

Supplementary Materials

Table S1. Standardization of measurement of short-chain fatty acids.

	Acetic acid	Propionic acid	Butyric acid	Isovaleric acid
R ²	0.9995	0.9995	0.9998	0.9999
Equation	$y = 0.0094x + 0.0057$	$y = 0.0163x - 0.0228$	$y = 0.0241x - 0.0081$	$y = 0.0308x - 0.0061$
Linear range (mmol L ⁻¹)*	5.08 - 101.62	1.83 - 73.34	1.45 - 46.38	0.70 - 22.30
LOD (mmol L ⁻¹)	1.27	0.92	0.72	0.35
LOQ (mmol L ⁻¹)	2.54	1.83	1.45	0.70
Accuracy	105.96	109.16	104.11	103.39
Repeatability (RSD)	2.76	4.30	1.66	2.54

*The linear range, LOD (limit of detection) and LOQ (limit of quantitation) were expressed in mmol of SFA for L of ruminal fluid.

Table S2. Treatment × day interaction for erythrogram and urea variables.

Variables	Crossbred	Angus	Holstein	SEM	P: treat x day*
Erythrocytes (x10 ⁶ µL)					0.01
d0	7.36	7.10	8.05	0.04	
d30	7.86	7.18	7.53	0.05	
d81	6.45b	8.02a	5.37b	0.05	
d130	6.52b	7.94b	7.03ab	0.05	
Hemoglobin (mg/dL)					0.01
d0	10.2	10.0	10.4	0.09	
d30	11.2	10.1	10.6	0.08	
d81	11.0b	12.6a	9.56c	0.09	
d130	11.9b	13.1a	11.8	0.08b	
Hematocrit (%)					0.01
d0	30.1	29.2	30.7	0.37	
d30	31.4	28.9	30.2	0.35	
d81	30.4ab	32.8a	27.8b	0.37	
d130	32.7a	36.3a	33.5a	0.36	
Urea (mg/dL)					0.03
d0	17.4	19.1	16.5	2.94	
d30	21.0ab	26.9a	16.0b	2.86	
d81	26.4ab	28.3a	21.4b	2.87	
d130	33.3ab	38.8a	29.5b	2.79	

*Different letters on each day (line - a,b) indicate difference between groups, represented the treatment × day interaction (P<0.01).

Table S3. Short-chain fatty acids (SCFAs) in ruminal fluid from Holstein, Angus, and Holstein × Angus crossbred steers; results presented as proportions (%).

Variables	Crossbred	Angus	Holstein	SEM ¹	P: treat ²	P: treat × day ³
Acetic acid (%)					0.75	0.22
D81	67.5	68.1	66.6	0.66		
D130	66.5	67.5	67.2	0.65		
Propionic acid (%)					0.81	0.03
D81	20.3a	19.8a	17.1b	0.39		
D130	20.7	19.0	18.8	0.40		
Butyric acid (%)					0.12	0.01
D81	9.50b	9.19b	12.3a	0.32		
D130	10.3	11.0	11.0	0.31		
Isovaleric acid (%)					0.21	0.01
D81	1.53b	1.46b	2.24a	0.08		
D130	1.33	1.27	1.50	0.07		
Valeric acid (%)					0.17	0.01
D81	0.98c	1.21ab	1.41a	0.06		
D130	1.06	1.02	1.17	0.06		

¹SEM – Standard Error of the mean. ² There was no treatment effect (P > 0.05). ³The treatment × day interaction was significant at P < 0.05; illustrated with different superscripts (a,b) in each row.

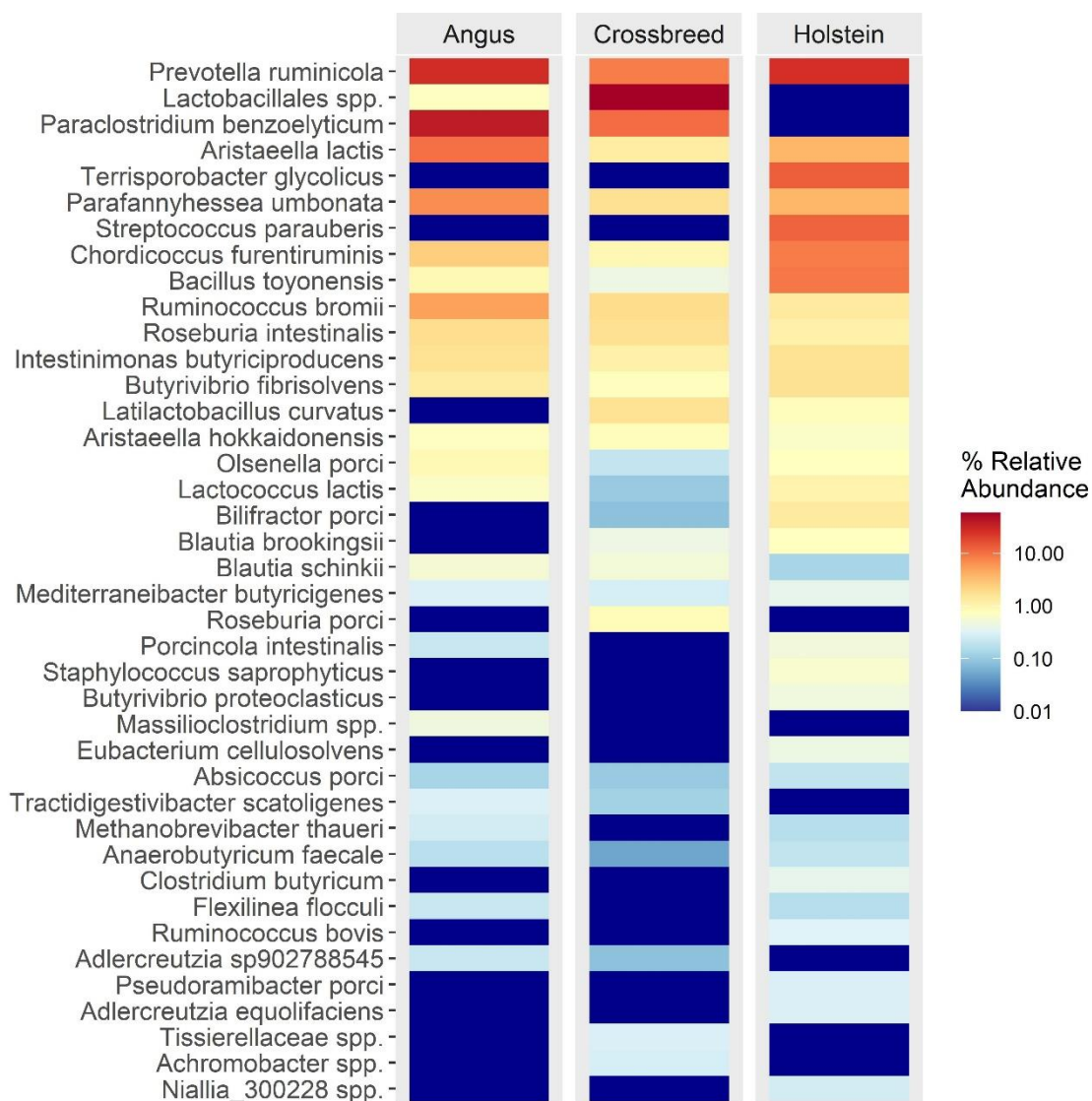


Figure S1. Heatmap of relative abundance of the 40 core microorganisms in the ruminal fluid of cattle from different experimental groups.