

Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures

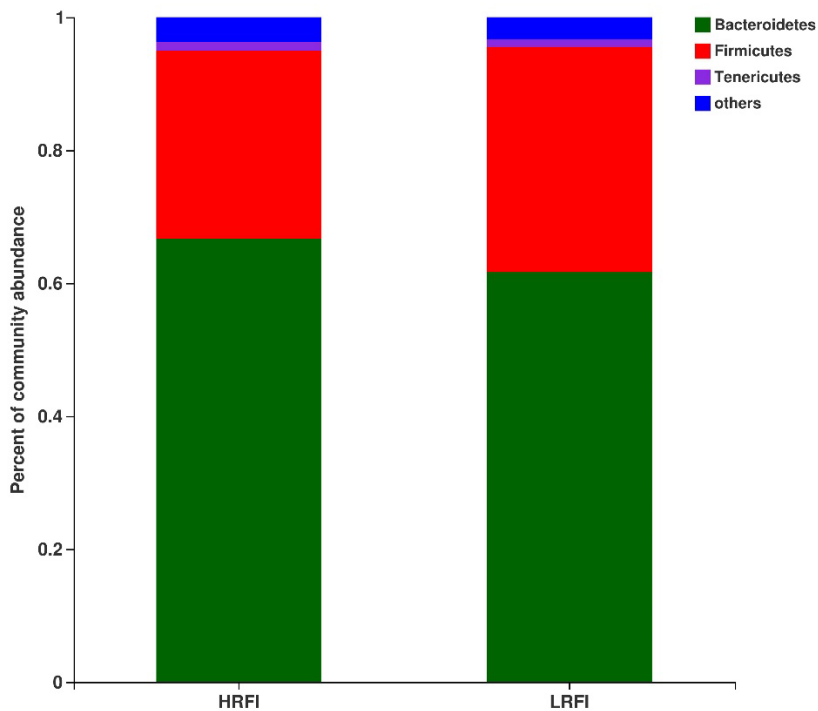
Supplementary Figure 1 shows the ruminal bacteria composition of HRFI and LRFI Angus heifers. (A) the average relative abundances of phyla. (B) the average relative abundances of families. (C) the average relative abundances of genera. (relative abundance > 1% for all samples).

Supplementary Figure 2 shows the overlap of the total ion chromatogram of the QC sample in (A) the positive and (B) the negative ion modes, respectively. The results confirm the reliable repeatability and precision of the data obtained in the present study.

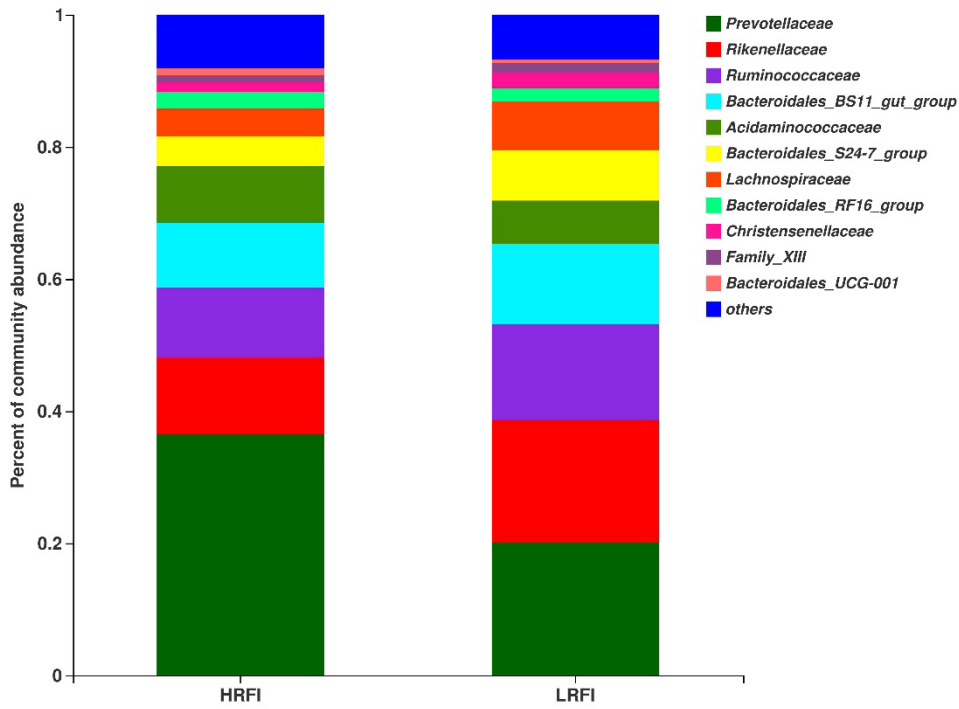
Supplementary Figure 3 shows samples examined by PCA following (A) the positive and (B) the negative mode ionization to provide a global overview of the differences among the metabolite data.

Supplementary Figure 1

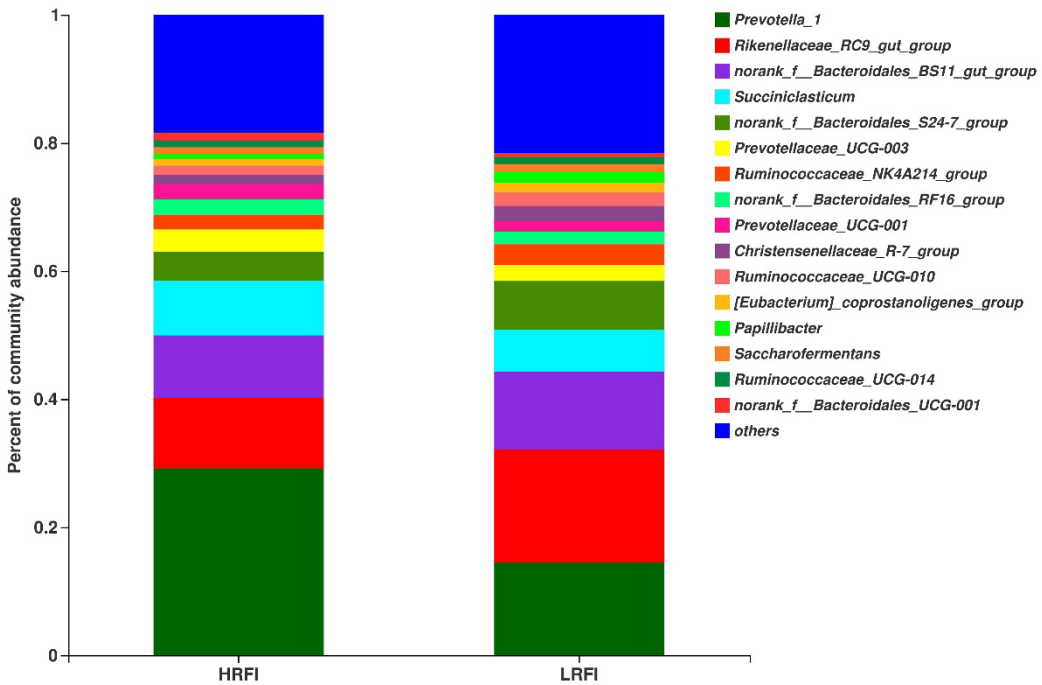
A



B



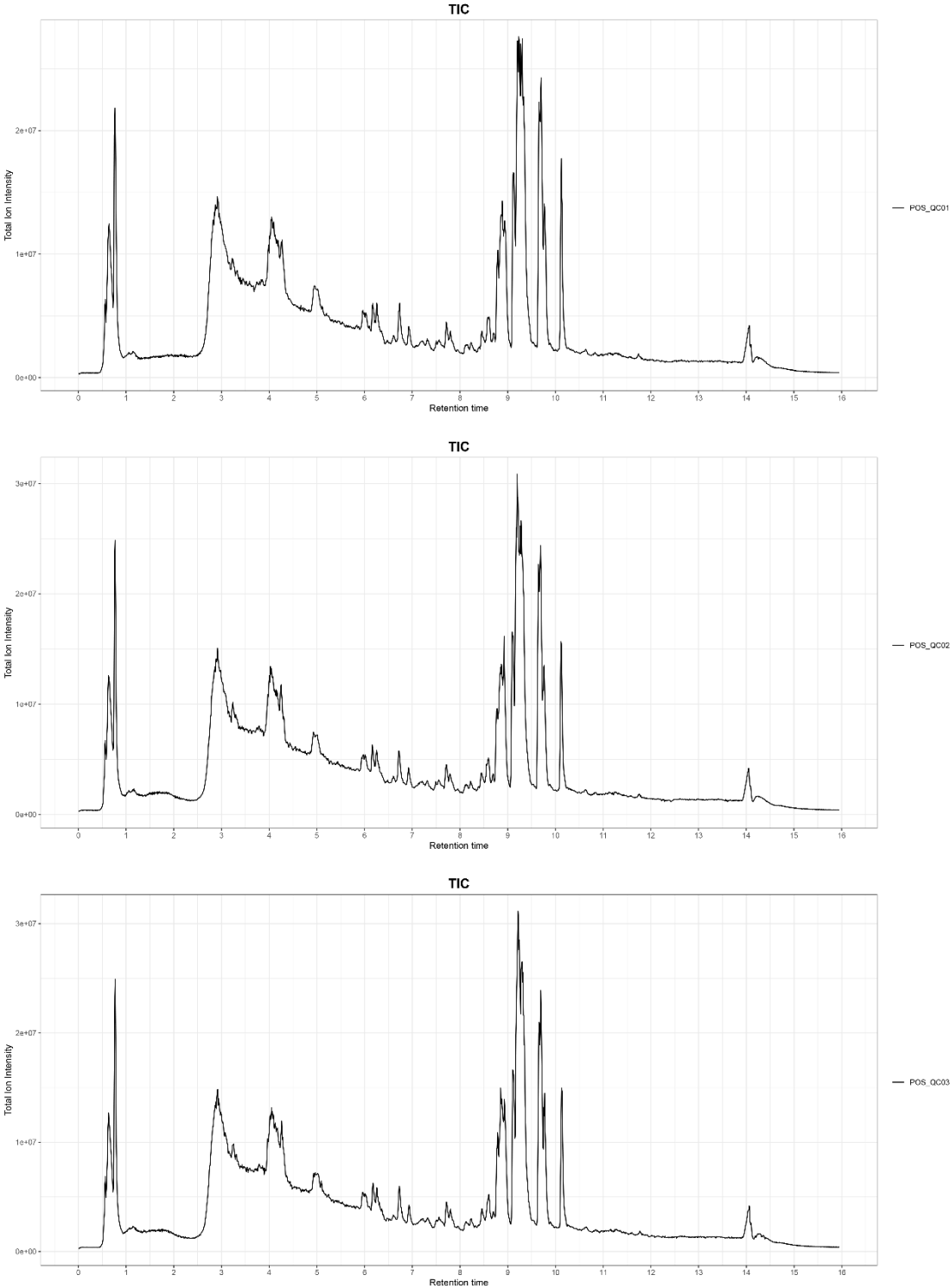
C

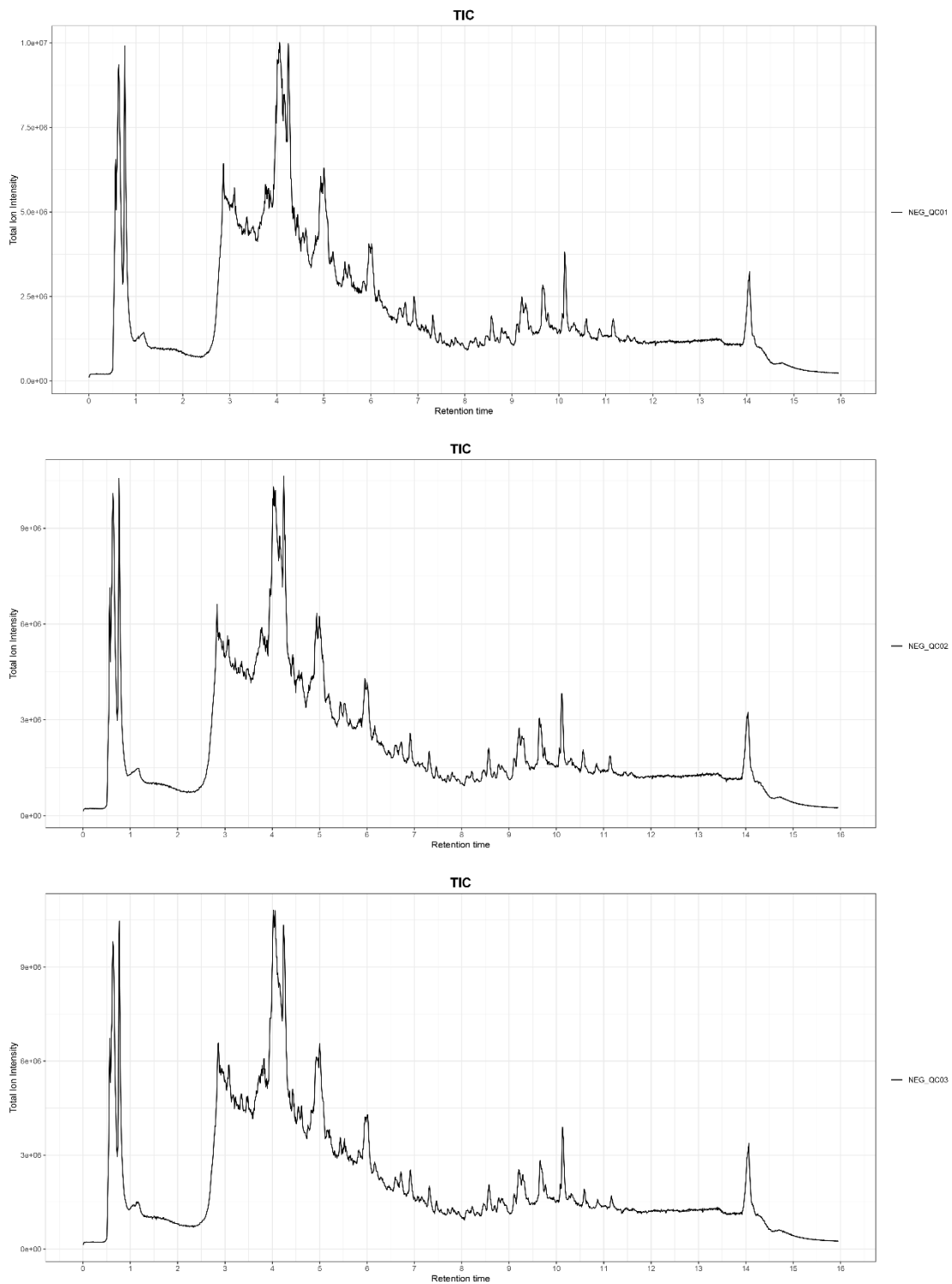


Supplementary Figure 1. Stacked bar graphs of the average relative abundances of phyla (A), families (B), and genera (C) (relative abundance > 1% for all samples).

Supplementary Figure 2

A

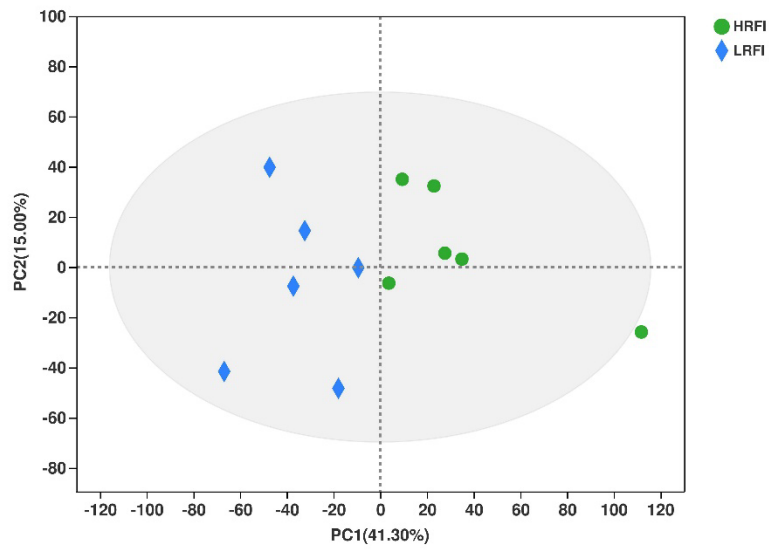


B

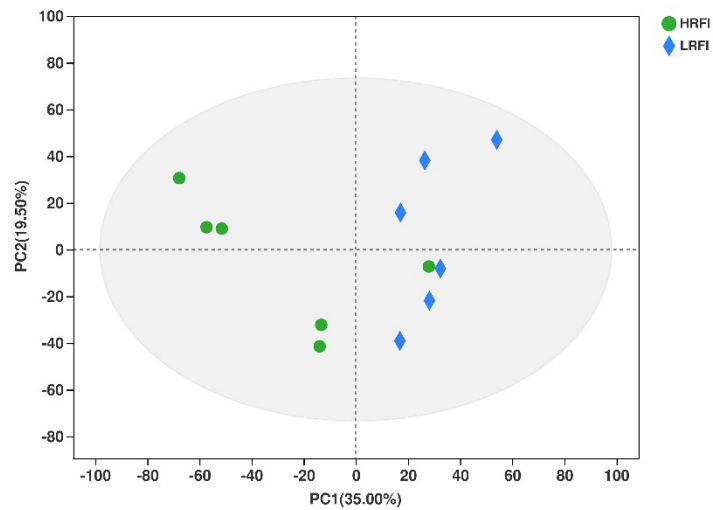
Supplementary Figure 2. LC-MS total ion chromatogram of the QC sample in (A) the positive ion mode and (B) the negative ion mode.

Supplementary Figure 3

A



B



Supplementary Figure 3. The PCA plots of the ruminal samples corresponding to the HRFI and LRFI groups following (A) the positive and (B) the negative modes ionization.

1.2 Supplementary Tables

Supplementary Table 1. Ingredients and chemical composition of the experimental diet.

Item	Value
Ingredient composition, % DM	
Whole corn silage	30.00
Wheat shell powder	20.00
Corn	28.00
Cottonseed meal	10.50
Corn germ meal	10.00
Premix ¹	0.20
Salt	0.50
MgO	0.20
Limestone	0.60
Chemical composition (DM basis), %	
CP	13.25
NDF	43.62
ME, Mcal/kg	2.59

¹Mineral supplement contains 0.198% cobalt as well as 0.9228% copper, 8.0376% iron, 0.0754% iodine, 5.8131% manganese, 0.0366% selenium, and 6.635% zinc.

Supplementary Table 2. Residual feed intake (RFI) values of the 42 Angus heifers evaluated in the present study.

Animal number	RFI	Group¹
30	-1.951	LRFI
51	-1.823	LRFI
17	-1.662	LRFI
40	-0.998	LRFI
49	-0.825	LRFI
66	-0.703	LRFI
57	-0.663	LRFI
3	-0.654	LRFI
62	-0.614	LRFI
1	-0.436	LRFI
86	-0.413	LRFI
15	-0.387	LRFI
10	-0.351	NS
11	-0.329	NS
56	-0.307	NS
44	-0.181	NS

72	-0.155	NS
5	-0.133	NS
71	-0.095	NS
80	-0.028	NS
8	0.008	NS
73	0.053	NS
12	0.217	NS
50	0.332	NS
14	0.350	NS
48	0.398	HRFI
24	0.411	HRFI
37	0.441	HRFI
13	0.461	HRFI
82	0.520	HRFI
55	0.541	HRFI
68	0.545	HRFI
2	0.614	HRFI
7	0.664	HRFI
55	0.709	HRFI
37	0.719	HRFI
28	0.729	HRFI
29	0.757	HRFI
39	0.843	HRFI
45	0.904	HRFI
36	0.907	HRFI
64	1.584	HRFI

¹ Bold lines represent the 12 heifers selected for evaluation in this study; NS: $-0.38 < \text{RFI} < 0.38$.

Supplementary Table 3. Animal performances of the 12 Angus heifers selected in the present study.

Animal number	Initial weight, kg	DMI³, kg/d	ADG⁴, kg/d	RFI, kg/d
LRFI¹				
30	420.45	7.01	1.34	-1.951
51	424.31	7.15	1.20	-1.823
17	415.42	6.58	0.66	-1.662
40	386.15	7.74	0.88	-0.998
49	417.10	8.77	0.98	-0.825
66	412.21	8.72	1.00	-0.703
HRFI²				
28	431.41	8.82	1.09	0.729
29	434.62	8.80	1.00	0.757
39	429.50	10.12	0.86	0.843
45	399.68	10.38	0.93	0.904
36	433.32	9.05	0.74	0.907
64	425.15	9.91	0.66	1.584

¹ LRFI: low residual feed intake; ² HRFI: high residual feed intake; ³ DMI: dry matter intake; ⁴ ADG: average daily gain.

Supplementary Table 4. Alpha diversity of ruminal microbial communities in composition of the HRFI and LRFI groups.

Items ¹	HRFI	LRFI	SEM ²	P-value ³
Chao 1	1453.60	1480.70	71.42	0.53
Shannon	5.57	5.64	0.241	0.63
Simpson	0.013	0.012	0.007	0.79

¹ HRFI, high residual feed intake; LRFI, low residual feed intake. ² SEM, standard error of the mean.

³ P-values are derived using a Student's t-test to assess the differences between the HRFI and LRFI groups.