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Growth performance and gene expression analysis of the white shrimp (*Litopenaeus vannamei*) cultured in zero-water exchange and supplemented with *Bacillus licheniformis* and molasses

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Super-intensive shrimp farming is characterized by high stocking densities. In these systems, there is zero-water exchange, feed intake and nutrients are controlled and the accumulation of flocculated particles (bioflocs) formed by aggregates of algae, bacteria, protozoa, feces and uneaten feed is favored. Several studies have pointed out that microorganisms present in biofloc systems contribute to stimulate shrimp immune response and growth. Therefore, the aim of this study was to determine the effect of *Bacillus licheniformis* BCR 4-3 and molasses on growth performance and gene expression of white shrimp (*Litopenaeus vannamei*) cultured with zero-water exchange. The effects *B. licheniformis* BCR 4-3 and molasses on growth performance and gene expression in L. vannamei, cultured at high stocking density and zero-water exchange, were evaluated in bioassays with treatments by triplicate. Bioassay 1 (growth): 1) Control, biofloc without additives, 2) Bioflocs+molasses, 3) Bioflocs+bacilli and 4) Bioflocs+ bacilli+molasses and Bioassay 2 (gene expression): 1) Control, bioflocs without additives and 2) Bioflocs+bacilli+molasses. Survival, growth in weight, nitrogenous wastes, total suspended solids (TSS), inorganic (IM) and organic matter (OM) and the expression of crucial immune, digestive and stress-related genes were determined. Survival was higher and the growth was increased at lower stocking density. Reduction of ammonium concentration was found in treatments with bacilli and bioflocs. TSS and organic matter were either within or above the optimal range for shrimp culture. The expression of superoxide dismutase, lysozyme, HSP90, chymotrypsin and trypsin genes were down-regulated, whereas mRNA HSP70 transcription was up-regulated in treatments with molasses, bacilli and bioflocs. Zero-water exchange, B. licheniformis and molasses promotes biofloc formation that decreases ammonium but stressing culture conditions such as high stocking density, high TSS and suboptimal temperature and oxygen concentration affect shrimp growth and stress control, digestive and immune capacity.

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