.37 ± 0.09[7]	1.39 ± 0.10	*NS*[8]
Consistency[2]	1.39 ± 0.09	1.17 ± 0.06	0.07
Smell[3]	1.13 ± 0.05	1.09 ± 0.04	*NS*
Scouring days[4]	1.57 ± 0.61	0.14 ± 0.14	0.03
Body condition[5]	4.84 ± 0.06	4.94 ± 0.04	0.10
Electrolyte therapies[6]	0.71 ± 0.36	0.14 ± 0.14	0.06
Antibiotic therapies	0.29 ± 0.18	0.14 ± 0.14	*NS*

[1]Scored in 4 point scale: 1-normal, 4-diarrhea; [5]Scored in 5 point scale: 1-normal, 2-frothy, 3-mucous, 4-sticky, 5-hard (constipation); [3]Scored in 3 point scale: 1 – normal, 3 – disgusting; [4]scouring days were considered when fecal fluidity was equal to 3 or 4; [5]Scored in 5 point scale: 1-bed, 5-very good; [6]Electrolyte therapies were started when fecal fluidity was equal to 3 or 4; [7]Mean ± Standard error; [8]Not significant

Table 3. The effect of sodium butyrate supplementation in milk replacer and starter mixture on rumen fluid pH, abomasum fluid pH, volatile fatty acid (VFA) concentration and ammonium concentration in rumen fluid in calves

Item	Group		P-value
	Control	NaB	
Rumen fluid pH	5.34 ± 0.23[1]	5.08 ± 0.07	*NS*[2]
Abomasum fluid pH	2.11 ± 0.15	1.64 ± 0.08	0.02
Sum of VFA (mmol\L)	76.2 ± 11.4	93.1 ± 7.8	*NS*
VFA (mmol/L)			
Acetic acid	38.1 ± 5.8	41.7 ± 3.2	*NS*
Propionic acid	28.7 ± 5.2	38.0 ± 3.6	*NS*
Butyric acid	5.70 ± 0.92	7.04 ± 1.09	*NS*
Valeric acid	2.29 ± 0.44	3.25 ± 0.79	*NS*
Isobutyric acid	0.60 ± 0.17	0.58 ± 0.09	*NS*
Isovaleric acid	0.84 ± 0.20	0.80 ± 0.14	*NS*
N-ammonium (mg%)	25.9 ± 5.7	18.5 ± 1.9	*NS*

[1]Mean ± Standard error; [2]Not significant

NaB group. Moreover, when mean starter diet intake in the whole trial was included in statistical analysis as a covariate, abomasum weight expressed as a percent of BW tended to be lower for NaB animals (P=0.13). The NaB supplementation positively affected papillae length (P<0.01) and width (P<0.01) (*Table 5*). There were no effects of NaB supplementation on tunica muscularis thickness.

Plasma GLP-2 concentration

There was a significant effect of group and time on plasma GLP-2 concentration in the whole trial period (P=0.02 and P=0.03, respectively) (*Table 6*). Plasma GLP-2 concentration increased from 5 to 12 days of age and, decreased thereafter. The NaB animals had lower plasma GLP-2 concentration at the beginning of the trial (P<0.10) and tended to have lower plasma GLP-2 concentration on day 19 of age (P≤0.15). When initial plasma GLP-2 concentration was used in statistical model as a covariate, we observed only tendencies toward group effect (P=0.11) and tendencies toward lower plasma GLP-2 concentration in NaB animals on day 5 of age (P=0.13). However, when plasma GLP-2 concentrations were expressed in relative values as percentage compared to mean value on 5 day of age, we observed opposing results (P=0.08). The NaB group had higher GLP-2 increment on 12 day of age (P<0.10) and tendencies toward higher GLP-2 increment in the whole trial period (P<0.15).

DISCUSSION

Previously, a positive effect of NaB supplementation as a feed additive was shown in neonatal piglets, veal calves and broiler chickens (24-26). In most studies supplementation of feed additives in newborn calves was restricted to the milk replacer or starter diet (11, 24, 33 - 36). To our knowledge, the effects of NaB supplementation both to the milk replacer and starter diet on newborn calf performances and GIT development have not been investigated. Because of a specific course of liquid and solid feed digestion in preruminant species this way of feed additive supplementation may be more effective. Our results suggest that concomitant addition of NaB to milk replacer and starter mixture may stimulate rumen development as well as positively affect lower GIT function (own unpublished data) and thus calf performances.

BW loss during first weeks of life is often observed in dairy calves fed milk replacers (14, 15, 37, 38). Such a situation may negatively affect future productivity of animals (39). Supplementation of feed additives like probiotics in milk replacers reduced body weight loss in the first weeks of life (37, 38).The positive effect of this way of feed additive supplementation to newborn calves is restricted mainly to the intestine (37). Thus NaB in the milk replacer could have positive effect on intestinal development and function as previously showed in newborn piglets and veal calves (24, 25), and by this way improve calves performances. We have observed that addition of NaB to the milk replacer exert a promitotic and antiapoptotic influences on calf intestinal epithelium (40). Higher mitotic:apoptotic ratio in NaB calves could increase villus height and intestinal absorptive surface. Moreover, intestinal brush border lactase activity in mid jejunum, aminopeptidase A and aminopepidase N activity in distal jejunum were positively affected in NaB calves (unpublished data). An effect of NaB on pancreas development in newborn calves was also demonstrated (24, 41). Taken together, it seems that the digestive processes in the intestinal lumen of NaB animals could be more efficient. However, besides a positive effect of NaB on calf growth rate in first 18 days of life, it should be pointed out that the daily BW gains were rather low. Even thought restricted amounts of milk replacer were used in this study, protein and energy intake by calves should provide BW gain in first days of life in a range of about 100-200 g/day (42). It seems that supplementation of NaB in the milk replacer positively affected GIT development and functions but did not fully compensate for the lack of milk bioactive components or presence of antinutritional factors in milk substitutes.

Table 4. The effect of sodium butyrate supplementation in milk replacer and starter mixture on structural stomach development in calves

Item	Group		P-value
	Control	NaB	
Weight (g)			
Whole stomach	622 ± 59[1]	728 ± 36	*NS*[2]
Reticulorumen	288 ± 39	365 ± 25	0.13
Omasum	79 ± 7	78 ± 6	*NS*
Abomasum	296 ± 20	286 ± 7	*NS*
Percent of body weight			
Whole stomach	1.46 ± 1.09	1.48 ± 0.09	*NS*
Reticulorumen	0.64 ± 0.09	0.74 ± 0.06	*NS*
Omasum	0.17 ± 0.02	0.16 ± 0.02	*NS*
Abomasum	0.65 ± 0.04	0.58 ± 0.03	*NS*
Percent of whole stomach weight			
Reticulorumen	42.6 ± 2.3	49.8 ± 1.1	<0.01
Omasum	11.9 ± 0.6	10.6 ± 0.4	*NS*
Abomasum	45.5 ± 2.4	39.5 ± 1.2	0.02

[1]Mean ± Standard error; [2]Not significant

Table 5. The effect of sodium butyrate supplementation in milk replacer and starter mixture on rumen wall development in calves

Item	Group		P-value
	Control	NaB	
Papillae length (μm)	314 ± 16[1]	516 ± 34	<0.01
Papillae width (μm)	150 ± 8	228 ± 12	<0.01
Tunica muscularis (μm)	1345 ± 142	1320 ± 113	*NS*[2]

[1]Mean ± Standard error; [2]Not significant

Table 6. The effect of sodium butyrate supplementation in milk replacer and starter mixture on blood plasma GLP-2 concentration in calves

Item	Group	
	Control	NaB
GLP-2 (pmol/l)[1]		
5 day of life	29.1 ± 8.3[3]	16.0 ± 4.2**
12 day of life	39.5 ± 6.4	34.4 ± 5.6
19 day of life	32.4 ± 8.1	3.0 ± 5.1*
26 day of life	26.4 ± 3.4	21.8 ± 4.3
Mean in the whole trial	33.5 ± 4.7	24.3 ± 2.3***
GLP-2 (% of values at day 5)[2]		
12 day of life	134 ± 22	215 ± 40**
19 day of life	111 ± 28	144 ± 36
26 day of life	115 ± 12	136 ± 32
Mean during the whole trial	120 ± 25	165 ± 20*

[1]Significant group effect and time effect (P=0.02 and P=0.03, respectively); [2]Significant group effect and time effect (P=0.08); [3]Mean ± Standard error; *P<0.15; **P<0.10; ***P<0.05

The starter diet intake is especially important when restricted amounts of liquid feed are offered to the calves and early weaning is planned. In such rearing systems, the solid feed intake determines growth rate of animals and possible weaning age (1, 3, 43). Despite free access of calves to the starter diet in the present study from 5 day of life, most animals started to consume it between 12 and 18 days of age. The highest starter diet intake was observed for NaB group mainly in the last period of the trial (19-26 days of life). It is well accepted that at least 3-4 weeks are needed for rumen epithelium development after the calves start consume solid feed (5). However, higher starter diet intake by calves from NaB group after 7-14 days could be adequate to initiate rumen function development and stimulation of high starter diet intake in the later age. Kristensen *et al*. (3) clearly showed that high solid feed intake in first days of life determines subsequent consumption around weaning period.

Our results suggests that the main part of calf stomach affected by NaB was reticulorumen rather than omasum and abomasum. Those results are further supported by tendencies toward lower abomasum weight in NaB calves when solid feed intake was used as a covariate in statistical analysis. We observed high correlation between daily BW of animals and solid feed intake between 19-26 days of life as well as for the whole trial period (r=0.75 and r=0.51, respectively). In order to provide adequate solid feed utilization for growth, rumen was the most important part of GIT.

Direct effect of butyric acid on rumen papillae development was previously shown by Tamate *et al*. (8) and Mentshel *et al*. (9). The NaB supplementation in the starter diet and its slow release from microcapsules allowed for direct and continuous exposition of rumen epithelial cells to its preferred energy source. The mechanisms behind the effect of butyric acid on rumen epithelial cell growth is not clear, however, and antiapoptic rather than promitotic action should be considered (9). It should also be taken into account that NaB supplementation significantly stimulated solid feed intake. Other VFAs may also affect rumen development. Zitnan *et al*. (44) showed that propionic acid may be even more important for papillae growth than butyric acid. The NaB did not significantly affect rumen fluid pH and rumen fermentation, however, the sum of VFAs, especially propionic, butyric and valeric acid concentration, was higher and ammonium concentration was lower in the rumen fluid of NaB calves suggesting enhanced rumen fermentation. The NaB supplementation in milk replacer could also have affected positively reticulorumen mass and rumen epithelium development. It was shown that growth, health and metabolic status of animals may affect solid feed intake and thus rumen development in newborn calves (45). This mechanism of NaB action is especially probable in a light of its positive effect on small intestine development. Efficient solid feed digestion in lower parts of the GIT, not only in forestomachs, may also determine its intake and rumen epithelium development.

An indirect effect of NaB action should be also considered. Infusion of butyric acid into the colon was shown to exert trophic and mitotic effects on illeal and jejunal epithelial cells (46, 47). Additionally, it was suggested that possible effect of NaB on GIT development may be exerted by its effect on bioactive peptides or hormones secretion into the blood and/or its local production in GIT (17, 24, Flaga *et al*., unpublished). Thus, plasma concentration of cholecystokinin (CCK) was increased and pancreatic polypeptide (PP) decreased in newborn piglets following NaB supplementation in milk replacer (25). Colonic infusion of butyric acid affected gastrin concentration in rat blood (47). Similar indirect actions of NaB on calf GIT development were especially possible when it is supplemented to milk replacer. However, in ruminants, solid feed allowance and composition may also modulate hormone secretion and expression of growth factors and their receptors in GIT (48-50). We showed for the first time that nutritive supplementation of NaB can modulate GLP-2 concentration in blood plasma of newborn calves. GLP-2 is considered as important endocrine signal activating intestinal adaptation, cell survival and proliferation in newborn mammals (51, 52). It is well known that luminal VFAs stimulate GLP-2 secretion from intestinal and colon enteroendocrine L cells (52–54). In pigs the intravenous infusion of butyrate increased plasma GLP-2 concentration (54). In the present experiment, GLP-2 plasma concentration increased with age in both groups, mainly during the first week of the trial is in accordance with other data (55, Guilloteau *et al*, unpublished). The GLP-2 plasma level was lower in NaB group than in C group, even before NaB supplementation. This aspect seems linked to the individual variability but not to the treatment. The effect of feeding on its plasma concentration in first day of experiment was eliminated because calves were fasted for 12 h before the onset of the trial and blood sampling. Colostrum intake was similar between treatment as well. In contrast, based on values obtained before any NaB supplementation, the relative increase (%) of GLP-2 plasma concentration was higher in NaB group than in C group during the whole experiment and mainly during the first week. Based on our unpublished results, addition of NaB to the starter diet, not to the milk replacer, stimulate GLP-2 secretion into the blood. These results are not unexpected because fermentation products of solid feed in the gut are the main stimulators of GLP-2 secretion and NaB supplementation in starter mixture stimulated solid feed intake. These results are in accordance with the higher length and width of rumen papillae. It also seems that stimulation of starter diet intake and GLP-2 secretion may be valuable for entire calf GIT development, not only rumen. On the other hand, to our best knowledge, GLP-2 and its receptor expression in the calf GIT were not shown so far. A complete explanation of mechanism of NaB action requires further studies.

Slowing down of intestinal development as result of milk replacer feeding instead of whole milk may especially predispose neonatal calves to diarrheas (12). The NaB supplementation in milk replacer partially reversed these negative effects. It also cannot be excluded that significantly lower abomasal fluid pH in NaB calves might reduce bacterial infections. A possible action of NaB on different gastric cells in pigs (56) was recently reported. On the other hand, faster rumen development may also positively affect health status of the calves. In many cases high solid feed intake, that reflects rumen function development, positively correlated with calf health (33, 34, 43). Especially feed additives that promote rumen development may restrict diarrheas problems in dairy calves (33, 34). It is well known that solid feed may accelerate GIT development in newborn mammals (57, 58, 59, 60). Lambs that had access to the solid feed had higher jejunum and ileum weight as compared to solely milk fed ones. Similar effects were observed when milk fed lambs received intraruminal infusion of VFAs (acetic, propionic and butyric acid) (57). Previously mentioned results of Baldwin (57) may suggest a positive effect of solid feed intake and fermentation process development in forestomachs not only on the rumen development but also on small intestine development. Additionally, some parts of microencapsulated NaB could avoid rumen digestion and by pass to the lower GIT. By this way, NaB action on abomasum and intestine function could have been strengthened and elongated to the periods between milk replacer feedings.

In conclusion, dietary addition of NaB may enhance solid feed intake, rumen development and health status of neonatal calves in first weeks of life. It predicts better health and animal performances around weaning period. Those effect seems to be

results of direct and indirect effect of NaB on forestomachs, abomasum and small intestine development. Elucidation of of the mechanisms of its action may allow for further improvement of calves GIT development in the future.

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