Overcrowding-Induced Stress in Catfish (*Heterobranchus longifilis*) Is Associated with Serum Chemistry Derangement

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Abstract

Even though several studies have reported how stress induces detrimental pathophysiological responses that are linked to increase in disease susceptibility, no study has evaluated the impact of overcrowding on the serum chemistry of *Heterobranchus longifilis*. This study aimed at evaluating the impact of overcrowding on serum chemistry in *Heterobranchus longifilis*. Briefly, 30 apparently healthy *Heterobranchus longifilis* weighing between 1 kg and 1.5 kg were randomly divided into 2 groups of 15 catfish, each designated as groups 1 and 2. Group 1 (control group) was not subjected to stress, while group 2 was subjected to 3 hours of overcrowding in a 120 L plastic water tank at 201 g/L of water. Blood samples were collected from the caudal vein into non-heparinized tubes before stress, immediately after stress and 24 hours after stress. Sera were collected, and serum chemistry and electrolytes were analyzed. At 24 hours after stress, alanine aminotransferase, aspartate aminotransferase, creatinine, uric acid, total bilirubin, conjugated bilirubin, total protein, albumin, total cholesterol, triglyceride, chloride, potassium, and sodium were significantly higher in group 2 compared to the control group. Based on the findings of this study, it was concluded that overcrowding of *Heterobranchus longifilis* for 3 hours has a significant impact on its health status.

Keywords: Catfish, *Heterobrochus longifilis*, overcrowding, serum chemistry, stress

Introduction

The consumption of fish as a major source of protein has led to reduction in the rate of undernutrition, most especially in developing countries (Pradeepkiran, 2019). As global productivity of captured fish is declining, there is a substantial increase in inland aquaculture production (Naylor et al., 2021). The aquaculture industry has become one of the fastest-growing food production industries worldwide (Gephart et al., 2020; Naylor et al., 2021). Analysis has shown that there will be a deficit in fish supply due to increased demand, and to forestall the expected increase in demand, it is critical to significantly increase fish production by 2030 (World Fish, 2015). The expansion and intensification of the aquaculture industry is hindered by several factors, including diseases and adverse environmental conditions, especially in developing countries (Naylor et al., 2021).

The increased intensification in aquaculture has led to consequential increase in the manipulation of fish such as handling, limiting confinement, fertilization, transportation and other farm operations from hatcheries to the final commercial stage constitutes source of stress to the fish (Portz et al., 2006; Tanko et al., 2023). These management procedures are known to induce stress, consequently leading to increased susceptibility to diseases as well as decrease in fish performance (Maule & Shreck, 1990; Tanko et al., 2023).

Biochemical parameters, on the other hand, provide information on the health status and magnitude of possible sickness in fishes; hence, understanding these parameters is critical as it is useful in monitoring pathological and physiological changes in fishes (Francesco et al., 2012). Biochemical constituents as well as certain enzymes in the blood have been employed as biomarkers since they are less variable, very sensitive, and conserved between species (Owolabi, 2010; Tanko et al., 2023). Enzymes that are intracellular, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), or creatine kinase, are released by death or damaged cells, and these enzymes can then be used as indicators of stress (Sopinka et al., 2017). There has been an increasing interest in studying biochemical parameters, and the parameters are regarded as vital for aquaculture purposes (Gayatri et al., 2014).

When indicators of stress are not classified, quantified, refined, and interpreted, the significance and implications of fish responses to external and internal environmental changes may not be well appreciated (Schulte, 2014). Measuring stress from an applied perspective is necessary to determine how the health performance and...
welfare of fishes are being influenced by interaction with humans (Sopinka et al., 2017; Tanko et al., 2023). Although there are several studies that have evaluated the effect of stress on fish health and performance (Ali et al., 2021; Nadirah et al., 2016; Noraini et al., 2013), no study has evaluated the impact of overcrowding-induced stress on the serum chemical parameters of *Heterobranchus longifilis*. Hence, this study aimed at evaluating the impact of 3 hours of overcrowding-induced stress on serum chemistry in *H. longifilis* and the recovery rate.

Materials and Methods

**Ethical Approval**
The experimental design was approved by the institutional ethical committee on animal use and care of the University of Jos in compliance with the animal use and care guidelines with reference number F17-00379. The catfish were handled in such a manner that they were not subjected to additional stress before and during sampling.

**Experimental Catfish**
The 30 apparently healthy adult catfish weighing 1–1.5 kg that were used for the experiment were procured from a reputable catfish farm within Jos metropolis with GPS coordinates 9.851095 (N9° 51’ 3.94164”) and 8.923327 (EB8° 55’ 25.21884”) at an altitude of 1275 m above sea level (Tanko et al., 2023). The catfish were acclimatized for one week in a section of the farm in a 5000 L plastic water tank (actual capacity = 5010 L, width = 1600 mm, length = 1600 mm, height = 2020 mm, weight = 110 kg, and color = black) before the onset of the experiment. The fish were exposed to natural day and night without any artificial light. The fish were fed commercial feed pellets (Coppens) in the morning and evening daily, at 10% of their body weight, but feed was withdrawn 24 hours before the experiment to ensure clean water during the experiment. The water in the tanks was changed every 3 days through partial draining to ensure clean water was sustained during the acclimatization as well as during the experiment, while the water temperature during the experiment ranged from 20°C to 21°C and the average environmental temperature was 26°C. For sampling, the water from the water tanks were partially drained, and the catfish were subsequently captured using dip nets. A soft, clean towel was used to hold the catfish in place during blood collection.

**Experimental Design**
The 30 catfish were divided into 2 groups designated as groups 1 (control group) and 2 of 15 catfish each. Group 1 was not exposed to any form of stress to serve as a control. Group 2 was subjected to overcrowding-induced stress for 3 hours in a 120 L plastic water tank at the rate of 201 g/L of water (about 1 kg/5 L of water) (Pakhira et al., 2015), as opposed to 1 kg/20 L of water for the control group. The fish were then returned to their normal water tanks (5000 L plastic water tanks) according to their respective groups. The stress experiment was done in a private catfish farm located in Lamingo-Haske, opposite Lamingo golf club, Jos, Plateau State, with coordinates 9.851095 (N9° 51’ 3.94164”) and 8.923327 (EB8° 55’ 25.21884”). Blood samples (5 mL) were collected via the caudal vein from five catfish in each group shortly before exposure to stress. Blood samples were also collected from five catfish in each group immediately following stress and at 24 hours post overcrowding-induced stress. The water used before and during the experiment was borehole water used to keep the fish in the farm. The experiment was carried out in November 2020, and the water temperature during the experiment ranged from 20°C to 21°C, the pH ranged from 7.0 to 7.1, and the average environmental temperature was 26°C.

**Drawing Blood Samples and Collection of Serum**
Blood samples collected from the caudal vein suggested by Campbell and Grant (2022) and Duman et al. (2019) were transported to the Microbiology and pathology laboratory of the Faculty of Veterinary Medicine, University of Jos, Plateau State, Nigeria, where they were centrifuged and the sera were separated after centrifugation for serum chemistry analysis using an automated serum chemistry analyzer, Cobas C111 (Roche Diagnostics GmbH, IN, USA), to evaluate the concentrations and/or levels of mean glucose, ALT, ALP, AST, creatinine, urea, uric acid, total bilirubin, conjugated bilirubin, serum total protein, albumen, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, bicarbonate, chloride, potassium, and sodium (Tanko et al., 2023).

**Statistical Analysis**
Data analysis was done using JMP version 10. All the data were found to have a normal distribution. Student t-test was used to identify statistically significant differences among the groups at each sampling time. Differences between the groups were considered statistically significant at *p < .05*. Results are expressed as the mean ± standard error (SE). Principal component analysis was also done for group 2 analytes immediately following overcrowding-induced stress and 24 hours post stress, and eigenvalue, percentage variance, and cumulative percentage were determined.

**Results**

**General Clinical Observation**
At the beginning of the experiment, the catfish were observed to be calm, with little or no movement. Thirty minutes later, the catfish in group 2 were observed to have an increased rate of movement, frequently jumping out of the water. This continued for about 2 hours, at which point calmness returned and there was excessive foam in the water. The catfish were all trying to raise their heads up.

**Mean Glucose Level**
Analysis of the mean glucose level prior to overcrowding-induced stress for the two groups showed no significant difference between the groups (Figure 1). However, group 2 showed a significantly increased blood glucose level (*p < .0001*) compared to group 1 immediately following stress to a value of 6.3 mmol/L from 5.0 mmol/L. Amazingly, at 24 hours post stress, the glucose level declined to 5.90 mmol/L, which was not significantly different (*p = .594*) compared to group 1 (control group). As expected, there was no significant alteration in blood glucose level in group 1 throughout the study period. Analysis of the overall results showed the glucose level of group 2 was significantly higher (*p < .0001*) compared to group 1.

**Mean Alanine Aminotransferase**
The mean transaminase enzyme (ALT) level prior to stress for the two groups showed an insignificant difference between the groups (Figure 2). However, the mean ALT in group 2 increased but insignificantly (*p = .081*) compared to group 1 immediately following stress. At 24 hours post stress however, the mean ALT level increased significantly (*p < .0001*) from 22.7 g/dL to 63 g/dL compared to the group. Group 1, on the other hand, recorded no significant change in ALT value throughout the study period. Generally, the increase in the
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level of ALT in group 2 was significantly higher ($p < .0001$) in group 2 compared to group 1.

**Alkaline Phosphatase**
The mean ALP level before stress for the two groups showed no significant difference between the groups (Figure 3). Analysis of the result showed there was a significant increase in ALP level ($p < .017$) in group 2 compared to group 1 immediately following stress. Thereafter, the ALP level in group 2 further increased significantly ($p < .0001$) compared to group 1 at 24 hours post stress. However, group 1 showed no significant change in the level of ALP throughout the period of study. The generality of the result showed the ALP level of group 2 was significantly higher ($p < .0001$) than that of group 1.

**Aspartate Aminotransferase**
The mean AST levels before and through 24 hours post stress is depicted in Figure 4. Analysis of the AST levels revealed that prior to stress, there was no significant difference between groups 1 and 2. The mean AST levels in group 2, however, showed a significant increase ($p < .0001$) compared to group 1 immediately following 3 hours of overcrowding-induced stress. Thereafter, the mean AST level for group 2 further increased significantly ($p < .0001$) to its peak.

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**Table 1**

<table>
<thead>
<tr>
<th>Time</th>
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**Table 2**

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<tr>
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<tr>
<td>24HAT</td>
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value of 163 U/L from 71 U/L. At 24 hours post stress, Group 1 showed no significant change in AST level throughout the period of study. The generality of the results again showed the increase in AST level was significantly higher ($p < .0001$) in group 2 compared to group 1.

**Creatinine**

Analysis of the creatinine level before stress showed no significant difference between the groups (Figure 5). However, the creatinine level in group 2 increased but not significantly ($p = .081$) compared to group 1 immediately following stress to a value of 31.0 µmol/L from the initial value of 29 µmol/L. Thereafter, the creatinine level increased to 43 µmol/L at 24 hours post stress which was significantly higher ($p < .0001$) compared to group 1. Group 1 showed no significant changes in creatinine level throughout the period of the study. Overall analysis of the results of the serum creatinine level showed there was significant differences ($p < .0001$) between groups 1 and 2, and the creatinine level in group 2 was significantly higher compared to group 1.

**Urea Level**

The mean urea concentration before and following stress is depicted in Figure 6. Analysis of the entire results for urea concentration revealed that there was no significant difference between the groups prior to the 3 hours of crowd-induced stress. The mean urea level in group 2 however, increased slightly higher compared to group 1 immediately following stress to 3.0 mmol/L from the initial value of 2.9 mmol/L. Thereafter, the urea level again, increased significantly ($p < .04$) to 3.30 mmol/L compared to group 1 which had 2.9 mmol/L at 24 hours post stress. Group 1 showed no significant change in urea level during the study period. The results of blood urea level generally depict significantly higher ($p < .0001$) levels in groups 2 compared to group 1 which is the control group.

**Uric Acid Concentration**

The mean uric acid concentration before, after and through 24 hours post crowd-induced stress is depicted in Figure 7. Analysis of the uric acid concentration showed that there was no significant difference between the groups prior to stress. The uric acid concentration in group 2 however, increased significantly ($p < .0001$) immediately following stress to a value of 104 µmol/L from the initial value of 94 µmol/L compared to group 1. Uric acid level in group 2 thereafter further increased significantly ($p < .0001$) to 128 µmol/L as against the value of 93.5 µmol/L at 24 hours post stress. Group 1 on the other hand recorded no significant change in uric acid level throughout the study period. The generality of the results showed there were significant differences ($p < .0045$) in mean uric acid concentration between the groups and Uric acid level of group 2 was significantly higher compared to group 1.
Mean Total Bilirubin Level
Analysis of the total bilirubin level showed that there was no significant difference in the level of total bilirubin between the groups prior to the overcrowding-induced stress (Figure 8). However, the mean total bilirubin level in group 2 increased significantly ($p < .0002$) from the initial value of 9.9 mg/dL to 13 mg/dL compared to group 1 immediately following stress. Thereafter, there was a further significant increase in the total bilirubin level ($p < .0001$) at 24 hours post stress to 19 mg/dL as against 9.8 mg/dL in group 1. Group 1 showed no significant change in the level of total bilirubin throughout the period of study. Generally, analysis of the overall results showed there was a significant difference ($p < .0158$) in total bilirubin levels between groups 1 and 2, and the level of total bilirubin was higher in group 2 compared to group 1.

Mean Conjugated Bilirubin
The mean conjugated bilirubin level for the two groups is depicted in Figure 9. Analysis of the results showed there was no significant difference between the groups prior to the overcrowding-induced stress. The level of conjugated bilirubin in group 2 increased significantly ($p < .0001$) compared to group 1 immediately following stress, from the initial value of 4.20 mg/L to 6.4 mmol/L. At 24 hours post stress, there was a further significant increase in conjugated bilirubin level ($p < .0001$) to 11.8 mmol/L compared to group 1 which had 4.20 mg/L at 24 hours post stress. Group 1 showed no substantial alteration in the level of conjugated bilirubin throughout the study period. Analysis of the overall results showed the conjugated bilirubin level in group 2 was significantly higher ($p < .0001$) compared to group 1.

Total Protein Level
The mean total protein level shortly before exposure to stress for the two groups showed no significant difference between the groups (Figure 10). However, analysis of the mean total protein level in group 2 showed a significant decrease ($p < .0039$) compared to group 1 immediately following stress, from the initial value of 70 g/dL to 67 g/dL. The mean total protein thereafter further declined significantly ($p < .001$) through 24 hours post stress to 61 g/dL as against the value of 70 g/dL in group 1. The total protein level in group 1 recorded no significant alteration throughout the study period of 24 hours. Statistical analysis of the total protein level showed that the total protein level of group 2 was significantly lower compared to group 1.

Albumin Level
The mean albumin level shortly before stress and through 24 hours post stress is shown in Figure 11. The mean albumin concentration prior to stress for the two groups showed no significant difference between the groups. However, the mean albumin concentration in group 2 showed a decreased level immediately after stress, from the initial value of 42 g/dL to 37 g/dL, and this was significantly higher ($p < .0011$) compared to group 1. There was a further significant

![Figure 8. Mean Total Bilirubin Level Shortly Before 3 Hours of Overcrowding-Induced Stress Through 24 Hours Post Stress. SBT = Shortly before stress; IAT = Immediately after stress; 24HAT = 24 hours post stress.](image1)

![Figure 9. Mean Conjugated Bilirubin Level Shortly Before 3 Hours of Overcrowding-Induced Stress Through 24 Hours Post Stress. SBT = Shortly before stress; IAT = Immediately after stress; 24HAT = 24 hours post stress.](image2)

![Figure 10. Mean Total Protein Level Shortly Before 3 Hours of Overcrowding-Induced Stress Through 24 Hours Post Stress. SBT: Shortly before stress; IAT: Immediately after stress; 24HAT: 24 hours post stress.](image3)
decrease in albumin level ($p < .0001$) at 24 hours post stress from the value of 37 g/dL to 31 g/dL as against the value of 41.5 g/dL in group 1. Group 1 showed no significant alteration in albumin concentration throughout the study period.

**Total Cholesterol**
The mean TC level shortly before stress and through 24 hours post stress for the two groups showed no significant difference between the groups (Figure 12). The mean TC level in group 2 revealed a significant decrease ($p < .0001$) in TC level compared to group 1 immediately after stress, from the initial value of 5.30 mg/dL to 4.00 mg/dL. Even though there was an increase in total cholesterol (4.7 mg/dL) at 24 hours post stress, it was still significantly lower ($p < .0222$) compared to group 1. Group 1 showed no significant difference in the TC level throughout the study period.

**High-Density Lipoprotein**
The mean HDL level revealed no significant difference between the groups before stress (Figure 13). The mean HDL level for group 2 increased but insignificantly ($p = .172$) compared to group 1 immediately following stress, from the initial value of 1.70 mg/dL to 2.10 mg/dL. Although the HDL level in group 2 decreased through 24 hours post stress, it was still slightly higher compared to group 1. There was no significant alteration in HDL level for group 1 throughout the study period.

**Low-Density Lipoprotein**
The mean LDL level shortly before exposure to stress for the two groups showed no significant difference between the groups, as depicted in Figure 14. However, the mean LDL level in group 2 declined significantly ($p < .0003$) to 2.10 mg/dL from the initial value of 2.7 mg/dL as against the value of 2.60 mg/dL in group 1. At 24 hours post stress, however, there was an increase in LDL level (2.50 mg/dL), even though it was still lower compared to group 1. Group 1, on the other hand, recorded no significant change in LDL value throughout the study period.

**Triglyceride**
The mean triglyceride level shortly before overcrowding-induced stress through 24 hours post stress is depicted in Figure 15. Triglyceride results analysis revealed that prior to the overcrowding-induced stress, there was no significant difference between the groups. Triglyceride levels in group 2 increased significantly ($p < .0001$) from the initial value of 1.20 mmol/L to 3.10 mmol/L as against the value of 1.30 mmol/L in group 1 immediately following overcrowding-induced stress. At 24 hours post stress however, the triglyceride level declined to 1.7 mmol/L which was still significantly higher ($p < .0014$) compared to group 1. Group 1 showed no significant alteration in triglyceride level throughout the study period. Analysis of the overall results showed the triglyceride level in group 1 was significantly higher ($p < .0001$) compared to group 1.
Mean Bicarbonate Concentration

The mean bicarbonate level before and following overcrowding-induced stress is shown in Figure 16. Analysis of the results showed that there were no significant alterations in bicarbonate concentration between the groups prior to stress. The mean bicarbonate concentration in group 2 revealed a significant decrease ($p < .015$) compared to group 1 immediately following stress. The bicarbonate level in group 2 increased but was lower compared to group 1 through 24 hours post stress. However, the bicarbonate level in group 1 expectedly recorded no significant alteration throughout the period of the study.

Mean Chloride Concentration

The mean chloride concentration before stress for the two groups revealed there was no significant difference between the groups (Figure 17). The mean chloride concentration in group 2 did not record any significant alteration compared to group 1 immediately following stress. At 24 hours post stress however, chloride concentration decreased significantly ($p < .0002$) to 91 mmol/L from the initial value of 103 mmol/L. Chloride concentration for group 1 on the contrary recorded insignificant alteration throughout the study period. General analysis of chloride concentration showed that the chloride concentration of group 2 was significantly lower ($p < .0001$) compared to group 1.
Potassium Concentration
The mean potassium concentration before stress for the two groups showed no significant difference between the groups (Figure 18). The mean potassium level in group 2, however, decreased significantly from the initial value of 3.5 mmol/L to 3.10 mmol/L compared to group 1, which recorded 3.5 mmol/L. Group 1 recorded no significant alteration in potassium concentration throughout the period of study.

Sodium Concentration
The mean sodium concentration prior to overcrowding-induced stress for the two groups showed no significant difference between the groups (Figure 19). The mean sodium concentration in group 2 increased but not significantly compared to group 1 immediately following 3 hours of overcrowding-induced stress. Twenty-four hours post stress, however, the sodium concentration increased significantly (p < .0266) from the initial value of 140 mmol/L to 145 mmol/L compared to group 1, which recorded 139 mmol/L. Group 1 recorded no significant alteration in sodium concentration throughout the study period.

Principal Component Analysis
Principal component analysis for group 2 analytes immediately after stress revealed in component 1, all analytes were significantly influenced by overcrowding-induced stress total bilirubin and triglyceride, with an eigenvalue of 12.2939 and a percentage variance of 64.705%. In component 2, analytes such as ALT, ALP, AST, protein, uric acid, potassium, conjugated bilirubin, glucose, and LDH are significantly influenced by overcrowding stress, while albumin, cholesterol, HDL, triglyceride, creatinine, urea, total bilirubin, sodium, chloride, and bicarbonate were not significantly influenced by the overcrowding stress. The second component has an eigenvalue of 4.0992 and a percentage variance of 21.575%. In component 3, only triglyceride, total bilirubin, and bicarbonate were significantly influenced by the overcrowding stress, and the eigenvalue was 1.9867 and the percentage variance was 10.456%. In component 4, however, only HDL was significantly influenced by the overcrowding-induced stress, with an eigenvalue of 0.6203 and a percentage variance of 3.264%. Principal component analysis for group 2 analytes 24 hours post stress showed in component 1, all the analytes were significantly influenced by overcrowding-induced stress except ALT, sodium, and glucose, with an eigenvalue of 11.58 and a percentage variance of 60.95% (Table 1). In the second component, however, ALT, albumin, uric acid, potassium, total bilirubin, and triglyceride were not significantly influenced by overcrowding-induced stress in the catfish, while the rest were significantly influenced by stress, with an eigenvalue of 6.39 and a percentage variance of 33.64%. In the third component, only ALT was significantly influenced by the stress, with an eigenvalue of 1.02 and a percentage variance of 5.38%. The cumulative variance was 99.97%.

Discussion
The findings of marked hyperglycemia in group 2 immediately after stress agree with the findings in earlier studies (Dobšíková et al., 2009; Rosa et al., 2019), where the authors reported that an immediate increase in glucose level observed during stress was induced by the secretion of catecholamines, particularly epinephrine, which subsequently stimulate glycogenolysis in carp fish subjected to transportation and stocking density-induced stress.

Alanine aminotransferase, which is a leakage enzyme produced by hepatocytes, is usually employed as an indicator of hepatocellular damage. Even though no study has evaluated ALT in *H. longifilis* under overcrowding-induced stress, the significant increase in ALT observed in group 2 compared to group 1 immediately after stress is in accordance with the findings of Pakhira et al. (2015), where *Labeo rohita* juveniles exposed to overcrowding, packing, and transportation stress were found to have an increased ALT level. In a similar manner, higher ALT levels were reported by Dobšíková et al. (2009) in common carp exposed to high-density-induced stress. The increase in the ALT level shows that overcrowding-induced stress is associated with hepatocellular injuries, which could have resulted in the
The significant increase in ALP level in this study both immediately after stress and at 24 hours post overcrowding-induced stress in group 2 could be attributed to the enhancement of fish basal metabolism during stress, and this corroborates with the results obtained by Dobsikova et al. (2006), who reported an increase in ALP level while loading fish in preparation for transportation. The increase in ALP level may be linked to cholestasis and cellular damage induced by the stress, which might have led to leakage of ALP into the circulation (Siddique & Kowdley, 2012).

The significant increase in the level of AST in group 2 compared to group 1 both immediately after overcrowding-induced stress and at 24 hours after stress could be linked to compromised hepatic function, consequently resulting in the accumulation of AST in the bloodstream due to hepatocellular damage (Pakhira et al., 2015). Our findings corroborate the findings of Pakhira et al. (2015), where the authors recorded an increased AST level in fish packed at rates of 67 g/L, 134 g/L, and 201 g/L, respectively, for 2 hours and 30 minutes and similarly found a significant increase in AST level. In a related study, AST was reported to increase significantly in common carp when they were exposed to 7- and 12-hour stress (Dobšikova et al., 2009). The significant increase in the creatinine level in group 2 compared to group 1 at 24 hours after overcrowding-induced stress could be an indication that the stress-induced renal insufficiency is consequently leading to inadequate clearance of creatinine by the kidney. The observed increase in the level of creatinine agrees with the findings of Chatterjee et al. (2004), who similarly observed increase in creatinine level in L. rohita juveniles following stress and linked the increase to renal impairment. The observed an significant increase in urea level at 24 hours after stress shows that overcrowding-induced stress has a significant impact on urea level. This increase could be attributed to increased arginase activity, resulting in urea buildup in circulation (Napulitano et al., 2021).

When fish are stressed, particularly at high density, their excretory products accumulate in the water, and consequently, huge volumes of carbon dioxide, ammonia, and a small amount of uric acid are continually produced (Smith & Ramos, 1980). The accumulated ammonia is detoxified in the fish by converting it into a relatively nontoxic form such as urea or uric acid, which is subsequently excreted by the kidney (Giordano et al., 2015). The observed significant increase in uric acid both immediately after stress and 24 hours after stress could be attributed to renal insufficiency, which could have led to its accumulation (Jalal, 2016). The observation of hyperbilirubinemia in group 2, which was significantly high both immediately after stress and 24 hours after stress, shows an imbalance between the breakdown of red blood cells to bilirubin and the production of conjugated bilirubin.
biliarubin by the liver using glucuronic acid (Tsai & Tarng, 2018). This could be due to hepatic insufficiency, consequently leading to the accumulation of bilirubin in circulation. This finding further correlates the findings of an increase in ALT and AST in this study, which were all attributed to hepatocellular injury (Pakhira et al., 2015). This study appears to be the first to evaluate the level of bilirubin in fish in association with overcrowding-induced stress.

Once bilirubin is conjugated, it is secreted into the gall bladder, from where it is released into the gastrointestinal tract via the bile duct and eventually excreted through feces and urine (Sticova & Jirsa, 2013). In this study, conjugated bilirubin in group 2 was found to increase significantly both immediately after stress and 24 hours after stress compared to group 1, implying overcrowding-induced stress has a significant impact on the level of circulation conjugated bilirubin. The increase could be attributed to bile duct injury, blockage, or cholestasis, and this would have led to the inflow of conjugated bilirubin back into circulation (Hastuti et al., 2019). The indication of renal insufficiency earlier opined based on increased serum creatinine levels in this study could perhaps explain the accumulation of conjugated bilirubin in circulation (Chatterjee et al., 2004). It was found in this study that there was marked hypoproteinemia in group 2, both immediately after stress and 24 hours after stress. The observed decrease (hypoproteinemia) could be a result of hydration in serum due to osmotic imbalance between the extracellular and intracellular compartments and the breakdown of protein (Milligan, 1982). In addition, since the liver is a major organ that synthesizes and exports serum protein, the opined hepatocellular injury indicated by the increase in AST and ALT levels could have played a role in the observed hypoproteinemia (Anderson et al., 2011; Pakhira et al., 2015). The findings further confirmed those reported by Dobsikova et al. (2009), where total protein was found to decrease when common carp fish were exposed to transportation stress.

In this study, there was marked hypoalbuminemia in group 2 both immediately after stress and 24 hours after stress. The observed marked hypoalbuminemia could be due to its degradation and utilization for metabolic purposes during stress (Adeyemo, 2005). Even though this study appears to be the first to evaluate serum albumin in catfish following overcrowding-induced stress, the observed hypoalbuminemia could be an indication of weak innate immunity (Kaleseswaran et al., 2012). Since albumin is predominantly synthesized in the liver, the hypoalbuminemia observed could be attributed to hepatocellular injury indicated by other biomarkers such as ALT and AST, as earlier reported in this study. In the presence of hepatocellular injury, the rate of synthesis of albumin will logically decline, leading to hypoalbuminemia (Pakhira et al., 2015). The observed hypercholesterolemia could be attributed to hepatocellular injury, as indicated by the concurrently marked increase in other biomarkers such as ALT and AST (Pakhira et al., 2015). Similarly, Olufemi et al. (2010) reported hypercholesterolemia in an experiment where fish were subjected to stress.

Lipoproteins are classified based on their densities, and they play vital roles in the transportation of cholesterol from peripheral tissues to the liver. Since this appears to be the first study to evaluate lipoproteins in H. longifilis subjected to overcrowding-induced stress, the increase in lipoprotein observed could be attributed to overcrowding-induced stress on the catfish, which could have led to a reduction in protein synthesis by the liver (Pakhira et al., 2015).

The observed increase in the level of HDL could be as a result of hepatocellular injury and/or cholestasis, as typified by the observed concurrent increase in ALT and AST (Baruch et al., 1984; Pakhira et al., 2015). The observed decrease in LDL in group 2 could again be attributed to hepatic insufficiency, which was indicated by the concurrent elevated levels of ALT and AST (Pakhira et al., 2015). This shows that stress via transportation plays a key role in reducing the level of LDL in the catfish. This implies that while overcrowding-induced stress has a tremendous detrimental effect on the health status of catfish as seen in this study, paradoxically, it also makes the catfish healthier for consumption (Froyen, 2021). The observed increase in triglyceride level in group 2 immediately after stress compared to group 1 may be attributed to enhanced lipid synthesis and/or reduced lipid catabolism. The increase in the level of triglyceride is in agreement with Refaey et al. (2017), in which experimental fish were subjected to transport stress for 0, 1, and 6 hours, and the level of triglyceride was found to increase, as similarly observed in this study.

The observed marked increase in bicarbonate level immediately after stress and 24 hours after stress in group 2 could be attributed to metabolic alkalosis. This metabolic alkalosis could be due to an increase in the excretion of carbon dioxide into the small water volume, consequently raising the bicarbonate proportion (Randall & Tsui, 2006). These findings agree with the findings of Randall and Tsui (2006), who noted a decrease in the pH of water and linked the decrease to carbon dioxide loss to the surrounding water through the gills of the fish. The marked hypochloremia observed in group 2 at 24 hours after stress could be attributed to a possible distortion in osmoregulation in the catfish due to the stress, as similarly reported by other scholars (Good et al., 2009). Stress due to overcrowding could lead to osmotic and ionic disorders owing to diuresis and loss of blood electrolytes, including chloride, and this could result in hypochloremia, as observed in this study (Refaey & Li, 2018). Diverse stress conditions, such as physical exhaustion, hypoxia, and high density, as in the case of this study, may cause ion imbalance in fish. In a related study, Urbinati and Carneiro (2006) observed a decline in serum chloride in Matrinixá (Brycon sp.) juveniles following transportation-induced stress, as similarly recorded in this study. This perhaps shows that distortions in electrolyte balance induced by stress may vary among species (Rosa et al., 2019).

The observed hypokalemia in group 2 could be attributed to the distortion of electrolyte balance induced by the stress (Rosa et al., 2019). This finding portrays the fact that stress is associated with distortions in electrolyte balance (Refaey & Li, 2018). The marked hypernatremia observed in group 2 at 24 hours after stress when compared to group 1 could be attributed to the loss of mucus during the overcrowding stress which might have led to an increase in electrolyte loss from the blood, consequently resulting in hypernatremia in the catfish. Stress has been reported to be associated with the loss of electrolytes from the blood and consequently hypernatremia, as similarly observed in this study (Becker et al., 2012; Rosa et al., 2019). In a related study (Urbinati et al., 2006), in which Brycon amazonicus was subjected to 4 hours of transportation-induced stress, it was found that the fish required more than 24 hours to recover from the stress, as similarly observed in this study. The changes in the level of serum potassium, sodium, and chloride observed in this study are an indication that there is a distortion of osmotic balance as a result of overcrowding-induced stress. Hence, the increase in sodium
and decrease in chloride levels in group 2 could be linked to stress-induced cations and anions distortion, respectively (Magnoni et al., 2018).

Large datasets are increasingly common and are often difficult to interpret. Principal component analysis is a technique for reducing the dimensionality of such datasets, increasing interpretability, and at the same time minimizing information loss. It does so by creating new uncorrelated variables that successively maximize variance. In this study, 19 variables were included, and the first three PCs accounted for 64.7%, 21.56%, and 10.3%, respectively, for IAT, while for 24HAT, the first three PCs accounted for 61.0%, 33.64%, and 5.4%, respectively, of the total variation in the data set. These are very good approximations, as they represent 96.6% and 87.9% of the total variations in the first and second PCs, respectively. This is in agreement with the analysis reported by Jolliffe and Cadima (2016), where it was reported that PCs represent 95% of the total variation.

Conclusion and Recommendations

Based on the findings of this study, it was concluded that 3-hour overcrowding-induced stress in catfish has a significant impact on the health status of the catfish, as revealed by serum biochemistry and electrolyte distortions in the catfish. Hence, effort should be made to mitigate the magnitude of stress induced by overcrowding.

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References


Tsai, M. T., & Tarrg, D. C. (2019). Beyond a measure of liver function - Bilirubin acts as a potential cardiovascular protector in chronic kidney disease patients. *International Journal of Molecular Sciences*, 20(1), 117. [CrossRef]