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Research Article

Effect of *Nigella sativa* on Growth, Haematological and Serum Biochemical Profiles of Grass Carp

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AUR collected data, conducted an investigation, and drafted the manuscript, while IU reviewed and edited the manuscript. OR analyzed data; MA developed methodology; SJ validated results and proofread. AR assisted in lab and field work, HY supported statistical analysis, SZ supervised the project and approved the final version, and AI interpreted data and prepared figures/tables.

Keywords
Nigella sativa, *Ctenopharyngodon idella*, Hematology, Black seed, Hematocrit growth

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Abstract | This study investigated the effects of *Nigella sativa* (black seed) on the growth performance, haematological profiles, and serum biochemical parameters of grass carp (*Ctenopharyngodon idella*). Three groups were established: a control group fed a basal diet, and two treatment groups (T1 and T2) receiving diets supplemented with 2.5% and 3.5% *N. sativa* seed extract, respectively. Each aquarium contained ten fish with an average weight of 39.00 ± 0.50 g and length of 11.6 ± 0.5 cm. At the end of the feeding period, both treatment groups showed significantly improved weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) compared to the control group. Haematological analysis revealed enhanced white blood cell (WBC) count (T1: 9.65 ± 0.01 $\times 10^3/mm^3$; T2: 10.37 ± 0.02 $\times 10^3/mm^3$), red blood cell (RBC) count (Control: 1.35 ± 0.03 $\times 10^6/mm^3$; T2: 1.52 ± 0.04 $\times 10^6/mm^3$), haemoglobin (Hb) levels (Control: 5.95 ± 0.06 g/dl; T2: 6.45 ± 0.03 g/dl), and hematocrit (Hct) percentage (Control: 26.95 ± 0.1%; T2: 28.20 ± 0.1%). Biochemical analysis showed that fish fed *N. sativa* had reduced levels of urea (Control: 3.75 ± 0.1 mg/dl; T2: 2.65 ± 0.1 mg/dl), creatinine (Control: 0.16 ± 0.1 mg/dl; T2: 0.11 ± 0.3 mg/dl), alkaline phosphatase (ALP) (Control: 103.58 ± 0.9 U/l; T2: 87.35 ± 0.7 U/l), alanine aminotransferase (ALT) (Control: 21.8 ± 0.9 U/l; T2: 18.9 ± 0.7 U/l), and aspartate aminotransferase (AST) (Control: 27.2 ± 0.6 U/l; T2: 22.7 ± 0.3 U/l). The results suggest that *N. sativa* supplementation improves growth, boosts immunity, and enhances the health status of grass carp without adverse effects. Given its natural origin, safety, and availability in local Pakistani markets, *N. sativa* may be a cost-effective dietary supplement in grass carp aquaculture.

Novelty Statement | This study provides novel evidence that *Nigella sativa* seed extract significantly improves growth, immunity, and biochemical health in grass carp (*Ctenopharyngodon idella*). A significant improvement in key immunological parameters, such as WBC count, haemoglobin levels, and hematocrit percentage, highlights *N. sativa's* potential as a natural immune stimulant in freshwater aquaculture. Dietary inclusion of N. sativa at 3.5% led to a notable reduction in liver and kidney stress biomarkers (ALT, AST, ALP, urea, and creatinine), indicating improved organ function and overall health status in grass carp.

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Introduction

Ctenopharyngodon idella belongs to the family *Cyprinidae* and is the largest among fish, comprising about

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367 genera and 3006 species. These fish are incredibly adaptable to different freshwater environments, yet it is uncommon to encounter them in saltwater (Wang *et al*., 2024). It endures various climates and inhabits large turbid rivers, floodplain lakes, and wetlands, enduring a broad temperature range (Li *et al*., 2024). A medicinal herb belongs to the *Ranunculaceae* family and is native to the Middle East and South Asia (Kooti *et al*., 2016). Its seeds contain bioactive compounds, including thymoquinone, oleic acid, and vital amino acids, exhibiting potent antioxidant, anti-inflammatory, and antimicrobial effects (Latif *et al*., 2020). Native to Pakistan, it is widely cultivated and accessible, making it a valuable natural source for potential therapeutic interventions.

In aquaculture research, *Nigella sativa* enhances fish growth and health (Ramadan *et al*., 2024). *Nigella sativa* powder in the *Nile tilapia* diets improved growth performance, weight gain, and feed conversion ratio (Abouelezz *et al*., 2020). Thymoquinone in *Nigella sativa* has antioxidant and immunomodulatory properties (Öz *et al*., 2024). Optimal dosages and long-term effects on different species and environmental impacts are yet to be determined. However, a 4% black seed inclusion improved growth and immunity in *Labeo rohita* (Ali *et al*., 2020). Haematology studies the impact of environment and physiology on blood cells and organs (Clark and Farrell, 2011). In fish, the blood carries nutrients and oxygen crucial to various parts of the body that need them. This transportation process is critical for fish survival since they depend on these resources (Yousefi *et al*., 2021). Haematology helps understand the interaction between blood characteristics, environment, and physiological conditions (Rosidah and Pratiwy, 2022). Fish blood components, including erythrocytes, leukocytes, and haemoglobin, are essential for monitoring toxicity and health (Oyawoye and Ogunkunle, 2004). Depending on species and form, *Nigella sativa* supplementation affects fish erythrocyte counts (Bektaş *et al*., 2019). Leukocytes are crucial in disease resistance; high levels indicate better adaptability (Rosidah and Pratiwy, 2022). Supplementation increased leukocyte numbers, hematocrit, and haemoglobin levels in various fish species (Abouelezz *et al*., 2020).

Nigella sativa has shown promise in aquaculture research, enhancing fish growth and health (Elkamel and Mosaad, 2012). Black seed powder in the *Nile tilapia* diets improved growth performance and feed conversion ratio (Abouelezz *et al*., 2020). Thymoquinone in black seeds has antioxidant and immunomodulatory properties (Öz *et al*., 2024). *Nigella sativa* may be a valuable feed supplement, but more research is needed to determine optimal dosages and long-term effects on different species and environmental impacts (Li *et al*., 2024). In *Labeo rohita*, 4% *Nigella sativa* inclusion led to the highest weight gain, length increase, and a favorable feed conversion ratio (Ali *et al*., 2020). The serum biochemistry analysis provides insights into a fish's metabolic state and overall health (Srivastava and Reddy, 2020). Serum biochemical parameters like ALT, AST, ALP, creatinine, urea, glucose, total protein, and albumin indicate fish health and metabolic processes (Nourian *et al*., 2019). Elevated ALT, AST, and ALP levels may indicate liver damage, tissue damage, or bone and liver health issues (Javed *et al*., 2017). Creatinine and urea levels indicate kidney function and protein metabolism (Yousefi *et al*., 2021). *Nigella sativa* has been shown to improve immune defenses, reduce lipid peroxidation, and decrease metabolic enzymes in fish (Liang *et al*., 2019). The given research aims to investigate the effect of *Nigella sativa* on the growth, haematological, and serum biochemical profiles of grass carp.

Materials and Methods

Grass carp *(Ctenopharyngodon idella)* of 11.6cm ± 0.5 lengths, 2 months of age, and 30 in number were obtained from a fish hatchery and acclimatized in 90 L fiber glass tanks for 2 weeks (Liang *et al*., 2019). The laboratory water temperature was maintained at 27 ± 2°C, pH at 7.2-7.7, dissolved oxygen at 4.5 ± 0.5 mg/L, and monitored and adjusted ammonia and nitrite levels. Fish were fed twice daily with a basal diet and acclimatized with continuous aeration, periodic water changes, and close observation.

Basal diet

For the present study, feed as a basal diet containing commercially available feed for floating carps and other ingredients, as described in Table 1 was formulated by a hit-and-trial method with the following composition:

Table 1: Basal feed ingredients and composition.

Ingredients (in grams)	Formulation (per 1000g)
Fish meal	350 g
Soya bean meal	300 g
Rice	100 g
Wheat bran	100 g
Sunflower meal	100 g
Premix mineral and vitamins*	50 g
N. sativa powder	-
	1000 g

The premix diet obtained from the market reflected the following constituents of vitamins and minerals per kg of the feed: A:1000IU; B1:20mg; B2:20mg; B4: 600 mg; B5:100mg; B6:20mg; B9:6mg; B12:1mg; C:50mg; Co:2.5 mg; Cu: 3 mg; D3:5000IU; Dicalcium phosphate:10g; E:20mg; Fe:13mg; H:1mg; Mg:350mg; NaCl:3g; Zn:60mg.

Formulation of N. sativa supplemented diet

N. sativa (black seeds) was purchased from the local grain market and cleaned thoroughly through manual inspection, rinsing with tap water, and drying on paper for 2 days (Liang *et al*., 2019). The seeds were then ground into a fine powder using a pestle and mortar and an electric grinder, mixed with the basal diet, and pelleted for fish consumption. The prepared diet was stored in the refrigerator at an optimal temperature range. The experimental diet consisted of a mixture of powder and basal diet, with an approximate composition.

Table 2: Formulation and composition of *N. sativa* experimental fish diet.

Ingredients (in grams)	Control group diet (per 1000g)	T1 (2.5%)	T2 (3.5%)
Fish meal	350	350	350
Soya bean meal	300	200	100
Rice	100	50	50
Wheat bran	100	50	50
Sunflower meal	100	50	50
Premix minerals and vitamins	50	50	50
N. sativa powder	-	250	350
	1000 g	1000 g	1000 g

Experimental groups and feeding trial

After acclimatization, grass carp were randomly divided into three groups: Control (0% Nigella sativa extract), T1 (2.5% extract), and T2 (3.5% extract), with seven fish per group (Liang *et al*., 2019). Fish were placed in 90L aquaria with aeration, covered with a nylon net, and received twice-daily hand feeding for 8 weeks. Water was changed every 2 days, regularly removing uneaten feed and debris.

Fish sampling and data collection

Before the experiment, fish weight was recorded (Latif *et al*., 2020). After 8 weeks, fish were anesthetized and weighed again (n= 10 per group), and blood samples were collected from the caudal veins (Öz *et al*., 2023). Blood samples were divided into two sets: One with anticoagulant for haematological analysis and another without anticoagulant for serum biochemical analysis. The latter was centrifuged to obtain serum.

Growth performance parameters

The data collected on fish weight (in grams) from each aquarium was analysed to calculate key growth and feed efficiency parameters, including feed consumption (FC) per fish (g) over 8 weeks (56 days), body weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) (Latif *et al*., 2020).

Hematological parameters analysis

Hematological parameters in fish are sensitive to environmental factors (Witeska *et al*., 2022). Manual methods were used to examine fish haematology due to nucleated cells. Red blood cell parameters included RBC, Hb, Hct, MCH, MCV, and MCHC. White blood cell parameters included WBC and DLC for neutrophils, lymphocytes, eosinophils, monocytes, and basophils. A laboratory technician expert in clinical diagnostics conducted the analysis using a Neubauer Hemocytometer, Giemsa staining, cyanmethemoglobin spectrophotometry, and capillary tube micro hematocrit method (Chen *et al*., 2019).

Serum biochemical parameters analysis

Fish serum biochemistry provides insights into metabolic processes (Congleton and William, 2001). Serum enzymes like ALP, ALT, and AST are vital health indicators (Zikić *et al*., 2001). Other markers like glucose, triglycerides, total protein, creatinine, and urea are used for health assessment (Öner *et al*., 2008). Ions are crucial indicators, and their imbalances can jeopardize survival. A laboratory technician expert in clinical diagnostics conducted the analysis using manual examination procedures and commercial kits (Samanta *et al*., 2014). The examined biochemical parameters included ALT, AST, ALP, blood urea, and creatinine.

Results

The results obtained from the experimentation for various growth performance parameters are described in the above Table 2. Fish in the control group were fed a basal diet containing 0% *N. sativa*. In contrast, the treatment groups T1 and T2 were fed basal diets containing the plant extracts in a proportion of 2.5% and 3.5%, respectively. The data recorded for each group in terms of length and weight at the beginning and end of the experimental period was computed and analysed according to the formulas discussed in for weight gain, the initial weight of all the fish in each group per aquarium was computed as a mean value of all the fish in the individual group. Similarly, the final weight of all the fish at the end of the experimental period of 56 days was re-recorded and taken as the mean value to calculate the final weight gain. In Table 3, S. No.1 specifies the mean initial weight of all the fish for each group, *i.e*., Control, T1, and T2 at the beginning of the experiment, which are 38.99g ± 0.62, 39.17g ± 0.59, and 39.04g ± 0.57, respectively. In Table 3, S. No. 2 demonstrates the mean final weight obtained of all the fish in each group at the end of the experimental trial after the feeding trials, which are 50.39g ± 0.43, 51.87g ± 0.46, and 52.44g ± 0.44 for control, T1, and T2, respectively. The results obtained for weight gain are demonstrated in S. No. 3, Table 3 shows a significant increase in weight for T2 and T3 nourished with 2.5% and 3.5% *N. sativa* in feed compared to the fish in the control group. The weight gain for fish in T3 is the highest, i.e., 13.4g, compared to T2 and control, which are 12.7g and 11.4 g, respectively. Also, the one-way

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Table 3: Results of growth performance parameters (WG and WG %).

S. #	Growth parameters	Experimental groups		
		Control	T1 (2.5%)	T2 (3.5%)
1	Avg. initial weight(g)	38.99±0.62	39.17±0.59	39.04±0.57
2	Avg final weight (g)	50.39±0.43	51.87±0.46	52.44±0.44
3	Weight gain (g)	11.4±0.09	12.7±0.05	13.4±0.03
4	Weight gain %	29.24	32.42	34.32

Table 4: Results of growth performance parameters (FC and SG %).

S. #	Parameters	Experimental groups		
		Control	T1(2.5%)	T2 (3.5%)
1	Feed consumed (g)	690	650	650
2	Feed conversion (FC) (g/fish for 56 days)	69	65	65
3	Feed conversion ratio (FCR)	5.96	5.12	4.85
4	Specific growth rate %/ day	0.46	0.50	0.53

ANOVA carried out for this measurement with alpha value set to 0.05 while computing results in MS Office Excel 2010 confirmed the results for a significant value, P< 0.05.

As shown in Table 4, S. No.1, the total feed consumed by fish in the Control group was 690g, and that for T1 and T2 was 650g each. The feed conversion (FC) and feed conversion ratio (FCR) values were calculated with the help of MS Office Excel 2010. In the same Table 4, S. No. 2 reflects the mean results of feed conversion in terms of g/fish per day for 56 days for each fish, which are 69g, 65g, and 65g for control, T1, and T2, respectively. The readings for feed conversion ratio at S. No. 3 calculated for fish in control, T1, and T2 groups resulted in 5.96, 5.12, and 4.85, respectively. This ratio is the highest in the control group compared to the T1 and T2 groups. For the specific growth rate percentage noted in Table 3, S. No. 8, the fish in the T3 group showed the highest value, i.e., 0.53, implying that the *N. sativa* mixed diet in proportion to 3.5% has a significant positive effect on the growth of fish in this group. Similarly, the black seeds mixed in the diet with a proportion of 2.5% for T2 also confirmed a positive effect on the specific growth rate of fish per day in this group, which is 0.50 compared to the control group, which is 0.46 for this growth parameter. From the results in Table 4, it may be concluded that the *N. sativa seed* extracts have imparted a positive effect on the overall growth performance parameters of the grass carp.

The results presented in Table 4 above encompass an investigation into various haematological parameters of grass carp across distinct experimental groups, namely the control, T1 (with a 2.5% concentration of Nigella sativa), and T2 (with a 3.5% concentration of *Nigella sativa*). In Table 5, S. No.1, the experimental group T2 reflects the highest mean increase in RBC count, i.e., 1.52 ± 0.04 million per mm3, followed by the T2 group compared to the control, which is 1.35 ± 0.03 million per mm3. However, in the Table 5, at S. No. 2, the WBC count for the T1 group fed with a 2.5% concentration of *N. sativa* has elevated to 10.60 ± 0.06 103 per mm^3 compared to the control and T2 groups. Further, the mean haemoglobin level noted at S. No. 3 also increased in fish of T1 (6.35 ± 0.04 g/dl) and T2 (6.45 ± 0.03g/dl) groups in proportion to the concentration of *N. sativa* diets in these groups compared to the control group (5.95 ± 0.06 g/dl). The mean hematocrit percentage at S. No. 4 also increased with elevated value for the T2 group, i.e., 28.20 ± 0.1, with a slight difference from the T1 group, i.e., 28.05±0.3, compared to the control group percentage of 26.95±0.1. The results from the experimentation show an increase in values for all the tested haematological parameters for the fish, however, they still fall within the normal ranges as reported by several researchers for this age group of grass carp. These results, therefore, collectively suggest that higher concentrations in the experimental groups correspond to increased counts of red blood cells, along with elevated haemoglobin and hematocrit levels, possibly indicating a concentration-dependent influence of *N. sativa* on the haematological parameters of fish.

Table 5: Results of hematological performance parameters.

Hematological parameters	Experimental groups		
	Control	T1 (2.5%)	T2 (3.5%)
RBC count ($10^6/mm^3$)	1.35±0.03	1.40±0.02	1.52±0.04
WBCs count ($x10^3/mm^3$)	9.65±0.01	10.60±0.06	10.37±0.02
Hb (g/dl or g/100ml)	5.95±0.06	6.35±0.04	6.45±0.03
Hct (%)	26.95±0.1	28.05±0.3	28.20±0.1

Table 6: Results of serum enzymes and biochemical performance parameters.

Serum enzymes/ biochemical parameters	Experimental groups		
	Control	T1 (2.5%)	T2 (3.5%)
ALP (u/l)	103.58±0.9	90.51±0.4	87.35±0.7
ALT (u/l)	21.8±0.9	19.25±0.6	18.9±0.7
AST (u/l)	27.2±0.6	21.85±0.4	22.7±0.3
Urea (mg/dl)	3.75±0.1	2.95±0.3	2.65±0.1
Creatinine (mg/dl)	0.16±0.1	0.12±0.1	0.11±0.3

The results from the analysis of serum blood samples obtained from all the experimental groups are reflected in the Table 6. 2x of fish blood samples were collected from the fish in the control, T1, and T2 groups, respectively. The results for serum enzyme alkaline phosphatase (ALP) show a decrease in fish fed with a 3.5% N. sativa diet in the T2 group compared to T1 with a 2.5% *Nigella sativa*

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diet and the control group with a 0% *Nigella sativa* diet. The values for alanine transaminase (ALT) also showed a decrease in the T2 (3.5%) group, i.e., 18.9 ± 0.7 (U/L), compared to the control group, having a value of 21.8 ± 0.9 (U/L). Similarly, the enzyme aspartate transaminase (AST) mean value in fish of the Control group was 27.2 ± 0.6 (U/L), which decreased to 22.7 ± 0.3 (U/L) in the T2 group fed with 3.5% *N. sativa.*

Discussion

Body weight gain is a primary indicator of growth efficacy in fish, reflecting the efficiency of the provided feed over a given period. Upon concluding the experiment in this study, the groups fed with *N. sativa* enriched feed (T1 and T2) exhibited superior growth compared to the control group, as shown by the evaluation and results of experimental groups initially weighing 39.90 ± 0.62g on average. Notably, the T2 group, fed with feed containing 3.5% black seed supplementation, displayed the most substantial growth of grass carp. The differences in growth among all groups were statistically significant ($p < 0.05$). This finding aligns with prior studies by Öz et al. (2024). Demonstrating the growth-enhancing effects of black seeds in rainbow trout. Similarly, the research by Samanta *et al*. (2014) explored the impact of rosemary and aloe Vera extract on Nile tilapia (*O. niloticus*), highlighting the favorable influence of plant extracts on fish growth. Previous studies indicate reduced growth may be attributed to the physiological stress induced by various toxicities stemming from inadequate accumulation of essential components like proteins and lipids in fish muscles (Abdel-Tawwab *et al*., 2021). The results obtained in this study, however, underscore the positive growth-promoting effects of incorporating *N. sativa* into the fish feed of grass carp, as these contain folic acid, biotin, niacin, vital fatty acids such as linoleic and oleic acid, along with vitamins A B1, B6, C, and essential minerals like calcium, zinc, magnesium, iron and selenium. Moreover, these seeds possess notable pharmacological properties, including antifungal, antibacterial, antiviral, antihistamine, antiprotozoal, antioxidant, anti-inflammatory, and immune stimulant attributes by (Öz et al., 2024; Yousefi *et al*., 2021) contributing to fish growth by stimulating appetite, reducing stress, enhancing digestive enzymes, and fostering growth. Herbal applications are envisioned to confer protective effects and enhance fish survival. In response to stressors and diseases, fish usually experience heightened activity and numbers of white blood cells (WBCs) (Yáñez *et al*., 2015). Elevated WBC counts may correspond to increased antibody production, aiding survival and recovery, as observed in a study on Nile tilapia (Joshi *et al*., 2002). Leukocytes are pivotal components of the immune system in fish defense mechanisms. Consequently, variations in leukocyte numbers, whether increased or decreased, are common reactions to chemicals. A study on common carp (*Cyprinus carpio*) showed that *N. sativa* supplementation increased the WBC count and percentage of lymphocytes, the key players in the fish immune system (Yousefi *et al*., 2021). The authors suggested that the immunomodulatory effect of *N. sativa* oil may be attributed to the high thymoquinone content, which has been reported to have anti-inflammatory and antioxidant properties. Notably, *N. sativa* may mitigate the impact of toxic agents and bacterial and fungal infections. Hemoglobin (Hb), which reflects erythrocyte count, red blood cell (RBC) count, and Hb value, offers insights into hypoxia and anoxia. Decreased RBC and Hb values may stem from the adverse impact of bacteria, fungi, or toxins on the production of erythrocytes within erythropoietic tissues (Chen *et al*., 2019). We have reported that adding 1% and 2% *N. sativa* to the feed of Nile tilapia (*Oreochromis niloticus*) with an average body weight of 101 ± 1g led to an increased Hb level compared to the control group. The Hb levels for the fish fed with 1% and 2% black seeds were 8.81 ± 0.37 g/dl and 8.88 ± 0.47 g/dl, respectively, while the fish in the control group had an Hb content of 7.63 ± 0.43 g/dl. Another study conducted by (Mahboub *et al*., 2022) investigated the dietary supplementation effect of *N. sativa* on the haematological and immunological responses of Nile tilapia (*Oreochromis niloticus*), witnessing significantly higher RBC count, Hb level and Hct compared to the control group. In the current study, the increased number of erythrocytes (WBCs) in the T1 and T2 groups and enhanced RBC count in grass carp within T1 and T2 groups may imply potential protective effects of *N. sativa* A reduction in RBC count can lead to decreased Hb levels as noted in *Labeo rohita* by (Das and Mukherjee, 2003) and in Oncorhynchus mykiss by Çakmak and Gorgon (2003). Hematocrit (Hct %) indicates the percentage of RBCs within the blood. Reduced RBC count leads to reduced Hct percentage (Adedeji *et al*., 2009). Elevated mean corpuscular volume (MCV) was found following diazinon treatment in African catfish (*Clarias gariepinus*). The elevated haemoglobin content per erythrocyte (MCHC) and quantity of erythrocyte Hb (MCH) are attributable to larger RBC size with lower Hb content (Kumar and Banerjee, 2016). Fish subjected to various stresses exhibited Hct reduction. Blood plasma levels substantially regulate Hematocrit. Increased plasma level (decreased Hct) facilitates electrolyte and protein movement through blood flow, compensating for tissue oxygen demands. This parameter uses dual and opposing effects on systemic oxygen transport, affecting cardiac output and oxygen-carrying capacity. A study on rainbow trout (*Oncorhynchus mykiss*) exposed to cold stress showed that *N. sativa* supplementation improved the RBC count, Hb concentration, and Hct compared to the control group (Dorucu *et al*., 2009). The authors suggested that the antioxidant and immune-modulatory properties may have contributed to the observed improvement in haematological parameters. In the current study, the Hct levels have decreased in the T1 and T2 groups. Further, the WBC, RBC and Hb levels in groups T1 and T2 fed with 2.5% and 3.5% *N. sativa,* respectively, show an increase in the values and levels compared to the control group; therefore, it may be suggested that the supplementation of these black seeds in the diet of fish have a positive effect on these haematological parameters of grass carp. The ALT, ALP, and AST enzymes in the blood offer insights into the liver condition, and increased enzyme activities indicate leakage into the bloodstream due to pesticide-induced liver harm, highlighting its hepatotoxic nature (Abhijith *et al*., 2016). In this study, groups supplemented with the *N. sativa* diet (T1 and T2) showcased reduced levels in grass carp compared to the control group, indicating a potential liver-protective effect of these black seeds. Similar findings were observed in *Oncorhynchus mykiss* Imani *et al*., 2015 and *Cyprinus carpio* (Olusola *et al*., 2023), which explored the effects of thorn apple (*Datura stramonium*) extract on *N. sativa*, noting increased albumin values in experimental groups and decreased AST and ALT compared to the control group. Black seed constituents, such as thiamine influencing growth, development, and cell functions, niacin impacting blood lipid levels, and pyridoxine affecting protein metabolism, contribute to their positive effects. Additionally, substances like selenium and folic acid in black seeds play roles in RBC production and possess antioxidant properties, safeguarding against tissue damage. These black seed components potentially exert a positive influence by participating in hepatocyte and blood cell production.

Conclusion

Including *Nigella sativa* (black seed) at 2.5% and 3.5% in the diet of grass carp (*Ctenopharyngodon idella*) significantly enhanced growth performance, including increased body weight and specific growth rate. Hematological parameters such as RBCs, WBCs, hemoglobin (Hb), and hematocrit (Hct) were also elevated. Additionally, serum biochemical enzyme levels (ALT, ALP, and AST) were reduced, likely due to the presence of thymoquinone. These findings suggest that *N. sativa* may be an effective natural feed additive for improving health and productivity in grass carp aquaculture.

Declarations

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IRB approval

This study was reviewed and approved by the Institutional Review Board (IRB) of Department of Zoology (KUST).

Ethical statement

All procedures performed in this study involving human/animal subjects were in accordance with the ethical standards of the institutional research committee.

Statement of conflict of interest

The authors have declared no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies wer used in the writing process.

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