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Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings

Swagatika Sahu, Basanta Kumar Das*, Jyotirmayee Pradhan, B.C. Mohapatra, B.K. Mishra, Niranjan Sarangi

Aquatic Animal Health Division, Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-2, Orissa 751002, India

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Abstract

The study evaluated the efficacy of dietary doses of *Magnifera indica* (mango) kernel on the immune response and disease resistance of *Labeo rohita* fingerlings against the bacterial pathogen *Aeromonas hydrophila* infections in. *L. rohita* fingerlings fed diet containing 0 (Control), 1 g, 5 g, 10 g mango kernel kg^{-1} dry diet for 60 days. Biochemical (serum total protein, albumin, globulin, albumin:globulin ratio, blood glucose), haematological (WBC, RBC, haemoglobin content) and immunological (superoxide anion production, lysozyme, serum bactericidal activity) parameters of fish were examined at 20, 40 and 60 days of feeding. Fish were challenged with *A. hydrophila* 60 days post feeding and mortalities were recorded over 10 days post-infection. The results demonstrate that fish fed with mango kernel showed enhanced superoxide anion production, lysozyme, serum bactericidal, serum protein, albumin ($P < 0.05$) compared with the control group. The mortality (%) was recorded up to 10th day post-challenge. Less survivability was observed in control group (50%) up to day 10 after infection. The survivability was higher in experimental diets. The group fed 5 g kernel kg^{-1} dry diet showed highest percentage survival (98%). These results indicate that mango kernel stimulates the immunity and makes *L. rohita* more resistant to *A. hydrophila* infection.

Keywords: *Magnifera indica*; *Labeo rohita*; *Aeromonas hydrophila*; Immune parameters

1. Introduction

The use of antibiotics and chemotherapeutics for prophylaxis and treatment in intensive aquaculture has been widely criticized for its negative impact [1] and research on interactions between immunity, growth and development of eco-friendly alternatives to antibiotics that may keep fish healthy such as probiotics and plant based immunostimulants has increased, Indigenous technological knowledge for treating diseases is receiving attention in fish health and disease management.

* Corresponding author. Tel.: +91 0674 2465 446x228/235; fax: +91 0674 2465 407.
E-mail addresses: basantadas@yahoo.com, dasb@marlab.ac.uk (B.K. Das).

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Mangifera indica L., which belongs to the Anacardiaceae family, is a large evergreen tree of tropical and subtropical regions. Different parts of the mango have a broad range of medicinal properties, such as antimicrobial [2,3], antiviral, antifungal [4], anti-inflammatory [5], anti-diarrhoeal [6], antioxidant activity [7,8], antitumor [9], as well as immunomodulatory [10].

Mango kernel which is the cheapest and useless material has been investigated for the first time in aquaculture sector for its immunostimulatory effects. Huge amounts of mango kernel which is not presently utilized can be utilized for feed to enhance the non-specific immunity in fish. The mango kernel is also used as a non-conventional feed source for growth and feed utilization of *Oreochromis niloticus* fingerling [11], broiler chicken [12] and rat [13].

The study was undertaken to evaluate the effects of mango kernel on immunological, serum biochemical and blood parameters of rohu (*Labeo rohita*), which is considered to be the major aqua-crop species contributing about 40% of the total freshwater aquaculture production in India [14]. Hence an approach is made by using an appropriate method of application relevant to farm conditions.

2. Materials and methods

2.1. Fish and management

Fingerlings of rohu (average wt. 10 ± 2 g) obtained from the Central Institute of Freshwater Aquaculture fish farm, Kausalyaganga, Bhubaneswar, India. The fish were transported in a circular container (500 L) with sufficient aeration to the wet laboratory. They were carefully transferred to another circular tank (1000 L) and left undisturbed overnight. In order to ameliorate the handling stress, the fish were given a mild salt treatment (3% NaCl) the next day. The stock was acclimatized under aerated conditions for a period of 15 days at 27–28 °C. Fish were fed with a formulated pelleted diet at 3% body weight once daily during the acclimatization period.

2.2. Mangifera indica

Mangifera indica kernel was collected from a local village and washed. It was dried in an oven at 60 °C, powdered by mortor and pestle and sieved. The powder was then incorporated into fish feed at a rate of 0, 1 g, 5 g and 10 g per kg of feed to prepare different experimental fish diet. The mango kernel free feed was used as a control diet.

2.3. Culture of pathogens

Aeromonas hydrophila (ATCC 49040) and *Micrococcus luteus* ATCC 49732 (DIFCO, BBL-Qualis lab) were cultured in nutrient broth (Himedia) for 24 h at 37 °C. The cultures were centrifuged at $3000 \times g$ for 10 min. The supernatants were discarded and the pellets were resuspended in phosphate buffered saline (PBS, pH 7.4) and the OD of the solution was adjusted to 0.5 at 456 nm which corresponded to 1×10^7 cells/ml. These bacterial suspensions were serially diluted with PBS and used for the challenge experiment, lysozyme and bactericidal activity.

2.4. Preparation of herbal diets

Three experimental diets were prepared by incorporating mango kernel powder at 1 g, 5 g and 10 g kg^{-1} feed (Table 1). First, dry ingredients were mixed thoroughly and 1% binder was added. Water was added and mixed thoroughly in a mixer for 20 min. The resulting dough was pelleted, dried at room temperature for 48 h and then stored in airtight containers until fed.

2.5. Experimental design and feeding diet

Rohu fingerlings ($n = 240$) were selected for the study and divided randomly into 4 groups (A, B, C and D) with 60 fish in each group, maintained in 8 tanks (500 L capacity) each containing 30 fish. Group A was fed with basal diet and acted as the control. The remaining groups were fed with mango kernel at 1 g kg^{-1} of feed (Group B), 5 g kg^{-1} of feed (Group C) and 10 g kg^{-1} of feed (Group D) for 60 days. Blood and serum samples were collected from fish in each group and examined for the following parameters such as total protein, albumin, globulin, albumin globulin ratio,