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Effect of ruminal administration of the lactate-utilizing strain *Megasphaera elsdenii* (*Me*) NCIMB 41125 on abrupt or gradual transition from forage to concentrate diets

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ABSTRACT

The objective in Trial 1 was to study feed intake and ruminal acidosis when the diet is rapidly transitioned from forage to concentrate and *Megasphaera elsdenii* (*Me*) NCIMB 41125 (strain CH4) is administered into the rumen. The objective in Trial 2 in addition was to investigate whether drenched CH4 can be measured in the rumen and will promote a viable lactate-utilizing population during transition. In Trial 1, 24 rumen-cannulated lambs were used and in Trial 2, 12 rumen-cannulated steers. The lambs were randomly allocated to one of four treatments in a 2 × 2 factorial design: drenched with either CH4 or a placebo, and fed forage *ad lib.* or restricted to 200 g/day. On day 1 of the trial, the lambs in addition to the forage were fed a concentrate mixture at 09:00 and 15:00, and dosed 10^{11} cfus at 12:00. Intake and lamb weight were measured for 50 days until slaughter to obtain dressing %. Rumen pH and lactic acid concentration were measured between days −1 and +14. In Trial 2, four treatments of three steers each received one of four dosages intra-ruminally: a placebo (Control), 10^9 cfus (low), 10^{10} cfus (medium) and 10^{11} cfus (high). The transition from forage to concentrate was in five steps, *i.e.* more gradual than in the lamb trial. Feed intake and weight gain were measured for 37 days. Rumen fluid was sampled for determination of pH, lactic acid concentration, VFAs and qrt-PCR analysis for *Me* presence between days −4 and +35. Lambs drenched with strain CH4 consumed more concentrate than Control ($P<0.006$), but less forage ($P<0.049$). Total feed intake was also higher and it fluctuated less ($P<0.023$). Lamb ADG and dressing % did not differ. Rumen pH declined less in CH4 than Control lambs ($P<0.001$) and lactic acid accumulated less ($P<0.001$) during days +2 and +3. In Trial 2, feed intake and ADG were higher in CH4 treatments than Control. The low, medium and high CH4 treatments did not differ in any parameter. qrt-PCR results reflect higher *Me* concentrations during days +2 and +3 ($P<0.06$) in CH4 treatments than in Control, coinciding with lower

Abbreviations: ADG, average daily gain; cfus, colony forming units; CV, coefficient of variation; DM, dry matter; DNA, deoxyribose nucleic acid; *Me*, *Megasphaera elsdenii*; MSE, mean standard error; ND, not detectable; NDF, neutral detergent fiber; qrt-PCR, quantitative real time polymerase chain reaction; SARA, sub-acute ruminal acidosis; SD, standard deviation; VFAs, volatile fatty acids.

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lactic acid concentrations (P<0.13). Rumen pH in Control approached pH = 5 between days +9 and +17, whereas pH in the CH4 treatments remained above 5.5. Total VFAs were similar in all treatments, but the proportional distribution shifted towards butyric acid in the CH4 treatments. It is concluded that strain CH4 should control ruminal acidosis during transition from forage to concentrate.

1. Introduction

In feedlots sheep and cattle are required to adapt quickly to high concentrate (starch) diets to support viable economic returns. During this transition phase acute or sub-clinical ruminal (lactic acid) acidosis can develop (Owens et al., 1998), primarily because the high level of readily fermentable carbohydrates increases the amount of lactate in the rumen (Dawson and Allison, 1988). This occurs because the population of lactate producers markedly exceeds the population of lactate utilizers (Slyter, 1976) and also grow faster (Oetzel, 2003). In theory, therefore, if the number of lactate utilizers can be increased the accumulation of lactate may be prevented (Nisbet and Martin, 1994; Kung and Hession, 1995) and the disorder prevented or alleviated. This has to be done by exotic introduction into the rumen (Klieve et al., 2003) which, amongst others, has lead to the recent advancement in direct-fed microbial (DFM) research, however, with limited success to reducing the risk of ruminal acidosis (Ghorbani et al., 2002).

Recently Horn et al. (2009) reported on bacterial isolates (strains) selected through stringent pH-auxostat criteria, some of them meeting the requirements to possibly prevent ruminal acidosis. Two of these with the most promising attributes were subsequently tested in batch culture and *in vivo*, using extreme lactate producing substrates of maltose and maize meal (Henning et al., 2010). The results were promising as the isolates effectively prevented both lactic acid accumulation and pH decline. However, since these substrates were rather unusual the present study was designed to simulate abrupt and gradual feed transitions of diets as would be found in commercial sheep and cattle feedlots. The bacterial strain tested was *Megasphaera elsdenii* (*Me*) NCIMB 41125, also known as CH4, and which is the term used in this presentation.

The objective of study 1 was to study feed intake trends and acidosis indicators in sheep required to change rapidly from a forage to a concentrate diet, if drenched or not with strain CH4.

The objective of study 2 was to establish whether dosing cattle at the outset of the transition phase with strain CH4 will show its presence in the rumen post-drenching, secondly, will enhance the presence of a viable lactate-utilizing population and if so, what should be the dose level. In addition, similar to Trial 1, intake patterns and critical ruminal indicators were studied.

2. Materials and methods

2.1. Trial 1

Animal ethics approval was obtained from the Agricultural Research Council Animal Ethics Committee for both Trial 1 and Trial 2.

Twenty-four South African Mutton Merino wether lambs (29.4 kg mean live weight, SD = 3.3 kg) were fitted with rumen cannulae (70 mm inner diameter), confined in individual pens and fed chopped *Eragrostis teff* hay *ad lib.*, supplemented with a commercial protein–mineral mix. The lambs were blocked by weight after 21 days on the forage diet and randomly allocated to four treatments of six animals each in a 2×2 factorial design. The treatments were *ad lib. vs* restricted forage intake and with or without strain CH4.

On the first day of the trial (day 1) all lambs received a commercial maize-based concentrate mixture *ad lib.*, fed twice daily in equal amounts at 09:00 and 15:00. The composition of the mixture is shown in Table 1.

Table 1
Composition of the concentrate mixture fed *ad lib.* to the lambs in Trial 1.

	g/kg as fed
Maize (whole kernel)	889
Slaked lime ($CaCO_3$)	11
'Kalori 3000' (molasses product)	69
Trace minerals[a]	0.6
Urea	16.6
Ammonium sulphate	3.9
Dicalcium phosphate	5.6
Sodium chloride	4.3

[a] *Composition:* iron, 55 g; zinc, 55 g; cobalt, 1.1 g; magnesium, 500 g; manganese, 33 g; iodine, 1.1 g; selenium, 0.11 g.

In addition to the concentrate mixture, two treatments were fed the forage diet *ad lib.* whereas the other two treatments were fed a restricted amount of 200 g (as fed basis) of the forage diet per animal per day. On days 1 and 2 of concentrate feeding, each lamb in one treatment from forage *ad lib.* and one treatment from forage restricted was administered intra-ruminal a dose of strain CH4 (10^{11} colony forming units [cfus]) in 100 ml/animal, which corresponds to *ca* 10^6 cfus/ml in the rumen) at 12:00, *i.e.* 3 h post-feeding. Their counterparts in the other two treatments were similarly drenched, but with ordinary tap water (100 ml/animal).

Concentrate and forage intake and incidences of diarrhoea (visual appraisal only) were monitored daily for the duration of the trial (50 days), *i.e.* until the lambs were slaughtered. The animals were weighed fortnightly and rumen samples for pH and lactic acid determination were taken each day at 08:00, 12:00, 15:00 and 19:00 on days −1, +1, +2, +3, +7 and +14 of concentrate feeding. The lambs were slaughtered at a commercial abattoir within 24 h after arrival. During this period they received water, hay and a mineral supplement. The cold carcass weight was measured to calculate dressing %.

Feed refusals were collected daily and oven-dried to calculate dry matter (DM) intake. To ensure representative sampling, six grab samples of digesta were obtained from different positions in the cranial and ventral sacs of the rumen. These were pooled and squeezed through cheese cloth into a glass beaker, mixed, the pH determined and a sub-sample taken for lactic acid analysis. The remaining material was returned to the rumen.

Strain CH4 cells were harvested from a continuous culture system (Horn et al., 2009) and the concentrations verified prior to drenching. Concentrations administered were controlled by enumeration by means of the plate count method on semi-defined lactate medium. Plate counts were performed under strict anaerobic conditions within a Forma anaerobic chamber containing an atmosphere of 5% H_2, 30% CO_2 and the balance N_2. Incubation was overnight at 39 °C for 48 h.

2.2. Trial 2

Fourteen Bonsmara steers of average live weight 192 kg (SD = 2.7 kg) were fitted with 81 mm inner diameter rumen cannulae. They were allowed to recuperate for 14 days, individually housed in stanchions and fed a combination of grass and lucerne hay only. Thereafter they were weighed, 12 were blocked by weight and allocated to one of four treatments: (1) Control, not drenched with strain CH4, (2) drenched with a low dose of strain CH4 (1.72×10^9 cfus/dose), (3) drenched with a medium dose of strain CH4 (1.72×10^{10} cfus/dose) and (4) drenched with a high dose of strain CH4 (1.72×10^{11} cfus/dose). These were contained in 1 l strained rumen fluid with the respective concentrations. The dosage levels translated in the rumen to respectively 4×10^4, 4×10^5 and 4×10^6 cfus/ml. In the case of the Control 1 l sterile strained rumen fluid was administered.

One day before the onset of the trial the steers were fasted for one day, where after the trial period of 37 days commenced. On day 1 of the trial they received the first composition of the concentrate diet and on day 2 the respective treatments were administered through the rumen cannula 3 h after feed was offered at 08:00. The transition consisted of a five step up program (Table 2) with increasing increments of concentrate and reducing increments of lucerne hay. Each step was fed for 4 days except step 5 which was fed for the remainder of the trial period.

Strain CH4 cells were harvested, controlled and verified in the same way as in Trial 1.

Rumen fluid was sampled once 3 h after feeding on days −4, −2, +2, +3, +5, +6, +7, +9, +12, +15, +21, +28 and +35 for determination of pH, volatile fatty acids (VFAs) and lactic acid, in addition to quantitative bacterial counts of strain CH4 by real time polymerase chain reaction (qrt-PCR). Strained rumen fluid samples were immediately frozen and stored at −20 °C until the respective analyses.

2.3. Analytical procedures

DL-Lactic acid and VFAs were determined by gas chromatography with a Carlo Erba GC4200 gas chromatograph with flame ionization detector and a Tupelo 1-1825 column (Supelco Inc., Bellefonte, PA, USA). The qrt-PCR analysis entailed: genomic DNA was isolated from pure cultures of strain CH4 and the 16S rDNA amplified and sequenced (Inqaba Biotec, Pretoria, South Africa). The sequence information was used to design an appropriate primer and probe set (TIB Molbiol Syntheselabor GmbH, Berlin, Germany), which was used to determine strain CH4 in rumen samples by qrt-PCR using the *Light Cycler Fast Start DNA MasterPlus Hybridization* kit of Roche Diagnostics (Roche Products (Pty) Ltd., Randburg, South Africa). DNA was extracted from rumen samples using the *MagNA Pure LC* method of Roche Diagnostics.

It could not be established whether the qrt-PCR analysis solely picked up strain CH4, or whether *Me* DNA with very similar genomic sequence is also included. Therefore, the results are presented as *Me* concentration rather than strain CH4 concentration.

Feed dry matter was determined by drying in a forced air oven (Labcon, Midrand, South Africa) at 105 °C for 18 h. Nitrogen was determined by the Kjeldahl method (AOAC 920.40, 1984).

Crude fat (ether extract) was analyzed by the Soxtec method (AOAC official method 2003.05).

Starch was analysed using the official method of the AOAC (AOAC 988.05, 1984) and neutral detergent fiber (NDF) was analysed by the Van Soest method, using alpha amylase (Van Soest et al., 1991).

2.4. Statistical analysis

Statistical analysis to separate treatment means was by ANOVA (Genstat, 2000) in Trial 1, with level of forage intake and +CH4 or −CH4 as variables. The test also accommodated the interaction between level of forage intake and +CH4 or −CH4. Least significant difference was accepted at the 5% level of probability. Parameters tested were DM intake, average daily gain (ADG) and dressing %. In the case of rumen pH and lactic acid concentration, the MIXED procedure for repeated measures of SAS was used with treatment and time as variables.

In trial 2 feed intake, VFAs, lactic acid, pH and *Me* concentrations were analyzed as repeated measures using the MIXED procedure of SAS (Genstat, 2000). The model statement included the effects of treatment and time of measurement. In most instances the strain CH4 treatment means did not differ from each other and were therefore considered together in comparison with Control means. Means were separated using Fishers' protected *t*-test with the least significant difference being accepted at P<0.05.

3. Results

3.1. Trial 1

Voluntary intake by the lambs of concentrate and forage at various stages of the trial is shown in Table 3.

Mean forage intake for the period prior to concentrate feeding was 840 g per lamb.

On the first day of being offered concentrate all lambs over-indulged (Table 3). From day +2 to +11, the period which simulates the unstable transient rumen, lambs drenched with strain CH4 consumed 46% (P<0.001) more concentrate than Control; a result which was maintained until slaughter with strain CH4 lambs consuming 11% (P<0.006) more concentrate than Control lambs. In contrast, forage intake was less for strain CH4 lambs than for Control in both measured periods (P<0.017 and P<0.049 respectively). Nevertheless, total intake was 29% higher for strain CH4 than for Control between days +2 and +11 and 6% from day 1 to slaughter.

Strain CH4 lambs visibly showed less signs of diarrhoea than Control lambs during the first week of concentrate feeding, which coincided with less variation in concentrate intake. From day +2 to +11 the coefficient of variation (CV) was 52% for strain CH4 lambs and 68% for Control (P<0.018), a result which was also carried through to slaughter, although smaller, respectively 40% and 47% (P<0.023).

Figs. 1 and 2 respectively display the changes in rumen pH and lactic acid concentration between days −1 and +14.

Rumen pH in strain CH4 lambs decreased less than in Control lambs (P<0.001) and correspondingly much less lactic acid (P<0.001) accumulated during days +2 and +3, where after the values became similar (Figs. 1 and 2).

Table 2
Dietary composition and chemical analysis results of the five step feeding program in Trial 2.

Diet ingredient	Composition, g/kg as fed				
	Step 1	Step 2	Step 3	Step 4	Step 5
Lucerne	450	350	250	150	60
Steam flaked maize	413	506	601	695	760
Cotton seed oilcake	30	30	30	30	40
Wheaten bran	100	100	100	100	110
NaCl	5	5	5	5	5
Feed lime ($CaCO_3$)	–	4	8	12.1	16
Urea	–	2	3.9	5.9	6.9
Mineral–vitamin premix[a]	2.5	2.5	2.5	2.5	2.5
Analysis	Composition, g/kg DM				
	Step 1	Step 2	Step 3	Step 4	Step 5
DM[b]	907	897	895	894	898
Crude fat	25	24	26	28	29
Nitrogen	2.6	2.2	2.2	2.0	2.0
Starch	320	420	428	498	546
NDF[c]	327	277	260	216	189
ME[d,e] (MJ/kg)	11.2	11.8	12.3	12.9	13.3
Calcium[e]	7.7	7.7	7.7	7.7	7.8
Phosphorus[e]	4.2	4.2	4.2	4.2	4.4

[a] *Composition:* Vit. A, 6×10^6 IU; Vit. B1, 3 g; antioxidant, 3.5 g; iron, 30 g; copper, 12 g; zinc, 50 g; cobalt, 1 g; magnesium, 200 g; manganese, 40 g; iodine, 1 g; selenium at 0.1 g and monensin at 22 mg.
[b] DM, dry matter.
[c] NDF, neutral detergent fiber.
[d] ME, metabolizable energy.
[e] Calculated from tabulated values.

Table 3
Intake by lambs of concentrate and forage in Trial 1.

	g/day, as fed						
	Forage level		Drench		P-value		
	Restricted	*Ad lib.*	Control	*Me* strain[a]	Forage	Drench	$F \times D$
Concentrate							
Day 1	2103	2089	2095	2097	0.909	0.992	0.959
Days 2–11	774	676	589	861	0.159	<0.001	0.064
Days 1–slaughter	1136	1081	1053	1164	0.142	0.006	0.120
Forage							
Days 2–11	164	178	192	150	0.377	0.017	0.967
Days 1–slaughter	139	231	203	168	<0.001	0.049	0.308

[a] *Me* strain, *Megaspheara elsdenii* strain.

There were no differences at any stage in ADG and dressing % between lambs on treatments fed restricted forage or *ad lib.*, or drenched with or without strain CH4, and therefore the results are not displayed.

3.2. Trial 2

Tables 4 and 5 provide results of counts of *Me* by qrt-PCR and ruminal lactic acid concentrations respectively.

Individual variation in both qrt-PCR counts and lactic acid analyses were substantial as can be deducted from the MSE's. The results nevertheless suggest that qrt-PCR counts for strain CH4 treatments at days 2 and 3 post-drenching were higher than for Control (P<0.06), where after endemic *Me* strains apparently caught up (Table 4). The higher counts of strain CH4 treatments coincided with lower lactic acid concentrations at days +2 and +3 in comparison to Control (Table 5). There were no differences between the three CH4 drench treatments in both qrt-PCR counts and lactic acid concentrations at any stage, and the results are therefore not shown.

Rumen pH for the Control and strain CH4 treatments is displayed in Fig. 3.

Rumen pH results fluctuated considerably due to individual variation but nevertheless indicate that Control primarily experienced lower pH than the three strain CH4 treatments. Control pH levels approached pH = 5 between days 9 and 17 post-drenching, whereas those of the three strain CH4 treatments averaged just above pH = 5.5 (Fig. 4). Again, the differences between the three strain CH4 treatments were not detectable.

Table 6 shows the results of rumen VFA concentration for Control and the means of the three strain CH4 treatments. The CH4 treatments did not differ and were consequently pooled for this display.

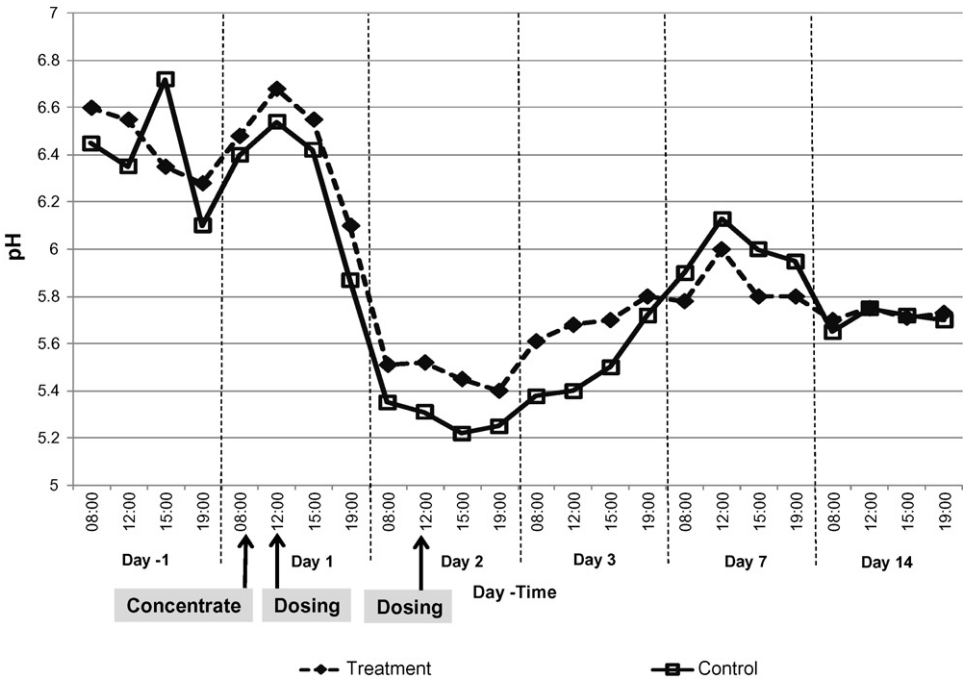

Fig. 1. Rumen pH in sheep dosed with CH4 (treatment) or water (Control) in Trial 1.

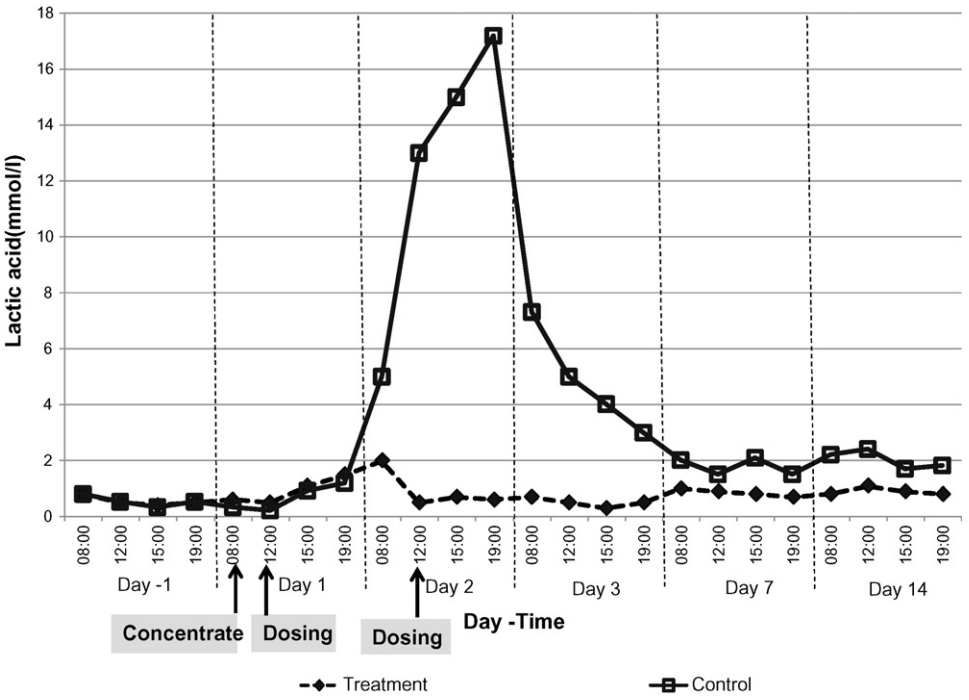

Fig. 2. Rumen lactic acid concentration in sheep dosed with CH4 (treatment) or water (Control) in Trial 1.

Table 4
qrt-PCR counts (log_{10}[Me]) for the Control and strain CH4 treatments in Trial 2.

Day	Control	Low	Medium	High	P-value[a]
−4	ND[b]	ND	ND	ND	–
−2	ND	ND	ND	ND	–
+2	1.33	3.31	3.87	4.65	0.06
+3	1.48	2.87	3.32	4.46	0.06
+5	4.66	3.93	5.12	5.11	0.93
+6	3.12	3.26	4.75	4.31	0.27
+7	4.95	4.42	4.71	4.24	0.17
+9	4.91	4.67	4.93	4.84	0.88
+12	5.55	5.37	4.40	5.61	0.59
+15	5.48	3.05	5.51	3.31	0.38
+21	4.54	3.69	4.81	2.72	0.45
+28	4.27	3.66	4.71	4.38	0.98
+35	3.96	3.51	4.76	3.20	0.92

MSE = 0.76.
[a] P-value between Control and strain CH4 treatments only.
[b] ND, not detectable.

Table 5
DL-Lactic acid concentrations (mmol/l) for the Control and strain CH4 treatments in Trial 2.

Day	Control	Low	Medium	High	P-value[a]
−4	0.39	0.32	0.48	0.40	0.89
−2	0.31	0.50	0.25	0.35	0.64
+2	20.8	0.28	0.42	0.33	0.13
+3	10.1	0.45	0.42	0.47	0.14
+5	0.90	0.45	0.59	0.65	0.26
+6	0.52	0.73	0.80	0.37	0.62
+7	0.83	0.65	0.64	0.29	0.26
+9	1.35	1.29	0.73	0.43	0.50
+12	1.28	0.90	0.88	4.62	0.59
+15	1.18	1.32	2.48	0.71	0.77
+21	1.79	1.33	1.58	1.59	0.52
+28	4.67	1.14	1.30	2.55	0.26
+35	1.36	1.25	1.19	1.02	0.38

MSE = 1.66.
[a] P-value between Control and strain CH4 treatments only.

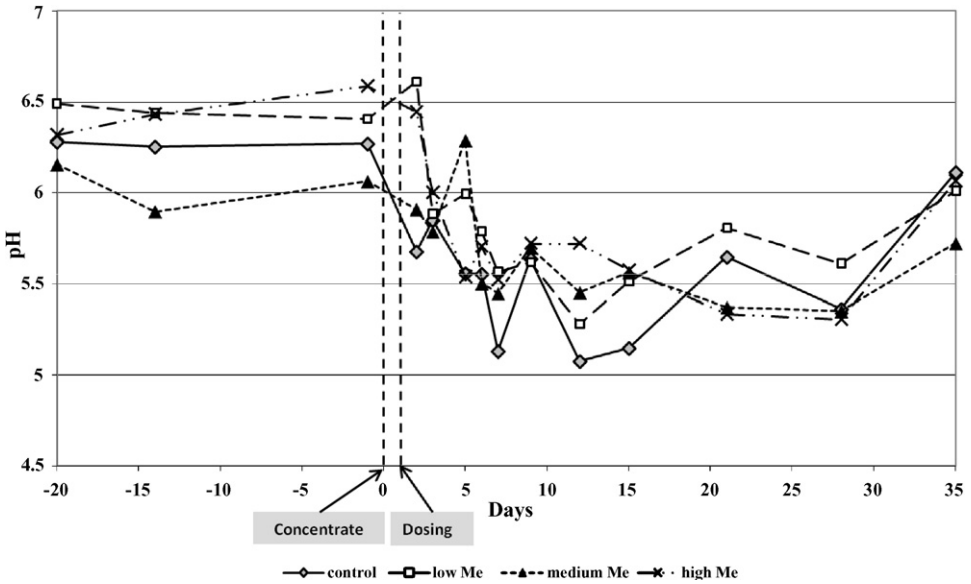

Fig. 3. Trends of rumen pH for the Control and low, medium and high strain CH4 treatments in Trial 2.

Total VFAs did not differ between Control and strain CH4 treatments, and among strain CH4 treatments. The mean values were respectively 80.4, 81.5, 96.1 and 77.1 mmol/l for the Control, low, medium and high strain CH4 treatments, with the overall MSE 27.6 mmol/l. VFA concentrations reflected the typical decline in acetic acid as concentrate feeding increased and a corresponding increase in propionic and butyric (*n* + iso) acids (Table 6). There were differences (P<0.05) in concentrations of individual VFAs on particular days: on day 3 (step1) acetic acid was lower in Control compared to strain CH4 treatments (P<0.046) and propionic acid correspondingly higher (P<0.002). A similar result was evident on day 9 (step 3) with acetic acid differing at P<0.017 and propionic acid at P<0.056. On day 15 (step 4) butyric acid was higher in strain CH4 treatments than Control (P<0.035), similar for day 35 (step 5) (P<0.043), whereas propionic acid was lower (P<0.014). The overall impression is that proportionally more acetic and butyric acid and less propionic acid resulted in the strain CH4 treatments compared to Control.

Daily feed intakes of the steers are displayed in Fig. 4 and given in Table 7 together with ADG results.

Feed intake (Fig. 4; Table 7) was sustainably higher for strain CH4 treatments than for Control (P<0.01 for days 12, 25, 33 and 34, and P<0.05 for days 10, 28, 32 and 35). This held true for overall means when the strain CH4 treatments were considered together. The three strain CH4 treatments did not differ. On average feed intake was about 21% lower in Control, a difference which was reflected in the ADG's of the steers (Table 7).

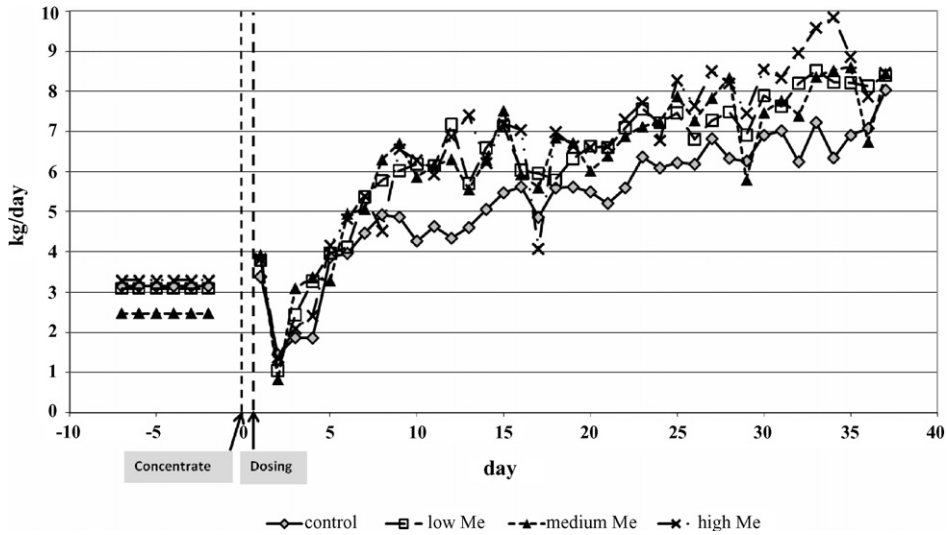

Fig. 4. Trends in daily feed intake for the Control and low, medium and high strain CH4 treatments in Trial 2.

Table 6
Concentrations of acetic, propionic and *n* + iso-butyric acids as affected by transition step and Control *vs* strain CH4 treatment in Trial 2.

Volatile fatty acid	mmol/mol total VFA[b]		
	Control	Strain CH4	MSE[c]
Acetic acid			
Pre-adaptation	785	776	0.61
Step 1	587	574	4.72
Step 2	449	469	2.73
Step 3	386	444	3.08
Step 4	404	405	5.65
Step 5	402	403	2.17
Average[a]	446	459	
Propionic acid			
Pre-adaptation	136	142	0.63
Step 1	324	272	4.29
Step 2	291	273	6.19
Step 3	361	252	5.66
Step 4	415	343	7.17
Step 5	460	417	3.57
Average[a]	370	311	
Butyric acid			
Pre-adaptation	75.7	78.3	0.21
Step 1	80.6	131	3.43
Step 2	206	210	5.12
Step 3	184	258	4.74
Step 4	105	185	2.53
Step 5	99.0	130	2.49
Average[a]	135	183	

[a] Excluding the pre-adaptation phase.
[b] VFAs, volatile fatty acids.
[c] MSE, mean square error.

Table 7
Steer weights, ADG[a] and feed intake of Control and the low, medium and high strain CH4 treatments in Trial 2.

	Control	Low	Medium	High	MSE
No. of steers	3	3	3	3	–
Days in trial	37	37	37	37	–
Initial weight (kg)	191.0	190.7	188.0	197.0	0.78
Final weight (kg)	235.6	243.8	237.3	249.1	1.30
ADG (kg/day)	1.21	1.44	1.33	1.41	0.05
Feed intake (kg/day)	5.33	6.35	6.33	6.63	0.34
Feed intake (% above Control)	–	19	19	24	–

[a] ADG, average daily gain.

4. Discussion

A number of measured parameters showed consistency in both trials: (1) feed intake was higher for strain CH4 drenched animals than for Control during initial exposure to concentrate feeding and the difference was sustained for the duration of the measured period. The difference in the case of the steers of about 21% corresponds with the 24% reported by Robinson et al. (1992), following intra-ruminal administration of *Me* 407A to steers. The commonality between the two trials was evident even though in the case of lambs the transition from forage to concentrate was abrupt whereas a step up procedure was applied for steers. (2) ADG's tended to be better for strain CH4 treatments than for Control. (3) Lactic acid concentrations were much less for strain CH4 drenched animals than for Control in the first 2–3 days following exposure to concentrates, supporting the results of McDaniel et al. (2008) after 24 h exposure, where after the difference disappeared. (4) Although more variable and less defined, supporting observations of Bevan et al. (2005), rumen pH in both trials did not decline to the same degree in strain CH4 drenched animals as in Control animals, and in fact never reached the critical acute acidosis indicator level of pH about 5 (Owens et al., 1998). This corresponds with the study of McDaniel et al. (2008). Henning et al. (2010) in this regard measured the time that rumen pH remained below pH = 6 and pH = 5 and found that strain CH4 reduced the time in comparison to Control by 7 and 6 h respectively, which in fact suggests that under these circumstances not even sub-acute ruminal acidosis (SARA), defined by Plaizier et al. (2008) as being when rumen pH < 5.6 for >3 h per 24 h, was experienced with the strain CH4 treatments.

Nagaraja and Titgemeyer (2007) defined SARA somewhat differently, as being corresponding to a ruminal pH of 5–5.5, VFA concentration of 150–225 mmol/l and by implication, lactic acid levels of about 10–40 mmol/l, since they consider concentrations of 0–5 mmol/l as normal and 50–150 mmol/l as acute acidosis. Apart from the VFA concentration indicator (about 80 mmol/l), one can deduct from Table 5 and Figs. 2 and 4 that Control conformed to this definition, but again it appears as if the strain CH4 treatments prevented development of SARA.

Sub-clinical or sub-acute lactic acid acidosis (Kleen et al., 2003) often results in more variable intake. This was observed in Trial 1 with rapid transition to the concentrate diet, but not in Trial 2 where the transition was more gradual. Since lambs on the Control treatment also experienced diarrhoea the condition apparently approached the acute form, even though the definitions above would suggest otherwise. Drenching with strain CH4 alleviated the impact. With the gradual transition the condition was less visible and more typical of the problem experienced by well-managed feedlots. Yet, the evolution of other critical lactic acid acidosis indicators was surprisingly similar in the two trials as discussed above. Taking into consideration the definitions above, the indications from the study are that drenching with strain CH4 should assist in controlling SARA, when acidosis–prone (high fermentable carbohydrate) diets are fed.

Indications from the literature are that in VFA production *Me* sometimes may ferment to favour propionic acid as end product, but sometimes butyric acid at the expense of propionic acid. The shift towards butyric acid in Trial 2 (Table 6) support reports from pure cultures (Marounek et al., 1989; Kettunen et al., 2008), but other results show that this will depend on dilution rate (Horn et al., 2009), rumen pH (Counotte et al., 1981) and fermentation substrate (glucose *vs* lactic acid) (Rogosa, 1984). In this regard, Horn et al. (2009) reported that strain CH4 in batch culture produced 58 mol% acetate and 42 mol% propionate from lactate, with <0.02 mol% butyrate and no valerate at pH = 5.5. Counotte et al. (1981) found in trials with dairy cows that up to 77% of lactic acid fermentation by *Me* was via the acrylate pathway with propionic acid as end product, but the fermentation shifted away from propionic acid towards butyric acid as the rumen pH declined. Recent results with high producing dairy cows at reading (Aikman et al., 2009) with strain CH4 showed an increase in propionic acid as end product when the rumen pH on average was around six. In Trial 2 rumen pH for strain CH4 treatments fluctuated around 5.5 (Fig. 3), which could be borderline for the propionate to butyrate shift. If so, the benefit of strain CH4 in the feedlot with similar diets as in Trial 2 will primarily be limited to the control of lactic acid accumulation in the forage to concentrate transition phase, whereas the dairy cow may benefit further from propionate as glucose precursor. If on the other hand rumen pH does restore to approach pH = 6 as in Trial 1 (Fig. 1), the propionate scenario may also be possible in the feedlot as the dose-dependent results of McDaniel et al. (2008) imply. This hypothesis clearly warrants further research.

The qrt-PCR method proved satisfactory in distinguishing between *Me* concentration in the rumen of Control and strain CH4 treatments in Trial 2. It could consequently show that lactic acid was controlled in the first 2–3 days following concentrate exposure because of the presence of larger numbers of these lactic acid utilizers, most probably as a result of strain CH4 drenching. As mentioned previously, however, we were uncertain whether the method, apart from the specific strain (*Me* NCIMB 41125), also picked up closely related DNA. This has recently been addressed when Apajalahti (2007, confidential) developed an exclusive primer for *Me* NCIMB 41125. The primer was included in a study by McDaniel et al. (2009, unpublished) at Kansas State University using the same dosage levels as the present. Their results show that in the transition phase all non-strain CH4 DNA accounted for less than one log unit/ml in the rumen, whereas strain CH4 was of the order of 10^6/ml. This enabled them to demonstrate that the control of lactic acid in this critical phase was indeed the result of the introduction of strain CH4.

Our results suggest that a viable *Me* population can be established in 4–5 days (Tables 4 and 5) following concentrate introduction, which is earlier than the 7–10 days reported by Klieve et al. (2003) and McDaniel et al. (2009, unpublished). The difference could be a function of transition procedure, but does suggest that under certain conditions successful adaptation to feedlot diets can be accomplished in a shorter period than current commercial practice.

One of the objectives in Trial 2 was to find the optimum dosage size of strain CH4. The medium size dose (10^{10} cfus/dose) is currently favoured in commercial application, but the results suggest that even the low dose (10^9 cfus/dose) might have been sufficient. However, this could also be due to the insensitivity of the qrt-PCR method employed. The study by McDaniel et al. (2009, unpublished) suggests that, based on population numbers and lactate utilization, a dosage size of the order of 10^{10} cfus/dose is probably optimal.

5. Conclusions

The study was designed to investigate whether the *Megasphaera elsdenii* (*Me*) strain NCIMB 41125 (CH4) can prevent ruminal acidosis or alleviate its effect *in vivo* on highly fermentable carbohydrate diets. The transition from forage to concentrate was either abrupt or gradual. The responses, whether abrupt or gradual, were rather similar. In both instances, feed intake remained at higher levels in CH4 drenched animals than in Control animals, and rumen pH and lactic acid concentrations immediately post-administration were maintained beyond the SARA zone. Feed intake was however more erratic on abrupt than gradual transition with accompanying digestive disturbances.

Volatile fatty acids in total did not differ between Control and CH4 treatments but the concentrations of acetic, propionic and butyric acids suggested that strain CH4 introduced a shift away from propionic acid in favour of butyric acid. Results from the literature indicate that the proportions are depended on pH, dilution rate and substrate, sometimes also favouring propionate.

qrt-PCR results for the presence of introduced *Me* in the rumen coincided with lactic acid concentration trends, suggesting that strain CH4 was responsible for the prevention of SARA.

It is concluded that strain *Me* NCIMB 41125 could be useful in preventing or alleviating ruminal acidosis during comparatively rapid transition from forage to highly fermentable carbohydrate diets.

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