Comparative effects of two multi-species direct-fed microbial products on rumen fermentation, bacterial community and metabolome of beef steers

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INTRODUCTION

In the last decade, efforts to improve ruminant productivity have primarily focused on manipulating the ruminal microbial community and fermentation (DiLorenzo, 2011). One of such efforts include the use of direct-fed microbials (DFM). In recent years, most commercial DFM products are formulated to contain several microbial species and their fermentation products in order to ensure multi-factorial response (McAllister et al., 2011). The objective of this study was to compare the effects of two DFM products containing multiple microbial species and their fermentation products on ruminal fermentation, bacterial community and metabolome of beef steer during a three 21-d periods receiving period.

MATERIALS AND METHODS

- Nine rumen-cannulated Holstein steers (mean ± SD body weight (BW): 243 ± 12.4 kg) were assigned to 3 treatments arranged in a 3 × 3 Latin square design with three 21-d periods and 10-d wash-out between periods.
- Dietary treatments were (1) Control (CON; basal diet without additive), (2) Commence (PROB; basal diet plus 19 g/d of Commence), and (3) RX3 (SYNB; basal diet plus 28 g/d of RX3).
- Commence is a blend of active *S. cerevisiae*, *Enterococcus lactis*, *Bacillus subtilis*, *Enterococcus faecium, and L. case*i, and their fermentation products ((PMI, Arden Hills, MN).
- RX3 is a blend of active *S. cerevisiae* and the fermentation products of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus licheniformis*, and *Bacillus subtilis* (PMI, Arden Hills, MN).
- On d 21 of each period, 150 mL of the ruminal contents were collected via the cannula at 5 different sites within the rumen at approximately 1, 3, 6, 9, 12, and 18 h after feeding.
- Ruminal fluid samples collected at different time points were used analyze the following;
  - VFA, lactate, and ammonia nitrogen concentrations
  - Untargeted metabolome profile using a chemical isotope labeling (CIL)/ liquid chromatography–mass spectrometry (LC-MS)-based technique to target the amine/phenol-containing submetabolome
  - Bacterial community composition using 16S rRNA sequencing

RESULTS

Table 1. Effects of dietary supplementation of direct-fed microbial containing multiple microbial species and their fermentation products on the rumen fermentation of beef steers

Item	CON	PROB	SYNB	SEM	P-value
Acetate, mM	25.8	27.8	30.5	2.02	0.32
Propionate, mM	8.32^{b}	11.1^{a}	12.3^{a}	0.95	0.03
Valerate, mM	0.99^{b}	1.76^{a}	1.55^{a}	0.18	0.01
Isovalerate, mM	0.43^{y}	0.55^{x}	0.58^{x}	0.05	0.10
Butyrate, mM	6.89	7.99	7.90	0.77	0.83
Isobutyrate, mM	0.32	0.35	0.39	0.04	0.58
Total VFA, mM	52.5^{b}	59.9^{a}	61.6^{a}	2.71	0.01
Lactate, mM	0.63	0.69	0.53	0.20	0.63
Ammonia-N, mM	3.72^{a}	2.13^{b}	3.21^{a}	0.42	0.05
pH	6.67	6.54	6.71	0.12	0.52

CON = control; PROB = a blend of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus subtilis*, *Enterococcus faecium, and L. case*i, and their fermentation products fed at 19 g/steer/day (PMI, Arden Hills, MN); SYNB = a blend of live *S. cerevisiae* and the fermentation products of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus licheniformis*, and *Bacillus subtilis* fed at 28 g/steer/day PMI, Arden Hills, MN).
SEM = standard error of mean;
^{a,b}Within a row, treatment means with different superscripts differ, $P \leq 0.05$.
^{x,y}Within a row, treatment means with different superscripts tend to differ, $0.05 < P \leq 0.10$.

Table 2. Effects of dietary supplementation of direct-fed microbial containing multiple microbial species and their fermentation products in the diet of beef steers on the relative abundance of ruminal bacteria at the genus level.

Genus (% of total sequences)	CON	PROB	SYNB	SEM	P-value
Prevotella 1	72.6^{a}	65.6^{b}	64.1^{b}	2.67	0.01
Prevotella 7	0.30^{b}	0.96^{b}	12.8^{a}	3.51	0.01
Rikenellaceae RC9 gut group	1.96^{b}	5.62^{a}	2.44^{b}	0.96	0.01
Succinivibrio	0.07^{b}	0.34^{b}	4.96^{a}	1.17	0.02
Succinivibrionaceae UCG-001	0.88^{b}	4.44^{a}	0.00^{b}	1.39	0.01
Succiniclasticum	0.19^{b}	1.34^{a}	1.69^{a}	0.42	0.01
Ruminococcaceae UCG-014	0.57^{b}	1.57^{a}	1.66^{a}	0.41	0.04
Ruminococcaceae UCG-002	0.83^{b}	1.43^{a}	0.79^{b}	0.23	0.05
Prevotellaceae UCG-001	1.14^{a}	0.60^{b}	0.44^{b}	0.17	0.01

CON = control; PROB = a blend of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus subtilis*, *Enterococcus faecium, and L. case*i, and their fermentation products fed at 19 g/steer/day (PMI, Arden Hills, MN); SYNB = a blend of live *S. cerevisiae* and the fermentation products of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus licheniformis*, and *Bacillus subtilis* fed at 28 g/steer/day PMI, Arden Hills, MN).
SEM = standard error of mean;
Only bacterial genera (≥ 0.1% of total sequences) that were different in any of the treatment groups ($P \leq 0.05$) are shown.
^{a,b}Within a row, treatment means with different superscripts differ, $P \leq 0.05$.

RESULTS

Table 3. Identified metabolites that were affected by supplemental PROB

Item	Normalized RT	FC	P-value	Identification level
Taurine	157.6	1.50	0.04	Tier 1
Glutamyl-proline	389.6	1.42	0.03	Tier 1
Valine	662.4	1.26	0.04	Tier 1
Phenylalanine	783.2	1.73	0.01	Tier 1
Cystathionine	830.8	0.70	0.04	Tier 1
Norleucine	837.8	1.63	0.01	Tier 1
Histidinyl-proline	1098.2	1.25	0.03	Tier 1
Tyrosine	1363.3	1.62	0.04	Tier 1
Para-cresol	1482.6	1.42	0.01	Tier 1
Isomer of (R)-1-aminopropan-2-ol	413	0.51	0.04	Tier 2
3-(2,3-dihydroxyphenyl)propanoate	756.9	0.72	0.02	Tier 2

Normalized RT (retention time).
FC: fold change relative to Control; Tier 1 - Positive Identification (CIL Library); Tier 2 - High Confidence Putative Identification (LI Library). No identified metabolites were affected by supplemental SYNB.
PROB = a blend of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus subtilis*, *Enterococcus faecium, and L. case*i, and their fermentation products fed at 19 g/steer/day (PMI, Arden Hills, MN); SYNB = a blend of live *S. cerevisiae* and the fermentation products of *S. cerevisiae*, *Enterococcus lactis*, *Bacillus licheniformis*, and *Bacillus subtilis* fed at 28 g/steer/day PMI, Arden Hills, MN).
Only metabolites with both fold-change ≥ 1.2 or ≤ 0.83, relative to Control and $P \leq 0.05$ are shown.

CONCLUSIONS

- This study demonstrated that supplementation of PROB or SYNB differentially altered the ruminal bacterial community towards increased relative abundance of bacteria (*Succinivibrio*, *Rikenellaceae*, and *Succinivibrionaceae*) that can produce succinate and that of *Succiniclasticum* that can ferment succinate to propionate.
- The differential bacterial community and metabolome shift caused by the additives resulted into similar concentrations of ruminal fermentation acids

REFERENCES

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