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Effect of dietary *Clostridium butyricum* on growth, intestine health status and resistance to ammonia stress in Pacific white shrimp *Litopenaeus vannamei*

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1 Effect of dietary *Clostridium butyricum* on growth, intestine health status and

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8 ABSTRACT The present study evaluated the effect of dietary *Clostridium butyricum* (CB) on growth, 9 intestine microstructure, intestine digestive and immune function, intestine short-chain fatty acids (SCFA) 10 content and body composition of Pacific white shrimp Litopenaeus vannamei. The shrimp was fed for 56 d with diets containing different levels of C. butyricum $(1 \times 10^9 \text{ cfu/g})$: 0% (Control), 0.25% (CB1), 0.5% 11 12 (CB2) and 1.0% (CB3) as treatment groups, followed by an acute ammonia stress test for 72 h. The results 13 indicated that dietary supplementation of C. butyricum decreased the feed conversion rate (FCR) and 14 increased the growth performance of shrimp. Compared with the control group, after shrimp fed with C. butyricum 56 d, intestine amylase and protease activity in the three C. butyricum group increased, while 15 16 lipase activity was only affected in the CB1 and CB2 group. Total antioxidant capacity (T-AOC) content, 17 lysozyme (LSZ) activity, and the relative expression level of Toll and immune deficiency (Imd) gene all 18 increased in three C. butyricum groups. Inducible nitric oxide synthase (iNOS) activity increased in the 19 CB2 and CB3 group, heat shock protein 70 (HSP70) gene expression level increased in the CB3 group, 20 while nitric oxide (NO) content was not affected by C. butyricum. After shrimp exposed to ammonia stress, 21 intestine immune biochemical parameters (T-AOC, LSZ, iNOS and NO) and genes (HSP70, Toll and Imd) 22 expression level of C. butyricum group was higher than that of the control. HE stain showed that C. butyricum increased the intestine epithelium height of L. vannamei. These results revealed that C. 23 24 butyricum could improve the growth performance, increased intestine SCFA content and body crude 25 protein content, modulated intestine digestive capacity, and enhanced intestine immune function of L. 26 vannamei against ammonia stress.

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