Rumen pH and fermentation characteristics in dairy cows supplemented with *Megasphaera elsdenii* NCIMB 41125 in early lactation

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ABSTRACT

This study investigated the effect of using the lactate-utilizing bacterium *Megasphaera elsdenii* NCIMB 41125 as a probiotic supplement on rumen fermentation and pH in dairy cows in the immediate postcalving period. Fourteen multiparous rumen-fistulated Holstein cows, blocked according to 305-d milk yield in the previous lactation, were used in a randomized complete block design. From d 1 to 28 postcalving, cows were fed ad libitum a total mixed ration with a forage to concentrate ratio of 392:608 and a starch concentration of 299 g/kg of dry matter. Treatments consisting of a minimum of 10^{10} cfu of *Megasphaera elsdenii* NCIMB 41125 or autoclaved *M. elsdenii* (placebo) were administered via the rumen cannula on d 3 and 12 of lactation ($n = 7$ per treatment). Mid-rumen pH was measured every 15 min, and eating and ruminating behaviors were recorded for 24 h on d 2, 4, 6, 8, 11, 13, 15, 17, 22, and 28. Rumen fluid for volatile fatty acid and lactic acid analysis was collected at 11 time points on each of d 2, 4, 6, 13, and 15. Yields of milk and milk protein and lactose were similar, but milk fat concentration tended to be higher in cows that received the placebo. Time spent eating and ruminating and dry matter intake were similar across treatments. Ruminal lactic acid concentrations were highly variable between animals, and no cases of clinical acidosis were observed. Both treatment groups had rumen pH <5.6 for more than 3 h/d (a commonly used threshold to define subacute ruminal acidosis), but the length of time with rumen pH <5.6 was markedly reduced in the days immediately after dosing and fluctuated much less from day to day in cows that received *M. elsdenii* compared with those that received the placebo. Ruminal total volatile fatty acid concentrations were similar across treatments, but the acetate:propionate ratio tended to be smaller in cows that received *M. elsdenii*. Despite the

lack of a measurable treatment effect on ruminal lactic acid concentration, supplementation of early lactation dairy cows with lactate-utilizing *M. elsdenii* altered the rumen fermentation patterns in favor of propionate, with potential benefits for energy balance and animal productivity.

Key words: *Megasphaera elsdenii*, dairy cow, rumen fermentation, probiotic

INTRODUCTION

Rapid ruminal fermentation of starch and sugars in the cereal-based, energy-dense diets typically fed to highly productive ruminants can cause rumen acidosis, a metabolic disorder that exists in both acute and subacute forms. Acidosis is characterized by a decrease in pH caused by an accumulation of organic acids in the rumen and a shift in the delicate balance of rumen microorganisms (Krause and Oetzel, 2006). In dairy cattle, the period around calving is critical: the rumen, having become accustomed to moderate intakes of a low-energy, high-forage diet in the dry period, must become adapted to large amounts of a high-energy, carbohydrate-rich diet over the course of a few days as the cow rapidly increases her DMI to meet the rising energy demands of lactation. The rate of microbial VFA production exceeds the rate at which VFA can be absorbed across the ruminal epithelium, leading to their accumulation in the rumen and a decrease in pH. This elicits a shift in the fermentation patterns of the rumen microbial population from VFA toward lactic acid, which is considerably stronger than VFA (Owens et al., 1998), and depresses pH further. Naturally occurring, lactate-utilizing bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminantium* ssp. *lactilytica* should begin to proliferate in response to lactic acid accumulation and remove it by converting it to VFA that can be absorbed from the rumen. Unfortunately, however, the proliferation of lactate-utilizing bacteria can be inhibited by the low rumen pH that results from lactate accumulation (Nagaraja and Titgemeyer, 2007).

The traditional management strategy to avoid acidosis is to provide dairy cows with small amounts of

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the lactation diet in late gestation followed by gradual increases in the amount of concentrate offered postcalving. In theory, this should allow the optimum profile of rumen microorganisms to proliferate but this does not always happen in practice because moderate intakes precalving mean that the amount of readily fermentable carbohydrate available is not sufficient to cause a meaningful shift in microbial species. Measures that allow dairy cows to be fed a high-concentrate, energy-dense diet from calving while preventing the development of acute or subacute acidosis would therefore be beneficial.

The use of probiotic bacteria to augment existing rumen populations until they are able to proliferate naturally is one such measure that has been explored. *Megasphaera elsdenii* is one of the most promising candidates for this type of intervention, because in vitro studies have shown that it is the most active of the lactate-utilizing bacteria, fermenting up to 95% of the total lactate present (Counotte et al., 1981) or preventing its accumulation and maintaining a higher pH (Kung and Hession, 1995). In vivo studies have produced variable results. Klieve et al. (2003) showed that inoculating the rumens of steers with *M. elsdenii* as they were introduced to a high-grain diet resulted in establishment of viable *M. elsdenii* populations 5 to 7 d earlier than in uninoculated controls, but no advantage in terms of increased rumen pH or reduced lactic acid concentrations was demonstrated. In contrast, in studies where lambs or steers received a single dose of *M. elsdenii* NCIMB 41125, reduced lactic acid accumulation and higher rumen pH in animals that received the *M. elsdenii* treatment were accompanied by higher DMI in the lambs and higher DMI and average daily BW gain in the steers (Henning et al., 2010). A study investigating the use of *M. elsdenii* NCIMB 41125 in early lactation dairy cows reported no overall advantage in terms of milk production or feed efficiency (Hagg et al., 2010). In a production study conducted at this laboratory, a 2-L milk yield increase in response to *M. elsdenii* NCIMB 41125 supplementation was observed in high-yielding animals (305-d milk yield >10,000 kg in previous lactation) fed a high-starch diet, but not in animals with lower milk yields (Aikman et al., 2009). A combination of higher DMI and a greater degree of metabolic stress due to their genetic predisposition for high milk yields probably meant that the high-yielding cows were less able to buffer their rumen acid load than the lower-yielding animals, and they would therefore have benefitted from changes in rumen fermentation patterns elicited by *M. elsdenii*, although these were not measured by Aikman et al. (2009).

The objective of the present study was therefore to use continuous rumen pH measurements and frequent

rumen samplings to assess the effect on rumen pH and fermentation patterns of dosing freshly calved, high-yielding dairy cows at high risk of developing rumen acidosis with *M. elsdenii* NCIMB 41125 at critical time points in the postpartum period.

MATERIALS AND METHODS

Animals, Housing, and Diets

The study was conducted at the Metabolism Unit, Centre for Dairy Research, University of Reading. All experimental procedures used were licensed, regulated, and inspected by the UK Home Office under the Animals (Scientific Procedures) Act, 1986. Fourteen pregnant, multiparous, rumen-fistulated Holstein dairy cows were enrolled on the study 21 d before expected calving date. Animals were housed in individual straw-bedded pens in the precalving period and were moved to tie-stalls with rubber mattresses and wood shavings within 48 h of parturition, where they stayed for the remainder of the study. Animals were fed a restricted amount of a late-gestation TMR (Table 1) in the precalving period. After parturition, an energy-dense lactation TMR (Table 1) was introduced over 3 d, after which the lactation TMR was offered ad libitum. The lactation TMR, which was designed to challenge the rumen buffering capacity, had a 392:608 forage to concentrate ratio and targeted starch and NDF concentrations of 313 and 277 g/kg of DM respectively. Two-thirds of the total feed offered was provided at 0900 h and one-third at 1600 h. Milking occurred twice daily at 0600 and 1600 h. Animals were blocked according to 305-d milk yield (mean $\pm$ SD: 11,247 $\pm$ 1,466 kg) in the previous lactation, and treatments (placebo or *M. elsdenii* NCIMB 41125) were randomly assigned within each pair.

Preparation and Administration of M. elsdenii and Placebo

Individual doses of *M. elsdenii* NCIMB 41125 (Biolifer Megamilk) were provided by Kemira Phosphates Ltd. (Umbogintwini, South Africa). Each dose was provided as 50 mL of *M. elsdenii* NCIMB 41125 inoculum (10^8 cfu/mL) and 200 mL of sterile growth medium containing sodium lactate, soy peptone, yeast extract, minerals, and vitamins anaerobically packed in separate compartments of a 2-compartment plastic bag. Doses were prepared by squeezing the contents of the growth medium compartment into the *M. elsdenii* inoculum, thus breaking the seal between the 2 compartments and mixing the solutions. The outer seal of the bag remained intact, ensuring that anaerobic conditions were maintained within the bag. The bag was then incubated

Table 1. Composition of the precalving and lactation period TMR

Item	Precalving	Lactation
Component (g/kg of DM)		
Corn silage	480	196
Grass silage	140	196
Wheat straw	170	—
Wheat	43	277
Barley	—	89
Rapeseed meal	24	52
Molasses	—	9
Soybean meal	24	100
Molassed sugar beet pulp	—	48
Calcium salts of fatty acids ¹	3	16
Concentrate mix ²	73	—
Minerals ³	43	17
Nutritional value		
CP (g/kg of DM)	114	176
ME (MJ/kg of DM)	10.3	12.2
Starch (g/kg of DM)	157	299
Water-soluble carbohydrate (g/kg of DM)	23	39
NDF (g/kg of DM)	512	260
Ash (g/kg of DM)	62	50

¹Megalac (Volac International Ltd., Royston, UK).

²Concentrate mix contained (g/kg of DM): palm kernel expeller meal (276), citrus pulp (299), distillers dark grains (253), wheatfeed (138), urea (23), calcium carbonate (11).

³Mineral supplement had the following composition: vitamin A, 500,000 IU/kg, vitamin D₃, 100,000 IU/kg, vitamin E, 1,000 IU/kg, Co, 50 mg/kg, Mn, 4,000 mg/kg, Cu, 1,500 mg/kg, Zn, 5,000 mg/kg, I, 500 mg/kg, Ca, 270 g/kg, P, 40 g/kg, Na, 40 g/kg, and Mg, 60 g/kg.

for 24 h in a 45-L insulated food carrier (Igloo Products Corp., Houston, TX) containing a thermostatically controlled heating element (Kemira Phosphates Ltd.) to maintain temperature at 39°C. Inflation of the bags during incubation indicated that the population of *M. elsdenii* was increasing, and only bags that were fully inflated after the 24-h incubation period were used. The entire 250-mL contents were removed through a small cut in the bag with a 250-mL syringe and immediately administered to the animal via the rumen cannula. Two bags were randomly selected from each batch of 12 doses received for determination of viable cell counts. These bags were incubated as described and then subjected to serial dilution and plate counts on a medium containing sodium lactate (5.3%) and agar (2%) in an anaerobic chamber (MACS-MG-1000 Anaerobic Workstation, Don Whitley Scientific Ltd., Shipley, UK). Counts ranged from 9.0×10^7 to 9.8×10^{10} cfu/mL, meaning that the 250-mL doses contained 2.25×10^{10} to 2.45×10^{13} cfu of *M. elsdenii*.

Placebo doses were prepared by mixing and incubating individual doses of *M. elsdenii* as described, and then autoclaving the suspension for 20 min at 121°C to kill all live *M. elsdenii* before administering the entire 250-mL dose through the rumen cannula.

Animals received the first dose (250 mL) of the placebo or *M. elsdenii* as appropriate on d 3 relative to

calving, when DMI was at least 8 kg/d, followed by a second dose on d 12 relative to calving.

Sampling, Measurements, and Laboratory Analysis

Dry matter intake was recorded daily for the duration of the study. Individual forage and concentrate components were sampled 3 times and 1 time per week, respectively. All feed samples were stored at −18°C until required for analysis. Starch and water-soluble carbohydrate concentrations were determined in monthly bulk samples of forage and nonforage dietary components according to the method of the Ministry of Agriculture, Fisheries and Food (MAFF, 1986). Monthly bulk samples of grass and maize silages were scanned by near-infrared spectroscopy to estimate the concentrations of other nutrients and ME using the principles reported by Barber et al. (1990). Further nutritional composition of nonforage components was determined by wet chemistry methods (MAFF, 1986), and ME concentration was calculated according to MAFF (1993).

Milk yield was recorded at each milking. Representative milk samples were taken at each milking, preserved with potassium dichromate (Lactabs, Thomson and Capper, Runcorn, UK), and stored at 4°C until analyzed for fat, protein, and lactose concentrations by infrared spectrophotometry. Milk production efficiency was calculated as milk yield (kg/d)/DMI (kg/d).

Mid-rumen pH was measured at a depth of 30 cm below the rumen cannula every 15 min for 24 h starting at 0900 h on each of d 2, 4, 6, 8, 11, 13, 15, 17, 22, and 28 of lactation using an indwelling pH electrode and portable data recorder (pH340i, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). These data were used to calculate the mean daily pH, the amount of time (h/24 h) that rumen pH was below threshold values of 6.0 and 5.6, and the area ($\text{min} \times \text{pH/d}$) below pH 6.0 and 5.6. All pH recording equipment was calibrated daily using pH 4.0 and 7.0 buffer solutions (Fisher Scientific UK, Loughborough, UK).

Samples of rumen fluid were obtained from each animal on d 2, 4, 6, 13, and 15 of lactation. On each of these days rumen fluid was obtained from each animal 0.5 h before morning feeding and 0.5, 1.0, 1.5, 2.0, and 4.0 h after the morning feeding, and at the same intervals in relation to the afternoon feeding time. Samples were obtained at a depth of 34 cm below the rumen cannula by pushing a metal tube with a coarse filter on the end down the side of the rumen cannula and withdrawing 100 mL of rumen fluid with a syringe. Subsamples (25 mL) were stored without preservative in screw-top polypropylene tubes at −18°C until analysis. For each cow, rumen VFA, lactic acid, and ethanol concentrations were determined in a pooled sample (0.5, 1.0, 1.5,

and 2.0 h after the morning feeding on d 2) obtained before the first dose of *M. elsdenii* or placebo, and in all rumen fluid samples collected on d 4, 6, 13, and 15 of lactation. Lactic acid, VFA, and ethanol concentrations were determined by gas chromatography using an adaptation of the method described by Fussell and McCalley (1987), which included use of a 4% Carbowax 20M column (Supelchem, Sawbridgeworth, UK), pivalic acid (2.5 mg/mL) as an internal standard, an oven temperature gradient between 180 and 200°C, and injector and detector temperatures of 220°C.

Total jaw movements were recorded for 24 h starting before the morning feed on d 2, 4, 6, 8, 11, 13, 15, 17, 22, and 28 of lactation using IGER Behavior Recorders (Rutter et al., 1997). Specialist software (Rutter, 2000) was used to identify periods of eating and ruminating, the total time spent in each activity, and the number of boluses produced during rumination as described by Aikman et al. (2006).

Statistical Analysis

All data were analyzed as repeated measures using the Mixed procedure of SAS 9.1 (SAS Institute, 2003). Block was initially included as a fixed effect in all of the statistical models described below, but was removed because it was not significant ($P > 0.1$) for any variable. Treatment and day were included as fixed effects and animal was included in the model as a random effect. Measurements made on d 2 were included as covariates as appropriate, but were removed from the model if not significant ($P > 0.1$). Different covariance structures, detailed below for each analysis, were tested for each variable, and the structure that yielded the smallest Bayesian information criterion was used for the results presented.

Daily DMI, milk yield, and milk composition variables for d 3 to 28, eating and ruminating behavior measurements, and the length of time and the area of rumen pH below 6.0 and 5.6 on d 4, 6, 8, 11, 13, 15, 17, 20, and 28 were analyzed using a model that assessed the effects of treatment, day, and the treatment $\times$ day interaction. Compound symmetry, heterogeneous compound symmetry, autoregressive, and heterogeneous autoregressive covariance structures were tested for the DMI, milk yield, and milk composition variables. Compound symmetry, heterogeneous compound symmetry, and spatial power covariance structures were tested for the eating and ruminating behavior and rumen pH variables.

The individual VFA concentrations, molar proportions, and ratios measured over 11 time points on d 4, 6, 13, and 15 were divided into 2 categories for analysis: before morning feed (one sample per cow per d) and

after morning feed (10 samples per cow per day). The prefeeding samples were analyzed using a model that assessed the effects of treatment, day, and the treatment $\times$ day interaction, with d 2 measurements used as covariates if $P < 0.1$. Spatial power, compound symmetry, and heterogeneous compound symmetry covariance structures were tested for all prefeeding VFA variables. The postfeeding samples were analyzed as repeated measures over day and time points within day, using a model with a spatial power covariance structure that assessed the effects of treatment, day, time point within day, and the treatment $\times$ day interaction. All time point interactions were initially included in the model, but were not significant ($P > 0.1$) and were removed. The individual VFA concentrations obtained from the d 2 pooled sample were used as covariates in the statistical analysis of VFA concentrations, but no covariates were used for the molar proportion or VFA ratio variables. Lactic acid concentrations were not statistically analyzed because of the large number of zero values in the data set; they are reported as arithmetic means.

RESULTS

In the lactation TMR, starch and NDF concentrations of 299 and 260 g/kg of DM, respectively, were achieved (Table 1), slightly below the targeted concentrations of 313 and 277 g/kg of DM.

Dry matter intake, yield of milk and milk components, and milk concentrations of protein and lactose were not affected ($P \geq 0.113$) by treatment (Table 2). Milk fat concentration tended to be reduced ($P = 0.067$) in cows dosed with *M. elsdenii* compared with those that received the placebo, and a significant ($P < 0.001$) treatment $\times$ day interaction occurred. Milk production efficiency was greater ($P = 0.038$) by almost 0.3 kg of milk per kilogram of DMI in cows that received *M. elsdenii* compared with those that received the placebo (Table 2). Cows spent just over 4 h eating and around 9.5 h ruminating in each 24-h measurement period, but eating and ruminating behaviors were not influenced ($P \geq 0.184$) by dosing with *M. elsdenii* or placebo (Table 3). Day relative to calving influenced total time spent eating ($P < 0.026$) and time spent ruminating per kilogram of DMI ($P < 0.001$), but did not affect total time spent ruminating ($P < 0.615$; Table 3). This occurred because cows spent longer eating as DMI increased after calving, but did not increase the total time spent ruminating; thus, time available to ruminate each kilogram of ingested feed decreased (data not shown).

Over the study as a whole, mean rumen pH was similar ($P < 0.153$) across treatments (Table 4); however,

Table 2. Dry matter intake and milk yield and composition between d 3 and 28 postcalving in cows dosed with *Megasphaera elsdenii* or a placebo on d 3 and 12 relative to calving

Item	Treatment (Trt)			P-value		
	Placebo	<i>M. elsdenii</i>	SEM	Trt	Day	Trt × Day
DMI (kg/d)	19.3	17.5	0.74	0.113	0.001	0.112
Milk yield (kg/d)	43.0	45.4	1.01	0.125	0.001	0.235
FCM yield ¹ (kg/d)	46.4	45.1	1.37	0.522	0.057	0.546
Milk production efficiency ²	2.31	2.60	0.082	0.038	0.016	0.214
Milk composition (g/kg)						
Fat	45.6	40.4	1.81	0.067	0.001	0.001
Protein	33.9	32.7	0.73	0.250	0.001	0.578
Lactose	44.4	45.0	0.49	0.407	0.001	0.007
Yield of milk components (g/d)						
Fat	1,949	1,796	81.3	0.208	0.496	0.128
Protein	1,458	1,450	45.5	0.910	0.088	0.435
Lactose	1,917	2,049	64.2	0.173	0.001	0.121

¹4% FCM yield = [0.4 × milk yield (kg/d) + 15 × fat output (kg/d)].

²Milk production efficiency = milk yield/DMI.

although no overall treatment × day interaction ($P < 0.174$) occurred, rumen pH was higher ($P < 0.05$) on d 8 and 20 in cows that received *M. elsdenii* (Figure 1). Mean rumen pH was below pH 6.0 or 5.6 for 92 and 73 min longer, respectively, in cows that received the placebo compared with those that received *M. elsdenii* (Table 4), although the effect of treatment was not statistically significant ($P > 0.291$). In cows that received *M. elsdenii*, the length of time with rumen pH below 5.6 declined markedly in the days immediately after dosing and fluctuated much less from day to day than in cows that received the placebo (treatment × day interaction, $P < 0.016$; Figure 2). The areas under the pH × time curves (Table 4), which give an indication of the severity of the pH depression, were generally smaller in animals that received *M. elsdenii*, but the effect of treatment was not statistically significant ($P > 0.226$) for the pH 5.6 or pH 6.0 threshold, and no treatment × day interactions ($P > 0.326$) were observed.

Total VFA and concentrations and molar proportions of individual VFA measured 0.5 h before the morning feeding time on d 4, 6, 13, and 15 of lactation (Table 5) were generally similar ($P \geq 0.229$) across treatments,

as were the ratios of acetate:propionate and acetate plus butyrate:propionate ($P \geq 0.154$). The only exception was caproic acid, which was less as an absolute concentration ($P < 0.050$) and as a proportion of total VFA ($P < 0.025$) in the prefeeding samples of cows that received *M. elsdenii* (Table 5). No treatment × day interactions ($P \geq 0.347$) were observed for any of the VFA measurements made 0.5 h before the morning feeding time (Table 5). Concentrations and molar proportions of individual VFA and total VFA measured 10 times over 11 h after the morning feeding time on d 4, 6, 13, and 15 of lactation were generally unaffected by treatment ($P \geq 0.120$) or treatment × day interactions ($P \geq 0.107$; Table 6). The exceptions were acetic acid, in which the total concentration declined ($P < 0.053$) by 4 mmol/L (7%) in cows that received *M. elsdenii*, and caproic acid, where both total concentration and molar proportion declined ($P < 0.008$) by approximately 50% in cows that received *M. elsdenii* (Table 6). The only significant treatment × day interaction ($P < 0.039$) was observed for propionic acid as a proportion of total VFA, which declined between d 4 and 6 before increasing again until d 15 in cows that received the

Table 3. Eating and ruminating behavior measured at d 4, 6, 8, 11, 13, 15, 17, 20, and 28 relative to calving in cows dosed with a placebo or *Megasphaera elsdenii* on d 3 and 12

Item	Treatment (Trt)			P-value		
	Placebo	<i>M. elsdenii</i>	SEM	Trt	Day	Trt × Day
DMI (kg/d)	18.8	16.8	0.86	0.191	0.001	0.223
Eating (min/kg of DMI)	14.4	16.0	1.04	0.349	0.326	0.215
Eating (min/d)	260	265	14.7	0.808	0.026	0.682
Ruminating (min/kg of DMI)	31.2	35.3	2.06	0.292	0.001	0.126
Ruminating (min/d)	567	575	12.5	0.693	0.615	0.942
Total boluses (n/24 h)	541	594	24.3	0.184	0.553	0.578
Boluses (n/kg of DMI)	31.9	33.9	2.00	0.542	0.001	0.121

Figure 1. Mean rumen pH per 24-h period in cows dosed with a placebo (black) or *Megasphaera elsdenii* (gray) on d 3 and 12 relative to calving (indicated by arrows). Overall treatment $\times$ day interaction, $P = 0.245$. Asterisks indicate that mean rumen pH differed ($P < 0.05$) between treatments on that day.

placebo. In contrast, cows that received *M. elsdenii* had a sharp increase in the molar proportion of propionic acid between d 4 and 6, followed by a slight decline until d 15 (data not shown). Nonsignificant declines ($P \geq 0.250$) in the ratios of acetic acid:propionic acid and acetic plus butyric acid:propionic acid were observed in cows that received *M. elsdenii* compared with those that received the placebo (Table 6). Treatment $\times$ day interactions were observed for the acetic acid:propionic acid ($P < 0.057$) ratio and the acetic acid + butyric acid:propionic acid ($P < 0.019$) ratio (Table

6). Both ratios declined between d 4 and 15 in animals that received placebo, but in animals that received *M. elsdenii*, declines between d 4 and 6 were followed by increases in each of these ratios on d 13 and 15 (Figure 3, only acetic acid:propionic acid ratio shown).

In total, 616 rumen samples were analyzed for lactic acid. Of these, 25.6 and 24.3% of samples from cows on the placebo and *M. elsdenii*, respectively, had lactic acid concentrations above the minimum detectable concentration (0.44 mmol/L). Lactic acid concentration varied greatly between cows: at the extremes, it was

Figure 2. Mean length of time with rumen pH < 5.6 per 24-h period in cows dosed with a placebo (black) or *Megasphaera elsdenii* (gray) on d 3 and 12 relative to calving (indicated by arrows). Overall treatment $\times$ day interaction, $P = 0.016$. Treatment difference: $P = 0.069$ on d 8, $P \geq 0.158$ on all other days.

Table 4. Mean rumen pH and length of time (min/d) and area (min $\times$ pH/d) below pH 6.0 and pH 5.6 on d 4, 6, 8, 11, 13, 15, 17, 20, and 28 relative to calving in cows dosed with placebo or *Megasphaera elsdenii* on d 3 and 12

Item	Treatment (Trt)			P-value		
	Placebo	<i>M. elsdenii</i>	SEM	Trt	Day	Trt $\times$ Day
Mean rumen pH	5.98	6.08	0.046	0.153	0.463	0.174
Time below pH 6.0	700	608	59.1	0.291	0.642	0.189
Time below pH 5.6	260	187	59.3	0.401	0.034	0.016
Area below pH 6.0	258	188	45.3	0.296	0.730	0.326
Area below pH 5.6	73	35	21.0	0.226	0.435	0.515

not detected in any samples from one animal (placebo), whereas another (*M. elsdenii*) had lactic acid in 21 out of 44 samples. Mean lactic acid concentrations of the d 2 pretreatment pooled rumen samples ($n = 7$ per treatment) were 1.30 (± 0.921) and 1.00 (± 0.999) mmol/L for placebo and *M. elsdenii* treatments, respectively. Mean lactic acid concentrations of the postfeeding samples ($n = 180$ per treatment) in the posttreatment period (d 4, 6, 13, and 15) were 2.13 (± 0.353) for the placebo and 2.23 (± 0.376) mmol/L for the *M. elsdenii* treatment. The maximum lactic acid concentrations observed at any single time point in the posttreatment period were 34.4 mmol/L for placebo and 32.1 mmol/L for *M. elsdenii*.

DISCUSSION

There has been much debate in the literature about how subacute ruminal acidosis (**SARA**) and acute acidosis should be defined. Acute acidosis is easier to diagnose because of the immediate presence of clinical signs such as inappetence, ruminal stasis, abdominal pain, and a sudden decrease in milk yield. In these cir-

cumstances, determination of the actual rumen pH is of secondary importance, but the clinical manifestation of acidosis is usually associated with a rumen pH lower than approximately 5.0 to 5.2 (Owens et al., 1998; Stone, 2004). Although rumen pH transiently fell below 5.0 to 5.2 in some individuals in this study, no cases of acute acidosis were diagnosed. Subacute ruminal acidosis has variously been defined in the literature as occurring when rumen pH is between 5.0 and 5.6 (Nagaraja and Titgemeyer, 2007), 5.0 and 5.8 (Beauchemin and Yang, 2005), or 5.0 and 5.5 (Krause and Oetzel, 2006), but it is now recognized that rumen pH may briefly decrease within the SARA range several times within a 24-h period, with no apparent ill effects for the animal. For this reason, it is becoming more common to define SARA in terms of length of time rumen pH is below a certain level. Gozho et al. (2005) used a threshold of rumen pH < 5.6 for more than 3 h/24 h to define SARA, because this was the point at which feed intake depression and the occurrence of an inflammatory response, in the form of increased blood concentrations of acute phase proteins, became apparent. According to this definition, SARA occurred in both placebo and *M. elsdenii* cows in

Table 5. Mean ruminal concentrations (mmol), proportions (mmol/mol of total VFA) and ratios (mol:mol) of VFA 0.5 h before morning feeding on d 4, 6, 13, and 15 relative to calving in cows dosed with placebo or *Megasphaera elsdenii* on d 3 and 12

Item	Treatment (Trt)			P-value		
	Placebo	<i>M. elsdenii</i>	SEM	Trt	Day	Trt $\times$ Day
Acetic acid	51.24	48.65	2.43	0.510	0.027	0.927
Butyric acid	13.18	12.31	1.01	0.576	0.059	0.703
Propionic acid	21.91	24.12	1.19	0.229	0.878	0.893
Caproic acid	0.51	0.19	0.10	0.050	0.454	0.624
Valeric acid	2.42	2.75	0.39	0.573	0.475	0.734
Total VFA	89.65	87.33	3.47	0.675	0.064	0.926
Molar proportions (mmol/mol of total VFA)						
Acetic acid	570	555	18.0	0.580	0.394	0.767
Butyric acid	145	141	8.5	0.765	0.095	0.369
Propionic acid	248	275	14.0	0.229	0.007	0.378
Caproic acid	5	2	0.9	0.025	0.300	0.735
Valeric acid	27	31	3.9	0.511	0.334	0.764
Ratios						
Acetic:propionic	2.44	2.05	0.19	0.202	0.007	0.347
(Acetic + butyric):propionic	3.02	2.53	0.22	0.154	0.004	0.360

Figure 3. Ratio of acetic acid:propionic acid measured over 11 h postfeeding in cows dosed with a placebo (black) or *Megasphaera elsdenii* (gray) on d 3 and 12 relative to calving. Overall treatment $\times$ day interaction, $P = 0.057$. Treatment difference: $P = 0.072$ on d 6, $P \geq 0.270$ on d 4, 13, and 15.

the present study, as shown by the overall means (Table 4). In terms of individual sampling days, 35 out of 70 sampling days fell into this category for placebo cows, and 31 out of 70 for *M. elsdenii*. Considerable variability was observed between cows, however, with one individual (#7191, placebo) fitting the criteria on 9 out of 10 sampling days and another (#81, placebo) with no sampling days fitting the criteria. This suggests that even though the experimental diet had a lower starch concentration than planned, it did present a substantial

acidosis challenge, which the animals met with varying degrees of success.

Many of the parameters measured in this study showed small differences that, although not statistically significant in all cases, collectively reveal a shift in fermentation patterns in response to *M. elsdenii* supplementation as the study progressed. The rumen acetate:propionate ratio was numerically decreased in cows that received *M. elsdenii* compared with those that received the placebo, and this tended to be most apparent on d 6 relative to calving, 3 d after the first dose of *M. elsdenii* (Figure 3). Butyrate was the main fermentation end product when *M. elsdenii* was grown on mixed media containing lactate and glucose (Marounek et al., 1989). Henning et al. (2010) also noted a shift toward butyrate production in steers that received *M. elsdenii* NCIMB 41125, but Counotte et al. (1981) found in vivo that up to 77% of lactic acid fermentation by *M. elsdenii* was via the acrylate pathway with propionate as the end product. Counotte et al. (1981) also showed that lactic acid fermentation by *M. elsdenii* shifted away from propionate toward butyrate as rumen pH declined. In the present study, mean pH of the rumen samples analyzed for VFA was around 6.0, thereby favoring production of propionate instead of butyrate (Counotte et al., 1981), whereas mean rumen pH was generally around 5.5 in the study of Henning et al. (2010).

In common with other studies (e.g., Bevans et al., 2005), lactic acid was not consistently detected in rumen fluid samples in the present study and, as with rumen pH, a large amount of between-animal variation was observed. The individual in which no lactic acid was detected (#7309, placebo) had rumen pH indica-

Table 6. Mean ruminal concentrations (mmol), proportions (mmol/mol of total VFA) and ratios (mol:mol) of VFA postfeeding¹ on d 4, 6, 13, and 15 relative to calving in cows dosed with a placebo or *Megasphaera elsdenii* on d 3 and 12

Item	Treatment (Trt)			P-value			
	Placebo	<i>M. elsdenii</i>	SEM	Trt	Day	Time	Trt $\times$ Day
Acetic acid	58.64	54.64	1.210	0.053	0.898	0.217	0.796
Butyric acid	18.09	17.20	0.621	0.356	0.319	0.001	0.147
Propionic acid	27.34	28.01	0.881	0.603	0.072	0.001	0.429
Caproic acid	0.93	0.44	0.103	0.008	0.023	0.161	0.168
Valeric acid	3.30	3.19	0.238	0.748	0.451	0.001	0.593
Total VFA	108.15	103.65	1.771	0.120	0.721	0.001	0.661
Molar proportions							
Acetic acid	542	528	9.1	0.303	0.118	0.001	0.557
Butyric acid	166	166	5.5	0.970	0.271	0.001	0.150
Propionic acid	253	271	14.8	0.423	0.002	0.001	0.039
Caproic acid	8	4	0.9	0.008	0.017	0.284	0.107
Valeric acid	29.2	32.1	2.0	0.331	0.284	0.010	0.623
Ratios							
Acetic:propionic	2.25	2.00	0.15	0.250	0.005	0.001	0.057
(Acetic + butyric):propionic	2.90	2.60	0.22	0.360	0.003	0.001	0.019

¹Sampling times were 0.5, 1.0, 1.5, 2.0, 4.0, 7.5, 8.0, 8.5, 9.0, and 11.0 h after morning feeding.

tive of SARA (as defined above) on 8 out of 10 sampling days, confirming that SARA is driven by accumulation of VFA, not lactic acid. The 2 highest lactic acid concentrations (32.1 mM, cow #254, *M. elsdenii*, and 34.4 mM, cow #203, placebo) were both recorded on d 4 of lactation at 0.5 h after the morning feeding time and were associated with rumen pH of 5.56 and 5.76 respectively. Despite this, detectable concentrations of lactic acid were more likely to be present on later sampling days, presumably because intakes, and thus availability of readily fermentable carbohydrate, were increasing as the study progressed. Ruminal lactic acid concentrations of 0 to 5 mM are considered normal, whereas concentrations of 50 to 120 mM are indicative of acute acidosis (Nagaraja and Titgemeyer, 2007). Only 16% of samples analyzed for each treatment had lactic acid concentrations above normal and none were close to the levels seen in acute acidosis. No treatment differences in terms of lactic acid removal were detected in the present study despite the sampling intensity at key points. Despite this, *M. elsdenii* had a generally positive effect both on mean rumen pH on specific days (Figure 1) and on total amount of time that rumen pH was below 5.6 throughout the study (treatment $\times$ day interaction, $P = 0.016$).

A nonsignificant ($P = 0.125$) 2.4 kg/d increase occurred in milk yield in cows dosed with *M. elsdenii* compared with those that received the placebo, but milk fat concentration was lower ($P = 0.067$) by 5.2 g/kg. Suboptimal rumen pH is often indicated in cases of milk fat depression (Nocek, 1997), but as time spent below pH 5.6 was generally less in the cows that received *M. elsdenii*, this is unlikely to be the cause of such a substantial reduction in the present study. The shift in rumen fermentation patterns from lipogenic acetate and butyrate toward glucogenic propionate in cows that received *M. elsdenii* probably contributed to the reduction in milk fat concentration. Furthermore, Palmonari et al. (2010) and Weimer et al. (2010), using molecular techniques to assess shifts in the composition of the rumen bacterial community, demonstrated an inverse relationship between milk fat concentration and the abundance of *M. elsdenii* in the rumen. These authors note that several strains of *M. elsdenii* have been shown to produce *trans*-10,*cis*-12 conjugated linoleic acid (Kim et al., 2002), which can be biohydrogenated in the rumen to produce C18 monounsaturated fatty acids that inhibit milk fat synthesis in the mammary gland (Bauman and Griinari, 2003). At present, it is not known whether *M. elsdenii* NCIMB 41125, the specific strain used in this study, behaves in a similar fashion.

Conversely, elevation of milk fat concentration in animals that received placebo rather than depression in those that received *M. elsdenii* should also be consid-

ered, because the mean milk fat concentration of 40.4 g/kg observed in the cows that received *M. elsdenii* cannot be regarded as excessively low for the Holstein breed. In a companion 14-wk production study (Aikman et al., 2009), high-yielding animals that received the *M. elsdenii* supplement tended to have lower milk fat concentrations, but they also tended to either lose less weight (when fed a standard dietary starch concentration) or gain more (when fed a high dietary starch concentration) compared with animals that received the placebo, indicating that less mobilization of body fat occurred in animals dosed with *M. elsdenii*. Animals with suboptimal rumen pH and in negative energy balance, and thus mobilizing body fat, can have elevated milk fat concentrations due to the higher concentrations of lipids circulating in the bloodstream (Stone, 1999). Body fat mobilization was not assessed in the present study, but it is possible that greater mobilization of body fat in animals that received the placebo contributed to milk fat concentrations above normal. Hagg et al. (2010) noted no difference in milk fat concentration between treatments when a diet containing 60% concentrate was fed but a decline in cows dosed with *M. elsdenii* when the diet contained 70% concentrate. A further South African study has observed an increase in milk fat concentration in cows dosed with *M. elsdenii* (P. H. Henning, unpublished data). It is clear that the effect of *M. elsdenii* supplementation on milk fat yield and composition is still not clearly understood and further work in this area would be useful.

The shift toward propionate production may also have contributed to the numerical reduction ($P = 0.113$) in DMI observed in cows on the *M. elsdenii* treatment. Allen et al. (2005) reviewed several studies in which propionate infusion in ruminants altered meal size and frequency by increasing satiety, resulting in a reduction in DMI. This reduction in DMI, accompanied by the nonsignificant increase in milk yield in cows that received *M. elsdenii*, led to an increase ($P = 0.038$) in milk production efficiency, as assessed by milk output per unit of DMI.

CONCLUSIONS

The diet fed to cows on this study challenged their ability to regulate rumen pH and, consequently, animals on both treatments experienced some degree of subacute ruminal acidosis. Lactic acid, the primary substrate for *M. elsdenii*, was only transiently present in the rumen but, despite this, evidence was found of a shift in rumen fermentation patterns in animals that received *M. elsdenii*. In these animals ruminal propionate production increased at the expense of acetate, thus reducing the acetate:propionate ratio, and this could

have a positive effect on energy balance and milk yield in early lactation dairy cows. Less day-to-day fluctuation occurred in the length of time that rumen pH was <5.6 in cows that received *M. elsdenii* compared with those that received the placebo, indicating that the probiotic exerted a stabilizing effect on rumen fermentation. Nonetheless, time spent with rumen pH <5.6 tended to be shortest in the days immediately after dosing with *M. elsdenii* before gradually increasing, suggesting that more frequent dosing with the probiotic in the critical period of diet adaptation and increasing feed intake after calving could be beneficial.

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