Short communication: Effect of inclusion rate of microencapsulated sodium butyrate in starter mixture for dairy calves

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ABSTRACT

The aim of this study was to determine the effect of different inclusion rates of microencapsulated sodium butyrate (M-SB) in the starter mixture (SM) on performance of dairy calves. Forty female Holstein calves with a mean ($\pm$ SD) age of 12.8 ($\pm$ 1.5) d were allocated to 1 of 4 treatments (10 calves/treatment) and fed SM without (M-SB-0) or with 0.3% (M-SB-0.3), 0.6% (M-SB-0.6), or 0.9% (M-SB-0.9) of M-SB (as fed) during a 49-d period of milk replacer feeding. The milk replacer was fed at 670 g/d divided into 2 equal meals. Starter mixture with or without M-SB was offered for ad libitum consumption beginning on the first day of the trial. Body weight of calves was recorded weekly, whereas intakes of milk replacer and SM and fecal fluidity were recorded daily. Intake of SM decreased linearly with increasing M-SB inclusion rate. Average daily gain decreased and body weight gain tended to decrease linearly with increasing amounts of M-SB in SM, but feed efficiency was not affected. Fecal score and number of days with diarrhea increased cubically with increasing M-SB inclusion rate in SM. Under the conditions of the current study, supplementation of SM with M-SB had a negative effect on performance of calves.

Key words: rearing calves, feed additive, solid feed

Short Communication

Butyric acid is the most potent stimulator of the rumen epithelium growth (Tamate et al., 1962). As its concentration in the rumen increases the mitosis:apoptosis ratio of the rumen epithelium increases, resulting in larger rumen papillae and a greater surface area of nutrient absorption (Tamate et al., 1962; Mentschel et al., 2001). As a result, nutritional strategies to increase butyric acid production in the rumen are widely used to speed up rumen epithelium development in newborn calves. This is achieved primarily by feeding calves

starter mixtures (SM) high in starch and sugar, which are both fermented in the rumen, mostly to propionic and butyric acid (Lesmeister and Heinrichs, 2005; Khan et al., 2008).

A positive effect on rumen development can also be obtained by microencapsulated sodium butyrate (M-SB) supplementation in SM, as a means of increasing the available butyric acid pool in the rumen (Górka et al., 2011a). Supplementation with M-SB in SM increased reticulorumen weight and positively affected papillae length and width and, consequently, SM intake. Furthermore, M-SB supplementation reduced the number of days that calves had diarrhea in the first weeks of life (Górka et al., 2011a).

Although in previous studies, an inclusion rate of 0.3% M-SB in SM (as fed) stimulated rumen epithelium growth and had a positive effect on performance of calves, the optimal inclusion rate in SM is unknown. We hypothesize that a positive effect of M-SB supplementation in SM on performance of calves, especially on SM intake, would increase with increasing M-SB inclusion rate in SM. The aim of this study was to determine the effect of inclusion rate of M-SB in SM on performance of dairy calves.

The trial was conducted at a commercial dairy farm (Top Farms Głubczyce Sp. z o.o., Nowe Gószowice, Poland) located in southern Poland between October and December 2012. Throughout the study period, calves were cared for according to the recommendation of the local ethical committee.

Forty Holstein female calves with mean ($\pm$ SD) BW of 40.7 $\pm$ 3.5 kg and age 12.8 $\pm$ 1.5 d were used in the study. Before the study began, routine procedures for newborn calves adopted at the farm were followed. Briefly, after birth, calves were immediately separated from the dams, transported to individual hutches bedded with straw and fed 3 L of colostrum within the first 3 h of life. Colostrum feeding was continued until the end of 48 h, and transition milk and whole-milk feeding was continued until d 7 of life, followed by milk replacer (MR) feeding (Polmass Milk, Polmass S.A., Bydgoszcz, Poland). Liquid feeds were offered in amounts equal to 5 L/d. No SM or hay was offered until calves were allocated to the study.

Received June 12, 2014.

Accepted December 23, 2014.

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Between d 11 and 15 of life, calves were transported to a ventilated barn, treated with a broad-spectrum antibiotic (Zactran, Merial, Lyon, France), and allocated to experimental treatments. The required number of calves was collected over a period of 2 wk, resulting in 3 blocks of 20, 16, and 4 calves each. Within a block, calves were randomly allocated to 1 of 4 treatments (10 calves/treatment) and fed SM without (**M-SB-0**) or with 0.3% (**M-SB-0.3**), 0.6% (**M-SB-0.6**), or 0.9% (**M-SB-0.9**) of M-SB (as fed). Only healthy calves were selected for the study.

Throughout the study period, calves were fed 670 g of MR/d (as fed). The MR was reconstituted at a rate of 134 g/L of water and offered in buckets at 0700 and 1400 h at 2.5 L/feeding. Commercial calf SM (Kälber Starter, Blattin Sp. z o. o., Siedlec, Poland) with or without M-SB was offered once daily for ad libitum consumption, beginning on the first day of the trial. Sodium butyrate microencapsulated within a triglyceride matrix (30:70 sodium butyrate: triglyceride; VetAgro, Reggio Emilia, Italy) was added to SM by the SM manufacturer and used to slow release of butyric acid in the rumen. During the study period, calves were kept in 1.5 × 1.2 m individual pens bedded with straw. Free access to fresh water was provided every day. Calves were in the study for 49 d.

Feed intake was monitored daily. Calves were weighed at the beginning and end of the trial and at weekly intervals before the morning MR feeding. Fecal fluidity (4-point scale, where 1 = normal; 4 = diarrhea) was determined daily according to Larson et al. (1977). All abnormal health conditions and veterinary treatments were documented.

Representative samples of feeds were sampled every other week, composited at the end of the study, and analyzed for DM, ash, CP, and ether extract contents using standard analytical procedures (methods 934.01, 942.05, 976.05, and 2003.05 for DM, ash, CP, and ether extract, respectively; AOAC International, 2000). Neutral detergent fiber (Van Soest et al., 1991), ADL (Robertson and Van Soest, 1981), starch (Faisant et al., 1995), and sugar (Dubois et al., 1956) were also analyzed.

Data were analyzed as a completely randomized block design using the MIXED procedure of SAS (version 9.2; SAS Institute, 2002). The statistical model included fixed effects of treatment and random effect of block. The statistical model for repeated variables included effect of time (day or week) and interaction between effect of time and treatment as fixed effects (Littell et al., 1998). Optimal covariance structure (autoregressive order 1, unstructured or compound symmetry) was chosen based on Akaike's information criterion. For all

analyzed parameters, initial age was used as a covariate; for ADG and SM intake analyses, initial BW was used as a covariate. Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Data are presented as least squares means and corresponding standard errors of the means.

Chemical composition of experimental feeds is presented in Table 1. No treatment × time interaction was detected for any of the analyzed parameters. Calves from all groups had similar BW at the beginning of the trial (Table 2). Milk replacer intake did not differ between treatments. Average daily gain decreased ($P = 0.02$) and BW gain tended to decrease ($P = 0.08$) with increasing dose of M-SB in SM. During the study, calves from groups M-SB-0.3, M-SB-0.6, and M-SB-0.9 gained 1.3, 3.2, and 4.7 kg less BW compared with calves from group M-SB-0, respectively (Figure 1A). The increasing inclusion rate of M-SB in SM linearly decreased SM intake ($P < 0.01$; Figure 1B). A negative effect of M-SB supplementation on SM intake was observed between d 1 and 24 and between 25 and 49 of the study (linear effect, $P \leq 0.04$). We detected no effect of M-SB supplementation on feed efficiency. Fecal score tended to increase ($P = 0.09$) and number of days with diarrhea increased ($P < 0.01$) cubically with increasing M-SB inclusion rate in SM.

Positive effects on performance of calves of M-SB supplementation in SM and of crystalline sodium butyrate in MR have been reported in earlier studies (Guilloteau et al., 2009; Górka et al., 2011a,b). These were mainly the result of the positive effect of sodium butyrate on gastrointestinal tract development and function, and consequently on efficiency of nutrient digestion and feed intake. In the current study, we investigated a dose effect of M-SB in SM on performance of calves. Although we hypothesized that performance of calves would be improved by M-SB supplementation, linear decreases in ADG and BW of calves with increasing M-SB inclusion rate in SM were observed, mainly because of the negative effect of M-SB on SM intake. This observation is difficult to explain. However, at least 2 negative aspects of M-SB use in SM on performance of calves can be considered. First, although butyric acid stimulates proliferation of rumen epithelial cells, it may also promote keratinization of the rumen papillae (McGavin and Morrill, 1976). This, in turn, may negatively affect absorption of short-chain FA from the rumen (Bull et al., 1965) and thus feed intake. Second, it has been shown that calves may be sensitive to the flavor of SM (Fathi et al., 2009). Inclusion of M-SB at 0.6 and 0.9% of SM noticeably changed its smell and probably also its taste. Furthermore, we cannot exclude the possibility that the effect of M-SB use in SM depends on

Table 1. Chemical composition of experimental feeds (on DM basis)

Item	Milk replacer	Starter mixture ¹			
		M-SB-0	M-SB-0.3	M-SB-0.6	M-SB-0.9
DM, %	96.71	90.35	90.01	90.24	90.12
CP, %	26.06	18.50	18.19	17.88	18.25
Crude fat, %	15.93	2.71	2.73	2.56	2.69
Crude fiber, %	ND ²	5.17	5.41	5.23	5.18
Ash, %	7.01	7.65	8.04	7.60	7.88
Lactose, ³ %	51.0	—	—	—	—
N-free extractives, ⁴ %	—	70.14	71.04	71.96	71.18
Starch, %	—	40.76	41.51	39.94	41.01
Sugars, %	—	7.87	7.78	7.99	7.12
NDF, %	—	16.77	16.92	16.69	16.07
ADF, %	—	6.93	6.87	7.16	6.75
ME, ⁵ Mcal	4.62	3.03	3.01	3.01	3.01
Butyrate, g/kg	—	0.00	0.79	1.58	2.37

¹Starter mixture (Kälber Starter, Blattin Sp. z o. o., Siedlec, Poland; 28% barley, 15% wheat, 15% corn, 10% wheat bran, 13% soybean meal, 10% corn dried distillers grain, 1% molasses, 2.5% whey, 2% glycerin, 0.5% NaCl, 2% limestone 1% vitamin/mineral premix) containing 0, 0.3, 0.6, or 0.9% of sodium butyrate microencapsulated within triglyceride matrix (M-SB).

²Not detected.

³Lactose = 100 – CP – crude fat – crude fiber – crude ash.

⁴N-free extractives = 100 – CP – crude fat – crude ash.

⁵Calculated from NRC (2001).

SM composition or its method of delivery. In the study of Górka et al. (2011a), which showed a positive effect of M-SB use in SM on performance of calves, SM with or without M-SB was blended with whole corn grain before being offered to calves. Moreover, the chemical and ingredient composition of the SM differed substantially between the current study and that of Górka et al. (2011a).

It is also worth noting that butyrate intake with the supplement was low in this study when endogenous butyrate synthesis was considered. When starter intake was 1 kg/d, dietary butyrate intake ranged from 0.8 to 2.4 g/d (9.1 to 27.2 mmol/d). Assuming that 5 mol of short-chain FA/1 kg of DM is produced in the rumen and that butyric acid accounts for 10% of that production (≈ 500 mmol; Bergman, 1990), this amount was

Table 2. The effect of inclusion rate of microencapsulated sodium butyrate in starter mixture on BW, BW gain, ADG, milk replacer and starter mixture intakes, fecal score, and days with diarrhea of calves

Item	Treatment ¹				SEM	Polynomial contrast		
	M-SB-0	M-SB-0.3	M-SB-0.6	M-SB-0.9		Linear	Quadratic	Cubic
Number of calves	10	10	10	10				
Initial BW, kg	39.8	39.9	39.5	40.2	0.55	0.89	0.76	0.75
Final BW, kg	73.1	70.9	68.9	67.8	1.37	0.14	0.83	0.95
BW gain, kg	33.3	31.0	29.4	27.6	0.03	0.08	0.91	0.93
ADG, ² g/d	684	638	608	567	29	0.02	0.94	0.86
Milk replacer intake, g/d	668	670	667	668	8	0.74	0.88	0.13
Starter intake, g/d								
d 1 to 24 ²	257	209	173	174	6.3	0.04	0.43	0.84
d 25 to 49 ²	737	671	574	508	12.2	<0.01	0.99	0.79
d 1 to 49 ²	495	437	370	338	8.5	<0.01	0.71	0.77
ADG:DMI, g/kg	640	625	636	596	9	0.39	0.70	0.57
Fecal fluidity ^{2,3}	1.04	1.22	1.16	1.19	0.01	0.06	0.09	0.09
Diarrhea, ⁴ d	0.30	1.97	0.49	1.32	0.22	0.41	0.33	<0.01

¹Starter mixture without (M-SB-0) or with 0.3% (M-SB-0.3), 0.6% (M-SB-0.6), or 0.9% (M-SB-0.9) of microencapsulated sodium butyrate (as-fed basis).

²Significant effect of time in the model ($P < 0.05$).

³Scored on a 4-point scale, where 1 = normal, 4 = diarrhea.

⁴Fecal fluidity = 4.

Figure 1. Least squares means for BW (A) and starter mixture (SM) intake (B) of calves fed SM without (M-SB-0) or with 0.3% (M-SB-0.3), 0.6% (M-SB-0.6), or 0.9% (M-SB-0.9) of microencapsulated sodium butyrate (as fed).

negligible compared with endogenous release. However, it was sufficient to affect performance of calves in this and other studies when sodium butyrate was used in SM or MR (Guilloteau et al., 2009; Górká et al., 2011a,b).

Altogether, this study showed a negative effect of supplementation of SM with M-SB on SM intake and performance of calves. Even the lowest inclusion rate

of 0.3% had a negative effect on performance of calves, and further studies are needed to establish the optimal inclusion rate of M-SB in starter diets for calves and strategies for its use. Additional studies are needed to better explain the mechanism of action of dietary butyrate on performance of calves, especially when used in starters.

ACKNOWLEDGMENTS

This study was funded by Ministry of Science and Higher Education of Poland (DS-3217/KŻŻiP/2012). The authors acknowledge Top Farms Głubczyce (Nowe Góleszowice, Poland) staff for help in collecting data.

REFERENCES

- AOAC International. 2000. Official Method of Analysis. 17th ed. AOAC International, Washington DC.
- Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 70:567–590.
- Bull, L. S., L. J. Bush, J. D. Friend, B. Harris, and E. W. Jones. 1965. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. *J. Dairy Sci.* 48:1459–1466.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Roberts, and F. Smith. 1956. Colorimetric methods for determination of sugar and related substances. *Anal. Chem.* 28:350–356.
- Faisant, N., V. Planchot, F. Kozłowski, M. P. Pacouret, P. Colonna, and M. Champ. 1995. Resistant starch determination adapted to products containing high level of resistant starch. *Sci. Aliment.* 15:83–89.
- Fathi, M. H., A. Riasi, and A. Allahresani. 2009. The effect of vanilla flavoured calf starter on performance of Holstein calves. *J. Anim. Feed Sci.* 18:412–419.
- Górka, P., Z. M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, J. J. Holst, R. Guilloteau, and R. Zabielski. 2011a. Effect of method of delivery of sodium butyrate on rumen development in newborn calves. *J. Dairy Sci.* 94:5578–5588.
- Górka, P., Z. M. Kowalski, P. Pietrzak, A. Kotunia, W. Jagusiak, and R. Zabielski. 2011b. Is rumen development in newborn calves affected by different liquid feeds and small intestine development? *J. Dairy Sci.* 94:3002–3013.
- Guilloteau, P., R. Zabielski, J. C. David, J. W. Blum, J. A. Morisset, M. Biernat, J. Woliński, D. Laubitz, and Y. Hamon. 2009. Sodium-butyrate as a growth promoter in milk replacer formula for young calves. *J. Dairy Sci.* 92:1038–1049.
- Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, S. B. Park, K. S. Baek, J. K. Ha, and Y. J. Choi. 2008. Starch source evaluation in calf starter: II. Ruminant parameters, rumen development, nutrient digestibilities, and nitrogen utilization in Holstein calves. *J. Dairy Sci.* 91:1140–1149.
- Larson, L. L., F. G. Owen, J. L. Albright, R. D. Appleman, R. C. Lamb, and L. D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. *J. Dairy Sci.* 60:989–991.
- Lesmeister, K. E., and A. J. Heinrichs. 2005. Effects of adding extra molasses to a texturized calf starter on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. *J. Dairy Sci.* 88:411–418.
- Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216–1231.
- McGavin, M. D., and J. L. Morrill. 1976. Scanning electron microscopy of ruminal papillae in fed various amounts and forms of roughage. *Am. J. Vet. Res.* 37:497–508.
- Mentschel, J., R. Leiser, C. Mülling, C. Pfarrer, and R. Claus. 2001. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. *Arch. Tierernähr.* 55:85–102.
- NRC. 2001. Nutrient Requirements of Dairy Cows. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis. Pages 123–158 in *The Analysis of Dietary Fiber in Food*. W. P. T. James and O. Theander, ed. Marcel Dekker, New York, NY.
- SAS Institute. 2002. The SAS System. Version 9.2. SAS Institute Inc., Cary, NC.
- Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. *J. Dairy Sci.* 45:408–420.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.