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Research Article

Growth performance and immune response of *Labeo rohita* under the dietary supplementation of black seed (*Nigella sativa*)

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Abstract

Black seed (*Nigella sativa*) is used as a natural immuno-stimulant in the feed of various farmed animals. In this study, the effect of three dietary inclusion levels of *N. sativa* was assessed on growth performance and immune response of *Labeo rohita* for 8 weeks. Growth parameters in terms of weight (g), length (cm), feed conversion ratio and specific growth rate (SGR) along with immunity response parameters including white blood cells, lysozyme activity, and globulins protein were measured in this study. All the recorded growth and immunity parameters showed a significantly different outcome at the end of the experimental trial. The group fed by 4% black seed showed the highest increase in weight gain (14.20±0.621 g) increase in length (2.26±0.11 cm) specific growth rate (1.34±0.45) and best value of feed conversion ratio (2.27±0.56) followed by 3% and 2%. Among the immunity parameters, group of fish fed by 4% black seed in the diet showed an increase in white blood cell count (198 10^3/µL ±3.78), globulin protein (23.66 mg/dl ±1.21) and lysozyme activity (60.66 µl/ml ±1.20) followed by 3% and 2%. Inferences of this study reveal that *N. sativa* improves the growth and immunity of *L. rohita*.

Keywords: Globulin; Length; Lysozyme activity; Weight; White blood cells

Introduction

The aquaculture industry has very commercial significance as it is a great source of earning that contribute into the economy of the country along with providing the essential nutrients to human. However, the growth and weight are the main points of interest [1] for which a perfect diet supplemented with all the essential nutrients is mandatory to give maximum growth and weight to fish [2]. Feed conversion ratio is primarily required to maximize fish growth and weight gain [3]. To increase economically viable production without affecting the growth and overall health of fish, proper herbal feed supplementation is a prerequisite to endure environmental fluctuations [4]. Using a feed supplement for the improvement of fish growth and health has attained widespread interest and appreciation [5].

Immuno-stimulants increase both the specific and non-specific immunity by enhancing the resistance to disease [6]. A lot of ways are used by these immuno-stimulants to enhance non-specific immunity parameters by increasing

complement and Immunoglobulin M (IgM) levels, the activity of natural killer, and phagocytic activity. The immuno-stimulants directly involved in the initiation of the innate immune system that acts on receptors and starts the activation of intracellular genes which result in the formation of antimicrobial particles [7]. The use of plants and their extracts is becoming more popular than chemical products. Therapeutic plants have been utilized in customary frameworks to treat numerous infections [8]. Biologically active substances of plant sources are a part of animal feed that effects the feed conversion ratio, growth activity of digestive enzymes, and immune system [9]. Black seed (*N. sativa*) is a member of family *Ranunculaceae*, which showed fungicidal and anti-bacterial effects in animals [10]. *N. sativa* has been used for improvement in growth and immune mechanism of several species of fish [11, 12]. The oil and the derivatives of black cumin has a significant role as antioxidant, anti-inflammatory, anticancer, analgesic and antimicrobial activities [13].

Among Indian major carps, *L. rohita* consider a good basis of protein and has beneficial status as commercial fish [14]. *L. rohita* is mostly used in polyculture [15]. It has maximum acceptability as a source of food for purchasers due to its good flesh and taste. It has maximum market need in term of food in Asia [16]. Because of the above reasons, we select *L. rohita* for research to achieve better outcomes in terms of per capita production.

In research, we use *N. sativa* in feed to check its effect on the growth and immunity of *L. rohita.*

Materials and methods

Experimental design

A total of 120 fishes of *L. rohita* (Rohu) having initial weight 15.48±0.11g (mean ± SD) were divided into four triplicate groups (each replicate contained 10 fish). Before the start of the trial, *L. rohita* fingerlings in glass aquarium were acclimatized to laboratory conditions for one week in tanks. During this period, fish were fed by basal feed. Aquaria were provided by aerators to supply proper oxygen. DO and water temperatures were checked daily. pH was measured by electronic pH meter weekly.

Feed preparation

Basal fish feed constituents contained soya bean meal, fish meal, wheat flour, rice polish, minerals, vitamins and sunflower oil. All the ingredients were grounded in mortar and pestle to get in the powder form. Black seed was obtained from the local market and dried it for 3 days and then ground by pestle and mortar in a powder form (Table 1) The experimental feed was prepared by adding black seed powder in a basal diet in the proportion of 2%, 3% and 4%. The control group feed was without black seed. Feed was given a rate of 3% body weight of fish for eight weeks of the experiment.

Table 1. Basal feed preparation ingredients

Ingredients	Weight (g)
Fish meal	45
Soya bean meal	35
Rice polish	4
Wheat flour	6
Mineral and vitamins	4
Sunflower oil	6
Total	100

Growth and immunity parameters

Fish weight (g), length (cm) Feed conversion ratio (FCR) and specific growth rate (SGR) were measured weekly. After completing the 08 weeks of the experiment, the immune parameters were determined

from blood samples obtained from the caudal vein of fish and separated into two parts. One was used for the count of white blood cells. The second part was kept overnight at 4ºC for clot formation. It was used then for serum collection. Heamocytometer was utilized for the count of WBCs. "Total protein" and "albumin" kits were used for the measurement of the levels of total proteins and albumins in serum respectively. After that by directing subtracting the values of albumin from that of total protein, the level of globulins was calculated. For the measurement of lysozyme activity of serum, a specific kit known as LZM test kit (Nanjing Jiancheng Bioengineering Institute, China) [17].

Statistical analysis

Data obtained after calculation of growth and immunological parameters were subjected to statistical analysis by using Minitab 18.0 statistical software. One-way ANOVA was used for the analysis of data. Tukey's pairwise multiple comparison tests were used to check differences among the treatments.

Results and discussion

Results of the research were indicated that black seed supplemented feed affected the growth and immunity of fish.

Growth parameters

Description of growth are shown in (Table 2). The final weight was maximum in fish that fed by diet with 4% *N. sativa* which was 29.80±0.64g. This showed that *N. sativa* containing feed has a productive effect on the growth of fish. The final length was also indicated a significant difference among treatments ($p<0.05$). The final length was showed a maximum in fish of 4% black seed group followed by 3% and then 2%.

Feed conversion ratio is increased by adding new ingredients in the feed that give better growth [3]. Feed conversion ratio also remained efficient in the group of fish treated with 4% black seed.

Table 2. Statistically demonstration at the end of trial; Average gain in wet weight (g±SD), average increase in length (cm±SD), feed conversion ratio and specific growth rate

	0% (Control)	2%	3%	4%
Initial average weight (g)	15.54±1.34	15.34±1.25	15.45±0.86	15.60±0.76
Final average weight (g)	22.28±0.64^{d}	23.86±0.56^{c}	26.86±0.67^{b}	29.80±0.64^{a}
Average Weight gain (g)	6.74±0.415 ^{d}	8.52±0.388 ^{c}	11.41±0.327 ^{b}	14.20±0.621 ^{a}
Initial average length (cm)	10.80±1.07	10.85±1.14	10.82±1.08	10.86±1.12
Final average length (cm)	11.57±0.66^{b}	12.21±1.29^{ab}	12.32±1.11^{ab}	13.12±0.57^{a}
Average gain in length (cm)	0.77±0.06 ^{d}	1.36±0.06 ^{c}	1.50±0.09 ^{b}	2.26±0.11 ^{a}
Feed conversion ratio	4.07±0.80^{a}	3.38±0.82^{ab}	3.01±1.86^{ab}	2.27±0.56^{b}
Specific growth rate	0.73±0.15^{b}	0.89±0.19^{ab}	1.12±0.50^{ab}	1.34±0.45^{a}

The specific growth rate showed significantly different values for black seed supplemented groups as compared to the control group ($p<0.05$). It revealed maximum values for a group of fish treated with 4% black seed followed by 3% then 2%. These results resembled Wafaa *et al.* [18] who estimated the effects of the extracts of green tea, propolis and black seed on the body composition, growth & the economic competence of the *O. niloticus*. Their results revealed that the weight gain (g), FCR and final body mass notably rise in the groups which were fed with the black seed.

Data were demonstrated as mean ± SD. Within the same row different superscripts, letters show statistically different results ($p<0.05$).

The gain in weight was showed a significant difference among black seed supplemented treatments ($p<0.05$). The maximum gain in weight was 14.20±0.621 in 4% black seed treated group followed by 11.41±0.327 in 3% and then 8.52±0.388 in 2%.

The final length was also indicated a significant difference among treatments ($p<0.05$). The final gain in length was showed a maximum 2.26±0.11 in fish of 4% black seed group followed by 3% and then 2%. Feed conversion ratio remained efficient in the group of fish treated with 4% black seed.

The finding has resemblance with Oz *et al*. [19] who estimated the growth performance and fatty acid composition of rainbow trout enhanced under the influence of black seed as a feed additive. The fish flourish well on feed artificially made or a productive converter of various types of foodstuff [20]. Using artificial feed in farming fish achieved good weight [2] increases fish activity and growth in limited time [21]. *N. sativa* has been used for improvement in growth and immune mechanism of several species of fish [22]. It is also recognized as the growth promotor for fish [23-25]. The response of *N. sativa* on the digestive system, showing betterment for absorption and performance, enhanced bile flow rate results in an increase of emulsification action which activated the enzymes like lipases, necessary for absorption and digestion of fat and fat-soluble vitamins [26].

Immunity parameters

Immunostimulants can increase specific and non-specific immunity by increasing the resistance for disease [27]. Oil of black seed have properties of immunostimulant and immunomodulatory [28, 29]. There is an increased of resistance in fishes for infectious disease using immunostimulants which regulate acquired immunity as well as innate immunity [30]. Fish received black seed revealed the highest increase in immunity parameters like white blood cell count, globulins proteins and lysozyme activity White blood cells are the base of the defense system in the body and play a significant role to keep the organism immune to external attacks [31].

In the present study, the average White blood cells were significantly higher in black seed supplemented groups. 4% showed higher values for white blood cell count than others (Fig. 1). The fish group supplemented by 4% black seed showed higher values for globulins than other supplemented groups (Fig. 2). The addition of plant originated immunostimulants in the diet of *L. rohita* resulted in an increased level of serum lysozymes. Black seed supplemented groups showed higher lysozyme activity as compared to control (Fig. 3).

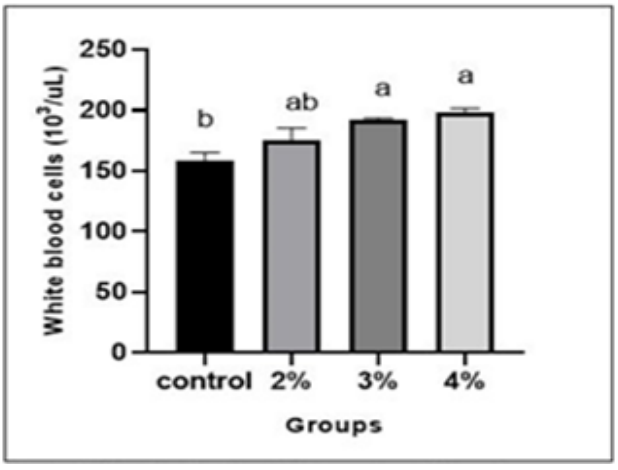

Figure 1. White blood cells of *Labeo rohita* fed by feed supplemented by 0%, 2%, 3% and 4% black sedd. Different superscript letters show statistical different results ($p< 0.05$). Bars= mean+SD

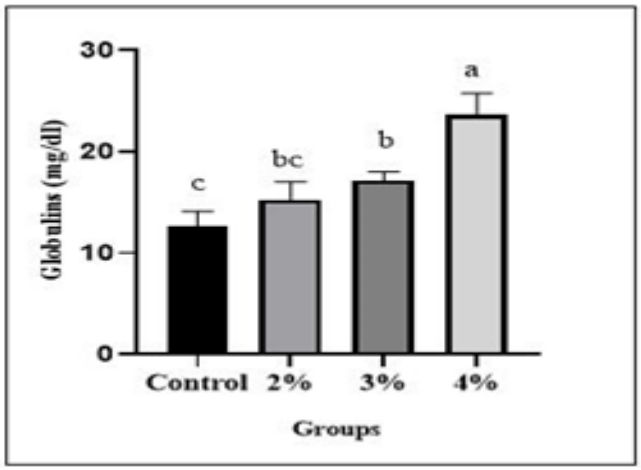

Figure 2. Globulins protein (mg/dl) of *Labeo rohita* fed by feed supplemented by 0%, 2%, 3% and 4% black sedd. Different superscript letters show statistical different results (p< 0.05). Bars= mean+SD

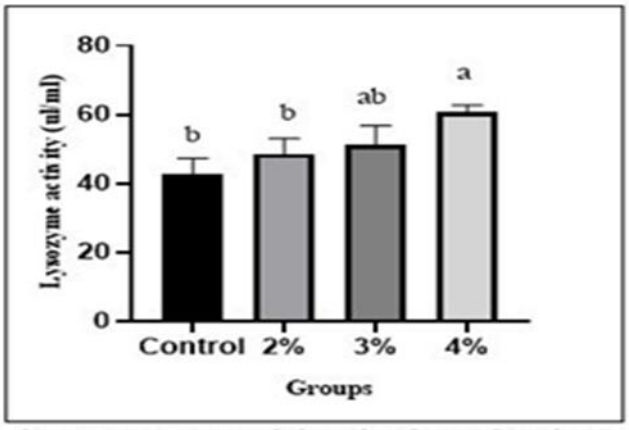

Figure 3. Lysozyme activity (ul/ml) of *Labeo rohita* fed by feed supplemented by 0%, 2%, 3% and 4% black sedd. Different superscript letters show statistical different results (p< 0.05). Bars= mean+SD.

Sahu *et al*. [32] revealed that there was present a large numbers of WBCs in *L. rohita* fingerlings which were fed by diet mixed with herbal ingredients. This increased level of WBCs indicated the improvement of the non-specific immune system of fishes. Higher values of white blood cells in fish showed that black seed has an effective result. *N. sativa* extract has a beneficial impact on leukocytes production [33]. Altinterim and Dorucu [34] investigated the fluctuations in a few specific immune parameters of *Oncorynchus mykiss* by using feed with *N. sativa* seeds. The hematological parameters including immunity parameters were improved in *N. sativa* treated groups. The same results were reported by Dorucu *et al.* [35] who concluded that the incorporation of different percentages of black seed in the basic diet of rainbow trout notably increase the immunoglobulins Lysozyme is a constituent of non-specific immune system [36]. Lysozyme count was higher in fish supplemented by 4% black seed. The current study was supported by the results of Chen et al. [37] who reported that the activity of plasma lysozyme in crucian carp was enhanced by using herbal feed additives.

Conclusion

It is observed that feed containing *N. sativa* is suitable to enhance the growth and immunity parameters of fish. The result

showed that feed having the highest percentage of *N. sativa* has productive outcomes for gaining weight and the size of fish. It is also effective for the white blood cells, globulins proteins and lysozyme activity enhancement to achieve better resistance against disease.

Authors' contributions

Conceived and designed the experiments: A Ali & J Bashir, Performed the experiment: M Naveed & A Raza, Analyzed the data: H Rehman & N Aslam, Contributed regents/materials/analysis tools: H Rehman & M Naveed, Wrote the paper A Ali & N Aslam.

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