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Short communication: Does early-life administration of a *Megasphaera elsdenii* probiotic affect long-term establishment of the organism in the rumen and alter rumen metabolism in the dairy calf?

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ABSTRACT

Megasphaera elsdenii is a bacterial species of the rumen that can utilize lactate to produce butyrate, a key volatile fatty acid often implicated in driving calf rumen development. Because lactate is abundant in the rumen of young calves, administration of *M. elsdenii* to increase butyrate production and thus promote calf rumen development is an appealing possibility. The main objective of this study was to determine whether *M. elsdenii* administration to calves via oral drench at 14 d of age affected its long-term establishment at 70 d post-administration. Ruminal volatile fatty acid and lactate profiles and blood glucose and β-hydroxybutyrate concentrations were also examined to determine potential influence on rumen metabolism. Six neonatal Holstein heifer calves were blocked on d 1 by body weight (41.3 ± 1.8 kg) and total serum protein (5.23 ± 0.16 g/dL) and assigned to either the *M. elsdenii* (n = 3) or control (n = 3) treatment groups. On d 14, calves in the *M. elsdenii* group orally received 25 mL of a commercially available *M. elsdenii* suspension, whereas calves in the control group received 25 mL of the same product that had been autoclaved. Rumen contents and blood samples were collected weekly from each animal until 84 d of age. The oral administration of *M. elsdenii* at 14 d did not increase the abundance of *M. elsdenii* 70 d postdosing, alter rumen fermentation, or change blood metabolites associated with butyrate. These results suggest that a single administration of the *M. elsdenii* probiotic may not affect the rumen establishment of the organism.
Key words: Lactipro, microbiome, rumen fermentation end product

Short Communication

Rumen development is influenced by microbial fermentation of ingested feedstuffs to VFA, and butyrate is most often implicated in promoting rumen papillae development (Sander et al., 1959; Mentschel et al., 2001). One way to potentially increase ruminal butyrate production would be to enrich the rumen ecosystem with bacteria that produce butyrate as a fermentation end product. *Megasphaera elsdenii* is known to utilize lactate and produce butyrate (Elsden et al., 1956; Counotte et al., 1981), making it a bacterial species that might be used to increase rumen butyrate production. Lactate is not limiting in the rumen of very young dairy calves, and it primarily contributes to the low rumen pH (5.0–6.0) reported for periruminant calves (Gentile et al., 2004; Yohe et al., 2015).

Research with *M. elsdenii* and rumen function is not limited to calves and has a long history in adult ruminants (Hobson et al., 1958; Klieve et al., 2003; Henning et al., 2010; Aikman et al., 2011; Weimer et al., 2015). Klieve et al. (2003) examined ruminal *M. elsdenii* establishment in steers and found success when *M. elsdenii* was inoculated in combination with *Butyrivibrio fibrisolvens*. In contrast, Weimer et al. (2015) did not observe *M. elsdenii* establishment in lactating cows. It is clear that the efficacy of *M. elsdenii* probiotic varies. Recent research involving *M. elsdenii* administration to calves has shown promise as *M. elsdenii*-dosed calves had larger and a greater number of rumen papillae (Muya et al., 2015), improved feed efficiency pre- and postweaning (Muya et al., 2017), and increased plasma BHB concentrations (Muya et al., 2015, 2017). However, as noted earlier, demonstration that *M. elsdenii* probiotics establish in the rumen is generally lacking. We hypothesized that *M. elsdenii* could be used as a probiotic and effectively establish itself in the calf rumen. The main objective of this pilot study was to determine whether oral *M. elsdenii* administration to dairy calves at 14 d of age would help establish a persistent population of the organism after dosing. We also examined the effect

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on rumen VFA and lactate concentrations and blood glucose and BHB concentrations.

This experiment was approved by the Virginia Tech Animal Care and Use Committee (protocol no. 15-166) and conducted from September 2015 to January 2016. Six Holstein heifers born at Virginia Tech (Blacksburg) were used. Heifers were enrolled as they were born and were individually housed and fed in outdoor hutches on gravel with sawdust bedding. Hutch placement prevented calf-to-calf contact. Within 8 h of birth, each calf received 4 L of colostrum over 2 feedings. A single blood sample was obtained from each calf within 24 h of birth to determine total serum protein concentrations (Yohe et al., 2015).

On d 1, calves were blocked by BW (41.3 ± 1.8 kg) and serum total protein taken within 24 h after colostrum administration (5.23 ± 0.16 g/dL) into 2 treatment groups: treatment (**PRO**; n = 3) and control (**CON**; n = 3). On d 14, calves assigned to PRO received 25 mL of a commercially available *M. elsdenii* suspension (Lactipro; MS Biotec, Wamego, KS), and calves assigned to CON received 25 mL of the same product that had been autoclaved. Lactipro contains approximately 2×10^8 cfu/mL of *M. elsdenii* NCIMB 41125 according to the manufacturer. Lactipro was received directly from the manufacturer, stored at room temperature, and used within 14 d of receipt. Because *M. elsdenii* is a strict anaerobe, anaerobiosis of the product was visually confirmed (lack of blue color) immediately before administration.

All calves were fed the same nonmedicated milk replacer (**MR**; Amplifier Max; Land O'Lakes Animal Milk Products Co., Shoreview, MN) containing 22.7% CP and 21.9% fat (DM basis). Milk replacer composition included milk protein sources and animal fat. The MR was reconstituted to 13% solids and fed by bottles until calves were bucket trained. Calves began a 5-d weaning process when 0.91 kg of starter was consumed for 3 consecutive days or at d 42, whichever came first. Calves were fed MR at morning (0700 h) but not evening (1900 h) feedings during the weaning process. Pelleted starter (Southern States Cooperative, Richmond, VA) and water were available ad libitum to all calves from d 0. Starter composition included approximately 26.3% high-protein soybean meal, 23.2% ground soybean hulls, 11.8% wheat middlings, 11.5% ground wheat, 11.5% ground barley, 7.0% cottonseed hulls, 3.4% ground corn, 1.8% ground limestone, and 1.2% molasses (23.6% CP, 31.4% NDF, and 17.0% starch on a DM basis). Weekly samples of MR and starter were combined, mixed, and sent to Cumberland Valley Analytical Lab (Hagerstown, MD) for analysis.

Heifer was the experimental unit. Feed intake and fecal and respiratory scores were recorded daily (Yohe et al., 2015) and did not differ by treatment (data not shown).

Rumen fluid samples (20–50 mL) were obtained weekly and at 15, 16, and 18 d of age, resulting in 15 rumen samples per calf. Samples were collected via oresophageal tube at 0900 h using an attached 60-mL syringe for aspiration; pH measures were taken at sampling. Approximately 10 mL of each rumen sample was immediately transferred into sterile 15-mL polystyrene conical tubes and stored at −20°C for rumen microbiome analysis. The remaining rumen fluid was strained through 4 layers of cheesecloth and stored at −20°C in glass screw-top tubes for VFA and lactate analyses. The VFA was analyzed using GC using an adapted method from Kristensen (2000); D- and L-lactate were analyzed using an enzymatic test kit (Boehringer Mannheim D/L-Lactic Acid Test Combination; R-Biopharm AG, Darmstadt, Germany).

Rumen sample metagenomic DNA was extracted using the method of Yu and Morrison (2004) to determine *Megasphaera* prevalence and to analyze changes in rumen bacterial populations. Deoxyribonucleic acid was quantified on a NanoDrop (Thermo Fisher Scientific, Waltham, MA), diluted to 10 ng/μL, frozen at −20°C, and shipped overnight to the Molecular Cell Imaging Center at The Ohio State University (Wooster, OH) for 16S rRNA gene amplicon sequencing. Briefly, the V4-V5 hypervariable region of the 16S rRNA gene of each sample was amplified using universal primers 515F and 806R, with each library having a unique barcode. Amplicon libraries were pooled at equal molar ratios and sequenced using the V3 kit (2 × 300 paired-end kit) on an Illumina (San Diego, CA) MiSeq platform. The sequencing data were analyzed using QIIME (Caporaso et al., 2010) as described by Kigerl et al. (2016). Bases with quality scores lower than 25 were removed, and then paired reads were joined together using the fastq-join algorithm (Aronesty, 2011). Clean sequences shorter than 250 bp were removed. Chimera sequences were identified using ChimeraSlayer (Haas et al., 2011) and filtered out. Species-equivalent operational taxonomic units (**OTU**) were clustered using the pick open reference method against the Silva_119_release reference sequences at 97% similarity using the uclust algorithm of QIIME (Edgar, 2010). Taxonomic assignment of OTU was performed by comparing the representative sequence of each OTU against the same Silva reference database using the uclust consensus taxonomy assigner of QIIME (Edgar, 2010). Minor OTU each represented by either <0.005% of total sequences or <0.1% in all the samples were discarded (Bokulich et al., 2013). The sequences were deposited in the GenBank SRA database with the accession number SRP092199.

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