Temperature impacts on Growth Performance, Hormonal Changes and Hematological Parameters of African Catfish (*Clarias Jarpinus*)

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**ABSTRACT**

This take a look at become directed to discover the effect of temperature on increase and survival rate of African catfish. Different temperatures as 24, 27, 30, 34°C and 36°C had been examined and 27°C become a control. Plasma cortisol, plasma glucose, osmotic strain, and increase overall performance parameters had been envisioned at some point of the experimental duration of 25 days. The outcomes discovered that increase and survival rate had been prompted with the aid of using excessive temperature stage. Moreover, cortisol, glucose, and osmotic strain had been without delay proportional to the temperature stage. Therefore, it become concluded that multiplied temperature stage impacts increase and survival and different hormones of African catfish. So that a few growth in water temperature might appear like useful to catfish subculture overall performance.

**Keywords:** Temperature, Growth, Hormones, Blood, African catfish

1. **Introduction**

The environmental temperature variety skilled via way of means of any animal may have a main effect on survival, overall performance, and reproduction, and that is a selected trouble for ectotherms which have constrained potential to modify their personal frame temperature. For maximum species of their regular temperature variety, a mild boom in temperature is possibly to be useful to boom as it consequences in greater electricity which ends up in better response charges for boom. Wootton (2011). This is frequently because of how the molecular shape of mitochondria is stricken by adjustments in temperature Guderley H (2004) inside the regular tolerance variety, the price of biochemical procedures kind of doubles for each 10°C boom in temperature Boyd C E. (1998). Raised temperature can frequently beautify metabolic pastime and boom charges in fish, at the same time as decrease temperatures usually lessen overall performance Kemp J O G (2009). Most tropical fishes display superior boom overall performance in temperatures that variety from 25-32°C however the methods where in character species adapt to new temperature ranges, however, can range significantly. Laboratory research regularly reveal that temperature can boom to a factor in which it will become negative for boom, and subsequently will become deadly at which boom starts off evolved to deteriorate, has a tendency to be species specific Porter H O (2011). The boom of the fish is a essential index which suffer from temperature; better or declined than the thermal tolerance reduces boom rate Stickney R R (1994). The correlation of chemical elements in fluctuating water temperatures and boom overall performance in subculture is un-predictable and wishes in addition studies. Many studies from India have performed many experiments on each wild and farmed butter catfish. Examples encompass studies on stocking density Debnath C, et al. (2016). Increasing gill blood flow, plasma glucose, locomotory activity, gluconeogenesis and declining meals intake, boom, reproduction, glycogen shop and muscle proteins are foremost responses to disturbing situations and are managed via way of means of the mind and endocrine system Wendelaar-S E, (2011). Cortisol is the foremost hormone for responding to pressure are suitable signs therefore, for assessing the results of temperature extrude on boom overall performance Jentoft S, et al (2005) temperature.

Extrude is likewise without delay correlated however, with dissolved oxygen attention and therefore, hematological parameters and plasma glucose ranges also are beneficial signs to evaluate the ability of fish to carry out below temperature extrude or different stresses Tucker C S, (1998). If will increase of water temperature can beautify fish juvenile increase as well, it might provide extra gain to fish farmers. Therefore, a few thrilling elements approximately this take a look at inclusive of water first-class in way of life and increase overall performance below temperature manipulation have been investigated Pyanuth Rem, et al.(2020). In the cutting-edge take a look at, we tested the reaction of replicated cohorts of sweet sixteen African catfish to temperatures overlapping with the variety expected below cutting-edge weather extrude fashions on imply water temperature. The number one goal become to perceive temperature situations that offer most advantageous increase and survival in cultured African catfish. Furthermore, we wanted to perceive the particular temperature reached at which the strain reaction of people become to divert strength far from increase and direct it to coping with strain.

2. **Material and methods**

2.1 **Experimental System and Animals**

African catfish juveniles (18–25g) were collected from private fish farm located in Edku, Beheira, Governate, Egypt. Fish were maintained in freshwater tanks at the laboratory of aquaculture in faculty of agriculture (Saba Basha) Alexandria University. Test individuals were acclimated to tank condition for 15 days prior to experimentation in 4000 L tanks (approximately 500 individuals per tank) in freshwater (1.27±0.44 mOsm) at 27°C equipped with a continuous supply of well aerated freshwater. Fish were fed commercial pellets (Aqua feed, 25% protein, d=2mm) twice daily to satiation. After the acclimation time, cohorts were used in grow out trials. The experiment begin with 810 individuals were placed randomly into six treatments with three replicate 500L tanks (45 individuals / tank / treatment) which found to be the optimal density for African catfish Islam M, Rahman M, Tanaka M (2006). The six temperature treatments were based on a pilot study that recorded 100% mortality at <21oC and >39oC. The treatments were: 24oC, 27oC, 30oC, 32oC, 34oC and
36oC. The 27oC treatment considered as a control as the ambient temperature during the experiment was 27-28oC. For the 24oC treatment, tanks were set up in a cold room using an air conditioner to maintain the desired temperature. Fish adjusted to higher temperature treatments (30, 32, 34, and 36oC) were acclimated gradually to their temperatures before the experiment commenced using thermostats (Mennekes System, Germany) in a stepwise at 2oC /day until all tanks had reached their aimed temperatures. Highest temperature treatment acclimations started first and in sequence such that all treatments achieved their experimental temperatures on the same day. When all tanks had reached their target temperatures, fish were sampled at 25 days. For sample collection, three individuals were sampled randomly for hormone samples of cortisol, glucose level, osmolality, and hematological measurements.

2.2 Sample Collections
Water quality measured daily using a YSI Professional plus meter that determined dissolved oxygen (DO), NH3 concentration, pH, and water temperature. 2mL water samples from each tank were also collected and stored in 2ml, plastic tubes at the time of every fish sample collection to measure osmotic pressure. Growth performance was determined per treatment as: weight gain (WG), daily weight gain (DGW), specific growth rate (SGR) and food conversion ratio (FCR) based on the following standard formulae: WG (g) = final mean weight – initial mean weight; LG (cm) = final length – initial length; SGR (% d-1) = [ln(final weight) – ln(initial weight)] x 100/Δt; FCR (g/g) = daily food intake x 25/WG.

Catfish were collected from tanks using a hand net. According to Grutter A. (2000), handling time can have a significant effect on measures of cortisol and glucose concentrations in fish blood plasma, so the samples from the caudal vein were taken immediately within 5 minutes of collection with 1 mL heparin coated syringes Becker AG, et al. 2012, prepared under ice. Samples were then transferred to 1.5 mL labelled tubes that were then stored on ice prior to centrifugation. To estimate differences in growth performance, one way ANOVAs and Tukey’s test, applying a 95% confidence level.

3. Results

3.1. Environmental Condition
There was no significantly a difference in pH value among treatments during the course of the experimental time while DO and NH3 concentrations both tended to be higher in the 24°C and 27°C treatments (Table 1). Despite of NH3 concentration in treatments varied, all records were lower than those seen under standard healthy pond conditions observed by Bosma RH, et al. (2009). More over the negative correlation between temperature and DO estimated, the variation seen in DO levels amongst treatments in this study (i.e., lower DO in warmer temperatures) was well within the standard daily range of DO fluctuations observed in catfish grow out ponds, Huong D, et al. 2014.

3.2. Survival Rate and Growth Performance
The survival percent observed in the 24oC treatment was lower than for all other treatments (P<0.05). There was no significant difference in fish survival rate among all other treatments except that survival in the 36oC treatment was lower when compared with 27oC (Table 2). Fish WG increased at 24°C and 27°C treatment, but were significantly different at higher temperature, from the lowest level at 24oC to the highest record at the 34oC treatment. There was then a significant decrease in WG from 34oC to 36oC of approximately 20-25% (Table 2). The same pattern was generally viewed for DGW and SGR while the effect was less for LG. The statistical significance of differences among LG, DGW and SGR measures largely reflects that seen in the WG analysis. In overall, high temperature increased performance in comparison with the low treatment temperature (24oC). Both DGW and SGR of African catfish maintained at 24°C were poorer than in all other temperature treatments (P<0.05) while fish in the 34°C treatment showed the highest DGW and SGR levels (P<0.05). Daily food intake was significantly different among treatments, with the highest consumption evident at 34°C while FCR values among treatments did not differ from 27oC to 36oC however, all were superior to that observed in the 24°C treatment.

3.3. Hematological Parameters
Estimates of the three hematological parameters (RBCs, hemoglobin, and hematocrits) in fish sampled from the various thermal conditions showed significant main effects among temperatures, time of collection and also their interaction (Table 3). Water temperature therefore had a clear effect on catfish during both short term and long-term exposure. Results show that RBC, Hct and Hb were significantly lower at 27°C. There was no difference between mean values at any other temperature (Table 3).

3.4. Biochemical and Hormonal Changes
No significant effects were evident for temperature, time, and their interaction on osmotic pressure in African catfish (Table 4) and there was no significant difference among treatments. African catfish plasma osmotic pressure was 267.18±31.09 mOsm (n=331). Glucose concentrations, however, were significantly affected by temperature and time of collection although there was no apparent interaction between the two variables (Table 4). The highest glucose concentration was seen in the 34 and 36oC treatment, significantly higher than in the 24oC and 27oC treatments. Levels of glucose in fish at the 34oC & 36oC. Cortisol levels increased at 24 to 32oC. The same pattern was generally observed variation in DO levels played a little role in estimation growth of catfish. In the current study, low temperature affected growth of catfish more significantly with individuals in these growth rates at higher temperatures. In the current study, low temperature increased performance in comparison with the low treatment temperature (24oC). Both DGW and SGR of African catfish maintained at 24°C were poorer than in all other temperature treatments (P<0.05) while fish in the 34°C treatment showed the highest DGW and SGR levels (P<0.05). Daily food intake was significantly different among treatments, with the highest consumption evident at 34°C while FCR values among treatments did not differ from 27°C to 36°C however, all were superior to that observed in the 24°C treatment.

4. Discussion
The results of this experiment clearly indicate that a moderate increase in water temperature in the tanks improved performance of African catfish farmed, and this effect could possibly translate to wild populations as well. However, it is well known that as temperature increases, we should see an associated decline in DO, with all other things being equal. This effect was evident in the current study with DO declining from almost 5 mg/L at 24°C to approximately 3.4 mg/L at 36°C. meanwhile these values were statistically different from each other, we think that the observed variation in DO levels played a little role in estimation growth of African catfish for a number of reasons: i) Cat fish is an air breathing species and can usually access at least 10% of its oxygen requirements directly from the air Roberts TR, Vidhyathanon C 1991. Low DO levels would normally result in some degree of stress and therefore the growth have been declined at higher temperatures, which was not the case here fish with the highest DO had the lowest survival and growth rate, while the reverse was evident for the lowest DO treatments; and iv) Ficke AD, et al. 2007 observed that each species of fish has an optimal temperature range for growth performance; for warm water fish or fish in tropical regions in general, optimal temperature for growth ranges generally from 20 to 32oC. The relationship between temperature and growth is represented by the thermal growth coefficient effect Schulle P 2011, whereby metabolic rates increase in raised temperature producing faster growth rates at higher temperatures. In the current study, low temperature affected growth of catfish more significantly with individuals in these conditions not only consuming approximately half the amount of food compared with the treatment with the highest feeding rate (34°C), but also showing a much higher FCR value (Table 2) than in all other treatments. Furthermore, this was evident in a low relative growth rate and reduced length gain. As most fish are true ectotherms, their body temperature and hence metabolic rate will essentially follow the temperature of the surrounding water, Wootton.R 2011.
Catfish is a warm water fish, not only showed poorer survival rate at 24°C, but also had a lower growth rate. In the current study, catfish showed optimal response to temperatures ranging from 27°C to 36°C, but 34°C provided the best thermal conditions for African catfish culture. At this temperature, fish explored twice the weight gain in comparison with the 27°C treatment. Meanwhile at the same time, had no difference in FCR value. Fish in this treatment consumed approximately double the amount of food daily. The daily growth rate in comparison with 27°C, what might be considered as control conditions. Together, these results suggest that increased temperature to at least 34°C did not result in a stress response and stress response started at 36°C.

Temperature can clearly affect ectothermic animals by impacting on their mitochondrial capacities for substrate oxidation and ADP re-phosphorylation. Portner H O (2011). Mitochondrial capacities fall at lower temperatures, following a simple Q10 relationship and, conversely, they increase at higher temperatures, so tropical fish can increase their metabolic rates by activating their mitochondrial capacity. There is assurance in this study that water temperature increased to 36°C, seem the beginning of a decline in growth performance in comparison with 34°C. Suggesting that thermal stress was becoming significant at this temperature and that some energy was now being diverted to coping with stress.

The observed decline in growth rate from 34°C to 36°C due to temperature stress. Lefevre et al. (2014) explained that hypoxic conditions can inhibit growth of catfish by reducing appetite; decreasing assimilation efficiency (i.e., increasing FCR); and a shift in energy balance due to the requirement for increased surface activity for air breathing. In this study, it explored that fish at 36°C had low appetite (compared with 34°C) despite the DO values were not significantly different from those at 34°C. Therefore, it is difficult to differentiate between temperatures and DO related hypotheses with respect to the observed decline in growth rate at this higher temperature.

While conducted that growth performance of butter catfish was negatively impacted by elevated water temperatures Pyanuth Rem, et al. (2020). Moreover Stickney R. R. (1994). Metabolic rate decrease at low temperatures and fish started growing slowly.

Dealing with thermal stress in either lower temperature (24°C) or raised temperatures (30-36°C), was reflected in sampled individuals showing significant increases in hematological parameters including RBC, Hb and Hct (Table 3). Increased RBC, Hb and Hct are a common response to hypoxia or anoxia and to dealing with stress Carvalho CS, Fernandes MN (2006). When individuals were exposed to either low or raised temperature environments in this study, RBC, Hb and Hct levels were all significantly increased when compared with the ambient 27°C treatment (Table 3). Hedayati A, Tarkhani R (2013) reported in their study the effect of diazinon and deltamethrin on tra catfish and subjected to these stressful conditions. RBC, Hct and Hb were raised to increase oxygen-carrying capacity of the blood. Osmotic and thermic stress both can affect fish blood parameters including Hb, Hct and cortisol levels. Roche H, Bogé G (1996). Temperatures can cause stress because increases in temperature decrease oxygen solubility in water and hence availability to fish Cech JJ, Brauner CJ (2011). The increase in quantity of red blood cells in treatments led to increases in Hb, Hct and MCH. The internal osmotic pressure was probably unchanged however so red blood cell volume and relative quantity of Hb in each red cell was not essentially affected by changes in water temperature. African catfish possibly respond to thermal stress by increasing RBC number that in turn increases Hb, Hct and MCH to ensure they meet higher oxygen demands (Table 3). African catfish therefore appear very suitable for high density culture as already observed by Phuong NT, Oanh DTH (2010) and, in particular, they possess not only an air bladder for air breathing Roberts TR, Vidhathyanan C (1991), they also can respond by changing hematological parameters to deal with raised temperature stress ensuring higher oxygen demands can be met efficiently. However, although mean Hb concentration of fish in the 36°C treatment were significantly higher than at 27°C, RBC and Hct tended to decline after reaching a peak at 34°C. This study showed that the limitations of hematological acclimation in thermal stress response, and this reflect a tendency towards reduction of growth in the 36°C treatment.

Furthermore, osmotic pressure in the experimental catfish was not substantially different from other freshwater fishes, including bowfin (279 mOsm), carp (274 mOsm), or euryhaline steelhead trout (260 mOsm) Evans DH (2011). In the current study, temperature appeared to have no effect on plasma osmotic pressure of African catfish (Table 4), and this result provides a similar conclusion to other studies on freshwater fish including Mozambique tilapia Oreochromis mossambicus, Yaneyce PH, et al. (2007) and Mozambique tilapia hybrids O. mossambicus x O. arolepis hornorum Sardella BA, et al. (2004) that temperature has little effect on fish osmotic pressure; osmolality levels, however, can change when combined with different salinity levels Sardella BA, et al. (2004). A single study observed a temperature-related impact on common carp plasma osmolality Metz J R, et al (2003) however the authors could provide no clear explanation for their observation.

When catfish subjected to stress, fish will use more power from food for swimming, regulation, and respiration instead of growth, reproduction, and storage Klein SE, Sheridan MA (2008), thereby leading to increases in plasma glucose concentration. In the current study, plasma glucose in high temperature treatments was mobilized at significantly higher levels than at 24 or 27°C, presumably to deal with thermal stress. Plasma glucose concentration has been reported to increase from hours to days under regulation of some stress response hormones including cortisol .Pankhurst N (2000) and Barton BA (2000). McCormick SD (2011), attempting to escape from high temperatures, or surfacing for air oxygen Huong D, et al. (2014); glucose levels then declined in the high thermal environments (34 and 36°C) after 4 days. From day 8, energy mobilization for swimming activity, were regulated and fish acclimated to their surroundings resulted in no significant differences in plasma glucose concentrations Natt MPH, McCormick SD (2011).

5. Conclusion

We provide information explored that water temperature has an important effect on the growth, Haematology and metabolism of African catfish, and these parameters are important when evaluating the physiological status of the species. The best temperature for the growth and feed conversion ratio of African catfish ranged from 27 to 32°C and we could recommend reducing the feeding rate during the winter when temperatures may decrease significantly. In this work, 34°C appeared to be the optimum temperature for African catfish with no significant difference on FCR values in comparison with lower temperature conditions but producing faster growth rates. Temperatures across the thermal tolerance range in African catfish do not have clear effect on fish osmoregulation but individuals do respond to rapid changes in temperature by increasing plasma glucose concentration finally that some increase in water temperature would appear to be beneficial to catfish culture performance.

6. References


Stickney R R (1994) Principles of aquaculture. In: John Wiley and Sons,
New York.
Table 1: Environmental factors of experiment.

<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>24 °C</th>
<th>27 °C</th>
<th>30 °C</th>
<th>32 °C</th>
<th>34 °C</th>
<th>36 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.80±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.02±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.11±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.03±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.95±0.58&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.08±0.63&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4.96±1.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.66±0.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.44±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.30±0.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.55±0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.30±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NH3 (mg/L)</td>
<td>0.10±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.07±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04±0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.04±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.06±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

Means ± SD in row have not the same letter are significantly different (p<0.05).

Table 2: Growth performance parameters and survival rate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial W (g)</th>
<th>Final W (g)</th>
<th>Survival (%)</th>
<th>WG (g)</th>
<th>LG (cm)</th>
<th>SGR(%/day)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 °C</td>
<td>21.99±0.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>39.21±2.54&lt;sup&gt;d&lt;/sup&gt;</td>
<td>70.37±3.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.21±2.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.40±0.49&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>27 °C</td>
<td>20.23±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.09±3.87&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>97.78±2.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.86±5.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.64±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15±0.19&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>30 °C</td>
<td>20.88±2.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.68±9.49&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>91.85±7.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.79±7.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.37±0.40&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.57±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47±0.13&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>32 °C</td>
<td>24.22±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.22±4.95&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>90.37±5.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.00±4.73&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.00±0.85&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.65±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59±0.16&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>34 °C</td>
<td>22.09±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.52±4.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.30±5.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.43±4.29&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.73±1.81&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.19±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.49±0.16&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td>36 °C</td>
<td>23.85±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.17±1.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.89±2.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.32±1.81&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.44±0.97&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.79±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.37±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
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Means ± SD in same column that have not the same letter are significantly different (p<0.05).

Table 3: Mean hematological parameters of African catfish under different temperatures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>24°C</th>
<th>27°C</th>
<th>30°C</th>
<th>32°C</th>
<th>34°C</th>
<th>36°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (106 cells/mm3)</td>
<td>3</td>
<td>2.78±0.10&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>2.42±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.79±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.75±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.87±0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.62±0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hb (g/ DL)</td>
<td>3</td>
<td>7.99±0.09&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>6.68±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.97±0.20&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.21±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.53±0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.52±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Het (%)</td>
<td>3</td>
<td>28.80±0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.40±0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.41±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.18±1.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.23±0.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.86±0.91&lt;sup&gt;ab&lt;/sup&gt;</td>
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Means ± SD in same row that do not share the same letter are significantly different (p<0.05).

Table 4: Changes of osmotic pressure, plasma glucose and cortisol under different temperatures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>24°C</th>
<th>27°C</th>
<th>30°C</th>
<th>32°C</th>
<th>34°C</th>
<th>36°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic pressure</td>
<td>3</td>
<td>271.98±3.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>264.36±3.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>264.35±6.19&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>269.98±3.65&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>268.02±3.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>265.79±3.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>3</td>
<td>0.62±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58±0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.56±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.46±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.56±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma cortisol</td>
<td>3</td>
<td>4.55±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.61±0.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.65±1.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.59±1.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.48±1.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.97±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± SD in same row that do not share the same letter are significantly different (p<0.05).