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Performance of Holstein calves having free access to milk and dosed with *Megasphaera elsdenii*

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ABSTRACT: *Megasphaera elsdenii* converts lactate and glucose into butyrate, the main volatile fatty acid responsible of papillae development and may benefit calf performance. Twenty-six Holstein calves (BW = 34.5 ± 1.65 kg) were randomly assigned at birth to a control group (Meg0) and a group that received an oral dose of *M. elsdenii* NCIMB 41125 at 14 d of age (Meg14). Calves received colostrum for the first 3 d followed by free choice access to whole milk until weaning at 56 d. From d 4 onward, starter and water were offered ad libitum. Intakes were measured daily and body weights (BW) weekly. Blood samples were collected on day 7, 21, 28, 42, and 56 for β-hydroxybutyrate (BHBA) analysis. Performance was measured for an additional 14 d post-weaning. Pre-weaning milk intake was lower ($p = 0.010$) and starter DMI (dry matter intake) greater ($p = 0.001$) for Meg14 than Meg0 calves. Total DMI, metabolisable energy (ME) intake and average daily gain (ADG) were similar ($p > 0.05$) for both groups, but Meg14 calves had greater weaning BW ($p = 0.012$) and feed efficiency ($p < 0.029$). The average BHBA between d 21 and 56 was greater for Meg14 ($p = 0.03$) compared to Meg0 calves. After weaning, Meg14 calves had greater DMI ($p = 0.027$), ME intake ($p = 0.023$) and ADG ($p = 0.002$) and tended to have better feed efficiencies ($p = 0.07$) than Meg0 calves. Administering *M. elsdenii* NCIMB 41125 improved starter intake and feed efficiency, which was associated with high blood BHBA.
Keywords: Beta-hydroxybutyrate, rumen development, weaning, probiotics

Introduction

The amount of milk consumed by young dairy calves influences gut development (Anderson et al., 1987) and determines intake of starter feed as well as health and growth of calves (Appleby et al., 2001). Studies to improve milk-feeding systems for dairy calves through *ad libitum* milk feeding have shown higher milk consumption and BW gain, with reduced starter intake, compared to restricted milk feeding (Appleby et al., 2001; Hammon et al., 2002; Jasper and Weary, 2002). Delayed solid feed intake because of *ad libitum* milk consumption during the pre-weaning period (Appleby et al., 2001; Hammon et al., 2002) results in delayed ruminal development with subsequent poor post-weaning performance (Baldwin et al., 2004) and poor welfare (Khan et al., 2007).

Rumen development is an important factor determining early solid feed intake and performance in cattle (Górka et al., 2009). Butyrate and propionate are the main volatile fatty acids (VFA) responsible for rumen epithelial and papillae development in the pre-weaned ruminant (Coverdale et al., 2004). Megasphaera elsdenii is thought to play a major role in production of branched-chain of VFA in the rumen (Wallace, 1986) and alter rumen fermentation in favour of propionate and butyrate as end products (Henning et al., 2010).

In a recent study, dosing *M. elsdenii* NCIMB 41125 to calves in restricted milk feeding system improved feed intake and rumen development, suggesting increased epithelium metabolism and improved absorption of digestive end products. These effects were associated with increasing ruminal butyrate concentration (Muya et al., 2015). Possibly *M. elsdenii* also plays a positive role in rumen development under condition where starter intake is suppressed due to a*d lib* or high volume milk feeding in accelerated growth-feeding systems. The objective of this study was to evaluate the influence of an oral dose of *M. elsdenii* NCIMB 41125 at 14 d of age on pre- and post-weaning intake, performance, and ruminal development of calves fed milk *ad libitum* in the morning and afternoon.

Materials and Methods

Animals and treatments

The experiment was conducted in Pretoria, South Africa (25°53'54.1" S 28°12'01.6" E: 1526 m altitude). Twenty-six Holstein calves (BW = 34.5 ± 1.65 kg) were blocked based on order of birth and sex and randomly assigned at birth to one of the two treatments. The treatments were a control group, which did not receive *M. elsdenii* (Meg0) and a *M. elsdenii* group (Meg14), which received a 50-mL oral dose of *M. elsdenii* NCIMB 41125 (10^8 CFU mL^{-1}) at 14 d of age. Calves were fed colostrum for the first 3 d of life followed by free choice access to whole milk (CP: 4 %; Fat: 4 %; Lactose: 5 %) during feedings at 08h00 and 14h00. Milk was offered in a 5 L bucket and if the calf consumed all of the 5 L, it was immediately refilled until voluntary intake ceased. From d 52 until weaning (d 56), milk intake was limited to 4 L d^{-1}, offered once daily at 08h00. A commercial calf

starter feed (19 % CP, 4 % fat, 13 MJ kg^{-1} of DM ME) was offered *ad libitum* starting at d 4 of age until weaning at d 56. Fresh water was available *ad lib* throughout the study.

Measurements and sample collection

Daily calf starter, milk and water consumption of each calf were measured throughout the experiment. Starter was offered at 08h00, with starter intake recorded daily for each calf. Calves were initially offered 250 g of starter, and the remaining feed was weighed back at each delivery time. Amounts of offered feed starter were increased by 250 g increments when calves refused less than 50 g of feed. Calves were weighed at birth and at 7-day intervals throughout the experiment before AM milk and starter feeding. Average daily gain was calculated from weekly weight gain. Average BW gain, total DM intake, feed efficiency (kg BW gain kg^{-1} of total DM intake) and ME conversion ratio (kg BW gain kg^{-1} of total ME intake) were calculated. Metabolisable energy concentration of whole milk was calculated using NRC (2001) equations as ME (MJ kg^{-1}) = 3.77 ((0.057 × CP %) + (0.092 × Fat %) + (0.0395 × Lactose %)) and predicted ADG calculated based on equations of energy requirements for calves fed milk (NRC, 2001). At 7, 21, 28, 42, and 56 d of age in the morning after milk feeding, blood was collected via the jugular vein from randomly selected calves in both groups (8 in Me0 and 9 from Me14 groups) by venipuncture for analysis of β-hydroxybutyrate (BHBA). Blood was collected in 10 mL tubes containing sodium heparin. Two mL of blood samples were then transferred to two clean test tubes in duplicate. Cold 30 % perchloric acid was added at 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 (4 °C) centrifuge at 2000 rpm, for 20 min. The clear supernatant was transferred to clean glass tubes and recapped with clean screw caps, as quickly as possible, to prevent evaporation of acetone. After, it was stored at –20 °C until analyses of BHBA. The BHBA analysis was performed through an enzymatic analysis (Williamson et al., 1962). At weaning, calves in both groups continued on the starter feed and remained in individual pens until 70 d of age when the experiment concluded.

Statistical analysis

Data were analysed as repeated measures for two periods (pre- and post-weaning) using the PROC MIXED model of SAS (Statistical Analysis System, version 9.2). Response variables were pooled by week for the analysis. The statistical model included calf as a random effect, and experimental group and its interaction with time as a fixed effect. The model was subjected to an autoregressive order one-covariance structure. The statistical model used was

$$Y_{cgt} = = \mu + \alpha_g + \beta_t + \iota_{gt} + \tau_{cg} + e_{cgt}$$

where Y_{cgt} = an observation value for response variables measured from calf c from group g at time t; μ = overall mean for the population; α_g = fixed effect of group g, where g = group Meg0 or Meg14; β_t = fixed effect of time t; ι_{gt} = fixed interaction of effect of group g and time t; τ_{cg} = random effect of calf c nested within group g; and e_{cgt} = error associated with the measurement taken from calf c from group g at time t.

Significance was declared at $p < 0.05$ and tendencies at $p < 0.10$. A linear mixed-effect model was also performed to compare predicted and observed ADG within groups.

Results and Discussion

Intake, growth and efficiency

Least squares means for average daily intakes, BW and ADG for both the pre-weaning period and two weeks after weaning are presented in Table 1. All calves had free access to milk during feeding time, but Meg14 calves consumed less milk ($p = 0.010$) than Meg0 calves did. The range of milk consumption for both groups was comparable to 8.1 kg d^{-1} reported by Borderas et al. (2009), but lower than 8.79 and 8.97 kg d^{-1} reported by Jasper and Weary (2002) and Moallem et al. (2010), respectively.

All calves had low starter DMI during pre-weaning due to increased milk supply and associated increase in nutrient availability (Appleby et al., 2001; Cowles et al., 2006; Hill et al., 2008). Dosed calves consumed more ($p = 0.001$) starter DMI than Meg0 calves did, but total DMI (calf starter + milk) and estimated ME intake were not different between treatments ($p = 0.358$ and $p = 0.153$; respectively).

Starter DMI for control calves (0.10 kg d^{-1}) was close to 0.09 kg d^{-1} reported by Jasper and Weary (2002) for calves fed whole milk *ad libitum*. Greater early starter intake by Meg14 calves could be adequate to initiate rumen function development and stimulate starter intake in the later age (Górka et al., 2011; Muya et al., 2015). These Meg14 calves consumed an average of 0.14 ± 0.011 kg d^{-1} starter DM during the pre-weaning period, representing 40 % more starter feed than control calves did. This may be attributed to more developed rumen papillae and associated improved digestion and increased absorption capacity of nutrients (Kristensen et al., 2007; Górka et al., 2011) allowing high intake of solid feed by these dosed calves when milk was not available. These calves reached 0.225 kg d^{-1} of starter DMI during week 6 (Table 2) when milk intake was 9 kg d^{-1}.

When compared to calf starter DMI within weeks and before weaning, the treatment difference occurred during week 7 and 8, when dosing *M. elsdenii* NCIMB 41125 increased DMI compared to Control calves ($p = 0.002$ and $p < 0.001$, respectively). This was indicated by the significant ($p = 0.005$) interaction between treatment and time (Table 2). After weaning, dosed calves

tended (p = 0.07) to consume more and consumed more (p = 0.01) starter DM during week 9 and 10, respectively. Pre-weaning average daily gain did not differ (p = 0.261) between treatments, but Meg14 calves were 5.8 kg heavier (p = 0.01) at weaning compared to Meg0 calves. Feed efficiency was greater for Meg14 calves compared to Meg0 calves (p = 0.029). Post-weaning DMI and estimated ME intake were greater (p < 0.05) for Me14 calves compared to Meg0 calves and Meg14 calves gained 0.37 kg more per day than Meg0 (p = 0.02) calves did and weighed 11.6 kg more than Meg0 calves at the end of the study.

The progressive change in milk and starter DMI over time are shown in Figures 1 and 2 respectively. In agreement with previous studies, all calves increased milk consumption during the first two weeks and were able to consume a large quantity of milk (De Passillé et al., 1992; Khan et al., 2007). There was fluctuation of milk intake throughout the pre-weaning period in both groups, with the average milk intake increasing with age. Starter DMI was very low in both treatments, but exponentially increased from day 52 to 70. This was related to milk withdrawal, as it was reduced to 4 L once in the morning until weaning at 56 days old. Differences between treatments in starter feed intake became apparent at week 7.

The average daily weight gain did not differ (p = 0.261) between treatments during the pre-weaning pe-

Table 1 – Least square means of intake, growth and efficiency of calves dosed (Meg14) or not (Meg0) with *M. elsdenii* NCIMB 41125.

	Treatment*		SEM[1]	*p*-value**		
	Meg0	Meg14		T	Time	T × Time
Pre-weaning						
Milk intake, kg d^{-1}	8.2	7.4	0.211	0.010	< 0.0001	0.4437
Starter DMI, kg d^{-1}	0.10	0.14	0.011	0.001	< 0.0001	0.0048
Tot DMI, kg d^{-1}	1.12	1.06	0.026	0.358	< 0.0001	0.7116
ME intake, MJ kg^{-1}	22.9	21.3	0.134	0.143	< 0.0001	0.6634
Initial BW, kg	34.3	34.8	1.654	0.820	-	-
Weaning BW, kg	69.7	75.5	2.320	0.012	-	-
ADG, kg	0.63	0.73	0.048	0.261	< 0.0001	0.5719
Gain:Feed	0.57	0.69	0.023	0.029	< 0.0001	0.2955
Post-weaning period						
Starter DMI, kg d^{-1}	1.46	1.73	0.065	0.027	< 0.0001	0.594
ME intake, MJ kg^{-1}	19.5	23.0	0.310	0.023	< 0.0001	0.594
Final BW, kg	77.4	89.9	2.012	< 0.0001	-	-
ADG, kg	0.55	1.03	0.123	0.002	0.048	0.2613
Gain:Feed	0.37	0.60	0.120	0.068	0.6060	0.1958

*Meg0 = control group, which did not receive *M. elsdenii*; Meg14 = received a 50-mL oral dose of *M. elsdenii* NCIMB 41125 (10^8 mL^{-1}) at 14 d of age; **T = Effect of treatment (dosing or not with *M. elsdeni*); T × Time = Interaction treatment × time; SEM = Standard error of mean; Tot DMI = Starter DM + Milk DM; ME intake: (Starter ME × Starter DMI) + (Milk ME × Milk intake); ADG = Average daily gain calculated from weekly ADG.

Table 2 – Least square mean of weekly starter dry matter intake of calves dosed (Meg14) or not (Meg0) with *M. elsdenii* NCIMB 41125.

	Treatment*		SEM[1]	*p*-value
	Meg0	Meg14		
Week 1	0.017	0.013	0.0029	0.915
Week 2	0.033	0.030	0.0460	0.937
Week 3	0.056	0.061	0.0063	0.874
Week 4	0.066	0.071	0.0069	0.903
Week 5	0.079	0.098	0.0098	0.526
Week 6	0.104	0.137	0.0104	0.161
Week 7	0.117	0.225	0.0234	0.002
Week 8	0.354	0.513	0.0456	< 0.001
Week 9	1.150	1.371	0.0624	0.071
Week 10	1.781	2.092	0.0668	0.012

*Meg0 = control group, which did not receive *M. elsdenii*; Meg14 = received a 50-mL oral dose of *M. elsdenii* NCIMB 41125 (10^8 CFU mL^{-1}) at 14 d of age; **T: Effect of treatment (dosing or not with *M. elsdeni*); T × Time = Interaction treatment × time; [1]SEM = Standard error of mean.

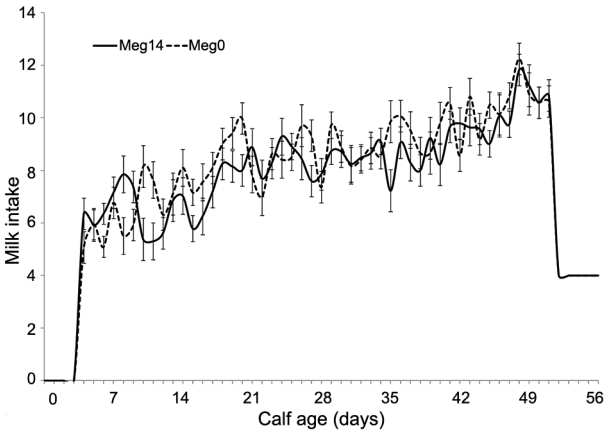

Figure 1 –Milk intake (± SE) (kg d^{-1}) over period for calves dosed (Meg14) or not (Meg0) with *M. elsdenii* NCIMB 41125 on day 14 of age.

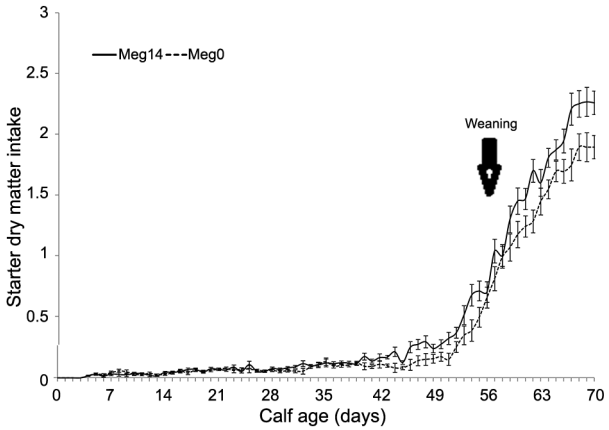

Figure 2 – Starter dry matter intake (± SE) (kg d^{-1}) over period for calves dosed (Meg14) or not (Meg0) with *M. elsdenii* NCIMB 41125 on day 14 of age.

riod, but it was higher (p = 0.002) in the Meg14 group during post-weaning (week 9 and 10). From d 21 to d 42, Meg0-calves increased their ADG from 0.51 to 1.0 kg d^{-1} (Figure 3), while Me14-calves increased it from 0.51 to 1.2 kg d^{-1} during the same period. In both groups, ADG decreased at weaning to less than 0.4 kg d^{-1}. Pre-weaning gain: feed was greater (p < 0.029) for Meg0 compared to Meg14, but tended (p < 0.068) to be greater for Meg14 post-weaning compared to Meg0. The increased post-weaning ADG within and between treatments provided a net growth advantage to Meg14 calves.

The decrease in ADG around weaning reported in previous studies (Bar-Peled et al., 1997; Jasper and Weary, 2002; Cowles et al., 2006; Terré et al., 2006) in calves fed high volumes of milk was also found in this study, as all calves decreased ADG a week before weaning. This is explained by the decrease in energy and protein intake when milk allowance was reduced. However, Meg0 calves presented a more pronounced decrease compared with Meg14 calves. A common pitfall encountered in high milk feeding programs is the low starter intake at weaning (Bar-Peled et al., 1997; Brown et al., 2005; Cowles et al., 2006). Conversely, increased starter feed in Meg14 group provided the calves a growth advantage after weaning as reflected in the high post-weaning ADG, suggesting improved intake of energy when calves were weaned from milk, as starter was the only source of energy available. This suggests that dosing *M. elsdenii* could be an efficient strategy for weaning calves adequately when fed larger volumes of milk or milk replacer. This may be attributed to a residual effect of early increased starter DMI and, therefore, increased gut fill. Early starter consumption increases nutrient digestibility, and consequently improves ruminal microbiota and fermentation activities (Anderson et al., 1987).

Dosed calves in the current study, gained 100 g d^{-1} and were 5 kg heavier than control calves at weaning at 56 d. The higher starter DMI was more pronounced during the last two weeks prior weaning, and was 108 and 159 g d^{-1} during week 7 and 8, respectively, when milk intake was similar between the two groups. The improved consumption of solid feed of calves dosed with *M. elsdenii*, and possibly related ruminal activity have mitigated negative effects of *ad libitum* milk intake and lessened the negative transitional effects of milk removal on growth and performance.

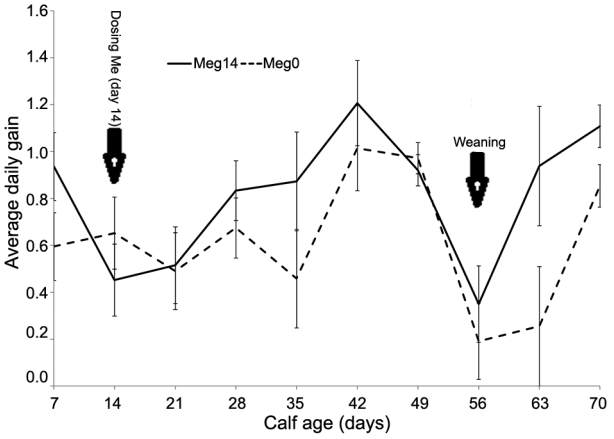

Figure 3 – Average daily gain (± SE) (kg d^{-1}) over period for calves dosed (Meg14) or not (Meg0) with *M. elsdenii*.

The average daily gain of pre-weaned calves was compared with predicted ADG (NRC, 2001) within groups to ascertain the efficiency of nutrient utilization, and results are presented in Figure 4. In Meg14 group, observed ADG was higher (p < 0.001) than predicted ADG, whereas in Meg0 group, observed ADG did not differ from the model (p = 0.721). Greater actual pre-weaned ADG observed in Meg14 group than predicted by the NRC (2001) model may indicate more efficient use of starter nutrients by calves that received *M. elsdenii*, supporting high ME intake and weaning BW observed in this group. This is related to improved starter DMI observed in Meg14-calves. They consumed more starter than Meg0 calves did, and therefore were more adapted to dry feed intake, suggesting improved absorptive capacity (Coverdale et al., 2004). This is believed to be the result of increased ruminal butyrate, which provides energy to thicken the rumen wall, form papillae, and increase capillary development (Weigand et al., 1975).

Blood beta-hydroxybutyrate

Results of effects on plasma BHBA concentration are presented in Table 3 and Figure 5. On d 7 (prior to treatment administration), BHBA concentrations tended (p = 0.09) to be greater for Meg0 calves compared to Meg14 calves; however, BHBA concentration increased

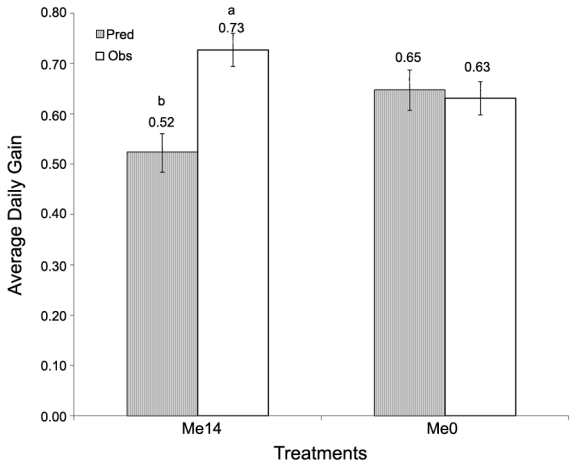

Figure 4 – Comparison of observed and predicted average daily gain (kg d^{-1}), using equation based on metabolisable energy requirement (NRC, 2001) during the pre-weaning period of calves dosed (Meg14) or not (Meg0) with *M. elsdenii*; [ab]Least square means differ significantly between treatment groups at the same time point (p < 0.01).

Table 3 – Least square mean of plasma beta-hydroxybutyrate (BHBA) of calves dosed (Meg14) or not (Meg0) with *M. elsdenii* NCIMB 41125.

	Treatment*		SEM[1]	*p*-value**		
	Meg0	Meg14		T	Time	T × Time
BHBA[2], mmol L^{-1}						
On day 7 (before dosing)	0.076	0.057	0.038	0.093	-	-
Average after dosing)	0.106	0.281	0.057	0.034	< 0.0001	0.009

*Meg0 = control group, which did not receive *M. elsdenii*; Meg14 = received a 50-mL oral dose of *M. elsdenii* NCIMB 41125 (10^8 CFU mL^{-1}) at 14 d of age; **T = Effect of treatment (dosing or not with *M. elsdenii*); T × Time = Interaction treatment × time; [1]SEM = Standard error of mean; [2]BHBA = beta-hydroxybutyrate.

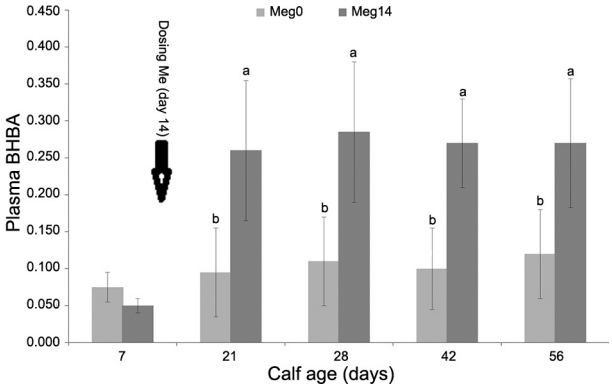

Figure 5 – Plasma beta-hydroxybutyrate (BHBA) (mmol L^{-1}) of calves dosed (Meg14) or not (Meg0) with *M elsdenii*; [ab]Least square means differ significantly between treatment groups at the same time point ($p < 0.01$).

drastically in Meg14 from d 21 and until d 56 ($p = 0.03$).

The potential of *M. elsdenii* to increase the proportion of rumen propionate and butyrate was previously reported (Marounek et al., 1989; Cruz et al., 2001; Henning et al., 2010; Muya et al., 2015). The particular role of these two VFA on stimulating rumen epithelial cells and papillae development and thereby, increasing the capacity of solid feed intake, has been previously discussed (Tamate et al., 1962; Coverdale et al., 2004; Lane and Jesse, 1997). The effects of VFA are reported to be most likely associated with the rate at which they are metabolized by mucosal cells during absorption, being over 90 % of butyric acid and approximately 50 % of propionic acid metabolized and oxidized to ketone bodies (Britton and Krehbiel, 1993). As it is oxidized by the rumen epithelial cells and passes through the rumen wall, butyric acid is converted to BHBA (Quigley, 1991; Lesmeister and Heinrichs, 2004), which is used as an indicator of the rumen development (Suarez et al., 2006).

Dosing *M. elsdenii* could have positive effects on rumen papillae development and function, as previously showed in calves (Kristensen et al., 2007; Górka et al., 2011), through increased rumen butyrate (Muya et al., 2015) and, thus, improve calves intake and growth. The increase rumen butyrate could have allowed for continuous exposition of rumen epithelial cells to its preferred energy source, and consequently stimulated rumen development. The more developed calf rumen observed in conventional rearing systems, compared to accelerated growth, is explained by higher ruminal butyrate concentration (Lesmeister and Heinrichs, 2004).

In the study of Muya et al. (2015), calves fed restricted milk and dosed with *M. elsdenii* had greater reticulum-rumen weight and papillae density, with greater papillae width compared to control calves. These improvements were associated with greater ruminal butyrate concentration and plasma BHBA. In this study, plasma BHBA concentration of dosed calves increased the week after it was dosed with *M. elsdenii*, when they consumed more calf starters feed than control calves did from two weeks before weaning until the end of the experimental period and kept their growth advantage after weaning.

Conclusion

Administering *Megasphaera elsdenii* NCIMB 41125 to Holstein calves at 14 d of age in accelerated growth program improved pre-and post-weaning performance, starter feed intake and feed efficiency. In addition, greater plasma BHBA concentration in these calves may be an indicative of prompt rumen physical development with concomitant increased metabolic activity of the rumen epithelium.

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