Effect of *Megasphaera elsdenii* NCIMB 41125 drenching on health and performance of steers fed high and low roughage diets in the feedlot

K-J. Leeuw[1,2#], F.K. Siebrits[2], P.H. Henning[3] and H.H. Meissner[1,4]
[1] Agricultural Research Council, Private Bag X2, Irene 0062, South Africa
[2] Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa
[3] Megastarter Biotech Pty LTD t/a MS Biotech, P.O. Box 10520, Centurion 0046, South Africa

Abstract

Lactate utilising bacteria (LUB) assist in reducing the risk of ruminal lactate acidosis when high concentrate diets are fed to feedlot cattle. Ruminal lactate acidosis can lead to lower animal performance and morbidity. Preliminary studies suggested that the strain, *Megasphaera elsdenii (M.e.)* NCIMB 41125, is a particularly potent LUB. The potential of *M.e.*NCIMB 41125 to improve the health and performance of feedlot cattle was investigated. Four hundred and forty eight Bonsmara steers (*ca*. 240 kg) were used in a 100-day feeding trial. Half the steers received at processing 200 mL *M*.e.NCIMB 41125 *per os* (LY) and the other half no LUB (LN). The diets in each of these treatments were divided into a low roughage (2%) (RL) and high roughage (8%) (RH) diet. The effects of LY *vs*. LN, RL *vs*. RH as main effects and their respective interactions (LYRL, LYRH, LNRL & LNRH) were compared in terms of morbidity, feedlot performance and carcass characteristics. The steers were weighed at two week intervals, feed was offered daily and the orts were removed weekly from each pen. The faecal consistency score and incidence of morbidity were recorded. At slaughter, carcass data were collected and the health status of the liver and rumen epithelium was assessed. Steers dosed with *M.e.*NCIMB 41125 had a 5.6% better average daily gain (ADG) during weeks 3 - 5 (2.09 kg/day *vs*. 1.98 kg/day for LY and LN, respectively). Feed conversion ratio (FCR, Weeks 1 - 13) was better for the steers fed the RL than the RH treatment (4.72 kg/kg *vs*. 4.99 kg/kg for RL and RH, respectively). Steers on the LNRH treatment during weeks 3 - 5 used more feed per kg gain than steers on the other treatments (5.39 kg/kg for LNRH *vs*. 4.74 kg/kg and 4.72 kg/kg for LYRL and LNRL, respectively). More steers (21) on the LNRL treatment were treated for morbidity than on the other treatments (8, 7 and 5 for LYRL, LYRH and LNRH, respectively). In general, animal performance was not improved by dosing with *M.e.*NCIMB 41125, but since ADG was improved in the immediate post-adaptation phase (weeks 3 - 5) and morbidity levels were lower on the low roughage diet, dosing of steers on low roughage, lactate acidosis-prone, diets with *M.e.*NCIMB 41125 should prove useful.

Keywords: Beef cattle, lactic acid utilising bacteria, acidosis, morbidity
[#] Corresponding author. E-mail: kleeuw@arc.agric.za
[4] Present address: 189 Van Riebeeck Avenue, Centurion 0157, South Africa

Introduction

Feedlot cattle that originate largely from pastures must adapt as quickly as possible to a high concentrate–low roughage diet. Diets containing high levels of grain have high levels of readily fermentable carbohydrates, which provide an excellent growth medium for ruminal bacteria (Stock *et al.*, 1990). Lactate producing bacteria grow well under these conditions, resulting in high levels of lactic acid in the rumen which in turn is the primary cause of ruminal or lactate acidosis (Owens *et al.*, 1998). The proliferation of these lactate producing bacteria is traditionally controlled by feed antibiotics (Berry *et al.*, 2004). However, the level of lactic acid can be kept low during the adaptation period by feeding higher levels of roughage (Owens *et al.*, 1998) until lactate utilising bacteria (LUB) have been established in the rumen, or through the dosing/supplementing of a LUB. It has been suggested that the presence of LUB in the rumen inhibits the accumulation of lactate in the rumen (Nisbet & Martin, 1994; Kung & Hession, 1995).

Hino *et al.* (1994) found that the bacterium, *Megasphaera elsdenii* (*M.e.*), strain NIAH 1102, prefers to utilise lactate above glucose. Horn *et al.* (2009a; b) selected from high producing dairy cows a number of

Megasphaera elsdenii strains which have the potential to utilise lactic acid. From these strains one was selected (Henning *et al.*, 2010a) and patented as *M.e.* strain NCIMB 41125. Kettunen *et al.* (2008) found that the *M.e.* strain NCIMB 41125 proved efficient against lactic acid accumulation. This is supported by Henning *et al*. (2010b) who found that this *M.e.* strain contributed in alleviating lactic acid acidosis when cattle were changed over a short period of time from a forage based to a concentrate based diet. This is further supported by research on the same strain by McDaniel *et al.* (2008) in the USA.

Available energy levels in high energy diets can be manipulated by changing the level of roughage in the diet. Schoonmaker *et al.* (2004) found that beef cattle on high energy concentrate diets were more efficient than ones on a high forage type of diet, and that high forage diets tended to increase the age of cattle at slaughter. Bartle *et al.* (1994) found that warm carcass weight decreased linearly with an increase in roughage level, with similar trends being suggested for dressing percentage. Thus, it would seem logical to determine whether roughage levels in feedlot diets can be lowered even further than what is currently used in commercial feedlot, especially by alleviating the risk of lactate acidosis by administering a potent lactate utiliser such as *M.e.*NCIMB 41125. A study was therefore conducted to evaluate the effects of administering *M.e.*NCIMB 41125 and changing the roughage content of the diet on ease of adaptation, morbidity and performance of weaner steers during the feedlot finishing period.

Material and Methods

Four hundred and forty eight Bonsmara weaner steers (220 kg ± 26 kg) were used in a complete randomized block design in a 100-day feeding trial at the South African Agricultural Research Council feedlot facility at Irene. The trial was approved by a duly appointed Animal Ethics Committee (APIEC02/01). Two hundred and twenty four steers received as a single dose, a suspension of *M.e.*NCIMB 41125 (200 mL) during processing (treatment LY) while 224 steers were dosed a placebo (200 mL water, treatment LN) as the control. The LN and LY treatments were collectively called Lz. Each dose of 200 mL *M.e.*NCIMB 41125 contained 10^9 colony forming units (cfu) per mL (Henning *et al.*, 2010b). One hundred and twelve steers from the LY and 112 from the LN treatment were fed low roughage (RL) diets containing 2% roughage, and the remaining 112 steers from each treatment high roughage (RH) diets containing 8% roughage. The RH and RL treatments were collectively called Rz. Furthermore, the diet fed to half of the animals in each treatment contained monensin at the normal monensin inclusion rate (33 mg/kg feed) and the other half no monensin. These treatments constitute a 2 x 2 x 2 factorial experimental design.

Upon arrival the steers were weighed individually, vaccinated against bacterial and viral infectious diseases (*Clostridium* ssp., anthrax, botulism, IBR), dosed against internal and external parasites, ear-tagged and given anabolic steroids (Ralgro®, on entry and Revalor-S® on day 35). The steers were housed in small open-air holding pens, each containing eight animals with a 12.5 m^2 floor area per animal and 68 cm feed trough space per animal. The troughs were covered by a roof. The steers had access to clean, fresh water.

Animals were blocked by weight and randomly allocated to the treatments. Within each treatment the 112 steers were allocated by weight to one of 14 replicates of eight animals each, thus minimizing weight variation within replicates. At a mean weight of 370 kg the animals received Zilmax® (a Beta-agonist, zilpaterol, from Intervet, calculated at: (((0.15* average animal weight) *0.048) *1000/average feed intake (kg/day per animal, of previous week))/1000 = gram Zilmax® per ton feed) supplementation for 30 days. After a two day withdrawal period of Zilmax® the steers were slaughtered at a commercial abattoir.

Adaptation to the finisher diet lasted 14 days and a five-step programme was followed (Table 1). The level of hay in the diet was reduced stepwise while the levels of hominy chop and maize meal were concomitantly increased. The finisher diets were fed from day 14 to the end of the trial (Table 2). The steers were fed once daily, using a clean feed trough management regime with only traces of feed remaining before the next morning's feeding. Feed refusals were removed daily from the feed bunk for the first 35 days and weekly thereafter. Daily feed offered and weekly orts removed from each pen were recorded. Animal weights were recorded at two-week intervals (weekly during the first five weeks). From these measurements, average daily gain (ADG), average daily intake (ADI, as is) and feed conversion ratio (FCR) were calculated. Faecal scores were recorded for each pen. During the feeding phase the measurements were grouped into adaptation period (weeks 1 - 2), immediate post adaptation period (weeks 3 - 5), whole adaptation period (weeks 1 - 5), finishing period (weeks 5 - 13) and whole feeding period (weeks 1 - 13).

Table 1 Feed adaptation regime followed from day 1 to 14

Day	Additional hay	Roughage level	
		High (%)	Low (%)
1 – 2	*Ad libitum*	18	12
3 – 4	none	18	12
5 – 7	none	14	8
8 – 10	none	12	6
11 – 13	none	10	4
14 - end	none	8	2

Table 2 Composition of the finisher diets of the low and high roughage treatments (as is basis)

Ingredient	Low roughage (g/kg)	High roughage (g/kg)
Eragrostis curvula hay	20	80
Molasses meal	120	120
Dried brewers grain	60	60
Wheaten bran	100	100
Maize meal (course)	300	270
Hominy chop	350	320
Cottonseed oilcake	20	20
Limestone	14	14
Urea	10	10
Salt	5	5
Vitamin/mineral. Premix*	1	1

*Containing: 6×10^6 IU vit A;, 3 g vit. B_1; 3.5 g antioxidant; 30 g iron; 12 g copper; 50 g zinc; 1 g cobalt; 200 g magnesium; 40 g manganese; 1 g iodine; 0.1 g, selenium.
Monensin at 33 mg/kg feed for the diet with monensin and a premix without monensin for the diets without monensin.

Faecal consistency was evaluated on a 1 to 5 point scale, daily for the first 35 days and twice weekly thereafter, where 1 was dry heaped manure with clear concentric rings typical of cattle eating dry roughage; 2 was manure less dry, starting to lose the concentric rings; 3 was manure that forms a consistent heap with some spreading, 4 was manure that spreads out on the ground, was watery which may indicate digestive disturbances, and 5 was watery manure that spreads out on the ground and is absorbed into the ground and may be bloody, and therefore is indicative of serious digestive disturbances. Faecal scoring was noted as the number of occurrences of each point score. These were added up and divided by the number of cattle to yield a pen average. Morbidity and mortality were noted daily for the duration of the trial. An animal was deemed morbid when signs of excessive nasal discharge were observed after the morning feeding while standing separately with a hanging head. Animals also assumed a hunched posture, walking with difficulty, had diarrhoea and showed signs of an empty stomach. During the trial a number of steers was observed as being morbid and taken to the crush for closer inspection. This included measuring of rectal temperature, observing of ruminal movements and, if required, listening to heart beat and lungs. Based on the inspection a decision was taken on possible treatment. If no treatment was required the animal was returned to its pen. If treatment was administered, this was recorded where after the animal was also returned to its pen.

At slaughter, carcass weight (warm) and grading (based on age, fat code and confirmation score) were obtained from the abattoir. The South African fat code is expressed on a 0 to 6 scale, where 0 = no fat cover (0 mm); 1 = very lean (<1 mm); 2 = lean (1 - 3 mm); 3 = medium (>3<5 mm); 4 = fat (>5<7 mm); 5 = slightly over fat (>7<10 mm) and 6 = excessively over fat (>10 mm) (Agricultural Products Standards Act

119 of 1990, regulation 7). Conformation score is on a 1 to 5 point scale, where 1 = very flat; 2 = flat; 3 = medium; 4 = round and 5 = very round (Agricultural Products standards Act 119 of 1990, regulation 8). Further post slaughter measurements were liver score (Brown *et al.*, 1975) and ruminal epithelial condition (on a five point scale). Ruminal epithelium condition was determined as follows: 0 = long (>1 cm) papillae present, very tightly packed indicative of a high fibre diet; 1 = short papillae (<1 cm) present, but still tightly packed; 2 = short papillae (<1 cm), spaces between papillae and even small areas where no papillae were present and signs of damage by the presence of visible connective tissue; 3 = short papillae (<5 mm) with large areas where there were no papillae present and even spots where the papillae could be removed from the rumen wall and large areas with highly visible connective tissue present on the rumen wall, and 4 = very short papillae, large areas devoid of papillae while the remainder could be scraped off easily and large areas of connective tissue present. Estimated carcass gain was calculated using the dressing percentage for young weaner calves as determined by Slabbert (1990).

Feed samples (1 kg per diet) were collected weekly during the trial period. At the end of the trial these were pooled, mixed and sub-sampled for proximate analysis. The dietary samples were analyzed for dry matter (AOAC, 2002), organic matter (Harris, 1970), fat, starch, protein (AOAC, 2002), neutral detergent fibre (NDF) (Mertens, 2002), acid detergent lignin (ADL) and acid detergent fibre (ADF) (Goering & Van Soest, 1970).

Initially results were compared statistically using a 2 x 2 x 2 factorial design. However, the monensin treatment did not show any significant differences in interactions with roughage and *M.e.*NCIMB 41125, and therefore a 2 x 2 factorial analysis of roughage and *M.e.*NCIMB 41125 treatments was used. Collected individual data (animal weights, carcass classification, carcass weights, fat code, conformation score, liver scores and rumen epithelial integrity scores), data per pen (feed consumption and faecal scores) and calculated data (daily gain, feed conversion ratio, dressing percentage and estimated carcass gain) were blocked by ANOVA to test for differences between treatments using the statistical programme, GenStat® (Payne *et al.*, 2007). Treatment means were separated using Fisher's protected LSD at the 5% level, using GenStat® (Payne *et al.*, 2007). Least significant difference (LSD) was established at the 5% level of significance (Snedecor & Cochran, 1980). Animal health data were analyzed using chi-square analysis (Payne *et al*., 2007).

Results and Discussion

Interactions between roughage level, *M.e.*NCIMB 41125 and monensin exposure did not differ (P >0.6). The difference on the monensin addition was similar to that reported in other studies (e.g. Goodrich *et al.*, 1984; Stock *et al.*, 1990), and since the interest in the present study was to investigate the effect of *M.e.*NCIMB 41125 on a high and low roughage diet, the monensin results are not reported. Monensin had no effect on *M.e.*NCIMB 41125, and *M.e.*NCIMB 41125 alleviated lactate acidosis despite the presence of tested antimicrobials (Apajalathi *et al.*, 2008), which is further supported by Callaway *et al.* (1999), who found that *M.e.* is highly resistant to monensin.

Table 3 Chemical analysis of the low and high roughage finisher diets (as is)

Analysed item	Low roughage diet	High roughage diet
Dry matter (g/kg)	897.7	899.5
Organic matter (g/kg)	934.4	934.8
Crude fat (g/kg)	48.2	46.2
Starch (g/kg)	358.5	334.7
Crude protein (g/kg)	133.6	144.2
Neutral detergent fibre (g/kg)	239.3	268.8
Acid detergent lignin (g/kg)	16.8	18.2
Acid detergent fibre (g/kg)	86.6	94.7

Dietary analysis (Table 3) showed that organic matter was similar for the treatments and that the protein level was somewhat higher in the higher roughage diet, while fat and starch levels were higher in the lower roughage diet and NDF, ADL and ADF higher in the high roughage diet.

Steers dosed with *M.e.*NCIMB 41125 had a 5.6 % better ADG (P <0.05) during the period, weeks 3 - 5 (Table 4). However, this effect was not significant (P = 0.27) for the whole adaptation period (weeks 1 - 5). For the period, weeks 1 – 13, no differences in ADG were observed between treatments LY and LN (Table 4). This is in agreement with Elam *et al.* (2003) and Beauchemin *et al.* (2003), who observed no differences in ADG for animals on control or direct fed microbial (DFM) treatments.

Table 4 Effect of *Megasphaera elsdenii* NCIMB 41125 on daily gain, feed intake and feed conversion of steers

Item / Period	n	Weeks 1 - 2	Weeks 3 - 5	Weeks 1 - 5	Weeks 5 - 13	Weeks 1 - 13
ADG LY	224	1.78	2.09[a]	1.97	2.32	2.19
ADG LN	224	1.83	1.98[b]	1.93	2.37	2.20
P[1]		0.485	0.049	0.267	0.161	0.697
e.s.e.[2]		0.052	0.038	0.030	0.022	0.019
ADI LY	28	7.01	10.07	8.83	11.81	10.66
ADI LN	28	6.94	10.00	8.76	11.82	10.63
P[1]		0.625	0.613	0.561	0.949	0.840
e.s.e.[2]		0.098	0.102	0.092	0.103	0.092
FCR LY	28	3.93	4.81[a]	4.48	5.08	4.87
FCR LN	28	3.79	5.04[b]	4.55	4.99	4.83
P[1]		0.555	0.044	0.345	0.109	0.384
e.s.e.[2]		0.108	0.082	0.048	0.039	0.026

LY - Animals single dosed with 200 mL 10^9 colony forming organisms/mL, *Megasphaera elsdenii* NCIMB 41125;
LN - Animals single dosed with 200 mL water.
ADG - average daily gain (kg/day); ADI - average daily intake (kg/day); FCR - feed conversion ratio (kg feed/kg gain);
[1] Probability level; [2] Estimated standard error of the mean.
[a,b] Means in columns with different superscripts differ significantly (P <0.05).

Average daily intakes did not differ (P >0.05) for steers between treatments during any of the periods (Table 4). This was also observed for DFM by Elam *et al.* (2003) and Beauchemin *et al.* (2003). The FCR (P = 0.044) showed a difference during weeks 3 - 5 (Table 4) in favour of the LY treatment. During the period, weeks 5 – 13, the difference in FCR between LN and LY was not significant (P = 0.11), and FCR for the whole feeding period (weeks 1 - 13) did not differ (P >0.05) between treatments. Similar observations were reported by Elam *et al.* (2003), who found no differences (P >0.05) for gain to feed ratio. Furthermore, no differences in carcass characteristics were recorded (Table 5).

As indicated previously, steers dosed with *M.e.*NCIMB 41125 had a better (P = 0.05) performance during the immediate post-adaptation period (weeks 3 - 5, Table 4) compared to the untreated steers. This, combined with a tendency (P = 0.097) of reduced morbidity (Table 10) in the LY treatment, indicates that dosing steers with *M.e.*NCIMB 41125 can be beneficial to feedlot operators. The better ADG and FCR during the period, weeks 3 – 5, of the adaptation period could be the result of inhibiting lactate accumulation (Nisbet & Martin, 1994; Kung & Hession, 1995) in the rumen by the animals dosed with *M.e.*NCIMB 41125; although no rumen samples were taken to confirm this.

Table 5 Carcass parameters of the main effects of *Megasphaera elsdenii* NCIMB 41125 and roughage level

Item / Treatment	LY	LN	P	RL	RH	P[1]	e.s.e.[2]
n	224	224		224	224		
Final weight (kg)	425.2	425.5	0.910	426.6	424.2	0.364	1.80
Carcass weight (kg)	244.5	244.9	0.797	247.2[a]	242.2[b]	0.001	1.026
Carcass gain (kg/day)	1.35	1.36	0.611	1.38[a]	1.32[b]	<0.001	0.012
Dressing %	57.48	57.52	0.742	57.95[a]	57.05[b]	<0.001	0.105
Fat code[3]	2.21	2.21	0.893	2.19	2.24	0.254	0.030
Conformation score[4]	3.22	3.23	0.783	3.28	3.17	0.013	0.031
Liver score[5]	1.91	1.86	0.555	1.91	1.86	0.534	0.058
Rumen score[6]	0.66	0.64	0.526	0.62	0.67	0.144	0.023

LY - Animals single dosed with 200 mL 10^9 colony forming organisms/mL, *Megasphaera elsdenii* NCIMB 41125; LN - Animals single dosed with 200 mL water; RL Roughage inclusion at 2% in the diet; RH - Roughage inclusion at 8% in the diet.
[1] Probability level; [2] Estimated standard error of the mean.
[3] 0 to 6 scale; 0 = no fat cover (0 mm), 1 = very lean (<1 mm), 2 = lean (1-3 mm), 3 = medium (>3-<5 mm), 4 = fat (>5-<7 mm), 5 = slightly over fat (>7-<10 mm), 6 = excessively over fat (>10 mm)
[4] 1 to 5 scale; 1 = very flat, 2 = flat, 3 = medium, 4 = round and 5 = very round.
[5] 4 point scale; 0 = no abscesses or lesions, A- (or 1) = few abscesses, A (or 2) = abscesses clearly present, A+ (or 3) = many abscesses present.
[6] 5 point scale; 0 = well developed long papillae present, 1 = well developed short papillae present, 2 = short papillae present, small areas with no papillae, 3 = short papillae with connective tissue present, 4 = very short papillae with large areas of connective tissue.
[a,b] Means in rows with different superscripts differ significantly (P < 0.05).

Table 6 Effect of roughage level on daily gain, feed intake and feed conversion of steers

Item/treatment / Period	n	Weeks 1 - 2	Weeks 3 - 5	Weeks 1 - 5	Weeks 5 - 13	Weeks 1 - 13
ADG RL	224	1.69[b]	2.09[a]	1.94	2.38[a]	2.21
ADG RH	224	1.93[a]	1.98[b]	1.96	2.31[b]	2.18
P[1]		0.003	0.043	0.649	0.029	0.211
e.s.e.[2]		0.052	0.038	0.030	0.022	0.019
ADI RL	28	6.73[a]	9.91	8.62[a]	11.58[a]	10.43[a]
ADI RH	28	7.23[b]	10.16	8.96[b]	12.05[b]	10.86[b]
P[1]		<0.001	0.092	0.009	<0.001	<0.001
e.s.e.[2]		0.098	0.102	0.092	0.091	0.085
FCR RL	28	3.98	4.73[a]	4.44[a]	4.86[a]	4.72[a]
FCR RH	28	3.75	5.18[b]	4.58[b]	5.22[b]	4.99[b]
P[1]		0.165	<0.001	0.030	<0.001	<0.001
e.s.e.[2]		0.108	0.082	0.048	0.039	0.026

RL - Roughage inclusion at 2 % in the diet; RH - Roughage inclusion at 8 % in the diet.
ADG - Average daily gain (kg/day); ADI Average daily intake (kg/day); FCR - Feed conversion ratio (kg feed/kg gain).
[1] Probability level; [2] Estimated standard error of the mean.
[a,b] Means in columns with different superscripts differ significantly (P < 0.05).

Steers on treatment RH had a higher (P <0.001) ADG during the first two weeks of adaptation (weeks 1 - 2, Table 6) than those on the RL treatment, though this was reversed during the period, weeks 3 - 5, when steers on treatment RL gained more (P = 0.043). No differences (P >0.05) in ADG for the period, weeks 1 – 5, were observed. This observation (Table 6) is supported by results of Galyean *et al.* (1994) who found that the ADG of steers on the RH treatment was higher (P <0.05) during most intervals. Steers on the RL treatment had a consistently lower (P <0.001) feed intake (Table 6), except during the period, weeks 3 – 5, where a numerical (P = 0.092) lower intake for RL was recorded. Bartle *et al.* (1994) also found that steers fed lower levels of roughage (10, 20 and 30% roughage equivalent) had lower feed intakes (P <0.001). The lower roughage level used in the present study caused a 4.1% reduction (P <0.001) in feed intake for the RL compared to the RH treatment during the whole feeding period (weeks 1 - 13, Table 6). With the exception of the periods of weeks 1 - 2 and weeks 1 - 5 (Table 6) the FCR was better (P <0.001) for steers on the RL treatment. The difference (P = 0.03) in ADG (Table 6) during the period, weeks 1 – 2, in favour of steers fed RL resulted in numerically better FCR during weeks 1 - 2 (Table 6) in favour of steers on the RH treatment. This can be due to the greater gut fill in steers on the higher level of roughage of treatment RH. This was reversed in the immediate post-adaptation period (weeks 3 - 5), where animals on the RL treatment showed an improved (P <0.001) FCR over the RH fed animals. During weeks 1 – 13 FCR was also better (P <0.001) for animals on the RL treatment. With a reduction of roughage from 20 to 10% in the diet, Zinn *et al.* (1994) found that feed efficiency increased by 11.6% (P <0.01). Improved feed efficiencies were still obtained, even at the lower roughage levels used in the present study compared to the levels used by Zinn *et al*. (1994).

The better performance on the RL than the RH treatments was also observed in the carcass parameters (Table 5), carcass weight, carcass gain and dressing percentage. Thus, it can be concluded that feeding at lower dietary roughage level is advantageous. The steers on the RH treatment had a lower incidence of morbidity (Table 10). Nine percent of the 224 steers of the RL treatment *vs.* 3.6% of the 224 steers on the RH treatment were treated for digestive disturbances. Berry *et al.* (2004) found with high energy diets and high starch diets that more animals were treated for morbidity, although the number of cattle treated for morbidity was much higher (70.9% reported by Berry *et al.* (2004) *vs*. 9% in this study).

Faecal scores were grouped according to number of incidences of diarrhoea (scores 4 and 5) and expressed as percentage occurrence for that group (Figures 1 and 2). Steers on the RH and RL treatments (Figure 1), were not different in terms of faecal scores during the first five days. From day 7 up to day 35 steers on treatment RH had on average a lower manure score than steers on treatment RL. From day 38 onward the scores for the occurrence of diarrhoea were quite similar. Intake during weeks 3 - 5 (Table 6) were higher for animals on the RL treatment, possibly due to some compensation for the first two week period when the animals on the RL treatment had a higher morbidity level (Table 10).

The higher intake (weeks 3 - 5, Table 6) may lead to increased rate of passage (proportionally less starch fermentation in the rumen) which might lead to the higher manure scores observed for the RL treatment during that period. Thus, the adaptation to low roughage diets might cause more loose manure in the later stages of the adaptation period. The higher incidence of diarrhoea, as indicator of digestive disturbances, during days 7 to 35 could have affected morbidity for the entire feeding period through the possible damage to the digestive tract caused by clinical and sub-clinical acidosis. Dosing steers with *M.e.*NCIMB 41125 had a mixed effect on manure score observations (Figure 2): From days 4 to 10 steers on the LY treatment had more diarrhoea incidences than in the LN treatment, this despite more steers being treated for digestive disturbances on the LN than on the LY treatment (Table 10). Therefore, faecal scoring apparently had no bearing on performance and health of steers dosed with *M.e.*NCIMB 41125.

*M.e.*NCIMB 41125 and roughage level interactions showed no differences (P >0.05) for ADG. However, during the period, weeks 1 – 2, the ADG for steers on the LzRH treatment was numerically (P = 0.095) higher, possibly indicating an increased gut fill, as discussed earlier (Table 7).

Average daily intake of steers on the different treatments were not different (P >0.05) (Table 7), although steers on LzRL had numerically lower intakes than those on LzRH. These differences resulted in differences in FCR (Table 7). Steers on LNRH during the period, weeks 3 – 5, used more (P = 0.025) feed per kg gain than steers on the other treatments. During the period, weeks 1 – 2, steers on LNRH tended to have a lower (P = 0.061) FCR than those on the other treatments.

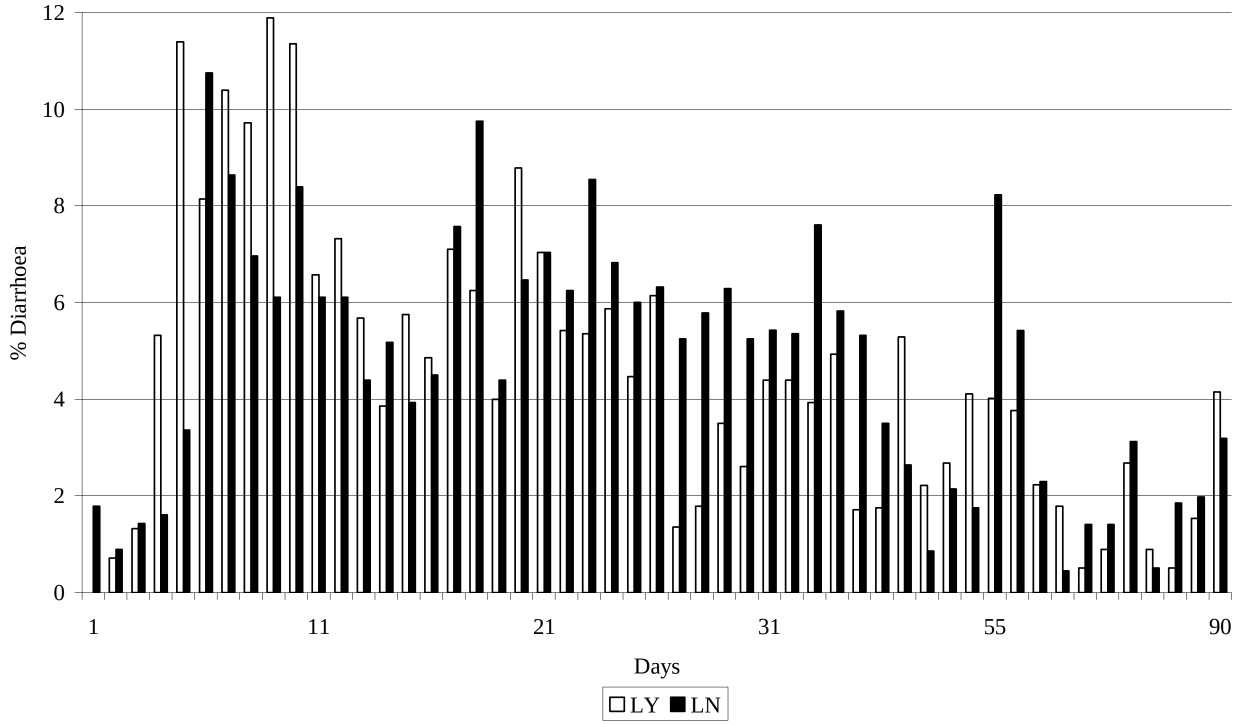

Figure 1 Percentage occurrence of diarrhoea of steers on treatments with (LY, n = 224) or without (LN, n = 224) *Megasphaera elsdenii* NCIMB 41125.

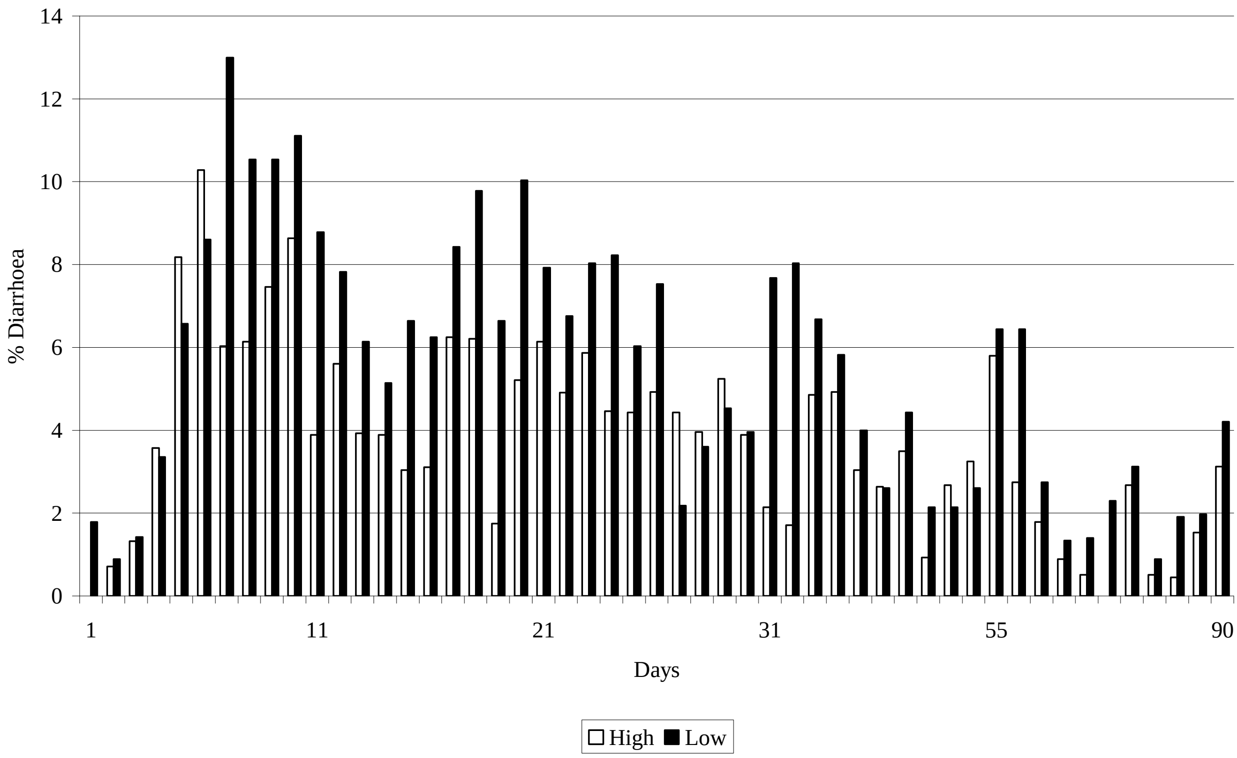

Figure 2 Percentage occurrence of diarrhoea of steers on treatments with high roughage (n = 224) or low roughage (n = 224) levels.

Table 7 Interactions of *Megasphaera elsdenii* NCIMB 41125 and roughage level on performance of steers

Period Item/treatment	n	Weeks 1 - 2	Weeks 3 - 5	Weeks 1 - 5	Weeks 5 - 13	Weeks 1 - 13
ADG LYRL	112	1.73	2.12	1.97	2.37	2.22
ADG LYRH	112	1.84	2.07	1.98	2.27	2.16
ADG LNRL	112	1.65	2.07	1.91	2.39	2.21
ADG LNRH	112	2.01	1.90	1.94	2.35	2.20
P[1]		0.095	0.274	0.809	0.316	0.365
e.s.e.[2]		0.073	0.054	0.043	0.031	0.028
ADI LYRL	14	6.80	10.05	8.74	11.62	10.51
ADI LYRH	14	7.23	10.09	8.93	11.99	10.81
ADI LNRL	14	6.66	9.77	8.50	11.53	10.36
ADI LNRH	14	7.23	10.22	9.02	12.10	10.91
P[1]		0.608	0.166	0.214	0.429	0.297
e.s.e.[2]		0.139	0.144	0.129	0.128	0.120
FCR LYRL	14	3.94	4.74[a]	4.44	4.90	4.74
FCR LYRH	14	3.95	4.88[a]	4.52	5.28	5.01
FCR LNRL	14	4.03	4.72[a]	4.45	4.83	4.70
FCR LNRH	14	3.59	5.39[b]	4.65	5.15	4.97
P[1]		0.061	0.025	0.322	0.522	0.969
e.s.e.[2]		0.152	0.115	0.068	0.054	0.037

LYRL - Steers dosed with *Megasphaera elsdenii* NCIMB 41125 and on a low roughage level diet; LYRH - Steers dosed with *Megasphaera elsdenii* NCIMB 41125 and on a high roughage level diet; LNRL - Steers dosed with a placebo and on a low roughage level diet; LNRH - Steers dosed with a placebo and on a high roughage level diet.
ADG - Average daily gain (kg/day); ADI - Average daily intake (kg/day); FCR - Feed conversion ratio (kg feed/kg gain).
[1] Probability level; [2] Estimated standard error of the mean.
[a,b] Means in columns with different superscripts differ significantly ($P < 0.05$).

Table 8 Interactions effect of *Megasphaera elsdenii* NCIMB 41125 dosing and roughage level on carcass parameters

Treatment Item	LYRL	LNRL	LYRH	LNRH	P[1]	e.s.e.[2]
Final weight (kg)	427.9	425.2	422.5	425.9	0.233	2.55
Carcass weight (kg)	247.9	246.6	241.2	243.3	0.238	5.86
Dressing %	57.91	57.98	57.04	57.06	0.854	0.424
Carcass gain (kg/day)	1.39	1.38	1.31	1.33	0.363	0.047
Fat code	2.20	2.17	2.21	2.26	0.349	0.042
Conformation score	3.26	3.30	3.17	3.16	0.612	0.044
Liver score	1.91	1.91	1.91	1.81	0.524	0.081
Rumen score	4.75	4.59	5.16	4.95	0.875	0.503

LYRL - Steers dosed with *Megasphaera elsdenii* NCIMB 41125 and on a low roughage level diet; LYRH - Steers dosed with *Megasphaera elsdenii* NCIMB 41125 and on a high roughage level diet; LNRL - Steers dosed with a placebo and on a low roughage level diet; LNRH - Steers dosed with a placebo and on a high roughage level diet.
[1] Probability level; [2] Estimated standard error of the mean.

No differences ($P > 0.05$) between treatments were observed for carcass characteristics (Table 8). The 2.7% difference in carcass weight ($P > 0.2$) and 5.7% difference in carcass gain ($P > 0.36$) between treatments LYRL and LYRH (247.9 *vs*. 241.2 kg and 1.39 *vs*. 1.31 kg/day, respectively, (Table 8) are therefore of no significance in this study.

Table 9 Number of animals treated for all illnesses (Treated) and for symptoms of digestive disturbances (diarrhoea and bloat), as affected by the interactions between *Megasphaera elsdenii* NCIMB 41125 dosing and roughage levels

Item Treatment	n	Treated	Diarrhoea and Bloat
LYRL[1]	112	8	5
LYRH[2]	112	7	4
LNRL[3]	112	21	15
LNRH[4]	112	5	4
Chi P[5]		0.001*	0.005*
Chi squared value		17.048	13.105

[1] Steers dosed with *Megasphaera elsdenii* NCIMB 41125 and on a low roughage level diet;
[2] Steers dosed with *Megasphaera elsdenii* NCIMB 41125 and on a high roughage level diet;
[3] Steers dosed with a placebo and on a low roughage level diet;
[4] Steers dosed with a placebo and on a high roughage level diet.
[5] Chi-square test approximate probability value at 5% test level, D.F. = 3.
* Statistically significant.

The occurrence of diarrhoea and bloat tended to be higher (P = 0.075) in the LN treatment (Table 10) (19 treated) than in the LY treatment (9 treated). For morbidity there was a numerical difference (P = 0.097) in the LY (15 treated) *vs*. LN (26 treated) treatment. Therefore morbidity other than diarrhoea and bloat are apparently not influenced by the dosing of *M.e.*NCIMB 41125 to cattle. Steers on treatment RL (Table 10) had more (P = 0.03) incidences of diarrhoea and bloat than steers on treatment RH.

Table 10 Number of animals treated for morbidity (Treated) and symptoms of digestive disturbances (diarrhoea and bloat), as affected by *Megasphaera elsdenii* NCIMB 41125 dosing and roughage levels

Treatment	n	Treated	Diarrhoea and Bloat
LY	224	15	9
LN	224	26	19
Chi P[1]		0.097	0.075
Chi squared value		2.685	3.086
RL	224	29	20
RH	224	12	8
Chi P[1]		0.009*	0.030*
Chi squared value		6.873	4.61

LY - Animals single dosed with 200 mL 10^9 colony forming organisms/mL *Megasphaera elsdenii* NCIMB 41125; LN - Animals single dosed with 200 mL water; RL - Roughage inclusion at a low level (2%) in the diet; RH Roughage inclusion at a high level (8%) in the diet.
[1] Chi-square test approximate probability value at 5% test level, D.F. = 3.
* Statistically significant.

In the LNRL treatment (Table 9) more (P = 0.005) animals were treated for morbidity and diarrhoea/bloat than in the other LzRz treatments. Dosing feedlot steers with *M.e.*NCIMB 41125 on low roughage diets (LYRL) reduced (P = 0.005) the incidence of bloat and diarrhoea. The interaction indicates (P = 0.001) that LNRL had more cases of morbidity than the other treatments (Table 9). Animal performance (Table 7) did not show a difference for the whole feeding period (weeks 1 - 13). The incidence of diarrhoea and bloat as indicator of lactic acidosis, was lower (P = 0.005) for steers on the LYRL treatment than steers on treatment LNRL. Therefore, the main benefit of dosing with *M.e.*NCIMB 41125 in this study appears to be the reduction in morbidity.

Conclusion

Performance differences for RH and RL treatments are indicative of the need to establish appropriate dietary roughage level where optimal animal health and maximum animal performance could be obtained. This will be difficult to achieve since the nutrient content of feedstuffs varies over time, but even more so social factors (objections to the use of antibiotics and animal derived products in animal feeds) will play a more decisive role in the formulation of feedlot diets. The use of *M.e.*NCIMB 41125 reduced the incidence of morbidity and improved animal performance during the immediate post-adaptation period. Therefore, the use of *M.e.*NCIMB 41125 is recommended when feeding low roughage type diets. Faecal observations must be used circumspectively when testing the influence of *M.e.*NCIMB 41125 or for that matter other substances, or a more sensitive method of faecal scoring in feedlot pens must be developed in order to detect digestive disturbances.

Acknowledgements

The authors wish to thank the personnel responsible for animal care at the Agricultural Research Council feedlot facility at Irene, specially J.H. Bothlolo, L.P. Loots, S.L. Maesela, D.M. Makhura, S.M. Masinga, K.W. Mashiane, J.K. Mokgase, P.M. Seboko. They also wish to thank the statisticians from the ARC Biometric Unit, especially M.F. Smith and E.H. van den Berg, for their assistance with the statistical analysis of the data.

References

AOAC, 2002. Official methods of analysis, 17th ed. Association of Official Analytical Chemists, Inc. Arlington, Virginia, USA.

Apajalahti, J., Henning, P.H., Horn, C.H., Alaja, S. & Kettunen, A., 2008. Effect of common antimicrobial agents on the growth and metabolism of *Megasphaera elsdenii* strain NCIMB 41125, a lactate utilising probiotic for ruminants. Abstract, RRI Symposium on Gut Microbiology, INRA.

Bartle, S.J., Preston, R.L. & Miller, M.F., 1994. Dietary energy source and density: Effects of roughage source, roughage equivalent, tallow level, and steer type on feedlot performance and carcass characteristics. J. Anim. Sci. 72, 1943-1953.

Beauchemin, K.A., Yang, W.Z., Morgavi, D.P., Ghorbani, G.R., Kautz, W. & Leedle, J.A.Z., 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical acidosis in feedlot cattle. J. Anim. Sci. 81, 1628-1640.

Berry, B.A., Krehbiel, C.R., Confer, A.W., Gill, D.R., Smith, R.A. & Montelongo, M., 2004. Effects of dietary energy and starch concentrations for newly received feedlot calves: I. Growth performance and health. J. Anim. Sci. 82, 837-844.

Brown, H., Bing, R.F., Greuter, H.P., McAskill, J.W., Coolley, C.O. & Rathmacher, R.P., 1975. Tylosin and chlortetracycline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J. Anim. Sci. 40, 207-213.

Callaway, T.R., Adams, K.A. & Russel, J.B., 1999. The ability of “Low G+C gram positive“ ruminal bacteria to resist monensin and counteract potassium depletion. Current Microbiology 39, 226-230.

Elam, N.A., Gleghorn, J.F., Rivere, J.D., Galyean, M.L., Defoor, P.J., Brashears, M.M. & Younts-Dahl, S.M., 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. J. Anim. Sci. 81, 2686-2698.

Galyean, M.L., Malcolm-Calis, K.J., Gunter, S.A. & Garcia, D.R., 1994. Performance by finishing steers as influenced by dietary roughage and fat levels. Clayton Livestock Research Center, Progress Report No. 90, May, USA.

Goering, H.K. & Van Soest, P.J., 1970. Roughage fibre analyses (apparatus, reagents, procedures and some applications). Agricultural Handbook No. 379, A.R.S., U.S. Dept. of Agric. Washington D.C., USA.

Goodrich, R.D., Garret, J.E., Gast, D.R., Kirick, M.A., Larson, D.A. & Meiske, J.C., 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58, 1484-1498.

Harris, L.E., 1970. Nutrition research techniques for domesticated and wild animals. Volume 1. Logan, U.T., USA.

Henning, P.H., Horn, C.H., Steyn, D.G. Meissner, H.H. & Hagg, F.H., 2010a. The potential of *Megasphaera elsdenii* isolates to control ruminal acidosis . Anim. Feed Sci. Technol. (accepted).

Henning, P.H., Horn, C.H., Leeuw, K-J., Meissner, H.H. & Hagg, F.H., 2010b. Effect of ruminal administration of the lactate- utilising strain *Megasphaera elsdenii* (Me) NCIMB 41125 on abrupt or gradual transition from forage to concentrate diets. Anim. Feed Sci. Technol. (accepted).

Hino, T., Shimada, K. & Maruyama, T., 1994. Substrate preference in a strain of *Megasphaera elsdenii*, a ruminal bacterium, and its implications in propionate production and growth competition. Appl. Environ. Microbiol. 60 (6), 1827-1831.

Horn, C.H., Kistner, A. & Fouche, G., 2009a. The Auxostat as isolation and rapid selection tool for strict anaerobic minority microorganisms in complex ecosystems. 6th Int. Symp. Anaerobic Microbiology; 17-20 June 2009 Liblice Chateau; Czech Republic.

Horn, C.H., Kistner, A. & Fouche, G., 2009b. Selective enrichment, isolation and characterization of fast-growing acid-tolerant and ionophore-resistant lactate utilizers from rumen contents of animals on high energy diets. XIth Int. Symp. Ruminant Physiology, Clermont-Ferrand (France) - September 6-9, 2009.

Kettunen, A., Henning, P.H., Horn, C.H., Alaja, S. & Apajalahti, J., 2008. Capability of *Megasphaera elsdenii* strain NCIMB 41125 and other probiotics to prevent accumulation of lactic acid in a rumen model. Abstract, RRI Symposium on Gut Microbiology, INRA.

Kung Jr , L. & Hession, A.O., 1995. Preventing *in vitro* lactate accumulation in ruminal fermentation by inoculation with *Megasphaera elsdenii*. J. Anim. Sci. 73, 250-256.

McDaniel, M.R., Higgens, J.J., Heidenreich, J.M., Shelor, M.K., Parsons, G.L., Henning, P.H. & Drouillard, J.S., 2008. Ruminal parameters of cattle drenched with a placebo, or live cultures of *Megasphaera elsdenii* strain CH4. Abstract, ASAS-ADSA Annual Congress, USA.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85 (6), 1217-40.

Nisbet, D.J. & Martin, S.A., 1994. Factors affecting L-lactate utilization by *Selenomonas ruminantium*. J. Anim. Sci. 72, 1355-1361.

Owens, F.N., Secrist, D.S., Hill, W.J. & Gill, D.R., 1998. Acidosis in Cattle: A Review. J. Anim. Sci. 76, 275-286.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. & Soutar, D.M., 2007. GenStat for Windows® (10th ed.). Introduction. VSN International, Hemel Hempstead, U.K.

Schoonmaker, J.P., Cecava, M.J., Fluharty, F.L., Zerby, H.N. & Loerch, S.C., 2004. Effect of source and amount of energy and rate of growth in the growing phase on performance and carcass characteristics of early- and normal-weaned steers. J. Anim. Sci. 82, 273-282.

Slabbert, N., 1990. The influence of dietary energy concentration and level of feeding on growth performance of steers. M.Sc. (Agric.) dissertation, University of Pretoria, South Africa.

Snedocor, G.W. & Cochran, W.G., 1980. Statistical methods (7th ed.). Iowa State University Press, USA.

Stock, R.A., Sindt, M.N., Parrott, J.C. & Goedeken, F.K., 1990. Effects of grain type, roughage level and Monensin level on finishing cattle performance. J. Anim. Sci. 68, 3441-3455.

Zinn, R.A., Plascencia, A. & Barajas, R., 1994. Interaction of roughage level and monensin in diets for feedlot cattle on growth performance and digestive function. J. Anim. Sci. 72, 2209-2215.