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Immunity Induction in Catfish (*Clarias gariepinus*) with Feed Containing Red Ginger Extract after High Temperature Treatment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to evaluate the effect of feeding feed containing red ginger extract on the immunity and survival of catfish (*Clarias gariepinus*) fingerlings exposed to high temperature conditions. The research was carried out experimentally with a Completely Randomized Design (CRD) consisting of four treatment doses of red ginger extract (A: 0 ml/kg, B: 30 ml/kg, C: 40 ml/kg, D: 50 ml/kg) and three replications. Parameters observed included survival rate, white blood cell count, red blood cell count. The results showed that the addition of red ginger extract to feed was able to increase

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catfish survival by up to 90% at a dose of 50 ml/kg. A significant increase in the number of white blood cells and red blood cells in fish given red ginger extract indicates a better immune response. Red ginger extract has proven effective in helping fish adapt to thermal stress by increasing immunity and resistance to high water temperatures.

Keywords: Catfish; hematology test; immunity; red ginger; thermal stress.

1. INTRODUCTION

The culture of catfish is often hindered by extreme temperature fluctuations that trigger thermal stress. This stress can disrupt metabolism, lower immunity, and impede growth Previous studies have shown the potential of red ginger extract in enhancing catfish resilience to stress. Bioactive compounds in red ginger, such as gingerol and shogaol, possess antioxidant and anti-inflammatory properties that can protect fish from cellular damage caused by thermal stress. A study conducted by the Department of Marine Affairs and Fisheries of Jember Regency in 2022 found that rising pond water temperatures, caused by prolonged dry seasons, led to stress in catfish. This resulted in decreased appetite and slow growth. In addition, extreme temperature fluctuations can cause diseases in catfish such as skin, fungal and bacterial diseases [1].

The hematological response of catfish to temperature conditions is a crucial aspect in understanding their adaptation to significant temperature changes. When exposed to extreme temperatures, catfish undergo alterations in their hematological parameters, including red blood cell count, hemoglobin levels, and cell size. High-temperature exposure in catfish leads to an increase in red blood cell count as a response to the rising oxygen demand in their bodies [2]. Moreover, studies indicate that extreme temperatures can also affect hemoglobin concentration in the blood of catfish, potentially impacting their ability to transport oxygen [3]. These hematological changes are important to understand in the context of the health and physical performance of catfish in environments experiencing high temperature fluctuations. They are also vital for planning appropriate adaptation measures to protect catfish populations from negative impacts. Ginger extract contains bioactive compounds that can improve the immune system. The addition of red ginger extract to feed can increase the survival of catfish at high temperatures because red ginger has antioxidant and antibacterial properties that can increase the body's resistance [4,5].

This research aims to examine the effectiveness of red ginger extract in enhancing the survival of catfish fingerlings exposed to high temperatures and analyze the fish's hematological response to this treatment.

2. MATERIALS AND METHODS

This research was conducted from April to June 2024, starting with preparation, implementation for 30 days, and data analysis stages. The study was conducted at the Aquaculture Laboratory, Building 4, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The preparation of the red ginger extract (*Zingiber officinale* var. rubrum) was independently carried out at the Biotechnology Laboratory, Building 3, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

The equipment used in this research includes 15L aquariums (30x25x20 cm), aeration equipment, 100-watt heater, water quality testing tools (temperature, DO, pH), centrifuge, hemocytometer, and microscope. The materials used are catfish fingerlings measuring 7-9 cm with an average weight of 3 grams/fish, put in 10 fish per aquarium, red ginger extract, and commercial feed Prima Feed (PF) 1000 three times a day, 3-5% biomass weight.

The research method follows an experimental design using a Completely Randomized Design, consisting of four treatments with three replications. The treatments involve the addition of red ginger extract to the feed of catfish fingerlings reared at high temperatures (32°C) with various doses, which are as follows:

Treatment A: commercial feed without the addition of red ginger extract (control)

Treatment B: addition of 30 ml/kg of red ginger extract to the feed

Treatment C: addition of 40 ml/kg of red ginger extract to the feed

Treatment D: addition of 50 ml/kg of red ginger extract to the feed

The red ginger extraction process begins with washing the ginger thoroughly with running water

to remove dirt, then thinly sliced. These slices are then dried in the sun for three days to remove water content. Next, the dried ginger is ground using a blender to obtain ginger powder. This ginger powder is mixed with 70% ethanol and allowed to soak for two 24-hour periods. The resulting mixture is then centrifuged at 3000 rpm for 15 minutes to facilitate the separation of the supernatant from the solid pellet. The supernatant is carefully collected and stored in a closed container for further analysis.

The parameters tested in this research include fish survival rate, white blood cell count, red blood cell count, and water quality. The data collected after the experiment will be analyzed using one-way ANOVA. If significant differences are found, further analysis will be conducted using Duncan's multiple range test with a 95% confidence level, using Excel and Sigmaplot 15 software.

The survival rate can be calculated using the following formula [6]:

$$SR = \frac{Nt}{No} x\ 100\%$$

Description :

SR = Survival Rate

Nt = Number of fish at the end of the experiment

No = Number of fish at the start of the experiment

The blood collection procedure initiates with the aspiration of a blood sample using a Thoma pipette pre-filled with EDTA solution. Subsequently, Turk's solution is added and the mixture is agitated by hand for 3 to 5 minutes to ensure complete homogenization. The resultant mixture is then aliquoted into a counting chamber for microscopic examination.

The total leukocyte count is calculated by counting the cells in four small squares, and the total is determined using the following formula [7]:

$$Leukocytes = \left(\frac{A}{N}\right) X\ \left(\frac{1}{V}\right) X\ Fp$$

Keterengan :

A = Number of cells counted

V = Hemocytometer box volume

N = Observed haemacytometer box

Fp = Dilution factor

The calculation of the number of erythrocytes (red blood cells) can be performed using the following formula [4]:

$$Erythrocytes = \left(\frac{A}{N}\right) X\ \left(\frac{1}{V}\right) X\ Fp$$

Description :

A = Number of cells counted

V = hemocytometer box volume

N = Observed haemacytometer box

Fp = Dilution factor

Monitoring water quality parameters serves as supporting data in determining the optimal conditions for rearing the test fish. The water quality parameters measured include water temperature, dissolved oxygen (DO), and pH [8].

3. RESULTS AND DISCUSSION

3.1 Survival Rate

The results of this statistical test indicate that the treatment factor significantly affects the survival of catfish fingerlings (Table 1). The differences in survival rates obtained from each treatment group support the hypothesis that different treatments can have different impacts on the ability of catfish fingerlings to survive.

Table 1. Survival rate of catfish

Treatment	Survival Rate
A (commercial feed 100%)	70%±0.10[a]
B (30ml/kg red ginger extract)	77%±0.15[b]
C (40ml/kg red ginger extract)	83%±0.06[c]
D (50ml/kg red ginger extract)	90%±0.10[d]

The average followed by a different letter indicates significance (p<0.05)

Based on Table 1, treatment A serves as a control to assess the effectiveness of adding red ginger extract to the feed. The survival rate in this treatment is 70%. This indicates that without the addition of red ginger extract, the survival rate of catfish is the lowest in this study.

The higher the dose of red ginger extract administered, the greater the survival rate observed. Treatment D (50 ml/kg) yielded the best results, achieving a survival rate of 90%. This indicates that a dosage of 50 ml/kg of red ginger extract is the most optimal for enhancing the survival of catfish in this study. This finding aligns with research indicating that the addition of natural supplements to catfish feed can increase survival rates up to 95% [9].

In the study results, the lowest survival rate was observed in treatment A (control), which is suspected to be due to the fish's immune system not functioning effectively under excessive stress from being in extreme temperature conditions. Changes in environmental temperature can inhibit the entire metabolic activity of catfish [10]. When water temperatures exceed 32°C, catfish tend to lose their appetite and experience digestive disturbances. This is caused by the denaturation of digestive enzymes such as protease, amylase, chitinase, and lipase due to excessively high temperatures. Similar findings have also shown that significant temperature fluctuations can trigger stress responses in fish and impede their growth [11].

3.2 White Blood Cell Count

The white blood cell count results from the study can be seen in Table 2.

The data analysis in Table 2 reveals that feeding treatments over a period of 30 days effectively stimulated an increase in the white blood cell count in catfish. This increase indicates a positive immune response to the treatments. The higher the concentration of red ginger extract added to the feed, the more significant the observed increase in white blood cell count, although it remains within the normal range (Fig. 1).

Table 2. Average white blood cell count of catfish

Treatment	Before Treatment	After Treatment	Normal Range of Leukocytes
A	146.93±0.00[a]	181.28±0.061[a]	
B	147.40±0.01[a]	220.95±0.007[b]	150.000-300.000
C	148.67±0.01[a]	237.97±0.003[c]	cell/mm^3 [12]
D	147.11±0.01[a]	245.73±0.006[d]	

The average followed by a different letter indicates significance (p<0.05)

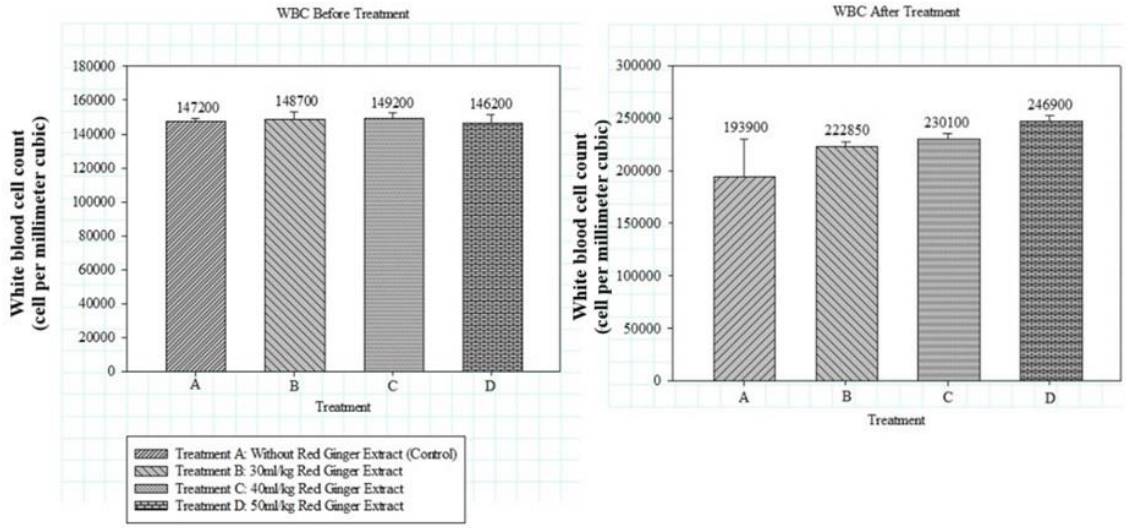

Fig. 1. Comparison of leukocyte count in Catfish

Based on Fig. 1, there is a comparison of white blood cell counts (WBC) in catfish before and after treatment with red ginger extract in the feed following exposure to high temperatures. In the pre-treatment condition, the white blood cell counts among the treatment groups were relatively uniform, with not much difference in range. This indicates that there were no significant differences among the treatment groups before exposure to high temperatures, reflecting a stable initial condition for the catfish. Exposure to extreme temperatures tends to induce stress in fish, which can trigger an increase in white blood cell production as part of the immune response [13].

After being provided with red ginger extract in the feed and subjected to extreme temperature treatment, a more significant change in the white blood cell count was observed. Based on the data, treatment D, which received the highest treatment (50 ml/kg feed), exhibited the highest increase in white blood cell count following the treatment, followed by treatment C and B. Meanwhile, treatment A, the control group, had the lowest white blood cell count after the treatment.

Studies have shown that the administration of red ginger extract to fish can significantly increase the white blood cell count, as evidenced by enhanced phagocytic activity and antibody production [14]. Additionally, the active compounds in red ginger possess antioxidant properties that protect immune cells from damage caused by oxidative stress, thereby strengthening the fish's immune system [15].

The increase in white blood cell (leukocyte) count in catfish fed with diets containing red ginger extract indicates a strong immune response. This aligns with the immunomodulatory properties of red ginger, which have been widely reported in the literature, as illustrated in Fig. 2. The bioactive compounds in red ginger, such as gingerol and shogaol, are believed to play a role in activating immune cells, thereby enhancing the fish's ability to combat infections. This increase in leukocyte count suggests that red ginger extract can function as an effective immunostimulant in catfish. Red ginger extract has been shown to be an effective immunomodulator in fish, enhancing immune responses and resistance to infections. Research indicates that the addition of red ginger extract to fish feed can improve phagocytic activity, increase antibody production, and reduce mortality rates in fish infected with pathogens [16].

Red ginger extract contains bioactive compounds such as gingerol, shogaol, and zingerone, which play a crucial role in increasing the white blood cell count in catfish. Research has shown that these compounds have immunostimulant effects, stimulating the production and activity of white blood cells, such as lymphocytes and neutrophils, which are essential for combating pathogen infections [17]. The active compounds in red ginger stimulate macrophage activation. These macrophages enhance phagocytic activity, which is the process of capturing and destroying foreign particles, bacteria, or other pathogens.

The bioactive compounds in red ginger extract, such as gingerol, shogaol, and zingerone, can enhance the white blood cell count in catfish through several mechanisms, including the stimulation of the innate immune system. Gingerol and shogaol are known to promote the proliferation of immune cells, which increases the number of leukocytes and accelerates the immune response to pathogens [18]. This activation leads to an increase in the production of cytokines and chemokines, which serve as signals to recruit more white blood cells to the site of infection or inflammation [19]. Bioactive compounds like gingerol can stimulate the bone marrow to enhance the production of blood progenitor cells, which then differentiate into various types of white blood cells, including lymphocytes and neutrophils, thereby increasing the number of white blood cells in the fish's bloodstream [20].

3.3 Red Blood Cell Count

The measurement of red blood cells in this study aims to evaluate the physiological condition of catfish after experiencing thermal stress. Hematological parameters such as the red blood cell count are important indicators in assessing the physiological condition and the fish's response to environmental stress. Based on the results of the study, the average red blood cell count before the addition of red ginger extract to the feed was 2.92×10^6 to 3.05×10^6 cells/mm³ (Table 3).

Fig. 3 shows a significant increase following the addition of red ginger extract to the feed over a 30-day period, with the average red blood cell count of the test fish rising to 3.33×10^6 to 3.86×10^6 cells/mm³.

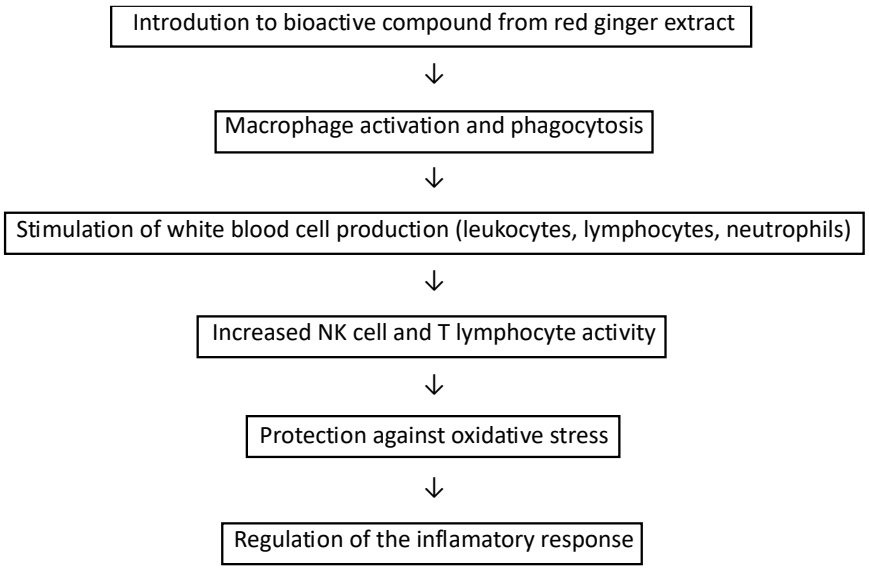

Fig. 2. Induction of Red Ginger Extract on Immune Response

Table 3. Average red blood cell count in catfish

Treatment	Before Treatment	After Treatment	Normal Range of Erythrocytes
A	3.05±0.005[a]	3.33±0.026[a]	
B	2.92±0.014[a]	3.60±0.009[b]	1.5 - 3.0 x 10^6
C	2.96±0.011[a]	3.77±0.005[c]	cell/mm^3 [21]
D	3.03±0.007[a]	3.86±0.009[d]	

The average followed by a different letter indicates significance (p<0.05)

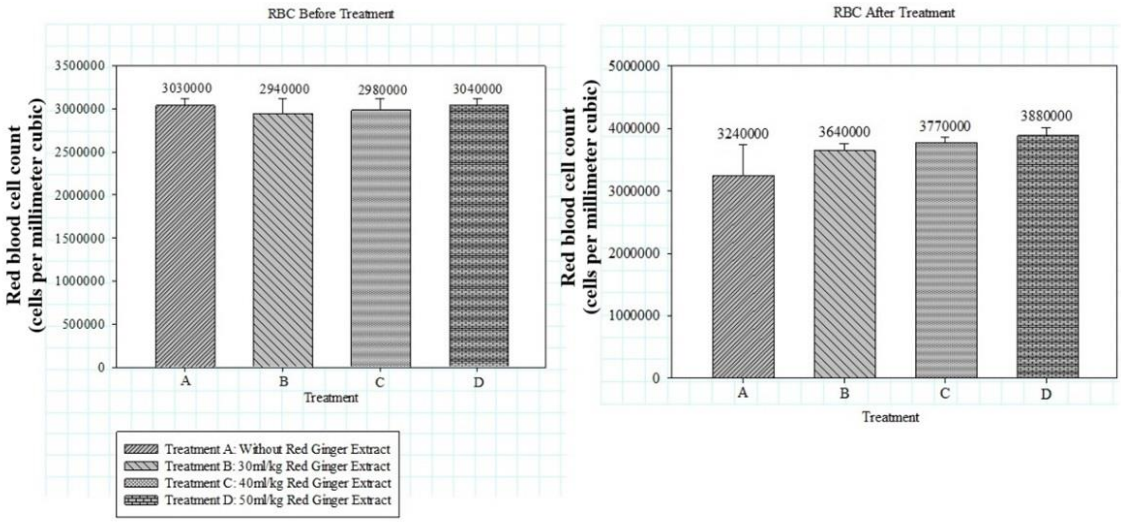

Fig. 3. Comparison of red blood cell count in catfish

The results of the ANOVA test after the maintenance period show that the calculated F value is greater than the F table value, indicating a significant difference or an effect of the changes in red blood cell count in catfish after treatment with the addition of red ginger extract. The highest erythrocyte count was observed in treatment D at 3.86 × 10^6 cells/mm^3, while

treatment A had the lowest erythrocyte count, still within the normal range, at 3.33 × 10^6 cells/mm^3. The increase in red blood cell count after treatment can be interpreted as an adaptive response of the fish to thermal stress. Red blood cells play a crucial role in transporting oxygen to tissues, which is particularly important under stress conditions, such as extreme temperatures, where oxygen demand increases. Red ginger extract acts as an adaptogen, which is a substance that helps organisms adapt to stress. The increase in red blood cells may indicate that this extract enhances the physiological response of fish to extreme temperatures, assisting them in maintaining or even improving their oxygen transport capacity under stressful conditions [22]. Based on Table 3, it can be observed that the increase in erythrocytes exceeds the normal range for catfish. An elevation in red blood cell counts beyond the normal limits can indicate a state of physiological stress in the fish [23]. Under stressful conditions, the fish's body may respond by increasing red blood cell production to enhance oxygen transport capacity, particularly in an effort to compensate for the reduced oxygenation efficiency due to extreme environmental changes [24]. In some cases, an elevated red blood cell count above normal could indicate an excessive adaptation to stress conditions such as extreme temperatures [25]. Red ginger extract may exert a strong adaptogenic effect, prompting excessive erythrocyte production in response to thermal stress.

Red ginger, containing bioactive compounds such as gingerol and shogaol, can function as an adaptogen that enhances the overall body's response to stress. Shogaol has been shown to reduce inflammatory processes and improve cellular health, potentially supporting growth and disease resistance in fish [26]. Additionally, shogaol can modulate growth signaling and immune activity, which are crucial for the production of red blood cells in catfish [27].

3.4 Response to Feed

Table 4 presents the various levels of response from the fish to the feed over a 30-day period, indicated by the symbols “+”, “++”, and “+++”, which represent the intensity of the response to the feed.

In Treatments A and B, there was a noticeable decline in response during the initial days (marked with “-” and “+”). This may indicate that the fish underwent an adaptation period to the feed containing red ginger extract, particularly after being subjected to extreme temperature treatments. After several days, the fish's response to the feed demonstrated a significant and consistent increase, indicating that the fish began to adapt well to the provided feed. This improvement in response suggests that the incorporation of red ginger extract in the feed could enhance palatability and acceptance, positively contributing to the overall health and performance of the fish.

The response pattern of catfish to feed containing red ginger extract after exposure to extreme temperatures demonstrates good adaptation, with a significant increase in feed consumption following the initial adaptation period. Herbal extracts, such as ginger, have been shown to enhance fish appetite and feed efficiency, particularly after the fish have experienced environmental stress [28].

Red ginger extract not only aids in maintaining fish survival under suboptimal conditions but also supports increased appetite and feeding activity. The active components in ginger act as immunostimulants that promote the overall health of the fish and enhance their response to feed [29]. This is crucial for the growth and overall health of the fish.

The increased response of fish to feed after the adaptation period indicates the effectiveness of red ginger extract in supporting the health and survival of catfish, particularly after experiencing stress conditions due to extreme temperatures [17, 28].

3.5 Water Quality

The results of water quality measurements during the research show the feasibility value for raising catfish (Table 5).

Based on Table 5, the water temperature in this study ranged from 33.04°C to 33.41°C, which is above the ideal range of 25-30°C. These high-temperature conditions have the potential to induce thermal stress in catfish, leading to decreased feed efficiency, inhibited growth, and increased mortality [30]. However, the measured dissolved oxygen (DO) levels (4.03 to 4.09 mg/l) remained above the minimum threshold necessary for the survival of catfish. The pH values of the water (6.8 to 7.1) also fell within a safe range according to Indonesian National Standards (SNI). The use of red ginger extract in the feed is believed to aid catfish in adapting to these extreme temperature conditions.

Table 4. Response to Fish Feed for 30 Days

Treatment	A			B			C			D		
Day -	Replication											
	1	2	3	1	2	3	1	2	3	1	2	3
1	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	-	++	-	++	+++	++	+++	++	+++	+++	+++	+++
3	+	+	+	++	++	+++	++	+++	+++	+++	++	+++
4	+	++	++	++	++	+++	+++	+++	+++	++	+++	+++
5	++	++	++	+++	+++	+++	+++	++	+++	+++	+++	++
6	+++	+++	+++	++	+++	++	++	+++	++	+++	++	+++
7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
10	+	+	+	++	+	+	++	++	+	++	++	+
11	+	++	+	++	+	++	++	++	++	++	++	++
12	++	++	++	++	++	+++	+++	++	+++	++	+++	+++
13	+++	+++	+++	+++	++	+++	+++	++	+++	+++	+++	+++
14	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
15	+++	+++	++	+++	+++	++	+++	++	+++	++	+++	+++
16	++	++	+++	+++	++	+++	+++	++	+++	+++	+++	+++
17	++	++	+++	++	+++	+++	++	++	+++	+++	++	+++
18	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
19	++	++	++	+++	++	+++	+++	++	++	+++	+++	++
20	+	++	+	++	+	++	++	+	+	++	+	+
21	+	+++	+	+++	++	++	++	++	++	+++	++	++
22	++	+++	++	++	+++	+++	+++	++	+++	++	+++	+++
23	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
24	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
25	++	+++	+++	+++	+++	++	+++	++	+++	+++	+++	+++
26	+++	+++	++	+++	++	+++	++	+++	+++	++	+++	+++
27	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
28	++	+++	+++	++	+++	++	+++	+++	+++	+++	++	++
29	++	++	++	++	+++	++	++	+++	++	+++	+++	+++
30	+++	+++	+++	+++	+++	++	++	+++	++	+++	++	+++

Table 5. Average water quality during the study

Treatment	Water Quality Parameters		
	Temperature (°C)	DO (mg/l)	pH
A	33.39	4.03	6.9
B	33.22	4.09	7.0
C	33.04	4.05	7.1
D	33.41	4.03	6.8

4. CONCLUSION

Red ginger extract has proven effective in helping catfish adapt to extreme environmental conditions. The best dosage from this study is treatment D (addition of red ginger extract at 50 ml/kg of feed), which resulted in the highest survival rate of 90%, increased erythrocytes to 8.3 × 10^5 cells/mm³, and leukocytes to 98.62 × 10^3 cells/mm³, as well as significantly enhancing the appetite of the catfish.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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