

**Table S1.** Ingredients and chemical composition of forage basal diet and concentrate.

Item	Concentrate	Basal Diet
Ingredients composition (g/kg DM)		
Maize silage		317
Grass silage		683
Maize	318	
Soybean meal	245	
Wheat middlings	174	
Palm kernel meal	120	
Hydrogenated palm fat	25	
Malt culms	30	
Cane bagasse	30	
Rapeseed meal	30	
Sunflower meal	10	
Barley	9	
Oil palm hydrogenated fat (99%)	6.8	
<sup>1</sup> Cows NUTEMIX	2	
Sodium chloride	0.2	
Chemical composition (% of DM)		
UFL	1.02	
DM	90	24
Crude ash	6.6	8.8
Starch	25	4.0
Crude protein	19	12
Neutral detergent fiber	21	49
Acid detergent fiber	10	27
Fat	6.2	

UFL: forage unit for lactation; DM: dry matter; <sup>1</sup>Contained: calcium (17 g/kg), magnesium (25 g/kg), sodium (40 g/kg), phosphorus (52 g/kg), zinc (15 g/kg), manganese (14 g/kg), copper (1.7 g/kg), iodine (130 mg/kg), cobalt (39 mg/kg), selenium (2 mg/kg).

**Table S2.** Main microbial phyla of rumen samples of cows fed a conventional concentrate and a concentrate with chitosan

Phylum (clr_RA)	HF		BS		SEM	Adjusted P-value		
	CTR	CHI	CTR	CHI		Treat	Breed	Treat x Breed
Euryarchaeota	1.10	0.53	0.75	0.87	0.198	0.704	0.988	0.361
Actinobacteria	0.030	0.253	0.302	0.193	0.1323	0.905	0.988	0.471
Bacteroidetes	4.84	5.20	5.24	5.17	0.149	0.751	0.988	0.398
Chloroflexi	-3.22	-3.27	-3.47	-3.62	0.233	0.905	0.988	0.846
Cyanobacteria	-2.57	-1.84	-1.86	-2.26	0.360	0.905	0.988	0.361
Elusimicrobia	-1.74	-1.30	-1.94	-1.75	0.220	0.582	0.988	0.696
Fibrobacteres	1.34	1.77	1.65	1.71	0.214	0.704	0.988	0.593
Firmicutes	3.86	3.80	3.85	3.97	0.120	0.905	0.988	0.596
Fusobacteria	-2.20	-2.53	-2.45	-2.29	0.142	0.905	0.988	0.361
Kiritimatiellaeota	0.83	1.11	1.20	0.97	0.239	0.933	0.988	0.575
Lentisphaerae	-0.229	0.096	-0.078	-0.272	0.2621	0.905	0.988	0.575
Planctomycetes	-2.54	-2.65	-2.63	-2.68	0.120	0.878	0.988	0.846
Proteobacteria	1.66	1.81	1.76	2.03	0.244	0.819	0.988	0.846
Spirochaetes	0.36	1.12	0.66	0.74	0.119	0.100	0.988	0.361
Synergistetes	-2.91	-2.68	-2.66	-2.85	0.282	0.933	0.988	0.596
Tenericutes	-1.16	-0.49	-0.84	-0.68	0.161	0.294	0.988	0.361
Verrucomicrobia	-1.67	-1.81	-1.85	-1.78	0.151	0.905	0.988	0.593
Ascomycota	0.37	-0.385	-0.084	-0.068	0.2307	0.504	0.988	0.361
Basidiomycota	-0.44	-1.35	-0.96	-0.92	0.217	0.504	0.988	0.361
Chytridiomycota	1.20	0.36	0.78	0.80	0.221	0.504	0.988	0.361
Ciliophora	3.08	2.27	2.66	2.72	0.223	0.504	0.988	0.361

RA\_clr: relative abundance CLR-transformed; HF: Holstein-friesian; BS: brown Swiss; CTR: control; CHI: chitosan; SEM: standard error of the mean; Treat: treatment; Adjust P-value: adjusted P-value using False Discovery Rate.

**Table S3.** Main microbial genera of rumen samples of cows fed a conventional concentrate and a concentrate with chitosan

Genus (clr_RA)	HF		BS		SEM	Adjusted P-value		
	CTR	CHI	CTR	CHI		Treat	Breed	Treat x Breed
Archaea								
Methanobrevibacter	2.05	1.64	1.63	1.85	0.217	0.686	0.991	0.281
Methanospaera	0.94	0.14	0.77	0.45	0.207	0.100	0.991	0.281
UC_Methanobacteriaceae	0.76	0.37	0.69	0.62	0.131	0.244	0.991	0.281
UC_Methanobacteriales	0.57	0.19	0.39	0.40	0.127	0.244	0.991	0.281
UC_Methanobacteria	0.138	-0.235	0.008	-0.003	0.1320	0.244	0.991	0.281
Bacteria								
Aeriscardovia	1.17	1.48	1.60	1.33	0.179	0.953	0.991	0.569
Bifidobacterium	-2.11	-0.43	-2.30	-1.93	0.471	0.651	0.991	0.682
UC_Bifidobacteriaceae	-0.99	-0.68	-0.86	-1.12	0.152	0.953	0.991	0.569
UC_Bifidobacteriales	-1.38	-1.09	-0.86	-1.09	0.125	0.906	0.991	0.569
UC_Actynomicetia	-0.96	-1.05	-0.84	-1.03	0.201	0.801	0.991	0.935
UC_Coriobacteriia	-1.69	-1.60	-1.50	-1.40	0.202	0.853	0.991	0.996
UC_Actinobacteria	-0.84	-0.68	-0.58	-0.64	0.146	0.887	0.991	0.847
Bacteroides	1.57	1.84	1.71	1.71	0.155	0.801	0.991	0.814
UC_Bacteroidaceae	3.79	4.19	4.18	4.11	0.143	0.750	0.991	0.569
Barnesiella	-1.85	-1.07	-1.37	-1.41	0.220	0.676	0.991	0.569
UC_Muribaculaceae	2.20	2.17	2.03	2.29	0.264	0.853	0.991	0.880
UC_Paludibacteraceae	3.32	3.46	3.45	3.48	0.149	0.853	0.991	0.928
Phocaeicola	-0.88	-0.22	-0.41	-0.42	0.182	0.676	0.991	0.569
Alloprevotella	-0.76	-0.34	-0.32	-0.43	0.155	0.758	0.991	0.569
Paraprevotella	-1.59	-1.52	-1.66	-1.34	0.251	0.801	0.991	0.893
Prevotella	5.90	6.47	6.47	6.26	0.207	0.790	0.991	0.569
UC_Prevotellaceae	3.67	4.27	4.33	4.08	0.249	0.801	0.991	0.569
Alistipes	-1.14	-0.83	-1.25	-1.05	0.180	0.676	0.991	0.928
UC_Rikenellaceae	0.10	0.51	0.36	0.43	0.146	0.676	0.991	0.693
UC_Salinivirgaceae	-0.79	-0.48	-0.68	-0.80	0.079	0.750	0.991	0.464
Parabacteroides	-1.25	-0.87	-1.05	-0.96	0.154	0.676	0.991	0.814
UC_Tanerellaceae	-1.85	-1.54	-1.90	-1.64	0.155	0.676	0.991	0.935
UC_Bacteroidales	5.25	5.63	5.58	5.51	0.125	0.750	0.991	0.569
UC_Bacteroidia	2.95	3.37	3.36	3.21	0.166	0.801	0.991	0.569
UC_Flavobacteriales	-1.18	-1.46	-1.16	-1.29	0.117	0.676	0.991	0.871
Chryseobacterium	-0.43	-0.78	-0.61	-0.66	0.210	0.758	0.991	0.871
UC_Flavobacteriia	-1.35	-1.38	-1.30	-1.36	0.145	0.875	0.991	0.944
UC_Bacteroidetes	3.02	3.34	3.34	3.20	0.147	0.822	0.991	0.569
UC_Cyanobacteria	-0.92	-0.06	-0.08	-0.62	0.446	0.875	0.991	0.569
UC_Elusimicrobiaceae	-1.10	-0.37	-1.44	-1.28	0.302	0.676	0.991	0.814
UC_Elusimicrobia	-0.51	-0.05	-0.56	-0.47	0.277	0.758	0.991	0.871
Fibrobacter	2.98	3.54	3.39	3.36	0.264	0.758	0.991	0.722
UC_Fibrobacteraceae	0.001	0.527	0.236	0.211	0.2031	0.750	0.991	0.689
UC_Fibrobacterales	-0.51	-0.17	-0.33	-0.29	0.170	0.758	0.991	0.814
UC_Fibrobacteria	-1.03	-0.77	-1.10	-0.89	0.183	0.750	0.991	0.935

UC_Fibrobacteres	-1.58	-1.04	-1.20	-1.34	0.130	0.676	0.991	0.464
Bacillus	-1.94	-1.66	-1.72	-1.87	0.242	0.922	0.991	0.814
Staphylococcus	-1.11	-0.32	-1.00	-1.04	0.371	0.758	0.991	0.722
UC_Bacillales	-1.30	-1.38	-1.03	-1.21	0.134	0.758	0.991	0.928
Leuconostoc	-3.64	-1.83	-3.10	-2.83	0.555	0.676	0.991	0.682
UC_Lactobacillaceae	-2.32	-2.41	-2.34	-2.56	0.255	0.847	0.991	0.935
Streptococcus	-1.32	-1.48	-1.46	-1.49	0.191	0.853	0.991	0.928
UC_Lactobacillales	-0.70	-0.98	-0.77	-0.75	0.169	0.801	0.991	0.815
UC_Bacilli	2.82	2.70	2.88	2.81	0.097	0.758	0.991	0.928
Clostridium	0.96	0.99	0.93	1.02	0.133	0.853	0.991	0.935
Sarcina	-1.01	-1.01	-1.02	-0.84	0.204	0.853	0.991	0.925
UC_Clostridiaceae	0.14	0.03	0.02	0.12	0.200	0.982	0.991	0.814
Eubacterium	0.93	1.35	1.43	1.30	0.174	0.801	0.991	0.569
UC_Eubacteriaceae	-0.38	-0.66	-0.40	-0.50	0.136	0.676	0.991	0.871
Agathobacter	-1.74	-1.30	-1.34	-1.30	0.354	0.807	0.991	0.880
Blautia	-1.39	-1.52	-1.89	-1.73	0.193	0.953	0.991	0.871
Butyrivibrio	1.83	2.01	1.88	2.00	0.178	0.790	0.991	0.935
Coproccoccus	-1.81	-1.51	-2.32	-1.71	0.220	0.651	0.991	0.871
Oribacterium	-0.518	0.164	-0.024	-0.047	0.209	0.676	0.991	0.569
Pseudobutyrvibrio	0.34	0.67	0.64	0.74	0.212	0.758	0.991	0.880
Roseburia	-0.74	0.33	-0.50	-0.11	0.359	0.651	0.991	0.814
Stomatobaculum	-0.398	-0.022	-0.580	-0.662	0.244	0.847	0.991	0.814
UC_Lachnospiraceae	3.16	3.31	3.30	3.34	0.098	0.758	0.991	0.878
Ruminiclostridium	-1.77	-2.72	-1.46	-1.72	0.319	0.676	0.991	0.748
Ruminococcus	2.15	2.75	2.58	2.62	0.139	0.651	0.991	0.569
UC_Oscillospiraceae	3.13	3.26	3.14	3.23	0.150	0.801	0.991	0.935
UC_Eubacteriales	4.01	4.00	4.01	4.07	0.097	0.887	0.991	0.928
UC_Clostridia	4.11	3.91	4.03	3.99	0.104	0.758	0.991	0.847
Sharpea	-3.78	-2.15	-3.13	-2.32	0.773	0.676	0.991	0.880
UC_Erysipelotrichaceae	0.68	1.18	0.81	0.99	0.120	0.464	0.991	0.689
UC_Erysipelotrichales	-0.76	-0.17	-0.56	-0.14	0.263	0.676	0.991	0.928
UC_Erysipelotrichia	-0.72	-0.64	-0.74	-0.59	0.107	0.758	0.991	0.928
Succiniclacticum	1.54	1.49	1.58	1.64	0.242	0.982	0.991	0.935
Acidaminococcaceae	1.38	1.36	1.45	1.53	0.200	0.946	0.991	0.928
Quinella	-0.83	-1.31	-0.98	-1.14	0.156	0.651	0.991	0.791
Anaerovibrio	-0.035	1.110	0.655	0.685	0.2871	0.651	0.991	0.569
Schwartzia	-0.003	0.376	0.488	0.580	0.1823	0.750	0.991	0.847
Selenomonas	0.79	1.85	1.63	1.58	0.312	0.676	0.991	0.569
UC_Selenomonadaceae	-0.595	0.094	-0.074	-0.102	0.2252	0.676	0.991	0.569
UC_Selenomonadales	-1.41	-0.92	-1.16	-1.16	0.208	0.750	0.991	0.693
UC_Negativicutes	-0.34	-0.26	-0.17	-0.18	0.089	0.868	0.991	0.885
Megasphaera	-3.78	-3.87	-3.36	-3.06	0.321	0.868	0.991	0.878
UC_Firmicutes	3.75	3.74	3.77	3.80	0.055	0.940	0.991	0.925
Fusobacterium	-1.69	-1.86	-1.71	-1.68	0.165	0.853	0.991	0.878
UC_Kiritimatiellae	2.54	2.95	2.99	2.67	0.301	0.946	0.991	0.693
UC_Kiritimatiellaeota	-0.72	-0.56	-0.51	-0.76	0.239	0.940	0.991	0.814
UC_Lentisphaeria	-0.37	0.15	-0.21	-0.34	0.275	0.801	0.991	0.693
UC_Victivallales	0.313	0.576	0.333	0.017	0.443	0.979	0.991	0.871

UC_Lentisphaerae	0.89	1.37	1.23	0.89	0.321	0.940	0.991	0.693
UC_Planctomycetes	-1.63	-1.99	-1.80	-1.94	0.179	0.676	0.991	0.878
Acetobacter	-1.026	0.010	-0.055	-0.238	0.495	0.800	0.991	0.693
UC_Alphaproteobacteria	0.55	1.29	1.00	0.77	0.224	0.758	0.991	0.569
UC_Betaproteobacteria	-1.90	-1.32	-1.94	-1.76	0.274	0.676	0.991	0.871
Desulfovibrio	-0.82	-1.00	-0.96	-0.84	0.245	0.953	0.991	0.878
Ruminobacter	0.020	0.483	0.229	0.440	0.602	0.853	0.991	0.935
Succinomonas	0.11	0.39	0.41	0.69	0.405	0.801	0.991	0.996
Succinivibrio	0.061	1.297	0.400	0.559	0.4146	0.676	0.991	0.693
UC_Succinivibrionaceae	2.19	2.34	2.46	2.75	0.398	0.853	0.991	0.935
UC_Aeromonadales	-0.392	0.322	-0.308	0.066	0.3909	0.676	0.991	0.925
Escherichia	-1.43	-1.56	-1.15	-1.30	0.275	0.853	0.991	0.987
Klebsiella	0.86	0.40	0.52	0.51	0.093	0.651	0.991	0.565
UC_Enterobacteriaceae	-0.10	-0.50	-0.43	-0.28	0.174	0.801	0.991	0.569
UC_Enterobacterales	-0.065	-0.623	-0.526	-0.213	0.1693	0.801	0.991	0.464
Acinetobacter	-0.75	-0.65	-0.69	-0.77	0.269	0.982	0.991	0.928
Pseudomonas	-1.96	-1.64	-2.09	-1.89	0.326	0.801	0.991	0.935
UC_Gammaproteobacteria	0.86	0.88	0.86	1.13	0.281	0.853	0.991	0.925
Vibrio	-2.20	-1.76	-1.99	-1.73	0.318	0.758	0.991	0.928
UC_Proteobacteria	1.21	1.31	1.21	1.26	0.148	0.853	0.991	0.935
Treponema	1.48	2.54	1.96	1.96	0.171	0.425	0.991	0.386
UC_Spirochaetaceae	0.064	0.940	0.426	0.428	0.1637	0.533	0.991	0.464
UC_Spirochaetales	0.10	0.85	0.42	0.38	0.106	0.406	0.991	0.348
UC_Spirochaetia	-0.197	0.069	-0.163	-0.085	0.1451	0.750	0.991	0.871
UC_Spirochaetes	-0.46	-0.18	-0.38	-0.49	0.118	0.801	0.991	0.569
Pyramidobacter	-2.11	-1.51	-1.52	-1.78	0.361	0.853	0.991	0.693
UC_Acholeplasmatales	-1.48	-0.79	-0.99	-1-10	0.200	0.676	0.991	0.569
Anaeroplasma	-0.85	0.57	-0.19	0.12	0.175	0.046	0.991	0.386
Mycoplasma	-2.08	-1.58	-1.61	-1.87	0.314	0.868	0.991	0.693
UC_Mollicutes	-1.21	-0.74	-1.00	-0.92	0.130	0.651	0.991	0.587
UC_Tenericutes	-1.18	-0.79	-0.94	-0.98	0.169	0.758	0.991	0.569
UC_Opitutae	-1.68	-1.55	-2.09	-1.92	0.312	0.853	0.991	0.976
UC_Verrucomicrobia	-0.41	-0.33	-0.37	-0.39	0.138	0.940	0.991	0.928
<i>Eukaryota</i>								
Sacharomyces	-1.28	-1.84	-1.67	-1.58	0.237	0.408	0.991	0.239
UC_Sacharomycetaceae	-1.22	-2.10	-1.88	-1.97	0.194	0.133	0.991	0.207
UC_Sacharomycetales	-0.81	-1.19	-1.30	-1.21	0.231	0.582	0.991	0.370
UC_Sordariomycetes	-1.59	-2.14	-1.89	-1.93	0.266	0.372	0.991	0.382
UC_Ascomycota	0.88	0.25	0.48	0.42	0.181	0.134	0.991	0.208
UC_Agarycomycetes	-0.18	-0.83	-0.45	-0.56	0.184	0.134	0.991	0.217
UC_Basidiomycota	-0.47	-1.29	-1.16	-0.92	0.180	0.189	0.991	0.207
Anaeromyces	0.71	-0.046	0.27	0.23	0.175	0.133	0.991	0.207
Neocallimastix	1.93	1.26	1.64	1.51	0.163	0.133	0.991	0.208
Piromyces	1.25	0.45	0.87	0.77	0.170	0.133	0.991	0.207
UC_Neocallimastigaceae	1.73	1.06	1.40	1.37	0.179	0.134	0.991	0.207
Nyctotherus	-1.63	-2.28	-2.41	-2.15	0.260	0.518	0.991	0.207
Stentor	2.68	1.95	2.31	2.27	0.168	0.133	0.991	0.207
Entodinium	2.48	1.79	2.05	2.05	0.162	0.133	0.991	0.207

Epidinium	-0.51	-3.04	-1.68	-1.98	0.565	0.133	0.991	0.207
Eudiplodinium	-1.51	-1.68	-1.45	-1.40	0.228	0.804	0.991	0.645
Polyplastron	-0.88	-2.34	-1.91	-1.53	0.527	0.397	0.991	0.207
Isotricha	-0.51	-0.98	-0.79	-0.76	0.185	0.356	0.991	0.239
Ichthyophthirius	2.54	1.73	2.03	2.07	0.181	0.133	0.991	0.207
Tetrahymena	1.67	1.06	1.45	1.34	0.163	0.133	0.991	0.208
UC_Hymenostomatida	0.536	-0.098	0.250	0.225	0.1508	0.133	0.991	0.207
Paramecium	2.36	1.71	2.08	2.00	0.167	0.133	0.991	0.207
Pseudocohnilembus	1.46	0.82	1.14	1.06	0.165	0.133	0.991	0.207
UC_Oligohymenophora	2.22	1.62	1.97	1.90	0.169	0.134	0.991	0.208
Halteria	1.82	1.08	1.38	1.41	0.160	0.133	0.991	0.207
Stylonichia	2.39	1.73	2.14	2.07	0.182	0.134	0.991	0.208
UC_Sporadotrichia	1.24	0.54	0.88	0.90	0.191	0.149	0.991	0.207
UC_Ciliophora	3.43	2.75	3.09	3.07	0.182	0.134	0.991	0.207

RA\_clr: relative abundance CLR-transformed; HF: Holstein-friesian; BS: brown Swiss; CTR: control; CHI: chitosan; SEM: standard error of the mean; T: treatment; Adjust P-value: adjusted P-value using False Discovery Rate; UC: unclassified.