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Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton)

Kusunur Ahamed Basha, Ram Prakash Raman*, Kurcheti Pani Prasad, Kundan Kumar, Ezhil Nilavan, Saurav Kumar

Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Mumbai 400 061, India

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ABSTRACT

The present study evaluated the effect of dietary andrographolide (EC 50%) on growth, non-specific immune parameters and disease resistance against *Aeromonas hydrophila* infection in Indian major carp, *Labeo rohita* fingerlings. Fishes were fed with formulated diet containing andrographolide as T0 (0.00%), T1 (0.05%), T2 (0.10%), T3 (0.20%), T4 (0.40%) and T5 (0.80%) for 42 days. Fishes were challenged with *A. hydrophila* 42 days post feeding and relative percentage survival (RPS) was recorded over 14 days post challenge. Blood and serum samples were collected for nonspecific immune parameters on 14, 28 and 42 days of feeding and growth performance was evaluated at the end of experiment. The results revealed that fishes fed with andrographolide showed significant ($p < 0.05$) increase in NBT levels, myeloperoxidase activity, phagocytic activity, serum lysozyme activity, and serum antiprotease activity when compared to the control group. The weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio of fishes fed with andrographolide were found to be significantly ($p < 0.05$) differed compared with control. Dietary andrographolide at the level of 0.10% showed significantly ($P < 0.05$) higher RPS (74.06%) against *A. hydrophila* infection than control. The results revealed that andrographolide supplemented diet has a stimulatory effect on non-specific immune parameters along with improved growth performance and increased disease resistance against *A. hydrophila* infection in *L. rohita* fingerlings.

1. Introduction

Aquaculture fish production increased significantly over the past few decades necessitating intensive fish culture practices. Since intensification is one of the prime necessities to cope with the present and future demand and hence, increase in productivity per unit space is performed by increasing the rearing density. Intensification leads to a number of other associated stressors like overcrowding, transport, handling, grading and poor water quality tend to adversely affect the health of the cultured fish [1]. These conditions produce poor physiological environment increasing the susceptibility of fish to infectious agents paving the way for the outbreak of a number of diseases due to an increasing range of pathogens. The disease outbreaks are increasingly being recognized as a potential constraint on aquaculture production trade, and cause massive economical loss through mortality or inferior meat quality, resulting in reduced profit margins [2]. Bacterial diseases have been reported to be a principal limiting factor in both wild and cultured fishes [3]. The most common and frequently encountered bacterial pathogen in India is *Aeromonas hydrophila* which causes severe damage to carp production [4]. *A. hydrophila* is associated with wide range of freshwater fishes. It is an important pathogen in causing stress related diseases in fish with the common symptoms of ulceration, exophthalmia and abdominal distension [5–7]. However, a potent drug and effective vaccine development against *A. hydrophila* continues to be a challenge to fish pathologists [8]. The most common approach to treat bacterial diseases is the application of antibiotics. However, accumulation of chemicals and antibiotics in the environment and in the fish flesh have led to the imposition of stringent regulations that limit the use of antibiotics [9]. A crucial approach in disease management should be based on application of preventive measures and one of the promising

* Corresponding author. Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Mumbai 400 061, India. Tel.: +91 9594073424; fax: +91 2226361573.
E-mail addresses: rpraman1@gmail.com, rpraman1@rediffmail.com (R.P. Raman).

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alternative or tool in fish disease management that have been evolved in response to these problems is "immunostimulants" which has overcome the lacuna of vaccines and probiotics [10].

An immunostimulant is a chemical, drug or action that enhances the defence mechanisms or immune response [11], thus rendering the animal more resistant to diseases. Non-specific defence mechanism plays an important role at all stages of fish infection. Fish particularly depend mostly on these non-specific mechanisms than do mammals [12]. Phytotherapy is the oldest form of health care known to man-kind. Bioactive substances present in herbs are well-known to have an antimicrobial and immunomodulatory properties. Herbs are an interesting alternative because they are inexpensive, renewable, locally available, user friendly and can be easily prepared [13]. Recently, there has been an increasing interest in the modulation of the non-specific immune system of fish, as a prophylactic measure against disease. Many of the medicinal plants such as *Ocimum sanctum* [14], *Acalypha indica*, *Phyllanthus niruri*, *Azadirachta indica*, *Piper betle*, *Mentha piperita* [15], *Allium sativum* [16], *Astragalus membranaceus*, *Lonicera japonica* [17] and *Withania somnifera* [18] have been shown to trigger innate immune system and enhance disease resistance against pathogenic organisms. An extensive work on the use of immunostimulatory herbs in fish was conducted by various researchers and they opined that the herbal extracts can be used in fish culture as an alternate to the chemotherapeutic agents [19–22].

Andrographis paniculata (AP) also called Kalmegh or "King of Bitters" belongs to family Acanthaceae, native to India and Sri Lanka. Mostly the leaves and roots are used for medicinal purposes. Diterpenoid lactone andrographolide ($C_{20}H_{30}O_5$, melting point 230–239 °C) is the principle medicinal compound found in *A. paniculata*, which is mainly concentrated in leaves and can be easily isolated from the crude plant extracts as crystalline solid [23]. The various bioactivities of andrographolide such as hepatoprotective against various inducers [24], antimicrobial [25], antioxidant [26], antidiabetic [27], anti-inflammatory [28,29], anticancer [30], antitumour [31] and immunomodulator [32] properties have been evaluated. The ethyl alcohol extract and purified diterpene andrographolides are reported to stimulate both specific and non-specific immune responses in mice [33]. In the recent years herbs based growth promoters, immunostimulants and therapeutics which are ecofriendly are given more importance in aquaculture system. Thus keeping these aspects in view, the present study was undertaken to investigate the effect of andrographolide on the growth, non–specific immune parameters and disease resistance against *Aeromonas hydrophila* in Indian major carp *Labeo rohita*.

2. Materials and methods

2.1. Experimental animal and regime

Labeo rohita fingerlings with an average weight of 9.50 ± 0.50 g were procured from Malad Fish Farm, Maharashtra, India. The fishes were acclimatized for 15 days in laboratory condition in 1000 L FRP tanks at 25–27 °C, under continuous aeration. The fishes were fed with control pelleted diet at the rate of 3% of body weight twice a day.

2.2. Preparation of experimental diet

The andrographolide (EC 50%) was procured from Neocare Naturals Limited, Hyderabad, India and used for preparation of six isonitrogenous experimental diet following the method of Rao et al. [34] with slight modification. Experimental diets were prepared by mixing all the ingredients in required quantity along with water to form dough and calculated amount of oil was added to the dough before cooking. Further, completely cooled dough was mixed with vitamins, mineral mixture and six of the experimental diets contained with andrographolide in different concentrations T0 (0.0%), T1 (0.05%), T2 (0.10%), T3 (0.20%), T4 (0.40%) and T5 (0.80%) as listed in Table 1. The dough was pressed through a hand pelletizer to get uniform sized pellets. These pellets were dried at 40 °C for 12 h. After drying, pellets were packed in polythene bags, sealed airtight and labelled according to the different concentrations of treatments, for further use. The feed was having (34.89–36.66%) of crude protein, (404.3–408.04 kcal 100 g^{-1}) of isocaloric, (9.40–10.8%) of lipid content, (25.05–26.14%) of carbohydrate and (3.01–3.58%) of ash content.

2.3. Experimental design

One hundred and eighty *L. rohita* fingerlings were equally and randomly distributed in six experimental groups in triplicate following a completely randomized design (CRD). The experimental trial for effects of andrographolide on growth, non-specific immune parameters in *L. rohita* was conducted with feeding the various level of andrographolide as control T0 (without andrographolide), T1 (0.05%), T2 (0.10%), T3 (0.20%), T4 (0.40%) and T5 (0.80%) for a period of 42 days. The fish were fed with the experimental diet at the rate of 3% of body weight twice a day at 09:00 and 17:00 h to approximate satiation for 42 days. The physicochemical parameters of water such as temperature (24.3–25.8 °C), pH (7.2 ± 0.4), dissolved oxygen (6.6–6.9 mg L^{-1}), ammonia (0.01 ± 0.005 mg L^{-1}), nitrate (0.02–0.06 mg L^{-1}) and nitrite (0.001–0.004 mg L^{-1}) were maintained in optimum condition during the experimental period. Six fish were sampled from each treatment group on 14th, 28th and 42nd day and blood was drawn for different immunological assays. After 42 days, nine fishes from each treatment were segregated for challenge study particular injection with *A. hydrophila* and post challenge study was continued up to 56 days. At the end of 56 days, growth parameters and relative percentage survival rate were estimated for disease resistance against *A. hydrophila*.

2.4. Collection of blood and serum

Each fish was anesthetized with clove oil (Merck, Germany) at 50 μl per litre of water before collecting blood samples from fish. Blood was drawn from caudal vein of fish by using 1.0 ml hypodermal syringe and 24 gauge needles, which was rinsed with 2.7% EDTA solution before use. The collected blood was immediately transferred to the test tube coated with thin layer of EDTA (as an anticoagulant) and shaked well in order to prevent clotting of blood. Serum was collected without using anticoagulant and was

Table 1
Composition of experimental diets (in 100 g).

Ingredients (g)	T0 (control)	T1	T2	T3	T4	T5
Fish meal	25	25	25	25	25	25
Soya flour	20	20	20	20	20	20
Corn flour	12	12	12	12	12	12
Wheat flour	15	15	15	15	15	15
Rice bran	10	10	10	10	10	10
GOC	12	11.9	11.8	11.6	11.2	10.4
CMC	1	1	1	1	1	1
Veg. oil	4	4	4	4	4	4
Vit & mineral[a]	1	1	1	1	1	1
Andrographolide (EC 50%)	–	0.1	0.2	0.4	0.8	1.6

[a] Composition of vitamin–mineral mix (Agrimin) (quantity kg^{-1}), Vitamin A – 6,25,000 IU; Vitamin D3 – 62,500 IU; Vitamin E – 250 mg; Nicotinamide – 1 g; Cu – 312 mg; Co – 45 mg; Mg – 6 g; Fe – 1.5 g; Zn – 2.13 g; I – 156 mg; Se – 10 mg; Mn – 1.2 g; Ca – 247.34 g; P – 114.68 g; S – 12.2 g; Na – 5.8 mg; K – 48.05 mg.