

## Aberystwyth University

### *Characterisation of the Faecal Bacterial Community in Adult and Elderly Horses Fed a High Fibre, High Oil or High Starch Diet Using 454 Pyrosequencing*

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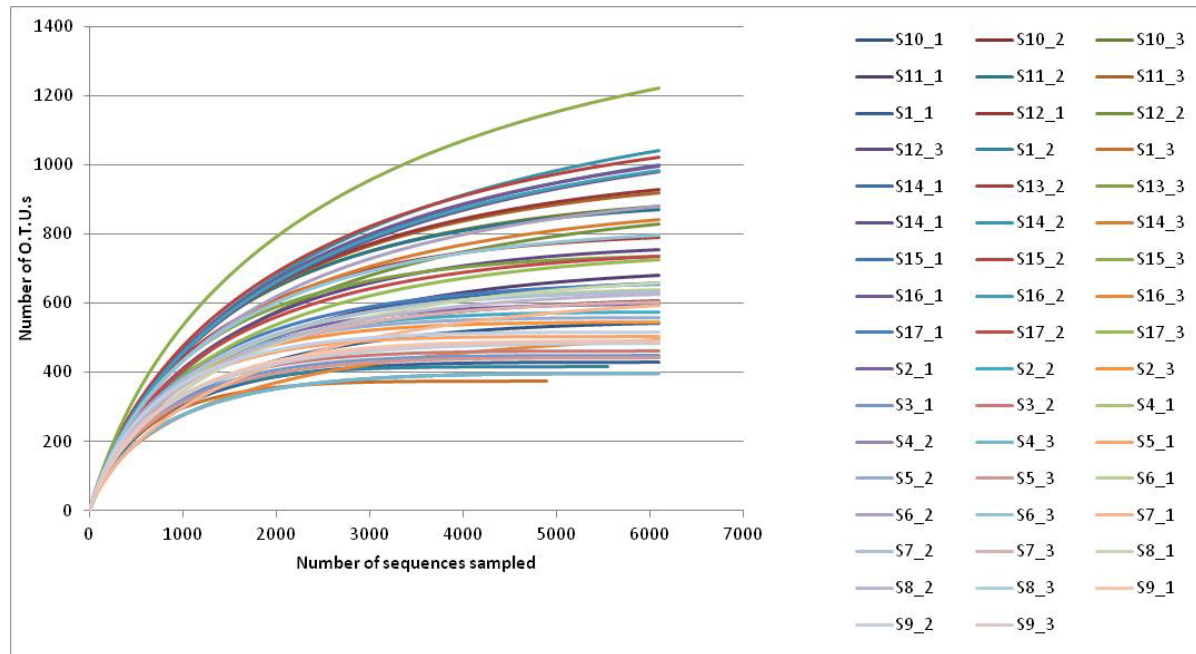
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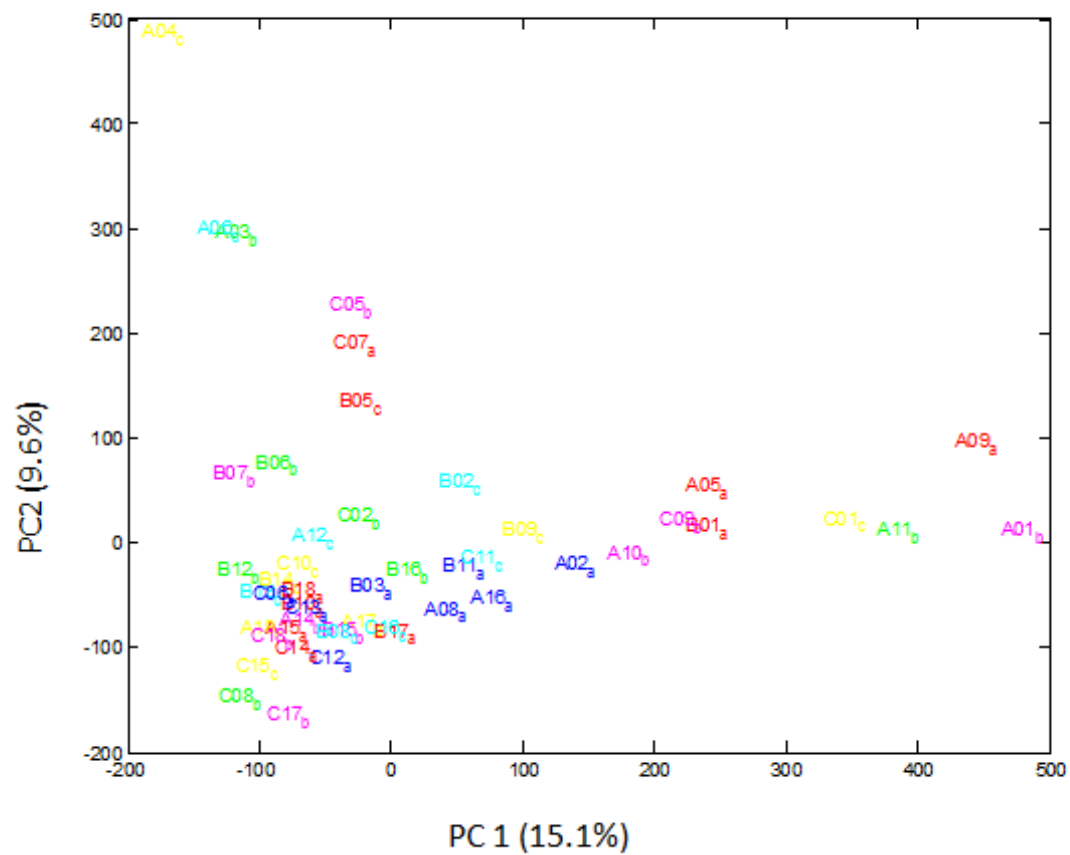
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**Figure S1.** Rarefaction Curves showing depth of sequencing of the microbial communities of faeces from eighteen horses fed three different diets (Calculated from non-normalised data)



**Figure S2**-Principle component analysis of relative abundance of OTUs identified from faecal samples from eighteen horses fed three different diets. Samples are coloured by age/diet combinations (Hay diet adult, hay diet elderly, fat diet adult, fat diet elderly, starch diet adult, starch diet elderly).



**Table S1.** Animal metadata

Animal number	Age	Breed	Bodyweight at start	Body condition score at start
1	20*	Stock-type	474	5.3
2	20*	Stock-type	491	5.0
3	10*	Stock-type	550	5.5
4	20*	Stock-type	461	5.0
5	11*	Stock-type	499	5.5
6	10*	Stock-type	514	5.0
7	20*	Stock-type	464	5.0
8	4*	Thoroughbred	511	4.0
9	30	Stock-type	461	5.5
10	20*	Stock-type	478	4.5
11	11*	Stock-type	544	5.5
12	10*	Stock-type	450	4.5
13	20*	Stock-type	434	5.0
14	19	Thoroughbred	523	5.0
15	10*	Stock-type	500	5.5
16	20*	Stock-type	425	5.0
17	8	Thoroughbred	470	4.5

\* Age estimated by Veterinary dental examination.

**Table S2.** MID barcode sequences used for multiplexed 454 pyrosequencing

MID number	Sequence	MID number2	Sequence3
MID-1	ACGAGTGCGT	MID-23	TACTCTCGTG
MID-2	ACGCTCGACA	MID-24	TAGAGACGAG
MID-3	AGACGCACTC	MID-25	TCGTCGCTCG
MID-4	AGCACTGTAG	MID-26	ACATACGCGT
MID-5	ATCAGACACG	MID-27	ACGCGAGTAT
MID-6	ATATCGCGAG	MID-28	ACTACTATGT
MID-7	CGTGTCTCTA	MID-29	ACTGTACAGT
MID-8	CTCGCGTGTC	MID-30	AGACTATACT
MID-10	TCTCTATGCG	MID-31	AGCGTCGTCT
MID-11	TGATACGTCT	MID-32	AGTACGCTAT
MID-13	CATAGTAGTG	MID-33	ATAGAGTACT
MID-14	CGAGAGATAC	MID-34	CACGCTACGT
MID-15	ATACGACGTA	MID-35	CAGTAGACGT
MID-16	TCACGTACTA	MID-36	CGACGTGACT
MID-17	CGTCTAGTAC	MID-37	TACACACACT
MID-18	TCTACGTAGC	MID-38	TACACGTGAT
MID-19	TGTACTACTC	MID-39	TACAGATCGT
MID-20	ACGACTACAG	MID-40	TACGCTGTCT
MID-21	CGTAGACTAG	MID-41	TAGTGTAGAT
MID-22	TACGAGTATG	MID-42	TCGATCACGT



**Table S4** Relative abundance of each OTU significant ( $P < 0.001$ ) for Diet, Age or Diet\*Age

O.T.U.	HAY		CHO		OIL		SED		
	Adult	Elderly	Adult	Elderly	Adult	Elderly	Diet	Age	Diet*Age
6	0.773	0.789	0.539	0.548	0.333	0.345	0.114**	0.090	0.231
8	1.183	1.158	0.943	1.097	0.323	0.466	0.221**	0.232	0.345
11	0.786	1.226	0.476	0.544	0.494	0.719	0.145**	0.180	0.246
18	0.020	0.026	0.370	0.269	0.000	0.019	0.048***	0.041	0.069
22	0.225	0.355	0.034	0.092	0.159	0.120	0.038***	0.047	0.064
26	0.000	0.017	0.575	0.700	0.000	0.014	0.125***	0.098	0.174
27	0.280	0.262	0.500	0.617	0.158	0.361	0.093**	0.090	0.140
31	0.222	0.198	0.103	0.003	0.051	0.021	0.055**	0.080	0.102
36	0.299	0.597	0.192	0.169	0.119	0.288	0.053***	0.080	0.101
62	0.028	0.051	0.132	0.304	0.008	0.000	0.052***	0.037	0.070
66	0.399	0.528	0.286	0.247	0.208	0.140	0.084**	0.126	0.160
68	0.001	0.000	0.299	0.512	0.001	0.000	0.117**	0.092	0.164
90	0.165	0.182	0.072	0.063	0.272	0.151	0.036***	0.031	0.052
103	0.196	0.064	0.002	0.006	0.028	0.041	0.026***	0.019	0.036
105	0.067	0.025	0.134	0.127	0.058	0.041	0.028**	0.016	0.064
107	0.144	0.171	0.628	0.315	0.674	0.498	0.121**	0.152	0.206
109	0.567	0.436	0.272	0.408	0.217	0.210	0.077**	0.118	0.147
116	0.121	0.029	0.098	0.103	0.228	0.341	0.051***	0.063	0.086
120	0.152	0.056	0.114	0.047	0.047	0.049	0.0239***	0.029	0.040
134	0.026	0.000	0.510	0.562	0.319	0.025	0.150**	0.143	0.225
138	0.192	0.115	0.007	0.027	0.186	0.095	0.039**	0.042	0.061
148	0.167	0.134	0.057	0.088	0.190	0.190	0.028***	0.029	0.043
153	0.200	0.156	0.075	0.057	0.151	0.115	0.027***	0.031	0.044
160	0.271	0.079	0.033	0.016	0.013	0.024	0.049**	0.055	0.079
162	0.099	0.110	0.091	0.125	0.513	0.267	0.084**	0.063	0.115
168	0.040	0.020	0.037	0.053	0.176	0.033	0.023**	0.015**	0.031**
169	0.008	0.004	0.144	0.379	0.008	0.004	0.060***	0.048	0.084
170	0.240	0.040	0.121	0.071	0.332	0.162	0.452**	0.081	0.097

188	0.075	0.136	0.037	0.027	0.263	0.248	0.048***	0.053	0.077
189	0.138	0.185	0.023	0.023	0.060	0.084	0.030***	0.038	0.051
191	0.040	0.000	0.000	0.038	0.072	0.213	0.039**	0.030	0.054
222	0.054	0.052	0.048	0.032	0.164	0.100	0.023***	0.015	0.031
224	0.024	0.065	0.024	0.020	0.042	0.083	0.011**	0.017	0.022
227	0.003	0.010	0.170	0.174	0.208	0.081	0.044**	0.041	0.065
268	0.242	0.082	0.051	0.067	0.059	0.037	0.028***	0.020	0.038
281	0.097	0.080	0.008	0.014	0.074	0.049	0.022**	0.020	0.032
300	0.069	0.085	0.028	0.024	0.037	0.017	0.016**	0.018	0.026
350	0.000	0.000	0.109	0.301	0.000	0.000	0.069**	0.060	0.100
396	0.101	0.063	0.012	0.040	0.006	0.010	0.023***	0.021	0.034
409	0.018	0.003	0.082	0.130	0.180	0.249	0.044***	0.041	0.065
413	0.029	0.070	0.153	0.384	0.017	0.014	0.048***	0.048	0.073
480	0.104	0.088	0.020	0.030	0.035	0.030	0.020**	0.022	0.032
531	0.380	0.233	0.151	0.083	0.163	0.113	0.057**	0.042	0.078
561	0.014	0.037	0.051	0.014	0.103	0.032	0.013**	0.020	0.025**
570	0.087	0.134	0.038	0.040	0.027	0.083	0.022**	0.022	0.034
668	0.029	0.059	0.034	0.124	0.005	0.038	0.017**	0.033	0.038
832	0.021	0.013	0.064	0.003	0.157	0.038	0.022**	0.015***	0.029
846	0.002	0.002	0.092	0.139	0.161	0.172	0.046**	0.038	0.065
993	0.129	0.088	0.007	0.020	0.028	0.022	0.031**	0.030	0.046
1089	0.044	0.091	0.027	0.020	0.128	0.055	0.017***	0.019	0.027**
1168	0.051	0.147	0.046	0.015	0.008	0.015	0.025**	0.020	0.036
1353	0.024	0.011	0.140	0.021	0.033	0.019	0.018**	0.020	0.02912**



**Table S5** Classification of each OTU significant ( $P < 0.001$ ) for Diet, Age or Diet\*Age

OTU	Phyla	Class	Order	Family	Genus
6	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
8	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Paraprevotella
11	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
18	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia
22	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
26	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Succinivibrio
27	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales incertae sedis	Phocaeicola
31	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
36	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
62	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
66	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenella
68	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
90	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus
103	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Pseudoflavonifractor
105	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
107	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae incertae sedis
109	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified
116	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
120	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
134	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter
138	Firmicutes	Unclassified	Unclassified	Unclassified	Unclassified
148	Firmicutes	Clostridia	Clostridiales	Unclassified	Unclassified
153	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
160	Proteobacteria	Alphaproteobacteria	Unclassified	Unclassified	Unclassified
162	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Unclassified
168	Firmicutes	Clostridia	Clostridiales	Clostridiales Incertae Sedis XIII	Mogibacterium
169	Proteobacteria	Gammaproteobacteria	Aeromonadales	Succinivibrionaceae	Unclassified
170	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Paraprevotella

188	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiracea incertae sedis
189	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
191	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
222	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Flavonifractor
224	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Pseudobutyrvibrio
227	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Unclassified
268	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema
281	Actinobacteria	Actinobacteria	Coriobacteridae	Coriobacteriales	Coriobacterineae
300	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
350	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
396	Firmicutes	Unclassified	Unclassified	Unclassified	Unclassified
409	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter
413	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium sensu stricto
480	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified
531	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified
561	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
570	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
668	Bacteroidetes	Unclassified	Unclassified	Unclassified	Unclassified
832	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
846	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter
993	Firmicutes	Unclassified	Unclassified	Unclassified	Unclassified
1089	Actinobacteria	Actinobacteria	Coriobacteridae	Coriobacteriales	Unclassified
1168	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
1353	Bacteroidetes	Bacteroidia	Bacteroidales	Unclassified	Unclassified

**Table S6.** Classification of the core bacterial community in the faeces of horses fed three different diets.

	O.T.U.	Phyla	Class	Order	Family	Relative Abundance (%)	Standard deviation
<b>ALL</b>	375	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.25	0.003
	237	Bacteroidetes	Unclassified	Unclassified	Unclassified	0.27	0.003
	15,38	Firmicutes	Clostridia	Clostridiales	Clostridiales_Incertae Sedis XIII	0.50	0.005
	6, 78	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	1.29	0.012
<b>HAY</b>	375	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.24	0.008
	221	Bacteroidetes	Bacteroidia	Bacteroidales	Unclassified	0.25	0.012
	531	Unclassified	Unclassified	Unclassified	Unclassified	0.28	0.014
	66	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	0.45	0.026
	15,38	Firmicutes	Clostridia	Clostridiales	Clostridiales_Incertae Sedis XIII	0.53	0.021
	21,82	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	0.58	0.017
	3	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	0.65	0.027
	92,237	Bacteroidetes	Unclassified	Unclassified	Unclassified	0.90	0.038
	8	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	1.13	0.049
	5	Firmicutes	Clostridia	Unclassified	Unclassified	1.20	0.044
	7	Fibrobacteres	Fibrobacteria	Fibrobacterales	Fibrobacteraceae	1.25	0.070
	4,9,13,96	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	3.21	0.107
	6,10,11,22,25,29,36,78,232,262,949,1047	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	5.26	0.109
	<b>CHO</b>	1414	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	0.17
6		Firmicutes	Clostridia	Clostridiales	Unclassified	0.21	0.038
47		Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	0.26	0.008
411		Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.30	0.026
96		Bacteroidetes	Unclassified	Unclassified	Unclassified	0.32	0.008
3		Unclassified	Unclassified	Unclassified	Unclassified	0.33	0.038
38,413		Firmicutes	Clostridia	Clostridiales	Clostridiales_Incertae Sedis XIII	0.44	0.016
78		Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	0.47	0.023
15		Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	0.75	0.004
18,21,109,237,375		Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	2.17	0.027
<b>FAT</b>	20	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	0.37	0.023
	109,531	Unclassified	Unclassified	Unclassified	Unclassified	0.37	0.014

	122,375	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.40	0.010
	15,38	Firmicutes	Clostridia	Clostridiales	Clostridiales_Incertae Sedis XIII	0.52	0.014
	63	Proteobacteria	Alphaproteobacteria	Rhizobiales	Unclassified	0.53	0.053
	145,365	Bacteroidetes	Bacteroidia	Bacteroidales	Unclassified	0.57	0.021
	17,92,237	Bacteroidetes	Unclassified	Unclassified	Unclassified	1.03	0.031
	5	Firmicutes	Unclassified	Unclassified	Unclassified	1.17	0.082
	4	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	1.40	0.078
	6,11,25,29,36,37,78,280,411,1047	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	3.96	0.079