Volume 3, Issue 8, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

EFFECT OF MELATONIN ON GROWTH, GONADOSOMATIC INDEX AND HEMATOLOGICAL PROFILE OF NILE TILAPIA

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DOI: https://doi.org/10.5281/zenodo.17947509

Keywords

Article History
Received: 17 August 2025
Accepted: 27 September 2025
Published: 10 October 2025

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Abstract

Development of the gonads and the pubertal development process in fish is closely intertwined with the prevailing light conditions. Findings revealed that the administration of melatonin have any significant effects on the growth of the experimental fish, as there were no notable differences in somatic growth compared to the control group. However, when we treated female tilapia with a high dose of melatonin (1.7 mg/kg BW), observed a reduction in spawning frequency, the number of eggs spawned, and the gonadosomatic index. In male tilapia, a similar high-dose melatonin treatment led to a decrease in sperm count, spermatocrit levels, and spermatozoa activity index. These outcomes suggest that exogenous melatonin may replicate the effects of a shortened light photoperiod, which typically suppresses reproductive activity in this particular fish species. In summary, the results indicate that carefully optimized melatonin treatment could potentially be employed as a means to control the spawning behavior of this species.

INTRODUCTION

Fisheries and aquaculture, recognized as one of the swiftest protein production sectors, is a pivotal role in addressing the paramount challenge(Pauly and Zeller 2017) providing sustenance for a global projected population of 9.6 billion by the year 2050.. The contemporary trajectory of fish farming underscores an unparalleled push for escalated production. On aworldwide scale, delving into the intricacies of fish physiology becomes imperative to amplify productivity. (Bromage, Porter et al. 2001) emphasize fish reproduction's seasonal nature, a well-established facet. In India, a nation marked by distinct seasonal fluctuations, the orchestration of reproductive timing appears linked to the hypothalamus-pituitary-gonadal axis (Barton 2002). Endocrine regulation of the reproductive cycle involves hormones originating from the hypothalamus, pituitary, and gonads (Maitra, Seth et al. 2006). Notably, the pineal organ emerges as the intermediary transmuting photoperiodic cues into melatonin(Paul, Zucker et al. 2008). While melatonin, a hormone, is associated with a range of organismal physiological processes, its involvement in fundamental functions within fish remains relatively underexplored. Limited insight exists concerning how melatonin contributes to regulating pivotal physiological aspects like the growth of organisms, their metamorphosis, reproduction, ability to respond to stress, and resistance to diseases are all crucial aspects of

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

their biological functions. This article compiles existing understanding of melatonin's role, particularly spotlighting the impact of external administration on fish physiological functions. This synthesis proposes melatonin as a potential candidate for future applications within the aquaculture industry. Aquaculture has witnessed a consistent growth in recent periods, emerging as a notable source of premium protein (Rana, Siriwardena et al. 2009). Nile tilapia has garnered significant research attention due to its rapid reproductive rate, adaptability to challenging environments, disease resistance, and suitability for varied cultivation techniques (Yosef 2009). Within breeding populations, female tilapia exhibit distinct patterns of ovarian development, leading to asynchronous spawning intervals of approximately in span of three to four weeks, contingent on ecological factors(Coward and Bromage 1999). Notably, tilapia has a tendency to attain sexual maturity and initiate reproduction at an early stage, often before reaching the desired market proportions(Longalong, Eknath et al. 1999). This phenomenon can also involve the suppression of maturation mechanisms (Ginés, Afonso et al. 2004).

Nile tilapia (Oreochromis Niloticus) holds significant economic importance as a food source, with its cultivation rapidly expanding worldwide due to its adaptability to diverse environmental conditions and intensified farming practices(Ponzoni, Nguyen et al. 2011). However, the introduction of stressors like silver nanoparticles into aquatic environments can jeopardize the health, well-being, and growth of this species. Therefore, the current investigation was directed towards to explore the effectt of melatonin administered through the diet on growth performance, plasma biochemical parameters, and liver enzyme activities, and assess the potential mitigating effects of melatonin on aquatic toxicity induced by varying concentrations of AgNPs, by evaluating plasma and liver biochemical indicators in Nile tilapia. Tilapia aquaculture is undergoing a dynamic phase of expansion globally, driven by the need to meet both local and international market demands. Following carp, tilapias have emerged as the second most prominent group of cultivated fish worldwide. In 2007, the global production of farmed tilapia surpassed 2.5 million tons, with cultivation spanning more than 85 countries. Notably, a significant majority of this production originates from Asia, contributing up to 80% of the global output. This growth is evident in the remarkable year-on-year escalation in tilapia production within leading nations. For instance, China (including Taiwan) has witnessed a remarkable surge, elevating tilapia production from 44,832 tons in 1980 to well over 1.2 million tons in 2007. The Philippines, once a notable tilapia producer, steadily increased its production to around 240,000 tons in 2007. Notably, the introduction of tilapia has triggered notable shifts in dietary preferences in some regions. For instance, in the Philippines, there has been a discernible transition towards favoring the consumption of tilapia over the traditional choice of milkfish. While various tilapia species find themselves under cultivation, the Nile tilapia (Oreochromis Niloticus Linnaeus, 1758) holds sway as the most extensively favored option in over 50 countries. In recent times, it has commanded a significant presence, contributing to more than 80% of the global tilapia production. However, it's worth noting that the precise portion of worldwide Nile tilapia production is estimated at around 50–60%. This estimation considers that China, a prominent tilapia producer, predominantly reports all of its production as Nile tilapia, whereas the reality encompasses the utilization of an important strain – the hybrid between Nile tilapia and blue tilapia (FRANCIS 2018).

Melatonin showcases a robust daily circadian pattern, with a substantial production surge occurring during the dark phase of the photoperiod. Within fish, melatonin's effects are mediated via receptors with high affinity(Falcon 2007). The oscillations of melatonin levels across organisms underscore its engagement in circadian rhythms and imply its role in governing a wide array of behavioral and physiological occurrences within vertebrate(Liebmann, Wölfler et al. 1997). Notably, the synthesis of melatonin has also been

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

observed in various human cell types, including peripheral blood mononuclear leukocytes(Carrillo-Vico, Calvo et al. 2004), bone marrow cells in both mice and humans (Conti, Conconi et al. 2000), as well as human lymphocytes. Nonetheless, the precise functional implications of melatonin derived from immune cells remain ambiguous, and comprehensive investigations into immune cell-derived melatonin within fish are still pending. In addition to the pineal gland, other sources of melatonin include the retinal and gastrointestinal tract (GIT), both of which contribute significantly to melatonin production. This has been established through studies on fish following pinealectomy. Among the non-pineal sources of melatonin, the gastrointestinal tract (GIT) stands out as the most plentiful producer (Bubenik 2008). The presence of melatonin-synthesizing enzymes, specifically NAT enzyme (Raikhlin and Kvetnoy 1974) and Hydroxyindole-O-methyltransferase present in the gastrointestinal tract (GIT) and retina validates the occurrence of extra-pineal melatonin synthesis. Recent validation via t-polymerase chain reaction further reinforces this finding(Hong and Pang 1995).

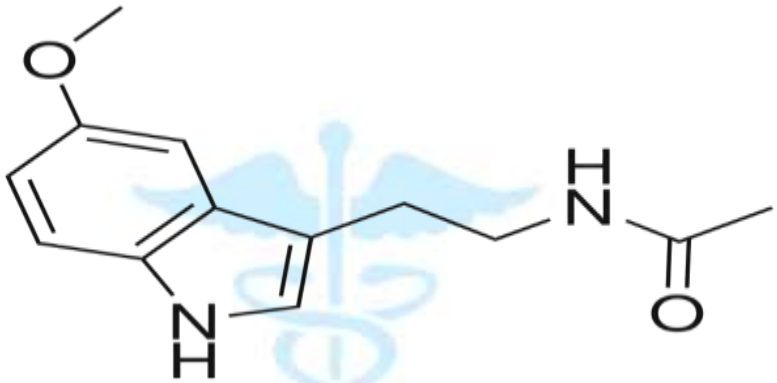

Figure 1: Structural formula of melatonin

2. Materials and methods

2.1. Study design and study area

The study took place in the city of Bahawalpur, situated within the Punjab province of Pakistan, at an altitude of 117 meters above sea level. Bahawalpur experiences a scorching, arid, and dry climate, with the majority of the region characterized by desert conditions. Rainfall in Bahawalpur is scarce throughout the year, with an average annual temperature of 78.3 degrees Fahrenheit and a mere 5.6 inches of rainfall. Feed for fishes collected from Fatima feed of generation, Lodhran.

2.2. Experimental animal and study design.

Oreochromis Niloticus (males and females) were selected for experiment. The weight of fishes was 70.2±5grams and their length was 8.01±1.5cm. Fishes were treated in equaponics for three month of trial/ experiment. Others equaponics occupied by controlled fishes.

2.3. How to make feed

- Wear the gloves and a face mask
- First I grind the pellets of rice protein and melatonin. Make it fine flour.
- Take a bowl.
- Add flour of melatonin and rice protein into the bowl. Then add water, 10 grams of sugar powder, 10 grams of salt, 300 grams of refined wheat flour and 20 grams of baking yeast.
- Mix all of these ingredients with water through hands.
- Make a dough of these ingredients.
- After making dough it would be spread on the slab and avoid water.

Dry the dough

First of all, I spread the dough on glass trays that were used to dry in the oven. With the help of a vacuum dry oven, I dried feed in the oven at a

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ISSN: (e) 3007-1607 (p) 3007-1593

temperature of 150 degrees Celsius. After drying the pellets of feed, the feed was ready.

Packing of feed

I preferred the packing of feed in polythene bags according to the body ratio of fish. Feed weight measured through digital weighing balance.

Feeding time

Feed was given to the fish twice a day. The time was 10: oo clock in the morning and 2: oo clock in the evening.

Growth

After giving the melatonin feed to the fish. First I measured the length and body mass then fishes were anesthetized with 50ppm benzocaine. Growth measurement was checked before feeding and after intervals of feeding.

Calculation of spawned eggs

The eggs released by female fish were carefully collected into a petri dish and counted. The frequency of spawning and the quantity of spawned eggs were investigated and documented following the commencement of melatonin feed. Finally, the gonadosomatic index (GSI) was determined by assessing the ratio of gonad weight to the body mass of the fish at the conclusion of the feeding trial.

2.4. Gonad somatic index

- The eight gonads of female fish were 2.4558gram
- The weight of female fish is 65gram
- The calculation of the gonadosomatic index (GSI) was performed using the formula: GSI = (GonW/BodW) x 100. Then the calculation is ...
- GSI = (Gon W / Bod W) x 100
- GSI = (2.4558/65) x 100
- GSI = 3.7781

Sperm and melatonin

In a male fish, check the effect of melatonin on it. Also check the number of sperm, and spermatozoa index (SI) spermatocrit at the last of the experiment based on the cut of the testes. The motility of sperm and spermatozoa index was evaluated through visual microscopy involving a combination of 1:10 of mixed extracted semen and cultured water.

2.5.1. How to check Spermatocrit

An indicator of spermatozoa density is determined by assessing the proportion of densely packed solid material in semen after undergoing centrifugation.

2.5.2. How to check spermatozoa index

Collect male fish specimens that were sexually mature and ready to release sperm. Choose healthy individuals that exhibit physical characteristics of maturity, such as coloration and body size.

Anesthesia:

To minimize stress and facilitate handling, anesthetize the fish using a suitable anesthetic solution. Follow recommended dosages and procedures to ensure the well-being of the fish.

Sperm Collection:

Once the fish was anesthetized, gently press the abdomen to release sperm. Use a clean, non-abrasive container to collect the sperm. The released sperm can be observed as a milky fluid.

Sperm Quantity:

Measure the volume of sperm collected. This can be done using a graduated pipette or a calibrated container. The quantity of sperm produced can provide insights into the reproductive health and readiness of the fish.

Sperm Quality Assessment:

Assess the sperm quality using various parameters, which may include:

Sperm Motility:

Observe the movement of sperm under a microscope. Motile sperm are generally more viable for fertilization.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Sperm Count:
Count the number of sperm cells per unit volume using a haemocytometer or specialized equipment.

Sperm Viability:
Use staining techniques to distinguish between viable and non-viable sperm cells.

Sperm Morphology:
Inspect the morphology and arrangement of sperm cells using a microscope. Abnormalities can affect fertilization success.

Sperm Concentration:
Protocol for hemoglobin concentration
Collecting Blood Sample:
Carefully collect a small amount of blood from the fish. This can be done by making a small incision and collecting the blood into a Micro centrifuge tube or another appropriate container.

This refers to the number of sperm cells present in a given unit volume of semen. It is often measured using a haemocytometer or automated sperm-counting equipment.

Blood and melatonin
After the one month of feeding the fishes were anesthetized. The benzocaine was added to their feed. After one hour they were all anesthetized completely. Take one by one of the fish. A 20 gauge of needles with 10cc syringes was used for taking blood. Blood was taken from the caudal fins of fish. Blood was stored in EDTA vials/tubes. Then blood sample was preserved in a refrigerator.
Check hemoglobin through equipment
Check the hemoglobin through Hematology analyzer sysmex kx 21

Results
4.1Growth
4.1.1 Weight of fishes
Weight of fishes were recorded and shown below in figure 12. The average weight of fishes is 148.5 ± 8.5g as compared to controlled fishes. Their average weight is 180.6± 4.5g.

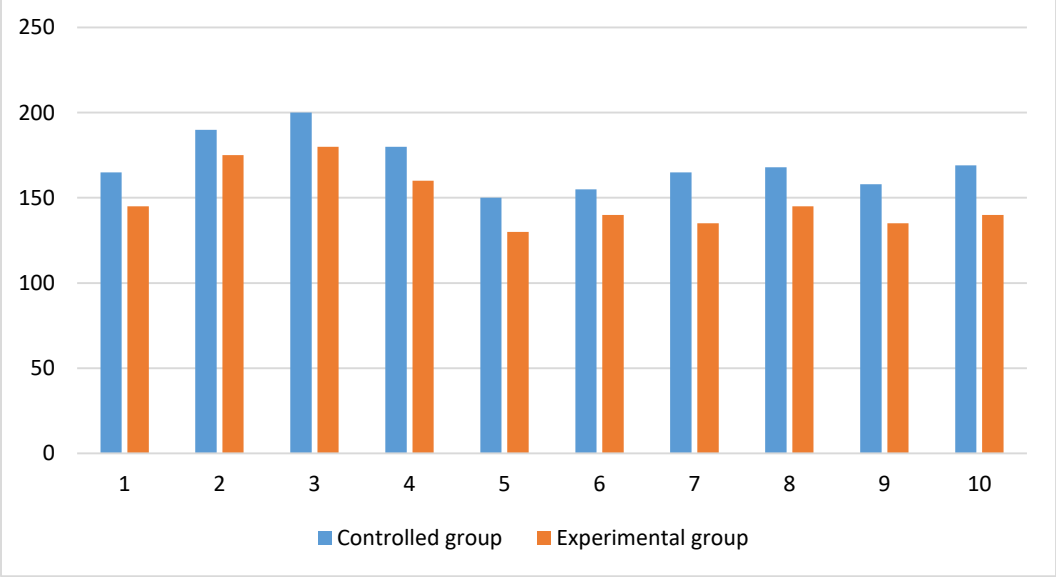

Figure 2: Weight of fishes by Graphical presentation

	Weight of fishes at 1st interval(1-30)	Weight of fishes at 1st interval(31-60)	Weight of fishes at 1st interval(61-90)

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Mean	80.65	120.98	134.40
Standard deviation	30.57	46.43	57.764
Significant value	0.024	0.02	0.016
Df	7	16	20

Table 1: statistical analysis of average weight of fishes

4.1.2 Length of fishes

The length of fishes were recorded and shown below in figure. Length average is decreased by mean of 23.24± 1.5cm as compared to controlled fishes. Their average weight is 30.34±0.5cm.

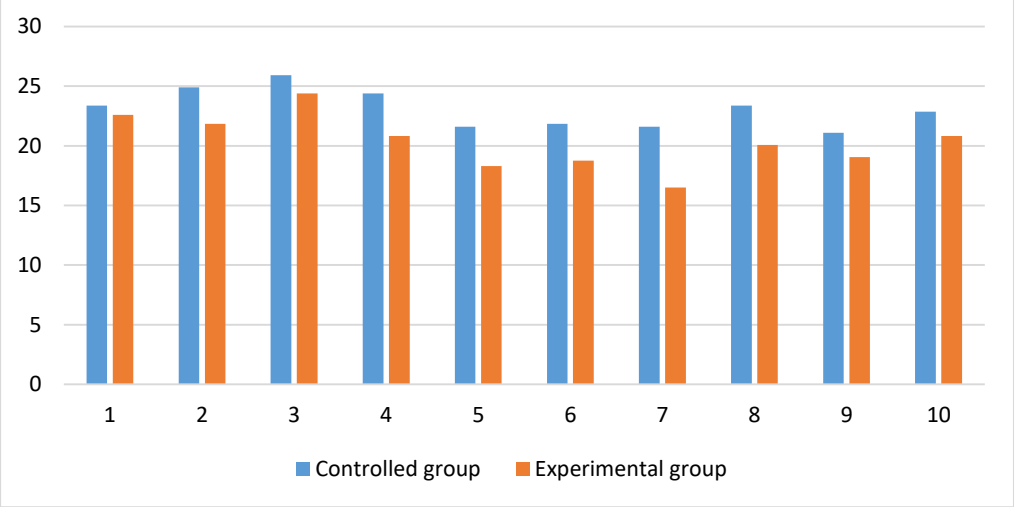

Figure3: length of fishes by graphical presentation

	Length of fishes at 1st interval	Length of fishes at 2nd interval	Length of fishes at 3rd interval
Mean	11.63	15.43	17.32
Standard deviation	1.76	2.065	3.036
Significant value	0.05	0.1	0.2
Df	12	18	22

Table 2: Statistical analysis of average length of fishes

4.2 Number of spawned eggs

Spawning was check every day of trial. There was no spawning at all. Then after end of trial (interval of 61-90) some fishes shows spawning and their results were given below in figure

Volume 3, Issue 8, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

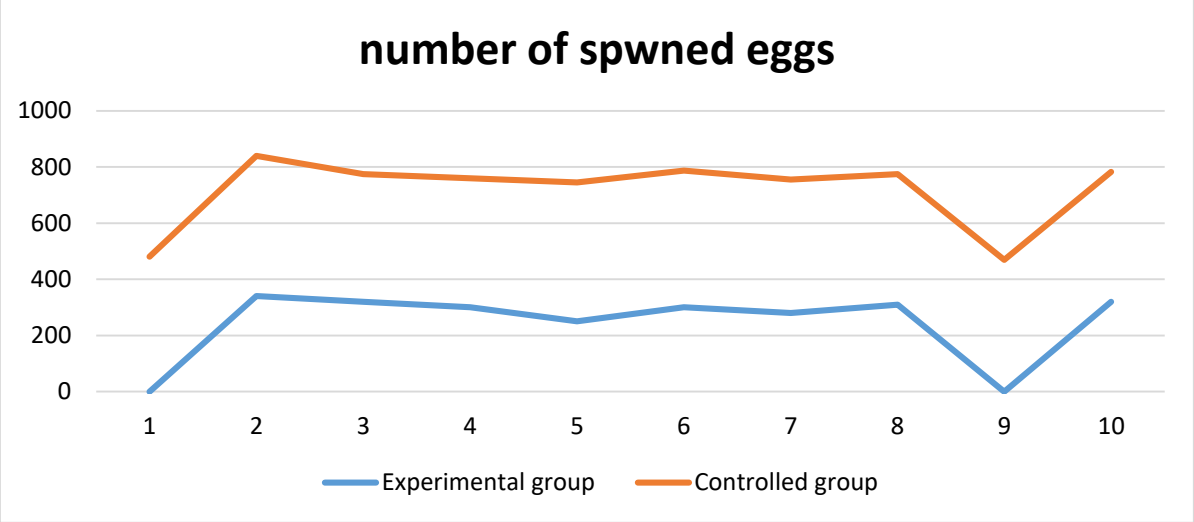

Figure 4: Spawning of fishes of graphical presentation

	No of spawned eggs
Mean	358.40
Standard deviation	149.5
Significant value	0.01
Df	16

Table 3: Statistical analysis of average spawning of fishes

4.3 Gonadosomatic Index (GSI) of female fishes

To check the gonad somatic index, cut the gonads of fishes and weigh the ovary of female fishes. Figure show the GSI of fishes below

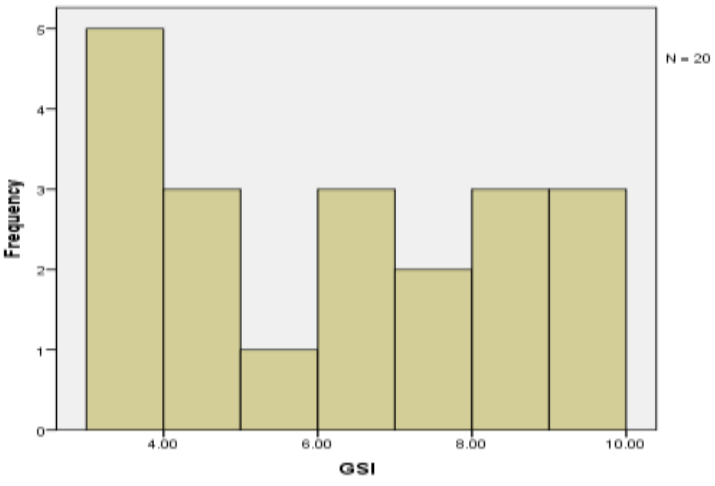

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Figure 5: Gonad somatic Index of fishes by Histogram Presentation

Volume 3, Issue 8, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

	Gonad somatic index of fishes
Mean	6.41
Standard deviation	2.24
Significant value	0.02
Df	22

Table 4: Statistical analysis of gonadosomatic index of fishes

4.4 Sperm and melatonin

The sperm count results for the control, Melatonin-treated, and HDM (High-Dose Melatonin) groups were as follows: 1.4334 ± 0.141 × 10^10, 1.372 ± 0.106 × 10^10, and 1.617 ± 0.003 × 10^10 sperm per ml, respectively. Notably, there was minimal variation in sperm count between the Melatonin group and the control group. Regarding spermatocrit levels, there was only a slight discrepancy between the control and Melatonin groups, with values of 61.4 ± 1.3 and 75.5 ± 1.5, respectively.

	SAI (score)	Spermatocrit (%)	Sperm concentration ($\times 10^{10}$ sperm/ml)
Experimental fishes	2.8	58.78 ± 0.6	1.558 ± 0.056
Control fishes	3.9	63.5 ± 1.1	1.634 ± 0.141

Table 5: Statistical analysis of sperm concentration, Spermatocrit and SAI

4.5 Blood and melatonin

The blood was taken after three month of trial/experiment. The figures show the hemoglobin concentration of controlled and experimental fishes.

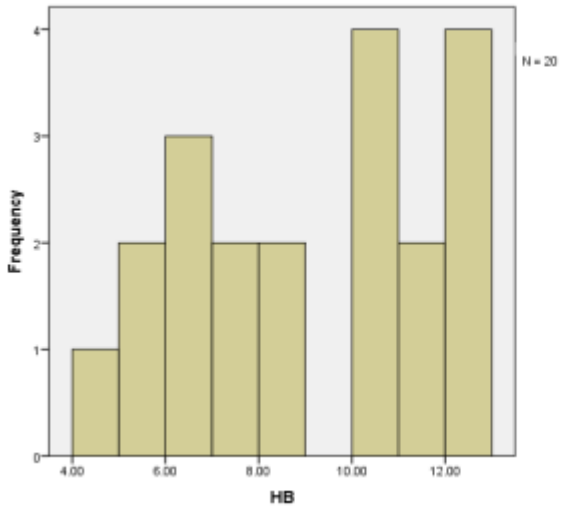

Figure 6: Hemoglobin concentration of fish's blood by Histogram Presentation

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

	Hb concentration of fishes
Mean	9.19
Standard deviation	2.60
Significant value	0.02
Df	21

Table 6: Statistical analysis of Hemoglobin concentration of fishes

Discussion

Numerous research studies have delved into the relationship between melatonin administration through oral intake and its impact on fish growth. In a specific instance (Porter et al., 1998), melatonin was orally administered to Atlantic salmon (Salmo salar), resulting in notable growth enhancement in comparison to both the control group and the group administered with melatonin. On the contrary, a 120-day experiment involving melatonin administration in masu salmon (Oncorhynchus masu), but they observed no observable influence on somatic growth. This absence of growth impact was further observed in a subsequent trial employing a substantial melatonin dosage (7.5 mg/kg) in masu salmon. The impact of melatonin introduction on growth was also investigated in bird species such as chickens (Gallus gallus) and quails (Coturnix japonica). This exploration unveiled that the administration of melatonin resulted in heightened growth rates in both the groups that received melatonin and those that were part of the control groups (Osei, Robbins et al. 1989).

In our current study, the lack of a notable difference in body length and weight among the three groups implies that melatonin did not negatively affect the somatic growth of Nile tilapia. The research centered on examining how melatonin influenced the gonad somatic index and hematological profile of Nile tilapia. The feed samples were sourced from Fatima Feed's Lodhran generation, and the feeding regimen extended over a period of three months. The growth performance was assessed after the first month, alongside the spawning rate after the same duration of feeding.

Figures 2 and 3 give detail on the growth performance of Nile tilapia in both controlled and experimental groups, including calculations of weight and length. Notably, the utilization of melatonin-infused feed exhibited a negative impact on the growth of the fish.

Figure 4 provides insights into the number of spawned eggs collected at the trial's conclusion. While daily spawning checks were performed, no spawning activity occurred due to two primary reasons. Firstly, the process of drawing blood from the fish's caudal fin for hematological profiling might have induced stress in the fish. Secondly, the trial period coincided with predominantly rainy weather, whereas fish typically require temperatures in the range of 45-60 degrees Celsius to trigger spawning. The desired temperature conditions were established during the months of June and July. Following the 60-day trial/experiment period, the eggs were examined in the fish's mouths, and any laid eggs were also checked in the surrounding water. A subset of fish exhibited spawning behavior, and the corresponding egg count was recorded numerically.

It's worth noting that the calculated p-values were significant for all parameters, including weight, number of spawned eggs, and gonad somatic index. However, for the parameter of length, the p-value did not yield significance.

The number of sperm produced by fish, including Nile tilapia, after receiving melatonin for almost three month can vary significantly depending on several factors, including the age and size of the fish, the dosage of melatonin administered, and the specific physiological and hormonal characteristics of the fish population in question. Melatonin influence fish reproduction,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

but its effects on sperm production can be complex and species-specific. Some studies have suggested that melatonin can have both stimulating and inhibitory effects on reproductive processes in different fish species.

5.2 Conclusion

Melatonin serves as a dietary supplement for fish, with a specific focus on regulating their spawning behavior. In the conducted experiment, fish feed from the Fatima feed generation Lodhran, were provided with feed infused with melatonin. This feed comprised not only melatonin but also rice protein, alongside minor components like sugar, salt, and yeast. Over the trial period, the fish were subject to daily monitoring post their consumption of the specialized feed. Following one month of the experiment, the fish's weights were documented through the utilization of a weighing balance. This conclusion suggested that the experimental group's growth was comparatively hindered in comparison to a control group of fish. A similar evaluation was performed after the second month of the experiment, reiterating that the experimental group displayed lesser growth both in terms of weight and length, when juxtaposed with the controlled fish group. The key influence of the melatonin-infused feed was observed in the spawning behavior of the fish. Interestingly, even a minimal dosage of melatonin exhibited a discernible impact on the spawning process. In the initial month of the experiment, the female fish did not exhibit egg spawning. However, in the subsequent month of melatonin administration, certain fish demonstrated signs of maturity and engaged in spawning activities. These activities were closely monitored by inspecting the fish's mouths for eggs, which were then further examined in a water environment. In contrast, the controlled fish group displayed comprehensive spawning behavior. It is notable that successful fish spawning and maturation typically hinge on specific environmental conditions, including a preferred temperature range of 24-26°C. This spawning behavior was predominantly observed during the months of June and July, coinciding with the maintenance of the specified temperature conditions. The impact of melatonin extended beyond spawning, as it was found to influence factors like the fish's hemoglobin concentration and various sperm-related activities, such as sperm motility,

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