

STANDARDIZATION OF PROTOCOL FOR *VIBRIO* CHALLENGE IN SPECIFIC PATHOGEN-FREE (SPF) SHRIMP (*LITOPENAEUS VANNAMEI*)

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This study was conducted to standardize the protocol for *Vibrio* challenge in specific pathogen-free (SPF) shrimp (*Litopenaeus vannamei*). Shrimp, from postlarvae15 to juvenile, were challenged with *Vibrio campbellii*, *V. harveyi* 642, *V. harveyi* E022, *V. harveyi* E2, and *V. penaeicida* by immersion and injection in normal as well as stress conditions. For immersion challenge, shrimp were immersed in seawater containing 10^3 , 10^5 , and 10^7 CFU mL⁻¹ of bacterial cells. They were fed with *Artemia* nauplii, *Artemia* charged with *V. campbellii*, a piece of shrimp meat which was injected with the *Vibrio campbellii* or artificial feed during five days experiment. Bacterial density in the water and shrimp samples was determined at the end of experiment. There was no significant difference in mortality between the control and *Vibrio* challenged groups in normal conditions ($p > 0.05$). Therefore, different stress factors were tested such as starvation, salinity and ammonium. 12h exposure to 40 and 50 mgL⁻¹ NH₄⁺ (pH = 7.9-8.1) were found as sublethal doses for postlarvae and juvenile shrimps respectively. Starvation, ammonium stress (before and during challenge periods), and salinity stress at 5, 10, 20 gL⁻¹ did not enhance the susceptibility of shrimp to *Vibrio* challenge by immersion.

In absence of stress, SPF shrimp is not susceptible to *Vibrio* either by immersion or injection. Only in ammonium stress, SPF shrimp is susceptible to *Vibrios* to a large extent by injection with 10^6 CFU shrimp⁻¹. *Vibrio campbellii* was found as the most virulent strain towards SPF shrimp among five tested strains.