

Determination of the Effect of Papaya (*Carica papaya*) Leaf Powder as a Low Cost, Immunogenic, Herbal Feed Additive on Zebrafish (*Danio rerio*)

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Fish diseases including bacterial diseases cause significant loss in aquaculture. Antibiotic treatments are expensive and lead to antimicrobial resistance. The objective of this study was to identify the potential of a low cost herb which enhance fish immunity using *Carica papaya* leaf powder as a feed additive on zebrafish infected with bacteria and determined the effect on immunity. Ninety healthy fish were divided into three groups with three replicates (30 fish per group) exposed to *Aeromonas hydrophila* in water for a week followed by feeding trial. Three groups were fed with commercial (control), antibiotic and papaya leaf incorporated fish feed with 2% of body weight per day. Differential white blood cell (WBC) count was taken to identify the effects on disease response. Antibiotic susceptibility test was performed to identify, antibiotic resistance of bacteria and antibacterial activity of aqueous papaya leaf extract. Growth parameters and growth performances of experimental fish were calculated. WBC counts were measured before exposure to bacteria, one week after infectious challenge, one week after feed trial. One way ANOVA followed Tukey Pairwise comparison test used for data analysis. Fish exposed to *A. hydrophila* showed haemorrhages on the base of pectoral fin, fin rot and body discoloration. As per the results, monocyte count was decreased and lymphocyte count was increased in all three tanks after infection but one week after receiving the antibiotic and papaya feed, lymphocyte counts were decreased significantly compared to the control, while monocyte count and neutrophil counts were increased compared to the control. Accordingly, initial increase of lymphocyte count was observed due to immune response against bacteria as they involved in antigen-antibody reactions. Lowered monocyte count was observed due to recruit them for wound healing. Lowered lymphocyte count was observed due to the reduction of pathogens in response to antibiotic and papaya feed. Neutrophils play a role in phagocytizing bacteria, increased in papaya fed fish indicated a positive immune response. The results indicated that papaya leaf incorporated feed increased the immune response of zebrafish against *A. hydrophila*, which can be used as a low cost, herbal feed additive in ornamental fish industry.

Keywords: Zebrafish; *Carica papaya*; *Aeromonas hydrophila*; Immunity; Herbal antibiotic