

SUPPLEMENTAL MATERIALS

Table S1. Frequency of occurrence of cows removed from the experiment fed a control diet (CON) or control diet plus direct-fed microbial (GF) from -21 ± 5 d relative to calving through 100 DIM.

Event	Treatment	
	CON	GF
Total	10	6
Lameness	1	0
Mastitis	0	1
Pneumonia	1	0
Twins	1	1
Calved ahead	2	0
Unadapted to Calan gate system	2	1
Displaced abomasum	2	1
Retained placenta	0	1
Abortion	0	1
Death	1	0

Table S2. Species-specific primers used in real-time qPCR assay for the quantification of selected rumen bacteria population.

Target bacterial species		Primer sequence (5'- 3')	Reference
<i>Anaerovibrio lipolytica</i>	F ^a	GAAATGGATTCTAGTGGCAAACG	(Abdelmegeid et al., 2018)
	R ^b	ACATCGGTCATGCGACCAA	
<i>Butyrivibrio fibrisolvens</i>	F	ACACACCGCCCGTACCA	(Klieve et al., 2003)
	R	TCCTTACGGTTGGGTCACAGA	
<i>Butyrivibrio proteoclasticus</i>	F	GGGCTTGCTTTGGAAACTGTT	(Abdelmegeid et al., 2018)
	R	CCCACCGATGTTCTCTCTAA	
<i>Eubacterium ruminantium</i>	F	CTCCCGAGACTGAGGAAGCTTG	(Abdelmegeid et al., 2018)
	R	GTCCATCTCACACCACCGGA	
<i>Fibrobacter succinogenes</i>	F	GCGGGTAGCAAACAGGATTAGA	(Abdelmegeid et al., 2018)
	R	CCCCCGGACACCCAGTAT	
<i>Megasphaera elsdenii</i>	F	AGATGGGGACAACAGCTGGA	(Abdelmegeid et al., 2018)
	R	CGAAAGCTCCGAAGAGCCT	
<i>Prevotella albensis</i>	F	GCGCCACTGACGCTGAAG	(Khafipour et al., 2009)
	R	CCCCAAATCCAAAAGGACTCAG	
<i>Prevotella brevis</i>	F	GGTTTCCTTGAGTGTATTCGACGTC	(Stevenson and Weimer, 2007b)
	R	CTTTCGCTTGCGCGCTG	
<i>Prevotella bryantii</i>	F	AGCGCAGGCCGTTTGG	(Abdelmegeid et al., 2018)
	R	GCTTCCTGTGCACTCAAGTCTGAC	
<i>Prevotella ruminicola</i>	F	GAAAGTCGGATTAATGCTCTATGTTG	(Stevenson and Weimer, 2007b)
	R	CATCCTATAGCGGTAAACCTTTGG	
<i>Rumicoccus albus</i>	F	ACGTCRTCCMCACCTTCCTC	(Koike and Kobayashi, 2001)
	R	CCTCCTTGCGGTTAGAACA	
<i>Rumicoccus flavefaciens</i>	F	CGAACGGAGATAATTTGAGTTTACTTAGG	(Denman and McSweeney, 2006)
	R	CGGTCTCTGTATGTTATGAGGTATTACC	
<i>Ruminobacter amylophilus</i>	F	CTGGGGAGCTGCCTGAATG	(Stevenson and Weimer, 2007b)
	R	GCATCTGAATGCGACTGGTTG	
<i>Selenomonas ruminantium</i>	F	CAATAAGCATTCCGCCTGGG	(Abdelmegeid et al., 2018)
	R	TTCACCTCAATGTCAAGCCCTGG	
<i>Succinimonas amylolytica</i>	F	CGTTGGGCGGTCAATTTGAAAC	(Abdelmegeid et al., 2018)
	R	CCTGAGCGTCAGTTACTATCCAGA	
<i>Succinivibrio dextrinosolvens</i>	F	TAGGAGCTTGTGCGATAGTATGG	(Abdelmegeid et al., 2018)
	R	CTCACTATGTCAAGGTCAGGTAAGG	
<i>Streptococcus bovis</i>	F	TTCCTAGAGATAGGAAGTTTCTTCGG	(Abdelmegeid et al., 2018)
	R	ATGATGGCAACTAACAATAGGGGT	
<i>Treponema bryantii</i>	F	AGTCGAGCGGTAAGATTG	(Tajima et al., 2001)
	R	CAAAGCGTTTCTCTCACT	
Bacteria general 1	F	GGATTAGATACCCTGGTAGT	(Abdelmegeid et al., 2018)
	R	CACGACACGAGCTGACG	
Bacteria general 2	F	GTGSTGCAYGGYTGTCTGTC	(Abdelmegeid et al., 2018)
	R	ACGTCRTCCMCACCTTCCTC	

^aF: forward primer

^bR: reverse primer

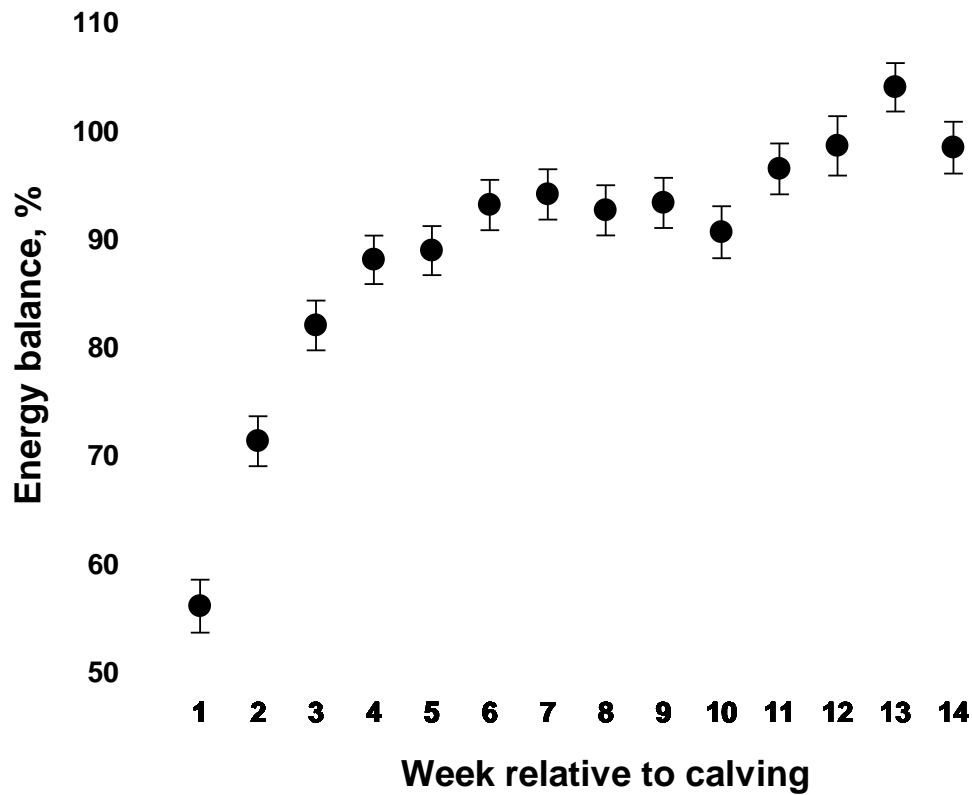


Figure S1. Energy balance as % of requirements for all cows enrolled in this study. Energy balance for multiparous cows was calculated as $NEB = [NE_I / (NE_M + NE_{Milk})] \times 100$, where NE_I is NE_L intake, NE_M is NE_L for maintenance, and ME_{milk} is NE_L for milk synthesis. From 1- 5 wk, energy balance is incremental, while it plateaus after 6 wk. Energy balance for primiparous cows was calculated as $NEB = [NE_I / (NE_M + NE_{Milk} + NE_G)] \times 100$.

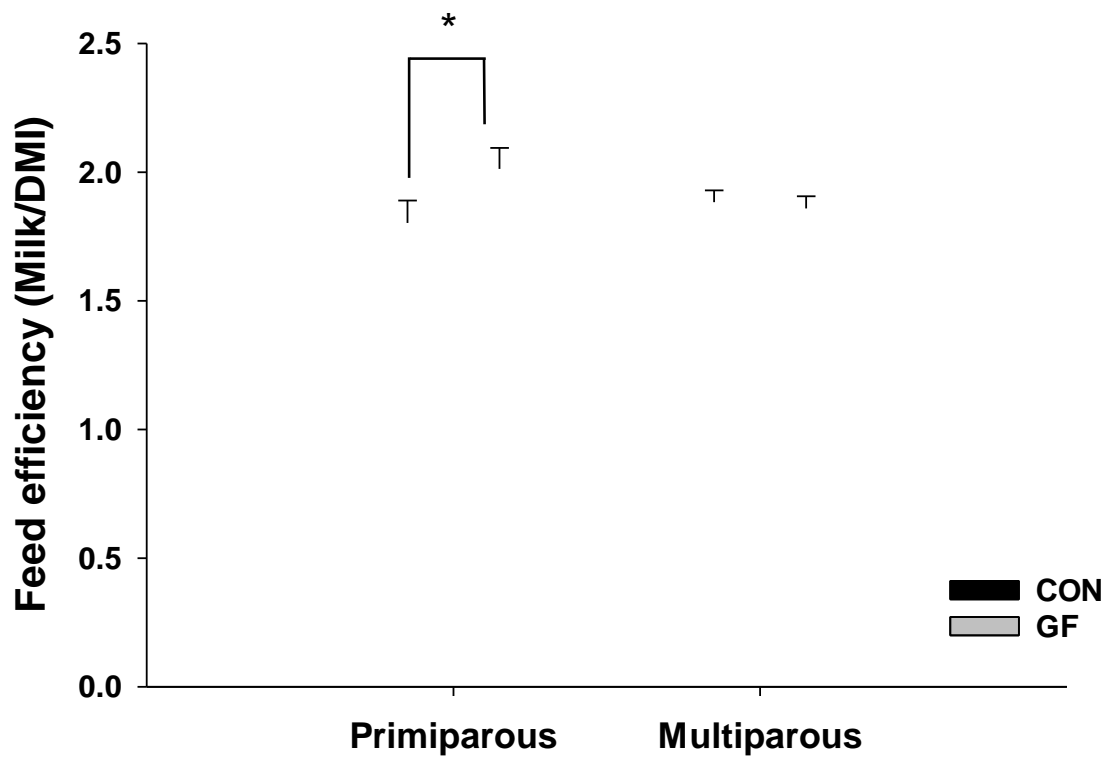


Figure S2. Feed efficiency in terms of milk/DMI across parity in dairy cows fed a control diet (CON) or control diet plus a rumen-derived DFM (GF) from -21 ± 5 d relative to calving through 100 DIM. Mean separations between diets at a given time point were evaluated when at least a trend for a treatment \times time (Trt \times T) interaction ($P \leq 0.10$) was observed, and differences (*) were declared at $P \leq 0.05$. Values are means, and the standard errors are represented by vertical bars.

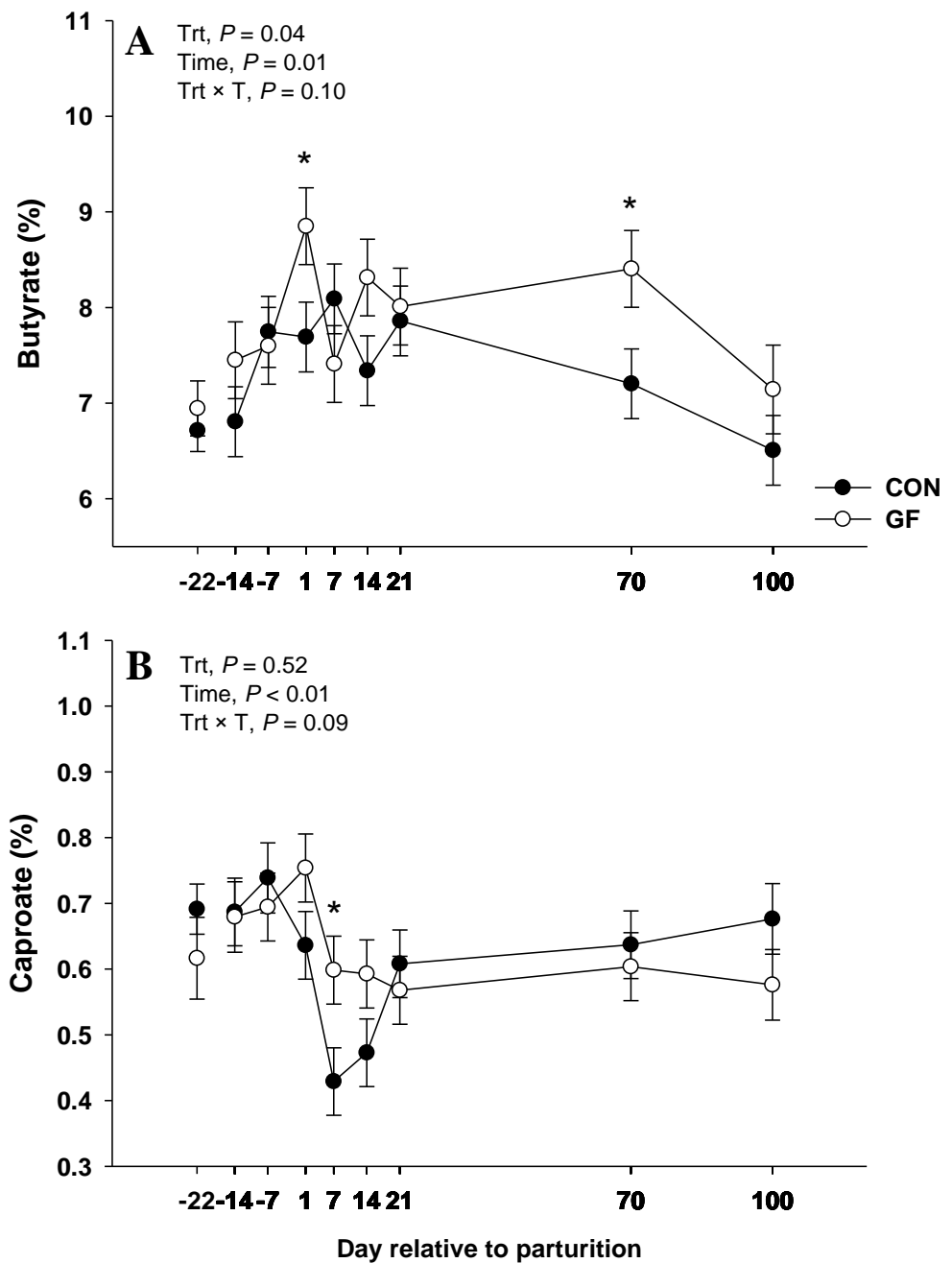


Figure S3. Ruminal butyrate (A) and caproate (B) in dairy cows fed a control diet (CON) or control diet plus a rumen-derived DFM (GF) from -21 ± 5 d relative to calving through 100 DIM. Mean separations between diets at a given time point were evaluated when at least a trend for a treatment \times time (Trt \times T) interaction ($P \leq 0.10$) was observed, and differences (*) were declared at $P \leq 0.05$. Values are means, and the standard errors are represented by vertical bars.

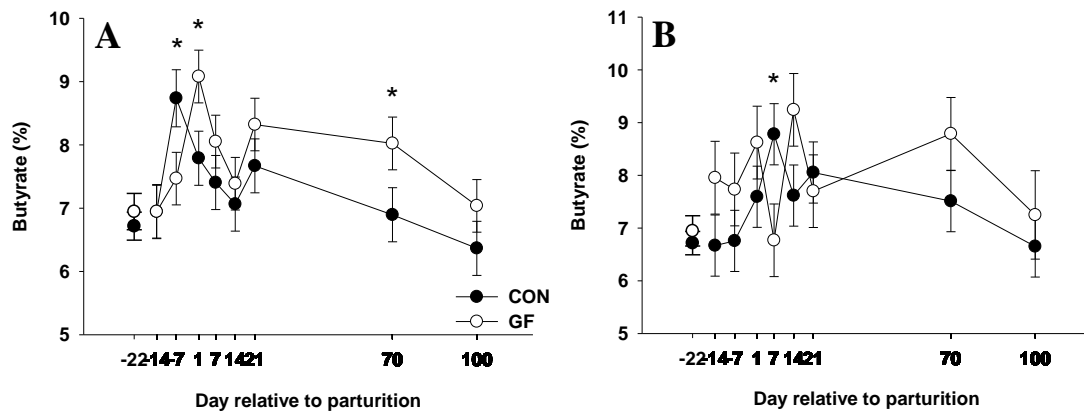


Figure S4. Ruminal butyrate in 2nd (A) and 3rd (B) lactation dairy cows fed a control diet (CON) or control diet plus a rumen-derived DFM (GF) from -21 ± 5 d relative to calving through 100 DIM. Mean separations between diets at a given time point were evaluated when at least a trend for a treatment × time (Trt × T) interaction ($P \leq 0.10$) was observed, and differences (*) were declared at $P \leq 0.05$. Values are means, and the standard errors are represented by vertical bars.

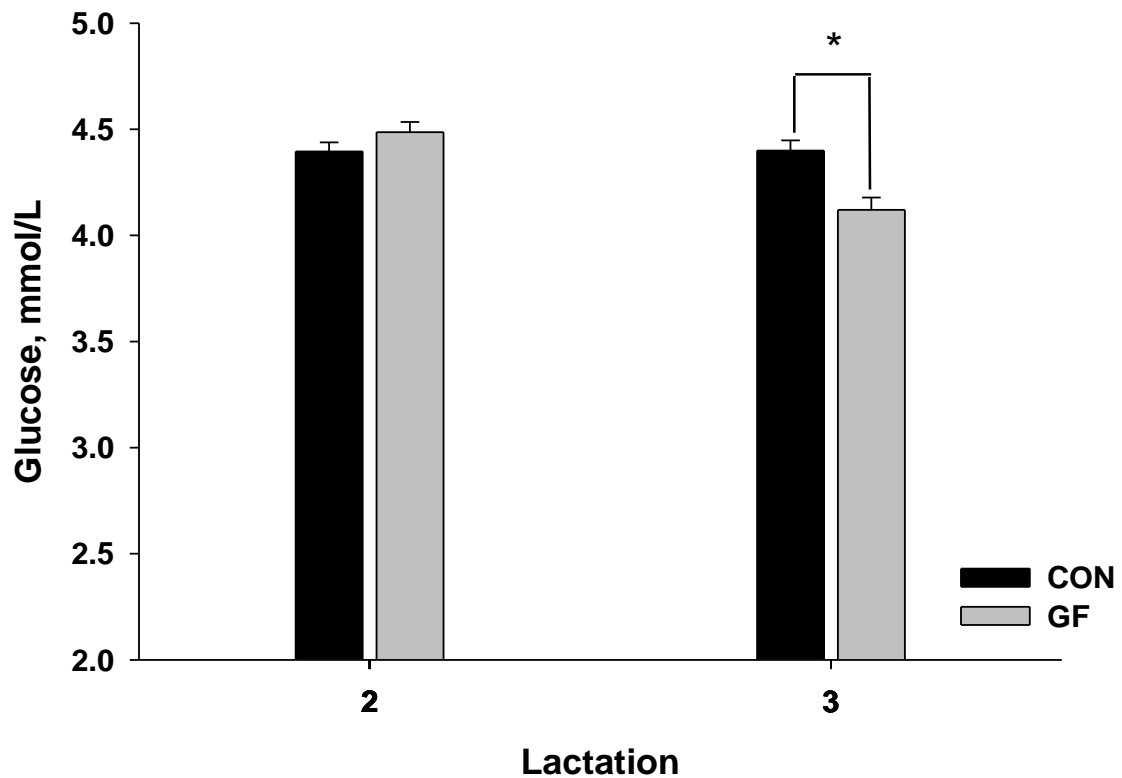


Figure S5. Blood glucose across 2nd and 3rd lactation dairy cows fed a control diet (CON) or control diet plus a rumen-derived DFM (GF) from -21 ± 5 d relative to calving through 100 DIM. Mean separations between diets at a given time point were evaluated when at least a trend for a treatment \times time (Trt \times T) interaction ($P \leq 0.10$) was observed, and differences (*) were declared at $P \leq 0.05$. Values are means, and the standard errors are represented by vertical bars.

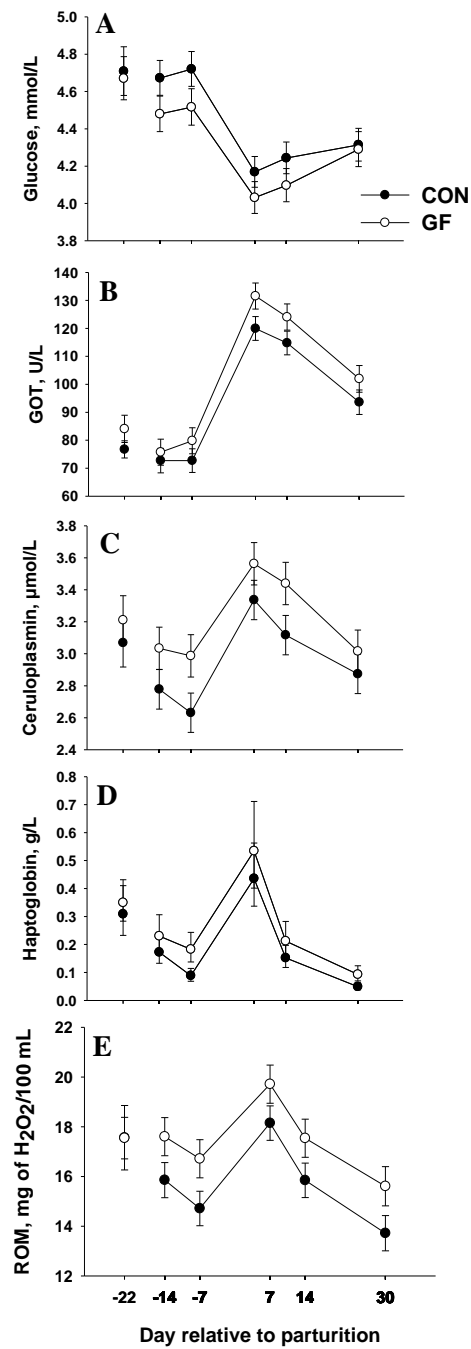


Figure S6. Blood glucose (A), glutamic-oxaloacetic transferase (GOT; B), ceruloplasmin (C), haptoglobin (D), and reactive oxygen metabolites (E) in dairy cows fed a control diet (CON) or control diet plus a rumen-derived DFM (GF) from -21 ± 5 d relative to calving through 100 DIM. Values are means, and the standard errors are represented by vertical bars.