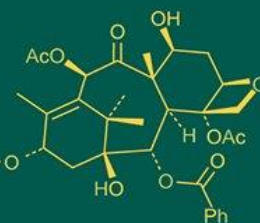
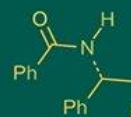
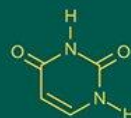
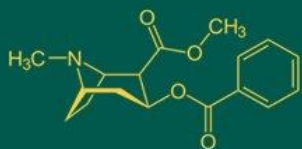


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Comparative evaluation of earthworm and poultry viscera diets on survival and growth of spotted murrel (*Channa punctata*) fingerlings

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Abstract

An experimental trial was conducted for 30 days to evaluate the growth and survival and haematological parameters of spotted murrel, *Channa punctata*, under different dietary treatments. The study comprised three experimental treatments with three replicates each, along with a control group. We have prepared 3 experimental feed such as Control, T₁ (poultry viscera waste) and T₂ (earthworm) raw diet. Fish were fed daily, and routine management practices including siphoning of uneaten feed and excreta, water replenishment, and water quality monitoring were consistently performed. Growth performance parameters was observed at the end of the experiment. The highest length gain (5.50±0.17), weight gain (13.76±0.15), percentage weight gain (223.24±0.84) and specific growth rate (1.69±0.003) was observed in T₂ group compared to the other treatment groups. The highest survivability (63.33±5.77) was observed in T₂ group while mortality (76.66±5.77), and cannibalism (56.66±5.77) in control group compared to the other treatment groups. The highest haematological parameters such as Haemoglobin (9.50±0.09), Red blood cells (2.36±0.15), White blood cells (29.43±0.25), haematocrit 37.46±0.25) were observed in T₂ group while the Mean Corpuscular Volume (178.40±0.30), Mean Corpuscular Haemoglobin (44.43±0.30) were observed in T₁ group and Mean Corpuscular Haemoglobin Concentration (27.36±0.25) were observed in T₂ group compared to the other treatment group. Water quality parameters were observed within acceptable limits throughout the experimental period. The results suggest that earthworm treatment groups enhanced the growth performance, haematological parameters and reduce cannibalism of *C. punctata*, indicating potential benefits for aquaculture nutrition strategies.

Keywords: *C. punctata*, Poultry viscera waste, Earthworm, Growth, Haematology

1. Introduction

Aquaculture has become one of the fastest-growing food-producing sectors globally, contributing significantly to food security, nutrition, employment, and economic development. In India, aquaculture plays a vital role in inland fish production, compensating for the stagnation and decline of capture fisheries. Freshwater aquaculture dominates the sector, with increasing emphasis on diversification toward high-value species to enhance farm income and sustainability (FAO, 2020) [16]. Air-breathing fishes, particularly murels (*Channa* spp.), are gaining importance due to their high market demand, fast growth, tolerance to poor water quality, and ability to survive under low dissolved oxygen conditions (Wee, 1982). The low availability of *Channa punctata* seed is mainly attributed to difficulties in captive breeding, larval rearing, and high cannibalism during early life stages. Although attempts have been made to standardize induced breeding techniques, challenges related to broodstock management, poor fertilization rates, and low larval survival continue to restrict large-scale seed production (Damle et al., 2023) [11]. Dependence on wild seed not only exerts pressure on natural populations but also increases seed cost and limits the expansion of murrel farming. Therefore, the development of reliable hatchery technologies and improved larval rearing protocols is essential for enhancing *C. punctata* seed availability and ensuring sustainable aquaculture production (Kumar et al., 2021) [25]. *Channa punctata* (Bloch, 1793), commonly known as the spotted snakehead or spotted murrel, is a freshwater fish belonging to the family Channidae.

It is widely distributed across South and Southeast Asia, including India, Bangladesh, Nepal, Sri Lanka, Myanmar, and Pakistan, inhabiting ponds, swamps, rivers, and brackish water systems. The species is of considerable economic importance due to its high nutritional value, medicinal properties, and role in local fisheries. Its hardy nature, tolerance to low oxygen conditions, and ability to survive in stagnant waters make it a suitable candidate for aquaculture development (Singh *et al.*, 2020)^[52]. In addition to its ecological adaptability, *C. punctata* is valued for its therapeutic potential, as its flesh is traditionally used in treating ailments such as anemia and post-operative recovery. The species exhibits unique reproductive behavior, including nest-building and parental care, which enhances its survival in natural habitats (Kumari *et al.*, 2011)^[52]. Given its commercial and medicinal significance, research on growth performance, survival, and haematological responses under different dietary and environmental conditions is essential for optimizing aquaculture practices. Such studies not only contribute to sustainable fish production but also provide insights into the physiological resilience of *C. punctata*, reinforcing its potential as a promising species in freshwater aquaculture.

Poultry viscera waste refers to the internal organs and associated by-products such as intestines, liver, gizzard, heart, and other offal generated during poultry slaughter and processing. With the rapid growth of the poultry industry, the volume of viscera waste has increased substantially, posing serious challenges related to disposal, environmental pollution, and public health. Improper handling and disposal of poultry viscera can lead to foul odors, attraction of pests, and contamination of soil and water resources. Therefore, effective management of this waste stream is essential for ensuring environmental sustainability and biosecurity in poultry processing operations (FAO, 2013; Nahm, 2003)^[35,15]. Despite being considered waste, poultry viscera are rich in proteins, lipids, essential amino acids, and minerals, making them a valuable resource for recycling and value addition. Processed poultry viscera have been explored as an alternative feed ingredient in aquaculture and livestock diets, helping to reduce feed costs and dependence on conventional protein sources such as fish meal (Sogbesan *et al.*, 2008)^[47].

Earthworms are important soil-dwelling invertebrates belonging to the phylum Annelida and class Oligochaeta, and they are widely distributed in agricultural, forest, and grassland ecosystems. They are commonly described as “ecosystem engineers” due to their significant role in modifying soil physical, chemical, and biological properties. The pioneering work of Charles Darwin highlighted the crucial contribution of earthworms to soil formation and the decomposition of organic matter, emphasizing their long-term impact on soil productivity (Darwin, 1881). The feeding and burrowing activities of earthworms enhance soil aeration, aggregation, and water infiltration while stimulating microbial activity and nutrient mineralization. Earthworm casts are rich in plant-available nutrients such as nitrogen, phosphorus, potassium, and calcium, which improve soil fertility and crop growth (Edwards & Bohlen, 1996). Several studies have demonstrated that soils with abundant earthworm populations show improved structure, reduced compaction, and greater biological activity, supporting sustainable agricultural practices (Lavelle *et al.*, 1997)^[28]. Beyond their ecological importance, earthworms have gained increasing attention for their applied value in vermiculture, vermicomposting, and animal nutrition. They efficiently convert organic wastes into nutrient-rich vermicompost,

contributing to environmentally friendly waste management systems (Hajam *et al.*, 2023)^[18]. Therefore, the present study was conducted to address the dual challenge of improving fish production and reducing dependence on costly conventional feeds. By systematically comparing two alternative protein sources, the study provides evidence-based insights into sustainable aquaculture nutrition strategies for *C. punctata*.

2. Materials & Methods

2.1 Location of work

The research work were carried out at the Live Fish Laboratory Department of Aquaculture, L.S.P.N. College of Fisheries, Kawardha, Chhattisgarh.

2.2 Experimental fish

The experimental fish were collected from the sakri river tributary nala majgaon at Kawardha, Kabirdham (C.G.), India.

2.3 Culture of Earthworm and Poultry viscera waste

Earthworm culture is the systematic rearing and multiplication of earthworms under controlled conditions to produce earthworms and vermicompost. It involves the use of suitable organic wastes such as cow dung, agricultural residues, kitchen waste, and leaf litter as feed substrate. Species like *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus* are commonly used due to their high reproduction rate and efficient waste conversion. Earthworm culture requires optimum moisture (60–70%), moderate temperature (20–30 °C), adequate aeration, and protection from direct sunlight and predators. Vermiculture plays a significant role in sustainable agriculture and aquaculture by recycling organic waste, improving soil fertility, and providing a high-quality protein source for fish and poultry feed. Poultry viscera waste can be used for direct fish feeding after thorough washing to remove blood, dirt, and undesirable contents, thereby improving its hygienic quality and palatability. After washing, the viscera are often chopped or minced to suit the mouth size of fish and fed fresh, particularly to carnivorous and omnivorous species. This washed poultry viscera waste serves as a rich source of animal protein, lipids, and essential nutrients that support rapid growth and better feed utilization. However, careful management is required to avoid water pollution, spoilage, and disease risks, including feeding in limited quantities and removing uneaten residues promptly. Direct feeding of washed poultry viscera waste offers a low-cost feeding strategy, especially in small-scale and experimental aquaculture systems.

2.4 Experimental design

One hundred twenty fingerlings of *C. punctata*, average weight ranging from 6.14g will be randomly stocked in four different experimental groups such as control, T₁, T₂ and T₃ with three replicates, following a completely randomized design (CRD).

2.5 Feeding trial

Both the diets were collected and offered to fish in raw form. *C. punctata* fingerlings showing average weight 6.14±0.05 g and average length 7.25±0.08 cm were collected from the Kawardha and were acclimatized in aquarium tanks (12×18×24cm) for 3 days then were divided into three groups; two groups received diets and the third group was kept on control diet. All the dietary treatments including control had three replicates. Fish weight and length were taken at the time of initiation of the experiment, and thereafter, the increase in fish weight and length were noted

on a weekly basis. All the fish were fed at 5% of their body weight twice a day at 9 am and 4 pm. Before feeding, the leftover feed and faecal matter were removed from water. At the end of the trial, all the fish were harvested, weighed and measured, and some fishes were dried for the determination of body composition. Water quality parameters like temperature, pH and dissolved oxygen (DO) of aquarium water were monitored on a daily basis.

2.6 Growth performance

After 60 days of the experimental period, the weight of every fish in the aquariums was measured separately. Growth performance was evaluated using the formulae given below.

Length gain (cm)

Body length gain = Final average length gain – Initial average length gain

Weight gain (g)

Body weight gain = Final average weight gain – Initial average weight gain

Percentage weight gain (%)

% weightgain=(Finalaverageweightgain-Initialaverageweight)/(Finalaverageweightgain) X100 %

Specific growth rate (%)

$$SGR = \frac{\text{ifinalweight} - \text{€ initialweight}}{\text{No. ofdays}} \times 100$$

Survivability (%)

$$\text{Survivability}(\%) = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \times 100$$

Mortality (%)

$$\text{Mortality}(\%) = \frac{\text{Number of fish that died during the experiment}}{\text{Total number of fish stocked}} \times 100$$

Cannibalism rate (%)

$$\text{Cannibalism rate}(\%) = \frac{\text{Number of fish lost or consumed}}{\text{Initial number of fish}} \times 100$$

2.7 Haematological parameters

Each fish was anaesthetized with clove oil (Merck, Germany) at the rate of 50 µl of clove oil per liter of water before collecting blood samples from fish. All the 5 fish from each tank were used for blood collection and pooled samples from each tank were used for analysis. Blood was collected from caudal vein of fish by using 1.0 ml hypodermal syringe and 24 gauge needles. The collected blood (approximately 400- 500µL from each fish) was immediately transferred into vials coated with thin layer of EDTA, as anticoagulant (for blood and plasma collection). Vials having EDTA coats were shaking gently in order to prevent haemolysis and clotting of blood. In the present

experiment, mainly the blood in collected and kept with EDTA for the study of different parameters (Lal *et al.*, 2025)^[53]. TEC was done by the method of Schaperclaus (1991). TLC was done with the help of Schaperclaus method (1991). PCV was measured by Schaperclaus method (1991) with slight modification. The Hb Total haemoglobin (Hb) content was estimated by using Sahli Haemometer (Marinefield, Germany). MCV and MCHC etimated by using Schaperclaus method (1991).

2.8 Water quality parameter analysis

The water physio-chemical parameters, viz., water temperature, dissolve oxygen (DO), pH, Turbidity, total dissolve solids, conductivity, alkalinity and hardness of the tank water were estimated and recorded fortnightly prior to sampling by the standard methods (APHA, 1998). DO and temperature is measured in early morning around 7-8:30 AM. Temperature, DO, Turbidity, total dissolve solids and conductivity was estimated using EXO multi parameter sonde. The pH was estimated in a digital pH meter 335 (Systronics). The total alkalinity and hardness of water was measured following the standard methodology of APHA (2005).

2.9 Statistical analysis

The data obtained were analysed by SAS (1999; statistical package; version 22.0 for Windows). Data obtained from studies based on completely randomized experimental design were subjected to one-way analysis of variance. Results were considered significant at $P < .05$. Means of each treatment including control then were compared using Duncan's multiple range test for level of statistical significance among treatments.

3. Results

3.1 The growth performance

The growth performance of *C. punctata* are repersented in the table No. 1 and Figure no. 1. The growth performance parameters of fish such as length gain, weight gain, percentage weight gain, specific growth rate, survivability, mortality and cannibalism. The highest length gain (5.50 ± 0.17) was observed in the T₂ group while lowest (3.06 ± 0.05) in the control group compared to the other treatment groups. The highest weight gain (13.76 ± 0.15) was observed in the T₂ group whreas the lowest (10 ± 0.10) in control group compared to the other treatment groups. The highest percentage weight gain (223.24 ± 0.84) was observed in the T₂ group and lowest (163.04 ± 0.82) in the control groups. The highest specific growth rate (1.69 ± 0.003) was observed in the T₂ group while the lowest (1.40 ± 0.004) in the control groups. There is a statistically significantly ($p > 0.05$) difference among the treatment groups. The highest survivability (63.33%) was observed in the T₂ group compared to the other treatment groups. The highest mortality (76.66%) was observed in the control group compared to the other treatment groups. The highest cannibalism rate (56.66%) was observed in the control group while the lowest (16.66%) in the T₂ groups. There is a statistically significantly ($p > 0.05$) difference in all among the treatment groups.

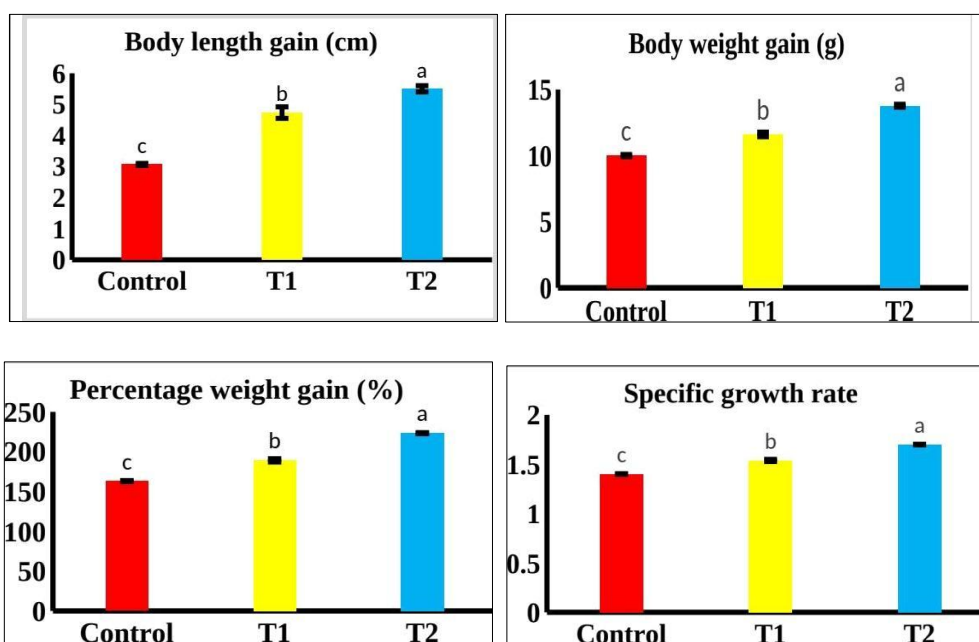


Fig 1: Growth performance of *C. punctata*; a. Body length gain; b. Body weight gain; c. Percentage weight gain; d. Specific growth rate (SGR); e. Food conversion ratio (FCR); f. Protein efficiency ratio (FCR); g. Hepato somatic index (HSI); h. Intestinal somatic index (ISI) \Data are presented as mean $\pm$ SE. Different superscripts indicate statistically significant difference ($p<0.05$) among the experimental group.

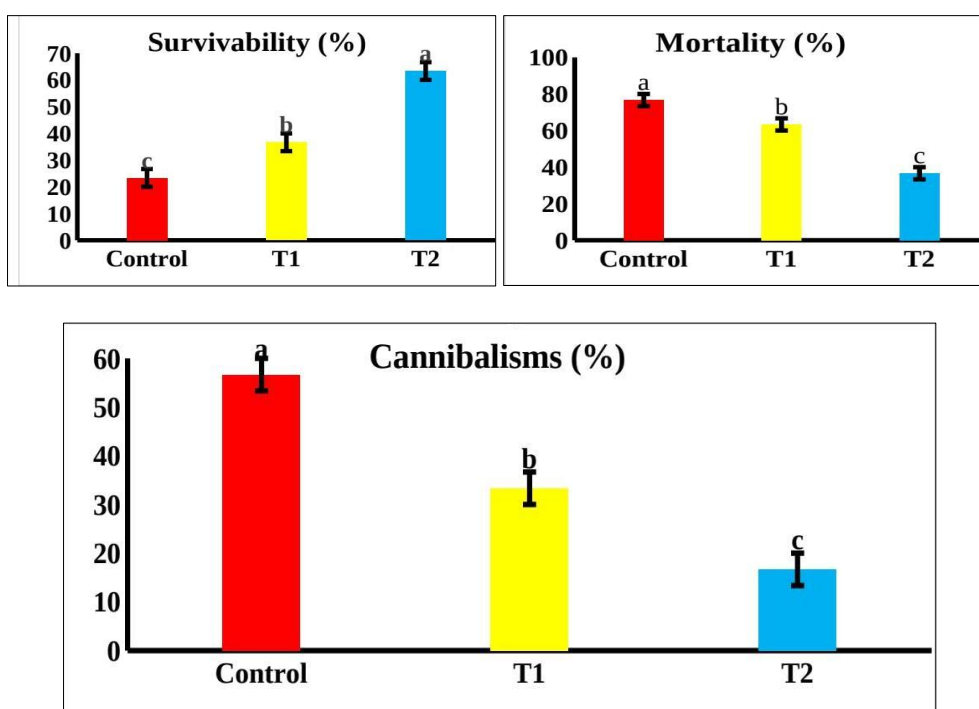


Fig 2: Survivability, Mortality and cannibalism of snakehead, *C. punctata* at end of the experiment. \Data are presented as mean $\pm$ SE. There is a statistically significantly difference ($p>0.05$) among the experimental groups.

3.2 Haematological parameters of fish

The haematological parameters of fish was observed at the end of the experiment are represented in table No. 2. The haematological parameters of fish such as haemoglobin, red blood cells, white blood cells, Haematocrit, MCV, MCH and MCHC. There is a no statistically significantly ($p<0.05$) difference among the treatment groups. The highest haemoglobin (9.50 ± 0.090) was observed in the T₂ group and lowest (8.30 ± 0.20) in the T₁ groups. The highest RBC (2.36 ± 0.152) was observed in the T₂ group whereas the lowest value (1.73 ± 0.208) in the T₁ groups. The highest WBC

(29.43 ± 0.25) was observed in the T₂ group whereas the lowest value (24.33 ± 0.25) in the T₁ groups. The highest haematocrit (37.46 ± 0.251) was observed in the T₂ group whereas the lowest value (32.36 ± 0.25) in the T₁ groups. The highest MCV (178.40 ± 0.30) was observed in the T₁ group whereas the lowest value (170.66 ± 0.32) in the T₂ groups. The highest MCH (44.43 ± 0.30) was observed in the T₁ group and whereas the lowest (43.66 ± 0.32) in the T₂ groups. The highest MCHC (27.36 ± 0.25) was observed in the T₂ group and whereas the lowest (25 ± 0.30) in the T₁ groups.

Table 1: The growth performance of *C. punctata* during the experimental periods

Treatment	Initial length (cm)	Final length (cm)	Initial weight (g)	Final weight (g)	Length gain (cm)	Weight gain (g)	% weight gain (%)	Specific growth rate (%)	Survivability (%)	Mortality (%)	Cannibalism (%)
Control	7.20±0.10	10.26±0.11 ^c	6.13±0.05	16.13±0.15 ^c	3.06±0.05 ^c	10±0.10 ^c	163.04±0.82 ^c	1.40±0.004 ^c	23.33±5.77 ^c	76.66±5.77 ^a	56.66±5.77 ^a
T ₁	7.30±0.10	12.03±0.25 ^b	6.13±0.05	17.73±0.30 ^b	4.73±0.32 ^b	11.60±0.26 ^b	189.12±3.43 ^b	1.53±0.017 ^b	36.66±5.77 ^b	63.33±5.77 ^b	33.33±5.77 ^b
T ₂	7.26±0.05	12.76±0.15 ^a	6.16±0.05	19.93±0.20 ^a	5.50±0.17 ^a	13.76±0.15 ^a	223.24±0.84 ^a	1.69±0.003 ^a	63.33±5.77 ^a	36.66±5.77 ^c	16.66±5.77 ^c
<i>F-Value</i>	1.00	148.633	0.333	204.750	101.927	311.258	620.038	602.281	37.333	37.333	36.333

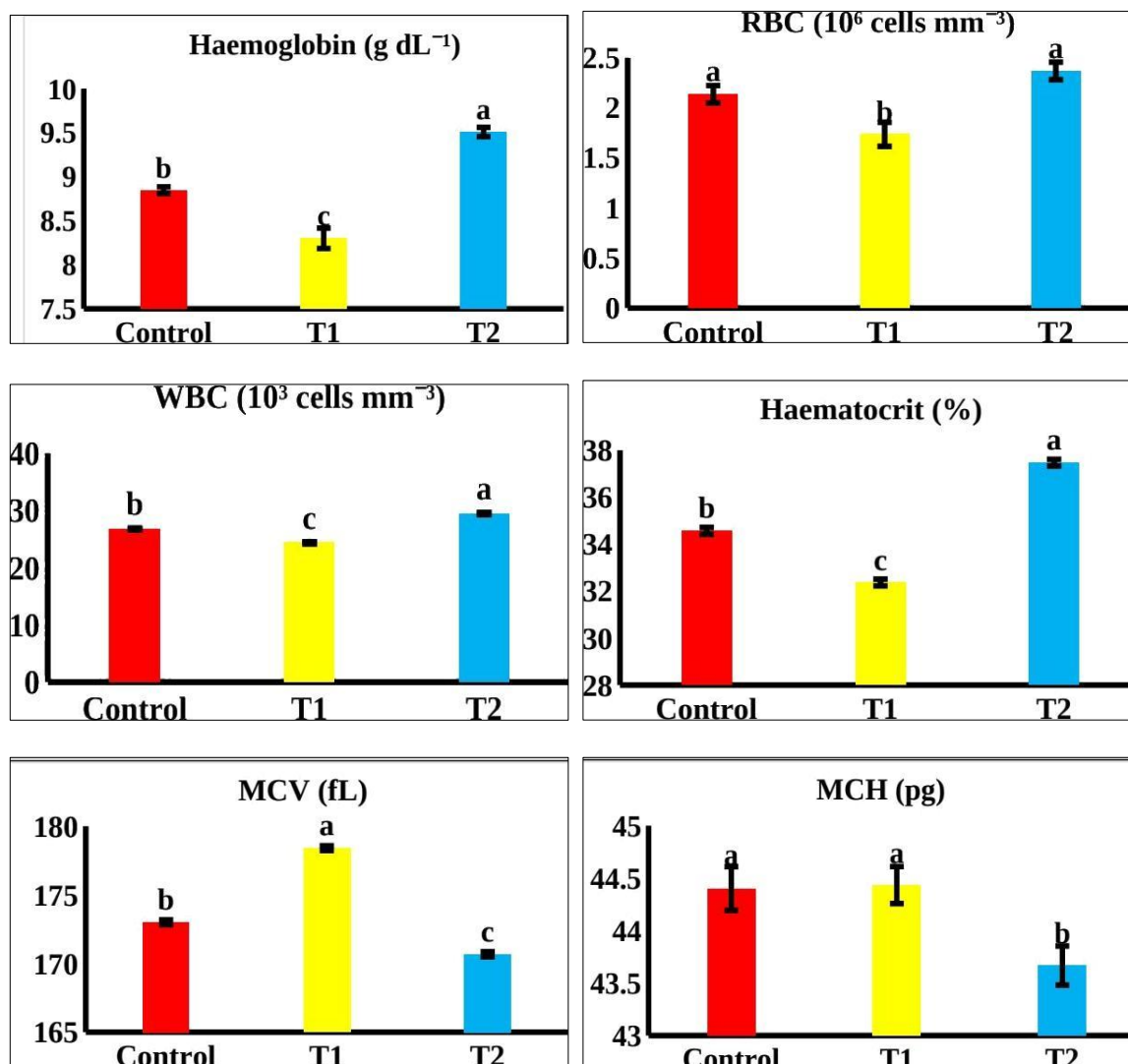
Values are means ± SD, *n* = 3 per treatment group. a-cMeans in a column without a common superscript letter differ (*P* < 0.05) as analyzed by229 one-way ANOVA and the DUNCAN test.230

Table 2: Haematological parameters of *C. punctata* at the end of 30th days of experiment.

Treatment	Haemoglobin (g dL ⁻¹)	RBC (10 ⁶ cells mm ⁻³)	WBC (10 ³ cells mm ⁻³)	Haematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g dL ⁻¹)
Control	8.84±0.065 ^b	2.13±0.152 ^a	26.73±0.208 ^b	34.56±0.251 ^b	173±0.30 ^b	44.40±0.36 ^a	25.60±0.30 ^b
T ₁	8.30±0.20 ^c	1.73±0.208 ^b	24.33±0.251 ^c	32.36±0.251 ^c	178.40±0.30 ^a	44.43±0.30 ^a	25±0.30 ^c
T ₂	9.50±0.090 ^a	2.36±0.152 ^a	29.43±0.251 ^a	37.46±0.251 ^a	170.66±0.32 ^c	43.66±0.32 ^b	27.36±0.25 ^a
<i>F-Value</i>	62.625	10.259	344.647	309.947	499.811	5.173	55.986

Values are means ± SD, *n* = 3 per treatment group. a-dMeans in a column without a common superscript letter differ (*P* < 0.05)

as analyzed by232 one-way ANOVA and the DUNCAN test.233



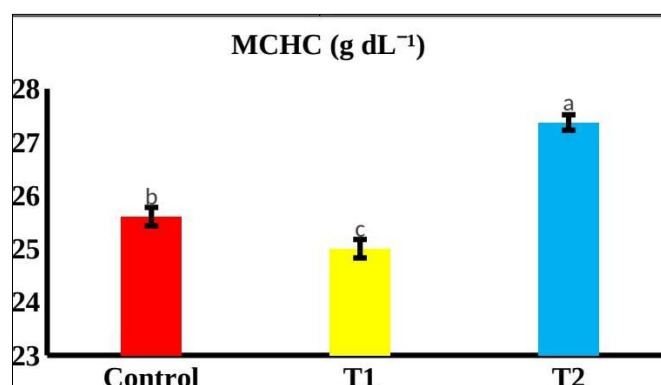


Fig 3: Haematological parameters of *C. punctata* at the end of the experiment; a. Total haemoglobin; b. RBC; c. WBC; d. Hematocrit; e. Mean corpuscular volume; f. Mean corpuscular haemoglobin, g. Mean corpuscular haemoglobin concentration. *Data are presented as mean±SE. Different superscripts indicate statistically significant difference ($p < 0.05$) among the experimental groups.

3.3 The water quality parameters

The water quality parameters are represented in the table No. 3. The acceptable range of water quality parameters was observed such as temperature, dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrite and nitrate. Water quality parameters are crucial for fish health, growth, and survival. Factors like dissolved oxygen, pH, temperature, and the presence of pollutants directly impact a fish's ability to perform essential functions such as breathing, feeding, and reproducing. Maintaining optimal water quality is essential for successful aquaculture and healthy fish populations. The highest water temperature range from (22.4-28.1) was observed in the T₁ group and the lowest value (21.6-27.8) in the control groups. The highest dissolved oxygen range from (4.3-6.1) was

observed in T₂ and lowest (4.2-4.9) in T₁ group during the experimental period in different treatment groups. The highest pH range from (6.5-7.6) was observed in T₂ and lowest (6.1-7.2) in T₁ group. The highest water alkalinity (130-142) was observed in T₁ group while the lowest (125-129) in control group during the experimental period in different treatment groups. The highest water hardness range from (129-146) was observed in T₁ group while the lowest (123-132) in control group. The highest ammonia range from (0.03-0.18) was observed in T₁ group while the lowest (0.02-0.05) in T₂ group. The highest water nitrite range from (0.01-0.12) was observed in T₁ group while the lowest (0.01-0.03) in T₂ group. The highest water nitrate range from (1.4-1.9) was observed in control group while the lowest (1.2-1.6) in control group.

Table 3: The water quality parameters of *C. punctata* during the experimental period.

Treatment	Temperature (°C)	Dissolved Oxygen (mgL ⁻¹)	pH	Alkalinity (mgL ⁻¹)	Hardness (mgL ⁻¹)	Ammonia (mgL ⁻¹)	Nitrite (mgL ⁻¹)	Nitrate (mgL ⁻¹)
Control	21.6-27.8	4.2-5.8	6.3-7.4	125-129	123-132	0.02-0.06	0.01-0.04	1.4-1.9
T ₁	22.4-28.1	4.2-4.9	6.1-7.2	130-142	129-146	0.03-0.18	0.01-0.12	2.9-3.8
T ₂	22.2-27.6	4.3-6.1	6.5-7.6	128-135	126-138	0.02-0.05	0.01-0.03	1.2-1.6

Values are represented as a range of water quality parameters during the experimental periods.

5. Discussions

5.1 Growth performance

The growth performance of *Channa* fish larvae is a crucial determinant of hatchery success and subsequent grow-out production. Larval stages are characterized by rapid physiological and morphological changes, making them highly sensitive to nutritional and environmental conditions. Several studies on *Channa striata* and other *Channa* species have demonstrated that growth performance during early larviculture is strongly influenced by feeding strategy, stocking density, water quality, and developmental readiness of the digestive system (Qin *et al.*, 1997) [38, 39]. Feeding regime plays a central role in determining larval growth and survival in *Channa* species. Early initiation of exogenous feeding after yolk sac absorption has been shown to significantly enhance weight gain, length increment, and specific growth rate (SGR). Delayed feeding beyond the critical period results in reduced growth and increased mortality due to irreversible starvation effects (Kalaiselvan *et al.*, 2024) [23]. Live feeds such as *Artemia nauplii*, rotifers, and *Moina* have consistently produced superior growth performance compared to formulated diets alone, owing to their high digestibility, appropriate size, and presence of exogenous digestive enzymes (Ramena *et al.*, 2025) [42]. Several studies have reported that co-feeding strategies combining live

feed and micro-diets improve larval growth performance and facilitate gradual weaning to artificial diets in *Channa* larvae. This approach supports better feed acceptance and nutrient assimilation, leading to higher weight gain and improved feed utilization efficiency (Chang *et al.*, 2006) [9]. The superior growth observed in livefeed-fed larvae is attributed to the underdeveloped digestive system of early larvae, which lacks sufficient endogenous enzyme activity to efficiently utilize complex formulated feeds (Kalaiselvan *et al.*, 2024) [23]. Stocking density is another important factor influencing growth performance of *Channa* larvae. Lower stocking densities generally result in higher growth rates and better survival, likely due to reduced competition for food and space, improved water quality, and lower stress levels. High stocking densities have been associated with growth retardation and increased cannibalism in *Channa* larvae, a species known for aggressive behavior during early life stages (Latifah and Rahardja, 2022) [27]. Reduced stress at optimal densities allows more energy to be allocated toward somatic growth rather than maintenance metabolism.

Environmental factors such as temperature, dissolved oxygen, and photoperiod also significantly influence larval growth. Optimal water temperature enhances metabolic activity, digestion, and nutrient absorption, thereby improving growth

performance. Poor water quality conditions, particularly low dissolved oxygen and high ammonia levels, negatively affect larval growth by increasing physiological stress and reducing feeding efficiency (Dai *et al.*, 2025)^[10]. Adequate light intensity and feeding frequency further contribute to improved growth by promoting better prey visibility and feed intake in *Channa* larvae (Qin, J. G., & Fast, 1997)^[39]. Physiological development of the digestive system strongly underpins growth performance in *Channa* larvae. The gradual increase in digestive enzyme activities such as protease, amylase, and lipase during larval development correlates positively with improved growth rates and feed conversion efficiency (Arenas-Pardo *et al.*, 2024)^[3]. Enhanced expression of growth-related hormones such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1) has also been linked to improved larval growth under optimal nutritional conditions (Duan, 1998)^[13].

5.2 Survivability, mortality and cannibalism

Survivability during the larval stage is one of the most critical challenges in the culture of *Channa* species such as *Channa striata*, *C. punctata*, and *C. micropeltes*. High larval mortality is commonly reported during the early post-hatch period, primarily due to nutritional stress, environmental fluctuations, and behavioral interactions such as cannibalism. The fragile physiological condition of larvae, coupled with an underdeveloped immune and digestive system, makes this stage particularly vulnerable, resulting in wide variations in survival rates across different rearing practices (War *et al.*, 2011)^[50]. Mortality in *Channa* larvae is strongly influenced by the timing and quality of first feeding. Failure to initiate exogenous feeding within the critical feeding window after yolk sac absorption often leads to starvation-induced mortality, which is irreversible even if feeding is resumed later. Several studies have demonstrated that delayed feeding significantly reduces survival due to depletion of endogenous energy reserves and impaired organ development (Arul, 1991)^[4]. Live feeds such as *Artemia*, rotifers, and cladocerans have been shown to enhance larval survival compared to artificial diets, as they are more readily ingested and digested, and stimulate feeding behavior (Samat *et al.*, 2020)^[46]. Cannibalism is a major behavioral cause of mortality in *Channa* larvae and juveniles and is considered a natural trait of this predatory species. Cannibalistic behavior usually begins shortly after the onset of exogenous feeding and intensifies with increasing size heterogeneity among larvae. Baras and Jobling, (2002)^[5] reported that size variation promotes aggressive interactions, where larger individuals prey upon smaller conspecifics, leading to significant stock loss. Cannibalism not only reduces survival but also creates further size disparity, thereby accelerating subsequent cannibalistic events (Bose, 2022)^[8].

Stocking density plays a dual role in influencing survivability and cannibalism in *Channa* larvae. High stocking densities often lead to increased stress, competition for food, and deterioration of water quality, which collectively elevate mortality rates. Conversely, extremely low densities may enhance encounter rates between individuals, increasing the likelihood of cannibalistic attacks. Studies on *Channa striata* larvae indicate that moderate stocking densities help minimize aggressive interactions while maintaining acceptable survival levels (Hutagalung *et al.*, 2019)^[22]. Environmental factors such as light intensity, water temperature, and dissolved oxygen also significantly affect larval survival and cannibalism. Elevated temperatures accelerate metabolism and aggression, which can increase cannibalistic tendencies if food availability is

insufficient. Similarly, high light intensity improves prey visibility but may also increase aggressive encounters among larvae. Reduced light conditions have been shown to lower cannibalism rates and improve survival in visually oriented predatory fish larvae like *Channa* (Mukai *et al.*, 2013)^[34]. Frequent grading and size sorting are effective management strategies to reduce cannibalism-related mortality in *Channa* larviculture. Removing larger individuals at regular intervals minimizes size dominance and aggressive predation. Additionally, increasing feeding frequency and ensuring feed size adequacy can reduce hunger-induced cannibalism by satisfying the high protein and energy requirements of rapidly growing larvae (Hseu *et al.*, 2003)^[21].

5.3 The haematological parameters

Haematological parameters are widely recognized as reliable indicators of nutritional status, health condition, and stress responses in fish. In the present study, variations in haemoglobin concentration, erythrocyte counts, leukocyte profiles, and erythrocyte indices among *Channa striata* fed different diets clearly demonstrate the influence of dietary quality on blood physiology. Similar observations have been reported in carnivorous fishes, where diet composition significantly alters haematological characteristics (Blaxhall, 1972)^[6]. The significantly higher haemoglobin (Hb) concentration and red blood cell (RBC) count observed in earthworm-fed *Channa striata* indicate improved oxygen-carrying capacity and enhanced metabolic activity. Earthworms are known to be rich in high-quality protein, iron, and essential amino acids, which are critical for erythropoiesis and haemoglobin synthesis. Sagada *et al.* (2017)^[45] reported improved haematological profiles in snakehead fish fed protein-rich natural diets, while Mekuria *et al.* (2022)^[32] emphasized that dietary protein quality directly influences haemoglobin levels and red blood cell production. In contrast, the comparatively lower Hb and RBC values in poultry waste-fed fish may be attributed to reduced digestibility and limited bioavailability of micronutrients required for blood formation. White blood cell (WBC) count, an important indicator of immune competence, was highest in earthworm-fed fish, suggesting enhanced immune responsiveness. Earthworms contain immunostimulatory compounds such as bioactive peptides and micronutrients that can stimulate leukocyte production. Khanjani *et al.* (2022)^[24] reported that nutritionally superior diets enhance leukocyte counts and immune function in fish. Conversely, lower WBC counts in poultry waste-fed fish may reflect mild physiological stress or inadequate immune stimulation, as reported by Martin and Król (2017)^[31], who linked nutritional stress to suppressed immune responses in fish. Haematocrit (Hct) values followed a similar trend, with earthworm-fed fish exhibiting higher percentages compared to control and poultry waste-fed groups. Elevated haematocrit values indicate increased blood volume and improved oxygen transport efficiency, which are often associated with better growth performance. Etim and Offiong, (2014)^[14] reported that optimal nutrition enhances haematocrit values, whereas stress and nutritional deficiencies reduce erythrocyte volume. The moderate haematocrit values observed in the control group suggest balanced nutrition, while lower values in poultry waste-fed fish indicate compromised physiological condition. Erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) provide insight into red blood cell morphology and functionality. Slightly lower MCV

and higher MCHC values in earthworm-fed fish suggest the formation of more efficient erythrocytes with better haemoglobin packing. He *et al.* (2023) [20] reported that nutritionally balanced diets result in optimized erythrocyte indices, improving oxygen delivery efficiency. The relatively stable MCH values across treatments indicate that haemoglobin content per cell remained consistent, while overall increases in Hb were primarily driven by higher RBC numbers. Differential leukocyte counts further support the superior physiological condition of earthworm-fed fish. Higher lymphocyte percentages indicate enhanced adaptive immune readiness, while lower neutrophil, monocyte, and eosinophil proportions suggest reduced stress and inflammatory responses. Yuan *et al.* (2017) [51] demonstrated that stress conditions in fish often lead to neutrophilia and lymphopenia, patterns that were more evident in poultry waste-fed fish. Thus, the leukocyte profile observed in earthworm-fed *Channa striata* reflects reduced stress and improved immune stability.

5.4 Water quality parameters

Water quality parameters play a decisive role in the survival, growth, and physiological stability of *Channa* fish larvae. During the larval stage, *Channa* species such as *C. striata*, *C. punctata*, and *C. micropeltes* are extremely sensitive to environmental fluctuations because of their high metabolic rate and underdeveloped osmoregulatory and detoxification systems. Even minor deviations from optimal water quality conditions can lead to stress, reduced feeding activity, impaired growth, and increased mortality (Kuppu *et al.*, 2018) [26]. Water temperature is one of the most influential factors governing larval performance in *Channa* species. The temperature measured in this experiment (table 4) is not much different from the results reported by Puspaningsih *et al.* (2019) [37], which stated that the snakehead can be cultured in temperatures from 20.5 °C to 28.8 °C. Optimal temperature ranges between 26-30 °C have been reported to support better metabolic efficiency, digestion, and growth in *Channa* larvae. Temperatures below or above this range adversely affect enzyme activity, oxygen consumption, and immune competence, leading to poor survival. Elevated temperatures may accelerate metabolism and aggression, thereby increasing stress and cannibalism, while lower temperatures can suppress feeding activity and growth (Volkoff and Rønnestad, 2020) [48]. Dissolved oxygen (DO) concentration is another critical water quality parameter affecting larval survival. Although *Channa* species possess accessory respiratory organs that enable them to tolerate hypoxic conditions as adults, larvae are still highly dependent on dissolved oxygen for respiration. DO levels below 4–5 mg L⁻¹ have been associated with increased stress, reduced feed intake, and higher mortality in larvae. Maintaining adequate aeration ensures efficient respiration, better feed utilization, and improved survival during larval rearing (Rosas *et al.*, 1998) [44]. Dissolved oxygen concentrations and pH values measured in this experiment are not much different from the ones reported by Rahman *et al.* (2012) [40], where dissolved oxygen concentrations ranged from 3.20 to 7.30 mg L⁻¹ and pH values ranged between 7.20 and 8.40. The pH of the rearing water significantly influences physiological processes such as respiration, ion exchange, and ammonia toxicity in *Channa* larvae. A near-neutral to slightly alkaline pH range (6.5–8.0) is considered optimal for larval rearing. Extreme pH levels can damage gill epithelium, disrupt acid–base balance, and reduce larval resistance to stress and disease. Moreover, higher pH levels increase the proportion of toxic unionized ammonia,

thereby intensifying its harmful effects on larvae (Wang and Leung, 2015) [49]. Nitrogenous wastes, particularly ammonia and nitrite, are among the most toxic water quality parameters in larval rearing systems. Larvae are especially sensitive to unionized ammonia (NH₃) : , which interferes with oxygen transport and damages gill tissues. Elevated ammonia levels often result from overfeeding, high stocking densities, and inadequate water exchange. Studies have reported that even low concentrations of ammonia can significantly reduce survival and growth in larval fish, including *Channa* species (Rodrigues *et al.*, 2007) [43]. Turbidity and accumulation of organic matter also influence larval performance by reducing water transparency and oxygen availability. Excess suspended solids may clog gill surfaces, impair respiration, and interfere with prey detection, especially in visually feeding larvae like *Channa*. Proper removal of uneaten feed and fecal matter through regular siphoning and water exchange is essential to maintain suitable water quality and prevent secondary stress-related mortality (Miner and Stein, 1993) [33].

6. Conclusions

The present study demonstrated that dietary treatments significantly influenced the growth, survival, and haematological responses of *C. punctata*. Among the tested diets, the earthworm-based feed (T₂) yielded superior growth performance, survivability, and haematological indices, while also reducing cannibalism compared to the control and poultry viscera waste diet (T₁). Water quality remained within acceptable limits throughout the trial, confirming that observed differences were attributable to dietary effects. These findings highlight the potential of earthworm-based diets as a sustainable and effective nutritional strategy for enhancing production and welfare in *C. punctata* aquaculture.

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