

**DETERMINATION OF THE EFFECT OF PAPAYA
(*CARICA PAPAYA*) LEAF POWDER AS A LOW
COST, IMMUNOGENIC, HERBAL FEED
ADDITIVE ON ZEBRAFISH (*DANIO RERIO*) AND
DETERMINING THE TOXICITY OF PAPAYA
LEAF EXTRACT ON ZEBRAFISH EMBRYO**

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by

HETTIHEWAGE ANUJI PRAMEESHA

**Department of Animal Science
Faculty of Animal Science and Export Agriculture
Uva Wellassa University**

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ABSTRACT

Determination of the effect of papaya (*Carica papaya*) leaf powder as a low cost, immunogenic, herbal feed additive on Zebrafish and determining the toxicity of papaya leaf extract on Zebrafish embryo

Fish diseases including bacterial diseases cause significant loss in aquaculture. Antibiotic treatments are expensive and lead to antimicrobial resistance. Objectives of this study were to identify the potential of a low cost herb which enhances fish immunity using Papaya (*Carica papaya*) leaf powder as a feed additive on Zebrafish (*Danio rerio*) infected with bacteria and determined the effect on immunity and to investigate the acute toxicity effect of papaya leaf extract on Zebrafish embryos. Ninety healthy fish were divided into three groups with three replicates exposed to *Aeromonas hydrophila* in water for a week and continued for a feeding trial. Three groups were fed with commercial (control), antibiotic or papaya leaf incorporated fish feed. Differential white blood cell 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 of Zebrafish were calculated. Zebrafish embryos were exposed to 200, 150, 100 and 50 mg mL⁻¹ of aqueous papaya leaf extracts. Distilled water was used as negative control while tetracycline (2 mg mL⁻¹) suspension was used as positive control. Lethality rate was recorded at 24, 48, 72 and 96 hours based on four apical observations. One way analysis of variance (ANOVA) followed Tukey Pairwise comparison test used for data analysis. WBC counts were measured before exposure to bacteria, one week after infectious challenge, and one week after feed trial. Fish exposed to *A. hydrophila* showed haemorrhages on the base of pectoral fin, fin rot and body discoloration. Elevated lymphocyte count and decreased monocyte and neutrophil counts were observed in all three groups after infection. One week after the feeding trial, lymphocyte count reduced and the neutrophil count was increased in papaya and antibiotic fed fish, while control group lymphocyte count remained high. Accordingly, initial increase of lymphocyte count was observed due to adaptive immune response which was controlled by the medicated papaya and antibiotic feeds later on. Increased neutrophil count in medicated feed indicates the positive immune response against bacteria. The results indicated that

papaya leaf incorporated feed increased the immune response of Zebrafish against *A. hydrophila*. Median lethal concentration (LC₅₀) of papaya leaf extract after 96 hours was around 257 mg mL⁻¹ which was higher than the tested concentrations. It was observed an increasing trend of mortality rate along with the concentrations. However, the results confirmed that papaya leaf extract below 100 mg mL⁻¹ has low toxicity effect on Zebrafish embryo compared to the tetracycline suspension.