

2.2 Experimental design

A study was performed to assess the effects of five dietary immunostimulants, namely vitamin C, vitamin E, chitin, chitosan, and levamisole, on the growth performance, morphometric characteristics, and haematological responses of *C. catla*. The experiment was carried out with four distinct groups as follows:

Group 1 (Control): The first group of fish was fed GrowFin fish feed without any immunostimulants and is referred to as the control group.

Group 2 (Immunostimulant Fed): The second cohort of fish was subsequently partitioned into five experimental sub-groups, each receiving a distinct diet supplemented with a particular immunostimulant.

Group 3 (*A. hydrophila* infected): In the third group, the fish were fed a control diet and infected with a concentrated *A. hydrophila* culture at a dosage of 200 µL/L (10⁶ CFU/mL).

Group 4 (*A. hydrophila* infected + Immunostimulant Fed/ Treated): In the fourth treatment group, the fish were further divided into five experimental sub-groups. Each group was infected with *A. hydrophila* like third group and fed independently with five selected immunostimulant mixed feeds.

In each experimental group ($n=10$), the fish were administered 3 gm of feed at 12-hour intervals. The water temperature and pH were calibrated at 25°C and 5.6, respectively. The photoperiod is established at 12 hours of light and 12 hours of darkness. Aerators were affixed to tubs to enhance aeration. Every day, half of the water in the tub was replaced with fresh, dechlorinated tap water, and the fish were maintained for one month in all the experimental groups. Following 30 days of exposure, fish were sacrificed with the objective of conducting growth performance, morphometric, and haematological assessments.

2.3 Measurements of growth performance

The initial measurement of length, weight, and other morphometric characteristics of all fish groups were done at the beginning of the experiment. Then the fish in all the treatment groups were allowed to grow for one month. Subsequently, the fish were sacrificed, and all growth parameters were recorded to determine their growth performance. The growth performance was assessed by measuring the total biomass gain (TBG), weight gain (WG), and feed conversion ratio (FCR) using the following formulas.

$$\text{Total biomass gain (Kg/m}^3\text{)} = \frac{\text{Final biomass}-\text{Initial biomass}}{\text{Water volume (m}^3\text{)}}$$

$$\text{Weight gain(g)} = \text{Final weight (g)} - \text{Initial weight(g)}$$

$$\text{Feed Conversion Ratio} = \frac{\text{Consumed feed (g)}}{\text{Weight gain (g)}}$$

2.4 Measurements of morphometric parameters

Morphometrics refers to the quantitative measurements of the dimension such as length, width, and height of the body or specific body portions of a fish. Morphometric measurements were obtained from fish in all the treatment groups at the beginning and end of the experiment. The morphometric characters were measured encompassing 14 specific traits including Body Length (BL), Body Width (BW), Body Depth (BD), Weight (W), Head Length (HL), Head Depth (HD), Eye Diameter (ED), Pupil Diameter (PD), Caudal Peduncle Length (CPL), Caudal Peduncle Depth (CPD), Caudal Fin Length (CFL), Dorsal Fin Length (DFL), Pectoral Fin Length (PcFL), Pelvic Fin Length (PIFN), and Anal Fin Length (Haryono, 2001).

2.5 Estimation of haematological parameters

2.5.1 Collection of blood

Following the end of the experiment, blood samples were obtained from the fish in all of the experimental groups. The blood sample was collected from the caudal vein, located just beyond the anal fin, by injecting a disposable