

Supplementary Material

Dietary Treatments of Competitive Exclusion Culture and Synbiotic Product Influenced Gut Microbiota Maturation in Broiler Chickens

Nikoletta Such^{1,†}, Valéria Farkas^{1,†}, Gábor Csitári¹, László Pál¹, Aliz Márton¹, László Menyhárt² and Károly Dublec^{1,*}

- ¹ Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences, Georgikon Campus, 8360 Keszthely, Hungary; Deák Ferenc Street 16.; farkas.valeria@uni-mate.hu (V.F.); such.nikoletta.amanda@phd.uni-mate.hu (N.S.); csitari.gabor@uni-mate.hu (G. CS.); pal.laszlo@uni-mate.hu (L. P.); marton.aliz@uni-mate.hu (A. M.); dublec.karoly@uni-mate.hu (K. D.)
- ² Institute of Technology, Hungarian University of Agriculture and Life Sciences, Georgikon Campus, 8360 Keszthely, Hungary; Deák Ferenc Street 16.; menyhart.laszlo@uni-mate.hu (L.M.)
- * Correspondence: dublec.karoly@uni-mate.hu; Tel.: Tel.: +36 30 6418597
- † These authors contributed equally to this work.

Table S1. Composition and calculated nutrient content of experimental diets (g/kg)¹.

Ingredients	Starter		Grower		Finisher	
	Basal diet	Sy diet	Basal diet	Sy diet	Basal diet	Sy diet
Maize	430.80	425.35	485.80	480.35	545.30	539.85
Extracted soybean meal	464.00	464.00	412.00	412.00	358.00	358.00
Sunflower oil	56.00	56.00	58.00	58.00	55.00	55.00
Limestone	18.00	18.00	15.00	15.00	14.00	14.00
MCP	16.00	16.00	15.00	15.00	14.00	14.00
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Sodium bicarbonate	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine-HCl	2.00	2.00	1.00	1.00	1.00	1.00
DL-Methionine	4.00	4.00	3.00	3.00	3.00	3.00
L-Threonine	-	-	1.00	1.00	0.50	0.50
Premix ²	5.00	5.00	5.00	5.00	5.00	5.00
Phytase ³	0.10	0.10	0.10	0.10	0.10	0.10
Xylanase ⁴	0.10	0.10	0.10	0.10	0.10	0.10
Probiotic supplement ⁵	-	0.40	-	0.40	-	0.40
Inulin supplement ⁶	-	5.00	-	5.00	-	5.00
Yeast supplement ⁷	-	0.05	-	0.05	-	0.05
<i>Sum</i>	<i>1000.00</i>	<i>1000.00</i>	<i>1000.00</i>	<i>1000.0</i>	<i>1000.00</i>	<i>1000.00</i>
Calculated nutrients						
AMEn (MJ/kg) ²	12.6	12.6	13.0	13.0	13.2	13.2
Crude protein	230.0	229.7	210.0	209.7	190.0	189.7
Crude fibre	28.5	28.4	27.5	27.4	26.5	26.4
Calcium	10.5	10.5	9.0	9.0	8.5	8.5
Phosphorus (available)	5.0	5.0	4.5	4.5	4.2	4.2
dig. Lysine	12.7	12.7	11.0	11.0	9.7	9.7
dig. Methionine	6.5	6.5	5.7	5.7	5.1	5.1
dig. Methionine + Cystine	9.4	9.4	8.4	8.4	7.6	7.6

¹ Basal diet was fed in the C (control) and Br (Broilact®) treatment groups; Sy diet was supplemented with the synbiotic mixture of probiotic, inulin and yeast supplements and fed in the Sy treatment group.

²Premix was supplied by UBM Ltd. (Pilisvörösvár, Hungary). The active ingredients in the premix were as follows (per kg of diet): retinyl acetate – 5.0 mg, cholecalciferol – 130 µg, dl-alpha-tocopherol-acetate – 91 mg, menadione – 2.2 mg, thiamin – 4.5 mg, riboflavin – 10.5 mg, pyridoxin HCl – 7.5 mg, cyanocobalamin – 80 µg, niacin – 41.5 mg, pantothenic acid – 15 mg, folic acid – 1.3 mg, biotin – 150 µg, betaine – 670 mg, Ronozyme® NP – 150 mg, monensin-Na – 110 mg (only grower), narasin – 50 mg (only starter), nicarbazin – 50 mg (only starter), antioxidant – 25 mg, Zn (as ZnSO₄·H₂O) – 125 mg, Cu (as CuSO₄·5H₂O) – 20 mg, Fe (as FeSO₄·H₂O) – 75 mg, Mn (as MnO) – 125 mg, I (as KI) – 1.35 mg, Se (as Na₂SeO₃) – 270 µg;

³ Quantum Blue® 5G (AB Vista, Marlborough, Wiltshire, SN8 4AN, United Kingdom)

⁴ Econase® XT 25P (AB Vista, Marlborough, Wiltshire, SN8 4AN, United Kingdom)

⁵ GalliPro® 200 (*Bacillus subtilis*, DSM17299 bacterial strain; 1.6x10⁶ CFU/g, Biochem Ltd., Küstermeyerstrasse 16. 49393 Lohne, Germany)

⁶ Orafti® HSI (Beneo Ltd., Aandorenstraat 1, B. 3300 Tienen, Belgium)

⁷ Levucell® SB20 (*Saccharomyces cerevisiae boulardii*, 2x10¹⁰ CFU/g, Lallemand Ltd., Ottakringer Str. 89, A-1160 Vienna, Austria)

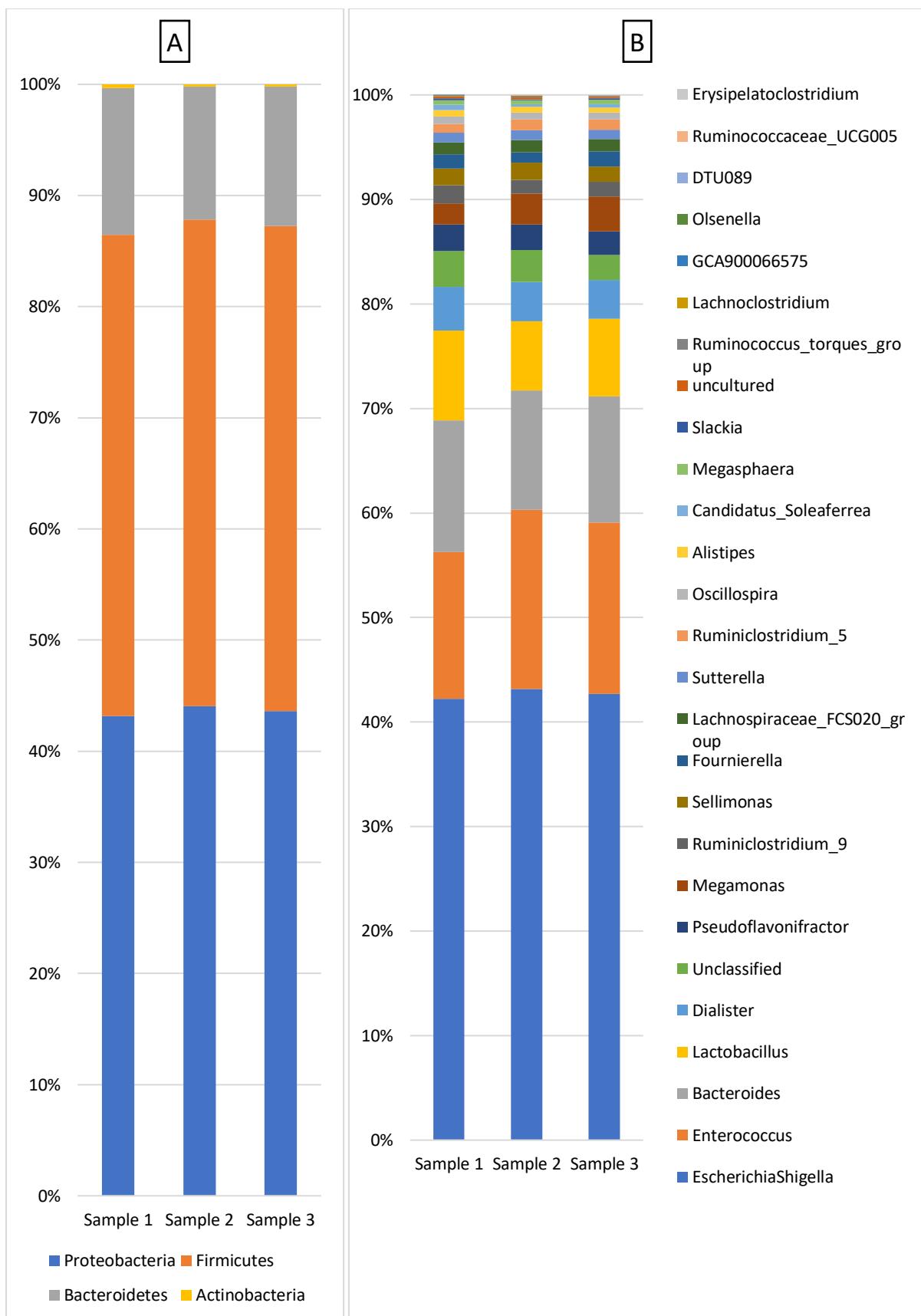


Figure S1. Microbial profile of Broilact®. Stacked column plots show the percent mean relative abundance of bacterial phyla (A) and genera (B).

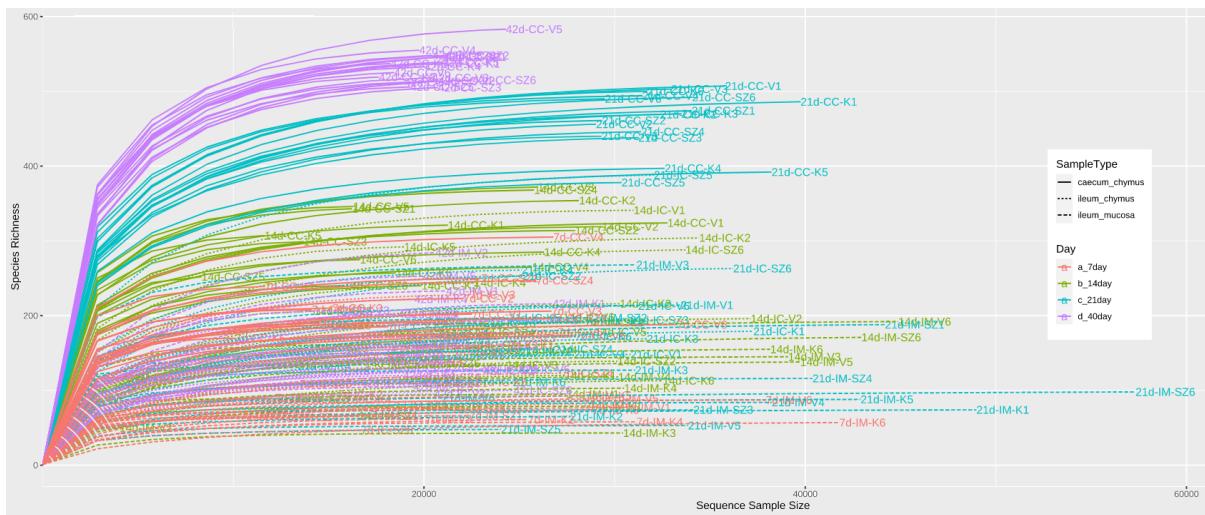


Figure S2. Rarefaction curves of Species richness. The observed metrics were number of Operational Taxonomic Units (OTUs). Each colored line represents the microbiota of a single chicken sample. Line color based on age of chicken; line type based on sample type (dashed: ileum mucosa; scored: ileum chymus; straight: caecum chymus). Sequence data were rarefied to a depth of 1000 sequences per sample.

Table S2. Alpha diversity indices of ileum chymus and effects of dietary treatments (C – Control, Br – Broilact®, Sy – Synbiotic feed additive) and age of birds on the microbiota diversity.

Diversity Indices	Ileum Chymus					p-Value		
	Dietary Treatment	Age of Birds			Mean (Dietary Treatment)	Dietary Treatment	Age	Interaction
		d 7	d 14	d 21				
Chao1	C	114.20 ^B	215.56	195.39	128.79	163.48	0.853	0.140
	Br	175.87 ^A	200.56	173.95	130.69	170.27		
	Sy	122.48 ^{AB}	188.36	234.62	138.96	171.11		
	Mean (Age)	137.52^a	201.5^b	201.3^b	132.8^a		0.001	
Shannon	C	1.94 ^B	2.60	3.02	2.95	2.63	0.678	0.011
	Br	2.96 ^A	2.61	2.86	2.46	2.73		
	Sy	2.37 ^{AB}	2.54	3.23	2.80	2.73		
	Mean (Age)	2.42^a	2.58^a	3.04^b	2.74^{ab}		0.002	
Simpson	C	0.69 ^B	0.79	0.89	0.90	0.82	0.501	0.024
	Br	0.89 ^A	0.82	0.87	0.81	0.85		
	Sy	0.81 ^{AB}	0.79	0.91	0.86	0.84		
	Mean (Age)	0.80^a	0.80^a	0.89^b	0.86^{ab}		0.009	

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S3. Alpha diversity indices in ileum mucosa and effects of dietary treatments and age of birds on the microbiota diversity.

Diversity Indices	Ileum Mucosa					p-Value		
	Dietary Treatment	Age of Birds				Mean (Dietary Treatment)	Dietary Treatment	Age
		d 7	d 14	d 21	d 40			
Chao1	C	73.34	113.38	99.91	186.95 ^A	118.39	0.051	0.060
	Br	75.32	123.31	143.84	226.45 ^A	142.23		
	Sy	80.40	122.56	120.66	128.18 ^B	112.95		
	Mean (Age)	76.36 ^a	119.75 ^a	121.47 ^b	180.53 ^c			0.001
Shannon	C	0.73	1.09	1.58	3.02 ^A	1.60	0.886	0.036
	Br	0.89	1.22	1.91	2.73 ^{AB}	1.69		
	Sy	1.73	1.45	0.96	2.62 ^B	1.69		
	Mean (Age)	1.12 ^a	1.25 ^a	1.48 ^a	2.79 ^b			0.001
Simpson	C	0.27	0.31	0.45	0.89	0.48	0.643	0.037
	Br	0.29	0.38	0.57	0.85	0.52		
	Sy	0.55	0.44	0.27	0.86	0.53		
	Mean (Age)	0.37 ^a	0.38 ^a	0.43 ^a	0.87 ^b			0.001

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S4. Alpha diversity indices in caecum chymus and effects of dietary treatments and age of birds on the microbiota diversity.

Diversity Indices	Caecum Chymus					p-Value		
	Dietary Treatment	Age of Birds				Mean (Dietary Treatment)	Dietary Treatment	Age
		d 7	d 14	d 21	d 40			
Chao1	C	201.74	294.96	453.91	530.71	370.33	0.124	0.983
	Br	216.81	317.95	483.32	542.08	390.04		
	Sy	207.32	303.37	449.33	529.75	372.44		
	Mean (Age)	208.63 ^a	305.43 ^b	462.19 ^c	534.18 ^d		0.001	
Shannon	C	3.79	4.18	4.34	4.77	4.27	0.119	0.372
	Br	3.51	4.12	4.34	4.59	4.14		
	Sy	3.82	4.14	4.24	4.70	4.23		
	Mean (Age)	3.71 ^a	4.15 ^b	4.31 ^b	4.69 ^c		0.001	
Simpson	C	0.948	0.963	0.962	0.978	0.963 ^A	0.024	0.232
	Br	0.930	0.962	0.955	0.966	0.953 ^B		
	Sy	0.952	0.960	0.950	0.977	0.960 ^{AB}		
	Mean (Age)	0.943 ^a	0.962 ^{bc}	0.955 ^b	0.974 ^c		0.001	

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S5. Relative abundance of ileum chymus microbiota at phylum level.

Phylum	Ileum Chymus (%)					FDR p-Value			
	Dietary Treatment	Age of Birds				Dietary Treatment	Age	Interaction	
		d 7	d 14	d 21	d 40				
Firmicutes	C	96.49 ^A	91.68	92.65	94.80	93.91	0.180	0.032	
	Br	83.95 ^B	75.59	95.09	96.31	87.74			
	Sy	92.01 ^{AB}	91.39	89.72	92.66	91.45	0.055		
	Mean (Age)	90.82 ^{ab}	86.22 ^b	92.49 ^{ab}	94.59 ^a				
Proteobacteria	C	1.48	2.08	0.73 ^A	0.19	1.12	0.060	0.083	
	Br	10.85	17.07	0.15 ^B	0.20	7.07			
	Sy	2.09	2.15	0.18 ^{AB}	0.43	1.21	0.026		
	Mean (Age)	4.81 ^{ab}	7.10 ^a	0.35 ^b	0.27 ^b				
Cyanobacteria	C	1.93 ^B	3.32	0.12 ^B	0.33	1.43	0.110	0.390	
	Br	4.35 ^{AB}	5.74	0.08 ^{AB}	0.11	2.57			
	Sy	5.49 ^A	5.39	0.18 ^A	0.24	3.16	0.001		
	Mean (Age)	3.92 ^a	4.82 ^a	0.13 ^b	0.23 ^b				
Actinobacteria	C	0.05	0.15	6.08	4.33	2.65	0.540	0.830	
	Br	0.65	0.09	4.44	3.11	2.07			
	Sy	0.12	0.26	8.74	5.05	3.54	0.001		
	Mean (Age)	0.27 ^b	0.17 ^b	6.42 ^a	4.16 ^a				
Bacteroidetes	C	0.03 ^B	2.53	0.15	0.00	0.68	0.360	0.008	
	Br	0.06 ^{AB}	0.19	0.12	0.04	0.10			
	Sy	0.12 ^A	0.39	1.08	0.01	0.40	0.024		
	Mean (Age)	0.07 ^b	1.04 ^a	0.45 ^{ab}	0.02 ^b				
Patescibacteria	C	0.00	0.20	0.08	0.34	0.16	0.360	0.036	
	Br	0.07	1.29	0.06	0.23	0.41			
	Sy	0.12	0.21	0.08	0.34	0.19	0.026		
	Mean (Age)	0.06 ^b	0.57 ^a	0.07 ^b	0.30 ^{ab}				
Verrucomicrobia	C	0.00	0.00	0.17	0.00	0.04	0.640	0.820	
	Br	0.00	0.00	0.06	0.00	0.02			
	Sy	0.00	0.00	0.16	0.00	0.04	0.003		
	Mean (Age)	0.00 ^b	0.001 ^b	0.13 ^a	0.001 ^b				
Tenericutes	C	0.01	0.03	0.02	0.00	0.02	0.610	0.650	
	Br	0.03	0.02	0.00	0.00	0.01			
	Sy	0.00	0.01	0.01	0.00	0.01	0.260		
	Mean (Age)	0.01	0.02	0.01	0.00				
Armatimonadetes	C	0.01	0.00	0.00	0.00	0.00	0.410	0.210	
	Br	0.04	0.00	0.00	0.00	0.01			
	Sy	0.04	0.00	0.00	0.00	0.01	0.001		
	Mean (Age)	0.03 ^a	0.00 ^b	0.00 ^b	0.00 ^b				
Chloroflexi	C	0.00	0.00	0.00	0.00	0.00	0.490	0.430	
	Br	0.00	0.00	0.00	0.00	0.00			
	Sy	0.00	0.00	0.00	0.00	0.00	0.400		
	Mean (Age)	0.00	0.00	0.00	0.00				

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S6. Relative abundance of ileum mucosa microbiota at phylum level.

Phylum	Ileum Mucosa (%)					FDR p-Value		
	Dietary Treatment	Age of Birds				Mean (Dietary Treatment)	Dietary Treatment	Age
		d 7	d 14	d 21	d 40			
Firmicutes	C	98.77 ^A	95.35	92.57	96.11	95.70	0.710	0.042
	Br	96.30 ^{AB}	93.21	95.09	97.72	95.58		
	Sy	91.84 ^B	92.72	96.13	97.86	94.64		
	Mean (Age)	95.64	93.76	94.60	97.23		0.140	
Proteobacteria	C	0.90 ^B	3.19	5.83	1.56	2.87	0.860	0.021
	Br	2.94 ^{AB}	5.30	2.78	0.52	2.89		
	Sy	6.03 ^A	5.07	2.15	0.61	3.47		
	Mean (Age)	3.29 ^{ab}	4.52 ^a	3.59 ^{ab}	0.90 ^b		0.030	
Actinobacteria	C	0.23 ^B	0.34	0.62	1.80	0.75	0.710	0.120
	Br	0.60 ^{AB}	0.63	0.32	0.92	0.62		
	Sy	1.26 ^A	0.58	0.47	1.10	0.85		
	Mean (Age)	0.70 ^{ab}	0.52 ^b	0.47 ^b	1.27 ^a		0.060	
Bacteroidetes	C	0.02	0.65	0.44	0.14	0.31	0.880	0.540
	Br	0.01	0.13	1.21	0.64	0.50		
	Sy	0.00	0.20	1.02	0.07	0.32		
	Mean (Age)	0.01	0.33	0.89	0.28		0.140	
Cyanobacteria	C	0.08 ^B	0.35	0.00	0.14	0.14	0.120	0.041
	Br	0.14 ^B	0.58	0.14	0.08	0.24		
	Sy	0.85 ^A	1.00	0.01	0.10	0.49		
	Mean (Age)	0.36 ^{ab}	0.64 ^a	0.05 ^b	0.11 ^b		0.001	
Patescibacteria	C	0.00	0.02	0.00	0.23	0.06	0.710	0.630
	Br	0.00	0.11	0.04	0.10	0.06		
	Sy	0.00	0.35	0.00	0.25	0.15		
	Mean (Age)	0.00	0.16	0.01	0.19		0.140	
Verrucomicrobia	C	0.00	0.00	0.33	0.01	0.09	0.880	0.990
	Br	0.00	0.00	0.36	0.01	0.09		
	Sy	0.00	0.01	0.19	0.00	0.05		
	Mean (Age)	0.00 ^b	0.00 ^b	0.29 ^a	0.01 ^b		0.060	
Tenericutes	C	0.00	0.04	0.16	0.01	0.05	0.710	0.780
	Br	0.00	0.04	0.05	0.01	0.03		
	Sy	0.00	0.07	0.02	0.00	0.02		
	Mean (Age)	0.00	0.05	0.08	0.01		0.210	
Deinococcus_Thermus	C	0.00	0.00	0.04	0.00	0.01	0.710	0.540
	Br	0.00	0.00	0.00	0.00	0.00		
	Sy	0.00	0.00	0.01	0.01	0.01		
	Mean (Age)	0.00	0.00	0.02	0.00		0.400	
Chloroflexi	C	0.00	0.01	0.00	0.00	0.00	0.790	0.610
	Br	0.01	0.00	0.00	0.00	0.00		
	Sy	0.00	0.00	0.00	0.00	0.00		
	Mean (Age)	0.00	0.00	0.00	0.00		0.210	

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S7. Relative abundance of caecum chymus microbiota at phylum level.

Phylum	Caecum Chymus (%)					FDR p-Value					
	Dietary Treatment	Age of Birds									
		d 7	d 14	d 21	d 40						
Firmicutes	C	88.60	86.21	83.19	85.66	85.92	0.940	0.740			
	Br	90.19	84.49	80.66	86.13	85.37					
	Sy	86.47	87.25	77.98	87.05	84.69	0.003	0.740			
	Mean (Age)	88.42^a	85.98^a	80.61^b	86.28^a						
Proteobacteria	C	5.50	1.65	0.59	1.02	2.19	0.940	0.730			
	Br	8.22	3.88	0.46	0.87	3.36					
	Sy	10.22	1.96	0.73	1.05	3.49	0.001	0.730			
	Mean (Age)	7.98^a	2.50^b	0.59^b	0.98^b						
Bacteroidetes	C	0.01	9.61	13.02	11.82	8.62	0.940	0.700			
	Br	0.06	7.10	15.29	11.26	8.43					
	Sy	0.00	8.62	17.60	9.92	9.04	0.001	0.700			
	Mean (Age)	0.02^c	8.44^b	15.30^a	11.00^b						
Tenericutes	C	5.86	2.01	0.34	0.33	2.14	0.940	0.160			
	Br	1.49	2.53	0.62	0.33	1.24					
	Sy	3.29	1.92	0.46	0.28	1.49	0.001	0.160			
	Mean (Age)	3.55^a	2.15^{ab}	0.47^b	0.31^b						
Cyanobacteria	C	0.00	0.45	0.18	0.59	0.31	0.940	0.280			
	Br	0.00	1.93	0.08	0.56	0.64					
	Sy	0.00	0.18	0.20	0.67	0.26	0.073	0.280			
	Mean (Age)	0.00	0.85	0.15	0.61						
Actinobacteria	C	0.03	0.07	0.10	0.07	0.07	0.940	0.230			
	Br	0.03	0.05	0.11	0.07	0.07					
	Sy	0.03	0.04	0.15	0.19	0.10	0.002	0.230			
	Mean (Age)	0.03^b	0.05^{ab}	0.12^a	0.11^a						
Verrucomicrobia	C	0.00	0.00	2.59	0.49	0.77	0.940	1.000			
	Br	0.00	0.01	2.78	0.77	0.89					
	Sy	0.00	0.04	2.89	0.82	0.94	0.001	1.000			
	Mean (Age)	0.00^b	0.02^b	2.75^a	0.69^b						
Patescibacteria	C	0.00	0.00	0.00	0.02	0.01	0.940	0.580			
	Br	0.00	0.00	0.00	0.01	0.00					
	Sy	0.00	0.00	0.00	0.02	0.01	0.001	0.580			
	Mean (Age)	0.00^b	0.00^b	0.00^b	0.02^a						

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S8. Relative abundance of ileum chymus microbiota at genus level.

Genus	Ileum Chymus (%)					FDR p-Value			
	Dietary Treatment	Age of Birds			Mean (Dietary Treatment)				
		d 7	d 14	d 21	Dietary Treatment	Age	Interaction		
<i>Lactobacillus</i>	C	79.43 ^A	61.30	63.68	60.68	66.27	0.820	0.030	
	Br	49.84 ^B	45.53	69.02	76.02	60.10			
	Sy	75.19 ^{AB}	56.34	51.58	67.25	62.59	0.150		
	Mean (Age)	68.15	54.39	61.43	67.99				
<i>Enterococcus</i>	C	1.74 ^B	0.47	1.42	1.14	1.19	0.800	0.011	
	Br	18.17 ^A	0.32	1.88	0.91	5.32			
	Sy	13.76 ^{AB}	1.69	2.19	1.74	4.84	0.001		
	Mean (Age)	11.22 ^a	0.82 ^b	1.83 ^b	1.26 ^b				
<i>Escherichia-Shigella</i>	C	0.80	1.49	0.58 ^A	0.17	0.76	0.710	0.079	
	Br	9.44	16.66	0.06 ^B	0.16	6.58			
	Sy	0.07	1.55	0.13 ^{AB}	0.30	0.51	0.081		
	Mean (Age)	3.44	6.57	0.26	0.21				
<i>Subdoligranulum</i>	C	3.19	0.14	0.07	0.01	0.85	0.820	0.730	
	Br	2.48	0.23	0.03	0.01	0.69			
	Sy	0.11	0.31	0.14	0.06	0.15	0.170		
	Mean (Age)	1.92	0.23	0.08	0.03				
<i>Streptococcus</i>	C	1.71	3.65	2.90	11.95	5.05	0.800	0.830	
	Br	1.26	0.80	2.68	6.39	2.78			
	Sy	0.16	1.54	2.96	7.24	2.97	0.001		
	Mean (Age)	1.04 ^b	2.00 ^b	2.84 ^b	8.53 ^a				
<i>Clostridium sensu stricto 1</i>	C	0.44	0.31	6.11	0.29	1.79	0.960	1.000	
	Br	0.14	0.08	5.30	0.35	1.47			
	Sy	0.34	0.30	5.50	0.10	1.56	0.001		
	Mean (Age)	0.30 ^b	0.23 ^b	5.64 ^a	0.25 ^b				
<i>Corynebacterium 1</i>	C	0.09	0.25	8.08	4.49	3.23	0.800	0.770	
	Br	0.05	0.11	5.63	3.22	2.25			
	Sy	0.56	0.07	3.49	2.01	1.53	0.001		
	Mean (Age)	0.23 ^b	0.14 ^b	5.73 ^a	3.24 ^{ab}				
<i>Candidatus-Arthromitus</i>	C	0.09	11.12	0.13	0.97	3.08	0.800	0.240	
	Br	0.47	0.58	0.58	0.09	0.43			
	Sy	0.11	0.99	0.14	0.01	0.31	0.230		
	Mean (Age)	0.22	4.23	0.28	0.36				
<i>Staphylococcus</i>	C	0.07	0.54	7.31	1.25	2.29	0.940	0.860	
	Br	0.04	0.71	5.24	1.60	1.90			
	Sy	0.20	0.19	7.75	0.70	2.21	0.001		
	Mean (Age)	0.11 ^b	0.48 ^b	6.76 ^a	1.18 ^b				
<i>Butyricicoccus</i>	C	0.04	0.96	0.10	0.00	0.28	0.820	0.820	
	Br	0.27	1.56	0.07	0.00	0.48			
	Sy	0.16	0.49	0.01	0.00	0.17	0.018		
	Mean (Age)	0.16 ^b	1.00 ^a	0.06 ^b	0.00 ^b				
<i>Weissella</i>	C	0.02	0.20	3.03	0.57	0.95	0.800	0.420	
	Br	0.05	0.29	1.74	0.23	0.58			
	Sy	0.40	0.28	0.63	0.11	0.36	0.014		
	Mean (Age)	0.16 ^b	0.26 ^b	1.80 ^a	0.30 ^b				
<i>Romboutsia</i>	C	0.02	3.30	1.85	3.06	2.06	0.950	0.640	
	Br	0.01	2.18	0.24	7.76	2.55			
	Sy	0.11	0.86	0.78	6.41	2.04	0.010		
	Mean (Age)	0.05 ^b	2.11 ^b	0.96 ^b	5.74 ^a				
<i>Turicibacter</i>	C	0.00	0.90	0.54	2.01	0.86	0.820	0.360	
	Br	0.00	0.01	0.15	6.38	1.64			
	Sy	0.00	0.02	0.12	3.37	0.88	0.001		
	Mean (Age)	0.00 ^b	0.31 ^b	0.27 ^b	3.92 ^a				
<i>Lactococcus</i>	C	0.00	3.17	3.54	0.55	1.82	0.990	0.160	
	Br	0.00	0.59	6.03	0.93	1.89			

	Sy	0.00	3.66	3.26	0.45	1.84			
	Mean (Age)	0.00^b	2.48^a	4.28^a	0.64^b			0.001	
<i>Leuconostoc</i>	C	0.00	0.01	1.50	0.16	0.42	0.920		0.200
	Br	0.00	0.00	0.89	0.52	0.35			
	Sy	0.00	0.00	1.06	0.37	0.36		0.001	
	Mean (Age)	0.00^b	0.00^b	1.15^a	0.35^b	66.27			
<i>Bacillus</i>	C	0.01 ^B	0.03	0.02	0.002 ^B	0.01	0.019		0.360
	Br	0.01 ^B	0.05	0.01	0.002 ^B	0.02			
	Sy	0.12 ^A	0.20	0.28	0.043 ^A	0.16		0.250	
	Mean (Age)	4.67	9.33	10.33	1.57				
<i>Ruminococcaceae</i> UCG-014	C	0.23	1.12	0.25	0.01	0.48	0.780		0.860
	Br	0.09	1.04	0.80	0.01	0.40			
	Sy	1.08	1.45	0.57	0.02	0.78		0.047	
	Mean (Age)	0.46^{ab}	1.20^a	0.54^{ab}	0.01^b				

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S9. Relative abundance of ileum mucosa microbiota at genus level.

Genus	Ileum Mucosa (%)					FDR p-Value		
	Dietary Treatment	Age of Birds			Mean (Dietary Treatment)	Dietary Treatment	Age	Interaction
		d 7	d 14	d 21				
<i>Candidatus Arthromitus</i>	C	83.76	82.20	70.15	3.51	59.90	0.660	0.021
	Br	72.26	78.03	51.15	3.93	51.34		
	Sy	42.19	72.73	84.89	11.62	52.86		
	Mean (Age)	66.07 ^a	77.65 ^a	68.73 ^a	6.35 ^b		0.001	
<i>Lactobacillus</i>	C	6.48	3.94	11.91 ^{AB}	41.35	15.92	0.660	0.025
	Br	6.00	5.93	22.64 ^A	64.41	24.74		
	Sy	16.25	9.96	3.90 ^B	47.03	19.28		
	Mean (Age)	9.58 ^b	6.61 ^b	12.82 ^b	50.93 ^a		0.001	
<i>Enterococcus</i>	C	6.47	0.05	0.45	3.37	2.58	0.770	0.950
	Br	3.86	0.06	1.51	1.13	1.64		
	Sy	11.95	0.79	0.25	3.61	4.15		
	Mean (Age)	7.42	0.30	0.74	2.70		0.320	
<i>Delftia</i>	C	0.35	0.01	0.15	0.01	0.13	0.660	0.110
	Br	1.08	0.00	0.06	0.00	0.29		
	Sy	2.46	0.00	0.07	0.00	0.63		
	Mean (Age)	1.30 ^a	0.00 ^b	0.10 ^b	0.00 ^b		0.001	
<i>Romboutsia</i>	C	0.05	1.32	0.80	10.33	0.04	0.990	1.000
	Br	0.13	1.84	0.91	9.71	0.11		
	Sy	1.27	0.81	0.47	10.84	0.25		
	Mean (Age)	0.48 ^b	1.32 ^b	0.73 ^b	10.29 ^a		0.001	
<i>Pseudomonas</i>	C	0.06	1.98	3.46	0.16	3.12	0.980	0.170
	Br	0.25	3.32	1.60	0.10	3.15		
	Sy	0.69	3.11	1.32	0.09	3.35		
	Mean (Age)	0.33 ^b	2.80 ^a	2.12 ^a	0.12 ^b		0.001	
<i>Streptococcus</i>	C	0.17	0.19	0.78	14.30	1.41	0.660	0.074
	Br	0.20	0.24	1.20	6.55	1.32		
	Sy	0.19	0.59	0.44	5.26	1.30		
	Mean (Age)	0.19 ^b	0.34 ^b	0.81 ^b	8.70 ^a		0.001	
<i>Turicibacter</i>	C	0.01	0.00	0.81	10.88	3.86	0.700	0.800
	Br	0.00	0.00	0.05	5.97	2.05		
	Sy	0.01	0.12	0.17	9.30	1.62		
	Mean (Age)	0.01 ^b	0.04 ^b	0.35 ^b	8.72 ^a		0.001	
<i>Lysinibacillus</i>	C	0.001	0.001	0.441	0.003	0.011	0.600	0.002
	Br	0.001	0.001	0.139	0.001	0.035		
	Sy	0.005	0.001	0.137	0.001	0.036		
	Mean (Age)	0.002 ^b	0.001 ^b	0.239 ^a	0.001 ^a		0.001	
<i>Ruminococcaceae UCG-008</i>	C	0.001	0.001	0.001	0.024 ^B	0.006	0.001	0.001
	Br	0.001	0.001	0.002	0.089 ^A	0.023		
	Sy	0.001	0.001	0.001	0.009 ^B	0.002		
	Mean (Age)	0.001 ^b	0.001 ^b	0.001 ^b	0.041 ^a		0.001	
<i>Bacillus</i>	C	0.061	0.154	0.166	0.008	0.097	0.660	0.220
	Br	0.085	0.064	0.136	0.042	0.082		
	Sy	0.243	0.210	0.094	0.012	0.140		
	Mean (Age)	0.130	0.143	0.132	0.021		0.110	

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S10. Relative abundance of caecum chymus microbiota at genus level.

Genus	Caecum Chymus (%)					FDR p-Value					
	Dietary Treatment	Age of Birds									
		d 7	d 14	d 21	d 40						
<i>Escherichia-Shigella</i>	C	5.46	1.52	0.49	0.16	1.91	0.910	0.770			
	Br	8.15	3.62	0.37	0.10	3.06					
	Sy	10.06	1.94	0.59	0.18	3.19					
	Mean (Age)	7.89^a	2.36^b	0.48^b	0.15^b		0.001				
	Mean (Age)										
<i>Subdoligranulum</i>	C	8.69	1.68	1.79	1.12	3.32	0.890	0.430			
	Br	10.32	1.23	0.99	0.80	3.33					
	Sy	1.43	0.88	1.56	1.09	1.24					
	Mean (Age)	6.81^a	1.26^b	1.45^b	1.00^b		0.015				
	Mean (Age)										
<i>Butyricicoccus</i>	C	4.85	2.86	2.56	1.31	2.89	0.890	0.370			
	Br	5.01	3.50	2.25	0.62	2.84					
	Sy	5.36	7.24	2.94	0.95	4.12					
	Mean (Age)	5.07^a	4.53^{ab}	2.58^{bc}	0.96^c		0.001				
	Mean (Age)										
<i>Ruminococcaceae UCG-014</i>	C	3.84	7.01	4.02	5.08	1.92	0.910	0.440			
	Br	2.79	4.54	4.98	5.64	1.99					
	Sy	3.49	4.68	4.33	5.39	1.76					
	Mean (Age)	3.37^b	5.41^a	4.44^{ab}	5.37^a		0.022				
	Mean (Age)										
<i>Anaeroplasma</i>	C	4.89	1.87	0.08	0.06	4.99	0.910	0.430			
	Br	1.31	2.37	0.31	0.08	4.49					
	Sy	2.88	1.76	0.08	0.04	4.47					
	Mean (Age)	3.03^a	2.00^{ab}	0.16^b	0.06^b		0.001				
	Mean (Age)										
<i>Ruminococcus torques group</i>	C	2.23	7.99	1.35	1.90	1.72	0.910	0.390			
	Br	3.14	3.19	1.79	1.73	1.02					
	Sy	2.90	5.00	1.88	1.44	1.19					
	Mean (Age)	2.75^{ab}	5.40^b	1.67^b	1.69^a		0.004				
	Mean (Age)										
<i>Lachnoclostridium</i>	C	2.08	1.66	0.82	0.36	3.37	1.000	1.000			
	Br	2.50	1.68	0.53	0.31	2.46					
	Sy	2.16	1.59	0.88	0.29	2.80					
	Mean (Age)	2.25^a	1.64^{ab}	0.74^{bc}	0.32^c		0.001				
	Mean (Age)										
<i>Faecalibacterium</i>	C	0.72	4.51	11.41	5.93	1.23	0.910	0.950			
	Br	1.53	6.37	13.58	6.79	1.26					
	Sy	3.29	4.64	12.47	5.93	1.23					
	Mean (Age)	1.85^{bc}	5.17^b	12.49^a	6.22^{bc}		0.001				
	Mean (Age)										
<i>Ruminiclostridium 5</i>	C	1.92	1.76	0.93	0.77	5.64	0.930	0.550			
	Br	1.16	1.93	0.91	0.91	7.07					
	Sy	1.80	1.70	0.80	0.67	6.58					
	Mean (Age)	1.63^a	1.80^a	0.88^b	0.78^b		0.001				
	Mean (Age)										
<i>Anaerotruncus</i>	C	2.12	0.46	0.24	0.08	1.35	0.910	0.250			
	Br	1.00	0.70	0.11	0.10	1.23					
	Sy	1.76	0.64	0.17	0.09	1.24					
	Mean (Age)	1.63^a	0.60^b	0.18^b	0.09^b		0.001				
	Mean (Age)										
<i>Negativibacillus</i>	C	1.58	1.69	0.78	0.69	0.72	0.910	0.120			
	Br	1.17	2.79	0.59	0.52	0.48					
	Sy	2.03	2.11	0.76	0.54	0.66					
	Mean (Age)	1.60^a	2.20^a	0.71^b	0.58^b		0.001				
	Mean (Age)										
<i>Lactobacillus</i>	C	1.67	3.03	10.95	15.45	1.18	0.930	0.020			
	Br	0.31	0.91	10.87	21.61	1.27					
	Sy	1.74	1.73	8.02	20.85	1.36					
	Mean (Age)	1.24^b	1.89^b	9.95^c	19.30^a		0.001				
	Mean (Age)										
<i>Bacillus</i>	C	1.53	0.89	2.07	0.95	7.78	0.890	0.980			
	Br	0.87	0.72	1.28	0.63	8.43					
	Sy	0.82	0.64	1.27	0.85	8.09					
	Mean (Age)	1.07	0.75	1.54	0.81		0.190				
	Mean (Age)										
<i>Oscillibacter</i>	C	0.73	1.66	0.27	0.28	1.36	0.890	0.089			
	Br	0.68	1.01	0.29	0.24	0.88					

	Sy	1.78	1.01	0.39	0.27	0.89			
	Mean (Age)	1.06^a	1.23^a	0.31^b	0.26^b			0.001	
<i>Ruminococcaceae</i> <i>UCG-005</i>	C	1.42	0.75	3.25	3.00	0.73	0.990		0.220
	Br	0.42	2.65	2.27	3.33	0.55			
	Sy	0.74	1.65	1.65	3.93	0.86			
	Mean (Age)	0.86^c	1.68^{bc}	2.39^{ab}	3.42^a			0.001	
<i>GCA 900066575</i>	C	1.20	1.74	0.53	0.34	2.11	0.910		0.210
	Br	0.55	2.24	0.49	0.24	2.17			
	Sy	0.76	0.76	0.72	0.26	1.99			
	Mean (Age)	0.84^{ab}	1.58^a	0.58^b	0.28^b			0.001	
<i>Erysipelatoclostridium</i>	C	0.94	0.38	1.63	1.79	0.95	0.450		0.100
	Br	0.22	0.27	1.36	0.81	0.88			
	Sy	0.93	0.29	1.66	0.84	0.62			
	Mean (Age)	0.70^c	0.31^{bc}	1.55^a	1.15^{ac}			0.001	
<i>DTU089</i>	C	1.01	1.47	0.46	0.24	1.19	0.990		0.890
	Br	0.31	2.16	0.64	0.27	0.67			
	Sy	0.40	1.76	0.34	0.27	0.93			
	Mean (Age)	0.57^b	1.80^a	0.48^b	0.26^{bc}			0.002	
<i>Blautia</i>	C	0.41	0.21	1.37	2.08	0.79	0.920		0.920
	Br	0.16	0.18	1.63	1.95	0.84			
	Sy	0.53	0.14	1.41	2.40	0.69			
	Mean (Age)	0.37^b	0.18^b	1.47^c	2.14^a			0.001	
<i>CHKC1001</i>	C	0.25	0.19	3.25	2.69	1.02	0.910		0.870
	Br	0.09	0.15	3.98	4.46	0.98			
	Sy	0.04	0.06	3.62	2.52	1.12			
	Mean (Age)	0.13^b	0.13^b	3.62^a	3.23^a			0.001	
<i>Christensenellaceae R</i> <i>7 group</i>	C	0.20	0.15	1.25	1.69	1.60	1.000		0.890
	Br	0.07	0.33	1.06	1.87	2.17			
	Sy	0.06	0.43	1.12	1.77	1.56			
	Mean (Age)	0.11^b	0.30^b	1.14^c	1.77^a			0.001	
<i>Romboutsia</i>	C	0.06	0.03	1.65	4.61	0.82	0.890		0.091
	Br	0.02	0.04	1.18	3.80	0.83			
	Sy	0.05	0.06	0.77	5.15	0.84			
	Mean (Age)	0.04^b	0.04^b	1.20^c	4.52^a			0.001	
<i>Alistipes</i>	C	0.01	8.90	10.41	5.37	1.59	0.990		0.830
	Br	0.06	7.10	8.52	7.33	1.26			
	Sy	0.00	8.62	8.15	6.61	1.51			
	Mean (Age)	0.02^b	8.21^b	9.02^c	6.44^a			0.001	
<i>Turicibacter</i>	C	0.00	0.00	0.22	2.89	6.17	0.890		0.180
	Br	0.00	0.00	0.12	2.26	5.75			
	Sy	0.00	0.01	0.34	3.28	5.84			
	Mean (Age)	0.00^b	0.00^b	0.23^b	2.81^a			0.001	
<i>Akkermansia</i>	C	0.00	0.00	2.59	0.49	0.78	0.990		1.000
	Br	0.00	0.01	2.78	0.77	0.60			
	Sy	0.00	0.04	2.89	0.82	0.91			
	Mean (Age)	0.00^b	0.01^b	2.75^a	0.69^b			0.001	
<i>Bacteroides</i>	C	0.00	0.71	2.61	6.44	0.77	0.940		0.230
	Br	0.00	0.00	6.77	3.93	0.89			
	Sy	0.00	0.00	9.45	3.31	0.94			
	Mean (Age)	0.00^b	0.24^b	6.28^a	4.56^a			0.001	
<i>Ruminococcaceae</i> <i>UCG-008</i>	C	0.00	0.00	0.00	2.08	2.44	0.910		0.900
	Br	0.00	0.02	0.00	2.38	2.68			
	Sy	0.00	0.00	0.00	1.78	3.19			
	Mean (Age)	0.00^b	0.01^b	0.00^b	2.08^a			0.001	
<i>Candidatus</i> <i>Arthromitus</i>	C	0.45	0.07	0.02 ^A	0.00	0.14	0.910		0.950
	Br	0.18	0.08	0.01 ^{AB}	0.00	0.07			
	Sy	0.23	0.05	0.00 ^B	0.00	0.07			
	Mean (Age)	0.29^a	0.07^{ab}	0.01^{ab}	0.00^b			0.062	

Averages of the columns with different capital letter superscripts and values within the mean rows with different lowercase letters are significantly different ($p < 0.05$).

Table S11. Spearman's correlation between gut microbiota composition and host IBD virus ELISA titers on day 7-21.

Phylum	Order	Family	Genus	IC	IM	CC
Actinobacteria	Corynebacteriales	Corynebacteriaceae	<i>Corynebacterium</i> 1	-0.61		
	Micrococcales	Micrococcaceae	<i>Rothia</i>	-0.69		
			<i>Kocuria</i>	-0.65		
Bacteroidetes	Bacteroidales	Propionibacteriaceae	<i>Cutibacterium</i>		0.69	
		Bacteroidaceae	<i>Bacteroides</i>			-0.72
		Rikenellaceae	<i>Alistipes</i>			-0.62
Firmicutes	Bacillales	Bacillaceae	<i>Anaerobacillus</i>		0.62	
		Planococcaceae	<i>Lysinibacillus</i>		-0.76	
		Staphylococcaceae	<i>Staphylococcus</i>	-0.68		-0.72
	Lactobacillales	Carnobacteriaceae	<i>Trichococcus</i>	-0.6		
		Lactobacillaceae	<i>Lactobacillus</i>			-0.66
		Leuconostocaceae	<i>Leuconostoc</i>	-0.76	-0.61	-0.66
			<i>Weissella</i>			-0.61
		Streptococcaceae	<i>Lactococcus</i>	-0.74		
			<i>Streptococcus</i>			-0.66
	Clostridiales	Christensenellaceae	<i>Christensenellaceae R7 group</i>			-0.77
		Defluviitaleaceae	<i>Defluviitaleaceae UCG-011</i>			-0.6
		Family XIII	<i>Eubacterium brachy group</i>			-0.78
			<i>Family XIII UCG-001</i>			-0.77
			<i>Family XIII AD3011 group</i>			-0.71
			<i>Eubacterium nodatum group</i>			-0.67
			<i>CHKC1001</i>	-0.61		-0.65
		Lachnospiraceae	<i>Marvinbryantia</i>			-0.66
		Peptostreptococcaceae	<i>Romboutsia</i>			-0.76
		Ruminococcaceae	<i>Ruminococcaceae UCG-004</i>			-0.78
			<i>Ruminococcaceae UCG-010</i>			-0.77
			<i>Ruminococcus</i> 1			-0.74
			<i>Ruminococcaceae NK4A214 group</i>			-0.67
			<i>Ruminiclostridium</i> 1			-0.65
			<i>Faecalibacterium</i>			-0.64
			<i>Anaerotruncus</i>			0.64
			<i>Papillibacter</i>			-0.61
	Erysipelotrichales	Erysipelotrichaceae	<i>Turicibacter</i>	-0.73		-0.71
Proteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	0.62		
	Betaproteobacteriales	Burkholderiaceae	<i>Aquabacterium</i>	0.6		
	Enterobacteriales	Enterobacteriaceae	<i>Escherichia-Shigella</i>			0.67
	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	0.76		
Verrucomicrobia	Verrucomicrobiales	Akkermansiaceae	<i>Akkermansia</i>	-0.65		-0.72

Table shows only strong R > 0.6 correlation coefficients based on Spearman's rank correlation (Spearman's rho).

Table S12. Spearman's correlation between gut microbiota composition and host Gumboro titer/ parameters on day 21-40.

Phylum	Order	Family	Genus	IC	IM	CC
Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	<i>Bifidobacterium</i>	0.79	0.85	0.62
			<i>Neoscardovia</i>		0.68	
	Micrococcales	Micrococcaceae	<i>Rothia</i>	-0.65	0.6	
	Coriobacteriales	Eggerthellaceae	<i>CHKCI002</i>			-0.75
Firmicutes	Bacillales	Planococcaceae	<i>Lysinibacillus</i>		-0.8	
		Staphylococcaceae	<i>Staphylococcus</i>	-0.61		
	Lactobacillales	Aerococcaceae	<i>Globicatella</i>	0.6		
		Carnobacteriaceae	<i>Carnobacterium</i>		-0.6	
		Lactobacillaceae	<i>Lactobacillus</i>		0.75	0.71
		Leuconostocaceae	<i>Leuconostoc</i>	-0.64		
		Streptococcaceae	<i>Lactococcus</i>	-0.68		
	Clostridiales	Clostridiaceae 1	<i>Candidatus Arthromitus</i>		-0.74	-0.67
			<i>Clostridium sensu stricto 1</i>	-0.83		0.73
			<i>Clostridium sensu stricto 10</i>	-0.66		
			<i>Clostridium sensu stricto 12</i>	-0.76		
		Family XI	<i>Gallicola</i>	0.68	0.72	
		Lachnospiraceae	<i>CHKCI001</i>	-0.64		
			<i>Eubacterium fissicatenae group</i>	-0.66		
			<i>Eubacterium hallii group</i>	-0.62		
			<i>Lachnoclostridium</i>	-0.61		
			<i>Ruminococcus torques group</i>	-0.63		
		Peptostreptococcaceae	<i>Romboutsia</i>	0.61	0.62	0.68
		Ruminococcaceae	<i>Butyricoccus</i>			-0.68
			<i>Faecalibacterium</i>			-0.61
			<i>Hydrogenoanaerobacterium</i>			-0.78
			<i>Oscillospira</i>			0.81
			<i>Ruminococcaceae UCG-008</i>		0.73	0.77
			<i>Ruminococcaceae UCG-010</i>			0.59
			<i>Ruminococcaceae UCG-014</i>	-0.78		
			<i>Ruminococcus 2</i>			0.72
Proteobacteria	Erysipelotrichales	Erysipelotrichaceae	<i>Turicibacter</i>	0.72	0.67	0.77
	Rhizobiales	Rhizobiaceae	<i>Ochrobactrum</i>		-0.8	
	Rhodospirillales	uncultured	<i>Azospirillum sp. 47 25</i>			0.76
	Desulfovibrionales	Desulfovibrionaceae	<i>Bilophila</i>			0.78
	Betaproteobacteriales	Burkholderiaceae	<i>Delftia</i>		-0.72	
			<i>Parasutterella</i>			0.85
	Enterobacteriales	Enterobacteriaceae	<i>Serratia</i>		-0.81	
	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>		-0.72	
Verrucomicrobia	Xanthomonadales	Xanthomonadaceae	<i>Stenotrophomonas</i>		-0.71	
	Verrucomicrobiales	Akkermansiaceae	<i>Akkermansia</i>	-0.75		

Table shows only strong R > 0.6 correlation coefficients based on Spearman's rank correlation (Spearman's rho).

