

Microbiota Composition of Mucosa and Interactions between the Microbes of the Different Gut Segments Could Be a Factor to Modulate the Growth Rate of Broiler Chickens

Supplementary Materials

* Corresponding author: Prof. Károly Dublec

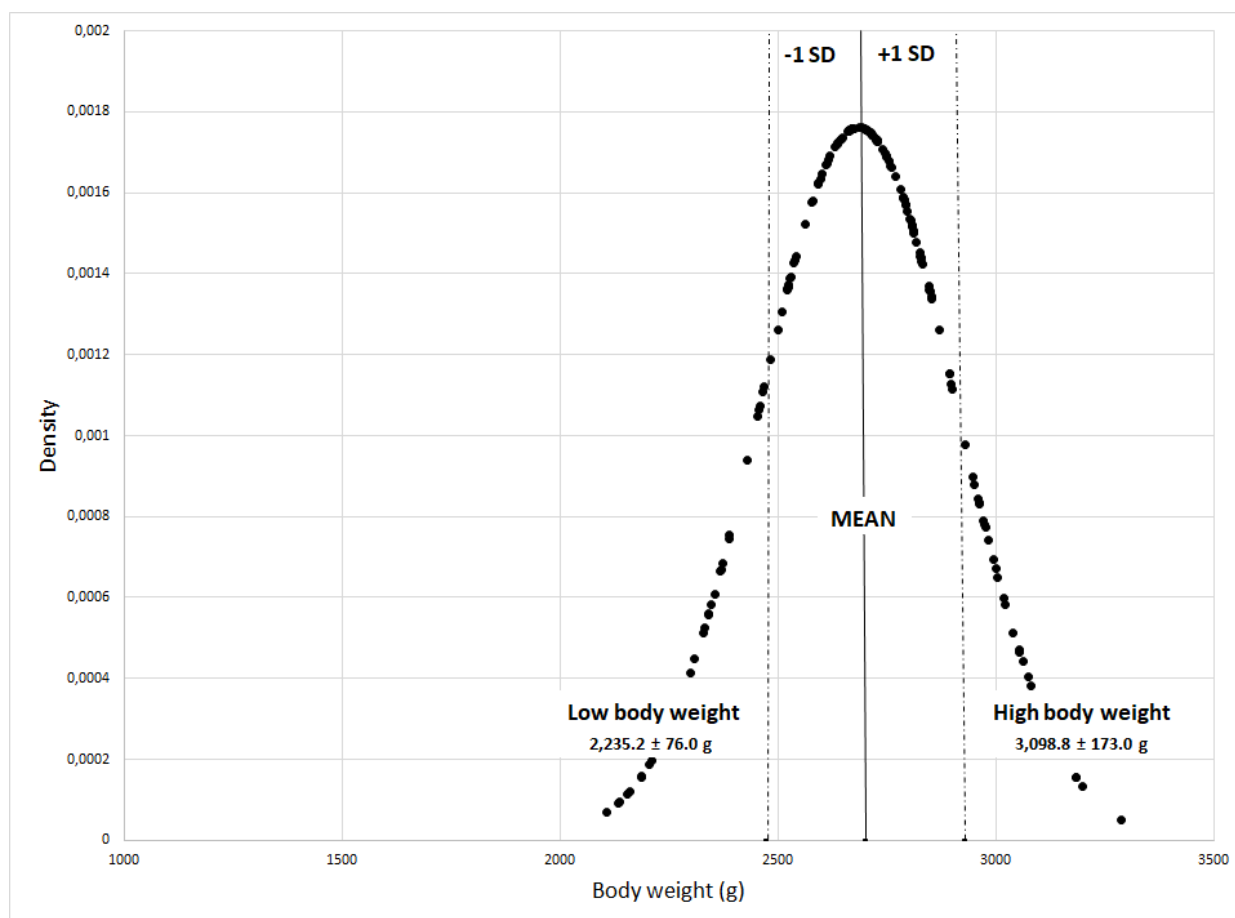
E-mail address: dublec.karoly@uni-mate.hu

Supplemental Table S1. Composition of diets (starter, grower and finisher) fed to the experimental chicks.

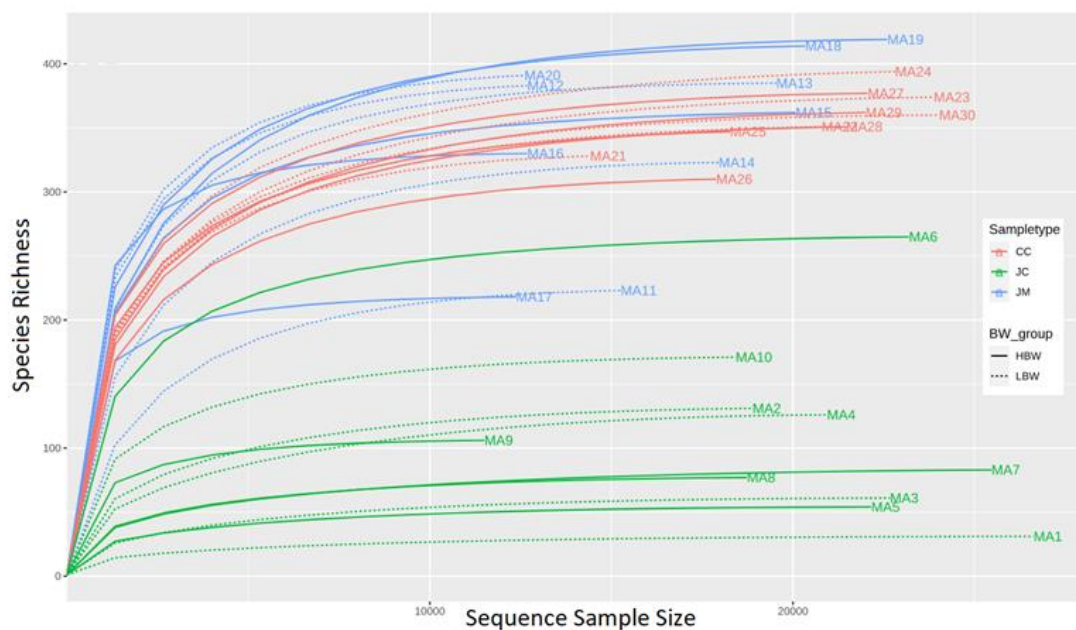
	Starter Diet (day 1-14)	Grower Diet (day 15-32)	Finisher Diet (day 33-37)
Ingredient (g/kg)			
Corn	412.50	442.62	468.10
Wheat	150	150	150
Soybean meal	370	243	178
Sunflower oil	25	28	32
Fullfat soybean	0	60	80
Sunflower meal	0	40	60
L-lizin	3.2	2.8	2.3
DL-Metionin	3.76	2.96	2.39
L-Treonin	1.34	0.92	0.51
Limestone	14.5	12	11
MCP	10	8	6.5
Salt	2.7	2.7	2.7
Sodium bicarbonate	1.5	1.5	1.5
Anticoccidian (Sacox 120)	0.5	0.5	0
Avizyme 1505 X	0	0	0
Premix ¹	5	5	5
Calculated nutrient content ()			
AME _n (MJ/kg) ²	12.50	12.96	13.26
Crude proteins	22.0	20.0	18.6
Lysine	1.44	1.25	1.10
Methionine + cysteine	1.07	0.95	0.86
Tryptophan	0.26	0.23	0.21
Threonine	0.97	0.84	0.74
Calcium	0.92	0.78	0.71
Available P	0.50	0.45	0.42

¹Vitamin-mineral premix contents per kg: 15 calcium 2.500.000 IU vitamin A, 1.000.000 IU vitamin D3, 16.800 mg vitamin E, 984 mg vitamin K3, 590.4 mg vitamin B1, 1.968 mg vitamin B2, 4.2 mg vitamin B12, 10.000 mg niacin, 2.952 mg pantothenic acid, 393.6 mg Folic acid, 40 mg biotin, 984 mg vitamin B6. 35.070 mg choline chloride, 29.008 mg betaine, 10.016 mg iron, 21.700 mg manganese, 3.200 mg copper, 20.160 mg zinc, 312.33 mg iodine, 63 mg selenium, 1.050 mg antioxidant, 200.000 FTU of Phytase (Aextra PHY).

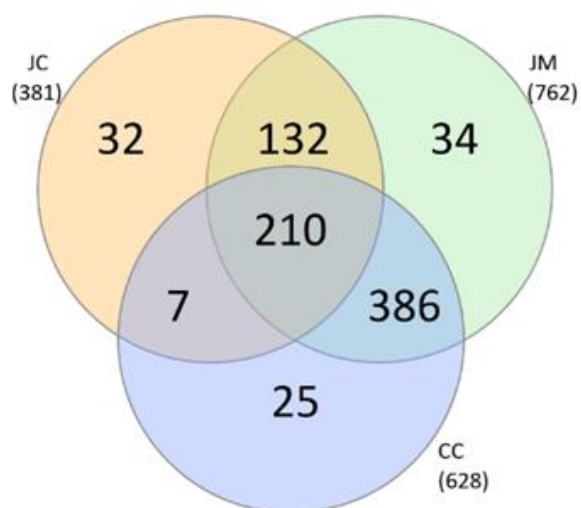
²AMEn values were calculated according to EU regulation 152/2009.



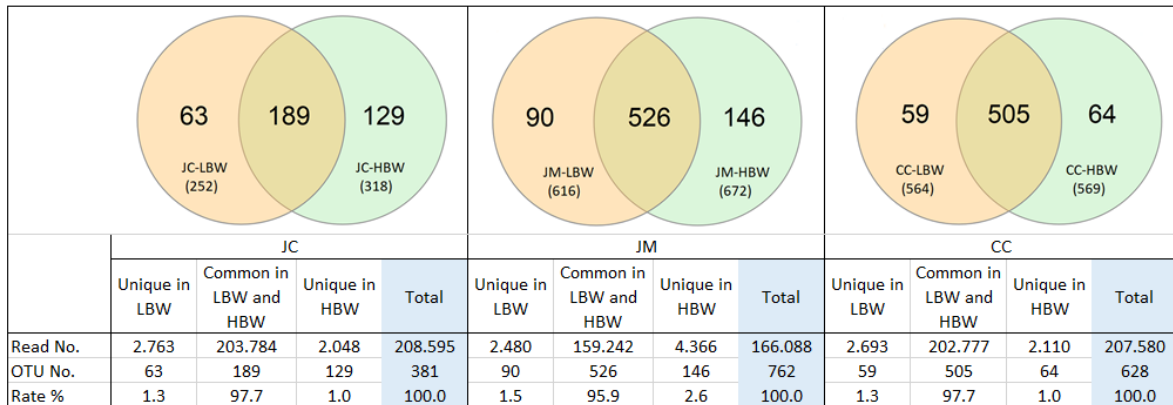
Supplemental Figure S1. Sampling strategy. A total of 160 male broiler chickens were weighed individually and average BW (mean \pm SD) were determined. For the selection. Five birds with heaviest body weight (mean BW +1 SD) and five lightest (mean BW -1 SD) were selected randomly and classify into two group with high or low live weight (HBW and LBW).



Supplemental Figure S2. The rarefaction curves of OTUs. The x-axis shows the number of valid sequences per sample and the y-axis shows the observed species (operational taxonomic units, OTUs). Each curve in the graph represents a different sample and the sample types shows in a different color, BW group indicates a solid (HBW) and dashed (LBW) line.



Supplemental Figure S3. Venn diagram show the number of OTUs that were shared or not shared in the jejunum mucosa (JM), jejunum content (JC), and caecum content (CC), or overlapped in the 3 examined groups.



Supplemental Figure S4. Venn diagrams shows the number of OTUs in different intestinal sampling places according to the body weight (low and high BW) of chickens indicating in table their read numbers and rates (%).

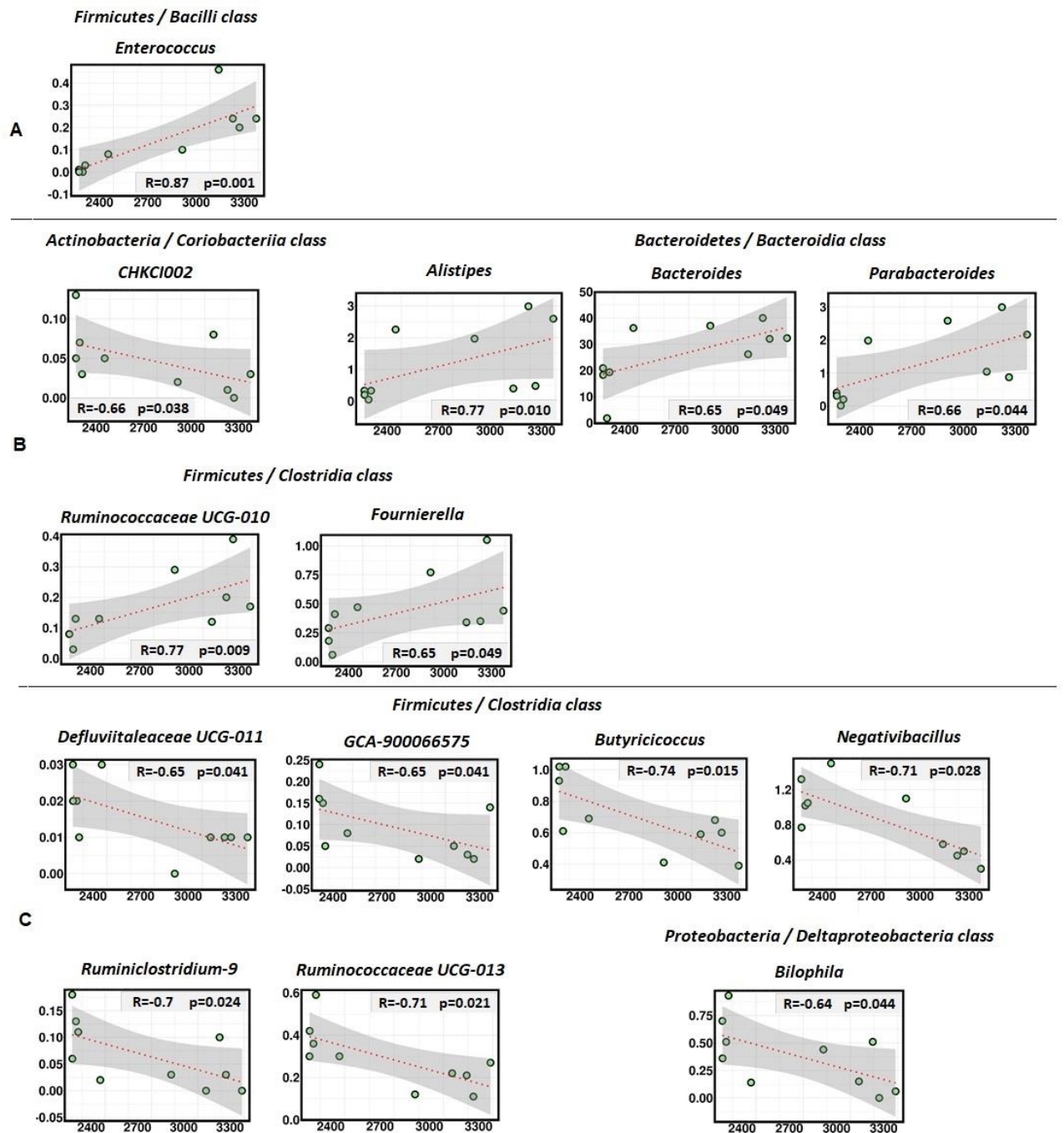
Supplemental Table S2. Relative abundance of bacterial genera in the different sampling places of broiler chickens as affected by BW (%).

Genus	Body Weight	Sampling Place			Mean (BW)	FDR p-Values		
		Jejunal Chymus	Jejunal Mucosa	Caecum Chymus		SP	BW	SP x BW
Actinobacteria								
<i>Corynebacterium-1</i>	LBW	2.06	0.97	0.00	1.01			0.747
	HBW	3.51	0.24	0.01	1.25		0.877	
	Mean (SP)	2.78	0.60	0.00		0.323		
Bacteroidetes (Class Bacteroidia)								
<i>Alistipes</i>	LBW	0.00	0.64	0.82 ^B	0.48 ^B			0.451
	HBW	0.03	1.71	2.48 ^A	1.41 ^A		0.081 ^T	
	Mean (SP)	0.01 ^b	1.17 ^a	1.65 ^a		0.017		
<i>Bacteroides</i>	LBW	0.11	19.06 ^B	42.39	20.52 ^B			0.280
	HBW	0.76	34.28 ^A	46.27	27.10 ^A		0.098 ^T	
	Mean (SP)	0.43 ^c	26.67 ^b	44.33 ^a		0.000		
<i>Parabacteroides</i>	LBW	0.00	0.58	1.99	0.86			0.509
	HBW	0.04	1.97	2.69	1.56		0.179	
	Mean (SP)	0.02 ^c	1.27 ^b	2.34 ^a		0.000		
Firmicutes (Class Bacilli)								
<i>Bacillus</i>	LBW	0.01	0.55	0.13	0.23			0.948
	HBW	0.03	0.65	0.09	0.25		0.907	
	Mean (SP)	0.02 ^b	0.60 ^a	0.11 ^b		0.024		
<i>Enterococcus</i>	LBW	0.02 ^B	0.03	0.00	0.02 ^B			0.001
	HBW	0.22 ^A	0.02	0.00	0.08 ^A		0.012	
	Mean (SP)	0.123 ^a	0.025 ^b	0.002 ^b		0.000		
<i>Lactobacillus</i>	LBW	65.94	21.87	2.90	30.24			0.721
	HBW	64.24	7.23	7.23	25.07		0.646	
	Mean (SP)	65.09 ^a	14.55 ^b	3.32 ^b		0.000		
<i>Streptococcus</i>	LBW	7.56	1.01	4.32	4.30			0.690
	HBW	6.77	1.05	0.96	2.93		0.585	
	Mean (SP)	7.17 ^a	1.03 ^b	2.64 ^b		0.022		
<i>Nosocomiicoccus</i>	LBW	0.15	0.06	0.00	0.07			0.906
	HBW	0.10	0.04	0.00	0.05		0.680	
	Mean (SP)	0.12	0.05	0.00		0.208		
<i>Lactococcus</i>	LBW	0.10	0.00	0.00	0.04			0.595
	HBW	1.36	0.00	0.00	0.45		0.585	
	Mean (SP)	0.73	0.00	0.00		0.513		
Firmicutes (Class Clostridia; Families Family XIII and Christensenellaceae)								
<i>Family XIII UCG-001</i>	LBW	0.00	0.06	0.08	0.05			0.624
	HBW	0.00	0.13	0.15	0.09		0.323	
	Mean (SP)	0.00 ^b	0.10 ^a	0.12 ^a		0.013		
<i>Christensenellaceae R-7</i>	LBW	0.03	0.64	1.39	0.68			0.476
	HBW	0.01	0.93	1.12	0.69		0.989	
	Mean (SP)	0.02 ^c	0.78 ^b	1.25 ^a		0.000		
Firmicutes (Class Clostridia; Family Lachnospiraceae)								
<i>Anaerostipes</i>	LBW	0.01	0.12	0.13	0.09			0.500
	HBW	0.00	0.17	0.06	0.08		0.821	
	Mean (SP)	0.00 ^b	0.14 ^a	0.10 ^a		0.007		
<i>Blautia</i>	LBW	0.03	0.23	0.59	0.28			0.417
	HBW	0.03	0.30	0.35	0.23		0.623	
	Mean (SP)	0.03 ^c	0.26 ^b	0.47 ^a		0.000		
<i>CHKC1001</i>	LBW	0.01	0.04	0.26	0.11			0.989
	HBW	0.02	0.03	0.28	0.11		0.955	
	Mean (SP)	0.01 ^b	0.04 ^b	0.27 ^a		0.024		
<i>Eubacterium hallii group</i>	LBW	0.02	0.17	0.56	0.25			0.985
	HBW	0.02	0.09	0.49	0.20		0.796	
	Mean (SP)	0.02	0.13	0.53		0.067		
<i>Eubacterium ventriosum</i>	LBW	0.00	0.02	0.10	0.04			0.919
	HBW	0.00	0.03	0.11	0.05		0.727	
	Mean (SP)	0.00 ^b	0.02 ^b	0.11 ^a		0.000		
<i>GCA-900066575</i>	LBW	0.00	0.26	0.14	0.13			0.680
	HBW	0.00	0.21	0.05	0.09		0.456	
	Mean (SP)	0.00 ^c	0.24 ^a	0.09 ^b		0.000		
<i>Lachnoclostridium</i>	LBW	0.02	0.65	1.12	0.59			
	HBW	0.05	0.62	1.10	0.59		0.989	

	Mean (SP)	0.03 ^c	0.64 ^b	1.11 ^a	0.000	0.989
<i>Ruminococcus torques</i> group	LBW	0.05	1.82	1.81	1.23	0.624
	HBW	0.08	2.39	1.12	1.20	0.970
	Mean (SP)	0.06 ^b	2.11 ^a	1.46 ^a	0.008	
<i>Sellimonas</i>	LBW	0.01	0.32	0.49	0.27	0.991
	HBW	0.02	0.31	0.49	0.27	0.995
	Mean (SP)	0.02 ^c	0.31 ^b	0.49 ^a	0.000	
<i>Tyzzereella</i>	LBW	0.00	0.19	0.29	0.16	0.610
	HBW	0.00	0.40	0.27	0.22	0.624
	Mean (SP)	0.00 ^b	0.29 ^a	0.28 ^a	0.025	
Firmicutes (Class Clostridia; Family Ruminococcaceae)						
<i>Anaerofilum</i>	LBW	0.00	0.17	0.09	0.09	0.885
	HBW	0.02	0.18	0.07	0.09	0.962
	Mean (SP)	0.01 ^b	0.17 ^a	0.08 ^b	0.003	
<i>Butyricoccus</i>	LBW	0.02	0.49	0.85	0.45	0.327
	HBW	0.05	0.45	0.52	0.34	0.346
	Mean (SP)	0.03 ^c	0.47 ^b	0.69 ^a	0.000	
<i>DTU089</i>	LBW	0.01	0.24	0.16	0.14	0.699
	HBW	0.00	0.15	0.10	0.08	0.372
	Mean (SP)	0.00 ^b	0.20 ^a	0.13 ^a	0.002	
<i>Eubacterium-</i> <i>coprostanoligenes-group</i>	LBW	0.00	1.07	1.03	0.70	0.810
	HBW	0.02	1.27	0.99	0.76	0.740
	Mean (SP)	0.01 ^b	1.17 ^a	1.01 ^a	0.000	
<i>Faecalibacterium</i>	LBW	0.01	3.15	3.08	2.08	0.683
	HBW	0.00	2.45	1.33	1.26	0.503
	Mean (SP)	0.01 ^b	2.80 ^a	2.20 ^a	0.031	
<i>Fournierella</i>	LBW	0.00	0.29	0.24	0.18	0.417
	HBW	0.02	0.61	0.28	0.30	0.323
	Mean (SP)	0.01 ^b	0.45 ^a	0.26 ^a	0.001	
<i>Negativibacillus</i>	LBW	0.00	0.17	1.13 ^A	0.44	0.027
	HBW	0.00	0.20	0.59 ^B	0.26	0.127
	Mean (SP)	0.00 ^b	0.19 ^b	0.86 ^a	0.000	
<i>Oscillibacter</i>	LBW	0.00	0.66	0.70	0.46	0.690
	HBW	0.00	0.89	0.69	0.53	0.660
	Mean (SP)	0.00 ^b	0.77 ^a	0.70 ^a	0.000	
<i>Ruminiclostridium 5</i>	LBW	0.00	0.28	0.44	0.24	0.260
	HBW	0.00	0.38	0.23	0.21	0.665
	Mean (SP)	0.00 ^b	0.33 ^a	0.34 ^a	0.000	
<i>Ruminococcaceae NK4A214</i>	LBW	0.01	0.35	0.82	0.40	0.342
	HBW	0.01	0.44	1.79	0.75	0.329
	Mean (SP)	0.01 ^b	0.39 ^b	1.31 ^a	0.001	
<i>Ruminococcaceae UCG-005</i>	LBW	0.01	1.03	0.70	0.58	0.727
	HBW	0.01	1.51	0.70	0.74	0.665
	Mean (SP)	0.01 ^b	1.27 ^a	0.70 ^{ab}	0.009	
<i>Ruminococcaceae UCG-010</i>	LBW	0.00	0.09 ^B	0.19	0.09 ^B	0.472
	HBW	0.00	0.24 ^A	0.29	0.18 ^A	0.083 ^T
	Mean (SP)	0.00 ^b	0.17 ^a	0.24 ^a	0.000	
<i>Ruminococcaceae UCG-013</i>	LBW	0.01	0.09	0.39 ^A	0.17 ^A	0.026
	HBW	0.01	0.06	0.18 ^B	0.08 ^B	0.020
	Mean (SP)	0.010 ^b	0.078 ^b	0.285 ^a	0.000	
<i>Ruminococcaceae UCG-014</i>	LBW	0.02	2.47	3.46	1.98	0.690
	HBW	0.15	3.70	4.79	2.88	0.341
	Mean (SP)	0.09 ^b	3.09 ^a	4.12 ^a	0.000	
<i>Subdoligranulum</i>	LBW	0.04	0.29	0.90	0.41	0.803
	HBW	0.14	0.64	1.11	0.63	0.371
	Mean (SP)	0.09 ^b	0.46 ^b	1.01 ^a	0.000	
Firmicutes (Class Negativicutes and Erysipelotrichia)						
<i>Phascolarctobacterium</i>	LBW	0.03	1.38	3.98	1.80	0.607
	HBW	0.03	1.01	2.06	1.03	0.475
	Mean (SP)	0.03 ^b	1.19 ^b	3.02 ^a	0.008	
<i>Erysipelatoclostridium</i>	LBW	0.00	0.22	0.68	0.30	0.381
	HBW	0.01	0.01	0.38	0.16	0.112
	Mean (SP)	0.01 ^b	0.15 ^b	0.53 ^a	0.000	
Proteobacteria						
<i>Burkholderia-Caballeronia-</i> <i>Paraburkholderia</i>	LBW	0.00	0.11	0.00	0.26	0.471
	HBW	0.00	0.10	0.00	0.19	0.630
	Mean (SP)	0.00 ^b	0.11 ^a	0.00 ^b	0.036	
<i>Enterobacter</i>	LBW	0.06	0.45	0.00	0.25	0.985

	HBW	0.02	0.02	0.00	0.20	0.796	
	Mean (SP)	0.02	0.13	0.53		0.067	
<i>Escherichia-Shigella</i>	LBW	0.98	0.65	1.79	1.14		0.157
	HBW	8.18	0.37	0.64	3.06	0.450	
	Mean (SP)	4.58	0.51	1.22		0.190	
<i>Paracoccus</i>	LBW	0.01	0.09	0.00	0.03		0.683
	HBW	0.12	0.09	0.00	0.07	0.646	
	Mean (SP)	0.064	0.089	0.00		0.577	
<i>Bilophila</i>	LBW	0.02	0.23	0.53	0.04		0.989
	HBW	0.00	0.33	0.23	0.03	0.929	
	Mean (SP)	0.01^b	0.28^a	0.38^a		0.017	

Bacterial genera differences between groups were assessed using two-way ANOVA test, with Benjamini-Hochberg false discovery rate (FDR) correction. FDR-corrected p-values below 0.05 were considered significant and results between 0.05 and 0.1 ($0.05 < p < 0.10$) were considered a trend (T). Body weight (BW) effect at each sampling places (SP) were also compared with one-way ANOVA test and the significance of Tukey's HSD multiple group comparison's Post hoc tests was indicated at $p < 0.05$. a,b,c: values within the mean (SP) rows with different lowercase letters were significantly different ($p < 0.05$). A, B: values within the mean columns with different capital letters superscripts were significantly different ($p < 0.05$).



Supplemental Figure S5. Correlation between body weight (g) and genus abundance in the different intestinal samples. (A) Correlation in jejunum chymus; (B) in jejunum mucosa and (C) in caecum chymus.