Supplementary Data:

Title: Significant alterations of intestinal symbiotic microbiota induced by intraperitoneal vaccination mediate changes in intestinal metabolism of NEW Genetically Improved Farmed Tilapia (NEW GIFT, *Oreochromis niloticus*)

Supplementary Table 1. Results of tilapia body weight (kg) in different groups

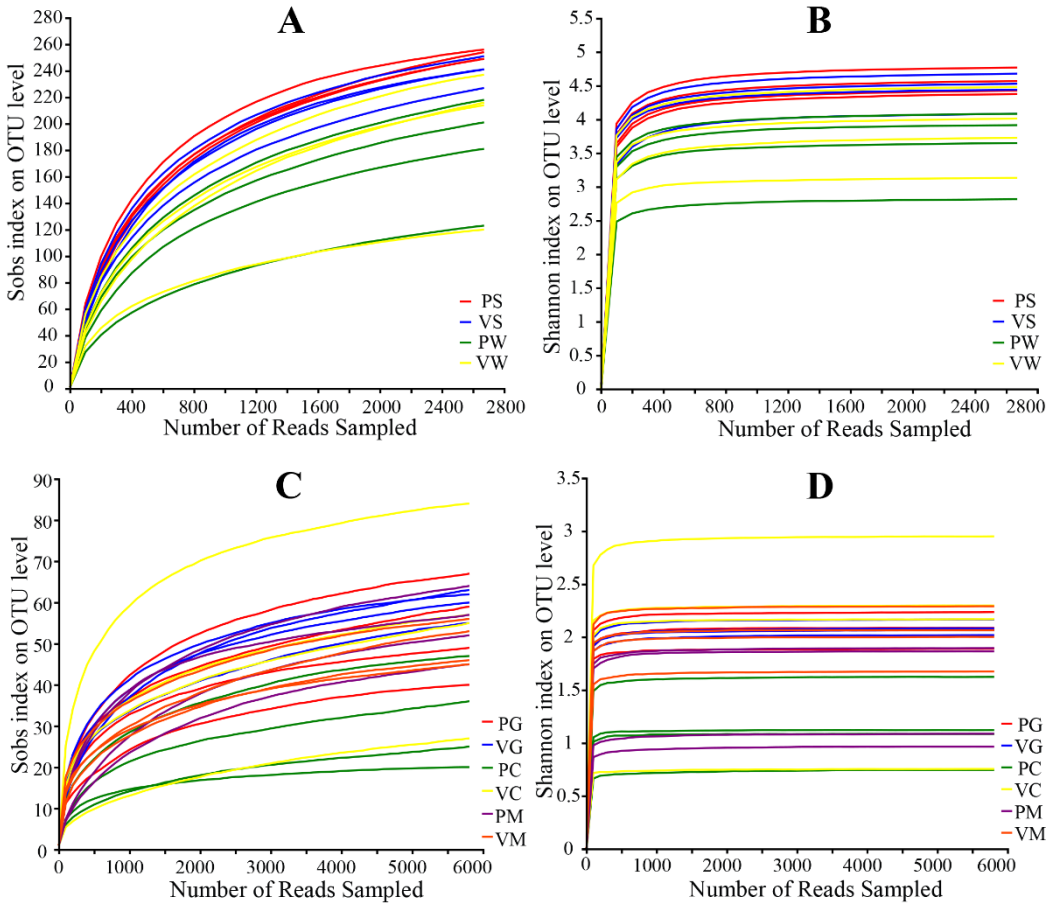
Initial group	Control group	Vaccinated group
0.26	0.28	0.28
0.26	0.38	0.27
0.25	0.34	0.38
0.21	0.3	0.32
0.25	0.32	0.37
0.2	0.28	0.34
0.26	0.29	0.39
0.25	0.3	0.35
0.22	0.31	0.31
0.23	0.35	0.33
0.26	0.37	0.36
0.24	0.36	0.29
0.24 ± 0.02	0.32 ± 0.04 kg	0.33 ± 0.04 kg

Supplementary Table 2. The distribution range of valid sequences obtained by high-throughput sequencing in different sample types

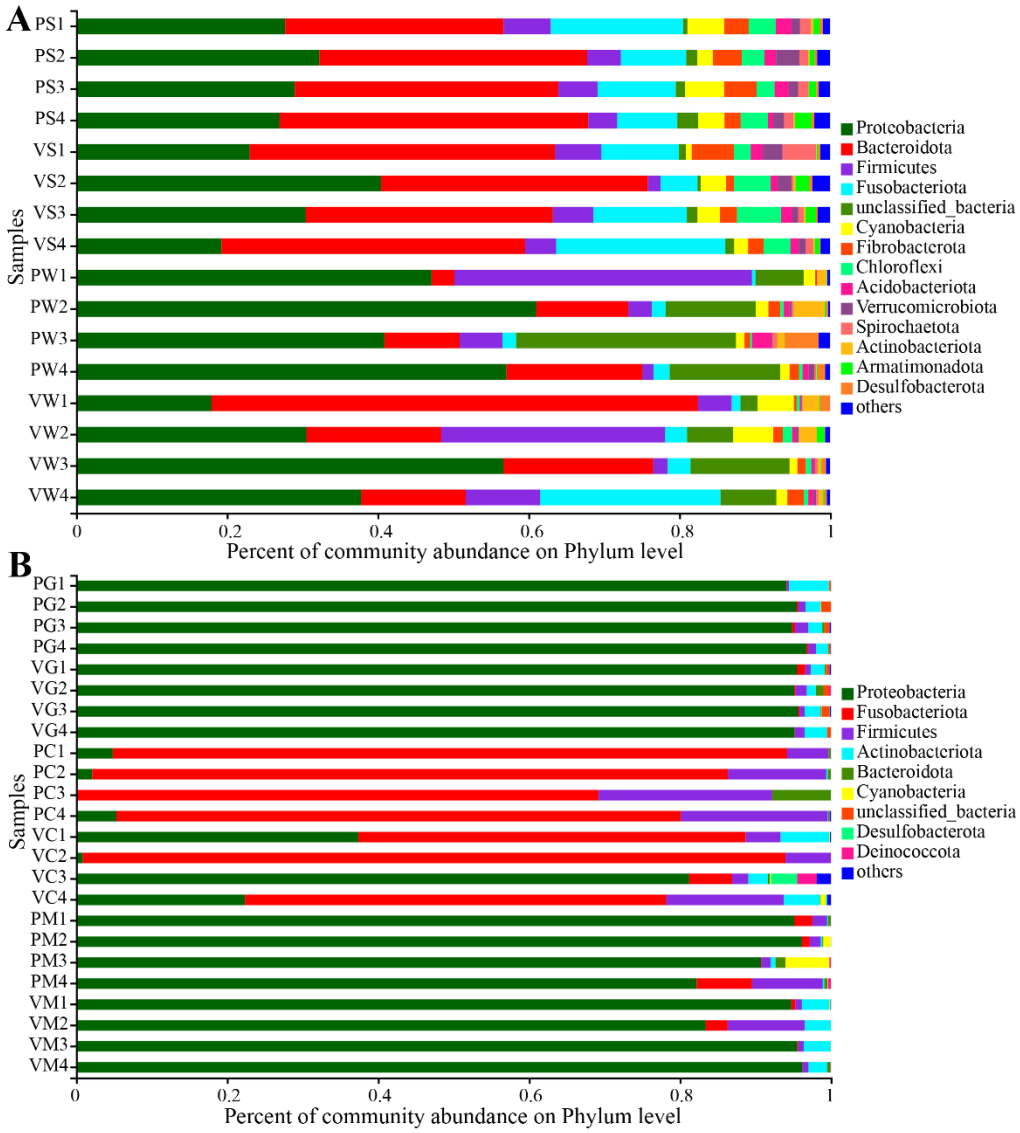
Sample types	Number of valid reads per sample
Stomach contents (S)	3400-8286
Stomach mucosae (W)	2671-5471
Gill mucosae (G)	6393-10160
Intestinal contents (C)	5810-23908
Intestinal mucosae (M)	11980-70742

Supplementary Table 3. Summary of alpha diversity indices calculated based on a cutoff of 97% similarity of 16S rRNA sequences

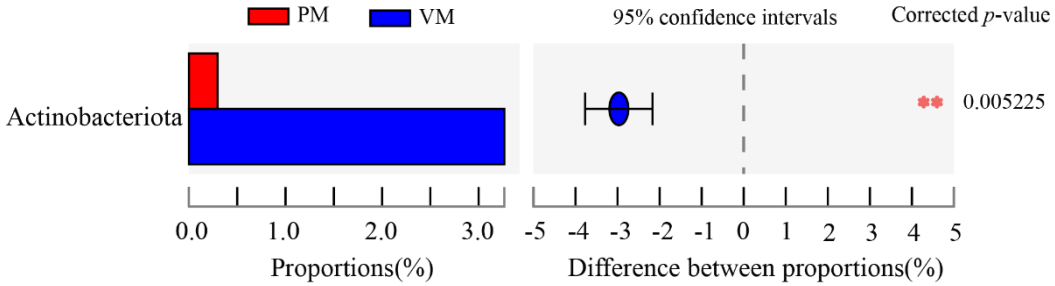
Group	**Indices**				
	OTUs	**Chao**	**Shannon**	**Simpson**	**Coverage**
PS	252 ± 3.56	285.04 ± 11.30	4.54 ± 0.18	0.03 ± 0.01	98.17 ± 0.40%
VS	240 ± 9.87	270.74 ± 9.76	4.43 ± 0.25	0.03 ± 0.02	98.23 ± 0.28%
PW	180.75 ± 41.36	223.60 ± 48.92	3.62 ± 0.56	0.08 ± 0.05	98.14 ± 0.34%
VW	196.75 ± 52.21	236.08 ± 67.33	3.84 ± 0.56	0.06 ± 0.04	98.11 ± 0.56%
PG	53.75 ± 11.76	64.19 ± 14.68	2.07 ± 0.14	0.21 ± 0.03	99.81 ± 0.06%
VG	60 ± 3.56	68.14 ± 6.35	2.08 ± 0.06	0.22 ± 0.02	99.79 ± 0.04%
PC	32 ± 12.03	42.19 ± 16.61	1.14 ± 0.36	0.49 ± 0.16	99.87 ± 0.07%
VC	55.75 ± 23.29	69.34 ± 22.30	2.04 ± 0.92	0.28 ± 0.25	99.78 ± 0.05%
PM	54.5 ± 8.02	80.53 ± 30.31	1.45 ± 0.50	0.38 ± 0.14	99.78 ± 0.03%
VM	50 ± 5.35	55.28 ± 6.23	2.01 ± 0.25	0.24 ± 0.05	99.82 ± 0.04%



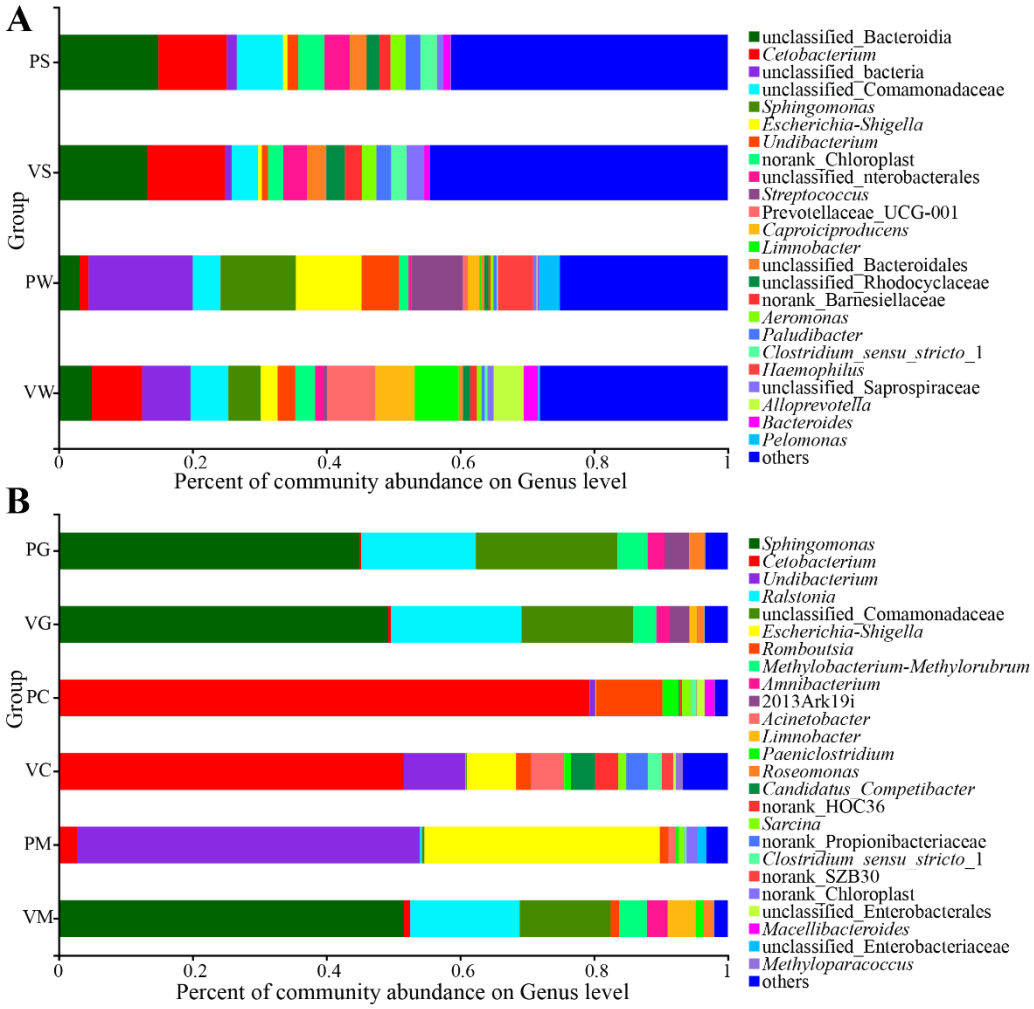
Supplementary Figure 1. Rarefaction analyses of OTUs clustered at 97% sequence identity of all samples: **(A)** Rarefaction curves on OTU level of the stomach contents and mucosae; **(B)** Shannon curves on OTU level of the stomach contents and mucosae; **(C)** Rarefaction curves on OTU level of the gill and intestinal samples; **(D)** Shannon curves on OTU level of the gill and intestinal samples. PS: stomach contents in the control group; VS: stomach contents in the vaccinated group; PW: stomach mucosae in the control group; VW: stomach mucosae in the vaccinated group; PG: gill mucosae in the control group; VG: gill mucosae in the vaccinated group; PC: intestinal contents in the control group; VC: intestinal contents in the vaccinated group; PM: intestinal mucosae in the control group; VM: intestinal mucosae in the vaccinated group.



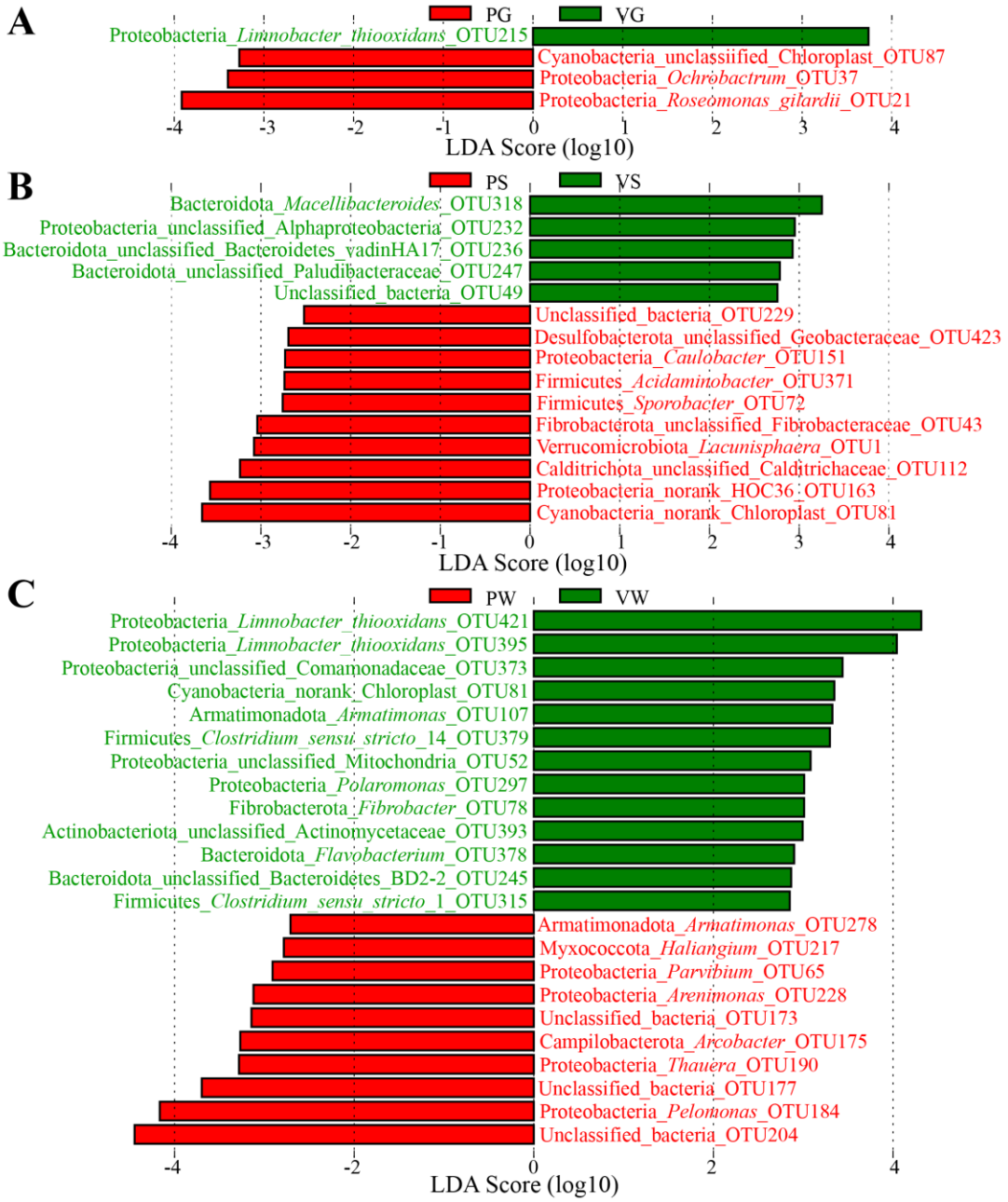
Supplementary Figure 2. Distribution of the bacterial communities in all samples at the phylum level: **(A)** Bacterial phyla in the stomach content and mucosa samples; **(B)** Bacterial phyla in the gill and intestinal samples. PS1-PS4: stomach contents in the control group; VS1-VS4: stomach contents in the vaccinated group; PW1-PW4: stomach mucosae in the control group; VW1-VW4: stomach mucosae in the vaccinated group; PG1-PG4: gill mucosae in the control group; VG1-VG4: gill mucosae in the vaccinated group; PC1-PC4: intestinal contents in the control group; VC1-VC4: intestinal contents in the vaccinated group; PM1-PM4: intestinal mucosae in the control group; VM1-VM4: intestinal mucosae in the vaccinated group.



Supplementary Figure 3. Comparison of the bacterial phylum in the intestinal mucosae between the control and vaccinated groups. PM: intestinal mucosae in the control group; VM: intestinal mucosae in the vaccinated group. The significance of Welch t-test: ** $p < 0.01$.

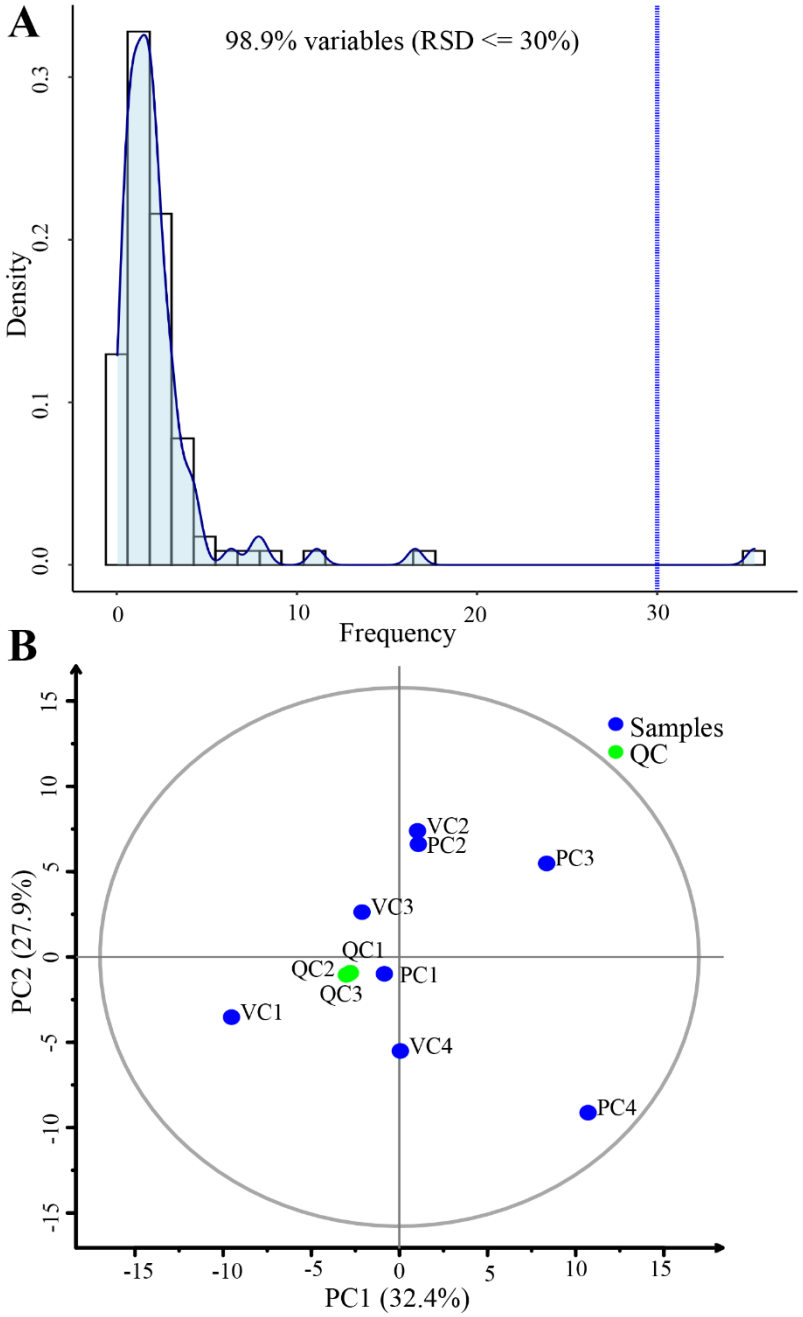


Supplementary Figure 4. Distribution of the bacterial communities in all samples at the genus level: **(A)** Bacterial genera in the stomach content and mucosa samples; **(B)** Bacterial genera in the gill and intestinal samples. PS1-PS4: stomach contents in the control group; VS1-VS4: stomach contents in the vaccinated group; PW1-PW4: stomach mucosae in the control group; VW1-VW4: stomach mucosae in the vaccinated group; PG1-PG4: gill mucosae in the control group; VG1-VG4: gill mucosae in the vaccinated group; PC1-PC4: intestinal contents in the control group; VC1-VC4: intestinal contents in the vaccinated group; PM1-PM4: intestinal mucosae in the control group; VM1-VM4: intestinal mucosae in the vaccinated group.

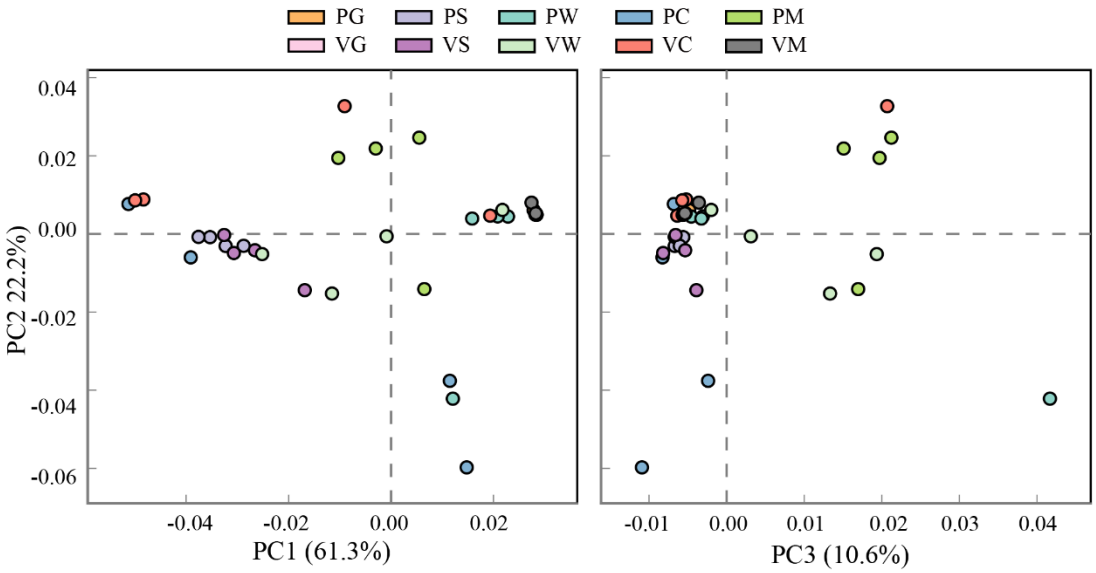


Supplementary Figure 5. Comparison of the bacterial communities in the gill and stomach samples at the OTU level by LEfSe: **(A)** Comparison of the bacterial communities in the gill mucosae between the control and vaccinated groups; **(B)** Comparison of the bacterial communities in the stomach contents between the control and vaccinated groups; **(C)** Comparison of the bacterial communities in the stomach mucosae between the control and vaccinated groups. The highlighted taxa are enriched in the group that

corresponds to each color; LDA scores can be interpreted as the degree of difference in the relative abundance of OTUs; PG: gill mucosae in the control group, VG: gill mucosae in the vaccinated group, PS: stomach contents in the control group, VS: stomach contents in the vaccinated group, PW: stomach mucosae in the control group, VW: stomach mucosae in the vaccinated group.



Supplementary Figure 6. Metabolome data processing results: **(A)** Result of quality assurance; **(B)** Result of quality control (QC). The relative standard deviation (RSD) of the QC characteristic peak, that is, the coefficient of variation should not exceed 30%.



Supplementary Figure 7. Principal component analysis (PCA) based on the Bray-Curtis distance visualizing the integral structure dissimilarities of microbial function.