At-hatch administration of probiotic to chickens can introduce permanent and beneficial changes in gut microbiota

Running Title: Early administration of probiotics

Stephen Baldwin[1], Robert J. Hughes[3,4], Thi Hao Van[2], Robert J. Moore[2] and Dragana Stanley[2§]

[1]Central Queensland University, Institute for Future Farming Systems, Rockhampton, Queensland, Australia.

[2]RMIT University, School of Science, Bundoora, Victoria, Australia.

[3]South Australian Research and Development Institute, Roseworthy, South Australia, Australia.

[4]The University of Adelaide, School of Animal and Veterinary Sciences Roseworthy, South Australia, Australia.

§Correspondence:

Dragana Stanley

Central Queensland University

Institute for Future Farming Systems

630 Ibis Avenue, CQIRP, North Rockhampton, QLD, Australia

Email: D.Stanley@cqu.edu.au

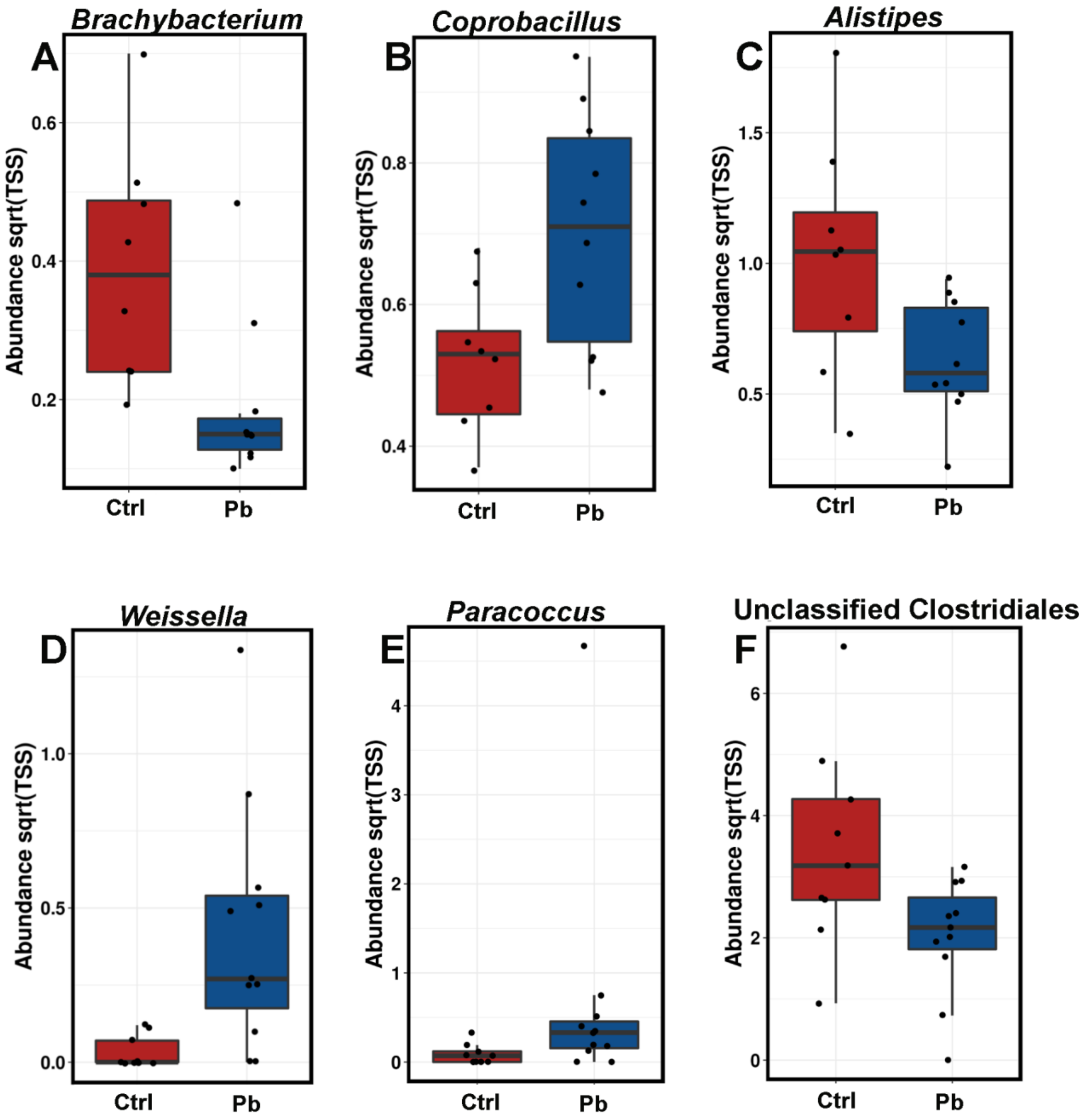

Figure A: Genera significantly different between probiotic inoculated birds and control in caecal content (A-C) and in ileal mucosa (D-E) microbial communities.

The full list of affected genera and OTUs with significance values is provided in Tables S5 to S8 below.

Table A: Excreta genera significantly altered by at-hatch administration at 14 days

Taxa	P value	Mean Ctrl	Mean Pb
Unclassified Planococcaceae	0.034	0.12	0.3
Unclassified Clostridiales	0.035	1.49	3.61
Lactococcus	0.038	0.16	0.07
Pseudoclavibacter	0.045	0	0.04
Nocardiopsis	0.045	0	0.1
Burkholderia	0.045	0	0.06
Brevundimonas	0.045	0	0.04

*The P-value was calculated using Wilcoxon test on square root transformed TSS normalised data. Mean values given for the control and probiotic group are also normalised and do not represent relative abundance.

Table B: Excreta OTUs significantly altered by at-hatch administration at 14 days

GreenGene annotation	OTUs by Qiime	Blast hit (16S Microbial NCBI)	% Id	P-value	Mean Ctr	Mean Pb1
Firmicutes_g_*Clostridium_s_Clostridium bifermentans*	*40448*	*Clostridium irregulare*	83	0.0035	0	0.14
Firmicutes_o_Clostridiales	*31016*	*Turicibacter sanguinis*	91	0.0087	0	0.28
Firmicutes_g_*Clostridium*	*58292*	*Clostridium chauvoei*	99	0.0087	0	0.37
Firmicutes_g_*Lactobacillus*	*92949*	*Clostridium bartlettii*	89	0.012	0.05	0.22
Firmicutes_g_*Clostridium*	*42048*	*Clostridium bartlettii*	89	0.02	0	0.04
Firmicutes_g_*Ruminococcus*	*110830*	*Ruminococcus torques*	95	0.025	0.05	0
Firmicutes_g_*Lactobacillus*	*990*	*Lactobacillus ingluviei*	88	0.025	0.14	0.4
Firmicutes_g_*Coprococcus*	*103119*	*Ruminococcus lactaris*	94	0.026	0.15	0
Proteobacteria_f_Enterobacteriaceae	*56839*	*Escherichia fergusonii/Shigella sonnei*	89	0.033	0.31	0.15
Firmicutes_g_*Lactobacillus*	*55066*	*Lactobacillus panis*	88	0.034	0.03	0.12
Firmicutes_g_*Clostridium*	*78276*	*Blautia luti*	92	0.034	0.06	0.21
Firmicutes_f_Planococcaceae	*28277*	*Bacillus odysseyi*	100	0.034	0.12	0.3
Firmicutes_f_Lachnospiraceae	*3791*	*Clostridium citroniae*	87	0.036	0.19	0.08
Firmicutes_g_*Lactococcus_s_Lactococcus lactis*	*65347*	*Lactococcus lactis*	98	0.038	0.16	0.07
Firmicutes_o_Clostridiales	*34656*	*Clostridium bartlettii*	91	0.043	0.03	0.1
Firmicutes_o_Clostridiales	*33821*	*Clostridium glycolicum*	97	0.043	1.14	3.33
Firmicutes_g_*Lactobacillus*	*2757*	*Lactobacillus ingluviei*	99	0.043	1	2.34
Proteobacteria_g_*Burkholderia_s_Burkholderia fungorum*	*7337*	*Burkholderia fungorum*	100	0.045	0	0.06
Proteobacteria_g_*Brevundimonas*	*115675*	*Brevundimonas nasdae*	100	0.045	0	0.04
Proteobacteria_f_Alcaligenaceae	*95362*	*Parasutterella excrementihominis*	87	0.045	0	0.04
Firmicutes_g_*Clostridium*	*61291*	*Clostridium lavalense*	92	0.045	0	0.05
Firmicutes_g_*Blautia*	*20413*	*Blautia producta*	97	0.045	0	0.03
Actinobacteria_g_*Pseudoclavibacter*	*14134*	*Curtobacterium flaccumfaciens*	98	0.045	0	0.04
Actinobacteria_g_*Nocardiopsis*	*26604*	*Nocardiopsis alba*	100	0.045	0	0.1
Firmicutes_g_*Paenibacillus*	*12572*	*Paenibacillus taiwanensis*	89	0.047	0.02	0.12
Firmicutes_o_Clostridiales	*4154*	*Clostridium glycolicum*	94	0.052	0.03	0.14

Table C: Excreta genera significantly altered by at-hatch administration at 28 days

Taxa	P value	Mean Ctrl	Mean Pb
Eubacterium	0.0013	0.58	0.34
Arthrobacter	0.018	0.06	0.01

Table D: Excreta OTUs significantly altered by at-hatch administration at 28 days

GreenGene annotation	OTUs by Qiime	Blast hit (16S Microbial NCBI)	% Id	P-value	Mean Ctr	Mean Pb1
Firmicutes_f_Lachnospiraceae	88432	*Ruminococcus lactaris*	96	0.00024	0.16	0.01
Bacteroidetes_g_*Bacteroides_s_bacteroides uniformis*	71588	*Bacteroides uniformis*	100	0.00041	0	0.35
Bacteroidetes_g_*Alistipes*	90591	*Alistipes onderdonkii*	100	0.00051	0.41	0.05
Bacteroidetes_g_*Bacteroides_s_Bacteroides uniformis*	999	*Bacteroides uniformis*	97	0.0012	0	0.15
Firmicutes_f_Lachnospiraceae	31167	*Ruminococcus lactaris*	95	0.0019	0.34	0.13
Bacteroidetes_g_*Alistipes*	13134	*Alistipes onderdonkii*	95	0.0019	0.2	0.04
Firmicutes_f_Lachnospiraceae	18967	*Ruminococcus lactaris*	96	0.0022	0.37	0.18
Firmicutes_g_*Eubacterium*	82056	*Blautia schinkii*	95	0.0033	0.16	0.04
Firmicutes_g_*Eubacterium*	65005	*Blautia luti*	97	0.0039	0.31	0.08
Firmicutes_g_*Eubacterium*	860	*Blautia luti*	95	0.0043	0.19	0.07
Firmicutes_f_Lachnospiraceae	3791	*Clostridium citroniae*	87	0.0043	0.14	0.03
Firmicutes_f_Lachnospiraceae	76773	*Ruminococcus lactaris*	95	0.0047	0.29	0.15
Firmicutes_f_Lachnospiraceae	90852	*Ruminococcus lactaris*	95	0.011	0.18	0.08
Firmicutes_g_Ruminococcus	110830	*Ruminococcus torques*	95	0.012	0.19	0.1
Firmicutes_f_Lachnospiraceae	52684	*Ruminococcus lactaris*	94	0.012	0.25	0.15
Firmicutes_o_Clostridiales	34656	*Clostridium bartlettii*	91	0.018	0.07	0.01
Firmicutes_g_Clostridium	60034	*Clostridium citroniae*	92	0.018	0.78	0.56
Firmicutes_f_Lachnospiraceae	64017	*Clostridium bolteae*	95	0.018	0.16	0.07
Actinobacteria_g_*Arthrobacter*	101679	*Clostridium bolteae*	95	0.018	0.06	0.01
Firmicutes_f_Lachnospiraceae	87732	*Clostridium hylemonae*	96	0.019	0.16	0.07
Firmicutes_o_Clostridiales	37819	*Clostridium cellulolyticum*	86	0.02	0.45	0.27
Bacteroidetes_g_*Bacteroides_s_Bacteroidesuniformis*	13244	*Bacteroides uniformis*	96	0.02	0	0.09

Bacteroidetes_o_Bacteroidales	32789	*Eubacterium ruminantium*	86	0.021	0.2	0.1
Firmicutes_f_Lachnospiraceae	72951	*Lactonifactor longoviformis*	95	0.022	0.47	0.32
Firmicutes_f_Lachnospiraceae	54704	*Ruminococcus lactaris*	94	0.022	0.27	0.15
Firmicutes_g_*Lactobacillus*_s_*Lactobacillusreuteri*	110349	*Lactobacillus reuteri*	100	0.024	0.42	0.03
Firmicutes_f_Lachnospiraceae	97957	*Ruminococcus lactaris*	97	0.024	0.22	0.12
Firmicutes_f_Lachnospiraceae	116114	*Clostridium phytofermentans*	92	0.024	0.23	0.12
Firmicutes_g_Ruminococcus	32851	*Blautia luti*	96	0.025	0.53	0.27
Firmicutes_g_*Lactobacillus*	52248	*Lactobacillus ingluviei*	96	0.025	0.03	0
Bacteroidetes_o_Bacteroidales	16	*Clostridium irregulare*	87	0.025	0.12	0.05
Firmicutes_o_Clostridiales	22180	*Clostridium phytofermentans*	92	0.027	0.61	0.42
Firmicutes_f_Lachnospiraceae	80564	*Ruminococcus lactaris*	97	0.027	0.19	0.11
Firmicutes_f_Lachnospiraceae	69308	*Ruminococcus torques*	94	0.029	0.23	0.12
Firmicutes_f_Lachnospiraceae	58606	*Ruminococcus lactaris*	96	0.033	0.16	0.08
Firmicutes_f_Lachnospiraceae	93936	*Ruminococcus torques*	95	0.037	0.29	0.16
Firmicutes_g_Clostridium	64502	*Ruminococcus lactaris*	97	0.038	0.16	0.08
Firmicutes_g_*Eubacterium*	80436	*Blautia luti*	94	0.04	0.15	0.06
Firmicutes_g_*Clostridium*	81771	*Clostridium lavalense*	93	0.044	0.22	0.15
Firmicutes_f_Lachnospiraceae	10489	*Ruminococcus lactaris*	93	0.044	0.16	0.07
Firmicutes_g_Ruminococcus	88419	*Ruminococcus torques*	95	0.045	0.2	0.13
Firmicutes_g_*Clostridium*	78276	*Blautia luti*	92	0.045	0.36	0.27
Firmicutes_o_Clostridiales	78661	*Shigella dysenteriae*	88	0.047	0.09	0.04
Firmicutes_g_*Lactobacillus*	37949	*Lactobacillus reuteri*	87	0.047	0.18	0.04
Firmicutes_g_*Ruminococcus*	845	*Clostridium xylanolyticum*	96	0.05	0.26	0.17
Firmicutes_f_Lachnospiraceae	32964	*Ruminococcus torques*	95	0.05	0.79	0.61

Table E: Caecal genera significantly altered by at-hatch administration at 28 days

Taxa	P value	Mean Ctrl	Mean Pb
Brachybacterium	0.0056	0.39	0.19
Arthrobacter	0.017	0.04	0
Eubacterium	0.034	0.57	0.47
Coprobacillus	0.036	0.52	0.7
Alistipes	0.045	1.02	0.63

Table F: Caecal OTUs significantly altered by at-hatch administration at 28 days

GreenGene annotation	OTUs by Qiime	Blast hit (16S Microbial NCBI)	% Id	P-value	Mean Ctr	Mean Pb1
Bacteroidetes_g_*Bacteroides_s_bacteroides uniformis*	71588	*Bacteroides uniformis*	100	0.00024	0	0.99
Bacteroidetes_g_*Bacteroides_s_bacteroides uniformis*	13244	*Bacteroides uniformis*	96	0.00024	0	0.34
Bacteroidetes_g_*Bacteroides_s_bacteroides uniformis*	999	*Bacteroides uniformis*	97	0.00034	0.02	0.45
Bacteroidetes_g_*Alistipes*	90591	*Alistipes onderdonkii*	100	0.00069	0.76	0.07
Bacteroidetes_g_*Alistipes*	13134	*Alistipes onderdonkii*	95	0.0047	0.33	0.07
Firmicutes_g_*Ruminococcus*	71066	*Ruminococcus torques*	96	0.0049	0.33	0.46
Actinobacteria_g_*Brachybacterium*	64941	*Brachybacterium faecium*	100	0.0056	0.39	0.19
Firmicutes_f_Lachnospiraceae	31167	*Ruminococcus lactaris*	95	0.0058	0.42	0.28
Firmicutes_f_Lachnospiraceae	42809	*Clostridium saccharolyticum*	96	0.0067	0.37	0.52
Firmicutes_g_*Ruminococcus*	46597	*Ruminococcus torques*	95	0.0087	0.47	0.63
Firmicutes_o_Clostridiales	16691	*Clostridium populeti*	93	0.013	0.32	0.11
Firmicutes_g_*Eubacterium*	65005	*Blautia luti strain*	97	0.014	0.32	0.18
Tenericutes_g_Coprobacillus	111142	*Clostridium xylanolyticum*	85	0.016	0.18	0.26
Firmicutes_f_Lachnospiraceae	62279	*Clostridium hylemonae*	94	0.016	0.14	0.19
Actinobacteria_g_*Arthrobacter*	101679	*Arthrobacter protophormiae*	100	0.017	0.04	0
Firmicutes_f_Lachnospiraceae	81021	*Clostridium* s. N6	94	0.021	0.3	0.41
Firmicutes_f_Lachnospiraceae	29697	*Clostridium hylemonae*	97	0.026	0.46	0.58
Firmicutes_f_Lachnospiraceae	81590	*Ruminococcus lactaris*	96	0.028	0.09	0.27
Firmicutes_g_*Clostridium*	22207	*Clostridium saccharolyticum*	96	0.032	0.17	0.22
Firmicutes_f_Lachnospiraceae	88432	*Ruminococcus lactaris*	96	0.032	0.18	0.09

GreenGene annotation	OTUs by Qiime	Blast hit (16S Microbial NCBI)	% Id	P-value	Mean Ctr	Mean Pb1
Firmicutes_f_Lachnospiraceae	60054	*Ruminococcus lactaris*	97	0.032	0.25	0.33
Actinobacteria_g_*Adlercreutzia*	83905	*Adlercreutzia equolifaciens*	96	0.032	0.14	0.2
Tenericutes_g_*Coprobacillus*	38777	*Clostridium cocleatum*	91	0.033	0.42	0.55
Actinobacteria_g_*Adlercreutzia*	45835	*Adlercreutzia equolifaciens*	99	0.033	0.17	0.25
Bacteroidetes_o_Bacteroidales	16	*Clostridium irregulare s.6VI*	87	0.036	0.21	0.13
Firmicutes_g_*Blautia*	77309	*Ruminococcus lactaris*	97	0.041	0.36	0.8
Firmicutes_f_Lachnospiraceae	29757	*Ruminococcus lactaris*	96	0.041	0.24	0.46
Actinobacteria_f_Dermabacteraceae	67140	*Brachybacterium rhamnosum*	97	0.041	0.25	0.12
Firmicutes_f_Lachnospiraceae	62881	*Clostridium hylemonae s.TN-272*	97	0.043	1.38	1.77
Firmicutes_f_Lachnospiraceae	62791	*Clostridium hylemonae s.TN-272*	96	0.043	0.22	0.29
Firmicutes_g_Turicibacter	93943	*Clostridium populeti*	89	0.045	0.33	0.18
Firmicutes_g_*Lactobacillus_s_lactobacillus reuteri*	79887	*Lactobacillus reuteri*	97	0.047	0.05	0
Firmicutes_g_*Turicibacter*	101382	*Clostridium haemolyticum s.ATCC9650*	89	0.048	0.06	0.02
Firmicutes_f_Lachnospiraceae	33535	*Ruminococcus lactaris*	93	0.049	0.18	0.1
Tenericutes_g_*Coprobacillus*	114607	*Clostridium cocleatum*	89	0.05	0.17	0.27

Table G: Ileum genera significantly altered by at-hatch administration at 28 days

Taxa	P value	Mean Ctrl	Mean Pb
Weissella	0.006	0.03	0.42
Paracoccus	0.025	0.09	0.68
Unclassified Clostridiales	0.031	3.46	2.03

Table H: Ileum OTUs significantly altered by at-hatch administration at 28 days

GreenGene annotation	OTUs by Qiime	Blast hit (16S Microbial NCBI)	% Id	P-value	Mean Ctr	Mean Pb1
Proteobacteria_f_Enterobacteriaceae	18699	*Shigella dysenteriae*	97	0.0023	0.08	0
Firmicutes_f_Lachnospiraceae	74903	*Turicibacter sanguinis*	87	0.0036	0.1	0.02
Firmicutes_g_*Clostridium*	49919	*Clostridium glycolicum*	99	0.006	0.27	0.04
Firmicutes_g_*Turicibacter*	52163	*Turicibacter sanguinis*	89	0.006	0.12	0.01
Firmicutes_g_*Weissella*	35028	*Weissella paramesenteroides*	100	0.006	0.03	0.42
Firmicutes_g_*Staphylococcus*	30640	*Staphylococcus cohnii*	97	0.018	0.01	0.13
Firmicutes_f_Ruminococcaceae	78501	*Clostridium sporosphaeroides*	91	0.019	0.04	0
Firmicutes_g_*Lactobacillus*_s_*lactobacillushelveticus*	56056	*Lactobacillus kitasatonis*	96	0.019	0.15	0
Firmicutes_o_Clostridiales	111588	*Clostridium glycolicum*	95	0.019	0.16	0.05
Firmicutes_g_*Lactobacillus*_s_*lactobacillushelveticus*	46075	*Lactobacillus crispatus*	96	0.02	0.09	0.02
Firmicutes_o_Clostridiales	31016	*Turicibacter sanguinis*	91	0.02	0.12	0.02
Firmicutes_o_Clostridiales	4154	*Clostridium glycolicum*	94	0.02	0.15	0.05
Firmicutes_o_Clostridiales	73173	*Clostridium glycolicum*	94	0.023	0.2	0.06
Proteobacteria_g_*Paracoccus*	49975	*Paracoccus aminovorans*	99	0.025	0.09	0.68
Firmicutes_f_Ruminococcaceae	40813	*Bacteroides capillosus*	95	0.031	0.09	0.01
Firmicutes_g_*Lactobacillus*	115894	*Lactobacillus kitasatonis*	89	0.031	0.08	0.01
Firmicutes_g_*Lactobacillus*_s_*lactobacillushelveticus*	28599	*Lactobacillus kitasatonis*	96	0.039	0.09	0.01
Firmicutes_f_Aerococcaceae	21216	*Globicatella sulfidifaciens*	99	0.04	0.54	0.19
Firmicutes_o_Clostridiales	33821	*Clostridium glycolicum*	97	0.046	3.29	1.91
Firmicutes_f_Ruminococcaceae	83533	*Faecalibacterium prausnitzii*	91	0.049	0.06	0.01
Firmicutes_f_Lachnospiraceae	16652	*Lactobacillus reuteri*	87	0.05	0.06	0
Firmicutes_f_Lachnospiraceae	44609	*Ruminococcus torques*	96	0.05	0.02	0
Firmicutes_f_Lachnospiraceae	81021	*Clostridium s.N6*	94	0.05	0.02	0
Firmicutes_g_*Clostridium*	81771	*Clostridium lavalense*	93	0.05	0.02	0