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EFFECTS OF IN VITRO MELATONIN EXPOSURE ON THE HEMATOLOGICAL AND PLASMA LIPID INDICATORS OF JUVENILE AFRICAN CATFISH

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Abstract: This study examined the impact of melatonin exposure on hematological and lipid indicators of juvenile African catfish. A total of 135 fish were randomized into three groups: 0-, 10-, and 30 mg/L of melatonin for 14 days, followed by 7 days of withdrawal. Hematological and lipid changes were determined using standard bioassays. The results showed that melatonin caused a significant elevation in total white blood cell counts and a significant reduction in red blood cell counts, packed cell volume, and hemoglobin concentration in a duration- and concentration-dependent manner ($p \leq 0.05$). Changes in WBC recovered after melatonin withdrawal, but RBC, PCV, and HB changes persisted. Furthermore, significant interactions were observed between melatonin concentration and exposure duration in all hematological indicators. However, no significant changes in plasma lipid indices were recorded ($p \geq 0.05$), which indicates that exogenous melatonin profoundly affects the hematology but not lipid metabolism of young catfish.

Keywords: Catfish, melatonin, circadian rhythm, hematology, lipid profile, supplementation.

INTRODUCTION

According to recent estimates, aquaculture and fisheries account for approximately 17% of the total animal-derived protein consumed by humans (FAO 1983; Boyd et al., 2022). The hope to increase capture based on current methods is declining as natural aquatic sources are currently being overexploited (Bodiguel et al., 2009; Ye and Gutierrez 2017; Costello et al., 2020), leaving farmed aquaculture to meet current demands. However, farmed aquaculture faces daunting challenges, including worsening climate change, availability of quality water supply, disease and parasite infestation, rising world population, increasing demand for seafood, the need for farming systems with less harmful effects on the environment, and availability of quality and affordable feed

(Chen et al., 2010; Kumar et al., 2018; Deng et al., 2020; Moyo and Rapatsa 2021; Yassien et al., 2022). Farmers have employed various approaches, including diet supplementation with synthetic hormones, such as melatonin, to boost productivity (Mosha 2018; Mustafa et al., 2020; Yu et al., 2022; Singh et al., 2024; Vijayaram et al., 2024).

Melatonin is the major neurohormone produced and secreted by the pineal gland in vertebrates (Gern et al., 1988; Bernard et al., 1997; Mandera et al., 1999; Somnez et al., 2023). With peak secretion coinciding with the onset of darkness (Grivas and Savvidou 2007; Kennaway et al., 2020; Qian et al., 2022), melatonin plays a central role in the induction and maintenance of the sleep-wake cycle in animals. Apart from its sleep-regulating functions, melatonin is a potent antioxidant and anti-inflammatory molecule (Korkmaz et al., 2012; Deng et al., 2020; Cho et al., 2021; Casper et al., 2024). Melatonin is secreted by the pineal gland and the eyes in fish (Cahil 1996; Besseau et al., 2006; Vuilleumier et al., 2007) and plays crucial roles in enhancing circulatory and immune functions (Acharyya et al., 2021; Adah et al., 2023).

Melatonin supplementation has been extensively used to improve aquaculture. For example, Samal et al. (2025) found that melatonin concentrations below 50 mg improved the reproduction and growth performance of ornamental fish (*Devario aequipinnatus*), whereas concentrations above 50 mg suppressed gonadosomatic indices. This study also revealed that melatonin supplementation significantly increased hematological variables, such as packed cell volume, hematocrit, and white blood cell counts. Another study by Lv et al. (2024) found that higher melatonin supplementation (up to 240 mg/kg) for 70 days significantly improved growth performance parameters and serum antioxidant enzyme markers in the rice field eel (*Monopterus albus*). In contrast, Amri et al. (2020) found that melatonin supplementation (40 mg/kg) strongly suppressed all growth performance indices in gilthead sea bream (*Sparus aurata* L.) reared under standard rearing conditions. Furthermore, feed supplementation with 50 and 200 mg/kg of melatonin did not affect the growth performance of juvenile Nile tilapia (*Oreochromis niloticus*) but reduced plasma glucose levels, total protein, and aspartate aminotransferase activities (Veisi et al., 2021). Feed supplementation with 329.2 mg/kg melatonin significantly decreased the total crude lipid content in Pacific White shrimp (*Penaeus vannamei*) (Ye et al., 2024). These earlier studies show that the effect of melatonin varies depending on the species, concentration, sex, biological system under investigation, or a combination of factors. Although melatonin is considered relatively nontoxic (Zetner et al., 2018), there are increasing reports of melatonin-associated toxicity in humans.

A recent study found that 2 mg/L of melatonin supplemented in fish for 1 month modulated hematological indices in response to transportation-induced stress (Adah et al., 2023). However, the impact of melatonin exposure on the hematology and lipid profile of juvenile catfish in the active growth and development stage has not been determined. We recently reported that in vitro exposure of juvenile catfish to melatonin caused profound changes in antioxidant and oxidative stress enzyme levels (Asogwa et al., 2025). However, it is unclear whether in vitro exposure of juvenile African catfish to melatonin has any impact on hematological and lipid profile indices. In the present study, we report additional data on the effects of in vitro melatonin exposure on the hematological and lipid profile parameters of juvenile African catfish for 14 days, followed by 7 days of melatonin removal. This adds to our growing body of data profiling the impacts of exogenous melatonin on juvenile African catfish (*Clarias gariepinus*) physiological, biochemical, and behavioral variables. Our findings further inform the use of melatonin supplementation in general aquaculture practices.

2.0 MATERIALS AND METHODS

2.1 Procurement and feeding of juvenile African catfish

The juvenile African catfish (*Clarias gariepinus*) used in this study weighed between 18-22 g and measured between 10-14 cm in length. They were purchased from Freedom Fisheries and Farms in Nsukka, Enugu State, Nigeria, and transported to the Fisheries Unit at the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The fish were placed in well-aerated plastic aquaria with a capacity of 500 L, filled with clean tap water at a temperature of 28.03 ± 0.6 ^oC. The fish were fed 5% of their body weight twice daily at 8 AM and 4 PM, with Aller Aqua Fish Feed containing 45% crude protein. The fish were acclimatized for 14 days prior to the study. Mortality was monitored in each experimental tank daily to prevent fouling.

2.2 Experimental design and melatonin exposure in fish

A total of 135 juvenile African catfish were used in this study and were randomly divided into three experimental groups that received 0, 10, and 30 mg/L of melatonin (Melatonin, 10 mg/capsule, Mason Vitamins Incorporated, USA). Each experimental group was further subdivided into three replicates with 15 fish per replicate. The fish were exposed in vitro to melatonin in a static renewal bioassay system where the test melatonin concentrations were renewed daily for the first 14 days, followed by a 7-day withdrawal period in which melatonin was removed from the aquaria (totaling 21 days of experimentation) (Asogwa et al., 2025). The water-borne route of melatonin exposure was employed to avoid potential changes in its chemical nature and increase the product's bioavailability to other organs, such as the gills, in which melatonin receptors have been identified (Confente et al., 2010). Throughout the melatonin exposure and withdrawal periods, the photoperiod was maintained at a 12-h light/dark cycle.

2.3 Blood sample collection and blood plasma separation

Blood samples were obtained on days 0 (baseline), 7, 14, and 21 (withdrawal) to assess the impact of in vitro melatonin exposure on various hematological and lipid profile indicators. Three fish were randomly selected from each of the three replicates in a group on each sampling day. The fish were gently wrapped in a damp cloth, with only the tail region exposed, and blood samples were taken from the caudal vein without anesthesia. Blood collection was completed within 2 min to reduce the potential stress caused by handling (Kole et al., 2022). After blood collection, plasma was separated as previously described (Asogwa et al., 2025).

2.4 Determination of changes in hematological variables

Changes in red blood cell (RBC) and white blood cell (WBC) counts in response to melatonin exposure were determined using an improved hemocytometer as described by Dacie and Lewis (1991). Red and white blood cell counts were determined using an improved Neubauer counting chamber. Blood samples containing heparin anticoagulant were drawn into the RBC pipette just below the 1.0 mark. Next, the dilution fluid (Rees-Ecker solution) was drawn into the pipette to create a dilution of 1:200 and 1:100 for RBC and WBC counts, respectively, before being introduced into the counting chamber of the Neubauer counter. RBCs were counted in five 1 mm squares, while WBCs were counted in four squares using the 10x objective of an inverted light microscope. Two readings were taken for each sample, and the mean values were calculated. The total RBC and WBC counts were then determined as follows:

$$RBC/WBC = \frac{Total\ number\ of\ cells\ counted}{Number\ of\ squares\ counted} * Dilution\ factor * (\frac{1}{volume\ of\ 1\ sqquare})$$

RBC/WBC = (Total number of cells counted)/ (Number of squares counted) dilution factor (1/ (volume of 1 square))

The PCV was determined using the microhematocrit method as previously described (Blaxhall and Daisley 1973; Wedemeyer and Yasutake 1977). This method is based on the differential sedimentation rate of various blood components. Blood samples were introduced into capillary tubes and centrifuged in a microhematocrit centrifuge for 10 min. Then, the packed cell volume was read off as a percentage. Finally, changes in hemoglobin concentration following melatonin exposure were determined using a diagnostic kit based on the cyanhemoglobin method (Vankampen and Ziglstra 1961). In this method, 20 µl of each blood sample was mixed with 5 mL of Drabkin's solution in a test tube and incubated at room temperature for 15 min. The optical absorbance of the sample reaction, blank, and a cyanhaemoglobin solution was then determined at 540 nm using a spectrophotometer. The total haemoglobin concentration was estimated by comparing the absorbance of the sample to that of the standard.

2.5 Determination of changes in plasma lipid levels

Blood samples were transferred to an ethylenediaminetetraacetic acid sample collection vial, and blood plasma was extracted by centrifugation at 3500 rpm and 4°C for 15 min. The lipid profile indices were quantified using commercial diagnostic kits from Randox in Germany, following established procedures: total cholesterol (Roeschlau et al., 1974). This method is based on the enzymatic quantification of both free and esterified cholesterol in tissue samples, which is first achieved by the enzyme cholesterol esterase, which hydrolyzes cholesterol esters into cholesterol. Next, cholesterol is oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. Briefly, 50 µl of diluted cholesterol standard or plasma sample was added into separate wells of a microplate reader. Next, 50 µl of a cholesterol Reaction Reagent was added to each microplate well, thoroughly mixed, covered to protect from light, and finally incubated at 37°C for 45 min. Then, the optical absorbance of the standards or samples was read at 540 nm using a microplate reader. The total cholesterol in each sample was calculated by comparing the samples' absorbance to a cholesterol standard.

Total plasma HDL-c was determined using a diagnostic kit based on the method described by Alberts et al. (1978). This method is based on solubilizing HDL with a detergent to allow HDL-c to react with cholesterol esterase, cholesterol oxidase, and a chromogen to produce a colored product. To do this, 300 µl of RI reagent was added to separate microplate wells containing 3 µl each of a standard and sample, while 3 ul of distilled water was added to a well that served as a blank. The contents of each well were thoroughly mixed, and the microplate was incubated at 37°C for 5 min. The optical absorbance of each reaction was read at 570 nm. The total HDL-c in each sample was estimated by comparing its optical absorbance to those of standards with known concentrations.

Furthermore, changes in plasma triglyceride concentration following melatonin exposure were determined using a diagnostic kit according to Tietz (1976). The principle involves the hydrolysis of triglyceride bonds by lipase, producing glycerol. Next, glycerol is phosphorylated and oxidized to produce hydrogen peroxide, which reacts with a colorimetric probe. To do this, 10 µl of diluted triglyceride standards or samples were added to separate wells of a microplate. Next, 90 µl of the Reaction Mix was added to the above wells, thoroughly mixed, shielded from light, and incubated at room temperature for 30 min on an orbital shaker. Thereafter, the optical absorbance of the resulting reaction was read at 570 nm, and the total TG concentration was calculated by comparing the optical absorbance of the sample to that of standards with known concentrations.

Finally, plasma low-density lipoprotein cholesterol was calculated using the following formula:

LDL=Total cholesterol-HDL/5-TAG

$$LDL = Total\ cholesterol - \frac{HDL}{5} - TAG$$

2.6 Statistical analysis of the data

Following the conclusion of juvenile catfish exposure and withdrawal from melatonin, the effects of exposure duration and melatonin concentration on changes in hematological and serum lipid profile indices of juvenile catfish were first separately analyzed using a one-way analysis of variance (ANOVA). The Duncan Multiple Range Test was then used to identify differences in mean values across different days and groups. Next, a two-way analysis of variance (ANOVA) was conducted to test the overall effect of time, dosage, and their interaction on the hematological and lipid profile parameters. All statistical comparisons were considered significant at $p \leq 0.05$, and the results are presented as mean ±standard error of the mean (SEM). All statistical analyses were performed using the Statistical Package for the Social Sciences software for Windows (SPSS, version 20.0 for Windows, IBM Statistics, USA).

3.0 RESULTS

3.1 Effects of melatonin on changes in red blood cell counts

Exposure of juvenile catfish to 0, 10, and 30 mg/L of melatonin resulted in concentration-dependent reductions in red blood cell counts (RBCs) on days 7 and 14 compared with values on day 0 (Figure 1A). This trend continued seven days after melatonin withdrawal (day 21). Interestingly, RBC values showed a tendency to increase, but the rise did not reach significant levels compared with the control group (day 21, Figure 1A). Similarly, there was a time-dependent decrease in the RBC values of juvenile fish in the 10 and 30 mg/L groups on days 7 and 14 compared with the day 0 values (Figure 1B). A two-way analysis of variance indicated significant effects of time ($p = 3.13 \times 10\text{-}13$), dosage ($p = 2.37 \times 10\text{-}15$), and the interaction between exposure duration and melatonin concentration ($p = 1.25 \times 10\text{-}10$).

3.2 Effects of melatonin on the changes in the packed cell volume

The effects of juvenile fish exposure to the test melatonin were evident in the significant concentration-dependent reduction in packed cell volume (PCV) on days 7 and 14 compared with that on day 0 (Figure 2A). However, after a 7-day withdrawal period, PCV values were restored to non-significant levels in the 10 and 30 mg/L groups compared with the control (Figure 2A). Across different groups, a similar tendency toward a reduction in PCV values was observed from day 7 to day 21; this is contrary to the gradual elevations in PCV values seen in the control group from day 7 to day 21 (Figure 2B). The two-way ANOVA showed statistically significant effects of exposure duration ($p = 2.47 \times 10\text{-}8$), melatonin concentration ($p = 2.08 \times 10\text{-}9$), and the interaction between exposure duration and melatonin concentration ($p = 1.05 \times 10\text{-}6$).

3.3 Effects of melatonin on hemoglobin concentration changes

Exposing juvenile fish to different concentrations of melatonin for 14 days resulted in concentration-dependent reductions in hemoglobin (Hb) levels in fish exposed to 10 and 30 mg/L on days 7 and 14 compared with the 0 mg/L group (Figure 3A). After 7 days of melatonin withdrawal, Hb values tended to restore toward day 0 levels, but the changes remained significant. However, no changes in Hb concentrations were observed on day 0 (Figure 3A). The impact of exposure duration on the Hb of fish was evident in a decline in Hb values in the 10 and 30 mg/L groups on days 7 and 14 compared with that on day 0. In contrast, Hb values appeared to increase in the control fish from day 7 to day 21 (Figure 3B). The two-way analysis of variance indicated significant effects of exposure duration ($p = 9.10 \times 10\text{-}9$), melatonin concentration ($p = 2.61 \times 10\text{-}7$), and an interaction between concentration and exposure duration ($p = 4.12 \times 10\text{-}6$) on Hb levels.

3.4 Effects of melatonin on WBC counts

Compared with changes in control fish, significant concentration-dependent elevations were observed in the total white blood cell (WBC) counts of fish exposed to 10 and 30 mg/L of melatonin on days 7 and 14 (Figure 4A). However, following a 7-day withdrawal period, WBC counts reversed to non-significant levels in the melatonin-exposed groups compared with the control values (Figure 4A). Similarly, significant duration-dependent elevations in the WBC values occurred in the melatonin-exposed fish on days 7 and 14 compared with day 0 counts (Figure 4B). However, the WBC count on day 21 was not significant compared with the day 0 values (Figure 4B). In contrast, the control juveniles did not experience significant changes in WBC counts for the entire duration of the experiment. Further investigation using a two-way analysis of variance showed significant effects of exposure duration ($p = 0.007$) and melatonin concentration ($p = 8.93 \times 10\text{-}5$), and an interaction between exposure duration and melatonin concentration ($p = 0.027$).

3.5 Effects of melatonin on juvenile catfish plasma lipid indices

Unlike the profound changes in most hematological indices analyzed in this study, the in vivo exposure of juvenile catfish to 10- and 30 mg/L of melatonin did not cause any significant changes in total cholesterol (CHOL), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol levels (Tables 1-4). However, there was a significant increase in the HDL values over time in fish exposed to 30 mg/L of melatonin on day 21 compared to day 0 (Table 3). Additionally, there was a concentration-dependent increase in LDL levels in fish exposed to 30 mg/L of melatonin on day 21 compared with the control group (Table 4).

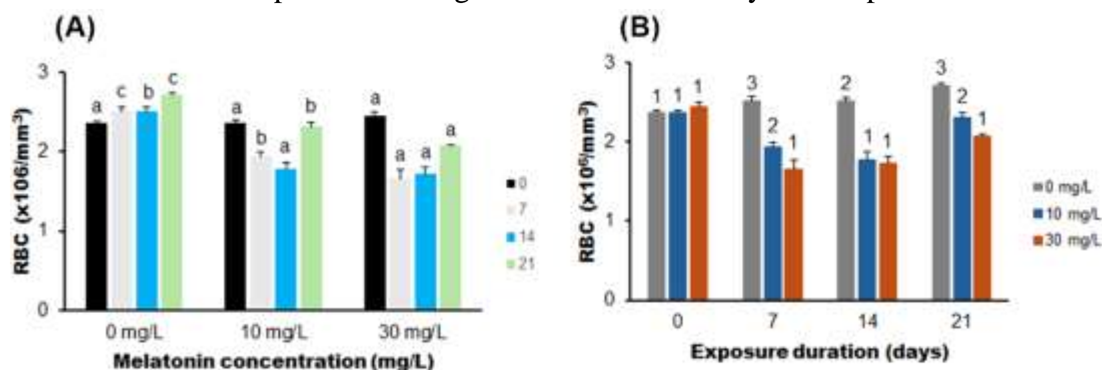

Figure 1: Changes in the red blood cells of juvenile melatonin-exposed catfish. All plotted data represent mean ± standard error of the mean (SEM). Top right: Exposure duration. Similar patterned bars with different numbers on top across groups are significantly different ($p \leq 0.05$), whereas different patterned bars with different letters within a group are significantly different ($p \leq 0.05$).

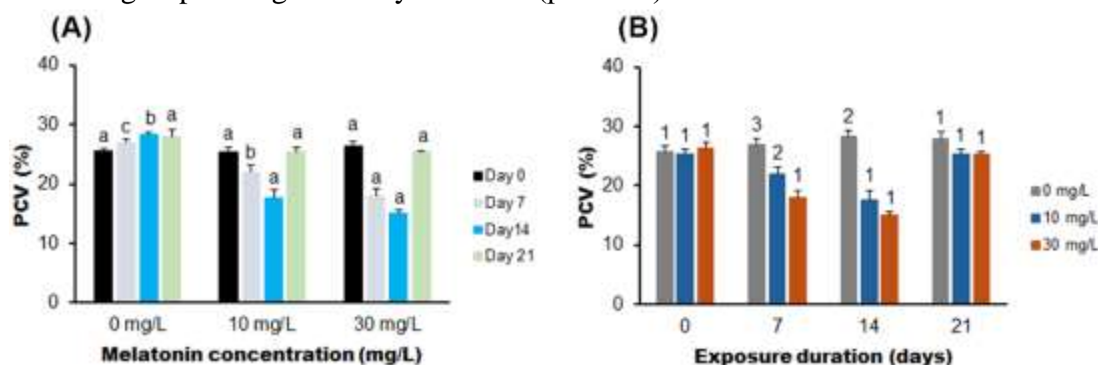

Figure 2: Changes in the packed cell volume of juvenile melatonin-exposed catfish. Top right: Exposure duration. Similar patterned bars with different numbers on top across groups are significantly different ($p \leq 0.05$), whereas different patterned bars with different letters within a group are significantly different ($p \leq 0.05$).

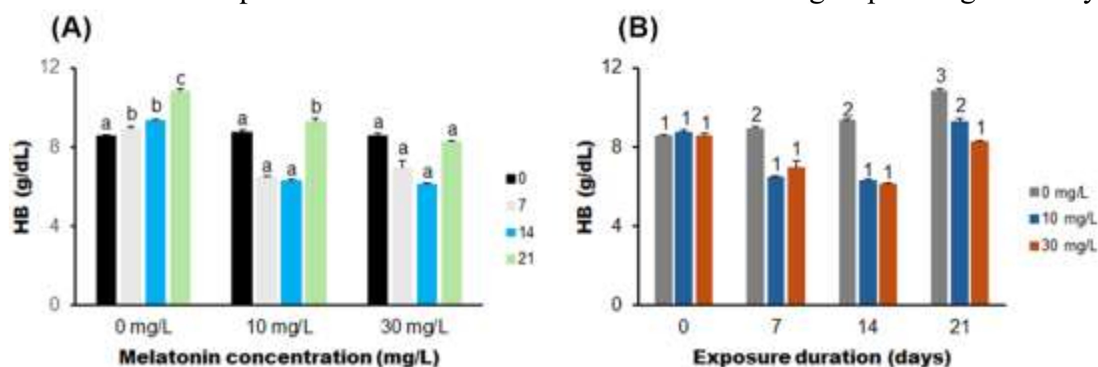

Figure 3: Changes in hemoglobin concentration of juvenile melatonin-exposed catfish. Top right: Exposure duration. Similar patterned bars with different numbers on top across groups are significantly different ($p \leq 0.05$), whereas different patterned bars with different letters within a group are significantly different ($p \leq 0.05$).

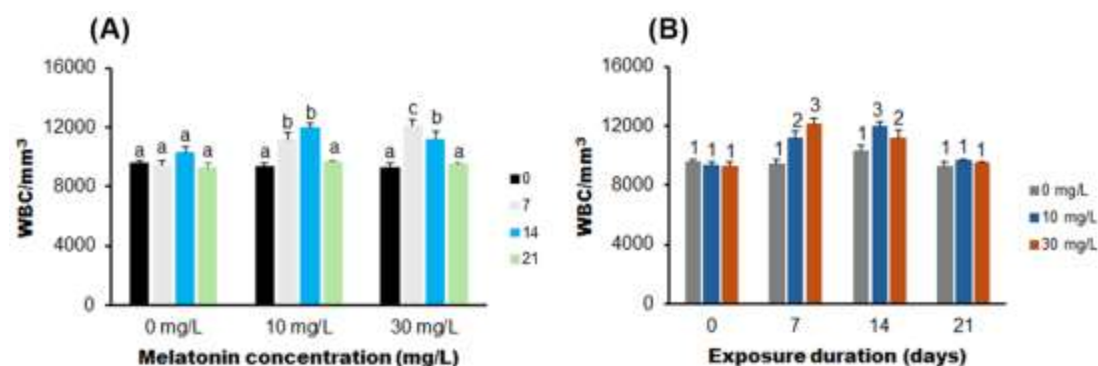

Figure 4: White blood cell count changes in juvenile catfish exposed to melatonin. Top right: Exposure duration. Similar patterned bars with different numbers on top across groups are significantly different ($p \leq 0.05$), whereas different patterned bars with different letters within a group are significantly different ($p \leq 0.05$).

Table 1: Changes in plasma total cholesterol concentration in juvenile catfish exposed to melatonin.

Concentration of melatonin (mg/L)	Total cholesterol concentration (mg/dL)			
	day 0	day 7	day 14	day 21
0	93.33 ± 1.76^{a2}	94.00 ± 2.08^{a1}	90.33 ± 3.93^{a1}	94.00 ± 4.16^{a1}
10	86.33 ± 2.08^{a1}	91.33 ± 2.91^{a1}	87.67 ± 1.86^{a1}	88.00 ± 1.15^{a1}
30	93.33 ± 2.40^{a2}	94.33 ± 2.20^{a1}	96.33 ± 0.67^{a1}	89.00 ± 3.61^{a1}

Values are expressed as mean ± standard error of the mean. Mean values with different letters in a row are significant ($p \leq 0.05$), whereas mean values with different numbers in a column are significant ($p \leq 0.05$). These statistical criteria hold for the data in Tables 3 and 4.

Table 2: Effects of melatonin on the plasma TG concentration of juvenile catfish.

Concentration of melatonin (mg/L)	TG concentration (mg/dL)			
	day 0	day 7	day 14	day 21
0	107.33 ± 4.37^{a1}	110.67 ± 4.37^{a1}	116.33 ± 5.36^{a1}	111.33 ± 2.91^{a1}
10	111.67 ± 2.03^{a1}	108.67 ± 1.76^{a1}	114.00 ± 2.52^{a1}	$111.67 \pm I.86^{a1}$
30	113.00 ± 2.31^{a1}	112.67 ± 3.38^{a1}	110.33 ± 3.38^{a1}	115.33 ± 4.70^{a1}

Table 3: Plasma high-density lipoprotein cholesterol (HDL-c) concentrations in juvenile catfish exposed to melatonin

Concentration of melatonin (mg/L)	HDL-c (mg/dL)			
	day 0	day 7	day 14	day 21
0	64.00 ± 1.15^{a1}	61.33 ± 0.88^{a1}	64.33 ± 4.10^{a1}	76.33 ± 1.67^{b1}
10	64.33 ± 2.33^{a1}	64.00 ± 1.15^{a1}	65.00 ± 4.51^{a1}	70.67 ± 4.26^{a1}
30	63.00 ± 0.58^{a1}	62.00 ± 0.58^{a1}	70.33 ± 0.88^{b1}	72.00 ± 1.15^{b1}

Table 4: Impact of melatonin exposure on plasma low-density lipoprotein cholesterol (LDL-c) concentration in juvenile catfish.

Concentration of melatonin (mg/L)	LDL (mg/dL)			
	day 0	day 7	day 14	day 21
0	16.00 ± 3.06^{a1}	17.00 ± 1.53^{a1}	23.00 ± 2.08^{a1}	18.33 ± 1.20^{a1}
10	19.33 ± 0.88^{a1}	20.67 ± 1.76^{a1}	22.00 ± 0.58^{a1}	18.00 ± 1.73^{a1}
30	20.00 ± 2.31^{a1}	20.33 ± 0.88^{a1}	21.00 ± 1.53^{a1}	23.67 ± 0.88^{a2}

4.0 DISCUSSION

As an extension of our earlier investigation (Asogwa et al., 2025), we examined the effects of in vitro melatonin exposure on the hematological and lipid indices of juvenile catfish for 14 days, followed by a 7-day withdrawal period. The results show that while there was no appreciable impact of melatonin on lipid profile indices, the tested melatonin concentrations reduced the red blood cell counts, packed cell volume, and hemoglobin concentrations. The reduction in red blood cell counts persisted for 7 days after melatonin removal. On the other hand, a pronounced elevation in the white blood cell counts was observed during the exposure period, a trend that returned to non-significant levels after melatonin removal.

Routine analysis of changes in hematological indices provides relevant information about the number, size, stage of maturity, and distribution of different cell types, enhancing the assessment of normal growth and development and the diagnosis of anemia, bleeding, cancer, dehydration, inflammation, and substance-induced organ damage (Stalling and Haine, 1982; Hao et al., 2017; López-Verdugo et al., 2020; Ghayyur et al., 2021). A direct examination of the impact of exogenous melatonin on fish hematology is largely lacking. Adah et al. (2023) conducted the closest study on the effects of exogenous melatonin on hematological indices of African catfish. They compared various hematological indices in two groups of adult catfish: one that received 2 mg/L of melatonin supplemented in feed and another that received no melatonin for one month. After melatonin supplementation, these fish were fasted overnight and transported for 100 km in 3 h, after which various hematological indices were compared pre- and post-transportation. They reported significantly higher post-transport red and white blood cell counts, packed cell volume, and hemoglobin concentration. Although they did not explicitly state whether hematological indices were compared between the two groups before transport, the

reported values suggested that melatonin supplementation for 1 month resulted in higher red and white blood cell counts, packed cell volume, and hemoglobin concentration, indicating a positive hematopoietic potential for melatonin in adult catfish. In contrast, our present results show that in vitro melatonin administration in juvenile catfish for 14 days persistently reduced RBC, PCV, and HB levels even after melatonin removal. These discrepancies could be due to differences in the age of the fish and the route of melatonin administration: since Adah et al. (2023) supplemented 2 mg/L of melatonin in the diet of adult catfish, whereas the present study adopted an in vitro assay in which 10 and 30 mg/L of melatonin was dissolved daily in aquarium water containing juvenile catfish for 14 days. Consequently, exposing juvenile catfish to the higher melatonin concentration used in our study could have created a toxic environment for young fish, leading to persistent negative hematopoietic responses. Similar to Adah et al. (2023), however, we found a significant elevation in the total white blood cell counts of juvenile catfish, which was restored to non-significant levels following melatonin withdrawal, suggesting that exogenous melatonin could have an immune system-boosting effect in young catfish. Contrary to our findings, Samal et al. (2025) reported that melatonin supplementation (up to 100 mg/100 g of feed) resulted in elevated RBC, PCV, and Hb levels in ornamental fish (*Devario aequipinnatus*). However, melatonin exposure caused significant leukocytosis. Apart from differences in the model species, brand, and melatonin concentration used, whether Samal et al. used juveniles or adults for their investigation remains unclear. A previous study found that RBC counts were higher in rainbow trout (*Oncorhynchus mykiss*) during winter when reductions in day length parallel prolonged melatonin secretion and an increase in oxygen solubility (Morgan et al., 2008). Their study indicated that the higher oxygen solubility in colder waters could explain the higher red blood cell concentration in the test fish. In contrast, there seems to be a consensus that white blood cell counts in salmonids and the tench, *Tinca tinca*, are higher in winter than in summer (Slater and Shreck 1998; Collazos et al., 1998; De Pedro et al., 2005). Our current findings that exogenous melatonin depressed RBC variables while elevating WBC counts mirror previous investigations in rainbow trout, salmonids, and the tench, suggesting that exposing juvenile catfish to the test melatonin in the current study modulated the actions of endogenously produced melatonin in a manner akin to recorded seasonal dynamics. However, changes in oxygen solubility following melatonin exposure were not measured in the current study; therefore, future investigations should ascertain a potential causal relationship between exogenous melatonin and oxygen solubility. In other studies, feed supplementation with 40 mg/kg of melatonin suppressed growth performance indices in the farmed gilthead sea bream *Sparus aurata* L. (Lv et al., 2024), whereas higher supplementation levels (50 and 200 mg/kg) had no significant impact on the growth performance of juvenile Nile tilapia (*Oreochromis niloticus*) while reducing plasma glucose levels, total protein, and aspartate aminotransferase activities (Veisi et al., 2021). However, unlike our current study, previous investigations on the impact of melatonin on the growth performance of various fish species did not examine changes in HIs. As a result, we do not know to what extent the melatonin concentrations used in those studies affected the hematological variables of fish. The impacts of melatonin exposure appear to depend on factors such as brand, concentration used, exposure time, test model and stage of development, and the mode of melatonin exposure. These concerns should be addressed.

In rodent models, diet supplementation with 50 mg/kg of melatonin reversed aluminum chloride-induced reduction in white blood cell count, red blood cell count, packed cell volume, and hemoglobin concentration (Almarzany 2020). Other studies have suggested that melatonin has anti-inflammatory/protective roles (Koc et al., 2002; Ozmerdivenli et al., 2011; El-Sayed et al., 2019; Hajam et al., 2020). However, El-Sayed et al. (2019) reported that oral administration of 10 mg/kg melatonin reduced lithium chloride-induced leukocytosis in adult

male albino rats. In chicken (Hubbard strain), diet supplementation with 20, 30, and 40 mg/kg caused a significant elevation in RBC count, PCV, Hb concentration, total white blood cell counts, and lymphocyte percentage (Essawy et al., 2011), further supporting the idea of a protective role for melatonin. Although the reduction in RBC, PCV, and Hb observed in our study seems not to agree with the hematoprotective hypothesis for melatonin, the pronounced leukocytosis recorded could be a beneficial anti-inflammatory response. In addition, this study revealed strong interactive effects of melatonin concentration and exposure duration, suggesting that the concentration of melatonin to which fish were exposed and the exposure duration contributed to the profound hematological changes observed in juvenile fish. The obvious discrepancies between our findings and previous studies could be due to the experimental design, test model, age of the test model, route/mode of administration, or the brand of melatonin used. For instance, most previous studies evaluated the impact of melatonin in the presence of substance-induced organ or hematological damage, whereas others had melatonin compounded in diets or injected intraperitoneally. In contrast, our study investigated the impact of melatonin on juvenile catfish in a static renewal bioassay system where the test substance was introduced afresh daily. Although we do not have sufficient evidence to suggest that the observed changes in hematological indices were due to inherent toxicity of the tested melatonin, there have been increasing cases of melatonin-associated poisoning reported to the US Food and Drug Administration (FDA) (Lelak et al., 2022). In addition, it was recently revealed that the actual amount of melatonin present in commercially available melatonin ranged from 74% to 347% (Cohen et al., 2023). This could result in unintended toxicity due to overdose or contamination with undeclared substances. Unlike the profound changes in hematological indices, we found no significant alterations in any of the examined plasma lipid indices examined. Previous studies have reported that melatonin has hypolipidaemic potential in various models (Aoyama et al., 1988; Mori et al., 1989; Chan et al., 1995; Barquilla et al., 2014; Mohammadi-Sartang et al., 2018; Loloei et al., 2019). Specifically, Mori et al. (1989) found that a daily intraperitoneal injection of 4 mg of melatonin had no significant effects on various lipid indices in rats fed a normal diet, but the same procedure prevented diet-induced hyperlipidemia. It is possible that acting alone, exogenous melatonin is not sufficient to elicit profound changes in cholesterol regulatory pathways in healthy subjects, but melatonin application is essential to trigger a restorative response in the presence of substance-induced hyperlipidemia. In line with this idea, Chan et al. (1995) reported that the protective functions of 12.5 mg/kg melatonin became evident upon the induction of hypercholesterolemia in rats. Although melatonin was administered in vitro in the present study, the observed effects on plasma lipid indices could not be attributed to the route of administration. However, we recommend that longer exposure periods be tested.

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Conflict of interest

We have no conflicts of interest to declare.

Ethical statement

Permission to conduct the experiments was obtained from the Faculty of Biological Sciences Ethics Committee on Animal Experiments. This committee ensures strict compliance with the regulations for animal welfare in Nigeria, which guarantees the humane treatment and management of experimental animals.

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