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Effects of a *Megasphaera elsdenii* oral drench on reticulorumen pH dynamics in lactating dairy cows under subacute ruminal acidosis challenge

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ABSTRACT

Subacute ruminal acidosis (SARA) is an important disorder in dairy cattle with economic and animal welfare implications. A preventive strategy for SARA control is oral drenching of probiotics, such as *Megasphaera elsdenii*. The aim of this study was to evaluate the effects of a *M. elsdenii* oral drench on reticulorumen pH, milk yield and components, and feeding behavior of lactating cows under a SARA challenge. This study consisted of two crossover trials with 8 cows each to determine the efficacy of drenching live culture of *M. elsdenii* NCIMB 41125. Experimental period of each trial lasted 8 d with a 4 wk washout interval between periods. The first 3 d of each period were considered baseline days. On day 4 of the period, feed delivered was reduced by half, based upon individual mean dry matter intake (DMI) during the baseline period. On day 5 of the period, all cows received a challenge diet rich in highly fermentable carbohydrates offered to the cows for 2 h to induce SARA. Orts were weighed and replaced with regular diet offered *ad libitum*. The last 3 d of each experimental period were considered recovery days. The difference between each trial was the administration time of *M. elsdenii* drench, where in Trial 1 it was delivered 4 d before (**PRO$_{-4}$**) and in Trial 2 it was delivered the day before (**PRO$_{-1}$**) the SARA challenge. Cows were randomly assigned to either a treatment (**PRO$_{-4}$** or **PRO$_{-1}$**) or control (**CON$_{-4}$** or **CON$_{-1}$**) drench, and reversed on the subsequent period. During Trial 1, **PRO$_{-4}$** cows produced more milk ($P < 0.01$), with greater protein percentage ($P = 0.03$) and lesser fat to protein ratio ($P = 0.01$) compared with control cows. **PRO$_{-4}$** cows also had greater overall reticulorumen pH and experienced shorter and less intense acidosis ($P < 0.05$) and greater DMI ($P = 0.04$) compared with control cows. Feeding behavior was not affected by treatment in Trial 1 ($P > 0.10$). In Trial 2, only feeding time was affected by the treatment, with **PRO$_{-1}$** cows spending more minutes per day feeding ($P < 0.01$) compared to control cows. The results indicate potential benefits of a *M. elsdenii* drench on rumen pH dynamics, acidosis resilience, and possibly milk production and feed intake. However, it appears that the time of drench administration might influence *M. elsdenii* establishment and drench efficacy.

1. Introduction

Subacute ruminal acidosis (SARA) is a disorder characterized by extended periods of ruminal pH below 5.6 (Garrett et al., 1999;

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Gozho et al., 2005; Plaizier et al., 2008). In grain-adapted cattle, normal rumen pH ranges from 5.5–6.5 (Nagaraja and Titgemeyer, 2007). However, cows fed diets rich in grains and highly fermentable carbohydrates have an increased risk of experiencing SARA (Krause and Oetzel, 2006). Around 19 % of early lactation and 26 % of mid-lactation dairy cows experience SARA (Garrett et al., 1997). The systemic impact of acidosis can have severe implications such as laminitis, which is highly associated with lameness (Nocek, 1997). Lameness is one of the most important causes of premature involuntary culling (Krause and Oetzel, 2006).

Megasphaera elsdenii is a gram-negative rumen microorganism known for metabolizing lactic acid and helping to stabilize ruminal pH (Counotte et al., 1981; Nocek, 1997; Nagaraja and Titgemeyer, 2007). It can ferment lactic acid to acetic and propionic acids (Hino et al., 1994). Some strains of *M. elsdenii* have been patented and are now used as acidosis control tools (Leedle et al., 1990; Horn et al., 2009). The prevention strategy consists of introducing lactate-utilizing bacteria into the rumen to increase existing populations of the bacteria until they are able to proliferate on their own. Klieve et al. (2003) demonstrated that the use of *M. elsdenii* allowed bacterial populations to establish 5–7 d earlier in inoculated animals when compared to non-inoculated animals. One study found that high yielding (> 10.000 kg milk/lactation) lactating dairy cows fed a high-starch diet and drenched with *M. elsdenii* had increased milk yield compared to non-drenched cows (Aikman et al., 2009), but this was not supported in a later study by Aikman et al. (2011), where early lactation cows drenched with *M. elsdenii* had similar yields to cows drenched with a placebo. However, in the study by Aikman et al. (2011), cows drenched with *M. elsdenii* spent less time with a rumen pH < 5.6 compared to cows drenched with a placebo.

Drenching cows with *M. elsdenii* may offer benefits to rumen health and production. However, there is a lack of evidence on how this tool improves rumen pH, and its effect on feeding behavior and milk production in cows under increased risk of SARA. The objective of this study was to evaluate the effects of a *M. elsdenii* oral drench on reticulorumen pH, milk yield, and milk components (fat and protein), as well as feed intake, feeding time and the number of feeder visits of lactating cows undergoing a ruminal acidosis challenge. We hypothesized that cows drenched with *M. elsdenii* would have a better ruminal environment, which could possibly increase the number of feeder visits and feeding time, consequently increasing DMI and performance.

2. Materials and methods

2.1. Animal housing and diet

The experiment was conducted at the University of Kentucky Coldstream Dairy Research Farm in Lexington, KY, between March and August 2017, under Institutional Animal Care and Use Committee protocol number 2017-2585. A total of 16 mid-lactation Holstein dairy cows (1.4 ± 0.9 lactations) averaging 670.0 ± 87.0 kg body weight and producing 42.1 ± 10.3 kg/d of milk were enrolled in this study.

The experimental pen comprised a compost bedded pack equipped with 8 automatic intake recording feeders (Insentec, Hokofarm Group, Marknesse, the Netherlands). These feeders precisely measure feed intake and behaviors such as number of visits to the feeder and time spent feeding as validated by Chapinal et al. (2007). We performed two cross-over trials with 8 cows each. Each cow had a reticulorumen bolus that recorded reticulorumen pH in 10-min intervals (iNVOTEC Animal Care, smaXtec Animal Care, Graz, Austria). The bolus measures 132 × 35 mm and has previously been validated in rumen-cannulated dairy cows (Klevenhusen et al., 2014). One week prior to the start of the experimental period, boluses were individually calibrated utilizing a pH 7.0 buffer solution and orally administered to the cows utilizing a bolus gun.

Cows were fed a TMR formulated following the National Research Council (NRC) guidelines (NRC, 2001) to meet or exceed the requirements of lactating dairy cows producing at least 39 kg of milk daily. Diet nutrient compositions for each trial are shown in Table 1. Cows were fed *ad libitum* twice per day at 0800 and 1430 h. Orts were removed once per day before the 1430 h feeding. Animals had *ad libitum* access to fresh water provided from a self-filling water trough located in the feeding alley. Milking occurred twice daily at 0700 and 1600 h. Daily milk yield was recorded using an automatic meter (AfiMilk, AfiMilk, Kibbutz Afikim, Israel). Milk fat and protein were measured twice daily using an in-line milk analyzer (AfiLab, AfiMilk, Kibbutz Afikim, Israel) validated by (Kaniyamattam and De Vries, 2014).

2.2. Experimental design

This study consisted of two crossover trials to determine the effectiveness of a drench containing a live culture of *M. elsdenii* NCIMB 41,125 (Lactipro Advance®, MS Biotec, Wamego, KS). The two trials were identical except for the administration time of the live culture of *M. elsdenii* which occurred at day 4 and day 1 prior to the acidosis challenge for trial 1 and 2, respectively. Each crossover consisted of two eight-day experimental periods separated by a 4 wk washout period. Each trial day was defined by the 24 h interval between the afternoon feedings. Therefore, study days started and ended at 1430 h. Cows were randomly assigned to one of two trial groups (Trial 1; n = 8; Trial 2; n = 8) and moved to the experimental pen 1 wk before the start of the study for habituation with the automatic feeders, pen mates, and the experimental pen. Each animal was assigned to one individual feeder using each cow's radio frequency identification (RFID) tag to operate and record the feed intake. Each animal ate from their assigned bin until the end of the experimental period.

The first 3 d of each experimental period (d_{-4}, d_{-3}, d_{-2}) were considered baseline days. On the fourth day of the experimental period (d_{-1}) cows had their feed allowance reduced by 50 %, based upon individual average dry matter intake during the baseline period. On the fifth day of the experimental period (d_0), cows received a challenge mix rich in highly fermentable carbohydrates to induce SARA. The challenge mix consisted of 2 kg of rolled barley, 2 kg of ground wheat and 0.9 kg of molasses that was combined

Table 1
Ingredients and chemical composition of the diets fed during Trial 1, Trial 2, and subacute acidosis challenge for cows (n = 8) drenched with distilled water (control) or *Megasphaera elsdenii* before an acidosis challenge.

Item	Trial 1	Trial 2	SARA challenge mix[1]
Ingredient (g/kg of DM)			
Grain mix[2]	463.0	471.0	
Corn silage	252.0	289.0	
Alfalfa silage	175.0	0.0	
Cottonseed	64.0	65.0	
Mineral mix[3]	24.0	17.0	
Alfalfa hay	22.0	158.0	
Chemical Composition (g/kg of DM)			
DM	569.4 ± 34.2	499.4 ± 35.3	699.4 ± 7.1
CP	147.0 ± 6.4	152.9 ± 4.9	131.4 ± 5.4
aNDF	314.1 ± 39.5	268.9 ± 17.9	184.1 ± 8.6
ADF	204.6 ± 34.4	171.1 ± 10.2	102.2 ± 7.5
Starch	270.2 ± 34.5	286.4 ± 22.0	418.8 ± 11.8
Ether Extract	36.4 ± 4.0	34.6 ± 5.3	34.2 ± 7.2
NFC	448.6 ± 32.4	475.7 ± 16.6	606.5 ± 6.3
Ash	74.5 ± 8.8	84.9 ± 3.5	59.9 ± 3.5
Ca	11.1 ± 2.2	11.0 ± 0.6	5.7 ± 0.4
P	6.2 ± 0.2	6.3 ± 0.4	5.0 ± 0.6

[1] The SARA challenge mix consisted of 2 kg of rolled barley, 2 kg of ground wheat and 0.9 kg, and 4.3 kg of TMR.
[2] The grain mix contained (%) ground corn (48.7), soft wheat middlings (14.5), dry corn gluten feed (11.7), SoyPlus (7.4), ground soybean hulls (5.5), soybean meal (4.9), calcium carbonate (2.3), molasses (1.7), sodium bicarbonate (1.6), white salt (0.8), magnesium oxide (0.7), urea (0.1), mineral mix[2] (0.1).
[3] The mineral supplement had the following composition: vitamin A (2,141,457 IU/kg), vitamin D3 (535,392 IU/kg), vitamin E (8,103 IU/kg), Zn (16,506 mg/kg), Mn (15,020 mg/kg), Cu (2720 mg/kg), I (351 mg/kg), Co (239 mg/kg), Se (106 mg/kg), Ca (109 g/kg), P (2 g/kg), Mg (1.8 g/kg), and Na (0.5 g/kg).

with 4.3 kg of TMR and offered to the animals for 2 h (Table 1). Orts were weighed and subsequently replaced with TMR offered *ad libitum*. The last 3 d of each experimental period (d_{+1}, d_{+2}, d_{+3}) were considered recovery days. Milk yield and components, dry matter intake (DMI) and feeding behavior (time spent feeding and number of visits to the feeder), and reticulorumen pH were recorded continuously during the entire experimental period. Total mixed ration samples were collected during the baseline days and analyzed. Samples for nutrient and dry matter (DM) analysis were oven dried at 55 °C for 48 h. Dried samples were ground to pass through a 1-mm screen and for analysis of acid detergent fiber (ADF) (AOAC International, 2000: method 973.18), neutral detergent fiber (NDF) with heat-stable α-amylase and sodium sulphite (Van Soest et al., 1991), and crude protein (CP) (N x 6.25; AOAC International 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI). Nutrient analyses of the offered feed are described in Table 1.

2.3. Trial 1

In Trial 1, six primiparous and two multiparous mid-lactation (238.5 ± 54.7 DIM; Mean ± SD) Holstein dairy cows producing 33.4 ± 3.4 kg of milk per day were used. Cows were randomly assigned to either a treatment (**PRO_{-4}**) or control (**CON_{-4}**) drench. At 1430 h of d_{-4}, **PRO_{-4}** cows received an oral 100 mL drench of *M. elsdenii* NCIMB 41,125 containing approximately 2 × 10^8 cfu/mL. At the same time, **CON_{-4}** cows were orally drenched with 100 mL of distilled water. At the end of the four-week washout period **PRO_{-4}** and **CON_{-4}** groups were crossed over and the experimental period started again. Cows that received **PRO_{-4}** during the first period, received **CON_{-4}** during the second period and *vice versa*. During the second period **PRO_{-4}** and **CON_{-4}** administration remained on d_{-4}.

2.4. Trial 2

In Trial 2, six primiparous and two multiparous mid-lactation (178.6 ± 35.9 DIM; Mean ± SD) Holstein dairy cows producing 50.9 ± 6.1 kg of milk per day were used. Cows were randomly assigned to either a treatment (**PRO_{-1}**) or control (**CON_{-1}**) drench. At 1430 h of d_{-1}, cows in the **PRO_{-1}** group received 100 mL of an oral drench of *M. elsdenii* containing approximately 2 × 10^8 cfu/mL. At the same time, **CON_{-1}** cows were orally drenched with 100 mL of distilled water. At the end of the washout period **PRO_{-1}** and **CON_{-1}** groups were crossed over and the experimental period started again. Cows that received **PRO_{-1}** during the first period, received **CON_{-1}** during the second period and *vice versa*. During the second period **PRO_{-1}** and **CON_{-1}** administration remained on d_{-1}.

2.5. Statistical analysis

All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). Before analysis, data were checked for normality using the UNIVARIATE procedure in SAS and probability distribution plots. No outliers were detected (data points that were beyond 3 standard deviations from the mean) and no transformations were deemed necessary. Reticulorumen pH, milk yield, milk fat and protein percentages, number of visits to the feeder, DMI, and time spent feeding were summarized by trial day using the MEANS procedure in SAS and expressed as daily means. Milk fat to protein ratio was obtained by dividing milk fat percentage by milk protein percentage and summarized by day. To measure SARA intensity, area under the curve (AUC) analyses were performed using two different reticulorumen pH thresholds, pH < 5.8 and pH < 5.6. Each observed pH value was subtracted from the thresholds and multiplied by the interval between pH readings. Area under the curve results were then summarized using the MEANS procedure and expressed as mean area under the curve below reticulorumen pH < 5.8 and pH < 5.6. Similarly, time spent below reticulorumen pH 5.6 or 5.8 was summarized by day and expressed as mean time below reticulorumen pH 5.6 or 5.8.

The effect of the *M. elsdenii* drench was determined by an analysis of variance (ANOVA) using mixed linear models (MIXED procedure) in SAS. The fixed effects in the model included treatment (**PRO** and **CON**), crossover sequence, challenge mix intake, milk yield and days in milk at experiment enrollment, lactation, and the interaction between treatment and study day (d_{-4} to d_3). Study day was specified as a repeated measure and cow as subject, using a compound-symmetry structure. Effects with a *p*-value above 0.30 were removed from the model using a stepwise backward elimination process starting with the least contributing effect. The final model for Trial 1 included treatment, crossover sequence, challenge mix intake, milk yield at experiment enrollment, lactation, and the interaction between treatment and study day. The final model for Trial 2 included treatment, challenge mix intake, milk yield at experiment enrollment, and the interaction between treatment and study day. Post-hoc comparison between treatments were carried out to determine differences between the treatments across experimental days utilizing the PDIFF option. Significance was declared at $P \leq 0.05$, and trends were defined as $0.05 < P \leq 0.10$.

3. Results

3.1. Reticulorumen pH

In Trial 1, **PRO$_{-4}$** and **CON$_{-4}$** drenches were administered 4 d before the acidosis challenge. Mean reticulorumen pH, area, and length of time below pH 5.8 and pH 5.6 are reported in Table 2. Briefly, when looking at the effect of the *M. elsdenii* drench during the whole experimental period, we found that treatment increased reticulorumen pH ($F_{1,7} = 28.47$; $P < 0.01$; Table 2), and reduced AUC ($F_{1,7} = 11.92$; $P = 0.01$; Table 2) and time below pH 5.8 ($F_{1,7} = 19.50$; $P < 0.01$; Table 2). Also, the treatment affected the

Table 2

Least square means (± SEM) of reticulorumen pH dynamics, DMI, feeding behavior, and milk yield and composition for dairy cows (n = 8) drenched with distilled water (control) or *Megasphaera elsdenii* 4 days (Trial 1) and 1 day (Trial 2) before an acidosis challenge.

Item	Treatment		SEM	*P-value*
	Control	*M. elsdenii*		
Megasphaera elsdenii 4 days before an acidosis challenge				
Reticulorumen pH Dynamics				
Mean reticulorumen pH average	6.11	6.23	0.05	< 0.01
Area below pH 5.8 (min × pH/d)	81.38	20.85	27.35	0.01
Area below pH 5.6 (min × pH/d)	37.31	6.77	13.89	0.03
Time below pH 5.8 (h/d)	4.68	1.82	1.42	< 0.01
Time below pH 5.6 (h/d)	2.65	0.64	0.88	0.01
DMI and Feeding Behavior				
DMI (kg/d)	21.43	23.50	1.45	0.04
Visits to the feeder (visits/d)	27.99	29.34	1.65	0.44
Time spent feeding (min/d)	191.65	200.62	27.72	0.59
Milk yield and Components				
Milk yield (kg/d)	26.07	28.75	1.62	0.01
Milk fat (%)	4.09	3.97	0.08	0.06
Milk protein (%)	3.19	3.28	0.15	0.02
Fat to protein ratio	1.30	1.22	0.06	0.01
Megasphaera elsdenii 1 day before an acidosis challenge				
Reticulorumen pH Dynamics				
Mean reticulorumen pH average	6.24	6.21	0.07	0.22
Area below pH 5.8 (min × pH/d)	21.54	36.26	11.60	0.22
Area below pH 5.6 (min × pH/d)	19.12	17.01	11.17	0.76
Time below pH 5.8 (h/d)	2.61	3.20	0.80	0.35
Time below pH 5.6 (h/d)	1.66	1.31	0.64	0.32
DMI and Feeding Behavior				
DMI (kg/d)	22.80	22.88	0.83	0.86
Visits to the feeder (visits/d)	37.42	35.98	4.86	0.67
Time spent feeding (min/d)	282.66	378.19	53.24	< 0.01
Milk yield and Components				
Milk yield (kg/d)	34.26	34.97	0.95	0.20
Milk fat (%)	3.78	3.82	0.04	0.37
Milk protein (%)	3.21	3.22	0.08	0.58
Fat to protein ratio	1.18	1.19	0.02	0.50

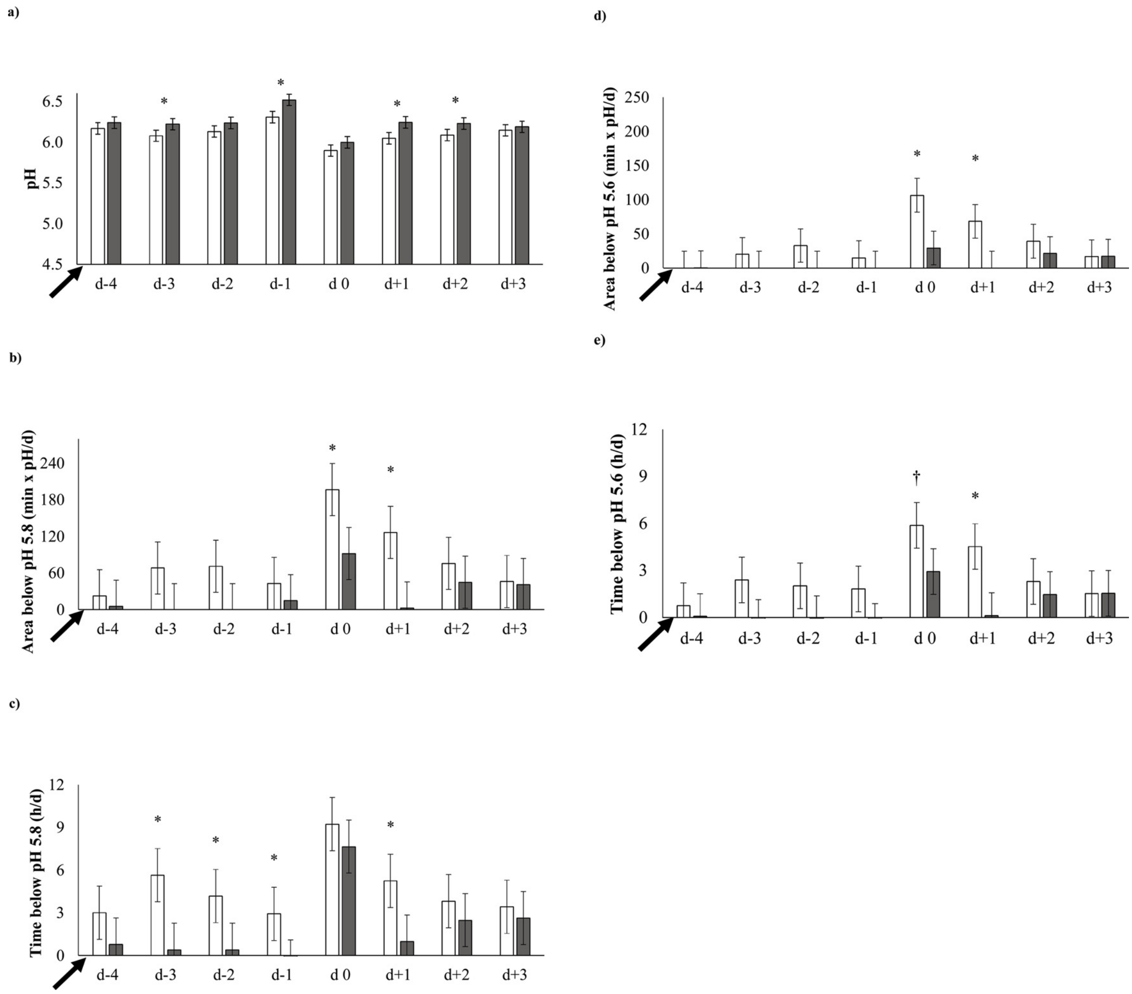

Fig. 1. Reticulorumen pH dynamics differences expressed as least square means ± SEM by experimental day relative to an acidosis challenge (d_0) in cows drenched with distilled water (white) or *Megasphaera elsdenii* (gray) 4 days before the acidosis challenge for.
a) reticulorumen pH average, **b)** area below pH 5.8, **c)** time below pH 5.8, **d)** area below pH 5.6, **e)** time below pH 5.6.
→ Indicates time of drenching.
* Indicates that treatments differed on that day ($P < 0.05$).
† Indicates that treatments tended to differ between treatments on that day ($P < 0.10$).

AUC ($F_{1,7} = 7.92$; $P = 0.03$; Table 2) and time ($F_{1,7} = 10.64$; $P = 0.01$; Table 2) below pH 5.6 in the reticulorumen. During Trial 1, *M. elsdenii* drench positively affected reticulorumen pH dynamics. Treatment cows had greater mean reticulorumen pH on d_{-3}, d_{-1}, d_{+1}, and d_{+2} ($t \geq -2.10$; $P \leq 0.04$; Fig. 1 – *a*). When the pH threshold was set as 5.8, **PRO$_{-4}$** cows had lesser area under the curve when compared to control cows on d_0 and d_{+1} ($t \geq 2.12$; $P \leq 0.04$; Fig. 1 - *b*). As expected, **CON$_{-4}$** cows spent more time below the pH 5.8 threshold on d_{-3}, d_{-2}, d_{-1}, and d_{+1} ($t \geq 2.01$; $P \leq 0.05$; Fig. 1 - *c*). When the pH threshold was set as 5.6, **CON$_{-4}$** cows had increased AUC d_0 and d_{+1} ($t \geq 2.28$; $P \leq 0.02$; Fig. 1 - *d*). In addition, **CON$_{-4}$** cows spent more time below the 5.6 pH threshold d_{+1} ($t = 2.53$; $P = 0.01$) and tended to spend more time under the threshold on d_0 ($t = 1.70$; $P = 0.09$; Fig. 1 - *e*).

In Trial 2 **PRO$_{-1}$** and **CON$_{-1}$** drenches were administered on the fourth day of the trial period, the same day the animals had their feed allowance reduced by 50 % and one day before the acidosis challenge day. However, reticulorumen pH was not affected by treatment ($F_{1,7} = 1.81$; $P \geq 0.22$; Table 2).

When analyzing the effect of the treatment by experimental day, we found that **PRO$_{-1}$** cows had lesser mean reticulorumen pH on d_{+1} ($t = 2.59$; $P = 0.01$; Fig. 2 - *a*). When the pH threshold was set as 5.8, **PRO$_{-1}$** cows tended to have increased AUC on d_{+2}, ($t = 1.83$; $P = 0.07$; Fig. 2 - *b*). No treatment by experimental day differences were found for time below the 5.8 threshold ($t \leq 1.36$; $P \geq 0.18$; Fig. 2 - *c*). Likewise, no treatment by day differences in AUC below pH 5.6 ($t \leq 1.63$; $P \geq 0.11$; Fig. 2 – *d*). However, **PRO$_{-1}$** cows tended to spend less time below pH 5.6 on d_{+1} ($t = 1.80$ $P = 0.08$; Fig. 2 – *e*) when compared to control.

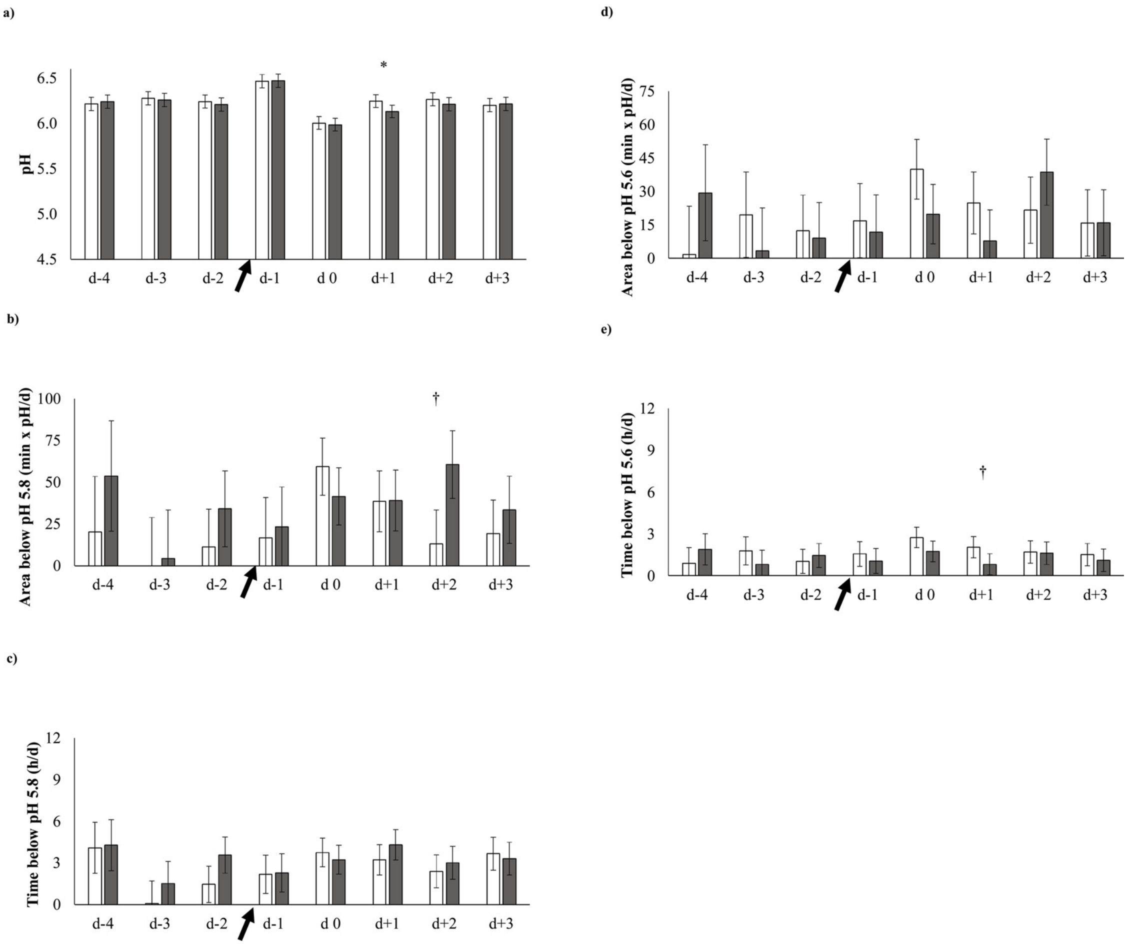

Fig. 2. Reticulorumen pH dynamics differences expressed as least square means ± SEM by day relative to an acidosis challenge (d_0) in cows drenched with distilled water (white) or *Megasphaera elsdenii* (gray) 1 day before the acidosis challenge for.
a) reticulorumen pH average, **b)** area below pH 5.8, **c)** time below pH 5.8, **d)** area below pH 5.6, **e)** time below pH 5.6.
→ Indicates time of drenching.
* Indicates that treatments differed on that day ($P < 0.05$).
† Indicates that treatments tended to differ between treatments on that day ($P < 0.10$).

3.2. DMI and feeding behavior

In Trial 1, treatment affected DMI ($F_{1,7} = 6.12$; $P = 0.04$; Table 2), but not feeding behavior ($F_{1,7} \leq 0.66$; $P \geq 0.44$; Table 2) throughout the experimental period. When analyzing the effect of the treatment by experimental day, there were no significant differences in the number of visits to the feeder (t ≤ 1.26; $P \geq 0.21$; *Supplementary* Fig. 1 - *a*) or time spent feeding (t ≤ 0.79;

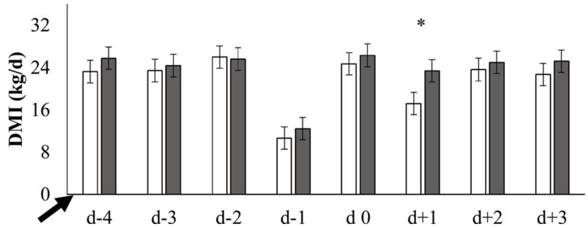

Fig. 3. Dry matter intake differences expressed as least Square means ± SEM by day relative to an acidosis challenge (d_0) in cows drenched with distilled water (white) or *Megasphaera elsdenii* (gray) 4 days before the acidosis challenge.
→ Indicates time of drenching.
* Indicates that treatments differed on that day ($P < 0.05$).
† Indicates that treatments tended to differ between treatments on that day ($P < 0.10$).

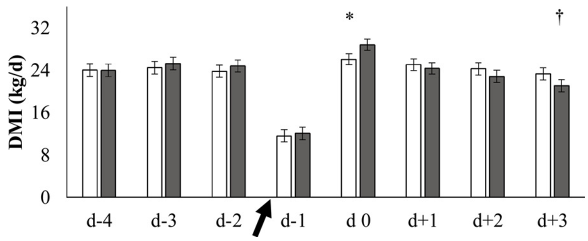

Fig. 4. Dry matter intake differences expressed as least Square means ± SEM by day relative to an acidosis challenge (d_0) in cows drenched with distilled water (white) or *Megasphaera elsdenii* (gray) 1 day before the acidosis challenge.
→ Indicates time of drenching.
* Indicates that treatments differed on that day ($P < 0.05$).
† Indicates that treatments tended to differ between treatments on that day ($P < 0.10$).

$P \geq 0.43$; *Supplementary* Fig. 1 -*b*) between **PRO-4** and **CON-4**. However, **PRO-4** cows had significantly greater DMI on d_{+1} (t = 2.61; $P = 0.01$; Fig. 3).

In Trial 2, DMI and number of visits to the feeder were not affected by treatment ($F_{1,7} \leq 0.19$; $P \geq 0.67$; Table 2). However, **PRO-1** cows spent more minutes per day feeding compared to control cows ($F_{1,7} = 13.25$; $P < 0.01$; Table 2). When looking at the effects of treatment by experimental day, **PRO-1** cows tended to visit the feeder less often on d_{-3}, and more often on d_0 (t ≥ 1.69; $P \leq 0.09$; *Supplementary* Fig. 2 - *a*). Also, **CON-1** cows spent less time feeding on d_{-4} and d_{-3} (t ≥ 2.51; $P \leq 0.01$; *Supplementary* Fig. 2 - *b*), and tended to spend less time feeding on d_{-2} (t = 1.91; $P = 0.06$; *Supplementary* Fig. 2 - *b*). Additionally, **PRO-1** cows had greater DMI d_0 (t = 2.86; $P < 0.01$; Fig. 4) and tended to have decreased intake on d_{+3} (t = 1.89; $P = 0.06$; Fig. 4)

3.3. Milk yield and components

In Trial 1, during the whole experimental period milk production was affected by treatment with **PRO-4** cows having greater daily milk yield compared to control cows ($F_{1,7} = 10.80$; $P = 0.01$; Table 2). The differences in reticulorumen pH dynamics and feed intake might have also influenced the milk components, where milk protein percentage was greater for **PRO-4** cows ($F_{1,7} = 8.08$; $P = 0.02$; Table 2) and **PRO-4** cows tended to have lesser milk fat percentage in comparison with **CON-4** cows ($F_{1,7} = 4.86$; $P = 0.06$; Table 2). Additionally, treatment influenced milk fat to protein ratio with **PRO-4** cows having a lesser milk fat to protein ratio compared to **CON-4** ($F_{1,7} = 11.37$; $P = 0.01$; Table 2).

When looking at the effects of treatment by experimental day, **PRO-4** cows tended to produce more milk on d_{-3} and d_{+2} (t ≤ 1.88; $P \leq 0.06$; *Supplementary* Fig. 3 - *a*). Additionally, **CON-4** tended to have greater milk fat percentage compared to **PRO-4** cows on d_{-4} (t = 1.76; $P = 0.08$; *Supplementary* Fig. 3 - *b*), and had greater milk fat on d_{-1}, (t = 1.95; $P = 0.05$; *Supplementary* Fig. 3 - *b*). Milk protein percentage tended to be greater for **PRO-4** cows on d_{-3}, d_{-1}, and d_{+2} (t ≤ 1.72; $P \leq 0.09$; *Supplementary* Fig. 3 - *c*). Consequently, **CON-4** cows had greater fat to protein ratio on d_{-1} (t = 2.40; $P = 0.02$; *Supplementary* Fig. 3 - *d*) and tended to have greater ratio on d_{+2} (t = 1.85; $P = 0.07$; *Supplementary* Fig. 3 - *d*).

In Trial 2, when looking at the whole experimental period, milk production and components were not affected by treatment ($F_{1,7} \leq 2.02$; $P \geq 0.20$; Table 2). When looking at the effect of the treatment by experimental day, **PRO-1** cows produced more milk on d_0 (t = 2.31; $P = 0.02$; *Supplementary Fig. 4 - a*) and tended to produce more milk on d_{-3}, (t = 1.66; $P = 0.10$ *Supplementary* Fig. 4 - *a*). Milk fat percentage was greater in **CON-1** cows on d_0 (t = 2.01; $P = 0.05$; *Supplementary* Fig. 4 - *b*). On the other hand, **PRO-1** cows had greater milk protein percentage on d_0 (t = 2.54; $P = 0.01$; *Supplementary* Fig. 4 - *c*). Consequently, **CON-1** cows had greater fat to protein ratio on d_0 (t = 3.05; $P < 0.01$; *Supplementary* Fig. 4- *d*).

4. Discussion

In this study, we tested the efficacy of an oral drench containing *M. elsdenii* in lactating cows under SARA risk. This study builds upon previous works showing benefits of utilizing a *M. elsdenii* oral drench on ruminal environment (Aikman et al., 2011), feed intake (Drouillard, 2004), and milk production (Aikman et al., 2011). However, the effects of drenching cows with *M. elsdenii* on feeding behavior of lactating dairy cows have not been previously investigated.

We found that drenching cows with *M. elsdenii* 4 d before an acidosis challenge improved reticulorumen pH throughout the experimental period. However, no improvement was seen when cows were drenched 1 d before an acidosis challenge. In Trial 1, cows that were drenched with *M. elsdenii* had greater mean daily reticulorumen pH following the acidosis challenge. In addition, **CON-4** cows spent almost 3 h/d with reticulorumen pH below 5.6 while **PRO-4** cows spent close to 1 h below the same threshold. These results indicate that **CON-4** cows were close to having SARA throughout the study period, given that Plaizier et al. (2008) defined SARA as when rumen pH stays below 5.6 for more than 3 h/d. A reduced AUC means that the pH drop experienced by treatment cows were less severe compared to control cows. Similarly, Henning et al. (2010) found that lambs drenched with *M. elsdenii* suffered fewer pH drops compared to control lambs. Aikman et al. (2011) reported that AUC for pH 5.6 or 6.0 was lesser in cows drenched with *M. elsdenii*, although treatment was not found to be statistically significant.

The lack of long lasting effects of *M. elsdenii* drenching in **PRO-1** cows may be due to the short time period between drenching and the acidosis challenge. Because the drench was administered one day before the acidosis challenge and the same day that cows had their feed allowance reduced in half, we hypothesize that there was not enough time and substrate to allow the introduced *M. elsdenii* to become established in the rumen. In one of the experiments reported by Weimer et al. (2015), the author hypothesizes that ruminal conditions might be suboptimal for development of the bacteria when dosing cows before feeding. In that same study, *M. elsdenii* populations returned to very low baseline levels within 24 h of dosing when the drench was administered before feeding. Henning et al. (2010) reported that viable *M. elsdenii* populations can be established in 4–5 d, and that drenched cows tended to have greater *M. elsdenii* populations on the first 2 d after dosing. However, it appears that the time of drench administration might influence *M. elsdenii* establishment and drench efficacy, which was not tested in this study and should be further investigated.

As rumen pH increased, treatment influenced feed intake as **PRO$_{-1}$** cows had a higher DMI over the experimental period. To our knowledge, the present study is the first to look at feeding behavior in lactating cows drenched with *M. elsdenii.* Our results agree with previous studies in lambs and beef cattle; for example Henning et al. (2010) reported that lambs and steers drenched with *M. elsdenii* showed increased feed intake compared to non-drenched individuals. Also, Drouillard (2004) reported that feedlot cattle tended to maintain greater and more consistent feed intake after being drenched with *M. elsdenii*. However, we cannot conclude that feeding behavior (number of visits to the feeder and feeding time), was affected by drenching the cows with a live culture of *M. elsdenii*, as the only significant differences between treatments were found in Trial 2 and occurred before the day treatment was administered to the animals. Therefore, future research should investigate other behavioral impacts of *M. elsdenii* drench in dairy cattle.

Our results agree with previous studies; for example daily milk yield increase was observed in high yielding lactating dairy cows fed a high-starch diet drenched with *M. elsdenii* (Aikman et al., 2009). Additionally, Aikman et al. (2011) observed a 2.4 kg/d numerical, but not statistically significant, increase in milk yield in cows drenched with *M. elsdenii.* Moreover, Henning et al. (2010) reported that lambs and steers drenched with *M. elsdenii* had increased feed intake and average daily gains compared to control animals. With the increased milk protein and decreased milk fat, it was expected that treatment cows would have decreased fat to protein ratio. This is in contrast to findings by Aikman et al. (2008) that showed cows receiving *M. elsdenii* had decreased milk protein percentages. On the other hand, the same authors reported a tendency for cows drenched with *M. elsdenii* to have reduced milk fat content. Some authors have associated the increase of ruminal *M. elsdenii* to decrease in milk fat concentration (*e.g.* Palmonari et al., 2010; Weimer et al., 2015) because some strains of *M. elsdenii* produce *trans*-10,*cis*-12, a conjugated linoleic acid capable of inhibiting milk fat synthesis in the mammary gland (Kim et al., 2002; Bauman and Griinari, 2003).

The *M. elsdenii* drench has the potential to be used as a strategic management tool to minimize ruminal acidosis and improve ruminal resiliency during the transition period or other nutritional challenging events in the dairy cow life. Drastic dietary changes between the dry period and parturition can increase the risk of ruminal acidosis (Nocek, 1997). Additionally, with the recent availability and adoption of in-line milk analyzers, farmers can utilize milk yield and components data to identify cows at risk of acidosis and possibly make management decisions (Rotz et al., 2003), such as utilizing a *M. elsdenii* drench. Future research should focus on the strategic uses of the drench and investigate how the establishment of the *M. elsdenii* population relates to rumen pH and health.

5. Conclusions

The results from this experiment indicate that there might be benefits of utilizing a *M. elsdenii* drench in lactating dairy cows. We found that the drench positively influenced reticulorumen pH dynamics, and increased feed intake and acidosis resilience, which consequently could have affected milk production and components. There was not sufficient evidence to indicate that drenching cows with *M. elsdenii* affects feeding behavior. Importantly, we found that the timing of drench administration may be an important factor for drench effectiveness and future research should investigate the dynamics of *M. elsdenii* population establishment relationship with rumen pH and health.

CRediT authorship contribution statement

G. Mazon: Investigation, Formal analysis, Writing - original draft. **M.R. Campler:** Data curation, Formal analysis, Writing - review & editing. **C. Holcomb:** Investigation, Resources. **J.M. Bewley:** Project administration, Conceptualization, Methodology, Funding acquisition, Writing - review & editing. **J.H.C. Costa:** Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

We confirm that there are no any conflicts of interests among authors and between authors and other people, institutions or organizations.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anifeedsci.2020.114404.

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