

Associating changes in the bacterial community of rumen and faeces and milk fatty acid profiles in dairy cows fed high-starch or starch and oil-supplemented diets

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SUPPLEMENTARY FILE

Supplementary Table 1 Ingredients and chemical composition of the four experimental diets¹ fed to dairy cows (from Bougouin et al., 2019)

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Items	Diets			
	Ca salts of palm oil	High starch	Extruded rapeseeds	Extruded sunflower seeds
Ingredients (% DM)				
Maize silage	56.0	56.0	56.0	56.0
Hay	4.0	4.0	4.0	4.0
Pelleted concentrate (% DM)	40.0	40.0	40.0	40.0
Maize grains		13.4	5.8	6.3
Ca salts of palm oil	12.3			
Extruded rapeseed			24.5	
Extruded sunflower seeds				24.5
Barley grains	2.6	4.0	9.6	
Wheat starch		12.0		
Wheat middlings	10.7	25.0		2.0
Wheat bran	28.8	20.0	12.6	10.5
Distillers dried maize grains				2.2
Soybeans	24.9		21.3	23.6
Beet pulp	5.1	18.8	20.0	25.0
Hay	10.0			
Molasses	4.0	2.0	2.0	2.0
Premix, vitamins and minerals	1.6	4.8	4.2	3.9
Composition (%)				
Organic matter	87.0	87.2	86.9	86.9
CP	13.0	12.9	13.3	13.3
NDF	41.6	40.6	40.1	41.2
ADF	19.2	17.9	19.2	19.2
Starch	18.2	22.9	18.5	16.7
Ether extract	6.3	3.3	7.1	7.4
Gross energy (MJ/kg DM)	18.4	19.1	19.2	19.2
Fatty acids (g/kg DM)				
C14:0	0.54	0.04	0.06	0.06
C16:0	23.87	4.06	4.95	5.2
C18:0	2.31	0.69	0.96	1.55
cis-9 C18:1	19.44	4.29	25.54	12.01
cis-11 C18:1	0.57	0.23	1.63	0.58
C18:2n-6	14.26	12.55	16.04	32.12
C18:3n-3	1.96	1.91	6.43	2.5

Supplementary Table 2 Concentrations of 4- to 24-carbon (not including 18:1, 18:2 or 18:3 isomers) fatty acids (FA) in milk fat in dairy cows fed the four experimental (from Bougouin et al., 2019) DOI: 10.1017/S1751731118003154

Items (% of total FA)	Diets				SEM	P-value
	Ca salts of palmoil	High starch	Extruded rapeseeds	Extruded sunflower seeds		
C4:0	2.01b	2.93a	1.86b	1.62b	0.169	< 0.001
C6:0	0.93b	1.81a	1.06b	0.84b	0.108	< 0.001
C8:0	0.41b	0.97a	0.55b	0.39b	0.071	< 0.001
C10:0	0.85b	2.11a	1.30b	0.88b	0.197	< 0.01
C12:0	1.13b	2.41a	1.74ab	1.33b	0.228	0.01
iso C14:0	0.05c	0.07a	0.05c	0.06b	0.004	< 0.001
C14:0	6.32b	9.95a	8.06ab	6.43b	0.763	0.03
cis-9 C14:1	0.74b	1.08a	1.16b	0.84b	0.219	0.4
iso C15:0	0.11b	0.18a	0.13b	0.12b	0.009	0.01
anteiso C15:0	0.25b	0.41a	0.31b	0.31b	0.032	0.01
C15:0	0.56	0.83	0.7	0.7	0.083	0.19
iso C16:0	0.14b	0.25a	0.16b	0.19b	0.013	< 0.01
C16:0	37.7a	29.6b	23.4bc	18.3c	2.47	< 0.001
cis-9 C16:1	2.70a	2.08b	1.96bc	1.41c	0.234	0.01
anteiso C17:0	0.29b	0.53a	0.38b	0.37b	0.029	< 0.001
C17:0	0.35c	0.62a	0.45b	0.45b	0.019	< 0.001
iso C17:0 ¹	0.66b	0.50c	0.62b	0.90a	0.045	0.0002
cis-9 C17:1	0.20b	0.33a	0.24b	0.21b	0.017	< 0.01
iso C18:0	0.03c	0.07a	0.04b	0.04b	0.003	< 0.001
C18:0	6.83	7.46	8.35	10.07	1.02	0.22
C20:4n-6	0.06b	0.14a	0.08b	0.08b	0.013	0.01
C20:5n-3	0.02	0.02	0.04	0.03	0.005	0.11
C22:5n-3	0.04b	0.11a	0.07b	0.06b	0.01	0.01
C22:6n-3	0.01	0.006	0.009	0.013	0.0024	0.14
Σ SFA ²	58.2a	60.6a	49.0b	42.4b	2.41	< 0.01
Σ MUFA ³	35.4b	32.1b	42.8a	46.3a	2.05	0.01
Σ PUFA ⁴	4.38b	5.21b	5.05b	8.29a	0.507	< 0.001
Σ trans FA	9.0c	8.4c	13.7b	19.3a	1.24	< 0.001
Σ OBCFA ⁵	1.89b	3.18a	2.40b	2.40b	0.164	< 0.01
Σ n-3	0.28c	0.48ab	0.57a	0.40b	0.032	0.001
Σ n-6	2.5bc	3.3b	2.4c	5.5a	0.38	< 0.001
Σ FA < 16 C ⁶	13.5b	23.2a	17.2b	13.7b	1.57	< 0.01
Σ FA 16 C ⁷	40.8a	32.3b	25.9c	20.1c	2.68	< 0.001
Σ FA > 16 C ⁸	44.6bc	43.5c	55.0ab	64.6a	3.66	< 0.01

^{a,b,c}Values within a row with different superscripts differ significantly at $P < 0.05$.

¹iso C17:0 coeluted with *trans*-9 C16:1.

²SFA = saturated FA (odd FA + branched-chain FA + even FA; from 4 to 26 carbon atoms).

³MUFA = monounsaturated FA from 10 to 22 carbon atoms

⁴PUFA = polyunsaturated FA from 18 to 26 carbon atoms.

⁵OBCFA = odd- and branched-chain FA.

⁶Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, *cis*-9 C10:1, C11:0, C12:0, *cis*-9 C12:1, C13:0, *iso* C13:0, *anteiso* C13:0, C14:0, *cis*-9 C14:1, *trans*-9 C14:1, *iso* C14:0, C15:0, *iso* C15:0, *anteiso* C15:0.

⁷Sum of C16:0, *iso* C16:0, *trans*-6,7,8 C16:1, *cis*-6,8 C16:1 + *trans*-11 C16:1, *cis*-9 C16:1, *cis*-11 C16:1.

⁸Sum of FA with more than 16 carbons except *iso* C17:0 because of coelution with *trans*-9 C16:1

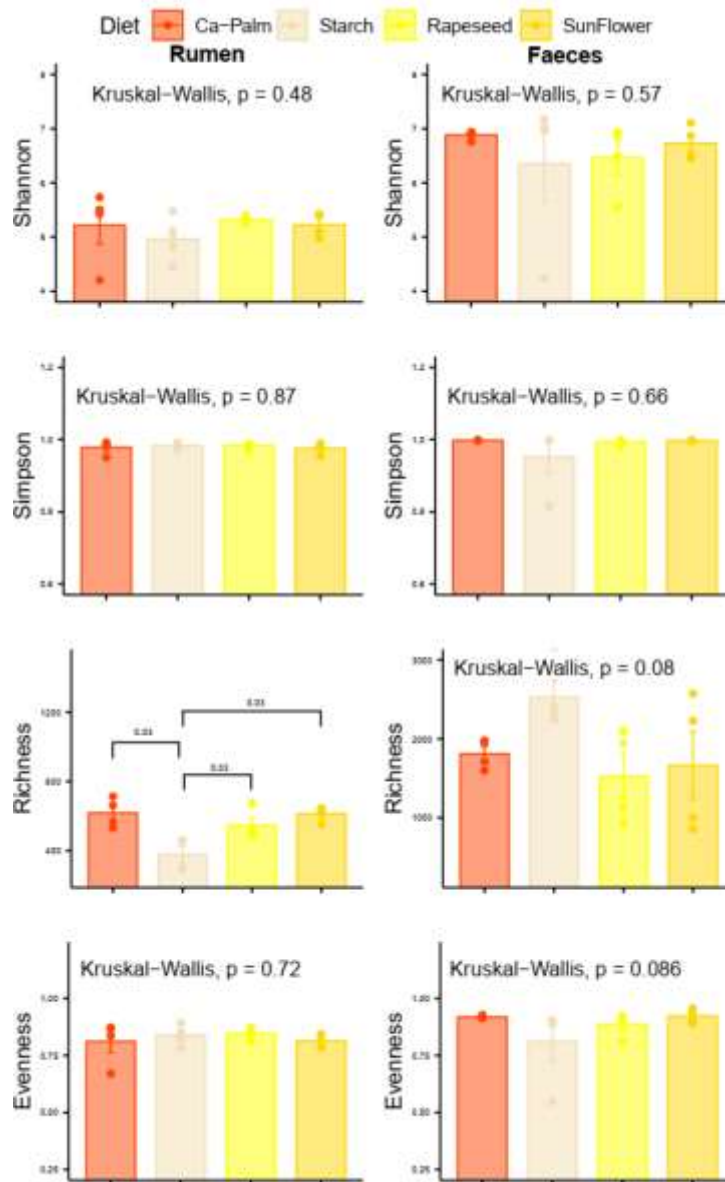
Supplementary table 3 Sequencing effort and summary of bioinformatics analysis metrics

	Rumen	Feces
Raw reads (R1+R2)	773486	915914
Reads passed quality control	158295	234418
Merged paired-ends	158295	234418
Identified chimeric sequences	24574	49549
No.of reads used to generate OTU table	132789	46557
Picked OTUs	4366	6408
Good's coverage (%)	91±2.7	78±8.1

Supplementary figure 1: Diversity_indices

Barplots of means with standard errors and individual values for Shannon, Simpson, Richness and Evenness diversity indices, computed on rumen (left column) and faecal (right column) OTU tables.

Cows received Starch (high starch diet), Palmoil (rumen protected SFA from Ca-salts of palm oil), Rapeseed diet (rich in MUFA from extruded rapeseeds) or Sunflower diet (rich in PUFA from extruded sunflower seeds). Statistical analysis was performed using non-parametric Kruskal-Wallis test and pairwise comparisons - using Wilcoxon-Mann-Whitney



Supplementary table 4a: Bacterial abundance at order level in the rumen contents of cows fed high starch or rapeseed, palmoil or sunflower oil supplemented diets. Values are means of four observations; statistical analysis was performed to evaluate the effect of diet and lipid supplementation (starch diet compared to all lipid supplemented diets). In both cases we used the non-parametric Kruskal-Wallis test with *fdr* *p* value adjustment followed by a Dunn test when appropriate.

	Diets				SEM	<i>Effect of Diet</i>	<i>Effect of lipid supplementation</i>
	Ca salts of palmoil	High starch	Extruded rapeseeds	Extruded sunflower seeds			
o__Bacteroidales	0.404	0.445	0.447	0.404	0.0414	0.442	0.396
o__Clostridiales	0.133	0.237	0.175	0.155	0.0168	0.326	0.115
o__Fibrobacteriales	0.048	0.101	0.074	0.074	0.0258	0.314	0.090
o__Aeromonadales	0.304ab *	0.101a	0.193ab	0.267b	0.0388	0.042	0.008
o__Erysipelotrichales	0.013ab	0.0250a	0.012ab	0.008b	0.0037	0.015	0.004
o__Spirochaetales	0.010	0.018	0.018	0.015	0.0044	0.647	0.808
o__RF32	0.007	0.013	0.006	0.010	0.0018	0.708	0.716
o__YS2	0.015	0.011	0.016	0.018	0.0048	0.518	0.146
o__Bifidobacteriales	0.006	0.008	0.001	0.004	0.0017	0.162	0.203
o__RF39 x 10 ²	7.522	6.210	5.340	4.424	0.0013	0.897	0.903
o__uncl_Bacteria x 10 ²	4.806	4.679	4.387	4.971	0.0015	0.927	0.544
o__WCHB1_41 x 10 ²	11.423	4.129	11.672	5.647	0.0047	0.134	0.090
o__Anaeroplasmatales x 10 ²	3.190	2.783	4.771	3.153	0.0018	0.589	0.628
o__uncl_SR1 x 10 ²	12.479	2.766	5.471	6.778	0.0028	0.206	0.069
o__Methanobacteriales x 10 ²	3.157	2.385	2.143	1.519	0.0002	0.917	0.903
o__Rhodospirillales x 10 ²	0.000	2.222	3.202	0.625	0.0001	0.274	0.688
o__uncl_Firmicutes x 10 ²	0.974	2.000	3.270	1.518	0.0014	0.218	0.275
o__uncl_Alphaproteobacteria.1 x 10 ²	1.331	1.740	1.664	1.254	0.0006	0.976	0.714
o__Elusimicrobiales x 10 ²	1.763	1.490	1.012	2.404	0.0002	0.544	0.628
o__PL_11B10 x 10 ²	1.015	1.048	1.017	0.713	0.0006	0.781	0.448
o__Victivallales x 10 ²	2.128	1.012	2.349	1.191	0.0018	0.568	0.181
o__Rickettsiales x 10 ²	0.826	0.958	1.884	0.584	0.0010	0.461	0.808
o__Burkholderiales x 10 ²	0.443	0.808	0.492	0.876	0.0002	0.975	0.704
o__E2 x 10 ²	0.672	0.798	0.458	0.660	0.0002	0.906	0.714
o__Rhizobiales x 10 ²	0.388	0.726	0.348	0.605	0.0004	0.811	0.448
o__HA64 x 10 ²	0.374	0.726	0.209	0.051	0.0002	0.549	0.688
o__Neisseriales x 10 ²	0.000	0.689	0.067	0.000	0.0001	0.243	0.075

o__Desulfovibrionales x 10 ²	0.184	0.641	1.013	0.154	0.0001	0.527	0.902
o__Pirellulales x 10 ²	1.196	0.572	1.512	1.174	0.0004	0.494	0.144
o__uncl_Gammaproteobacteria x 10 ²	0.000	0.523	0.000	0.000	0.0000	0.094	0.011
o__Mycoplasmatales x 10 ²	1.592	0.440	1.580	0.397	0.0007	0.148	0.076
o__Coriobacteriales x 10 ²	0.172	0.424	0.108	0.479	0.0000	0.497	0.376
o__Synergistales x 10 ²	0.105	0.403	0.095	0.247	0.0001	0.738	0.560
o__uncl_Mollicutes x 10 ²	0.000	0.242	0.000	0.047	0.0000	0.542	0.343
o__uncl_Proteobacteria x 10 ²	0.529	0.220	0.635	0.833	0.0005	0.714	0.271
o__Cardiobacteriales x 10 ²	0.325	0.174	0.676	1.689	0.0009	0.150	0.065
o__Xanthomonadales x 10 ²	0.130	0.161	0.288	0.249	0.0001	0.992	0.937
o__Pseudomonadales x 10 ²	0.234	0.081	0.096	0.996	0.0002	0.992	0.811
o__uncl_WPS_2 x 10 ²	0.208	0.081	0.522	0.225	0.0002	0.935	0.658
o__Streptophyta x 10 ²	0.446	0.058	0.000	0.000	0.0000	0.203	0.859
o__Lactobacillales x 10 ²	0.000	0.058	0.000	0.071	0.0000	0.542	0.461
o__Campylobacterales x 10 ²	0.026	0.058	0.258	0.219	0.0002	0.615	0.531
o__Desulfobacterales x 10 ²	0.026	0.044	0.000	0.056	0.0001	0.756	0.722
o__Sphingobacteriales x 10 ²	0.000	0.000	0.000	0.142	0.0000	0.392	0.564
o__uncl_Bacteroidetes x 10 ²	0.078	0.000	0.000	0.213	0.0001	0.542	0.399
o__Anaerolineales x 10 ²	0.329	0.000	0.130	0.257	0.0001	0.127	0.038
o__Bacillales x 10 ²	0.052	0.000	0.000	0.092	0.0001	0.243	0.286
o__uncl_Clostridia x 10 ²	0.216	0.000	0.075	0.234	0.0001	0.257	0.140
o__uncl_Alphaproteobacteria x 10 ²	0.216	0.000	0.067	0.328	0.0002	0.532	0.202
o__Caulobacterales x 10 ²	0.000	0.000	0.034	0.208	0.0000	0.203	0.286
o__unck_Betaproteobacteria x 10 ²	0.000	0.000	0.101	0.000	0.0001	0.392	0.564
o__GMD14H09 x 10 ²	0.148	0.000	0.075	0.051	0.0001	0.422	0.202
o__uncl_Deltaproteobacteria x 10 ²	0.182	0.000	0.000	0.000	0.0002	0.392	0.564
o__Enterobacteriales x 10 ²	0.026	0.000	0.254	0.071	0.0000	0.422	0.202
o__uncl_Mollicutes.1 x 10 ²	0.373	0.000	0.641	0.341	0.0002	0.108	0.023
o__ML615J_28 x 10 ²	0.130	0.000	0.067	0.047	0.0001	0.756	0.286
o__CW040 x 10 ²	0.140	0.000	0.034	0.000	0.0000	0.542	0.399
Unclassified x 10 ²	0.209	0.000	0.000	0.000	0.0000	0.392	0.564

* different letters in the same row indicate significant difference in order level abundance between diets

Supplementary table 4b: Bacterial abundance at the genus level in the rumen contents of cows fed high starch or rapeseed, palmoil or sunflower oil supplemented diets. Values are means of four observations; statistical analysis was performed to evaluate the effect of diet and lipid supplementation (starch diet compared to all lipid supplemented diets). In both cases we used the non-parametric Kruskal-Wallis test with *fdr* p value adjustment followed by a Dunn test when appropriate.

	Diets				SEM	<i>Effect of Diet</i>	<i>Effect of lipid supplementation</i>
	Ca salts of palmoil	High starch	Extruded rapeseeds	Extruded sunflower seeds			
g__Prevotella	0.271	0.328	0.300	0.278	0.0338	0.766	0.808
g__Fibrobacter	0.048	0.101	0.074	0.074	0.0258	0.314	0.090
g__uncl_Succinivibrionaceae1	0.245	0.061	0.132	0.168	0.0372	0.062	0.011
g__uncl_Bacteroidales	0.071	0.054	0.085	0.075	0.0152	0.687	0.396
g__uncl_Clostridiales	0.020	0.039	0.026	0.034	0.0136	0.553	0.332
g__Ruminococcus	0.016	0.030	0.027	0.019	0.0024	0.787	0.628
g__Succinivibrio	0.043	0.027	0.032	0.050	0.0095	0.867	0.544
g__Butyrivibrio	0.008	0.020	0.005	0.007	0.0018	0.206	0.146
g__uncl_Prevotellaceae1	0.019	0.019	0.017	0.014	0.0043	0.877	0.544
g__Treponema	0.009	0.018	0.018	0.015	0.0043	0.718	0.808
g__Selenomonas	0.003	0.016	0.004	0.003	0.0003	0.677	0.396
g__Megasphaera	0.002	0.015	0.002	0.004	0.0004	0.427	0.115
g__uncl_Clostridiales.1	0.012	0.014	0.013	0.011	0.0013	0.867	0.716
g__uncl_RF32	0.007	0.013	0.006	0.010	0.0018	0.708	0.716
g__RFN20 x 10 ²	6.99	13.24	9.67	4.48	0.0010	0.248	0.146
g__Succiniclasticum x 10 ²	9.26	12.93	11.84	7.56	0.0009	0.687	0.628
g__Clostridium x 10 ²	12.96	12.91	12.17	15.92	0.0015	0.867	0.808
g__Ruminobacter x 10 ²	13.73	12.84	26.46	45.87	0.0064	0.453	0.332
g__Coproccoccus x 10 ²	7.31	12.34	7.41	8.05	0.0009	0.912	0.467
g__uncl_Lachnospiraceae x 10 ²	11.61	11.56	15.26	13.14	0.0014	0.766	0.467
g__uncl_RF16 x 10 ²	20.95	11.33	21.72	13.77	0.0087	0.657	0.332
g__Shuttleworthia x 10 ²	3.15	10.82	0.94	3.29	0.0028	0.518	0.396

g__uncl_YS2 x 10 ²	15.24	10.50	16.16	17.89	0.0048	0.518	0.146
g__uncl_S24.7 x 10 ²	4.40	10.30	4.90	3.79	0.0005	0.897	0.716
g__Sharpea x 10 ²	4.745a	6.234a	0.973b	2.573ab	0.0037	0.034	0.225
g__uncl_RF39 x 10 ²	7.52	6.21	5.34	4.42	0.0013	0.897	0.903
g__uncl_Ruminococcaceae x 10 ²	6.98ac	5.25c	13.64b	10.35abc	0.0020	0.016	0.039
g__uncl_Paraprevotellaceae1 x 10 ²	4.41	5.22	4.70	5.53	0.0008	0.750	0.903
g__Roseburia x 10 ²	0.56	5.14	0.32	1.02	0.0002	0.292	0.218
g__Acidaminococcus x 10 ²	0.32	4.79	0.27	0.05	0.0000	0.553	0.437
g__uncl_Bacteria x 10 ²	4.81	4.68	4.39	4.97	0.0015	0.927	0.544
g__uncl_p.2534.18B5 x 10 ²	0.11	4.61	0.16	0.05	0.0001	0.877	1.000
g__uncl_Lachnospiraceae x 10 ²	1.91	4.15	3.15	2.92	0.0005	0.562	1.000
g__Bifidobacterium x 10 ²	3.25	4.04	1.09	3.80	0.0010	0.528	0.808
g__02d06 x 10 ²	3.52	4.00	3.13	1.95	0.0005	0.446	0.467
g__uncl_RFP12 x 10 ²	11.32	3.97	11.55	5.61	0.0047	0.134	0.090
g__CF231 x 10 ²	5.35	3.89	5.57	3.47	0.0021	0.766	0.808
g__uncl_Bacteroidales.1 x 10 ²	2.98	3.81	4.54	4.21	0.0016	0.907	0.716
g__uncl_Bifidobacteriaceae x 10 ²	2.34	3.78	0.10	0.44	0.0016	0.436	0.517
g__.Eubacterium. x 10 ²	0.13	3.24	0.34	0.31	0.0002	0.308	0.093
g__Clostridium.1 x 10 ²	1.84	2.93	4.52	2.73	0.0003	0.218	0.544
g__YRC22 x 10 ²	3.24	2.81	2.58	3.84	0.0007	0.687	0.628
g__uncl_SR1 x 10 ²	12.48	2.77	5.47	6.78	0.0028	0.206	0.069
g__uncl_Ruminococcaceae.1 x 10 ²	0.81	2.64	0.95	1.50	0.0001	0.583	0.671
g__Anaeroplasma x 10 ²	3.00	2.62	4.22	3.15	0.0018	0.846	0.903
g__Pseudobutyrvibrio x 10 ²	3.07	2.31	2.64	3.62	0.0009	0.457	0.275
g__Methanobrevibacter x 10 ²	3.13	2.30	2.01	1.33	0.0002	0.682	0.808
g__Acetobacter x 10 ²	0.00	2.22	3.20	0.63	0.0001	0.274	0.688
g__uncl_Paraprevotellaceae ² x 10 ²	1.16	2.09	0.49	1.15	0.0007	0.856	0.712
g__Anaerostipes x 10 ²	0.86	2.07	11.34	1.11	0.0003	0.391	0.160
g__uncl_Firmicutes x 10 ²	0.97	2.00	3.27	1.52	0.0014	0.218	0.275
g__Bulleidia x 10 ²	0.08	1.88	0.00	0.10	0.0001	0.107	0.033
g__uncl_Alphaproteobacteria.1 x 10 ²	1.33	1.74	1.66	1.25	0.0006	0.976	0.714
g__uncl_Elusimicrobiaceae x 10 ²	1.76	1.49	1.01	2.40	0.0002	0.544	0.628

g__Schwartzia x 10 ²	0.23	1.44	1.13	0.17	0.0006	0.840	0.806
g__uncl_Veillonellaceae x 10 ²	0.32	1.19	0.54	0.19	0.0002	0.059	0.020
g__uncl_PL.11B10 x 10 ²	1.01	1.05	1.02	0.71	0.0006	0.781	0.448
g__uncl_Victivallaceae x 10 ²	2.13	1.01	2.35	1.19	0.0018	0.568	0.181
g__Mitsuokella x 10 ²	0.16	0.99	0.10	0.09	0.0000	0.616	0.185
g__Sporobacter x 10 ²	0.17	0.78	0.25	0.25	0.0001	0.554	0.164
g__uncl_Mogibacteriaceae x 10 ²	0.60	0.76	0.52	0.48	0.0003	0.993	0.808
g__uncl_Rhizobiales x 10 ²	0.13	0.73	0.25	0.60	0.0004	0.658	0.560
g__uncl_HA64 x 10 ²	0.37	0.73	0.21	0.05	0.0002	0.549	0.688
g__uncl_Rickettsiales2 x 10 ²	0.13	0.72	1.48	0.25	0.0007	0.047	0.389
g__Alysiella x 10 ²	0.00	0.65	0.00	0.00	0.0000	0.392	0.083
g__Desulfovibrio x 10 ²	0.18	0.64	1.01	0.15	0.0001	0.527	0.902
g__uncl_Pirellulaceae x 10 ²	1.20	0.57	1.51	1.17	0.0004	0.494	0.144
g__uncl_Gammaproteobacteria x 10 ²	0.00	0.52	0.00	0.00	0.0000	0.094	0.011
g__vadinCA11 x 10 ²	0.23	0.52	0.10	0.16	0.0001	0.644	0.560
g__BF311 x 10 ²	0.33	0.45	0.03	0.11	0.0002	0.379	0.350
g__Moryella x 10 ²	1.13	0.45	1.39	1.03	0.0003	0.405	0.089
g__uncl_Mycoplasmataceae x 10 ²	1.59	0.44	1.58	0.40	0.0007	0.148	0.076
g__Olsenella x 10 ²	0.17	0.42	0.11	0.48	0.0000	0.497	0.376
g__uncl_Peptostreptococcaceae x 10 ²	0.03	0.42	0.00	0.00	0.0004	0.542	0.343
g__Mogibacterium x 10 ²	0.03	0.40	0.32	0.13	0.0001	0.323	0.688
g__Bordetella x 10 ²	0.00	0.40	0.00	0.09	0.0000	0.542	0.343
g__Pyramidobacter x 10 ²	0.10	0.40	0.10	0.25	0.0001	0.738	0.560
g__Oscillospira x 10 ²	0.00	0.35	0.21	0.09	0.0001	0.462	1.000
g__Dialister x 10 ²	0.00	0.35	0.00	0.00	0.0000	0.392	0.083
g__Lachnobacterium x 10 ²	0.05	0.35	0.00	0.00	0.0000	0.542	0.343
g__Lachnospira x 10 ²	0.33	0.35	0.07	0.24	0.0001	0.841	0.834
g__uncl_Methanomassiliicoccaceae x 10 ²	0.44	0.28	0.36	0.49	0.0001	0.911	0.618
g__uncl_Rickettsiales1 x 10 ²	0.69	0.24	0.40	0.34	0.0005	0.610	0.400
g__uncl_Mollicutes x 10 ²	0.00	0.24	0.00	0.05	0.0000	0.542	0.343
g__Lamproedia x 10 ²	0.00	0.23	0.10	0.00	0.0000	0.542	0.343
g__uncl_Erysipelotrichaceae.1 x 10 ²	0.15	0.22	0.17	0.05	0.0002	0.877	1.000

g__uncl_Proteobacteria x 10²	0.53	0.22	0.64	0.83	0.0005	0.714	0.271
g__uncl_Veillonellaceae.1 x 10²	0.48	0.21	1.03	0.16	0.0002	0.061	0.110
g__uncl_Spirochaetaceae1 x 10²	0.78	0.21	0.07	0.09	0.0007	0.992	0.937
g__Blautia x 10²	0.03	0.21	0.06	0.09	0.0000	0.715	0.403
g__uncl_Spirochaetaceae² x 10²	0.00	0.18	0.46	0.17	0.0000	0.058	0.476
g__Suttonella x 10²	0.32	0.17	0.68	1.69	0.0009	0.150	0.065
g__uncl_Christensenellaceae x 10²	0.09	0.16	0.38	0.00	0.0000	0.142	0.593
g__Oribacterium x 10²	0.14	0.16	0.90	0.36	0.0001	0.165	0.311
g__Anaerovibrio x 10²	1.03	0.16	1.24	0.70	0.0004	0.182	0.054
g__uncl_Xanthomonadaceae x 10²	0.00	0.16	0.00	0.04	0.0000	0.542	0.343
g__uncl_Anaeroplasmataceae x 10²	0.03	0.16	0.00	0.00	0.0000	0.542	0.343
g__uncl_WCHB1.41 x 10²	0.00	0.16	0.06	0.00	0.0000	0.542	0.343
g__uncl_Erysipelotrichaceae x 10²	0.77	0.15	0.80	0.25	0.0003	0.092	0.085
g__Comamonas x 10²	0.00	0.12	0.29	0.60	0.0000	0.756	0.859
g__Methanosphaera x 10²	0.03	0.08	0.14	0.19	0.0000	0.728	0.728
g__Syntrophococcus x 10²	0.29	0.08	0.07	0.05	0.0001	0.246	0.626
g__Clostridium.2 x 10²	0.17	0.08	0.29	0.05	0.0001	0.504	0.728
g__Succinimonas x 10²	0.74	0.08	2.24	3.28	0.0017	0.074	0.019
g__Pseudomonas x 10²	0.23	0.08	0.10	0.75	0.0002	0.992	0.811
g__uncl_WPS.2 x 10²	0.21	0.08	0.52	0.22	0.0002	0.935	0.658
g__uncl_Streptophyta x 10²	0.45	0.06	0.00	0.00	0.0000	0.203	0.859
g__uncl_Leuconostocaceae x 10²	0.00	0.06	0.00	0.07	0.0000	0.542	0.461
g__Sutterella x 10²	0.21	0.06	0.07	0.00	0.0000	0.756	0.859
g__Campylobacter x 10²	0.03	0.06	0.26	0.22	0.0002	0.615	0.531
g__uncl_Succinivibrionaceae² x 10²	1.01	0.06	0.15	0.21	0.0001	0.618	0.365
g__L7A_E11 x 10²	0.07	0.04	0.07	0.04	0.0000	0.992	0.937
g__p.75.a5 x 10²	0.12	0.04	0.03	0.14	0.0001	0.313	0.350
g__uncl_Neisseriaceae x 10²	0.00	0.04	0.07	0.00	0.0001	0.542	0.461
g__Desulfobulbus x 10²	0.03	0.04	0.00	0.06	0.0001	0.756	0.722
g__uncl_BS11 x 10²	0.41	0.00	0.03	0.66	0.0001	0.125	0.094
g__uncl_Prevotellaceae² x 10²	0.99	0.00	1.04	1.16	0.0004	0.057	0.007
g__Sphingobacterium x 10²	0.00	0.00	0.00	0.14	0.0000	0.392	0.564

g__uncl_Bacteroidetes x 10²	0.08	0.00	0.00	0.21	0.0001	0.542	0.399
g__SHD.231 x 10²	0.33	0.00	0.13	0.26	0.0001	0.127	0.038
g__Bacillus x 10²	0.05	0.00	0.00	0.09	0.0001	0.243	0.286
g__uncl_Clostridiaceae1 x 10²	0.19	0.00	0.10	0.00	0.0000	0.243	0.286
g__uncl_Clostridiaceae1.1 x 10²	0.37	0.00	0.29	0.05	0.0001	0.422	0.202
g__Robinsoniella x 10²	0.10	0.00	0.20	0.34	0.0001	0.225	0.061
g__Papillibacter x 10²	0.10	0.00	0.00	0.00	0.0000	0.392	0.564
g__uncl_Clostridia x 10²	0.22	0.00	0.07	0.23	0.0001	0.257	0.140
g__Asteroleplasma x 10²	0.00	0.00	0.11	0.06	0.0001	0.243	0.286
g__uncl_Alphaproteobacteria x 10²	0.22	0.00	0.07	0.33	0.0002	0.532	0.202
g__Brevundimonas x 10²	0.00	0.00	0.03	0.21	0.0000	0.203	0.286
g__Bosea x 10²	0.21	0.00	0.00	0.00	0.0000	0.392	0.564
g__Ochrobactrum x 10²	0.05	0.00	0.10	0.00	0.0001	0.542	0.399
g__Alcaligenes x 10²	0.16	0.00	0.00	0.00	0.0002	0.392	0.564
g__Brachymonas x 10²	0.08	0.00	0.03	0.00	0.0001	0.542	0.399
g__uncl_Comamonadaceae x 10²	0.00	0.00	0.00	0.18	0.0000	0.392	0.564
g__uncl_Betaproteobacteria x 10²	0.00	0.00	0.10	0.00	0.0001	0.392	0.564
g__uncl_GMD14H09 x 10²	0.15	0.00	0.07	0.05	0.0001	0.422	0.202
g__uncl_Deltaproteobacteria x 10²	0.18	0.00	0.00	0.00	0.0002	0.392	0.564
g__uncl_Enterobacteriaceae x 10²	0.03	0.00	0.25	0.07	0.0000	0.422	0.202
g__Acinetobacter x 10²	0.00	0.00	0.00	0.25	0.0000	0.392	0.564
g__Solimonas x 10²	0.10	0.00	0.00	0.00	0.0001	0.392	0.564
g__Stenotrophomonas x 10²	0.03	0.00	0.29	0.11	0.0000	0.756	0.286
g__Xanthomonas x 10²	0.00	0.00	0.00	0.11	0.0000	0.392	0.564
g__Anaeroplasmataceae_gut x 10²	0.17	0.00	0.55	0.00	0.0001	0.178	0.202
g__uncl_Mollicutes.1 x 10²	0.37	0.00	0.64	0.34	0.0002	0.108	0.023
g__uncl_ML615J.28 x 10²	0.13	0.00	0.07	0.05	0.0001	0.756	0.286
g__uncl_F16 x 10²	0.14	0.00	0.03	0.00	0.0000	0.542	0.399
g__uncl_WCHB1.25 x 10²	0.10	0.00	0.06	0.04	0.0001	0.756	0.286
Unclassified x 10²	0.21	0.00	0.00	0.00	0.0000	0.392	0.564

Supplementary Table 5: Bacterial abundance at order level in the faecal contents of cows fed high starch or rapeseed, palmoil or sunflower oil supplemented diets. Values are means of four observations; statistical analysis was performed to evaluate the effect of diet and lipid supplementation (starch diet compared to all lipid supplemented diets). In both cases we used the non parametric Kruskal-Walis test with fdr p value adjustment followed by a Dunn test when appropriate.

Order	Diets				SEM	P-value	
	Ca salts of palmoil	High starch	Extruded rapeseeds	Extruded sunflower seeds		<i>Effect of Diet</i>	<i>Effect of lipid supplementation</i>
o__Clostridiales	0.524	0.450	0.527	0.540	0.021	0.37	0.09
o__Bacteroidales	0.330	0.269	0.294	0.322	0.014	0.90	0.54
o__Aeromonadales	0.015	0.158	0.095	0.025	0.005	0.56	0.23
Unclassified	0.011	0.020	0.004	0.008	0.000	0.12	0.81
o__Spirochaetales	0.025	0.011	0.017	0.019	0.005	0.14	0.09
o__Erysipelotrichales	0.005	0.011	0.005	0.007	0.001	0.20	0.05
o__RF39 x 10 ²	4.731	8.084	2.007	3.826	0.004	0.03	0.05
o__YS2 x 10 ²	6.658	8.067	6.369	4.956	0.003	0.81	0.47
o__Victivallales x 10 ²	10.274	7.728	1.215	8.503	0.001	0.09	0.81
o__Bifidobacteriales x 10 ²	4.512	7.469	8.164	9.804	0.002	0.49	0.72
o__Desulfovibrionales x 10 ²	11.737	7.356	7.255	8.718	0.001	0.46	0.47
o__M2PT2.76 x 10 ²	7.212	4.583	2.812	3.264	0.001	0.38	0.36
o__Burkholderiales x 10 ²	5.761	4.395	3.578	4.161	0.001	0.41	0.72
o__WCHB1.41 x 10 ²	6.406	4.173	1.015	3.430	0.000	0.18	0.86
o__Verrucomicrobiales x 10 ²	6.316	3.493	3.680	4.649	0.002	0.38	0.47
o__uncl_Firmicutes x 10 ²	2.948	3.146	3.678	2.701	0.001	0.92	0.54
o__Anaeroplasmatales x 10 ²	1.921	2.337	1.142	2.090	0.001	0.59	0.54
o__RF32 x 10 ²	1.086	2.277	0.204	0.724	0.000	0.09	0.11
o__Pirellulales x 10 ²	1.635	1.960	1.293	2.176	0.001	0.88	1.00
o__uncl_Alphaproteobacteria x 10 ²	0.000	1.861	0.112	0.245	0.000	0.22	0.58
o__Campylobacteriales x 10 ²	0.879	1.261	3.029	0.551	0.002	0.58	0.90
o__uncl_Bacteroidetes x 10 ²	0.727	1.058	0.499	0.297	0.000	0.62	0.32
o__Turicibacteriales x 10 ²	0.250	0.901	2.332	0.574	0.000	0.59	0.81
o__Elusimicrobiales x 10 ²	0.313	0.829	0.000	0.298	0.001	0.15	0.25
o__uncl_Tenericutes x 10 ²	0.807	0.722	0.729	0.625	0.000	0.93	1.00
o__ML615J.28 x 10 ²	1.395	0.718	1.521	1.345	0.001	0.47	0.11
o__Fibrobacteriales x 10 ²	3.650	0.717	0.636	2.910	0.000	0.01	0.07
o__Coriobacteriales x 10 ²	0.311	0.628	0.396	1.288	0.000	0.50	0.32
o__Cerasiococcales. x 10 ²	0.813	0.620	0.158	0.748	0.000	0.09	1.00

o__uncl_Clostridia x 10 ²	0.322	0.438	0.234	0.214	0.000	0.97	0.71
o__uncl_Mollicutes x 10 ²	0.000	0.423	0.000	0.069	0.000	0.02	0.01
o__uncl_Proteobacteria x 10 ²	0.507	0.386	0.560	0.892	0.000	0.77	0.95
o__Rickettsiales x 10 ²	0.626	0.322	0.644	0.398	0.000	0.54	0.39
o__Enterobacteriales x 10 ²	2.135	0.235	0.112	0.411	0.000	0.69	0.42
o__Z20 x 10 ²	0.061	0.222	0.000	0.115	-	0.76	0.59
o__HA64 x 10 ²	0.065	0.182	0.000	0.182	0.000	0.26	0.08
o__Sphingobacteriales x 10 ²	0.970	0.169	0.000	0.000	-	0.28	0.11
o__Flavobacteriales x 10 ²	0.000	0.146	0.092	0.107	-	0.28	0.14
o__uncl_Bacilli x 10 ²	0.099	0.084	0.000	0.000	0.000	0.54	0.46
o__Bacillales x 10 ²	0.000	0.064	0.076	0.000	0	0.28	0.11
o__Rhizobiales x 10 ²	0.117	0.040	0.204	0.358	0.000	0.41	0.17
o__Rhodospirillales x 10 ²	0.104	0.011	0.000	0.000	-	0.54	0.46
o__Lactobacillales x 10 ²	0.000	0.000	0.000	0.091	0	0.39	0.56
o__GMD14H09 x 10 ²	0.182	0.000	0.000	0.000	-	0.39	0.56
o__Acholeplasmatales x 10 ²	0.000	0.000	0.130	0.000	-	0.09	0.40
