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ORIGINAL ARTICLE

Effect of *Megasphaera elsdenii* NCIMB 41125 dosing on rumen development, volatile fatty acid production and blood β-hydroxybutyrate in neonatal dairy calves

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Summary

Thirty calves were randomly assigned to two treatments and fed until weaning [42 days (d) of age]. Treatments were a control group (n = 15), which did not receive *Megasphaera elsdenii* (Me0) and a *M. elsdenii* group, which received a 50-ml oral dose of *M. elsdenii* NCIMB 41125 (10^8 CFU/ml) at day 14 day of age (Me14). Calves were given colostrum for the first 3 day followed by limited whole milk feeding. A commercial calf starter was offered *ad libitum* starting at day 4 until the end of the study. Fresh water was available throughout the study. Feed intake and growth were measured. Blood samples were collected via jugular venipuncture to determine β-hydroxybutyrate (BHBA) concentrations. Fourteen male calves (seven per group) were euthanised on day 42 and digestive tracts harvested. Reticulo-rumen weight was determined and rumen tissue samples collected from the cranial and caudal sacs of the ventral and dorsal portions of the rumen for measurements of papillae length, papillae width and rumen wall thickness. Dosing with *M. elsdenii* NCIMB 41125 improved starter dry matter intake (DMI), weaning body weight (BW) and tended to improve average daily gain. Calves in Me14 group had greater plasma BHBA concentration than Me0-calves during the last 3 weeks of the trial and had at day 42 greater reticulo-rumen weight, papillae width and papillae density compared to Me0. No differences in rumen wall thickness or papillae length were observed between the two groups. Total volatile fatty acids, acetate and propionate production did not differ between treatments, but butyrate production was greater in Me14 than Me0. Dosing *M. elsdenii* NCIMB 41125 showed benefit for calves with improved feed intake and rumen development suggesting increased epithelium metabolism and improved absorption of digestive end products.

Keywords *Megasphaera elsdenii*, β-hydroxybutyrate, rumen development, neonatal calves, starter intake, weaning

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Introduction

Many dairy producers wean their calves from whole milk or milk replacer early (3–6 weeks). Rapid rumen development is therefore critical before weaning as it influences post-weaning intake of solid feed, growth rates and health (Greenwood et al., 1997; Baldwin et al., 2004). Rumen development is dependent on intake of solid feed (Lesmeister and Heinrichs, 2004), which is fermented to volatile fatty acids (VFA's) that stimulate rumen papillae development in calves (Sander et al., 1959). Starter feeds that promote production of butyrate are the preferred feed type for triggering papillae growth in the rumen (Stobo et al., 1966). Amongst VFA's, Butyrate has the greatest effects on ruminal epithelium. Butyrate is reported to increase blood flow during nutrients absorption and metabolism and directly affects ruminal epithelium gene expression (Baldwin et al., 2004).

Megasphaera elsdenii, a lactate-utilising bacteria, plays a major role in production of branched-chain VFA's in the rumen (Wallace, 1986). This bacteria convert lactic acid to propionate and butyrate and converts glucose to butyrate (Marounek et al., 1989; Henning et al., 2010). The potential of *M. elsdenii* to increase molar proportions of ruminal propionate and/or butyrate was reported (Marounek et al., 1989; Aikman, 2008; Aikman et al., 2009; Henning et al., 2010) and the role of these two VFA's in stimulating rumen epithelial cells and papillae development

increasing the capacity of solid feed intake was reviewed (Warner et al., 1956; Tamate et al., 1962; Lane and Jesse, 1997; Coverdale et al., 2004). Butyrate provides energy for thickening of the rumen wall, formation of papillae and increasing capillary development (Weigand et al., 1975). In young calves, the physical development of the rumen is associated with changes in blood β-hydroxybutyrate (BHBA) produced from butyrate as a result of ruminal fermentation of carbohydrate (Quigley et al., 1991). Dosing calves with *M. elsdenii* may increase production of butyrate and accelerate rumen development during the neonatal phase. The objectives of this experiment were to determine the effects of dosing calves with *M. elsdenii* NCIMB 41125 on solid feed intake, growth, serum BHBA and rumen development (Figs 1–3).

Materials and methods

Animal and diets

The experimental protocol and procedures were approved by the Animal Ethics Committee (APIEC11/028) of the Agricultural Research Council at Irene, Pretoria, South Africa.

Thirty healthy male and female Holstein calves (34.6 ± 5.04) from the Agricultural Research Council/Animal Production Institute in Pretoria (South Africa) were randomly allocated post-partum to two treatment groups [males (n = 7), females (n = 8) per treatment]: control group (Me0), which did not receive *Megasphaera elsdenii* (*M. elsdenii*) NCIMB 41125 and (Me14), which received a 50-ml (10^8 CFU/ml) oral dose of *M. elsdenii* NCIMB 41125 at 14 day of age. All bull calves were euthanised on day 42 using captive bolt stunning and exsanguination for rumen evaluation. The product containing *M. elsdenii* NCIMB 41125 is commercially available as Megamilk, supplied by Afrivet, Office Park, 195 Dawie Str, Silver Lakes Road, Hazeldean 0081, South Africa. Calves were given colostrum for the first 3 days of life before receiving cow's milk as shown in Table 1. A commercial calf starter feed (18.9% CP, 3.9% fat) was offered *ad libitum* starting at day 4 of age until weaning at day 42. Fresh water was available throughout the study.

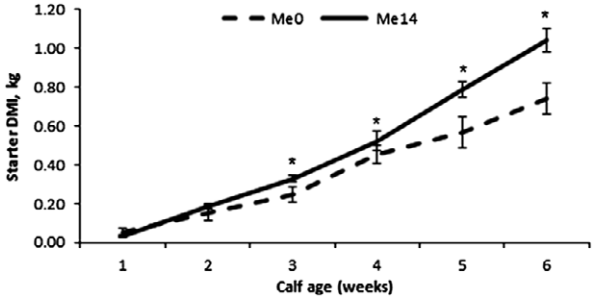

Fig. 1 Starter dry matter intake for calves with (Me14) or without (Me0) *Megasphaera elsdenii* NCIMB 41125 at 14 day of age. *Significant difference between groups (p = 0.05).

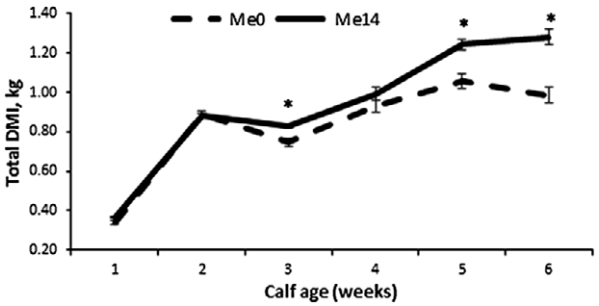

Fig. 2 Total dry matter intake for calves with (Me14) or without (Me0) *Megasphaera elsdenii* NCIMB 41125 at 14 day of age. *Significant difference between groups (p = 0.05).

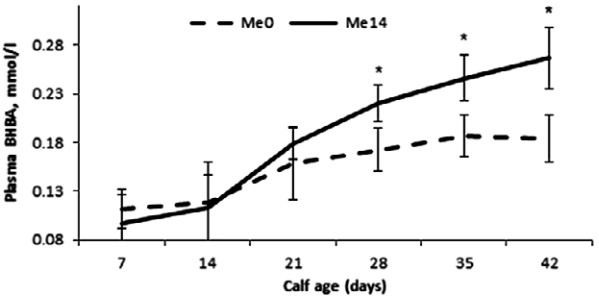

Fig. 3 Plasma β-hydroxybutyrate (BHBA) of calves with (Me14) or without (Me0) *Megasphaera elsdenii* NCIMB 41125 at 14 day of age. *Significant difference between groups (p = 0.05).

Measurements and analysis

Consumption of starter, milk and water by each calf was recorded daily throughout the experiment to estimate intake. Calves were initially offered 250 g of starter, and remaining feed was weighed at each delivery time the next day. Daily starter feed offerings were increased in 250 g increments when calves refused

Table 1 Milk feeding regime

		Morning	Afternoon
Day 4 to day 7	Milk	2 l	2 l
Day 8 to day 14	Milk	3 l	3 l
Day 15 to day 35	Milk	2 l	2 l
From day 36 to weaning (day 42)	Milk	4 l	–

<50 g of feed. The DM contents of the starter were determined by oven drying at 60° C for 48 h, and milk DM was determined by freeze-drying. Calves were weighed at birth and at 7-day intervals throughout the experiment and the average weight gain (ADG) was determined weekly. Weighing was carried out before morning milk and starter feeding. Jugular blood was collected from all male calves at 7, 14, 21, 28, 35 and 42 day of age in the morning 1 h post-feeding by venipuncture for determination of BHBA. Blood was collected into 10-ml collection tubes containing sodium heparin. Two ml of blood was then transferred to two clean test tubes. Cold 30% perchloric acid was added in a 1:1 ratio to the blood samples, for the precipitation of protein. After thorough mixing, the precipitated protein was removed by centrifuging in a refrigerated centrifuge (RCF: 1500) for 20 min. The clear supernatant was quickly transferred to clean glass tubes and recapped with clean screw caps, to prevent evaporation of acetone and stored at −20° C, until analyses for BHBA. The determination of BHBA was carried out by means of enzymatic analysis (Williamson et al., 1962).

After euthanised, digestive tracts were harvested, emptied and rinsed with cold water. Rumen tissue samples were collected from the cranial and caudal sacs of the ventral and dorsal portions of the rumen (area $n = 4$). Three random tissue samples were collected from each area (sample $n = 12$). Measurements for papillae length, papillae width and rumen wall thickness were performed in duplicate according to Lesmeister et al. (2004).

Statistical analysis

Average daily gain, starter and total DMI, and plasma BHBA were analysed as repeated measures using the PROC MIXED statement of SAS (SAS Institute, 2009). Parameters measured daily were pooled by week for analysis. The statistical model included calf as a random effect, and treatment group and its interaction with time as a fixed effect. A significant effect of treatment by week was observed for DMI and BHBA, so each week of these data was analysed separately. The model was subjected to an autoregressive order one. Significance was declared at $p < 0.05$ and tendencies were accepted if $0.05 < p < 0.10$. Differences between treatment means across time were assessed with a multiple test comparison using the Tukey's test.

The statistical model used for repeated-measure analyses was

$$Y_{cit} = \mu + \alpha_i + \beta_t + T_{it} + \delta_{ci} + e_{cit},$$

where Y_{cit} = an observation value for ADG, starter DMI, total DMI and plasma BHBA measured from calf c from treatment i at time t;

μ = overall mean for the population;

α_i = fixed effect of treatment i, where i = Me0 or Me14;

β_t = fixed effect of time t, where $t = w$ 1, 2, 3, 4, 5 or 6 for starter DMI, total DMI, ADG and BHBA;

T_{it} = fixed interaction of effect of treatment i and time t;

δ_{ci} = random effect of calf c nested within i treatment; and

e_{cit} = error associated with the measurement taken from calf c from group g at time t.

Means for gain:feed, initial and weaning BW, reticulo-rumen, papillae length, papillae width and rumen wall thickness were subjected to ANOVA using PROC GLM (SAS Institute, 2009). Significance was declared at $p < 0.05$ and tendencies were accepted if $0.05 < p < 0.10$. The statistical model used was

$$Y_{ci} = \mu + T_i + \delta_c + e_{ci}$$

where Y_{ci} = observation value taken from calf c at t time.

μ = overall mean of the population,

T_i = fixed effect of the ith treatment (Me0 or M14),

δc = random effect of calf and

e_{ci} = error associated with the measurement taken from calf c from ith treatment.

Results

Intake and body weight gain

There were effects ($p < 0.001$) of treatment, week and their interaction for starter and total DMI (Table 1). Calves in Me14 group consumed 29.7 and 13.2% more starter feed and total dry matter (milk + starter), respectively, than Me0 calves ($p < 0.05$), but gain numerically 17% more kg/day weight than Me0. Weaning BW was also higher on Me14 compared to Me0 ($p = 0.03$). The gain:feed ratio was not different between treatments but ADG tended ($p = 0.10$) to be greater for Me14 calves.

Plasma BHBA did not differ ($p > 0.05$) between Me0 and Me14 groups from day 7 to day 21 averaging 0.10, 0.12 and 0.17 mmol/l respectively. From day 28 to day 42, plasma BHBA of Me14 calves were higher than Me0 ($p = 0.003$).

Least square means of total and individual VFA concentrations are presented in Table 2. Total VFA (96.7 ± 5.6 μmol/l), acetate (53.7 ± 3.9 μmol/l) and propionate (24.2 ± 1.2 μmol/l) did not differ between treatments ($p > 0.10$), but butyrate concentration was

Table 2 Least square means for performance and β-hydroxybutyrate of calves with (Me14) or without (Me0) *Megasphaera elsdenii* NCIMB 41125 at 14 day of age

	Treatments			p-value		
Items	Me0	Me14	SEM	*T*	Time	*T* × Time
BW, kg						
Initial	33.4	35.8	1.7	0.18	–	–
Weaning	50.5	55.8	2.2	0.03	–	–
ADG, kg/day	0.41	0.48	0.05	0.10	0.018	0.20
Starter DMI, kg/day	0.37	0.48	0.01	<0.001	<0.001	<0.001
Total DMI, kg/day	0.83	0.94	0.01	<0.001	<0.001	<0.001
Gain: Feed	1.07	1.02	0.14	0.68	–	–
Plasma BHBA, mmol/l	0.17	0.22	0.01	<0.003	<0.001	0.23

Values in the same row differ if $p \leq 0.05$ and tend to differ if $0.05 \geq p \leq 0.10$. Gain: Feed: calculated as final body weight – initial body weight/starter DMI. *T*, treatment; SEM, standard error of mean.

greater in Me14 than Me0 ($p = 0.04$). Calves in Me14 treatment had greater reticulo-rumen weight ($p = 0.01$) and papillae density ($p = 0.02$), with greater papillae width ($p < 0.01$) compared to Me0. No differences in rumen wall thickness (1.58 ± 0.1 cm) and papillae length (1.48 ± 0.1 cm) were observed between the two groups ($p > 0.10$).

Discussion

Greater solid feed intake is very important in early weaning management systems because it determines growth rates and calf health post-weaning (Greenwood et al., 1997). In neonatal calves, solid feed intake is conditioned by rumen (Kristensen et al., 2007), which affects digestion and absorption of nutrients (Górka et al., 2011). Dosing *M. elsdenii* NCIMB 41125 stimulated feed intake in agreement with Miller et al. (2013), leading to Me14 calves consuming more starter than Me0 calves, suggesting a more developed absorptive capacity (Coverdale et al., 2004). Administering *M. elsdenii* NCIMB 41125 improved growth of the calves, as they gained 17% more kg/day and were 5.3 kg heavier than Me0 at weaning. This can be attributed to increased nutrient digestibility due to early increased starter DMI (Anderson et al., 1987; Terré et al., 2007), and consequently improved ruminal fermentation activities in Me14 treatment. Increased feed intake was previously reported when lambs (Henning et al., 2009) and steers (Henning et al., 2010) were drenched with *M. elsdenii* NCIMB 41125. Increasing in DMI detected in this study is similar to that of Drouillard (2004), who found that cattle drenched with strain 41125 tended to maintain higher intakes throughout the experiment than control cattle. In contrast, no difference in feed intake was observed by Leeuw et al. (2009) with the same strain of *M. elsdenii* on feedlot steer fed high and low roughage diets, but ADG was improved from week 3 after dosing (Table 3).

Plasma BHBA concentration and its precursor rumen butyrate were found greater in Me14. The increased blood BHBA production from butyrate by rumen epithelial cells increased as age increases in both groups of calves (Me0 and Me14) as also reported by Giesecke et al. (1970)and Bush (1988), but was more pronounced for Me14 calves than for Me0 and can be associated with the greater starter intake by Me14 calves. Higher butyrate production could have increased ketogenesis in the rumen (Quigley et al., 1991) leading to greater plasma BHBA. Rumen butyrate is oxidised by the rumen epithelial cells as it passes through the rumen wall (Quigley et al., 1991; Lesmeister and Heinrichs, 2004). Dosed

Table 3 Least square means for measurements taken at 42 days of age of calves with (Me14) or without (Me0) *Megasphaera elsdenii* NCIMB 41125 at 14 day of age

	Treatments			
Items	Me0	Me14	SEM	p-value
Ruminal volatile fatty acid production, μmol/l				
Total VFA	96.2	97.2	8.681	0.882
Acetate	53.8	53.6	3.883	0.962
Butyrate	10.7	15.9	1.279	0.037
Propionate	24.3	24.1	1.175	0.915
Rumen development				
Reticulo-rumen, kg	1.05	1.17	0.0246	0.010
Rumen wall thickness, cm	1.60	1.56	0.102	0.826
Papillae length, cm	1.34	1.62	0.105	0.182
Papillae width, cm	0.86	1.17	0.027	<0.001
Papillae density, number/cm^2	87.1	97.6	2.343	0.023

Values in the same row differ if $p \leq 0.05$ and tend to differ if $0.05 \geq p \leq 0.10$. *T*, treatment; SEM, standard error of mean.

calves had heavier reticulorumen and more dense and longer papillae. These results indicate that dosing *M. elsdenii* NCIMB 41125 stimulated rumen butyrate and plasma BHBA production and resulted in a faster rumen function development, suggesting more energy for thickening of the rumen wall, formation of papillae and increasing capillary development (Weigand et al., 1975).

The association of rumen epithelial cell proliferation with increased plasma butyric acid was previously reported with dietary supplementation (Górka et al., 2011) and with infusions (Mentschel et al., 2001) of sodium salt of butyrate to dairy calves. These reports suggested that the greatest effect on rumen papillae development was due to high rates of butyric acid metabolism in mucosa cells. Reticulum weight increases with increased BW (Tamate et al., 1962). Górka et al. (2011) observed that dietary supplementation with butyric acid increased reticulorumen weight and papillae length and width in dairy calves fed limited amount of milk (10% BW). Dosing *M. elsdenii* NCIMB 41125 resulted in higher starter intake, which could have additionally stimulated reticulorumen development (Tamate et al., 1962; Kristensen et al., 2007) as a result of consuming greater volume of feed and higher nutrient intake (Górka et al., 2011). Direct effects on gene expression within the rumen (Glauber et al., 1991) and an increase in blood flow through the rumen (Sander et al., 1959) are also suggested as mechanism by which butyrate may stimulate papillae development.

However, mechanisms regulating rumen development and nutrient accessibility for developing calf tissues need further investigation. The biochemistry of plasma BHBA and rumen butyrate, as well as mechanisms of rumen epithelial metabolic activities and induction of papillary development are not well defined. More scientific clarity on these mechanisms would improve understanding when predicting growth rates and management of dairy calves. The long-term effects of dosing *M. elsdenii* NCIMB 41125 on rumen microbial populations and gut development and animal performance are also pertinent areas of study.

Conclusion

Dosing *M. elsdenii* NCIMB 41125 increased ruminal butyrate and plasma BHBA and improved starter DMI and rumen development, suggesting increased epithelium metabolism and ketogenesis resulting in greater absorptive area and improved absorption of digestive end products. Nevertheless, biochemical and physiological feedback pathways and the long-term survival of *M. elsdenii* NCIMB 41125 in the rumen should be investigated as well as long-term effects on performance.

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