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Supra dietary levels of vitamins C and E enhance antibody production and immune memory in juvenile milkfish, *Chanos chanos* (Forsskal) to formalin-killed *Vibrio vulnificus*

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Abstract

Juveniles of milkfish, *Chanos chanos* (Forsskal), were fed two independent supra dietary levels of vitamins C (500 and 1500 mg kg^{-1} feed, T1 and T2) and E (50 and 150 mg kg^{-1}, T3 and T4). Milkfish fed diets with supra (in addition to the vitamins present in the control diet) and normal levels (T5 containing 90 and 1.2 mg of vitamins C and E, respectively, kg^{-1} of feed) of vitamins were immunized (ip) with formalin-killed *Vibrio vulnificus* (FKVV). Priming and booster antibody responses to the injected bacterin were significantly ($P < 0.05$) better in the milkfish juveniles fed supra dietary levels. Survival response of the experimental fish fed supra dietary levels of vitamins (T1, T2 and T3) was significantly ($P < 0.01$) better than that of the control set. Protective response against virulent bacterial challenge of the vaccinated fish fed vitamin-supplemented diets (T2 and T3) was better than the control (T5) and T1 and T4. Memory factor reflecting immunological memory was superior in the fish fed vitamin-supplemented diets. Diets supplemented with either 1500 mg of Vitamin C or 50 mg of Vitamin E kg^{-1} produced the best antibody responses, final survival and protective response upon challenge. No conclusive inferences could be drawn on the growth responses from the experiment.

Keywords: Milkfish; Immune response; *V. vulnificus*; Vitamins C and E; Memory factor

1. Introduction

The biological role played by vitamins C and E is very vital for the sustained growth and health of many living organisms. These vitamins exhibit antioxidant properties that scavenge reactive oxygen species in membranes [1] and biological fluids [2]. Vitamin deficiencies in fish under aquaculture are known to produce biochemical dysfunction leading to tissue and cellular level clinical manifestations. Several morphological and functional abnormalities have been reported in various fish species deprived of vitamins. Properties of disease resistance in fish fed ascorbic acid and Vitamin E have been reported by several researchers [3–7]. Dietary vitamins were reported to have antibody

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enhancement effects in salmon [4,7]. Disease resistance and humoral antibody production in rainbow trout was directly and positively related to the levels of Vitamin C in the trout diet [8]. Interaction between these vitamins is also known to influence the beneficial effects they induce in cultured fish. Vitamin C/E sparing action in channel catfish was studied to explain the variability observed in its sensitivity to Vitamin E deficiency [9]. A dose dependant protection of dietary Vitamin C against dietary deficiency of Vitamin E was demonstrated in Atlantic salmon [10].

With the persistent losses due to diseases in shrimp aquaculture, coastal aquaculture farmers in India are constantly on the look-out for sustainable aquaculture and mixed farming of fish with shrimp. Milkfish is one such fish species that is traditionally harvested from extensive paddy–fish culture systems [11]. Information generated on nutrition and disease management will not only help enhance productivity from milkfish aquaculture but make it possible to tackle the disease problems that are increasingly becoming a part of aquaculture. Private shrimp farms in India use commercial feeds with vitamins C and E supplementations. The present investigation was carried out with an aim of obtaining information on the immune response of milkfish to supra dietary vitamins C and E. This study also is aimed at obtaining the information on the protective response and immunological memory.

2. Materials and methods

2.1. Fish

Fingerlings of milkfish (0.87 ± 0.01 to 1.08 ± 0.04 g) collected from the coastal waters off north Chennai, India, were stocked in 10-tonne cement tanks supplied with filtered aerated seawater (Salinity – 32–34 ppt; DO – 6.2–7.4 ppm) for acclimatisation.

The experiments were conducted in two sets of rearing systems. Set-I was used for evaluating the effect of supra dietary levels of vitamins C and E on the growth and survival after 6 weeks of feeding. Set-II was used to immunize (priming and booster) and evaluate the efficacy of supra dietary vitamins on the antibody production and protective response.

Set-I: Fish were stocked (30 per tank) in fibre glass reinforced plastic (FRP) tanks of 0.5 tonne capacity and conditioned to experimental environment and control diet for a week. Five treatments were randomly laid out each with three replicates.

Set-II: Fish were stocked in 200-l FRP tanks. Duplicates of primed and booster sets ($5 \times 2 \times 2$) containing 12 fish in each tank were immunized and fed as stated above. All the tanks were supplied with filtered aerated seawater with more than 80% daily replenishment.

2.2. Feed preparation

Vitamin incorporated feed was prepared using locally available feed ingredients (Table 1). The ingredients such as the dry fish (*Anchovy* sp.), squid (*Loligo* sp.), mantis shrimp (*Oratosquilla nepa*), Acetes and soya cake were ground in a micropulveriser, passed through a 300-μm mesh screen and mixed with binder (Aquastab) in an electric blender. Fish oil was added into the blender and thoroughly homogenized. Feed ingredients were mixed with additional levels of stable Vitamin C (SD Fine Chemicals, India, T1 and T2 with 500 and 1500 mg kg^{-1} feed, respectively) and Vitamin E (Merck, India, T3 and T4 with 50 and 150 mg kg^{-1} feed, respectively) for the supra dietary supplementation. Control feed, as per the ingredients (Table 1), contained 90 mg of Vitamin C and 1.2 mg of Vitamin E kg^{-1} in the prepared diet. The ingredients were kneaded into a dough (to an approximate moisture level of 30%), steamed at atmospheric pressure for 5 min and pelletized (2 mm diameter) in a bench top pelletizer. The pellet was dried in a hot air oven at 40 °C for 2–3 days to a uniform moisture level of 9–10%.

Proximate composition of the feed was analysed as per the AOAC [12] methods.

2.3. Immunization

V. vulnificus, isolated from diseased wild collections of gray mullet collected from the backwaters of Muttukadu, south of Chennai, India was grown in brain–heart infusion broth (Hi Media, India) with a final salt concentration of 1.5% at 32 °C for 34 h. The bacterium was harvested by spinning the suspension at $13{,}000 \times g$ for 10 min; the process was repeated three times with sterile phosphate buffered saline (PBS, pH 7.2) as the resuspension medium. The final