

1 **Tables S-S7;Fig.S1**

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4 **TABLE S1.** Summary of dietary treatment comparisons for unique OTUs, richness estimates, and diversity indices. The minimum
 5 number of unique OTUs in each population was determined with a 10 % difference level.

	Forage	Mixed forage	High grain	Acidotic challenge	Challenge recovery	SEM	<i>P</i> -value
Number of sequences	5045	3731	4349	4148	4389	579	0.68
Coverage (%)	99.0	98.8	99.1	98.9	99.0	0.2	0.64
Total # of unique OTUs	161	147	144	148	149	11	0.90
Richness estimate							
Chao1	198	181	183	192	181	16	0.94
ACE	192	180	179	192	189	15	0.94
Diversity indices							
Shannon-Weiner	3.72	3.36	3.31	3.43	3.19	0.14	0.12
Simpson's	0.95	0.92	0.91	0.93	0.89	0.02	0.16

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10 **TABLE S2.** Percent contribution of genus level epithelial taxa to the rumen microbial populations averaged over all treatments for
 11 individual animals. Treatments include forage, mixed forage, high grain, acidotic challenge and challenge recovery. Remaining genera
 12 not shown due to non-significant differences between treatments.

Genera	Individual animals								SEM	P-Value
	7	41	43	143	153	156	315	346		
<i>12-18</i>	0.0	0.0	0.0	0.0	1.6	2.5	8.1	1.2	0.7	0.07
<i>adhufec405</i>	2.6	0.0	1.1	0.0	0.0	2.3	8.1	4.5	0.8	0.11
<i>Atopobacter</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.2	0.06
<i>Carboxydibrachium</i>	0.0	1.1	0.0	2.6	6.3	0.0	0.0	1.4	0.6	0.11
<i>Comamonas</i>	15.9a	5.6a	38.1b	40.8b	29.8b	34.2b	35.4b	29.4b	3.1	0.04
<i>Dialister</i>	0.0	1.3	1.3	0.0	1.4	3.5	10.6	3.2	0.9	0.07
<i>F24-B10</i>	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	5.4b	0.0a	0.3	<0.001
<i>Guggenheimella</i>	4.3ab	2.7a	6.8ab	10.9b	5.7ab	11.8b	2.8ab	9.4ab	0.8	0.03
<i>IS Eub. cellulosolvans</i>	5.3	2.1	0.0	0.0	1.3	0.0	0.0	1.4	0.5	0.09
<i>Oxobacter</i>	11.4	5.7	11.3	5.2	1.4	2.8	0.0	9.6	1.2	0.06
<i>p-4496-6Wb3</i>	2.5ab	0.0a	1.6ab	9.7b	2.2ab	0.0a	5.7ab	2.5ab	0.7	0.02
<i>Pelospora</i>	15.4ab	15.2ab	15.8ab	31.6b	3.2a	21.2ab	11.1ab	10.4ab	2.0	0.03
<i>rc1-13</i>	4.2	2.9	5.2	13.4	8.5	4.3	12.5	3.9	1.1	0.07
<i>RC20</i>	0.0a	0.0a	0.0a	0.0a	7.0b	0.0a	0.0a	1.4ab	0.6	0.01
<i>Succinivibrio</i>	0.0	0.0	1.3	0.0	0.0	3.1	9.2	3.9	0.8	0.07
<i>Thermotalea</i>	0.0a	4.9b	4.1b	4.0b	4.2b	0.0a	0.0a	0.0a	0.6	0.05
<i>U29-B03</i>	0.0a	0.0a	0.0a	0.0a	0.0a	3.5b	0.0a	0.0a	0.2	<0.001
<i>uncultured</i>	9.4ab	8.6ab	13.3b	4.2a	16.5b	4.3a	2.7a	10.9ab	1.2	0.05

<i>Verminophrobacter</i>	0.0a	0.0a	0.0a	6.4b	1.9ab	0.0a	1.8ab	4.2b	0.6	0.03
<i>wet75</i>	9.1ab	3.1a	7.5ab	11.4ab	11.8ab	19.01b	2.5a	7.8ab	1.2	0.05

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15 **TABLE S3.** Correlation of all calculated pH variables from the acidotic challenge treatment to epithelial genera. Only those genera
 16 found to be significant are shown. acidotic challenge pH variables are the mean for all animals on that dietary treatment.

Genera		pH variables								
		pH min	pH mean	pH max	Duration under pH 5.8 (min)	pH area under 5.8 (pH×min)	Duration under pH 5.5 (min)	pH area under 5.5 (pH×min)	Duration under pH 5.2 (min)	pH area under 5.2 (pH×min)
<i>Acetitomaculum</i>	Correlation	-0.32	-0.31	0.19	0.20	0.45	0.24	0.52	0.24	0.57
	<i>P</i> -Value	0.09	0.10	0.32	0.29	0.01	0.20	0.00	0.22	0.00
<i>Acidaminococcus</i>	Correlation	-0.32	-0.33	0.16	0.27	0.39	0.26	0.42	0.24	0.42
	<i>P</i> -Value	0.09	0.08	0.41	0.16	0.04	0.17	0.03	0.21	0.02
<i>Anaerophaga</i>	Correlation	-0.38	-0.61	0.12	0.25	0.62	0.32	0.71	0.41	0.80
	<i>P</i> -Value	0.04	0.00	0.52	0.18	0.00	0.09	0.00	0.03	0.00
<i>Anaerovorax</i>	Correlation	0.34	0.44	0.12	-0.43	-0.40	-0.45	-0.36	-0.38	-0.31
	<i>P</i> -Value	0.07	0.02	0.53	0.02	0.03	0.02	0.05	0.04	0.11
<i>Atopobacter</i>	Correlation	-0.30	-0.17	0.16	0.38	0.30	0.36	0.25	0.24	0.14
	<i>P</i> -Value	0.11	0.39	0.41	0.04	0.11	0.06	0.19	0.20	0.47
<i>Atopobium</i>	Correlation	-0.61	-0.58	-0.37	0.76	0.61	0.73	0.51	0.60	0.35
	<i>P</i> -Value	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.06
<i>Azonexus</i>	Correlation	0.39	0.40	-0.06	-0.50	-0.39	-0.48	-0.32	-0.43	-0.25
	<i>P</i> -Value	0.04	0.03	0.75	0.01	0.04	0.01	0.09	0.02	0.18
<i>Bacteroides</i>	Correlation	-0.11	-0.34	-0.07	0.07	0.29	0.13	0.35	0.20	0.39
	<i>P</i> -Value	0.57	0.08	0.72	0.72	0.13	0.52	0.06	0.30	0.03
<i>Butyrivibrio fibrisolvens</i>	Correlation	0.55	0.49	0.20	-0.59	-0.52	-0.57	-0.46	-0.51	-0.38
	<i>P</i> -Value	0.00	0.01	0.31	0.00	0.00	0.00	0.01	0.00	0.04
<i>Carboxydibrachium</i>	Correlation	0.40	0.27	0.26	-0.34	-0.27	-0.34	-0.23	-0.30	-0.16
	<i>P</i> -Value	0.03	0.15	0.18	0.07	0.15	0.07	0.23	0.11	0.40
<i>cc142</i>	Correlation	-0.43	-0.26	-0.17	0.43	0.24	0.38	0.15	0.31	0.03
	<i>P</i> -Value	0.02	0.18	0.38	0.02	0.21	0.04	0.43	0.11	0.87
<i>Comamonas</i>	Correlation	0.17	0.17	-0.06	-0.07	-0.30	-0.20	-0.36	-0.27	-0.39
	<i>P</i> -Value	0.38	0.37	0.78	0.71	0.11	0.29	0.06	0.16	0.04
<i>Dialister</i>	Correlation	-0.32	-0.36	0.06	0.37	0.34	0.36	0.31	0.39	0.28
	<i>P</i> -Value	0.09	0.06	0.74	0.05	0.07	0.05	0.11	0.03	0.13
<i>Eub. brachy</i>	Correlation	0.48	0.35	0.00	-0.37	-0.26	-0.31	-0.22	-0.28	-0.17

Genera		pH variables								
		pH min	pH mean	pH max	Duration under pH 5.8 (min)	pH area under 5.8 (pH×min)	Duration under pH 5.5 (min)	pH area under 5.5 (pH×min)	Duration under pH 5.2 (min)	pH area under 5.2 (pH×min)
	<i>P</i> -Value	0.01	0.06	0.99	0.05	0.17	0.10	0.26	0.14	0.37
<i>Fervidicola</i>	Correlation	0.43	0.34	-0.06	-0.33	-0.28	-0.34	-0.24	-0.35	-0.18
	<i>P</i> -Value	0.02	0.07	0.75	0.08	0.15	0.08	0.22	0.06	0.35
<i>Fusobacterium</i>	Correlation	0.48	0.37	0.61	-0.31	-0.25	-0.27	-0.22	-0.21	-0.18
	<i>P</i> -Value	0.01	0.05	0.00	0.10	0.19	0.16	0.26	0.28	0.36
<i>Incertae Sedis C. viride</i>	Correlation	0.47	0.33	-0.06	-0.35	-0.24	-0.29	-0.20	-0.27	-0.16
	<i>P</i> -Value	0.01	0.08	0.76	0.06	0.20	0.12	0.31	0.15	0.42
<i>IS B. fibrisolvens H15</i>	Correlation	-0.36	-0.54	0.11	0.34	0.56	0.36	0.61	0.47	0.66
	<i>P</i> -Value	0.05	0.00	0.55	0.07	0.00	0.05	0.00	0.01	0.00
<i>IS cTPY-17 adhufec52</i>	Correlation	-0.49	-0.45	-0.04	0.33	0.47	0.40	0.48	0.55	0.54
	<i>P</i> -Value	0.01	0.01	0.84	0.08	0.01	0.03	0.01	0.00	0.00
<i>IS R. gnavus</i>	Correlation	-0.38	-0.61	0.12	0.25	0.62	0.32	0.71	0.41	0.80
	<i>P</i> -Value	0.04	0.00	0.52	0.18	0.00	0.09	0.00	0.03	0.00
<i>Lactobacillus</i>	Correlation	-0.57	-0.66	0.09	0.36	0.74	0.43	0.84	0.53	0.93
	<i>P</i> -Value	0.00	0.00	0.64	0.05	0.00	0.02	0.00	0.00	0.00
<i>Marvinbryantia</i>	Correlation	0.45	0.42	-0.15	-0.45	-0.40	-0.45	-0.36	-0.41	-0.30
	<i>P</i> -Value	0.01	0.02	0.44	0.01	0.03	0.01	0.06	0.03	0.12
<i>Megasphaera</i>	Correlation	-0.52	-0.63	0.09	0.33	0.72	0.40	0.82	0.48	0.90
	<i>P</i> -Value	0.00	0.00	0.64	0.08	0.00	0.03	0.00	0.01	0.00
<i>Mitsuokella</i>	Correlation	-0.38	-0.61	0.12	0.25	0.62	0.32	0.71	0.41	0.80
	<i>P</i> -Value	0.04	0.00	0.52	0.18	0.00	0.09	0.00	0.03	0.00
<i>Olsenella</i>	Correlation	-0.61	-0.51	-0.05	0.41	0.58	0.45	0.61	0.57	0.67
	<i>P</i> -Value	0.00	0.00	0.79	0.03	0.00	0.01	0.00	0.00	0.00
<i>rc1-13</i>	Correlation	0.49	0.50	-0.09	-0.50	-0.52	-0.54	-0.49	-0.51	-0.42
	<i>P</i> -Value	0.01	0.01	0.63	0.01	0.00	0.00	0.01	0.01	0.02
<i>RC25</i>	Correlation	0.35	0.30	-0.29	-0.39	-0.32	-0.39	-0.26	-0.36	-0.21
	<i>P</i> -Value	0.06	0.11	0.13	0.03	0.09	0.04	0.17	0.05	0.28
<i>RC39</i>	Correlation	-0.75	-0.71	-0.08	0.64	0.79	0.69	0.79	0.70	0.77
	<i>P</i> -Value	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00
<i>RF21</i>	Correlation	0.44	0.45	-0.02	-0.35	-0.35	-0.31	-0.34	-0.29	-0.34
	<i>P</i> -Value	0.02	0.01	0.91	0.07	0.06	0.10	0.07	0.13	0.07

Genera		pH variables								
		pH min	pH mean	pH max	Duration under pH 5.8 (min)	pH area under 5.8 (pH×min)	Duration under pH 5.5 (min)	pH area under 5.5 (pH×min)	Duration under pH 5.2 (min)	pH area under 5.2 (pH×min)
<i>RF38</i>	Correlation	0.52	0.38	0.08	-0.43	-0.35	-0.41	-0.30	-0.35	-0.24
	<i>P</i> -Value	0.00	0.04	0.67	0.02	0.06	0.03	0.11	0.06	0.20
<i>RFN71</i>	Correlation	0.30	0.35	-0.36	-0.36	-0.42	-0.44	-0.40	-0.50	-0.35
	<i>P</i> -Value	0.12	0.06	0.06	0.05	0.02	0.02	0.03	0.01	0.07
<i>RFN8-YE57</i>	Correlation	0.42	0.42	0.06	-0.41	-0.42	-0.42	-0.40	-0.38	-0.32
	<i>P</i> -Value	0.02	0.02	0.77	0.03	0.02	0.02	0.03	0.04	0.09
<i>Ruminococcus 1</i>	Correlation	0.43	0.46	0.07	-0.42	-0.40	-0.42	-0.37	-0.42	-0.32
	<i>P</i> -Value	0.02	0.01	0.73	0.02	0.03	0.02	0.05	0.02	0.09
<i>Saccharofermentans</i>	Correlation	0.61	0.52	-0.04	-0.59	-0.51	-0.58	-0.44	-0.50	-0.36
	<i>P</i> -Value	0.00	0.00	0.86	0.00	0.01	0.00	0.02	0.01	0.05
<i>Selenomonas</i>	Correlation	-0.38	-0.61	0.12	0.25	0.62	0.32	0.71	0.41	0.80
	<i>P</i> -Value	0.04	0.00	0.52	0.18	0.00	0.09	0.00	0.03	0.00
<i>Solobacterium</i>	Correlation	-0.63	-0.59	-0.01	0.55	0.57	0.60	0.52	0.62	0.46
	<i>P</i> -Value	0.00	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.01
<i>Streptococcus</i>	Correlation	-0.43	-0.63	0.12	0.28	0.66	0.35	0.76	0.44	0.85
	<i>P</i> -Value	0.02	0.00	0.55	0.14	0.00	0.06	0.00	0.02	0.00
<i>Succiniclasticum</i>	Correlation	-0.43	-0.42	-0.29	0.60	0.47	0.57	0.39	0.42	0.27
	<i>P</i> -Value	0.02	0.02	0.12	0.00	0.01	0.00	0.04	0.02	0.16
<i>Succinivibrio</i>	Correlation	-0.41	-0.43	-0.07	0.49	0.39	0.53	0.32	0.61	0.23
	<i>P</i> -Value	0.03	0.02	0.74	0.01	0.04	0.00	0.10	0.00	0.23
<i>Thermodesulfobium</i>	Correlation	0.58	0.49	0.44	-0.51	-0.48	-0.52	-0.43	-0.49	-0.36
	<i>P</i> -Value	0.00	0.01	0.02	0.00	0.01	0.00	0.02	0.01	0.05
<i>uncultured</i>	Correlation	0.56	0.60	0.04	-0.48	-0.53	-0.49	-0.52	-0.52	-0.51
	<i>P</i> -Value	0.00	0.00	0.83	0.01	0.00	0.01	0.00	0.00	0.00
<i>vadinHA42</i>	Correlation	0.40	0.37	0.08	-0.28	-0.35	-0.32	-0.35	-0.34	-0.31
	<i>P</i> -Value	0.03	0.05	0.69	0.14	0.06	0.10	0.06	0.08	0.10
<i>wet75</i>	Correlation	0.22	0.20	-0.17	-0.05	-0.32	-0.22	-0.38	-0.38	-0.41
	<i>P</i> -Value	0.25	0.30	0.37	0.81	0.09	0.26	0.04	0.04	0.03

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19 **TABLE S4.** Percent contribution of phyla level epithelial taxa to the rumen microbial populations averaged over all treatments for
 20 individual animals. Treatments include forage, mixed forage, high grain, acidotic challenge and challenge recovery.

Phyla	Treatment					SEM	P-Value
	forage	mixed forage	high grain	acidotic challenge	Challenge Recovery		
<i>Actinobacteria</i>	2.05a	1.89a	3.39ab	4.93b	2.60a	0.51	0.0001
<i>Bacteroidetes</i>	9.86	7.87	9.05	8.42	12.81	1.60	0.34
<i>Candidatus TM7</i>	1.58c	1.30bc	0.26a	0.46ab	0.28a	0.12	0.0003
<i>Chloroflexi</i>	0.00	0.00	0.00	0.09	0.00	0.00	0.49
<i>Fibrobacteres</i>	0.44	0.11	0.16	0.16	0.07	0.03	0.63
<i>Firmicutes</i>	66.88	73.65	74.67	74.56	72.03	12.12	0.11
<i>Fusobacteria</i>	2.58b	0.11a	0.10a	0.00a	0.00a	0.07	0.01
<i>Planctomycetes</i>	0.30	0.31	0.00	0.00	0.00	0.02	0.36
<i>Proteobacteria*</i>	13.36	12.28	9.99	9.91	9.83	1.82	0.17
<i>Spirochaetes</i>	0.59	0.74	0.50	0.06	0.52	0.08	0.37
<i>Synergistetes</i>	0.17ab	0.10ab	0.63b	0.08a	0.25ab	0.04	0.05
<i>Tenericutes</i>	0.92b	0.38ab	0.11ab	0.00a	0.14ab	0.04	0.05

21 *Significant animal effect noted for *Proteobacteria* ($P=0.04$)

22 Letters in each row indicate significant difference between treatments.

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24 **TABLE S5.** Correlation of pH variables to epithelial phylum. Only those phyla found to be significant are shown

Phyla		pH parameters								
		pH min	pH mean	pH max	Duration under pH 5.8 (min)	pH area under 5.8 (pH×min)	Duration under pH 5.5 (min)	pH area under 5.5 (pH×min)	Duration under pH 5.2 (min)	pH area under 5.2 (pH×min)
<i>Actinobacteria</i>	Correlation	-0.75	-0.60	-0.29	0.70	0.68	0.72	0.63	0.68	0.54
	<i>P</i> -value	<0.001	0.00	0.13	<0.001	<0.001	<0.001	<0.001	<0.001	0.00
<i>Candidata division TM7</i>	Correlation	0.53	0.57	-0.09	-0.54	-0.60	-0.60	-0.57	-0.56	-0.50
	<i>P</i> -value	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.01
<i>Fusobacteria</i>	Correlation	0.34	0.38	0.59	-0.25	-0.20	-0.17	-0.18	-0.15	-0.17
	<i>P</i> -value	0.07	0.04	0.00	0.19	0.30	0.39	0.34	0.44	0.38
<i>Planctomycetes</i>	Correlation	0.47	0.33	-0.06	-0.35	-0.25	-0.30	-0.20	-0.27	-0.16
	<i>P</i> -value	0.01	0.08	0.77	0.06	0.20	0.12	0.30	0.15	0.41
<i>Tenericutes</i>	Correlation	0.39	0.32	-0.01	-0.31	-0.30	-0.33	-0.28	-0.32	-0.23
	<i>P</i> -value	0.03	0.09	0.96	0.11	0.11	0.08	0.14	0.09	0.22

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27 **TABLE S6.** Rumen fermentation variables measured in heifers during dietary transition. Transition treatment diets included forage,
 28 mixed forage, high grain, acidotic challenge and challenge recovery*.

Rumen Fermentation Variable	Treatment					SEM	P-value
	Forage**	Mixed forage	High grain	Acidotic challenge	Challenge recovery		
Mean nadir		5.78b	4.96a	4.61a	5.08a	0.12	<0.001
Mean daily pH		6.36b	5.99b	5.47a	6.09b	0.12	<0.001
Mean maximum pH		6.95b	6.62a	7.00b	6.56a	0.07	<0.001
Rumen pH \leq 5.8							
Duration (min day ⁻¹)		115c	828ab	1023a	512b	103	<0.001
Area under (pH \times min)		25c	448ab	833a	186bc	106	<0.001
Rumen pH \leq 5.5							
Duration (min day ⁻¹)		25b	616a	879a	232b	88	<0.001
Area under (pH \times min)		4b	229ab	550a	77b	85	<0.001
Rumen pH \leq 5.2							
Duration (min day ⁻¹)		0c	414ab	715a	101bc	99	<0.001
Area under (pH \times min)		0b	78ab	338a	29b	68	<0.001
Total VFA, mM	76.8a	91.8ab	144.2c	128.1abc	129.5bc	5.8	0.002
Acetate (A), mmol/100mol	67.8b	61.8b	46.6a	51.5a	50.6a	1.4	<0.001
Propionate (P), mmol/100mol	17.8a	19.7a	37.5b	32.3b	33.1b	1.5	<0.001
Butyrate, mmol/100mol	3.84b	3.27b	1.27a	1.69a	1.84a	0.17	<0.001
Lactic acid, mM ***			0.71a	2.96b	0.03a	0.41	0.02

29 *Letters in each row indicate significant difference between treatments. The pH variables are a mean values for all animals within a
 30 dietary treatment for the 24 h period starting at 08:00 h on the day of bacterial sample collection. The VFA and lactic acid
 31 concentrations are mean values for all animals on a dietary treatment for samples taken 4 h post-challenge.

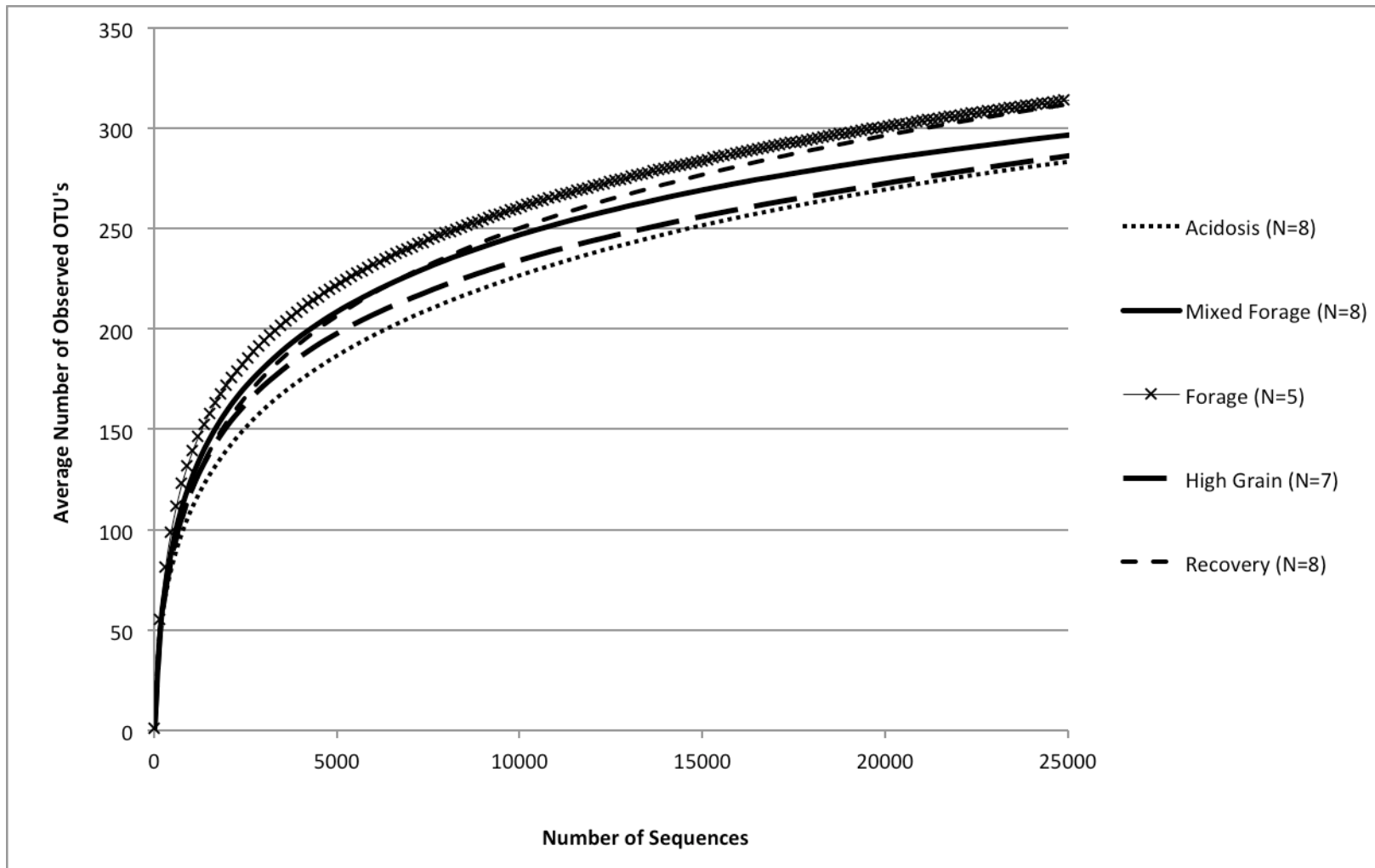
32 ** pH values were unavailable during the forage treatment

33 *** Lactic acid values were unavailable for forage and mixed forage treatment.

34 **TABLE S7.** Rumen fermentation parameters including pH, volatile fatty acids and lactic acid averaged in individual cattle over diet
 35 transition. Transition treatment diets included forage, mixed forage, high grain, acidotic challenge and challenge recovery.*

Fermentation variables	Individual Animal								SEM	<i>P</i> -value
	7	41	43	143	153	156	315	346		
Mean nadir	4.79	5.23	4.90	5.21	5.46	5.08	5.09	5.08	0.10	0.26
Mean daily pH	5.84	5.88	5.65	6.14	6.25	5.98	5.96	6.13	0.08	0.31
Rumen pH \leq 5.8										
Duration (min day ⁻¹)	788	494	1002	427	480	651	535	581	78	0.06
Area under (pH x min)	509	557	625	131	167	319	319	356	77	0.27
Rumen pH \leq 5.5										
Duration (min day ⁻¹)	554	455	787	195	272	408	391	446	75	0.05
Area under (pH x min)	311	415	348	43	53	164	180	206	57	0.38
Rumen pH \leq 5.2										
Duration (min day ⁻¹)	342	372	577	57	65	274	454	320	69	0.09
Area under (pH x min)	178	290	141	7	3	61	122	88	41	0.51
Total VFA, mM	127.2	119.8	121.2	136.2	107.6	136.5	137.1	107.9	5.8	0.43
Acetate (A), mmol/100mol	50.4a	57.1b	53.9ab	50.8a	53.9ab	51.2ab	50.9a	53.3ab	1.4	0.01
Propionate (P), mmol/100mol	32.5	28.2	31.3	33.1	26.1	31.8	33.4	30.3	1.5	0.18
A:P (acetate:propionate)	1.65	2.43	2.07	1.57	2.39	2.06	1.84	2.03	0.17	0.17
Butyrate, mmol/100mol	10.92	9.54	10.82	11.11	13.49	11.43	10.54	10.83	0.47	0.78
Lactic acid, mM	1.00	3.02	0.36	0.22	0.61	0.16	0.25	2.57	0.41	0.27

36 *Letters in each row indicate significant difference between treatments. The pH variables are a mean values for all animals by dietary
 37 treatment for the 24 h period starting at 08:00 h on the day of bacterial sample collection. The VFA and lactic acid concentrations are
 38 mean values for all animals on a dietary treatment for samples taken 4 h post-challenge.



39

40 **Fig. S1.** Rarefaction curves for rumen epithelial bacterial communities for each animal treatment group. Each curve represents a
 41 treatment average based on multiple animals. Unique OTU's are estimated at a 10% difference level.