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RESEARCH ARTICLE

Dietary Supplementation of Black Pepper (*Piper nigrum*) Enhances Growth Performance and Hematological Parameters in Fingerling Spotted Snakehead (*Channa punctatus*)

Alvia Farheen *, Mukhtar Ahmad Khan, Noorin Zafar

ABSTRACT: Aquaculture nutrition plays a pivotal role in optimizing fish growth and health, with feed additives increasingly explored for their growth-promoting and immunostimulatory effects. This study investigated the effects of dietary black pepper (*Piper nigrum*) supplementation on growth performance, feed utilization, and hematological parameters in fingerling *Channa punctatus*. Six iso-nitrogenous (45% crude protein) and iso-energetic (18.40 kJ/g) experimental diets were formulated with varying black pepper levels (0%, 0.2%, 0.4%, 0.8%, 1.6%, and 3.2%). Fish (initial weight: 8.82 ± 0.5 g) were stocked in 70-L tanks with a continuous water flow-through system (1–1.5 L/min) and fed twice daily to satiation for eight weeks. Results indicated that dietary black pepper at 0.4% significantly enhanced absolute weight gain (AWG), specific growth rate (SGR), protein retention efficiency (PRE), and protein gain (PG), while reducing feed conversion ratio (FCR). Beyond 0.4%, growth performance declined, likely due to reduced palatability and feed intake. Carcass protein content improved with 0.4% supplementation, whereas moisture, fat, and ash remained unaffected. Hematological parameters, including hemoglobin, red blood cell count, and hematocrit, also peaked at 0.4% inclusion, indicating improved oxygen transport and metabolic efficiency. These findings suggest that black pepper, particularly at 0.4% dietary inclusion, enhances growth, nutrient utilization, and hematological health in *C. punctatus*. The study highlights the potential of black pepper as a natural growth promoter in aquaculture, offering an eco-friendly alternative to synthetic additives while supporting sustainable fish farming practices.

Keywords: Black pepper (*Piper nigrum*), *Channa punctatus*, Hematological parameters, Feed efficiency, Aquaculture nutrition.

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1. INTRODUCTION

Aquaculture has emerged as a vital sector for global food security, providing nearly half of the world's fish supply for human consumption. Among the various factors influencing aquaculture productivity, feed constitutes the most critical component, accounting for 60–70% of total production costs [1]. A nutritionally balanced diet is essential for optimal fish growth, health, and disease resistance, requiring precise proportions of macronutrients (proteins, fats, carbohydrates) and micronutrients (vitamins, minerals) [2]. Imbalances in these nutrients can lead to stunted growth, metabolic disorders, and increased susceptibility to diseases, ultimately affecting survival rates [2]. Therefore, formulating cost-effective yet nutritionally adequate feeds remains a priority for sustainable aquaculture. Feed optimization not only ensures better growth performance but also minimizes waste, reducing environmental impacts associated with excess nutrient discharge [3].

Conventional fish feeds often rely on fishmeal and

Section of Fisheries Science and Aquaculture, Department of Zoology, Aligarh Muslim University, Aligarh, 202002, India

* Author to whom correspondence should be addressed: alviashakil@gmail.com (Alvia Farheen)

synthetic additives to meet protein and growth enhancement requirements. However, the rising cost and ecological concerns associated with fishmeal have driven research toward alternative protein sources, including plant-based ingredients [4]. While plant-derived proteins are more economical, they often contain antinutritional factors (e.g., phytates, tannins, and protease inhibitors) that impair nutrient absorption and digestion in fish [5]. Additionally, the use of synthetic growth promoters, antibiotics, and chemotherapeutics in aquaculture raises significant concerns regarding environmental pollution, antibiotic resistance, and bioaccumulation of toxic residues in fish tissues [6]. These residues can persist in the food chain, posing risks to human consumers [7]. Consequently, there is a pressing need to explore natural, eco-friendly feed additives that enhance growth and immunity without adverse effects.

Black pepper (*Piper nigrum*), a widely used spice, has gained attention as a potential functional feed additive in aquaculture due to its bioactive compound, piperine. Piperine exhibits multiple pharmacological properties, including antioxidant, anti-inflammatory, and digestive-enhancing effects [8]. In fish nutrition, dietary piperine has been shown to improve growth performance by stimulating digestive enzyme activity (e.g., lipase, trypsin, and amylase), enhancing nutrient absorption, and increasing intestinal villi surface area [9]. Studies on olive flounder (*Paralichthys olivaceus*) and sea bream (*Pagrus major*) have demonstrated that piperine supplementation reduces feed conversion ratio (FCR), boosts protein efficiency, and enhances innate immunity by elevating lysozyme activity and immunoglobulin levels [10, 11]. Furthermore, piperine's ability to modulate oxidative stress and inflammation suggests its potential in mitigating metabolic stressors in intensively farmed fish [12, 13]. These findings highlight black pepper as a promising alternative to synthetic growth promoters, aligning with the global shift toward sustainable aquaculture practices.

The present study focuses on the spotted snakehead (*Channa punctatus*), a commercially valuable freshwater fish species native to the Indian subcontinent. *C. punctatus* is renowned for its high protein content (78.32%), balanced amino acid profile, and beneficial omega-3 and omega-6 fatty acids, making it a nutritious food source [14]. Beyond its dietary value, this species holds medicinal significance in traditional practices, with reported anti-inflammatory, wound-healing, and analgesic properties [15, 16]. Despite its economic and nutritional importance, research on optimizing feed formulations for *C. punctatus* remains limited. Given the species' sensitivity to poor water quality and stress, identifying dietary strategies to enhance its growth and resilience is crucial for improving aquaculture yields.

This study is the first to comprehensively evaluate the effects of dietary black pepper supplementation on growth performance, feed utilization, and hematological parameters in fingerling *Channa punctatus*. While previous research has explored piperine's benefits in marine and other freshwater species, its application in *C. punctatus* aquaculture remains unexplored. The investigation provides critical insights into the optimal inclusion level of black pepper (0.4%) for maximizing growth and metabolic efficiency while avoiding the negative effects of over-supplementation. Additionally, the study correlates hematological improvements (e.g., increased hemoglobin and RBC count) with enhanced oxygen transport and stress resilience, offering a holistic assessment of black pepper's role in fish health. By demonstrating the efficacy of a natural, cost-effective feed additive, this research contributes to sustainable aquaculture practices and reduces reliance on synthetic additives, addressing both environmental and food safety concerns.

The growing demand for *C. punctatus* in domestic and international markets necessitates innovative approaches to improve its aquaculture productivity. By integrating black pepper into feed formulations, this study not only addresses nutritional optimization but also supports the broader goal of eco-friendly aquaculture. Future research should explore the long-term immunological and fillet quality benefits of black pepper, as well as its synergistic effects with other functional ingredients. Ultimately, this work underscores the potential of plant-based bioactive compounds in advancing sustainable fish farming, ensuring both economic viability and environmental stewardship.

2. EXPERIMENTAL DETAILS

2.1. Preparation of experimental diets

Six experimental diets containing the same level of protein (45%) and energy (18.40 kJ/g) and different concentrations of black pepper (0.2, 0.4, 0.8, 1.6 and 3.2%) were formulated (Table 1). These diets were designated as D0, D1, D2, D3, D4 and D5. All the ingredients were ground and weighed carefully according to the dietary formulations. All the weighed ingredients were blended in a Hobart electric mixer thoroughly and then cooked at 80ºC. After that oil premixes were added. Black pepper powder was added with other ingredients at the same time, while vitamin and mineral premixes were added only after cooling the mixture. The final diet with bread-dough consistency was then poured into a Teflon coated pan, cut into small cubes and stored in plastic bags at -20°C until used.

2.2. Experimental design

Fingerlings of *Channa punctatus* were procured from a local fish market, Rasalganj, Aligarh. These were brought to the Fish Breeding and Larval Rearing Facility, Department of Zoology, Aligarh Muslim University, Aligarh, India. They were then given a prophylactic dip in $KMnO_4$ solution (1:3000) and stocked in indoor circular aqua-blue coloured, plastic lined fish tanks for a fortnight. During acclimatization fish were fed a basal diet. For conducting the trial, fish (8.82±0.5 g) were sorted out from the above acclimatized lot and distributed at the rate of 10 fish per treatment in 3 triplicate groups in 70L circular aqua-blue coloured polyvinyl tanks. These were fitted with a continuous water flow-through system (1-1.5L min^{-1}).

2.3. Feeding Trial

The fingerlings were fed with the test diets twice a day to apparent satiation at 0800h and 1600h daily. No feed was given on the day mass-weight of the fish was being taken. Initial and weekly weights were recorded on a top-loading balance (Precisa 120A; 0.1 mg sensitivity, Oerlikon AG, Zurich, Switzerland). The feeding trial lasted for 8 weeks. Faecal matter and unconsumed feed, if any, were siphoned off. The unconsumed feed was filtered on a screen soon after active feeding, dried and weighed to measure the amount of feed consumed. Tanks were scrubbed without detergent and washed with water followed by $KMnO_4$ solution once every week, preferably on the day of taking mass weight.

2.4. Water Quality Analysis

The amount of oxygen and other substances dissolved in the water determine its quality and pH level. Besides physical factors such as atmospheric temperature, water temperature, and air-flow influence the water quality and its dissolved oxygen content. The pH of water in the tanks was taken once every day at 1500h using pH strips. Temperature was measured using a thermometer. The tests for dissolved oxygen (DO), free carbon dioxide content and total alkalinity were conducted using standard protocols [17].

The average water temperature, dissolved oxygen, free carbon dioxide, pH, and total alkalinity over the 8-weeks feeding trial, based on daily measurements were 24.8-26.5 °C, 6.8-7.7 mg/L, 7-9 mg/L, 7.2-7.5 and 73- 81.5 mg/L, respectively.

2.5. Proximate composition analysis

Assessment of proximate composition of ingredients, diets and carcass was made using standard techniques [18]. All the analyses were based on triplicate samples.

2.5.1. Moisture content

A known quantity of sample was taken in a pre-weighed crucible and placed in a hot air oven at 105±1 °C for 24 hours. After complete drying, the sample was cooled at room temperature in a desiccator and was reweighed. The loss in weight gave an index of water from which its percentage was calculated.

2.5.2. Ash content

A known quantity of dried powdered sample (2-5 g) was taken in pre-weighed silica crucible and incinerated in a muffle furnace (600 °C) for 2-4 hours or till the sample became carbon-free and completely white. The crucible was cooled in a desiccator and reweighed to estimate the quantity of ash. The result was expressed as percentage on a dry weight basis.

2.5.3. Fat content

Crude fat was estimated by continuous Soxhlet extraction technique using petroleum ether (40-60 C B.P.) as solvent. Finely powdered and dried samples (2-4 g) were taken in Whatman filter paper and introduced into the Soxhlet apparatus.

Table 1. Composition of the experimental diets.

	Dietary black pepper levels (%)					
INGREDIENTS	**0.0 (D0)**	**0.2 (D1)**	**0.4 (D3)**	**0.8 (D3)**	**1.6 (D4)**	**3.2 (D5)**
Fish meal	12	12	12	12	12	12
Soybean meal	35	35	35	35	35	35
Groundnut oil cake	30	30	30	30	30	30
Wheat bran	5	4.8	4.6	4.2	3.4	1.8
Mustard oil cake	14	14	14	14	14	14
Oil	2	2	2	2	2	2
Mineral premix[1]	1	1	1	1	1	1
Vitamin premix[2]	1	1	1	1	1	1
Black pepper	0	0.2	0.4	0.8	1.6	3.2

[1]Mineral mixture (g $100g^1$) calcium biphosphate 13.57; calcium lactate 32.69; ferric nitrate 02.97; magnesium sulphate 13.20; potassium phosphate (dibasic) 23.98; sodium biphosphate 08.72; sodium chloride 04.35, aluminium chloride.$6H_2O$ 0.0154; potassium iodide 0.015; cuprous chloride 0.010, mangnous sulphate H_2O 0.080; cobalt chloride. $6H_2O$ 0.100; zinc sulphate.$7H_2O$ 0.40 (Halver, 2002). [2]Vitamin mixture (1g vitamin mix+2 g α-cellulose) choline chloride 0.500; inositol 0.200; ascorbic acid 0.100; niacin 0.075; calcium pantothenate 0.05; riboflavin 0.02; menadione 0.004; pyridoxine hydrochloride 0.005; thiamin hydrochloride 0.005; folic acid 0.0015; biotin 0.0005; alpha-tocopherol 0.04; vitamin B12 0.00001; Loba Chemie, India (Halver, 2002).

The extraction was carried out for about 1-2 h. At the end of extraction, the solvent was recollected and the flask gave the quantity of crude fat extracted from the known weight of the sample.

2.5.4. Crude protein content

100 mg of dry powdered sample was treated with 5 ml diluted (1:1) sulphuric acid in the Kjeldahl flask and boiled for a few minutes till fumes disappeared. After cooling, 5 ml saturated potassium persulphate solution was added to oxidize the digesting mixture. The digestion was continued till the solution in the Kjeldahl flask became water clear, indicating that all the nitrogenous material present in the sample has been converted into ammonium sulphate. The clear solution was diluted to 50ml with distilled water. 0.5ml aliquot of the digested sample was mixed with 0.1ml each of dilute (1:1) sulphuric acid and saturated potassium persulphate. The content was raised to 3 ml with distilled water. This was then nesslerized with 7ml Nessler's reagent. The solution was kept at room temperature for 10 min for complete colour development. A blank was prepared, side-by-side, substituting the aliquot with distilled water. The colour was read on a spectrophotometer at 480 nm. The intensity of colour developed was proportional to the amount of ammonium sulfate contained in the solution. The values of optical density obtained for various samples were read off against a standard calibration curve prepared by taking readings of a series of different dilutions containing different grades of known amount of nitrogen in the stock solution. Crude protein was calculated by multiplying the nitrogen value with protein factor (6.25). The values were recorded as percentage on a dry weight basis.

2.6. Growth Performance Evaluation

The effects of dietary supplementation of black pepper on *Channa punctatus* were evaluated on the basis of growth, conversion efficiency indices and hematological indices.

Absolute weight gain (AWG; g fish^{-1}) = Final body weight - Initial body weight.

Feed conversion ratio (FCR) = Dry feed intake (g) / Wet weight gain (g).
Specific growth rate (SGR; %day^{-1}) = ln (Final body weight) - ln (Initial body weight) / No. of days ×100

Protein gain (PG; g fish^{-1}) = Final body protein (%) × Final body weight (g) - Initial body protein (%) × Initial body weight (g).

2.7. Hematological analysis:

Fishes up to the fingerling size of <20g are recommended by FAO Guidelines to be sampled using cardiopunction method in which the fish is lifted, fixed head down to the level of the eyes, and an injection needle is pierced at an angle of about 60° in the cardiac region ventrally. Heparinized insulin needle was used to draw out the blood. Thereafter, the blood was immediately transferred to EDTA tubes to prevent coagulation and stored at -20°C.

Sahli's method is the most commonly used method for Hb estimation which is based on the colour development. It uses a Hemometer that consists of a pipette, Hb tube with Gram Hb percentage marking and percentage Hb marking, and a colour comparison box with standard colouration. To conduct the test 0.1 N HCl solution and 20 µl blood was taken and mixed thoroughly. It was then left to stand for 6-8 minutes and diluted with distilled water as needed to match it with the standard colouration in the comparison box.

For RBC count, 20µl blood was mixed with 3980µl of diluting fluid (Hayem's solution). A drop of this well-mixed solution was put under the counting chamber of the Neubauer Hemocytometer. The cells were then observed and counted under a light microscope.

The haematocrit value was determined by centrifuging ethylenediaminetetraacetic acid (EDTA) treated blood drawn into microhematocrit tubes, one end of which was sealed with Critaseal. The tubes were centrifuged in a microhematocrit centrifuge (RM 12C, Microcentrifuge, Remi, RemiMotors, Bombay, India) at 3600g for 6 minutes. The hematocrit value was measured within a standardized time interval and expressed as percentage.

2.8. Statistical analysis

All growth data were subjected to analysis of variance [19, 20]. Differences among treatment means were determined by Duncan's Multiple Range Test at a $P<0.05$ level of significance [21].

3. RESULTS

Table 2 reflects the effects of feeding diets containing various levels of black pepper on growth and conversion efficiencies of fingerling *Channa punctatus*. Absolute weight gain (AWG), specific growth rate (SGR), protein retention efficiency (PRE), and protein gain (PG) were found to increase significantly ($p<0.05$) with increasing dietary black pepper up to the inclusion of 0.4% black pepper in the diet after that a significant decline was recorded in above mentioned parameters. On the other hand, feed conversion ratio showed the reverse trend and found to decrease with increasing levels of black pepper in the diets up to 0.4% and increased thereafter.

Results pertaining to the effects of experimental diets on the carcass composition of *C. punctatus* are presented in Table 3. Carcass protein was found to increase with the increasing levels of black pepper in the diets up to 0.4% and a significant decline was recorded on higher dietary levels.

Carcass moisture showed the reverse trend and decreased significantly with increasing levels of black pepper in the diets. However, carcass fat and ash did not show any significant difference among the dietary treatments.

Table 4 shows the hematological parameters of *C. punctatus* fed diets containing various levels of black pepper. Hemoglobin content, hematocrit value and RBC count were found to increase with increasing levels of black pepper in diets up to the level of 0.4%. Further inclusion of black pepper in the diets of *C. punctatus* resulted in reduction of hematological parameters.

4. DISCUSSION

While the world is searching for a quality protein to combat hunger and malnutrition, fishes are an excellent source. Fish proteins are highly digestible and contain all the essential amino acids required for human nutrition [22]. Compared to plant-based proteins, fish proteins are considered superior due to their balanced amino acid profile and higher bioavailability [23]. In addition to high-quality protein, fish also provides beneficial omega-3 fatty acids, vitamins, and minerals that are important for a healthy heart [24] and brain function [25, 26] in humans. Therefore, the aquaculture system is developed to meet the growing demand for fish proteins globally.

Major prerequisite for any aquaculture industry is meeting the quality and quantity of feed requirements of the fish species for their proper growth and survival [4]. This can only be achieved when right feed formulation is used and optimum quantity of all the required macro and micro-nutrients such as protein, fats, vitamins, minerals, etc. are provided to the fishes. Besides the basic dietary requirement, fishes also need to be supplemented with other compounds such as growth enhancers, drugs, hormones, etc.

Table 2. Growth performance and conversion efficiencies of fish fed experimental diets[1,2]

	Dietary black pepper levels (%)					
	0.0 (D0)	**0.2 (D1)**	**0.4 (D3)**	**0.8 (D3)**	**1.6 (D4)**	**3.2 (D5)**
Average initial weight (g/fish)	8.83±0.06	8.84 ±0.05	8.85±0.08	8.10 ±0.02	8.20±0.04	8.40 ±0.05
Average final weight (g/fish)	33.75±1.7^{e}	42.84 ±1.82^{b}	58.73±1.79^{a}	46.89 ±2.47^{b,c}	40.74±2.38^{c}	38.14 ±1.81^{d}
Absolute weight gain (g/fish)	27.92±1.43^{e}	34.00 ±1.31^{b,c}	49.88±1.72^{a}	38.79 ±1.53^{b}	32.54±1.69^{c}	29.74 ±1.73^{d}
Specific growth rate (%/day)	2.55±0.08^{e}	2.82 ±0.95^{c}	3.38±1.27^{a}	3.14 ±1.11^{b}	2.86±0.78^{c}	2.70 ±0.75^{d}
Feed conversion ratio	3.82±0.32^{a}	3.15 ±0.39^{b}	2.19±0.45^{e}	2.43 ±0.09^{d,e}	2.74±0.08^{d}	2.91 ±0.07^{c}
Protein gain (g/fish)	3.10±0.35^{e}	4.89±0.49^{c}	8.17±0.76^{a}	5.59±0.63^{b}	4.15±0.38^{c,d}	3.48 ±0.29^{d}

[1]Mean values of 3 replicates ± SEM; [2]Mean values sharing the different superscripts in the same row are significantly different (P<0.05)

Table 3. Carcass composition of fish fed experimental diets[1,2]

Dietary black pepper levels (%)						
	0.0 (D0)	**0.2 (D1)**	**0.4 (D3)**	**0.8 (D3)**	**1.6 (D4)**	**3.2 (D5)**
Moisture (%)	74.22±2.62^{a}	72.35 ±1.65^{b}	70.54±2.33^{d}	71.13 ±2.45^{c}	73.28±2.62^{b}	74.59±2.79^{a}
Crude protein (%)	11.38±0.68^{e}	13.94 ±0.72^{c}	15.76±1.12^{a}	14.04 ±1.04^{b}	12.67±1.23^{d}	11.84±0.98^{e}
Crude fat (%)	6.54±0.08	6.21 ±0.04	6.39±0.09	7.02 ±0.12	6.93±0.08	6.25±0.05
Ash (%)	4.89±0.06	5.02 ±0.09	5.12±0.06	4.18 ±0.05	5.04±0.04	5.22±0.06

[1]Mean values of 3 replicates ± SEM; [2]Mean values sharing the different superscripts in the same row are significantly different (P<0.05)

Table 4. Hematological parameters of fish fed experimental diets[1,2]

	Dietary black pepper levels (%)					
	0.0 (D0)	**0.2 (D1)**	**0.4 (D3)**	**0.8 (D3)**	**1.6 (D4)**	**3.2 (D5)**
Hemoglobin (g/dL)	4.42±0.62^{e}	5.52.24 ±0.73^{c}	8.23±0.87^{a}	7.71 ±0.74^{b}	6.49±0.57^{c}	5.54±0.58^{d}
RBCs (10^6/mm^3)	1.86±0.02^{d}	2.21 ±0.02^{c}	2.91±0.03^{a}	2.74 ±0.05^{b}	2.31±0.02^{c}	1.97±0.01^{d}
Hematocrit (%)	17.45±2.5^{e}	22.03 ±2.92^{d}	32.44±3.17^{a}	30.58 ±2.13^{b}	25.49±2.85^{c}	21.76±2.78^{d}

[1]Mean values of 3 replicates ± SEM; [2]Mean values sharing the different superscripts in the same row are significantly different (P<0.05).

In this experiment, the feed was supplemented with black pepper. Piperine is a compound found in black pepper that has shown to have beneficial effects on the growth, hematological, and immunological parameters of the freshwater fish [27, 28]. Black pepper supplementation improved the growth and conversion efficiencies of fingerling *C. punctatus*. Piperine has been found to increase pancreatic digestive enzyme secretion such as lipase, trypsin, chymotrypsin, and amylase, stimulating nutrient digestion [29]. It alters the passive permeability and fluidity of the brush border membrane, which is connected to lengthened microvilli and increased intestinal absorptive surface area. These changes enhance the absorption and bioavailability of nutrients, leading to improved protein synthesis and growth [29]. There are also proposed mechanisms by which black pepper and its active compound piperine enhance growth in other fishes [9, 30].

Piperine has also been demonstrated to activate the jejunum epithelial cells and membranes, further enhancing nutrient absorption. It can also improve enzyme activities due to its protein folding ability, thereby modifying the ultrastructure of enterocytes and increasing intestinal villi length [10]. This might be the reason for improved feed utilization on dietary supplementation of black pepper [31].

Decreased growth at the higher dietary levels of black pepper may be due to reduced feed intake at those levels and reduced palatability of the diet can be one of the reasons for that. The lower feed intake at higher levels could be due to pungent characteristics of piperine [32] in black pepper. The reduced feed consumption could also be ascribed to the strong taste produced by black pepper (black pepper tastes hot) in the feed. It was reported that spices produced feed aversion in rodents due to their pungent odour [32].

The alkaloid nature of piperine has also been reported for poor palatability and lower feed utilization in various farmed terrestrial and aquatic species [12]. In the present study, the growth performance and feed utilization were lower in higher than 0.4% dietary level of black pepper.

Piperine has been shown to have antioxidant, anti-inflammatory properties that could help protect fish from the oxidative stress, inflammation, and cell damage induced by exposure to toxicants like deltamethrin [33]. Dietary supplementation of piperine has been found to mitigate the toxic effects of the insecticide deltamethrin in snakehead fish [8]. Piperine was able to attenuate the deltamethrin-induced changes in biochemical parameters, antioxidant status, and immune responses in *Channa argus* [34, 35].

The combination of piperine with other active compounds has also been found to be more effective than either compound alone in mitigating toxicity [36, 37]. In the present study also the black pepper supplementation improved the protein deposition in tissues as well as hematological parameters.

The changes in blood parameters induced by piperine and other compounds from black pepper are dose and duration-dependent, and can serve as useful biomarkers to assess the physiological status of the fish [38]. In the present study, black pepper supplementation in the diets showed an improvement in the hematological parameters of *C. punctatus*. A study on the effect of dietary leaf extracts of black pepper (*Piper nigrum*) on the growth, hematological, and immunological parameters of the fish *Labeo rohita* found that the black pepper extract had a positive impact on these parameters [39].

5. CONCLUSION

The present study demonstrates that dietary supplementation of black pepper (*Piper nigrum*) significantly influences the growth performance, feed utilization, and hematological health of fingerling *Channa punctatus*. The optimal inclusion level of 0.4% black pepper in the diet yielded the highest absolute weight gain, specific growth rate, protein retention efficiency, and protein gain, while simultaneously reducing feed conversion ratio. These improvements can be attributed to the bioactive compound piperine, which enhances nutrient absorption, stimulates digestive enzyme activity, and improves intestinal morphology, as evidenced in previous studies. The decline in growth performance at higher inclusion levels (≥0.8%) suggests a threshold beyond which the pungency of piperine may reduce feed palatability and intake, aligning with findings in other aquatic and terrestrial species. Carcass composition analysis revealed that 0.4% black pepper supplementation significantly increased body protein content without altering fat or ash levels, indicating enhanced protein deposition and muscle development. Furthermore, hematological parameters such as hemoglobin, RBC count, and hematocrit were markedly improved at this inclusion level, reflecting better oxygen-carrying capacity and overall metabolic efficiency. These physiological enhancements suggest that black pepper not only promotes growth but also bolsters the fish's resilience to stress and disease. The findings underscore the potential of black pepper as a sustainable, plant-based feed additive in aquaculture, reducing reliance on synthetic growth promoters and antibiotics. Future research should explore the long-term effects of black pepper supplementation on immune responses, disease resistance, and fillet quality in *C. punctatus* and other commercially important fish species. Additionally, synergistic effects of black pepper with other functional feed ingredients could be investigated to optimize feed formulations. In conclusion, 0.4% dietary black pepper is recommended for enhancing growth performance and hematological health in *C. punctatus*, offering a viable strategy for sustainable aquaculture production.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally to this work.

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