

PROBIOTIC EFFECTS ON COBIA *Rachycentron Canadum* LARVAE REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Cobia (*Rachycentron canadum*) is a marine finfish with good potential for mariculture. This study evaluated the effects of probiotic *Bacillus* spp. on the performance of cobia larvae reared in recirculating aquaculture systems (RAS). Many studies have demonstrated the beneficial effects of probiotic *Bacillus* spp. in aquaculture, but there are few studies on the effects of probiotics in RAS. The trial was conducted at Virginia Tech VSAREC (USA). Larvae were stocked at a density of 15 larvae L⁻¹ into two independent RAS comprised of three tanks of 300 L each. One of the systems (PT) received the addition of commercial probiotic consisting of *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus* (10¹⁰ CFU g⁻¹) directly into the water and by live feed, according to the manufacturer, this was continued for the larviculture period of 26 days. The other system (CT) received no probiotic additions. Water quality was analysed daily by measurement of temperature, salinity, DO, pH, alkalinity, nitrate-N, nitrite-N and total ammonia-N. Water samples were also plated on culture medium for daily enumeration of total *Bacillus* and total *Vibrio*. At the end of the experiment we performed biometrics, larvae counts, stress test (salinity: 60 g L⁻¹, n = 30). Larvae were also fixed for morphometric analysis of head kidney and for immunohistochemistry of the thymus (n = 30) to evaluate the expression of complex antigens CD3 and CD4 in T-lymphocytes. The results were analyzed using the Student's T-test, with 95% significance (Software: Statistica 7.0). Survival was 14.9 ± 2.4% and 15.1 ± 0.9% for PT and CT, respectively ($P > 0.05$). The final weight was 170 ± 10 mg for PT and 160 ± 10 mg CT, ($P > 0.05$). The analysis of water microbiology had no significant differences between treatments in total counts of *Vibrio* (PT: 3366 ± 522 CFU mL⁻¹; CT: 4964 ± 902 CFU mL⁻¹) ($P > 0.05$). In the total count of *Bacillus* treatments differed statistically, with a higher concentration in PT because of the addition of *Bacillus* spp. in this group (901 ± 88 CFU mL⁻¹ and 61 ± 24 CFU mL⁻¹, $P < 0.05$). The salinity stress test was expressed by stress sensitivity index (SSI), which demonstrated a greater resistance to salt stress by larvae from the PT ($P < 0.01$). Immunohistochemical analysis showed a higher expression of CD4 in PT (PT: 43.7±6.16% CD4 cell/mm²; CT: 25.4±3.5% CD4 cell/mm², $P < 0.05$), which may indicate better immune response against pathogens. The morphometric analysis of head kidney indicates that this organ is larger in the PT (PT: 0.97±0.33mm; CT: 0.69±0.22mm, $P < 0.05$), probably due to an increase in the quantity of B-lymphocytes. These results indicate that *Bacillus* spp. probiotics used in a RAS may stimulate the immune system and increases stress resistance of cobia larvae.

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