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The effect of sodium butyrate on calf growth and serum level of β-hydroxybutyric acid

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ABSTRACT

The experiment was carried out on 40 Polish Black-and-White HF bull calves (52-87% of HF blood) aged from 9 and 12 days at the beginning of the experiment to 90 days at its end. From the beginning of the trial the calves were offered restricted liquid feed to 56 days of age and concentrates *ad libitum* according to IZ-PIB-INRA recommendations. The concentrates were without sodium butyrate (control group, C), or with 1% Na-butyrate (group B1), 3% Na-butyrate (group B3) and 0.3% Na-butyrate (group B0.3), and included meadow hay from 0.10 kg/day during the liquid feeding period to 0.20 kg/day after weaning at 57 days of age. Na-butyrate at 3% in the diet reduced feed intake and had a beneficial effect on calf growth and nutrient utilization. The dietary level of Na-butyrate did not cause significant changes in serum β-hydroxybutyric acid concentration of the calves.

KEY WORDS: calves, sodium butyrate, β-hydroxybutyric acid

INTRODUCTION

Early transition from simple gastric digestion to functional ruminal digestion in dairy calves is essential for their health and growth (Khan et al., 2007). This transition involves a number of anatomical and physiological changes in the forestomach, especially in the rumen. These processes affect rumen development in pre-weaned calves and are essentially caused by nutrients supplied by solid feed (cereal grains and hay). Forage consumption promotes muscular development of

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the rumen and stimulates rumination and flow of saliva into the rumen (Hodgson, 1971; Hamada et al., 1976). Forage digestion by microorganisms does not provide sufficient concentrations of rumen volatile fatty acids (VFA), especially butyrate and, to a lesser degree, propionate, required for papillae development (Coverdale et al., 2004). The necessary butyrate concentration required to stimulate rumen mucosa (papillae) development is provided by concentrate fermentation, but on the other hand, large amounts of ground concentrate increase keratinization of the ruminal wall (Mc Gavin and Morrill, 1976), which reduces VFA absorption (Nocek et al., 1980; Greenwood et al., 1997), and rumen papillae branch to compensate for the loss in metabolically active tissue (Greenwood et al., 1997; Beharka et al., 1998).

Rumen mucosa development is stimulated by concentrates with a significant potential for fermentation to butyric and propionic acids (Greenwood et al., 1997; Beharka et al., 1998; Baldwin et al., 2004). This effect is most likely associated with the rate at which these acids are metabolized by mucosal cells. Around 50% of propionic acid is metabolized during absorption and over 90% of butyric acid is oxidized to ketone bodies (Britton and Krehbill, 1993). Poor mucosa development was observed in animals fed for a longer time (up to 12 weeks) with milk or milk replacers (Stobo et al., 1966). The addition of aqueous VFA solutions, in particular butyric and propionic acids or their salts, to the rumen of milk-fed calves stimulated the development of rumen mucosa (Lane and Jesse, 1997). Butyric acid salts (Na-butyrate or Ca-butyrate) are often used instead of the acid in animal nutrition because the salts are more stable and less odorous. Supplementing liquid feed with Na-butyrate enhanced the growth performance of young calves and villus size of the intestinal tract and modified the activity of digestive enzymes (Guilloteau et al., 2009). Lane et al. (2002) showed that the development of the ketogenic capacity of ruminal epithelium occurs as the animals ages and that the genes encoding the enzymes controlling ketogenesis are expressed independently of intraruminal VFA concentrations. There is no conclusive evidence, however, whether sodium butyrate can affect these processes.

The aim of the experiment was to determine if supplementing different amounts of sodium butyrate to concentrates for calves has differing effects on the serum levels of β-hydroxybutyric acid (an indicator of ketogenesis) and calf rearing performance.

MATERIAL AND METHODS

Animals, feeding and management

The experiment was carried out on 40 Polish Black-and-White HF bull calves

(52-87% of HF blood) aged from 9 to 12 days at the beginning, to 90 days at the end of the experiment. Calves were assigned to four groups of 10 animals each according to day of birth and liveweight. The calves were kept in individual cages (1.8 × 0.9 m) on a perforated wooden floor without litter.

Before the experiment the calves received only colostrum and milk. From the beginning of the trial all of the calves were offered liquid feed (to 56 days of age) and concentrates *ad libitum* (Table 1) that contained no sodium butyrate (control group, C), 1% Na-butyrate (group B1), 3% Na-butyrate (group B3), or 0.3% Na-butyrate (group B0.3). Meadow hay was fed from 0.10 kg/day during the liquid feeding period to 0.20 kg/day after weaning.

Table 1. Composition of concentrate, %

Feed component	Group			
	C	B1	B3	B0.3
Wheat, ground	30.0	30.0	30.0	30.0
Maize, ground	30.0	29.0	27.0	29.7
Oat, rolled	15.0	15.0	15.0	15.0
Soyabean oilmeal	20.0	20.0	20.0	20.0
Na-butyrate	-	1.0	3.0	0.3
Premix [1]	3.0	3.0	3.0	3.0
Limestone	1.0	1.0	1.0	1.0
$CaHPO_4$	1.0	1.0	1.0	1.0

[1] *Kalber Gold* Mineral (Sano firm), in 1 kg: g: P 40, Ca 20, Mg 25, Na 80; mg: Cu 1000, Zn 12000, Mn 4000, vit. E 3000; IU: vit. A 1000000, vit. D 100000

Liquid feed was prepared from milk replacer powder that contained, according to the manufacturer's specification, milk protein concentrate, whey, animal fat, coconut oil and Soycomil. The concentration of solid milk replacer in liquid feed was 140 g/l liquid. The calves were fed milk replacer solution according to IZ-PIB-INRA (2009) recommendations. The protein and energy value of feeds, and the proportion of ingredients in concentrates were formulated according to these standards using INRAtion and PrévAlim version 3.x (2005) software based on our own chemical analysis of feeds and using coefficients of rumen protein degradability (deg) and intestinal digestibility (dsi) for concentrate components and hay. For milk replacer, the deg=0.01 (due to the function of the reticular groove) and dsi=0.95 (IZ-PIB-INRA, 2009) values were assumed.

The liveweight of calves was monitored over two successive days at the beginning of the experiment, at weaning, and at the end of the experiment.

Starting from 7 days of age, blood samples were taken from the jugular vein into 9-ml tubes with clot activator (Vacuette) and at 14, 42 and 90 days of age (4 h after the morning feeding) and centrifuged; the obtained serum was refrigerated at -18°C.

The animals were fed individually. Feed intake was monitored daily by weighing feed refusals, and representative samples were analysed at one-month intervals at the Central Laboratory of the National Research Institute of Animal Production. Liquid feed was provided from plastic buckets with nipples; water was offered *ad libitum*.

Chemical and statistical analysis

Proximate chemical analysis of feeds and refusals was performed according to AOAC (1990). β-hydroxybutyric acid (BHBA) was determined by a kinetic enzymatic reaction using a Cobas-Bio analyser (Roche) and a high-sensitivity reagent kit (RANDOX). Glucose was determined by dry chemistry using the glucose oxidase method with a VITROS 950 analyzer (Ortho-Clinical Diagnostic) as described in the Test Methodology Manual (1996), the instructions for use of this analyzer.

Calculations and statistical analysis

Liveweight, daily weight gains and serum concentration of BHBA and glucose in respective periods of the experiment were analysed statistically using the MIXED procedure (SAS, 2001) of one-way analysis of variance with repeated measures based on the following model:

$$Y_{ijk} = \mu + \alpha_i + d_{ij} + \tau_k + (\alpha\tau)_{ik} + e_{ijk},$$

where: Y_{ijk} - dependent variable, μ - overall mean, α_i - fixed effect of group, d_{ij} - random effect of j animal in i group, τ_k - fixed effect of time k, $(\alpha\tau)_{ik}$ - effect of the fixed interaction between i group and k age, e_{ijk} - random error i = C, B-1, B = -3 or B-0.3 k - days of age (7, 14, 42, 90).

Statistical analysis of daily feed and nutrient intakes during particular periods and feed and nutrient conversion efficiency for all periods of the experiment was performed using one-way analysis of variance according to the GLM procedure of SAS (2002) by estimating the significance of differences between groups with Duncan's multiple range test. Differences were considered significant at $P \leq 0.05$ and tendencies at $P < 0.15$.

RESULTS

The chemical composition and nutritive value of the feeds are given in Table 2. The energy and protein levels were similar in all concentrates. At different periods of the experiment, compared with groups B1 and B0.3, calves from groups C and B3 were characterized by a tendency towards lower ($P<0.1$) or significantly lower ($P=0.05$ or $P<0.01$) feed and nutrient intakes (Table 3), except the intake of milk replacer and PDIN before weaning and the intake of hay for the entire experimental period ($P>0.05$).

Table 2. Chemical composition and nutritive value of the feeds

Components	Dry matter %	Crude protein %	Ether extract %	Crude fibre %	Ash %	PDIN g·kg^{-1}	PDIE g·kg^{-1}	UFL in 1 kg
Wheat, ground	87.85	13.22	2.17	2.66	1.60	88	93	1.02
Maize, ground	89.61	9.34	3.82	2.03	1.30	73	91	1.09
Oat, rolled	87.93	10.55	2.44	9.34	2.61	65	69	0.80
Milk replacer, powder[1]	91.77	21.40	12.00		6.2	211	-	1.33
Na-butyrate[2]	99							1.74
Meadow hay	83.75	8,34	2.36	28.96	4.47	18	43	0.65
Concentrates for group								
C-0	88.70	17.50	2.63	3.50	2.58	125	112	0.97
B-1	88.7	17.40	2.60	3.40	2.52	125	111	0.98
B-3	88.7	17.20	2.52	3.40	2.55	123	109	0.99
B-0.3	88.7	17.5	2.57	3.50	2.62	125	112	0.97
			2.90	3.90	2.95	141	126	1.09

[1] PDIN as PDI corresponds to digested crude protein; [2] net energy value (NEL) expressed as UFL was evaluated taking into account gross energy (GE) in calorimetric bomb estimated (6026 kcal•1 kg^{-1}) and GE of animal fat utilization for NEL (49.6%) and value of 1 UFL according to IZ PIB-INRA standards (2009)

The age of calves (Table 4) had a significant effect on liveweight and daily weight gains ($P<0.01$), with no significant differences among the groups, although calves receiving Na-butyrate showed a tendency towards higher liveweight and higher weight gains ($P=0.087$ and $P=0.13$). Compared with the control group (C), calves from all B groups had significantly higher liveweight at 57 and 90 days of age. In all periods of the experiment, calves receiving feeds with Na-butyrate (groups B) had higher daily weight gains ($P<0.05$ and $P=0.053$) compared with those from group C. Compared with the other groups, calves receiving concentrates with 3% Na-butyrate concentrate utilized nutrients ($P=0.02$) better and showed slightly better nutrient conversion efficiency, but differences in relation to group C were not significant (Table 5).

Table 3. Daily intake of feeds and nutrients during different periods of the experiment

Items	C	B-1	B-3	B-0.3	P	SE
Before weaning						
milk replacer, powder, kg·day^{-1}	0.97	0.97	0.97	0.97	0.64	0.001
concentrate, kg·day^{-1}	0.61	0.83	0.73	0.86	0.07	0.04
hay, kg·day^{-1}	0.05	0.07	0.06	0.06	0.08	0.002
dry matter, kg·day^{-1}	1.47	1.68	1.58	1.70	0.052	0.03
crude protein, g·day^{-1}	317.7	358.5	339.4	361.8	0.058	6.48
PDIN, g·day^{-1}	281.0	309.6	294.7	312.2	0.20	5.09
PDIE, g·day^{-1}	274.4	300.5	288.1	302.6	0.057	4.13
UFL, day^{-1}	1.91	2.5	2.04	2.15	0.052	0.04
After weaning						
concentrate, kg·day^{-1}	2.61^{a}	3.23^{b}	2.77^{ac}	2.99^{bc}	0.004	0.07
hay, kg·day^{-1}	0.20	0.19	0.20	0.19	0.055	0.003
dry matter, kg·day^{-1}	2.48^{a}	3.02^{b}	2.62^{ac}	2.81^{bc}	0.05	0.06
crude protein, g·day^{-1}	473.5^{a}	577.6^{b}	492.9^{ac}	539.4^{bc}	0.004	11.85
PDIN, g·day^{-1}	329.7^{a}	406.8^{b}	344.2^{ac}	377.2^{bc}	0.003	8.57
PDIE, g·day^{-1}	300.9^{a}	366.5^{b}	310.4^{ac}	343.2^{bc}	0.004	7.60
UFL, day^{-1}	2.66^{a}	3.29^{b}	2.87^{ac}	3.03^{bc}	0.004	0.07
Whole experimental period						
concentrate, kg·day^{-1}	1.48^{a}	1.87^{b}	1.64^{ac}	1.79^{bc}	0.008	0.05
hay, kg·day^{-1}	0.12	0.12	0.12	0.12	0.81	0.002
dry matter, kg·day^{-1}	1.91^{a}	2.27^{b}	2.05^{ac}	2.19^{bc}	0.006	0.04
crude protein, g·day^{-1}	386.7^{a}	453.2^{b}	407.6^{ac}	440.8^{bc}	0.006	7.97
PDIN, g·day^{-1}	302.1^{a}	351.9^{b}	317.9^{ac}	340.8^{bc}	0.005	5.72
PDIE, g·day^{-1}	287.0^{a}	328.8^{b}	297.9^{ac}	320.5^{bc}	0.005	5.07
UFL, day^{-1}	2.24^{a}	2.64^{b}	2.42^{ac}	2.54^{bc}	0.005	0.04

^{a,b,c} - P≤0.05

Table 4. Liveweight and daily liveweight gains

Liveweight	Group				P			Contrast
	C	B1	B3	B0.3	group	age	G xA	C vs B
Initial, kg	48.3	50.1	50.4	49.1	0.087	<0.01	0.26	0.47
At weaning, 57 days of age, kg	66.4	72.9	72.2	71.4				0.03
Final, kg	98.4	107.8	108.1	103.5				0.02
Daily gains, g·day^{-1}								
from beginning of experiment to weaning	390. 1	488.2	477.0	484.8	0.13	<0.01	0.73	0.03
from weaning to the end of experiment[1]	914.3	997.1	1025.7	917.1				0.053
from beginning to the end of experiment[1]	617.1	708.8	717.7	680.0				0.04

Table 5. Concentrates and nutrient conversion efficiency, per kg gain

Item	C	B-1	B-3	B-0.3	SE	P
Concentrates, kg	2.40[ab]	2.64[a]	2.28[b]	2.67[a]	0.31	0.02
Dry matter, kg	3.09	3.20	2.87	3.22	0.07	0.18
Crude protein, kg	624.4	639.4	567.0	646.8	14.59	0.13
PDI, g	465.1	463.9	415.1	471.3	9.87	0.12
UFL	3.63	3.72	3.37	3.73	0.08	0.26

SE - standard error for the population; [a,b,c] - P≤0.05

The serum level of the analysed metabolite varied according to calves' age (P<0.01), with no statistically significant differences between individual groups or between group C and all B groups (Table 6). As the calves grew, the concentration

Table 6. Concentration of β-hydroxybutyric acid (BHBA) and serum glucose, mmol·l^{-1}

Age, days	Group				P			Contrast
	C	B1	B3	B0.3	group	age	G x A	C vs B
BHBA concentration					0.21	<0.01	0.81	
7	0.07	0.06	0.06	0.06				0.50
14	0.19	0.21	0.22	0.18				0.60
42	0.21	0.22	0.23					0.93
90	0.30	0.31	0.35	0,19				0.23
Glucose concentration					0.91	<0.01	0.99	
7	3.65	3.78	3.77	3.60				0.82
14	2.20	2.27	2.48	2.18				0.70
42	2.40	2.50	2.55	2.73				0.50
90	3.80	3.72	3.70	3.61				0.67

of β-hydroxybutyric acid was found to increase in all experimental groups. Before the start of the experiment when the colostrum and milk feeding periods were over (7 days of age), serum levels of BHBA were low (0.06-0.07 mmol·l^{-1}) but gradually increased four-fold (group C), five-fold (groups B1 and B0.3), and six-fold (group B3) at 90 days of age (P<0.01). The highest numerical values for serum glucose concentrations were found at 7 and 90 days, and the lowest, at 14 days of age.

There was no significant interaction between group and age for liveweight, daily weight gains, and serum BHBA and glucose concentration.

DISCUSSION

These results indicate that Na-butyrate given together with concentrates under the conditions of this study stimulated feed intake and growth of the calves. Compared with groups B1 and B0.3, however, a 3% proportion of Na-butyrate

in concentrate caused daily feed intake to decrease by about 10% (± 2 percentage units). This Na-butyrate concentration probably increased the odour offensiveness of the diets compared with the groups of calves fed diets with 1.0 or 0.3% Na-butyrate. Nonetheless, calves from group B3 grew slightly more rapidly and were more efficient in feed conversion. It can be assumed that this was due to the higher intake of Na-butyrate and thus to the better development of rumen mucosa in terms of rumen absorption surface area and rumen cell metabolism. Although calves from group B1, and particularly those from group B0.3, had greater feed intake, they grew less and utilized feed less efficiently compared with calves from group B3.

Lane and Jesse (1997) observed rumen papillae to elongate in milk-fed lambs receiving short-chain fatty acids. Not all acids stimulated epithelial growth to the same extent, however. The greatest effect was shown by butyric acid, followed by propionic and acetic acids, which is most probably due to different rates of mucosa cell apoptosis under the influence of these acids (Mentschel et al., 2001) or different rates of acid metabolism in mucosa cells. After being absorbed by ruminal epithelium, VFA are converted into ketoacids that provide energy during synthesis of different substrates in the liver (Beharka et al., 1988). Because ruminal mucosa cells metabolize about 90% of butyric acid, it can be assumed that changes in serum concentration of BHBA will be a good indicator of metabolic changes in these cells (Baldwin and Jesse, 1992).

Determination of the serum β-hydroxybutyric acid concentration in calves is regarded as a noninvasive indicator of rumen development, but the results are not always consistent. Some authors observed the serum concentration of β-hydroxybutyric acid to increase with age and feed intake (Quigley et al., 1992). Quigley and Bernard (1992) report that between days 28 and 42 of age, the blood concentration of BHBA ranges from 0.22 to 0.62 mM. Similar findings were obtained in the present study for all groups of calves at 42 days of age. This relationship was not observed by Coverdale et al. (2004). Lane et al. (2000) found BHBA production to increase in 42-day-old lambs receiving milk alone or milk and concentrate. These observations suggest that the presence of VFA may not be the only factor inducing the development of ketogenesis, which may occur through ontogenesis or the action of other factors (Lane et al., 2000). This is perhaps the reason why no differences in serum BHBA concentrations were found between the groups, despite different proportions of Na-butyrate in the calf diets. It is likely, however, that Na-butyrate could pass into the small intestine and have a positive effect on the development of intestinal mucosa and intestinal enzyme activity. Guilloteau et al. (2009) believe that Na-butyrate is a specific stimulant of calf growth and is largely active in the intestines, where it has a favourable effect on villus length, crypt depth, mitotic index (Kotunia et al., 2004) and enzyme

activity. Böcker et al. (2003) reported a favourable effect of Na-butyrate on the expression of genes regulating the development of intestinal mucosa.

Changes in serum glucose concentrations from 7 to 14 and 42 days of age indicate that glucose oxidation decreased in ruminal mucosa cells, with increases in the use (by mitochondria of mucosal cells) of VFA from microbiological fermentation for ketone body synthesis, and of Na-butyrate from feed. Although no significant differences were found among the groups in serum glucose concentrations, its numerical values in the B groups were slightly higher at 42 days compared with the control group. The glucose concentration at 90 days, which was similar to that at 7 days, suggests that gluconeogenesis intensified during this period.

CONCLUSIONS

Adding 1-3% Na-butyrate to calf diets could result in better rearing performance, since adding 3% Na-butyrate reduced feed intake while having a beneficial effect on calf growth and nutrient utilization. The dietary level of Na-butyrate did not significantly change serum β-hydroxybutyric acid concentration in the calves.

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