

# The influence of *Megasphaera elsdenii* on rumen morphometrics of cull cows immediately stepped up to a high-energy finishing diet

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**ABSTRACT:** Forty-five beef cull cows [body weight (BW) = 503 ± 58 kg; body condition score (BCS) = 2.1 ± 0.6] were randomized into two treatments to compare the effects of oral drenching of no probiotic vs. 100 mL of *Megasphaera elsdenii* NCIMB 41125 (*M. elsdenii* culture; Lactipro Advance; 2 × 10<sup>8</sup> cfu/mL; MS Biotec, Inc., Wamego, KS) on the realimentation of cull cows. The study featured a rapid 0-d step-up of concentrate-naïve cull cows to a 90% concentrate diet (1.43 Mcal/kg of NE<sub>g</sub>). The cows were finished for 35 d and were fitted with a wireless rumination tag (Allflex Flex Tag; SCR Engineers, Ltd, Netanya, Israel), which tracked head movement to record eating and chewing activity. Rumen morphometrics was recorded on the harvest floor, with each carcass assigned a rumenitis score, and a fragment of the cranial sac removed for further papillae analysis. An additional 23, thin, non-fed cull cows were harvested at the same abattoir to compare the effects of concentrate realimentation on ruminal morphometrics. *Megasphaera elsdenii* culture-drenched cattle registered a 13.3% increase in rumination time (39.27 min/d, *P* = 0.03) during

the first week of the trial compared to controls. A numerical rumination advantage for *M. elsdenii* culture-administered cattle was observed during week 2 of trial (*P* = 0.17), with no differences between treatments from weeks 3 to 5 (*P* ≥ 0.40). Subjective rumenitis evaluations approached a tendency (*P* = 0.12), with non-*M. elsdenii* culture-drenched concentrate-fed cattle logging twice the score of their day 0 cohorts (2.52 vs. 1.17) suggesting considerable lactic insults occurred to the ruminal epithelium in the short 35-d trial. Despite the short feeding duration, concentrate realimentation prompted a significant improvement in mean papillae area (*P* < 0.01). Among concentrate-fed treatments, *M. elsdenii* culture-drenched cattle posted superior absorptive surface area (*P* = 0.01) and a greater ratio of papillae area of absorptive surface area (*P* = 0.05), suggesting that *M. elsdenii* culture is favorably altering the ecology of the rumen and promoting papillae growth perhaps by mitigating lactate-driven pH drops. In conclusion, *M. elsdenii* culture application in a 0-d step-up protocol to finishing diets can help mitigate the effects of ruminal acidosis.

**Key words:** cull cow, Lactipro, *Megasphaera elsdenii*, papillae, rumen morphometrics, rumination

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## INTRODUCTION

Always a prevalent threat, acidosis underscores the importance of a successful diet adaptation strategy (Owens et al., 1998). With processed grains comprising the majority of carbohydrate-dense feedlot diets (Samuelson et al., 2016), ruminal organic acid production can exceed absorption and passage of digesta, fostering an inhospitable drop in pH (Nagaraja and Titgemeyer, 2007). Although considerable efforts have been made to alter fermentation by tempering ruminal pH (Russell and Rychlik, 2001), enhancing absorption of organic acids could also mitigate acidosis. With 65% to 75% of metabolizable energy absorbed via papillae (Sutton, 1979; Bergman, 1990), dietary transition strategies should not only focus on cultivation of amylolytic and lactate-utilizing bacteria within the microbiome, but also on proper development of the microbiota to improve timely volatile fatty acids (VFA) absorption. Failure to adapt the sensitive ecosystem of the rumen can lead to continued acid insults and the surreptitious state of subacute acidosis. Although liver abscesses are often utilized for post hoc detection of acidosis bouts, over time these ulcerations may heal, concealing prior insults (Itabisashi, 1987). In contrast, healed ruminal lesions are barren of papillae and appear as tangible scars (Thomson, 1967). Consequently, rumenitis scores can serve as an objective log, denoting prior acidotic insults which may have occurred during the dietary transition phase.

Lactipro Advance (*M. elsdenii* culture; MS Biotech, Inc., Wamego, KS) is a commercially available patented strain (NCIMB 41125) of the bacterium *Megasphaera elsdenii*. During in vitro acidosis challenges, mediation of ruminal pH has routinely been achieved by *M. elsdenii*, which is credited with vanquishing 60% to 80% of lactate in vivo (Counotte et al., 1981). Although *M. elsdenii* NCIMB 41125 has demonstrated an ability to halt acidosis in controlled in vitro settings (ARC/Kemira Phosphates, 2006; Kettunen et al., 2008; Horn et al., 2009; McDaniel et al., 2009), it has failed to consistently trigger improvements in vivo (Drouillard et al., 2012; Thieszen et al., 2015). It is possible that the efficacy of *M. elsdenii* culture is only distinguishable in severe acidosis challenges. It is our contention that a cull cow (presumably on a forage-based diet) is a formidable model for an acidosis challenge when placed directly on a concentrate diet for realimentation prior to slaughter. Cull cows have an exorbitant intake potential, compliments of a greater ruminal capacity, and

elevated circulating insulin-like growth factor-1 (IGF-1) concentrations (Yambayamba et al., 1996), provoked by the compensatory state's drive to replenish catabolized tissues. Collectively, the objective of this project is to clarify the effect of *M. elsdenii* NCIMB 41125 (Lactipro Advance) on animal health and rumen morphometrics in beef cull cows immediately transitioned to a calorically dense finishing diet.

## MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the Texas Tech University Institutional Animal Care and Use Committee (IACUC 18030-03). The experiment was conducted at the Texas Tech University Beef Cattle Research and Teaching Unit located 9.7 km east of New Deal, TX.

### *Pretrial Animal Selection and Management*

On May 18, 2018 (day 18), five semi-loads of beef cull cows were procured from sale barns located in southern Texas and transported to the Texas Tech University's Beef Center Teaching and Research Unit in New Deal, TX ( $n = 160$ ). Cattle were unloaded and housed with pot load groups (5 loads; 32 cows per load) in soil surface pens (27 × 37 m) and given ad libitum access to water and hay (native grass round bales). Pens featured cement fence line bunks which rested over a 3-m concrete apron. Cattle were allotted 27 m<sup>2</sup> of pen space and approximately 0.70 m of linear bunk space. Each pen was outfitted with a shade canvass which was 6.4 m overhead designed to help alleviate heat stress. Cattle were palpated by a professional embryologist to determine pregnancy status and were assigned a unique identification tag. Prior to trial initiation, cows were fed 6.8 kg of a starch-free maintenance diet comprised of wet corn gluten feed (Sweet Bran; Cargill Corn Milling, Dalhart, TX) and cotton burrs (Table 1) to determine each cow's willingness to consume a concentrate base diet. Only proven bunk broke cattle with requisite docility and general health parameters were considered as trial candidates. On May 25, 2018, (day 11) possible trial candidates were processed (Silencer chute; Moly Manufacturing, Lorraine, KS; mounted on Avery Weigh-Tronix load cells, Fairmount, MN; readability ± 0.45 kg). Processing procedures included documentation of body weight (BW), subjective assignment of body condition score (BCS) and chute disposition score, and allocation of a wireless rumination ear tag (Allflex Flex Tag; SCR Engineers,

**Table 1.** Ingredient and analyzed chemical composition (DM basis) of diets fed prior and during the trial period

Ingredient	Pretrial	Trial
	Maintenance diet	Finisher diet <sup>1</sup>
Corn grain, steam flaked	0.00	62.05
Wet corn gluten feed <sup>2</sup>	57.00	20.00
Ground cotton burrs	40.00	10.00
Tallow	0.00	3.00
Limestone	2.00	2.00
TTU supplement, <sup>3</sup> %	1.00	2.00
Urea	0.00	0.95
Analyzed composition <sup>4</sup>		
Dry matter % as-fed <sup>5</sup>	68.13	76.01
Crude protein %	17.66	14.33
Net energy for maintenance, Mcal/kg	1.45	2.08
Net energy for gain, Mcal/kg	0.86	1.43
Neutral detergent fiber, %	40.43	17.74
Fat, %	1.69	6.21
Calcium, %	1.03	0.82
Phosphorus, %	0.71	0.43
Potassium, %	1.48	0.78
Magnesium, %	0.39	0.23

<sup>1</sup>The finisher diet was fed through the duration of the 35-d trial with no step up.

<sup>2</sup>Supplement composition (DM basis): 67.755% cottonseed meal, 15.000% NaCl, 10.000% KCl, 3.760% urea, 0.986% zinc sulfate, 0.750% monensin (Rumensin-90; Elanco, Greenfield, IN), 0.506 Tylan-40 (Elanco), melengesterol acetate (MGA 500; Pfizer, New York, NY; 0.4 mg per animal per day), ractopamine hydrochloride (21.0 g/ton; Actogain 45, Zoetis, Parsippany, NJ), 0.500% Endox (Kemin Industries, Des Moines, IA), 0.196% copper sulfate, 0.167% manganese oxide, 0.157% vitamin E (500 IU/g), 0.125% selenium premix (0.2% Se), 0.083% iron sulfate, 0.010% vitamin A (1,000,000 IU/g), 0.003% ethylenediamine dihydroiodide, and 0.002% cobalt carbonate.

<sup>3</sup>Sweet Bran (Cargill, Dalhart, TX).

<sup>4</sup>Composition of weekly composite samples (two maintenance diet and five finisher diet) analyzed at a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX). DM calculated weekly (forced-air oven for 24 h at 100 °C).

<sup>5</sup>Diet DM was calculated using weekly ingredient sample DM records.

Ltd, Netanya, Israel). A trained evaluator assigned a visual BCS to each cow, in accordance with the guidelines published by [Rasby et al., \(2014\)](#). Cows selected for inclusion in the study ( $n = 45$ ) were open (nonpregnant), ranged in visual BCS from 2 to 5-, had manageable disposition scores, demonstrated a willingness to consume concentrate feed, appeared structurally sound, healthy, and not physically weak. Cows designated to the trial were housed together in a pair of soil surface pens previously described (27 × 37 m). Once fitted with electronic Allflex Flex Tag, cows were given a 10-d period to monitor daily rumination and activity, with the resulting data stored in SCR DataFlow II software

system (SCR Engineers, Ltd, Netanya, Israel). This period served as the baseline and was used to quantify the effects of the rapidly fermentable carbohydrate challenge.

### Experimental Design

The study utilized a direct comparison of treatments organized as a completely randomized design. Selected cows ( $n = 45$ ) were randomly assigned to treatments: 1) **Control**, 23 cattle were allocated to the negative control group and received 0 mL probiotic and 2) **Lactipro**, 22 cows received an oral drench of 100 mL of Lactipro Advance (*M. elsdenii* NCIMB 41125,  $2 \times 10^8$  cfu/mL). Cows received the same ration throughout the trial. The finisher diet was introduced on day 0 and continuously fed throughout the 35-d finishing period.

### Cattle Management

On the morning of June 5, 2018 (day 1), cows were reweighed and sorted from the Silencer chute into their respective treatments. Additional processing methods included all cows receiving a trenbelone acetate–estradiol combination implant (Synovex Plus; Zoetis, Parsippany, NJ; lot 234103 expiration 07/2020) and an oral anthelmintic (Valbazen; Zoetis, Parsippany, NJ, lot 128635, expiration 11/2018). Additionally, the 22 cows stratified to the *M. elsdenii* culture treatment received an oral drench with 100 mL of Lactipro Advance (*M. elsdenii* NCIMB 41125,  $2 \times 10^8$  cfu/mL; lot 5052018 expiration 04/2018; MS Biotec, Inc.). Once processed, cattle were moved back to the soil surface pens previously described (27 × 37 m) and penned by treatment. These adjacent treatment pens shared a common water source. Cattle were managed in treatment pens to improve the accuracy of feed delivery.

The trial diet was formulated to exceed the National Research Council (1996) requirements and was milled at the Texas Tech Burnett Center Feed Mill. Diet composition and nutrient analysis of diets utilized during the pretrial and trial periods are presented in [Table 1](#). Both diets included a supplemental premix, which was blended with a ground corn carrier with a commercial micro-mixer and was batched with the total mixed ration. The finishing diet contained monensin (Rumensin-90; Elanco, Greenfield, IN, targeted at a rate of 300 mg per animal per day), tylosin phosphate [Tylan-40; Elanco, Greenfield, IN, formulated at a rate of 12.1 mg/kg dry matter (DM)], melengesterol acetate

(MGA 500, Zoetis Animal Health, Parsippany, NJ, targeted at a rate of 0.4 mg per animal per day), and ractopamine hydrochloride (Actogain 45, Zoetis, Parsippany, NJ, targeted at a rate of 300 mg per animal per day).

Cattle were program-fed with feed deliveries predetermined, regardless of bunk calls during the first 5 d. On day 1 of the trial, cows were fed approximately 1.75% of their BW on an as-fed basis. Subsequently, from days 2 to 5 cattle were challenged with an additional 0.25% of their BW ending with an intake of 2.75% of their BW on an as-fed basis. Following day 5, diet delivery was dependent on feed bunk calls. Feed bunks were evaluated daily at approximately 0730 h. Refusals were estimated, recorded, and used to inform daily feed deliveries. The slick bunk management technique employed in this study was extensively reviewed by Pritchard and Bruns (2003). Briefly, feed calls were decreased if feed remained in the bunk by a similar amount. In contrast, if no refusals were found in the bunk for two consecutive days then the feed call was increased by 0.45 kg per cow per day.

Feed was mixed daily, and a skid steer was used to add ground cotton burrs and corn gluten feed to a tractor-pulled mixer (Roto-Mix, Dodge City, KS). The remaining ingredients were batched in a paddle type mixer and delivered by a drag chain conveyor system to the Rotomixer. A standard, stationary 5-min mix was applied to complete the diet. In an attempt to trigger ruminal pH declines, feed was distributed to cattle once daily beginning at 0800 h.

Daily feed samples were obtained from the Rotomix and stored at  $-15.6^{\circ}\text{C}$  for further analysis. Following trial completion, composites were generated for each week. Samples were thawed, forced through a separator and placed in a bucket, and mixed thoroughly for 1 min to create weekly composites. Composites were submitted for proximate analysis (Servi-Tech Laboratories, Amarillo, TX) using the methodology outlined by AOAC (1995). Diet composition and chemical analysis can be found in Table 1. Additionally, weekly dietary and ingredient samples were collected for DM analysis (forced-air oven for at  $100^{\circ}\text{C}$  for 24 h). With storage of cotton burrs and corn gluten feed in outdoor concrete bunkers, these ingredients were sampled and dried using the methodology previously described following any precipitation event. Collectively, ingredient DM records were the basis of diet formulation and dry matter intake (DMI) computations. Finally, all daily feed refusals greater than approximately 5 kg were removed from the

bunk, weighed, sub-sampled, and dried (forced-air oven for at  $100^{\circ}\text{C}$  for 24 h) to adjust DMI records. Individual BW measurements were recorded on days 11, 0, 1, and 35. Automatic water troughs were cleaned twice weekly. A daily log was maintained to track health and monitor heat stress.

An acidosis intervention protocol was established for the study period. Bloat scores were monitored daily and recorded 2, 4, and 6 h after feeding. Assignment of bloat scores was congruent to the work of Paisley and Horn (1998). Surprisingly, no cow exhibited any signs of bloat throughout the trial, and only minimal clinical signs of acidosis were observed on two separate occasions, each for cattle designated to the control group. Furthermore, no cattle revealed signs of bovine respiratory disease or any other ailment. As such, no animals were treated or removed from the study.

### **Rumination Data Collection**

Cessation of rumination and a reduction of animal activity are recognized as warning signs of poor animal health (Liboreiro et al., 2015). Both rumination and general movement/activity were recorded with a wireless rumination monitoring system, as cows were assigned an Allflex Flex Tag (SCR Engineers, Ltd, Netanya, Israel) on day 11. This waterproof tag (25 g, height = 68 mm, width = 38 mm, diameter = 15 mm) tracks the cyclical head motions made during chewing and is used to estimate total rumination. Activity is measured as general animal movement and is among the parameters used by the system to monitor animal health. At 20-min intervals the SCR Radio Frequency Base Unit collected data from the rumination tag and stored the downloaded information on the herd management software (Heatime Pro+; SCR Engineers, Ltd, Netanya, Israel). The summation of daily activity and rumination duration was generated by the software and used to monitor cattle during the trial.

### **Postmortem Data Collection**

Two harvest events were used to compare the effect of concentrate realimentation and *M. elsdenii* culture on rumen morphometrics. On May 22, 2018, a group of forty thin, beef, cull cows were sourced from several West Texas Ranches and harvested at Preferred Beef in Booker, TX. These open cows had recently weaned calves and would be classified as either “lean and light” (BCS  $\leq 3$ ) or “boners” (BCS 4–6). Visually, they were a

reasonable facsimile of the cows placed on feed at the Texas Tech Beef Teaching and Research Unit. Additionally, each carcass generated from these cows, which was subsequently utilized in the day 0 analysis, displayed yellow subcutaneous fat, validating a lack of concentrate feeding (Dunne et al., 2008; Woerner, 2010). A random number generator was used among cows with non-condemned livers, to select a subset of cattle ( $n = 23$ ) for trial inclusion. These cows were used to establish a baseline of effects of concentrate feeding on ruminal morphometric computations.

Rumen morphometric data was captured on the harvest floor. Plant employees, under the direction of a Texas Tech personnel, opened the rumens of selected cows and removed all digesta. Rumens were then transferred to wash tables and cleaned by a Texas Tech personnel. Two trained university evaluators independently assessed the entire rumen epithelium with each assigning a subjective score. Each university evaluator was blind to treatments. These scores were later averaged to create a composite estimate. The methodology utilized to define rumenitis scores was described by Bigam and McManus (1975), where scores are linearly arranged: 0 denotes no ulcerations and 10 represents severe lesions. In addition, a 3-cm<sup>2</sup> tissue sample was removed from the left side of the cranial dorsal sac in the method previously described by Lesmeister et al. (2004). Each biopsy sample was placed in a sterile, general purpose specimen container, filled with 70% ethanol, and placed on ice. Following fragment removal, plant personnel disposed of the rumens as inedible waste.

The second group of cattle that participated in concentrate realimentation were harvested on July 11, 2018 at Preferred Beef (Booker, TX). At the time of harvest, liver scores and estimated percentage of kidney heart and pelvic fat (KPH) were recorded. Liver scores were evaluated in accordance with the Eli Lilly Liver Check System (Elanco, Greenfield, IN) as published by Brown and Lawrence (2010). Rumen morphometric data were captured in the method previously described. Considerable effort was undertaken to collect ruminal data, which precluded direct recording of hot carcass weight (HCW) on the harvesting floor. As such, a plant personnel provided HCW information from an internal data tracking system. The following day, carcasses were cut open between the 12th and 13th rib interface by plant employees and routine carcass data were evaluated by a Texas Tech University Gordon W. Davis Meat Laboratory personnel. Carcass measurements taken were loin muscle area

(LMA), 12th rib fat, marbling score, lean maturity, and skeletal maturity. Subsequently, these measurements were used to calculate yield grade using the standards set forth by the USDA (1997).

Completion of rumen morphometric data analysis was conducted using the methodology outlined by Resende Júnior et al. (2006), Melo et al. (2013), Murillo et al. (2014), and Hoffmann et al. (2018). Ruminal tissue samples were arrived at the Texas Tech Animal and Food Science Building on May 22, 2018 and July 12, 2018 for non-fed and concentrate-fed cattle, respectively. Samples were removed from their cooler transporters and preserved at approximately 2 °C. Prior to analysis, samples were washed with alcohol and transferred to new containers immersed with clean 70% ethanol. In order to accurately measure the sample surface of the rumen wall, epithelium tissue preparation was completed by removal of excess connective and adipose tissues, and subsequent cutting with a scalpel into approximately 1-cm<sup>2</sup> fragments. Manual counting by five trained personnel was used to establish the number of papillae per cm<sup>2</sup> (NOP) of rumen wall. All personnel were blinded to treatments and only aware of plant-assigned sample number. Sample numbers were only matched to live animal tags following analysis. Subsequently, 12 papillae were randomly selected and removed from each fragment with a scalpel. Each removed papillae and their corresponding tissue fragment were placed into a petri dish. A premeasured, 1-cm line was added to each dish to calibrate the software. Samples were frequently cleaned with distilled water to improve resolution and to help adhere papillae to the petri dish surface. The petri dish containing the samples was subsequently scanned (HP Deskjet 3510; Hewlett-Packard, Palo Alto, CA) with images analyzed by the Image Tool software package (University of Texas—Health Science Center, San Antonio, TX). Following software calibration using the standard 1-cm line in each petri dish, fragments and papillae specimens were traced by hand and surface area measurements were recorded. For all computations, fragments were adjusted to 1 cm<sup>2</sup>.

### Statistical Analysis

Data were analyzed using a one-way analysis of variance to compare treatments (SPSS Statistics 25.0; IBM; Armonk, NY). *Megasphaera elsdenii* culture application was considered an independent variable while animal was declared the experimental unit. Several post hoc tests were conducted to evaluate the assumptions of the model. The

normality of errors assumption was solidified with the Shapiro–Wilk’s test, homogeneity of variances assumption was confirmed with Bartlett’s test, and the sphericity assumption was confirmed using a Mauchly’s test. For the results of the current study, significance was declared at  $P \leq 0.05$ , while all probabilities of  $P \geq 0.06$  to  $P \leq 0.10$  are discussed as tendencies.

Weights were shrunk 2% and 4% for initial and final measurements, respectively. Initial weights were derived from averaging measurements recorded during days 0 and 1. To improve BCS precision, all subjected scores were recorded on a scale of 1 to 9, with + signifying the top 1/3 and – representing the bottom 1/3. For statistical analysis, these data were transformed to a 27-point scale. Following data analysis, values were divided by 3 and reported on a 1 to 9 scale.

A standard dressing yield of 44% was applied to the shrunk starting BW of each individual cow to calculate carcass average daily gain (ADG), carcass G:F, and the ratio of carcass ADG:live ADG. The standard dressing yield (44%) was established following consultation with Travis Herod of Preferred Beef (Booker, TX; personal communication, Travis M. Herod). Travis estimated that thin, non-fed cows would dress approximately 44% using the plant methodology of Preferred Beef. Individual carcass G:F was computed as the quotient of carcass gain divided by the treatment average DMI and was not replicated as cattle were community fed. As such, DMI and both G:F values are merely provided to add additional context to the average of each treatments.

Daily activity and rumination values were averaged over each weekly period in the trial. These composite averages were expressed as deviations from the “baseline value” established during the pretreatment period. During this time, cattle were consuming ad libitum native grass hay and 6.8 kg of a starch-free maintenance diet comprised of wet corn gluten feed and cotton burrs (Table 1). The delta of these values was used to establish the change in activity and rumination due to the rapidly fermentable carbohydrate challenge.

Rumenitis scores were independently assessed by two trained evaluators with their scores subsequently averaged. Five trained personnel were used to establish the NOP/cm<sup>2</sup> of rumen wall. If the coefficient of variation for the independent counts exceeded 5%, the fragment was reexamined by all counters. Correspondingly, the mean papillae area (MPA) was generated by averaging the value of 12 randomly removed papillae using the Image Tool

software. Absorptive surface area (ASA)/cm<sup>2</sup> was calculated using the following computation:  $1 + (\text{NOP} \times \text{MPA}) - (\text{NOP} \times 0.002)$ , where 1 = 1-cm<sup>2</sup> fragment collected from the cranial sac (Daniel et al., 2006) and 0.002 is the approximate basal area of papillae (area unable to absorb nutrients). Finally, the formula used to estimate the percent of papillae comprising the absorptive surface was  $(\text{NOP} \times \text{MPA})/(\text{ASA}) \times 100$ .

## RESULTS AND DISCUSSION

### *Live Performance Parameters*

Summarized feedlot performance parameters are presented in Table 2. Live animal gain was not the primary focus of this study, as cattle were commingled in group housing, precluding the ability to analyze DMI and G:F. Oral drenching of *M. elsdenii* culture elicited a 15% numerical improvement in ADG (2.41 vs. 2.09 kg,  $P = 0.32$ ) over the 35-d trial. Although the value was not significant, it echoes the 9.7% improvement (2.9 vs. 2.7 kg,  $P = 0.08$ ) in ADG documented over a 42-d finishing period observed by DeClerck et al. (2019). The cattle in both these trials were managed in an analogous fashion with a few caveats. The cattle in the referenced trial were housed three per pen, with a 10-d step-up period and 42-d finishing phase. The diet employed in the current study was the same as the low roughage finisher (LRF treatment) from DeClerck et al. (2019). With performance not the primary focus of this study, failing to achieve significant power was not surprising.

Available literature features a diversity of live animal performance responses to oral drenching with Lactipro. Regardless of cattle age, studies with aggressive diets (low roughage, high caloric density) and expedited step-up protocols have reported augmented live ADG as a result of *M. elsdenii* culture application (Henning et al. 2010a). It appears that more conventional approaches to diet adaptation may conceal the benefits of *M. elsdenii* culture inoculation (McDaniel et al., 2010; Thieszen et al., 2015).

Regarding the current study, the carcass transfer (ratio of carcass ADG:live ADG) demonstrated by both treatments underscores the metabolic efficiency of cull cows experiencing compensatory gain. These means are likely somewhat inflated, due to the rigorous pretrial selection process, where cattle had to demonstrate the docility and the willingness to consume bunk delivered feed. Additionally, no cow was treated for any ailment during the trial further promoting compositional gain. Finally, no carcass

**Table 2.** Effects of *Megasphaera elsdenii*<sup>1</sup> on feedlot performance of cull cows finished for 35 d following a 0-d step-up

Item	Probiotic <sup>1</sup>	Control	SEM <sup>2</sup>	<i>P</i>
Initial body weight, <sup>3</sup> kg	502.9	503.4	8.71	0.98
Final body weight, <sup>4</sup> kg	587.2	576.5	10.34	0.61
Initial body condition score	2.11	2.10	0.28	0.98
Final body condition score	4.50	4.41	0.52	0.79
Dry matter intake, <sup>5</sup> kg	13.83	13.47	—	—
Average daily gain, kg	2.41	2.09	0.16	0.32
Gain:Feed <sup>5</sup>	0.174	0.155	—	—
Carcass average daily gain, kg	2.30	1.99	0.13	0.17
Carcass average daily gain:Live average daily gain	95.5	95.2	12.22	0.97
Carcass gain:Feed <sup>5</sup>	0.166	0.148	—	—

<sup>1</sup>Cows were orally drenched with either 0 mL (Control) or 100 mL (Lactipro) of *Megasphaera elsdenii* NCIMB 41125 (Lactipro Advance; MS Biotech, Inc., Wamego, KS) on day 0 of the trial.

<sup>2</sup>Pooled standard error of treatment means (Lactipro, *n* = 22; Control, *n* = 23).

<sup>3</sup>A 2% pencil shrink was applied to initial BW measurements.

<sup>4</sup>A 4% pencil shrink was applied to final BW measurements.

<sup>5</sup>Dry matter intake and Gain:Feed values are presented for descriptive purposes only. Due to cattle being grouped housed no replication and thus analysis was possible.

**Table 3.** Effects of *Megasphaera elsdenii*<sup>1</sup> on the change in rumination and activity time of beef cull cows finished for 35 d following a 0-d step-up relative to the baseline period

Period	DMI <sup>2</sup> , kg		Rumination <sup>3</sup> , min/d			General activity <sup>3</sup> , min/d			<i>P</i> -values	
	Probiotic <sup>1</sup>	Control	Probiotic <sup>1</sup>	Control	SEM <sup>4</sup>	Probiotic <sup>1</sup>	Control	SEM <sup>4</sup>	Rumination	Activity
Baseline <sup>5</sup>	—	—	465.57	474.76	8.58	346.34	329.11	6.52	0.60	0.19
Wk 1	9.79	9.24	-280.58	-319.85	9.11	-6.60	-3.43	5.30	0.03	0.77
Wk 2	12.7	12.95	-192.66	-218.48	9.26	-4.99	2.21	5.93	0.17	0.55
Wk 3	14.15	14.42	-158.38	-153.17	10.75	37.05	15.2	6.45	0.81	0.09
Wk 4	15.31	14.92	-144.91	-159.33	8.52	-10.47	-10.88	6.68	0.40	0.98
Wk 5	17.21	15.80	-159.20	-159.35	10.21	-57.36	-50.44	8.13	0.99	0.68

<sup>1</sup>Cows were orally dosed with either 0 mL (Control) or 100 mL (Probiotic) of *Megasphaera elsdenii* NCIMB 41125 (Lactipro Advance; MS Biotech, Inc., Wamego, KS) on day 0 of the trial.

<sup>2</sup>Dry matter intake values for each period were calculated by pen intake and not replicated. Presented here for descriptive purposes for the reader to relate it to rumination times.

<sup>3</sup>Cattle were fitted with rumination tags (Allflex Flex Tag; SCR Engineers, Ltd., Netanya, Israel) to track daily time in minutes ruminating and general activity.

<sup>4</sup>Pooled standard error of treatment means (Lactipro, *n* = 22; Control, *n* = 23).

<sup>5</sup>Cows were fitted with rumination tags and given 10 d to establish a baseline level of rumination and activity while consuming a basal roughage-based diet. Weekly computations were established by the delta ( $\Delta$ ) between each week and the baseline period.

in the current study was subject to significant plant trim as a result of bruising. However, past restoring previously catabolized organ tissue, these findings suggest that over a short duration nearly all the live animal gain is transferred to the carcass.

### Daily Rumination and General Activity Times

Additional resolution of the effect of oral drench of *M. elsdenii* culture during the rapidly fermentable carbohydrate challenge is presented in Table 3. Treatment means for activity and rumination were generated by establishing daily averages by week and reported as deviation from the baseline

period. The delta ( $\Delta$ ) for each period elucidates the effect of transitioning from a roughage-based diet to a concentrate ration. Based on ranges provided by the NRC (2016), cull cows were ruminating at a level commensurate with fiber intake during the baseline period, where no differences were observed between treatments for either rumination or activity (*P*  $\geq$  0.19). A significant difference between treatments was distinguished during the first week of the trial, where *M. elsdenii* culture-drenched cattle documented a 13.3% increase in rumination time (-280.58 vs. -319.85 min/d, *P* = 0.03). Although previous research has portrayed ambivalent performance responses to Lactipro, numerous

studies have touted *M. elsdenii* NCIMB 41125 ability to vanquish ruminal lactate (Kettunen et al., 2008; Henning et al. 2010a, 2010b; Ellerman et al., 2017) and accordingly mediate pH (ARC/Kemira Phosphates, 2006; McDaniel et al., 2009; Henning et al., 2010b; Meissner et al., 2010). The results of the current study suggest that *M. elsdenii* culture acted as a counterbalance to the copious amounts of rapidly fermentable substrate introduced to the rumen of concentrate naïve cull cows in this 0-d step-up protocol. By averting digestive upset, cattle drenched with *M. elsdenii* culture were able to spend additional time ruminating a precursor to saliva production. Responsible for neutralizing 40% of the acid generated in the rumen (Allen, 1997; Aschenbach et al., 2011), the salivatory buffer, sodium bicarbonate, also mediates pH and enables additional intake. This premise is validated by the additional 0.51 kg of DMI consumed by *M. elsdenii* culture-drenched cattle during week 1 of the experiment compared to controls. Considering cattle were program fed during the first 5 d of the trial, and thus received equivalent feed deliveries, the 0.51 kg of DMI is the product of delivery differences from days 6 to 7. Correspondingly, it is likely that in an ad libitum protocol the differences in rumination and DMI would be amplified between *M. elsdenii* culture-drenched cattle and controls.

Differences in rumination time were insignificant during the remainder of the trial (weeks 2 to 5,  $P \geq 0.17$ ). Although *M. elsdenii* culture cattle had a numerical increase of 25.82 min/d in rumination time during week 2, they had lower DMI compared to controls, which may help explain the insignificant value (-192.66 vs. -218.48 min/d,  $P = 0.17$ ). It appears by week 3 rumination activity reached equilibrium and cattle were adapted to highly digestible carbohydrate dense diets. Although, rumination time never reached baseline values, this discrepancy is most assuredly the result of the substantial difference in physically effective fiber between the diets.

A tendency was distinguished in week 3 for increased activity frequency for *M. elsdenii* culture-drenched cattle compared to controls (37.05 vs. 15.2 min/d,  $P = 0.09$ ). This was the only period in the trial, where activity characteristics exceeded the baseline period when cattle were consuming a roughage-based diet. This period also coincides, with rumination activity leveling off to normal range for concentrate diets. With no weather anomaly observed during week 3, it is possible that following the intense diet acclimation, improved ruminal health facilitated additional live animal activity. This could also help explain the improvement registered among

*M. elsdenii* culture-drenched cattle compared to controls in this period. However, it is perplexing that even after adapting to a cereal grain rich diet, which produces considerable enteric heat via fermentation, cattle would increase activity compared to a basal diet, especially during the summer.

### *Ruminal Health Characteristics*

Treatment means for rumen morphometrics are presented in Table 4. A 0-d harvest is included in the analysis to provide insight into the effects of concentrate feeding. However, these open cows utilized in the 0-d harvest are not from the same source as the cattle on trial. As such, caution should be used when evaluating the 0-d harvest. Analysis of variance failed to distinguish a difference between treatments for number of papillae ( $P = 0.39$ ) or rumenitis score which approached a tendency ( $P = 0.12$ ). Numerically, control cattle that did not receive *M. elsdenii* culture registered twice the rumenitis score of their day 0 cohorts, suggesting considerable lactic acid insults to the ruminal epithelium in a 35-d period (2.52 vs. 1.17,  $P = 0.12$ ). In response to high circulating IGF-1 concentrations, Yambayamba et al. (1996) found cattle have a voracious appetite. While combined with access to rapidly fermentable carbohydrates, can foster the conditions needed for ruminal acidosis. It is possible that extending the feeding period, would override the large variation in the cull population. However, cattle drenched with *M. elsdenii* culture posted intermediate values for rumenitis scores, insinuating the effectiveness of the probiotic to vanquish lactate and foster appropriate ruminal pH.

As expected, concentrate realimentation predicted an improvement in MPA (0.93 and 1.04 vs. 0.64 cm<sup>2</sup>,  $P < 0.01$ ). Considering that diet and VFA production are the principal factors in ruminal epithelial development (Dirksen et al., 1985), it is not surprising both *M. elsdenii* culture and control treatments logged superior papillae area's compared to cattle not fed concentrate diets. The current findings concur with those of Bannink et al. (2008), who noted that maximum papillae area could be obtained in 3 to 4 wk with high-intensity feeding regimens, compared to 7 to 8 wk with conservative diets in transitioning dairy cows. Given the documented improvement in VFA absorption resulting from increased papillae area (Dirksen et al., 1985), the rapid dietary adoption of caloric-rich grain diets utilized in this protocol may help explain the uncharacteristically high ADG and G:F



**Table 4.** Effects of *Megasphaera elsdenii*<sup>1</sup> drenching and concentrated feeding on rumen morphometrics of beef cull cows harvested at various endpoints

Item	Treatment <sup>2</sup>			SEM <sup>3</sup>	P
	Day 0	Probiotic <sup>1</sup>	Control		
Mean papillae area, <sup>4</sup> cm <sup>2</sup>	0.64 <sup>b</sup>	1.04 <sup>a</sup>	0.93 <sup>a</sup>	0.04	<0.01
Number of papillae, <i>n</i>	26.82	27.89	23.31	1.41	0.39
ASA/cm <sup>2</sup> of wall <sup>5</sup>	17.68 <sup>c</sup>	29.62 <sup>a</sup>	23.03 <sup>b,c</sup>	1.64	0.01
Papillae area:ASA, <sup>6</sup> %	93.57 <sup>b</sup>	96.20 <sup>a</sup>	92.56 <sup>b</sup>	0.65	0.05
Rumenitis score <sup>7</sup>	1.17	1.73	2.52	0.27	0.12

<sup>a-c</sup>Means within a row that do not have common superscripts differ ( $P \leq 0.05$ ).

<sup>1</sup>Cows were orally drenched with either 0 mL (Control) or 100 mL (Probiotic) of *Megasphaera elsdenii* NCIMB 41125 (Lactipro Advance; MS Biotech, Inc., Wamego, KS) on day 0 of the trial.

<sup>2</sup>Treatments were as follows: day 0 = non-fed cows harvested before the start of the trial for comparisons to the fed cattle treatments; Lactipro = cows drenched with Lactipro before a 0-d step-up to a finishing ration for a 35-d finishing period; and Control = cows not drenched with Lactipro before a 0-d step-up to a finishing ration for a 35-d finishing period.

<sup>3</sup>Pooled standard error of treatment means (day 0,  $n = 23$ ; Lactipro,  $n = 22$ ; Control,  $n = 23$ ).

<sup>4</sup>Determined by averaging the measurements of 12 random papillae from each fragment.

<sup>5</sup>ASA = absorptive surface area, calculated with the following equation:  $1 + (\text{NOP} \times \text{MPA}) - (\text{NOP} \times 0.002)$ , where number 1 = 1-cm<sup>2</sup> fragment collected from cranial sac, NOP = number of papillae, MPA = mean papillae area, and 0.002 = estimated basal area of papillae.

<sup>6</sup>Calculated with the following equation:  $(\text{NOP} \times \text{MPA})/(\text{ASA}) \times 100$ , where NOP = number of papillae, MPA = mean papillae area, and ASA = absorptive surface area.

<sup>7</sup>Rumenitis incidence was extrapolated with a scale of 0 (no lesions noted) to 10 (severe ulcerative rumenitis), according to [Bigham and McManus \(1975\)](#).

ratios observed in [Table 2](#) as well as by [DeClerck et al. \(2019\)](#).

Not surprisingly, concentrate realimentation provoked an ASA (29.62 and 23.03 vs. 17.68 cm<sup>2</sup>,  $P = 0.01$ ) and the ratio of papillae area to ASA (96.20 and 92.56 vs. 93.57%,  $P = 0.05$ ) response compared to day 0 harvest group; however, these parameters also verify the efficacy of *M. elsdenii* culture on a ruminal level. Previous studies have documented the effect of *M. elsdenii* culture on ruminal ecology, principally achieved by disposing lactate and subsequently governing pH. However, a pair of studies found increased butyrate levels following *M. elsdenii* culture inoculation ([Henning et al. 2010a](#); [Ellerman et al. 2017](#)). Considering that nearly 90% of butyrate is metabolized by the microbiota ([Dijkstra et al., 1993](#)), it is not surprising that papillae growth is strongly correlated to the ruminal availability of the VFA ([Blottière et al., 2003](#); [Hoffman et al., 2018](#)). Another theory explaining the improvement in ASA and the quotient of papillae area compared to ASA is *M. elsdenii* culture application promoted higher ruminal pH, enabling greater intake and rumination ([Table 3](#); week 1), provoking more papillae growth at the onset of the feeding phase.

### Carcass Parameters

Treatment means for carcass traits are presented in [Table 5](#). Analysis of variance was unable

to classify a significance for any carcass parameter ( $P \geq 0.16$ ). Interestingly, despite *M. elsdenii* culture-drenched cattle achieving a numerically enhanced carcass ADG, the reduction in subcutaneous BF (0.53 vs. 0.60 cm,  $P = 0.56$ ) and improvement in LMA (74.9 vs. 71.8 cm<sup>2</sup>,  $P = 0.51$ ) insinuate a shift in compositional growth. These characteristics lead to a 0.26-unit decrease in calculated yield grade, although like all other parameters it was insignificant (2.28 vs. 2.54,  $P = 0.20$ ). These findings are congruent with the work of previous investigators ([Leeuw et al., 2009](#); [Miller, 2013](#); [Ellerman et al., 2017](#)), who also failed to distinguish differences among *M. elsdenii* culture-drenched and non-treated controls.

### Implications

The intention of the current study was to examine the efficacy of *M. elsdenii* culture by immediately challenging cull cows with rapidly fermentable carbohydrates, which may lead to unfavorable ruminal conditions. Oral drenching of *M. elsdenii* culture appears to bolster ruminal health parameters, via enhancement of absorptive surface area. Previous experiments that feature improvements in G:F ratio are potentially the result of *M. elsdenii* culture fostering papillae growth leading to enhanced VFA absorption. Successful diet adaptation strategies are designed to exploit the low metabolic requirements of concentrate naïve cattle and to

**Table 5.** Effects of *Megasphaera elsdenii*<sup>1</sup> on carcass traits of cull cows finished for 35 d following a 0-d step-up to the finisher diet

Item	Treatment		SEM <sup>2</sup>	P-value
	Probiotic <sup>1</sup>	Control		
Hot carcass weight, kg	301.8	291.1	7.20	0.66
Dressing percent	51.4	50.5	0.56	0.65
Loin muscle area, cm <sup>2</sup>	74.9	71.8	2.31	0.51
12th rib fat, cm	0.53	0.60	0.06	0.56
Marbling score <sup>3</sup>	3.63	3.29	0.14	0.23
Calculated yield grade	2.28	2.54	0.10	0.20
Kidney pelvic heart fat, %	2.07	1.96	0.14	0.69
Skeletal maturity <sup>4</sup>	53.91	60.39	3.76	0.40
Lean maturity <sup>4</sup>	18.95	17.91	0.37	0.16
Liver score <sup>5</sup>	1.55	1.87	0.25	0.52

<sup>1</sup>Cows were orally dosed with either 0 mL (Control) or 100 mL (Probiotic) of *Megasphaera elsdenii* NCIMB 41125 (Lactipro Advance; MS Biotech, Inc., Wamego, KS) on day 0 of the trial.

<sup>2</sup>Pooled standard error of treatment means (Lactipro,  $n = 22$ ; Control,  $n = 23$ ).

<sup>3</sup>3.00 = Slight<sup>00</sup>, 4.00 = Small<sup>00</sup>.

<sup>4</sup>10 to 19 = A Maturity; 20 to 29 = B Maturity; 30 to 39 = C Maturity; 40 to 49 = D maturity; 50 to 59 = E Maturity.

<sup>5</sup>1 = normal liver; 2 = A - (one to two small abscesses); 3 = A (two to four small active abscesses); 4 = A + (more than four small active abscesses).

program the microbiome for efficient carbohydrate digestion and absorption. Despite the acidotic insults that occurred during a high-intensity 0-d step-up protocol, *M. elsdenii* culture is proficient at promoting ruminal health and development.

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