

Supplementary information for:

Rumen bacteria influence milk protein yield of yak grazing on the Qinghai-Tibet Plateau

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Table S1. Ingredients and chemical composition (based on dry matter) of the diet offered the yaks

Ingredient	Content %	Nutrient indices ¹	Content %
<i>Cyperaceae</i>	28.6	DM	38.36
<i>Poaceae</i>	19.4	CP	11.86
<i>Ranunculaceae</i>	15.3	EE	1.85
<i>Compositae</i>	16.8	OM	89.32
<i>Fabaceae</i>	3.4	NDF	54.13
Others	16.5	ADF	32.22

¹ Nutrient levels are measured values. DM, dry matter; CP, crude protein; EE, ether extract; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber.

Table S2. Milk yield and composition of yaks

Item	Group ¹		SEM	<i>P</i> -value
	HH	LL		
Yield, kg/d				
Milk	2.96	1.48	0.154	<0.01
Protein	0.17	0.06	0.011	<0.01
Composition, %				
Fat	5.75	5.70	0.024	0.285
Protein	5.84	4.69	0.119	<0.01
Lactose	5.59	5.54	0.022	0.401
Total solids	16.64	16.66	0.018	0.059
Ash	0.84	0.79	0.018	0.151

SEM, standard error of the mean.

¹ HH, yaks with high milk yield and high protein content; LL, yaks with low milk yield and low protein content.

Table S3. Comparison of rumen bacterial phyla and genera¹ between HH and LL yaks

Bacterial taxa	Relative abundance, ² %		SEM	<i>P</i> -value
	HH	LL		
Phylum level				
<i>Bacteroidetes</i>	59.18	37.14	2.501	<0.01
<i>Firmicutes</i>	32.64	54.83	2.525	<0.01
<i>Tenericutes</i>	2.65	1.68	0.128	<0.01
<i>Actinobacteria</i>	1.14	2.88	0.205	0.163
<i>Proteobacteria</i>	1.02	1.26	0.052	0.438
Genus level				
<i>Firmicutes</i>				
<i>Christensenellaceae R-7 group</i>	5.45	16.19	1.219	0.013
<i>Ruminococcaceae NK4A214 group</i>	3.88	8.64	0.532	0.138
<i>Ruminococcaceae UCG-014</i>	1.29	0.75	0.068	0.651
<i>Ruminococcaceae UCG-010</i>	1.18	1.27	0.028	0.397
<i>Eubacterium coprostanoligenes group</i>	1.14	0.68	0.053	0.263
<i>Succiniclasticum</i>	1.07	0.14	0.106	<0.01
<i>Butyrivibrio 2</i>	2.40	0.96	0.170	0.012
<i>Ruminococcaceae UCG-005</i>	0.87	1.98	0.128	0.194
<i>Saccharofermentans</i>	0.91	0.61	0.041	0.352
<i>Anaerovorax</i>	0.62	0.93	0.041	0.183
<i>Lachnospiraceae AC2044 group</i>	0.58	0.28	0.048	0.235
<i>Coprococcus 1</i>	0.15	0.67	0.057	0.042

Actinobacteria

<i>Olsenella</i>	0.62	0.89	0.060	0.622
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Bacteroidetes

<i>Prevotella 1</i>	28.77	14.86	1.742	0.018
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<i>Rikenellaceae RC9 gut group</i>	7.67	8.01	0.143	0.248
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<i>Prevotellaceae UCG-003</i>	1.51	2.91	0.179	0.413
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<i>Prevotellaceae UCG-001</i>	2.66	1.28	0.220	0.024
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<i>Prevotellaceae NK3B31 group</i>	1.08	0.56	0.094	0.536
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Tenericutes

<i>Anaeroplasma</i>	0.77	0.39	0.051	0.742
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SEM, standard error of the mean.

¹ Bacterial phyla and genera with relative abundances > 0.1% were regarded as detected and used for comparison.

² HH, yaks with high milk yield and high protein content; LL, yaks with low milk yield and low protein content.

Table S4. Comparison of Degree, Closness centrality, and Betweenness centrality between HH and LL yaks

HH				LL			
Id	Degree	Closness centrality	Betweenness centrality	Id	Degree	Closness centrality	Betweenness centrality
<i>Prevotella 1</i>	15	0.540984	8.8528	<i>Christensenellaceae R.7 group</i>	25	0.639344	16.270756
<i>Christensenellaceae R.7 group</i>	11	0.428571	6.259113	<i>Prevotella 1</i>	25	0.639344	20.342076
<i>Ruminococcaceae NK4A214 group</i>	15	0.540984	35.47137	<i>Ruminococcaceae NK4A214 group</i>	27	0.672414	29.746946
<i>Prevotellaceae UCG.001</i>	15	0.6	21.47697	<i>Butyrivibrio 2</i>	21	0.557143	4.678828
<i>Ruminococcaceae UCG.014</i>	20	0.673469	89.29058	<i>Ruminococcaceae UCG.005</i>	22	0.557143	7.793046
<i>Eubacterium coprostanoligenes group</i>	17	0.578947	43.50984	<i>Anaerovorax</i>	22	0.609375	7.973822
<i>Saccharofermentans</i>	14	0.568966	33.20013	<i>Ruminococcaceae UCG.014</i>	22	0.573529	9.569304
<i>Ruminococcaceae UCG.005</i>	14	0.532258	34.66896	<i>Coprococcus 1</i>	23	0.573529	5.654305
<i>Anaerovorax</i>	15	0.540984	8.8528	<i>Saccharofermentans Family XIII AD3011 group</i>	32	0.735849	74.58248
<i>Lachnospiraceae XPB1014 group</i>	16	0.559322	12.36309	<i>Defluviitaleaceae UCG.011</i>	19	0.541667	23.196338
<i>Papillibacter</i>	16	0.611111	26.61981	<i>Defluviitaleaceae UCG.011</i>	23	0.6	10.900387
<i>Lachnospiraceae UCG.006</i>	10	0.417722	0.611111	<i>Eubacterium hallii group</i>	23	0.609375	9.068266
<i>Anaerotruncus</i>	11	0.507692	11.7765	<i>Lachnospiraceae UCG.006</i>	22	0.6	8.100012
<i>Ruminococcaceae UCG.007</i>	12	0.5	8.372922	<i>Ruminococcus gauvreauii group</i>	14	0.5	40.095274
<i>Prevotellaceae UCG.003</i>	9	0.532258	3.420868	<i>Anaeroplasma</i>	17	0.534247	3.828954
<i>Fibrobacter</i>	4	0.392857	0	<i>Anaerotruncus</i>	26	0.661017	34.246495
<i>Phocaeicola</i>	18	0.578947	43.80598	<i>Quinella</i>	22	0.6	55.393745
<i>Ruminococcus 1</i>	16	0.559322	37.88039	<i>Phocaeicola</i>	22	0.609375	44.631367
<i>Lachnospiraceae ND3007 group</i>	15	0.6	61.70759	<i>Marvinbryantia</i>	21	0.6	13.808135
<i>Candidatus Hepatincola</i>	17	0.568966	33.04405	<i>Prevotellaceae UCG.003</i>	22	0.590909	12.622021
<i>Prevotellaceae NK3B31 group</i>	13	0.434211	0.650392	<i>Prevotellaceae UCG.001</i>	18	0.565217	19.527861
<i>Butyrivibrio 2</i>	13	0.5	6.542305	<i>Roseburia</i>	12	0.527027	40.007875
<i>Anaeroplasma</i>	12	0.417722	0.465909	<i>Senegalimassilia</i>	14	0.4875	1.008171
<i>Erysipelotrichaceae UCG.004</i>	10	0.4125	0.184483	<i>Streptococcus</i>	15	0.52	2.994827

<i>Pseudobutyrvibrio</i>	12	0.428571	0.184483	<i>Arthrobacter</i>	20	0.619048	152.088095
<i>Eubacterium ruminantium</i> group	14	0.532258	10.14865	<i>Lachnospiraceae</i> NK3A20 group	3	0.232143	0
<i>Ruminococcus gauvreauii</i> group	13	0.434211	0.650392	<i>Olsenella</i>	7	0.4875	0
<i>Ruminiclostridium 9</i>	13	0.434211	0.650392	<i>Carnobacterium</i>	4	0.29771	0
<i>Succiniclasicum</i>	3	0.666667	0	<i>Lachnospiraceae</i> XPB1014 group	5	0.410526	108
<i>Lachnospiraceae</i> AC2044 group	15	0.55	23.56759	<i>Solobacterium</i>	5	0.3	38
<i>Lachnospiraceae</i> NK3A20 group	7	0.44	6.797534	<i>Acetitomaculum</i>	9	0.46988	0.761905
<i>Olsenella</i>	3	0.666667	0	<i>Desulfovibrio</i>	13	0.493671	5.621068
<i>Quinella</i>	4	1	1	<i>Eubacterium coprostanoligenes</i> group	9	0.46988	0.125
<i>Acetitomaculum</i>	5	0.464789	0.973011	<i>Syntrophococcus</i>	10	0.47561	0.285714
<i>Defluviitaleaceae</i> UCG.011	6	0.392857	32	<i>Shuttleworthia</i>	6	0.419355	38.076923
<i>Desulfovibrio</i>	3	0.284483	0	<i>Blautia</i>	6	0.464286	1
<i>Veillonellaceae</i> UCG.001	4	0.354839	0	<i>Prevotellaceae</i> NK3B31 group	3	0.29771	0
				<i>Lachnospiraceae</i> ND3007 group	3	0.336207	0
				<i>Clostridium sensu stricto 1</i>	2	0	0
				<i>Anaerorhabdus furcosa</i> group	3	0.378641	0
				<i>Erysipelotrichaceae</i> UCG.004	3	0.348214	0

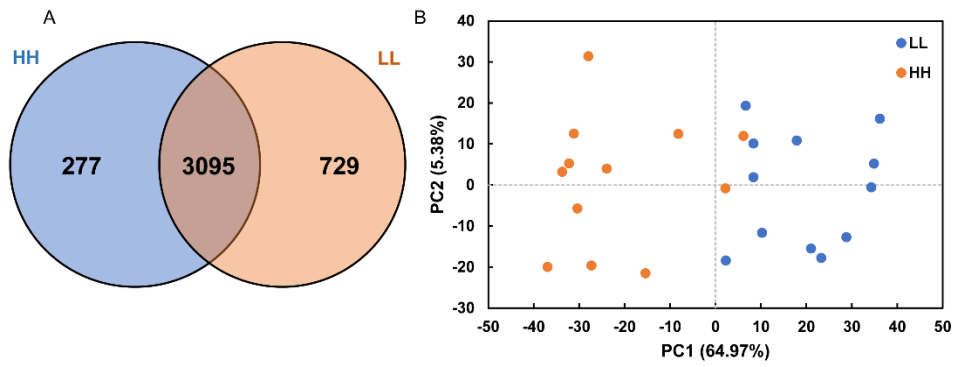


Figure S1. Differences in bacterial community diversity, richness, and OTUs between the two groups. (A) A Venn diagram showing the different and similar OTUs between both groups. (B) A principal coordinate analysis (PCoA) of the yak ruminal microbiota between both groups. HH, yaks with high milk yield and high protein content; LL, yaks with low milk yield and low protein content. OTU, operational taxonomic unit.