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Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*

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ABSTRACT

We investigated effects of dietary administration of stinging nettle (*Urtica dioica*) on growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) against *Aeromonas hydrophila*. Fish were divided into 4 groups and fed for 4 and 16 weeks with 0%, 1%, 2% and 5% of *U. dioica* incorporated into the diet. Use of *U. dioica* in the diet resulted in improved biochemical, hematological and immunological parameters. Among the biochemical parameters; plasma cortisol, glucose, triglyceride and cholesterol decreased while total protein and albumin in fish increased with increasing dietary inclusion of *U. dioica*. Among the haematology parameters: red blood cell (RBC), white blood cell (WBC) counts, haematocrit (Htc), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and netrophiles increased with increasing dietary inclusion levels of *U. dioica*, some depending on the fish age. Serum immunoglobulins, lysozyme activity and respiratory burst were the main immunological parameters in the adult and juvenile *L. victorianus* measured and they all increased with increasing herbal inclusion of *U. dioica* in the diet. Dietary incorporation of *U. dioica* at 5% showed significantly higher relative percentage survival (up to 95%) against *A. hydrophila*. The current results demonstrate that using *U. dioica* can stimulate fish immunity and make *L. victorianus* more resistant to bacterial infection (*A. hydrophila*).

1. Introduction

Due to increasing demands for more proteins, aquaculture the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants has increased worldwide between 1970 and 2010 at approximately 7% per annum [1]. Responding to the increased demand for fish, many previously riverine fish species are now being cultured. For instance, the riverine potamodromic Labeo Victoria (*Labeo victorianus* Boulenger 1901), which is endemic to Lake Victoria basin [2] is now being cultured at intensive levels [3,4] and therefore the health management of the cultured species is critical for the sustainable growth of the industry. During intensive fish culture, a variety of microbial agents (viruses, bacteria, fungi and parasites etc.) cause diseases in aquaculture system.

Bacterial infections are responsible for most of the mortality in cultured fishes throughout the world. *Aeromonas hydrophila*, a widespread, opportunistic pathogen, is a facultative anaerobic, chemo-organotrophic gram-negative microorganisms causing motile aeromonas septicemia [5]. Motile aeromonas septicemia, caused by various strains of *A. hydrophila,* is one of the most common and challenging diseases, causing high mortality in freshwater fishes in the aquaculture units [6]. Antibiotics have been extensively used as growth and immunity enhancer and treatment of bacterial diseases in fish. However, the application of antibiotics and other chemotherapeutics has negative aspects such as risk of

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creating resistant pathogens, problems of antibiotic residues accumulating in treated fish, and unfavorable impact on the environment [7–9]. Therefore, the demand for replacement of natural products has been rising with a focus on plant products as alternative to antibiotics. In this regard, several plants herbs and their extracts such as: *Nyctanthes arbortristis* in *Oreochromis mossambicus* [10]; *Origanum heracleoticum* in *Ictalurus punctutus* [11]; *Lactuca indica* in *Epinephelus bruneus* [12]; *Astragalus radix* and *Ganoderma lucidum* in *Cyprinus carpio* [13]; *Allium sativum* in *Labeo rohita* [14]; *Prunella vulgaris* in *Paralichthys olivaceus* [15]; *Sauropus androynus* in *Epinephelus coioides* [16]; *Azadiracta indica* on *Oreochromis niloticus* [17] among others have elicited immunological responses in fish.

The stinging neetle, *U. dioica* (nettle; Urticaceae family) is a native herb in Europe, Asia, North America, as well as in the northern and Eastern Africa [18]. It is an herbaceous perennial plants and chemical composition shows the presence of iron, vitamin A, vitamin B, vitamin B12, acetylcholine, histamine, serotonine, formic acid, salicylic acid, lecithin, carotenoids, flavonoids, sterols and thymol [19–21]. It possesses marked immunostimulation, anticarcinogenic, anti-inflammatory, antioxidant, anti-analgesic, antiulcer, antiviral, antibacterial and antifungal activities [20–23]. Studies conducted in juvenile and adult fish with the extract of *U. dioica* are scanty and scattered. Few researches have reported the beneficial effects of dietary nettle on rainbow trout [24,25]. There are also very few reports on the effects of dietary supplementation and administration of stinging nettles in biochemical, haematology and immunology in fish (e.g. [26], more particularly in bacteria challenged fish. More studies are thus needed therefore to fulfill this gap. Therefore, the objectives of the present study was to evaluate the effects of different dietary inclusion levels of *U. dioica* on the *L. victorianus* juvenile and adults concerning their growth performance, haematology and biochemical profile as well as on the immune response following experimental challenge with *A. hydrophila*.

2. Materials and methods

2.1. Fish culture and laboratory setup

The experiment was carried out under controlled hatchery conditions at Mwea Fish Farm, Kenya. Mature *L. victorianus* were collected from River Mara in Kenya during the breeding season and reared at Mwea Fish Farm broodstock tanks. Three mature female broodstock (mean weight = 3402 ± 5.5 g) and two mature males (mean weight = 470 ± 11.1 g) were netted from the tanks and transferred to the hatchery. Larvae were obtained through induced breeding and semi-natural spawning. Initially about 2500 larvae were hatched. During the culture period, the larvae were fed *Artemia nauplii*. The larvae were cultured for a period of 21 days to an initial mean weight of 25.0 ± 2.2 g in a flow-through raceway-type 2500−L open water tanks, supplied with filtered dechlorinated tap water at a rate of approximately 50 L h^{-1}. The water was continuously aerated, and temperature controlled thermostatically at 26.0 ± 1.5 °C. Before the beginning of the experiment, fish were fed a commercial extruded tilapia feed (Raanan Fish Feed Co., Israel: crude protein 280 g kg^{-1}; crude lipid 56 g kg^{-1}; crude fibre 61 g kg^{-1}; ash 62 g kg^{-1}; NFE, 541 g kg^{-1}). After acclimation, juveniles were netted from the nursery ponds and transferred to 12 fiberglass open flow-through tanks of capacity 500 L containing tap water that had been stocked in an intermediate holding tank for at least 48 h to remove chlorine and well aerated by means of electric pump with air stone diffusers. The water salinity of the tank as determined by salinometer (Model IC/SB-1 Salinity Cell) was 0.3 psu; $NO_2^- < 0.06$ mg L^{-1}; $NO_3 < 0.01$ mg L^{-1}; $NH_3 < 0.02$ mg L^{-1}; pH 7.2). The fish were held in the tanks for a period of six days before the start of the experiment. The health of the juvenile fish (changes in behavior and physical appearance) was checked before starting the experiment. No mortality was reported during this initial acclimation period. Fish were held under natural light, with a photoperiod regime of 12-h light and 12-h dark (0°34′13.8″N and 35°18′49.8″E) at a constant temperature 26.5 ± 0.5 °C maintained using thermostat heaters. The flow-rate was constantly regulated at about 20 L h^{-1} to maintain dissolved oxygen above 80% of the saturation level. Testing conditions included 500 fish (1 fish L^{-1}) (to simulate intensive culture conditions), with each formulated diet being experimentally tested in triplicate. The fish were then cultured for 16 weeks (when the fish reach table size and thus assumed to be adult) under experimental diet. During the culture period fish were fed 3–4% body weight twice on daily basis.

2.2. Plant materials and preparation

Fresh leaves of *U. dioica* were procured near Karatina Town, in Kenya. Authentication and identification of the plant was carried out at the Department of Biological Science, Karatina University. The plants were cleaned and cut into small pieces, and then air dried. The dried samples were then pulverized into fine powder in a grinder, which was then stored at −4 °C until use.

2.3. Culture of bacterial pathogens

A virulent strain of *A. hydrophila* (B2/12) was obtained from the Department of Microbiology, University of Eldoret, cultured in nutrient broth (composition Peptic digest of animal tissues: 5 g L^{-1}, Beef extract: 2 g L^{-1}, Yeast extract: 2 g L^{-1}) for 24 h at 37 °C. The bacterial culture was centrifuged at 3000 *g* for 10 min. The supernatant was discarded and the pellets were resuspended in phosphate buffered saline (PBS, pH 7.4). The optical density (OD) of the bacterial suspension was adjusted to 0.5 at 456 nm corresponding to 1×10^7 and kept in the water bath at 60 °C for 2 h. This bacterial suspension was used for the challenge test. Sterility was confirmed by lack of growth on the nutrient agar.

2.4. Diet formulation

Four experimental diets were formulated to contain approximately 30% crude protein, 7.8% crude lipid, 42.3–42.8% NFE and 17.3 MJ kg^{-1} as shown in Table 1. The diet without *U. dioica* was used as the control group. The *U. dioica* was added to the test diets at levels of 10, 20 and 50 g (kg $diet^{-1}$) with a corresponding decrease in the amount of cellulose. Dietary ingredients were ground and passed through a 0.05−mm mesh sieve and homogenized for 3 min in a blender (Hobart M-600; Hobart Corp., Troy, OH, USA). Simon-Heese pelleting machine (Boxtel, The Netherlands) was used to pelletize the wet mixture after addition of cassava as binders. The 500−μm pellets obtained were dried in a forced-air oven at 45 °C for 4 h. The pellets were packed in plastic bags and refrigerated at −4 °C until use.

2.5. Blood sampling

At week four and week 16, 10 fish from each tank were randomly captured with dip nets and quickly anesthetized with benzocaine (5 mg L^{-1}) for 2–3 min. Blood was withdrawn from the caudal vein of each sampled fish into 2 mL sterilized hypo-dermal syringe. One half of each blood sample was transferred to microtube containing heparin anti-coagulant and immediately used for respiratory burst assay and hematological examination, while the other half was transferred to non-heparinized micro tube, placed at room